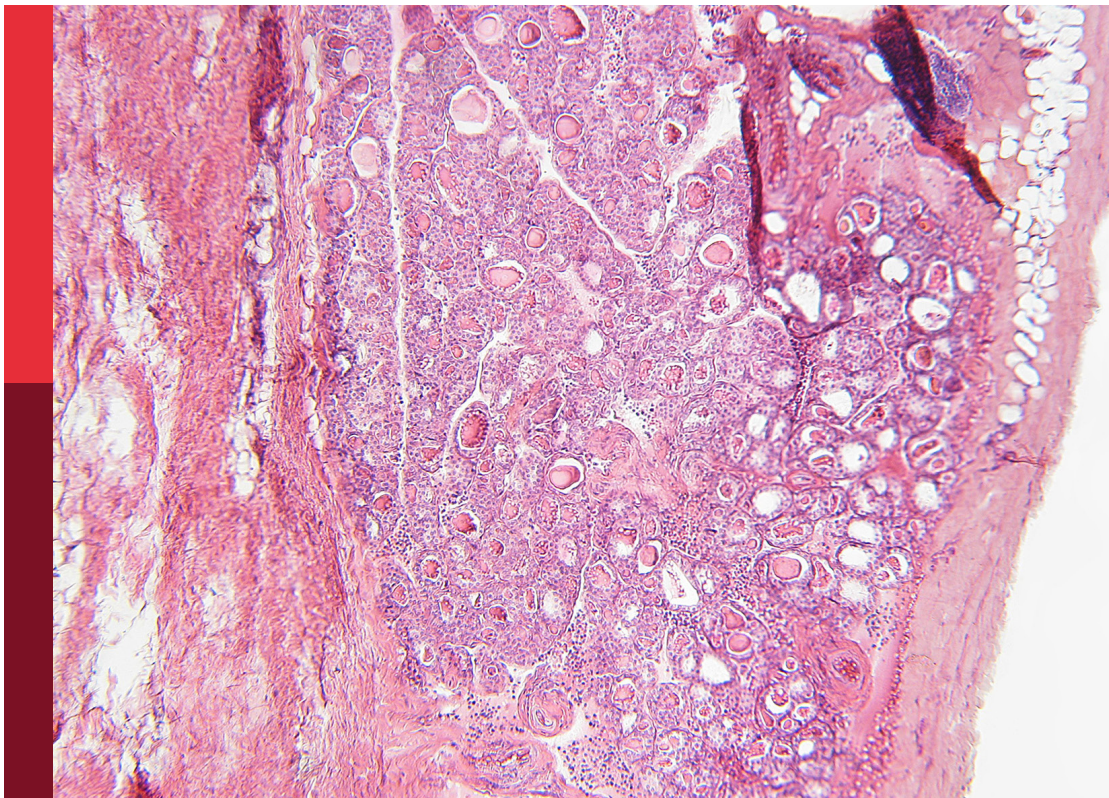
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Novel treatment strategies for thyroid autoimmunity and thyroid cancer

Edited by
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Novel treatment strategies for thyroid autoimmunity and thyroid cancer

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Age-based factors modulating the required thyroxine dose to achieve thyrotropin suppression in intermediate-and high-risk papillary thyroid cancer

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Background: Guidelines widely recommend thyrotropin suppression to reduce the risk of recurrence in intermediate- and high-risk papillary thyroid cancer (PTC) after total thyroidectomy. However, an insufficient or excessive dosage may result in a number of symptoms/complications especially in older patients.

Patients and methods: We constructed a retrospective cohort including 551 PTC patient encounters. Using propensity score matching and logistic regression models, we determined the independent risk factors affecting levothyroxine therapy at different ages. Our outcomes included: expected TSH level and an unexpected TSH level, which was based on the initial thyroid-stimulating hormone (TSH) goal< 0.1 mIU/L with usual dosage of L-T4 (1.6 μg/kg/day).

Results: From our analysis, more than 70% of patients undergoing total thyroidectomy did not achieve the expected TSH level using an empirical medication regimen, and the effect of the drug was affected by age (odds ratio [OR], 1.063; 95% CI, 1.032-1.094), preoperative TSH level (OR, 0.554; 95% CI, 0.436-0.704) and preoperative fT3 level (OR, 0.820; 95% CI, 0.727-0.925). In patients with age < 55 years old, preoperative TSH level (OR, 0.588; 95% CI, 0.459-0.753), and preoperative fT3 level (OR, 0.859; 95% CI, 0.746-0.990) were two independent protective factors, while, in patients with age ≥ 55 years old, only preoperative TSH level (OR, 0.490; 95% CI, 0.278-0.861) was the independent protective factors to achieve expected TSH level.

Conclusion: Our retrospective analysis suggested the following significant risk factors of getting TSH suppression in PTC patients: age (≥55 years), lower preoperative TSH and fT3 levels.

KEYWORDS

thyrotropin suppression, intermediate-and high-risk differentiated thyroid cancer, individualized medication regimens, age stratification, thyroid cancer

1 Introduction

Over the past several decades, there has been an increase in the incidence of thyroid cancer, with the vast majority being papillary thyroid cancer (PTC) (1). Although the 5-year survival rate of PTC is 90% or more, the risk of cancer recurrence is 5-20% or even higher in patients judged to be at intermediate to high risk. Total thyroidectomy is recommended by the American Thyroid Association (ATA, 2015 edition) to reduce the risk of recurrence (2). After total thyroidectomy, levothyroxine (LT4) is prescribed to replace the function of the removed thyroid gland. Meanwhile, a supraphysiological dose of LT4 is applied to suppress the secretion of thyroid-stimulating hormone (TSH), which further reduces the risk of recurrence (3, 4).

For patients with different levels of predicted risks, different postoperative TSH targets are recommended. In addition, the benefits of TSH suppression treatment could be outweighed by the side effects as age advances. It is widely acknowledged that iatrogenic thyrotoxicosis resulting from aggressive suppression therapy is associated with an increased risk of fracture and cardiovascular events (5, 6), 2- to 4-fold higher in elderly patients (7, 8). Organ declines and adaptive changes in the hypothalamic-pituitary-thyroid axis of aged individuals, who are at high risk of thyroid cancer (2, 9) and consequently the main group receiving thyroidectomy (1), complicate the selection of the individual dosage of LT4. Even though optimal TSH goals were set to balance the adverse effects of TSH suppression and recurrence risk, individual variations in responses to LT4 remain unresolved, and there is no appropriate LT4 dosage prediction strategy for patients, especially for the aged.

In clinical routine, patients are usually prescribed an initial dosage LT4 of 1.6 μg/kg/day at the beginning of suppression therapy (10). However, the required LT4 dosage is affected by more than just body weight (BW). It has been suggested that body mass index (BMI) and body surface area (BSA) may predict the initial dosage more effectively (11, 12). Sex, age, and autoimmune diseases have also been reported to affect the LT4 requirement (13–16). Drugs and comorbidities can interfere with the treatment effect by disturbing the absorption or metabolism of LT4 (17–19). In addition, LT4 requirements are apparently affected by environmental temperature since thyroid hormones play an important role in regulating metabolism. The metabolism rate and function of the endocrine system vary with advancing age (20, 21), which accounts for different responses to LT4 in people of different ages. It is necessary to develop a specific strategy for LT4 dosage selection for elderly patients.

Abbreviations: PTC, papillary thyroid cancer; ATA, the American Thyroid Association; LT4, levothyroxine; TSH, thyroid-stimulating hormone; BW, body weight; BMI, body mass index; BSA, body surface area; PSM, propensity score matching; HT, Hashimoto's thyroiditis; TPOAb, thyroid peroxidase antibodies; TgAb, anti-thyroglobulin antibodies; fT3, free T3; fT4, free T4; SD, standard deviation; CI, confidence interval; ROC, receiver operating characteristic curve; AUC, the area under the ROC curve; AJCC, American Joint Committee on Cancer.

In this study, we sought to identify the effect that age has on the treatment efficiency of LT4. Patients undergoing initial total thyroidectomy with intermediate to high risk were recruited and treated with LT4 following the guidance of the American Thyroid Association (ATA, 2015 edition) (2). We conducted propensity score matching (PSM) to diminish the effect of BW and ambient temperature. Other characteristics of the LT4-sensitive groups were explored, including age, sex, BMI, BSA, thyroid thickness, preoperative thyroid function, Hashimoto's thyroiditis (HT), hypertension and diabetes mellitus to offer a theoretical basis for individual medicine care during suppression therapy.

2 Materials and methods

2.1 Patients and procedures

This study is a single-center retrospective analysis that received approval from the medical ethics committee at Xiangya Hospital (20211245). Patients who met the following requirements were included in the study: (1) initially undergoing total thyroidectomy for thyroid cancer with an intermediate or high risk for recurrence (ATA, 2015 edition) (2); (2) taking LT4 per day based on the 1.6 μg/kg dosing regimen; (3) clinicopathologic data complete; and (4) no thyroid hormones or iodine given before surgery. The cohort was followed from January 2016 to October 2019 at the Department of General Surgery, Xiangya Hospital of Central South University.

A total of 551 patients met the criteria and were enrolled in this study. All the patients completed a physical examination, thyroid function test, ultrasonography of the thyroid, and measurements of serum thyroid peroxidase antibodies (TPOAb) and anti-thyroglobulin antibodies (TgAb) before surgery and were followed up at 1 month for postoperative TSH measurement. All tests used immunochemiluminometric assays conducted at the same laboratory. The reference ranges are 2.8-7.1pmol/l for serum free T3 (fT3), 12~22pmol/l for serum free T4 (fT4), and 0.27-4.2mIU/L for TSH.

According to the American Thyroid Association, initial TSH suppression to below 0.1 mIU/L is recommended among thyroid cancer patients at high risk of recurrence (ATA, 2015 edition) (2). At the first follow-up after surgery, the TSH suppression treatment effect was classified into the expected level or an unexpected level, corresponding to a serum TSH level <0.1 mIU/L and ≥0.1 mIU/L. We further divided the unexpected level into two groups, the replacement group and the hypothyroidism group, and the corresponding TSH levels were 0.1~4.2 mIU/L and ≥4.2 mIU/L, respectively.

2.2 Clinicopathological characteristic

To investigate the clinicopathological characteristics of patients who obtained different treatment effects, the variables collected included age, sex, BMI, BSA, average temperature during operation month, preoperative serum TSH, preoperative serum fT3, preoperative serum fT4, thyroid thickness, HT, and comorbidities

including hypertension and diabetes mellitus. The mean temperature during the operative month is available on the website of the China Meteorological Administration. The thyroid thickness was calculated by the average of the left and right glands obtained from the preoperative ultrasound report.

2.3 Statistical analysis

SPSS 26.0 software was used to conduct the statistical analyses. Continuous data are presented as the mean and standard deviation (SD), and the Mann–Whitney U test was used for comparisons between groups. The binary data are expressed as the patient number with their percentages, and the chi square test was used for comparisons between two groups. PSM was performed by using the nearest-neighbor matching method with a caliper distance of 0.02 without replacement between chosen groups. Weight and ambient temperature were used to calculate the propensity score to perform the matching. Significant variables with P <0.05 after PSM were entered into the multivariate binary logistic regression analysis to identify the factors affecting the treatment outcome. The effect of the factors was presented with the OR and 95% confidence interval (CI). A level of P<0.05 was used to indicate significance, and all statistical tests were two-tailed. A receiver operating characteristic curve (ROC) analysis was carried out to quantify the prediction performance of the identified factors, and the value of the area under the ROC curve (AUC) was calculated.

3 Results

3.1 More than 70% of patients did not achieve the expected TSH level using an empirical medication regimen

We selected 551 patients from the database who took LT4 (Euthyrox, Merck KGaA) at 1.6 μg/kg per day after total thyroidectomy, including 425 women (76.2%) and 126 men (23.8%) at a ratio of 3.4:1. Their mean age was 43.93 ± 11.18 years, ranging from 18 to 73 years. The average weight, height, BMI, and BSA of the 551 patients were 61.61 ± 10.48 kg, 161.05 ± 6.75 m, 23.70 ± 3.26 kg/m^2, and 1.66 ± 0.16 m2, respectively. Preoperative thyroid function included TSH (3.01 ± 2.57 mIU/L), fT3 (7.12 ± 5.02 pmol/L), and fT4 (13.67 ± 6.44 pmol/L). The average thyroid thickness was 16.38 ± 4.04 mm before surgery. Hashimoto's thyroiditis, hypertension and diabetes mellitus were found in 127 patients (23%), 75 patients (13.6%), and 21 patients (3.8%), respectively (Table 1).

The detailed characteristics of the different treatment effects are summarized in Table 1. A total of 23.6% of patients had their TSH suppressed under 0.1 mIU/L, 46.8% of the patients were euthyroid, and 29.8% were hypothyroid at the first follow-up. There were significant differences between the two groups with different TSH suppression levels, including age, sex, height, weight, BMI, BSA, ambient temperature, preoperative serum fT3 and preoperative serum TSH (all p<0.001), and preoperative serum fT4(p=0.013). Among patients who obtained the expected level, women distinctly made up the majority compared with patients who had an unexpected level (expected level 94.4% women vs. unexpected level 71.7% women). Patients in the unexpected level group had higher fT3 (expected level 4.92 ± 1.73 pmol vs. unexpected level 7.82 ± 5.48 pmol) and higher TSH (expected level 1.84 ± 1.31 mIU/L vs. unexpected level 3.37 ± 2.76 mIU/L) and lower fT4 (expected level 16.12± 4.02 pmol/l vs. unexpected level 12.91 ± 6.85 pmol/l). Their weight showed an increasing trend, while ambient temperature showed a decreasing trend as the treatment effect weakened. There were no significant differences in the categorical data except for sex between the two levels (diabetes mellitus, p = 0.306; hypertension, p = 0.430; Hashimoto's thyroiditis, p = 0.155) (Table 1).

3.2 Age, preoperative TSH level and fT3 level were independent risk factors after PSM

Ambient temperature (p <0.001) and weight (p <0.001) had significant differences between the expected level and unexpected level before PSM. All the other variables also showed significant differences between the two levels. After 1:1 PSM, weight and mean temperature reached an equilibrium (weight, p =0.338; ambient temperature, p = 0.236). Sex and BSA were not significantly different between the matched groups, while age, height, BMI and preoperative fT3, fT4, and TSH remained significantly different between the two groups. Age seemed to have a higher possibility of being different between the two groups (p<0.001).

We further explored the potential prognostic factors for obtaining the expected level using univariate binary logistic regression analysis. Age (p<0.001, OR=1.063, 95% CI, 1.032-1.094), preoperative TSH (p<0.001, OR=0.554, 95% CI, 0.436-0.704) and preoperative fT3 (p=0.001, OR=0.820, 95% CI, 0.727-0.925) were all significantly associated with the treatment effect (Table 2). ROC analyses were performed to quantify the prediction performance of the three identified factors. Preoperative fT3 was identified as the most effective prediction factor, with an AUC of 0.776, followed by age, with an AUC of 0.703. Using age and preoperative fT3 as co-prediction factors had the highest AUC of 0.825 (Figure 1).

3.3 Male sex and preoperative fT3 levels were independent risk factors leading to inadequate efficacy of LT4 dosage

Between the replacement group and hypothyroid group, ambient temperature showed a significant difference (p<0.001), while weight was already equilibrated (p=0.065). After 1:1 PSM, the ambient temperature reached equilibrium. Other variables,

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TABLE 1 Demographics and clinical characteristics of 551 PTC patients who underwent total thyroidectomy.

Variables	Total	Expected level	Unexpected level			*p*
			Total	Replacement	Hypothyroidism	
	n (%)/mean ± SD	n (%)/mean ± SD	n (%)/mean ± SD	n (%)/mean ± SD	n (%)/mean ± SD	
Number	551 (100)	130 (23.6)	421 (76.4)	258 (46.8)	163 (29.8)	
Sex, Female	425 (76.2)	123 (94.4)	302 (71.7)	201 (77.9)	101 (61.8)	**<0.001**[a]
Age, year	43.93 ± 11.18	47.80 ± 11.06	42.73 ± 10.96	42.78 ± 10.71	42.67 ± 11.18	**<0.001**[b]
Height, m	161.05 ± 6.75	158.12 ± 5.53	161.95 ± 6.84	161.25 ± 6.67	163.06 ± 6.97	**<0.001**[b]
Weight, kg	61.61 ± 10.48	56.33 ± 6.99	63.24 ± 10.84	62.36 ± 10.18	64.62 ± 11.71	**<0.001**[b]
BSA, m^2	1.66 ± 0.16	1.57 ± 0.11	1.68 ± 0.17	1.67 ± 0.16	1.71 ± 0.18	**<0.001**[b]
BMI	23.70 ± 3.26	22.53 ± 2.60	24.06 ± 3.36	23.96 ± 3.33	24.20 ± 3.42	**<0.001**[b]
Thyroid thickness, mm	16.38 ± 4.04	16.22 ± 4.37	16.43 ± 3.94	16.27 ± 3.80	16.68 ± 4.14	0.202[b]
Ambient temperature, °C	17.33 ± 8.93	20.38 ± 7.65	16.39 ± 9.10	19.10 ± 8.38	12.11 ± 8.55	**<0.001**[b]
Preoperative TSH, mIU/L	3.01 ± 2.57	1.84 ± 1.31	3.37 ± 2.76	2.78 ± 1.92	4.30 ± 3.52	**<0.001**[b]
Preoperative fT3, pmol/L	7.12 ± 5.02	4.92 ± 1.73	7.82 ± 5.48	5.46 ± 2.97	11.56 ± 6.40	**<0.001**[b]
Preoperative fT4, pmol/L	13.67 ± 6.44	16.12 ± 4.02	12.91 ± 6.85	15.58 ± 4.67	8.69 ± 7.62	**0.013**[b]
Post-operative fT3, pmol/L	8.44 ± 7.60	5.76 ± 1.34	9.27 ± 8.49	5.66 ± 3.84	14.99 ± 10.48	**<0.001**[b]
Post-operative fT4, pmol/L	17.48 ± 9.12	24.94 ± 4.56	15.18 ± 8.95	20.08 ± 5.11	7.42 ± 8.21	**<0.001**[b]
Type II diabetes						
With	21 (3.8)	3 (2.4)	18	11 (4.2)	7 (3.3)	<0.306[a]
Without	530 (96.2)	127 (97.6)	247	247 (95.8)	156 (96.7)	
Hypertension						
With	75 (13.6)	15 (12.1)	60	42 (15.8)	18 (11.2)	<0.430[a]
Without	476 (86.4)	115 (87.9)	361	216 (84.2)	145 (88.8)	
Hashimoto's thyroiditis						
With	127 (23.0)	24 (17.7)	103	63 (23.3)	40 (25.0)	<0.155[a]
Without	424 (77.0)	106 (82.3)	318	195 (76.7)	123 (75.0)	

Participants were grouped according to the TSH levels at 1 month on suppression therapy with a daily L-T4 dose of at least 1.6 μg/kg after total thyroidectomy. The expected level was defined as TSH levels below physiological levels (<0.1mIU/L), the replacement group was TSH levels equal to physiological levels (0.1~4.2 mIU/L), and the hypothyroidism group was defined as TSH levels above physiological levels (≥4.2 mIU/L). Variables with statistical significance are shown in bold, [a] chi-square test, [b] Mann–Whitney test.

including sex, height, BSA, BMI, preoperative serum TSH, preoperative serum fT3 and preoperative serum fT4 (all p<0.001), were significantly different before PSM. After PSM, only sex, preoperative serum TSH, preoperative serum fT3 and preoperative serum fT4 (all p<0.001) were distributed differently between the replacement and hypothyroidism groups. The multivariate binary logistic regression analysis showed that sex and preoperative fT3 were the two factors that affected the treatment effect. Male sex (p<0.001, OR=3.248, 95% CI, 1.708-6.177) and patients with higher preoperative fT3 (p=0.001, OR=1.248, 95% CI, 1.101-1.158) were more likely to be in above-target TSH state (Table 3).

3.4 Patients with low preoperative TSH and fT3 require lower dosage if aged under 55 years old

It has now been established that age is a factor that influences whether the expected TSH level is met, and we identified additional factors that influence the treatment effect of LT4 in different age groups. We divided the patients into two age groups based on age, using 55 years as the cut-off value according to the 8th American Joint Committee on Cancer (AJCC) staging system (18). We also conducted PSM before multivariate binary logistic regression. In the group under 55 years old, sex, height, BMI,

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TABLE 2 Variables before and after PSM between the expected and unexpected level.

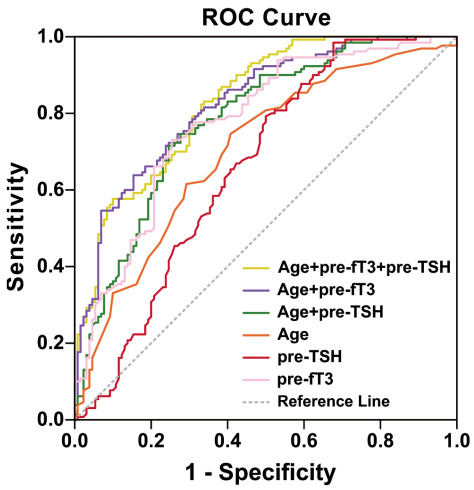
Variables	Before PSM			After PSM			p*	OR (95% CI)
	Unexpected level (n=421)	Expected level (n=130)	p	Unexpected level (n=130)	Expected level (n=130)	p		
Weight, kg	63.24 ± 10.84	56.33 ± 6.99	<**0.001**[b]	55.32 ± 6.07	56.33 ± 6.99	0.338[b]		
Ambient temperature, °C	16.39 ± 9.10	20.38 ± 7.65	<**0.001**[b]	18.52 ± 9.24	20.38 ± 7.65	0.236[b]		
Age	42.73 ± 10.96	47.80 ± 11.06	<**0.001**[b]	39.74 ± 11.19	47.80 ± 11.06	<**0.001**[b]	<**0.001**	1.063 (1.032,1.094)
Sex								
Male	119 (28.3%)	7 (5.4%)	<**0.001**[a]	14 (10.8%)	7 (5.4%)	0.111[a]		
Female	302 (71.7%)	123 (94.6%)		116 (88.2%)	123 (94.6%)			
Height, m	161.95 ± 6.84	158.12 ± 5.53	<**0.001**[b]	159.65 ± 5.03	158.12 ± 5.53	**0.020**[b]	0.137	
BMI	24.06 ± 3.36	22.53 ± 2.60	<**0.001**[b]	21.71 ± 2.19	22.53 ± 2.60	**0.010**[b]	0.372	
BSA, m^2	1.68 ± 0.17	1.57 ± 0.11	<**0.001**[b]	1.56 ± 0.10	1.57 ± 0.11	0.791[b]		
Preoperative TSH, mIU/L	3.37 ± 2.76	1.84 ± 1.31	<**0.001**[b]	3.93 ± 3.95	1.84 ± 1.31	<**0.001**[b]	<**0.001**	0.554 (0.436,0.704)
Preoperative fT3, pmol/L	7.82 ± 5.48	4.92 ± 1.73	<**0.001**[b]	8.62 ± 6.01	4.92 ± 1.73	<**0.001**[b]	**0.001**	0.820 (0.727,0.925)
Preoperative fT4, pmol/L	12.91 ± 6.85	16.12 ± 4.02	<**0.001**[b]	12.09 ± 7.95	16.12 ± 4.02	**0.006**[b]	0.579	

Variables with statistical significance were shown in bold. Weight and mean temperature were matched between groups. [a] chi-square test, [b] Mann–Whitney test. p* was the result of binary logistic regression analysis.

preoperative serum TSH and preoperative fT3 were included in the multivariate regression analysis. Only preoperative serum TSH (p<0.001, OR=0.588, 95% CI, 0.459-0.753) and preoperative fT3 (p=0.035, OR=859, 95% CI, 0.746-0.990) were significantly different (Table 4).

3.5 Patients with low preoperative TSH require lower dosage if aged 55 and older

In the group 55 years of age and older, all of the variables were significantly different between the unexpected level and expected



Model	AUC
Age+pre-fT3+pre-TSH	0.832
Age+pre-fT3	0.825
Age+pre-TSH	0.781
Age	0.703
pre-TSH	0.661
pre-fT3	0.776

FIGURE 1
ROC curve and AUC for different model. age (orange line), preoperative TSH (red line), preoperative fT3 (pink line), age+preoperative fT3 (purple line), age+preoperative TSH (green line), age+preoperative TSH +preoperative fT3 (yellow line).

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TABLE 3 Variables before and after PSM between replacement and hypothyroidism groups.

Variables	Before PSM			After PSM			p*	OR (95% CI)
	Replacement (n=258)	Hypothyroidism (n=163)	p	Replacement (n=116)	Hypothyroidism (n=116)	p		
Weight, kg	62.36 ± 10.18	64.62 ± 11.71	0.065[b]	62.54 ± 10.18	63.99 ± 12.25	0.356[b]		
Ambient temperature, °C	19.10 ± 8.38	12.11 ± 8.55	**<0.001**[b]	14.10 ± 7.75	14.46 ± 8.93	0.864[b]		
Age	42.78 ± 10.71	42.67 ± 11.18	0.866[b]	42.45 ± 10.76	41.53 ± 11.62	0.423[b]		
Sex								
Male	57 (22.1%)	62 (38.0%)	**<0.001**[a]	25 (21.6%)	42 (36.2%)	**0.014**[a]	**<0.001**	3.248 (1.708,6.177)
Female	201 (77.9%)	101 (62.0%)		91 (78.4%)	74 (63.8%)			
Height, m	161.25 ± 6.67	163.06 ± 6.97	**<0.001**[b]	161.31 ± 6.08	163.15 ± 7.00	0.059[b]		
BMI	23.96 ± 3.33	24.20 ± 3.42	**<0.001**[b]	24.05 ± 3.22	23.92 ± 3.60	0.710[b]		
BSA, m^2	1.66 ± 0.16	1.71 ± 0.18	**<0.001**[b]	1.67 ± 0.15	1.70 ± 0.19	0.276[b]		
Preoperative TSH, mIU/L	2.78 ± 1.92	4.30 ± 3.52	**<0.001**[b]	2.90 ± 2.26	4.14 ± 4.10	**<0.001**[b]	0.148	
Preoperative fT3, pmol/L	5.46 ± 2.97	11.56 ± 6.40	**<0.001**[b]	5.84 ± 3.60	10.03 ± 6.27	**<0.001**[b]	**0.001**	1.248 (1.101,1.158)
Preoperative fT4, pmol/L	15.58 ± 4.67	8.69 ± 7.62	**<0.001**[b]	14.80 ± 4.79	10.51 ± 7.58	**0.001**[b]	0.370	

Variables with statistical significance are shown in bold. Weight and mean temperature were matched between groups. [a] chi-square test and [b] Mann–Whitney test. p* was the result of binary regression.

level groups before PSM. After PSM, weight (p=0.775) and ambient temperature (p=0.870) were equilibrated. Preoperative TSH was slightly higher after PSM 3.57 ± 2.10 mIU/L vs. before PSM 3.27 ± 1.74 mIU/L in the unexpected level group after PSM and was the only variable that remained significantly different after PSM (p=0.010). In binary logistic analysis, preoperative TSH was an independent protective factor (p=0.013, OR=0.490, 95% CI, 0.278-0.861) (Table 5).

TABLE 4 Variables before and after propensity score matching among patients under 55 years old.

Variables	Before PSM			After PSM			p*	OR (95% CI)
	Unexpected level (n=358)	Expected level (n=95)	p	Unexpected level (n=95)	Expected level (n=95)	p		
Weight, kg	62.74 ± 10.93	56.27 ± 7.40	**<0.001**[b]	55.64 ± 8.18	56.27 ± 7.40	0.322[b]		
Ambient temperature, °C	16.57 ± 9.07	20.24 ± 7.80	**<0.001**[b]	19.13 ± 8.97	20.24 ± 7.80	0.634[b]		
Sex								
Male	97 (27.1%)	4 (4.2%)	**<0.001**[a]	15 (15.8%)	4 (4.2%)	**0.008**[a]	0.114	
Female	261 (72.9%)	91 (95.8%)		80 (84.2%)	91 (95.8%)			
Height, m	162.06 ± 6.87	158.66 ± 5.46	**<0.001**[b]	160.63 ± 5.82	158.66 ± 5.46	**0.034**[b]	0.253	
BMI	23.82 ± 10.84	22.34 ± 6.63	**<0.001**[b]	21.55 ± 8.12	22.34 ± 6.63	**0.009**[b]	0.136	
BSA, m^2	1.68 ± 0.17	1.57 ± 0.12	**<0.001**[b]	1.57 ± 0.13	1.57 ± 0.12	0.707[b]		
Preoperative TSH,mIU/L	3.38 ± 2.90	1.87 ± 1.28	**<0.001**[b]	3.69 ± 4.35	1.87 ± 1.28	**<0.001**[b]	**<0.001**	0.588 (0.459,0.753)
Preoperative fT3, pmol/L	7.72 ± 5.47	4.98 ± 1.89	**<0.001**[b]	7.36 ± 5.26	4.98 ± 1.89	**<0.001**[b]	**0.035**	0.859 (0.746,0.990)
Preoperative fT4, pmol/L	13.20 ± 6.82	16.14 ± 4.23	**0.092**[b]	14.23 ± 7.30	16.14 ± 4.23	0.960[b]		

Variables with statistical significance are shown in bold. Weight and mean temperature were matched between groups. [a] chi-square test, [b] Mann–Whitney test. p* was the result of binary regression analysis.

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TABLE 5 Variables before and after PSM among patients aged 55 and older.

Variables	Before PSM			After PSM			p*	OR (95%CI)
	Unexpected level (n=63)	Expected level (n=35)	p	Unexpected level (n=15)	Expected level (n=15)	p		
Weight, kg	66.07 ± 9.97	56.47 ± 5.82	**<0.001**[b]	59.17 ± 6.96	58.60 ± 6.60	0.775[b]		
Ambient temperature, °C	15.38 ± 9.27	20.77 ± 7.35	**0.006**[b]	18.77 ± 8.25	19.60 ± 8.28	0.870[b]		
Sex								
Male	22(34.92%)	3(8.6%)	**0.004**[a]	4(15.8%)	2(4.2%)	0.361[a]		
Female	41(65.08%)	32(91.4%)		11(84.2%)	13(95.8%)			
Height, m	161.32 ± 6.68	156.66 ± 5.53	**<0.001**[b]	160.13 ± 6.36	156.20 ± 6.55	0.061[b]		
BMI	25.40 ± 3.48	23.06 ± 2.61	**0.001**[b]	23.18 ± 3.37	24.08 ± 2.95	0.285[b]		
BSA, m^2	1.72 ± 0.15	1.57 ± 0.10	**<0.001**[b]	1.62 ± 0.11	1.59 ± 0.11	0.325[b]		
Preoperative TSH, mIU/L	3.27 ± 1.74	1.76 ± 1.41	**<0.001**[b]	3.57 ± 2.10	1.67 ± 1.31	**0.010**[b]	**0.013**	0.490 (0.278,0.861)
Preoperative FT3, pmol/L	8.37 ± 5.47	4.74 ± 1.22	**<0.001**[b]	8.13 ± 5.63	5.18 ± 1.72	0.089[b]		
Preoperative FT4, pmol/L	11.28 ± 6.88	16.06 ± 3.40	**0.004**[b]	11.71 ± 7.38	15.78 ± 4.14	0.367[b]		

Variables with statistical significance are shown in bold. Weight and mean temperature were matched between groups. [a] chi-square test, [b] Mann–Whitney test. p* was the result of binary regression analysis.

4 Discussion

For patients with an intermediate to high risk of recurrence, suppression of TSH is required to reduce the risk of recurrence (22–24). In elderly patients, it is essential to balance the benefits of suppression treatment with the side effects of iatrogenic hyperthyroidism. At present, the factors influencing LT4 medication based on age stratification remain unclear. In this study, we analyzed the factors influencing postoperative LT4 medication based on age in PTC patients with intermediate to high risks of recurrence, which has important value for the formulation of personalized LT4 medication regimens.

Consistent with a previous study, we observed that several factors affected the treatment effect, such as ambient temperature and BMI. Since thyroid hormone is required for normal development as well as regulating metabolism in the adult (25, 26), previous studies usually focused on the effects of body weight (27, 28). In our study, the patients all took LT4 at an empirical regimen of 1.6 μg/kg, and more than 70% did not achieve the expected TSH suppression level. This suggests that there are many factors other than body weight that affect the effect of LT4. Ambient temperature is an important factor affecting the body's metabolism. Therefore, our study considered the influence of ambient temperature on the effect of LT4 medication. We used BW and ambient temperature in the calculation of the propensity score to minimize their impact on the results. Surprisingly, although the effects of weight and ambient temperature were diminished, age was still an independent factor that affected the treatment effect of suppressing TSH levels among patients with an intermediate to high risk of recurrence.

It has been proven that the homeostasis of the endocrine system changes with the advancement of age, as does thyroid hormone regulation. The suppression of TSH by thyroid hormone is greater in elderly individuals (7, 29), which means that under a state of hypothyroidism, elderly individuals have a smaller rise in TSH concentration. In other words, a given dose suppresses the secretion of TSH more with advancing age. Age-related differences in treatment outcomes may be associated with changes in the hypothalamic-pituitary-thyroid axis in different organ hierarchies (20). For such changes, there is no specific age at which the effect begins. Several previous studies indicated that 97.5th percentile of serum TSH was noted to increase by 0.1-0.3 mU/L every 10 years after 30~39 years of age (21, 30, 31). The functional decline of the thyroid gland, causing it to secrete less thyroid hormone, could be the cause of upper reference limit of TSH in elderly individuals. The longer period of elimination of thyroid hormone in elderly individuals seems to result in a better suppression effect of LT4 (32).

Preoperative TSH was an independent factor in both elderly and younger patients after we stratified patients based on age. Our results showed that patients with lower preoperative TSH levels are more likely to achieve a state of TSH suppression after surgery. We suspect that variable changes in the sensitivity or numbers of thyroid hormone receptors in the pituitary and hypothalamus explain this correlation.

The preoperative fT3 level was also shown to be an independent factor among patients under 55 years old. In our study, patients with higher preoperative fT3 were less likely to suppress the

secretion of TSH, which may be the result of the adaptation of the pituitary to higher fT3. An interesting correlation between presurgical fT3 and the treatment effect was also seen in Vincenzo Di Donna's study (11). This result indicates that iodothyronine deiodinases may also function in the suppression of TSH secretion. Since the secretion of TSH is suppressed by fT4 and fT3, the meaning of fT3 or TSH as a predictor may be the same. It is also interesting that fT3 did not show significance among patients aged 55 and over, which is expected due to their decreased metabolism of thyroid hormone (33, 34) or the changes in the sensitivity of the hypothalamic-pituitary-thyroid axis during aging.

Previous studies have shown that age-related thyroid changes differ by sex (35) and sex was indicated to be relative with lymph node metastasis in PTC (36). So, is the age-related level of TSH suppression after LT4 medication also related to sex? In our analysis, sex was not an independent influencing factor for either people aged 55 years older or those younger than 55 years old. We considered that this was because sex hormones have little effect on the response to thyroid hormones in the hypothalamus and pituitary gland.

Even though sex was not identified as an independent factor affecting the TSH suppression level, it was shown to be a factor that led to a worse treatment effect. Among patients with unexpected levels, men were more likely to have TSH levels beyond the physiological range. This means that men require a larger LT4 dose if they cannot suppress TSH in the first month after surgery on the usual dose.

There are some limitations in our study. First, although we tried to determine the relationship between treatment outcome and some comorbidities, the number of patients who had comorbidities was too small and even smaller after PSM to reveal any relationship. In addition, patient adherence to LT4 is uncertain, which may bias our results. In our study, we only tried to determine the predictors of TSH suppression degree. It remains to be studied further how to adjust the initial levothyroxine dose according to known factors to obtain a satisfactory targeting suppression degree.

5 Conclusion

For PTC patients with intermediate to high risk of recurrence, age is an independent risk factor for achieving the target TSH levels through usual dosage of LT4. Under the condition of the same body weight and ambient temperature, it is easier to achieve the target TSH suppression level in patients aged less than 55 years old with lower preoperative TSH levels and fT3 and in patients aged 55 and older with lower preoperative TSH levels (Table 6).

TABLE 6 Identified factors leading to higher LT4 requirement to achieve suppression TSH level.

factors	OR(95%CI)	
	<55 years old	≥55 years old
preoperative fT3	0.859(0.746,0.990)	
preoperative TSH	0.588(0.459,0.753)	0.490(0.278,0.861)

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

This study received approval from the medical ethical committee at Xiangya Hospital (20211245).Written informed consent to participate in this study was waived because of the retrospective and anonymous nature of the study.

Author contributions

Study concepts, R-YZ and PH. Study design, PH and SC. Data acquisition, LA, NT, and NL. Quality control of data and algorithms, PC, ML, and D-JO-Y. Data analysis and interpretation, LA, BW, and Y-XZ. Statistical analysis, Z-EQ and H-LT. Manuscript preparation, R-YZ and PH. Manuscript editing, R-YZ and PH. Manuscript review, PH and SC. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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The efficacy and safety in radioactive iodine refractory thyroid cancer patients treated with sorafenib

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Background: Sorafenib included in Chinese medical insurance is the earliest targeted drug for radioactive iodine refractory differentiated thyroid cancer (RR-DTC). This study is to further demonstrate the clinical efficacy and safety of sorafenib used in Zhujiang Hospital of Southern Medical University.

Methods: RR-DTC patients treated at our Department of Nuclear Medicine in Zhujiang Hospital of Southern Medical University (October 2017–May 2020) were retrospectively analyzed. Treatment effects, progression-free survival (PFS), and adverse effects (AEs) during medication were evaluated.

Results: Of the 31 patients included, 26 patients were evaluated for efficacy with a median follow-up time of 17.5 months (4.0–51.0 months). The disease control rate (DCR) was 57.7% (n = 15) and the objective response rate (ORR) was 26.9% (n = 7). Most patients with disease control had thyroglobulin decreases of more than 60% (p = 0.004), ORRs were favorable in patients with lung metastasis and lung-only metastasis (p = 0.010 and 0.001, respectively). The PFS of the 26 patients analyzed was 16.5 months (95%CI: 14.41 –23.90 months). In the subgroup analysis, female, patients with lung-only metastasis, hand-foot skin syndrome (HFS), and thyroglobulin response ≥ 60% observed longer PFS (p = 0.038, 0.045, 0.035, and 0.000, respectively), while patients with bone metastasis had lower PFS (p = 0.035). The most common toxicity profile was HFS (93.5%), followed by diarrhea (83.9%), alopecia (74.2%). All the side effects were mainly grade 1–2. Grade 3–4 adverse reactions were more common in diarrhea and HFS.

Conclusions: Sorafenib has promising efficacy in RR-DTC, especially in patients with lung metastasis and lung-only metastasis. The AEs of sorafenib were generally mild, and the main AE was HFS.

KEYWORDS

differentiated thyroid cancer, sorafenib, radioactive iodine refractory, real-world clinical study, efficacy and safety

Introduction

Thyroid cancer is a common endocrine tumor, ranking seventh among all cancer types reported in China in 2022, with the fastest-growing incidence (1, 2). Differentiated thyroid cancer (DTC) is accounted for 95% of all histological types of thyroid cancer, including papillary thyroid cancer (PTC), follicular thyroid cancer (FTC), and Hürthle cell cancer (3). In general, thyroid cancer presents an excellent prognosis with standard management including surgery, radioactive iodine (RAI) treatment, and thyroid-stimulating hormone suppression therapy (4–6). However, about 20% and 10% of DTC patients are at risk of local recurrence and distant metastases respectively, and two-thirds of them lose the ability of RAI uptake initially or gradually, which was known as radioactive iodine refractory differentiated thyroid cancer (RR-DTC) (7). As limited treatment options result in poor prognosis, the 10-year survival rate is less than 10% in RR-DTC patients (8, 9).

Sorafenib, approved by the U.S. Food and Drug Administration and the State Food and Drug Administration of China for the treatment of RR-DTC, is an oral tyrosine kinase inhibitor (TKI) inhibiting vascular endothelial growth factor receptors (VEGFRs), RAF, RET, and platelet-derived growth factor receptor beta signaling (10–12). Compared with the placebo, sorafenib improved the progression-free survival (PFS) of RR-DTC patients showing efficacy and safety in DECISION trial (13). Real-world studies not only can reflect the effects of drugs and provide more important health condition information about patients in their daily life but also provide a more scientific basis guiding physicians' clinical decision. Further demonstration of the clinical efficacy and safety of sorafenib used in different populations, centers, and age groups is essential.

Although many real-world studies on the treatment of RR-DTC with sorafenib have been reported (14–19), there are a few studies on the efficacy and adverse effects (AEs) of sorafenib at a standard dose of 400mg twice daily in Zhujiang Hospital of Southern Medical University. Our research not only analyzed the efficacy and AEs of sorafenib at a standard dose of 400mg twice daily for the treatment of RR-DTC patients at our Department of Nuclear Medicine from October 2017 to May 2020 but also summarized the real-world studies on sorafenib reported to date in the Discussion section.

Material and methods

Patients diagnosed with RR-DTC at our Department of Nuclear Medicine in Zhujiang Hospital of Southern Medical University from October 2017 to May 2020 were retrospectively analyzed. All patients were given sorafenib at the standard dose of 400mg, twice a day, and then the dose was adjusted according to the patient's tolerance to side effects. Patients who received other treatments while taking sorafenib or were interrupted in follow-up were excluded. RR-DTC was defined as follows (if one of the followings is satisfied): 1) at least one target lesion without RAI uptake (never or ever); 2) progression of a target lesion despite significant RAI concentration; 3) cumulative RAI dose ≥ 22.3 GBq. Imaging examinations were performed every 3 to 6 months to assess efficacy, AEs were assessed monthly. The Response Evaluation Criteria in Solid Tumors version 1.1 was used to evaluate treatment efficacy (20): disease control rate (DCR) referred to complete response (CR) plus partial response (PR) plus stable disease (SD), objective response rate (ORR) was CR plus PR, and progression-free survival (PFS) was defined as the time from initiation of sorafenib to progression or death. Common Terminology Criteria for Adverse Events Version 5.0 was used to evaluate AEs and guide patients to adjust their medication in response to side effects: if patients had grade 1–2 AEs, the dose could be appropriately adjusted according to the patient's ability to tolerate the AEs, if the patients had grade 3 or above AEs, the medication would be suspended until the AEs returned to grade 1–2. (Available from: https://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm#ctc_50. Accessed 15 May 2020).

Baseline characteristics of patients were described: quantitative data were reported as mean or median, and categorical variables as numbers with percentages. Waterfall plots were used to present the best tumor response. The PFS was calculated using the Kaplan–Meier method, and a log-rank test was performed to compare the difference in the PFS of variables. Cox proportional hazard model was used to estimate prognostic factors associated with PFS. All data were analyzed using SPSS version 26.0, and a p-value < 0.05 was considered statistically significant.

Results

Baseline characteristics

Table 1 summarizes the clinicopathological profiles of 31 retrospective patients with a median age of 58 years (18–79 years) when starting sorafenib, most of whom had PTC (61.3%). Twenty-four (77.4%) patients had distant metastases, half of whom had bone metastases and 16 had lung metastases. Of the 31 patients, five were excluded for the following reasons: three had only one radiological examination, one was absent of any measurable target lesion, and one underwent surgery during sorafenib therapy. The other 26 patients were eligible for radiological response assessment with a median follow-up of 17.5 months (4.0–51.0 months). AEs during sorafenib administration were recorded in 31 patients with a median follow-up of 18 months (2.0–51.0 months).

Efficacy of sorafenib

As presented in Table 2, no one achieved CR, 26.9% ($n = 7$) of patients achieved PR and all exceeded 12 months, and 30.7% ($n = 8$) had SD. The DCR was 57.7% ($n = 15$), with 14 cases controlled for ≥ 12 months, nine cases for ≥ 18 months, and three cases for ≥ 24 months. Eleven (42.3%) had documented disease progression at the end of follow-up, three of whom recorded new metastases rather than enlargement of target lesions. Figure 1 demonstrates the best changes in target lesions.

TABLE 1 Baseline clinicopathologic characteristics of patients treated with sorafenib.

	Median (range) or *N* (%)
Age (years)	58 (18–79)
≥ 55	19 (61.3)
< 55	12 (38.7)
Gender	
Male	12 (38.7)
Female	19 (61.3)
Histologic types	
FTC	10 (32.3)
PTC	19 (61.3)
FTC + PTC	2 (6.4)
RAI uptake	
Yes	20 (64.5)
No	11 (35.5)
Distant metastases	
Yes	24 (77.4)
No	7 (22.6)
Metastasis sites	
Lung	16 (66.7)
Lung-only	7 (29.2)
Bone	12 (50.0)
Pleural	1 (4.2)
Target lesions	
Lung	14 (45.2)
Bone	9 (29.0)
lymph node	1 (3.2)
Neck	7 (22.6)
Cumulative RAI dose (GBq)	11.7 (0.1–58.8)
Definitions of RAI refractoriness	
At least one target lesion without RAI uptake	10 (32.3)
RAI uptake,but progressive target lesions	17 (54.8)
Disease progression even with RAI therapy or cumulative RAI dose ≥ 22.3GBq	4 (12.9)

FTC, follicular thyroid cancer; PTC, papillary thyroid cancer; RAI, radioactive iodine.

As illustrated in Table 3, there were no significant differences in DCRs and ORRs according to age, gender, histologic types, RAI uptake, distant metastases, bone metastasis, previous treatment modality, residual lesions, and hand-foot skin syndrome (HFS). Notably, DCRs were superior in patients with a reduction of ≥ 60% in thyroglobulin (Tg) (p = 0.004). ORRs were favorable in patients with lung metastasis and lung-only metastasis (p = 0.010 and 0.001, respectively).

TABLE 2 Treatment efficacy of patients treated with sorafenib.

	N (%)
Disease response	
CR	0
PR	7 (26.9%)
SD	8 (30.7%)
Disease control (CR+PR+SD)	15 (57.7%)
Disease progression	11 (42.3%)
Disease control duration	
≤ 6months	1 (3.8%)
6–12 months	0
12–18 months	5 (19.2%)
≥ 18months	9 (34.6%)
Objective response ≥ 12 months	7 (26.9%)

CR, complete response; PR, partial response; SD, stable disease.

The median PFS of all 26 evaluable patients was 16.5 months (95% CI: 14.41–23.90 months) (Figure 2). There were no significant differences in PFS concerning age, histologic type, RAI uptake, distant metastases, lung metastasis, previous treatment modality, and residual lesions as indicated in Table 4. PFS was better in female and patients with lung-only metastasis, HFS, and Tg response ≥ 60% (p = 0.038, 0.045, 0.007, and 0.000, respectively); however, it was worse in patients with bone metastasis (p = 0.035). Nineteen patients with distant metastasis were divided into groups with and without bone metastasis, and survival analysis showed a worse PFS with the former (p = 0.062) (Table 4; Figure 3).

The dose was adjusted according to the patient's tolerance to side effects. Five patients discontinued the drug for AEs and resumed the drug after the AEs were alleviated, with the longest interval of 6 months. The others' minimum maintenance dose was 200mg daily. There were no significant differences in DCR, ORR, and PFS between patients with dose reduction or withdrawal of sorafenib and patients with continuous treatment (Tables 3, 4).

Safety and AEs

AEs were documented in all patients as revealed in Table 5. The most frequent AE of any grade was HFS (93.5%), followed by diarrhea (83.9%), alopecia (74.2%), and so on. These AEs were mainly grades 1–2. Similarly, diarrhea (16.1%) and HFS (9.7%) were the most prevalent among grades 3–4. Moreover, a few uncommon AEs such as weight loss (n = 1), myodynia (n = 4), and thrombocytopenia (n = 4) were reported. Most side effects were persistent, especially HFS, diarrhea, and alopecia, with the longest lasting 44 months. The median duration of HFS was 18 months (range: 0.5–44 months), the median duration of diarrhea was 16.5 months (range: 1–44 months), and the median duration of alopecia was 12.0 months (range: 2–44 months). Grade 3-4 AEs were common with HFS, diarrhea and abnormal liver function for a maximum duration of 38 months. Nineteen (61.3%) patients had drug reductions due to AEs

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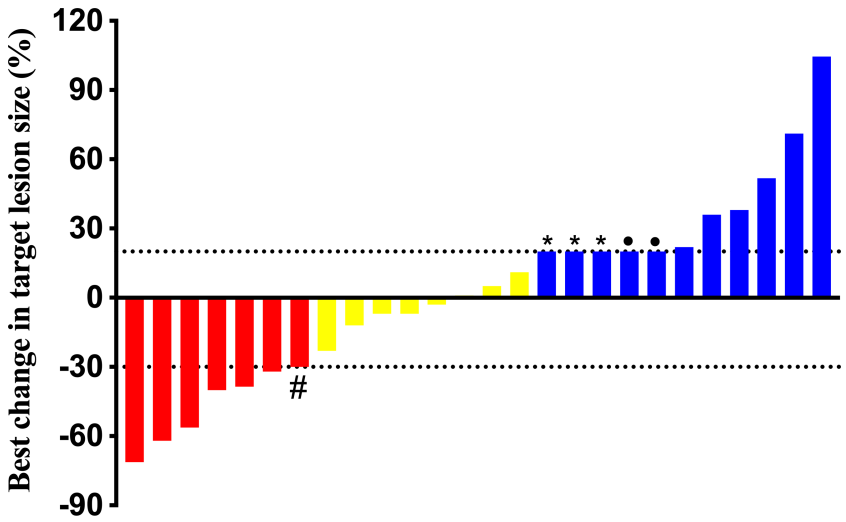


FIGURE 1
Best change in target lesions of 26 patients. #: This patient's chest computed tomography scan showed micro-nodules scattered throughout the lungs which couldn't be determined by the Response Evaluation Criteria in Solid Tumors version 1.1, but we found a significant reduction in his lung micro-nodules and a 91.4% reduction in thyroglobulin after follow-up, thus we considered a partial response. *: All three patients were found with new lesions. •:The two patients were died to pulmonary infection and bone related events, respectively.

TABLE 3 The association between prognostic features and response to sorafenib.

Variables	DCR, %	*p*-value	ORR, %	*p*-value
Age (years)		1.000		0.661
≥ 55	58.8		23.5	
< 55	55.6		33.3	
Gender		0.233		1.000
Male	41.7		25.0	
Female	71.4		28.6	
Histologic types		1.000		0.661
FTC	55.6		33.3	
PTC	58.8		23.5	
RAI uptake		1.000		0.665
Yes	57.1		21.4	
No	58.3		33.3	
Distant metastases		1.000		0.134
Yes	57.9		36.8	
No	57.1		0	
Lung metastasis		1.000		**0.010**
Yes	60.0		46.7	
No	54.5		0	
Lung-only metastasis		0.084		**0.001**
Yes	87.5		75.0	
No	44.4		5.6	

(Continued)

TABLE 3 Continued

Variables	DCR, %	*p*-value	ORR, %	*p*-value
Bone metastasis		0.228		0.190
Yes	40.0		10.0	
No	68.8		37.5	
Previous therapies		1.000		1.000
RAI-only therapy	58.3		25.0	
Other therapy	57.1		28.6	
Residual lesions		1.000		0.629
Yes	57.1		14.3	
No	57.9		31.6	
Hand-foot syndrome		0.423		1.000
Yes	60.0		28.0	
No	0		0	
Thyroglobulin response		**0.004**		0.081
≥ 60%	85.7		42.9	
< 60%	25.0		8.3	
Medication status		0.683		0.635
Reduction/discontinuation	61.1		22.2	
Standard dose	50.0		37.5	

DCR, disease control rate; ORR, objective response rate; FTC, follicular thyroid cancer; PTC, papillary thyroid cancer; RAI, radioactive iodine.
The bold values are statistically significant.

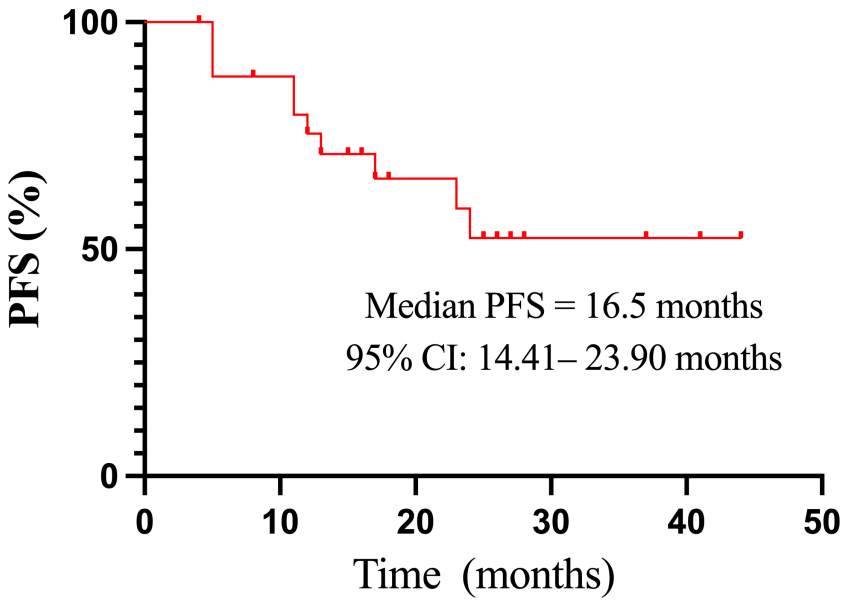


FIGURE 2
The Kaplan–Meier curve of PFS in 26 patients treated with sorafenib. CI, confidence interval; PFS, progression-free survival.

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TABLE 4 Univariate analyses of potential prognostic factors associated with the PFS of patients treated with sorafenib.

Variables	*N* (%)	PFS, months	*p*-value
Age (years)			0.744
≥ 55	17 (65.3)	29.607	
< 55	9 (34.6)	30.130	
Gender			**0.038**
Male	12 (46.2)	21.691	
Female	14 (53.8)	34.286	
Histologic types			0.776
FTC	9 (34.6)	28.075	
PTC	17 (65.4)	28.995	
RAI uptake			0.644
Yes	14 (53.8)	29.587	
No	12 (46.2)	28.483	
Distant metastases			0.621
Yes	19 (73.1)	28.900	
No	7 (26.9)	31.000	
Lung metastasis			0.696
Yes	15 (57.7)	29.214	
No	11 (42.3)	29.156	
Lung-only metastasis			**0.045**
Yes	8 (30.8)	39.875	
No	18 (69.2)	23.045	
Bone metastasis			**0.035**
Yes	10 (38.5)	16.347	
No	16 (61.5)	35.201	
Previous therapies			0.655
RAI therapy	12 (46.2)	29.917	
No-RAI therapy	14 (53.8)	28.573	
Residual lesions			0.894
Yes	7 (26.9)	26.667	
No	19 (73.1)	30.080	
Hand-foot skin syndrome			**0.007**
Yes	25 (96.2)	30.705	
No	1 (3.8)	5.000	
Disease control			**0.000**
PR + SD	15 (57.7)	–	
Disease progression	11 (42.3)	–	
Objective response			**0.017**
PR + CR	7 (26.9)	–	
SD + disease progression	19 (73.1)	–	

(Continued)

TABLE 4 Continued

Variables	*N* (%)	PFS, months	*p*-value
Thyroglobulin response			**0.000**
≥ 60%	14 (53.8)	39.611	
< 60%	12 (46.2)	13.727	
Medication status			0.396
Reduction/discontinuation	18 (69.2)	31.688	
Standard dose	8 (30.8)	18.406	

PFS, progression-free survival; FTC, follicular thyroid cancer; PTC, papillary thyroid cancer; RAI, radioactive iodine; CR, complete response; PR, partial response; SD, stable disease;–, no data; Distant metastases[1], Distant metastasis of all patients; Distant metastases[2].
The bold values are statistically significant.
–, not reached.

(some patients had more than one AEs: HFS, n = 19; diarrhea, n = 5; abnormal liver function, n = 1; proteinuria, n = 1).

Typical cases

Patients 1, 2, and 3 were diagnosed with RR-DTC on 08/09/2018, 06/20/2018, and 08/30/2019, respectively, and started taking sorafenib using 400 mg twice daily, followed by regular follow-up. Only lung metastases were found in all of them, and all target lesions were lung metastases. As depicted in Figure 4, at the end of the follow-up, their chest computed tomography scans (12/21/2021, 03/01/2022, and 12/18/2019, respectively) showed that their target lesions significantly reduced by ≥ 30% or even disappeared compared with the baseline data, hence they were evaluated as PR.

Discussion

As shown in Table 6, we summarize the real-world studies reported so far and the DECISION trial (13, 14, 16, 18, 19). The DCR of our study was 57.7%, similar to most of the above studies. However, the ORR in our study was slightly improved to 26.9% compared with those reported by Benekli et al., Massicotte et al., Kim et al., and Cheng et al. (14.0%,15.0%, 10.3%, and 21.7%, respectively). Furthermore, the median PFS in our study was 16.5 months, longer than those of DECISION, Kim et al., and Massicotte et al. (10.8, 9.7, and 7.2 months, respectively) (13, 14, 18), which may be due to the different tumor burdens of patients included such as those with advanced disease or distant metastases (14, 16, 18). However, the median PFS of the studies by Cheng et al. and Benekli et al. (17.6 and 21.3 months, respectively) was longer than ours, and a longer follow-up was found in the study of Cheng et al. than ours (median follow-up: 25.1 months versus 17.5 months) possibly resulting in longer PFS. Overall, both the clinical trial and real-world studies have demonstrated considerable efficacy in treating RR-DTC with sorafenib.

Studies have shown that soft tissue metastases such as lymph nodes, pleura, and pulmonary lesions presented better outcomes (16, 18, 21). Similarly, our study discovered that Sorafenib is more effective in lung metastases, all patients with PR were lung metastasis and patients with lung-only metastasis generally had a better prognosis such as typical cases shown in Figure 4, while those with bone metastasis presented a worse prognosis (Table 4). Besides, in the subgroup analysis of the relationship between PFS and clinicopathological features, as shown in Table 4, PFS was significantly better in patients with lung-only metastases and significantly worse in patients with bone metastases, in the subgroup analysis of PFS between the presence and absence of bone metastasis groups, the bone metastasis group tended to have worse PFS. We believe that the reasons are as follows: VEGF stimulates endothelial cell proliferation and tumor angiogenesis, playing an important role in the development and progression of thyroid cancer, and its expression level correlates with advanced disease (22, 23). Sorafenib is a multitargeted TKI whose core mechanism is to block the activity of VEGFR-2 and VEGFR-3 to inhibit tumor angiogenesis and cell proliferation. VEGFR is mainly expressed in soft tissues such as heart, lung, kidney, and skeletal muscle (24). Therefore, we estimate that lower VEGFR signal transduction in bone may be responsible for the poor efficacy of bone metastases in line with the study of Cheng et al. (16). Beyond that, pathophysiological processes in the bone microenvironment promote the rapid progression of bone lesions, and complex bone composition and structure make it difficult to be targeted, which leads to the rapid development of bone metastases (25–27). Therefore, patients with lung metastases respond relatively well to sorafenib treatment. For bone metastasis, anti-angiogenic therapy alone may be less effective, and a multimodal approach including local and systemic therapies should be considered. However, in this cohort, patients with bone metastasis just tended to have worse PFS, and the p value was still greater than 0.05, which still needs more data to further confirm.

In our cohort, male had worse PFS than female (Table 4; Figure 3). There are also various opinions on the relationship between gender and thyroid cancer prognosis. Some studies have shown that the prognosis of thyroid cancer in male patients is worse than that in female patients (28, 29), it may be related to the higher risk of radiation exposure and aggressive characteristics of male (28). However, there is no relevant study on the relationship between the efficacy of targeted drugs in RR-DTC treatment and gender, and we will further explore in our follow-up study.

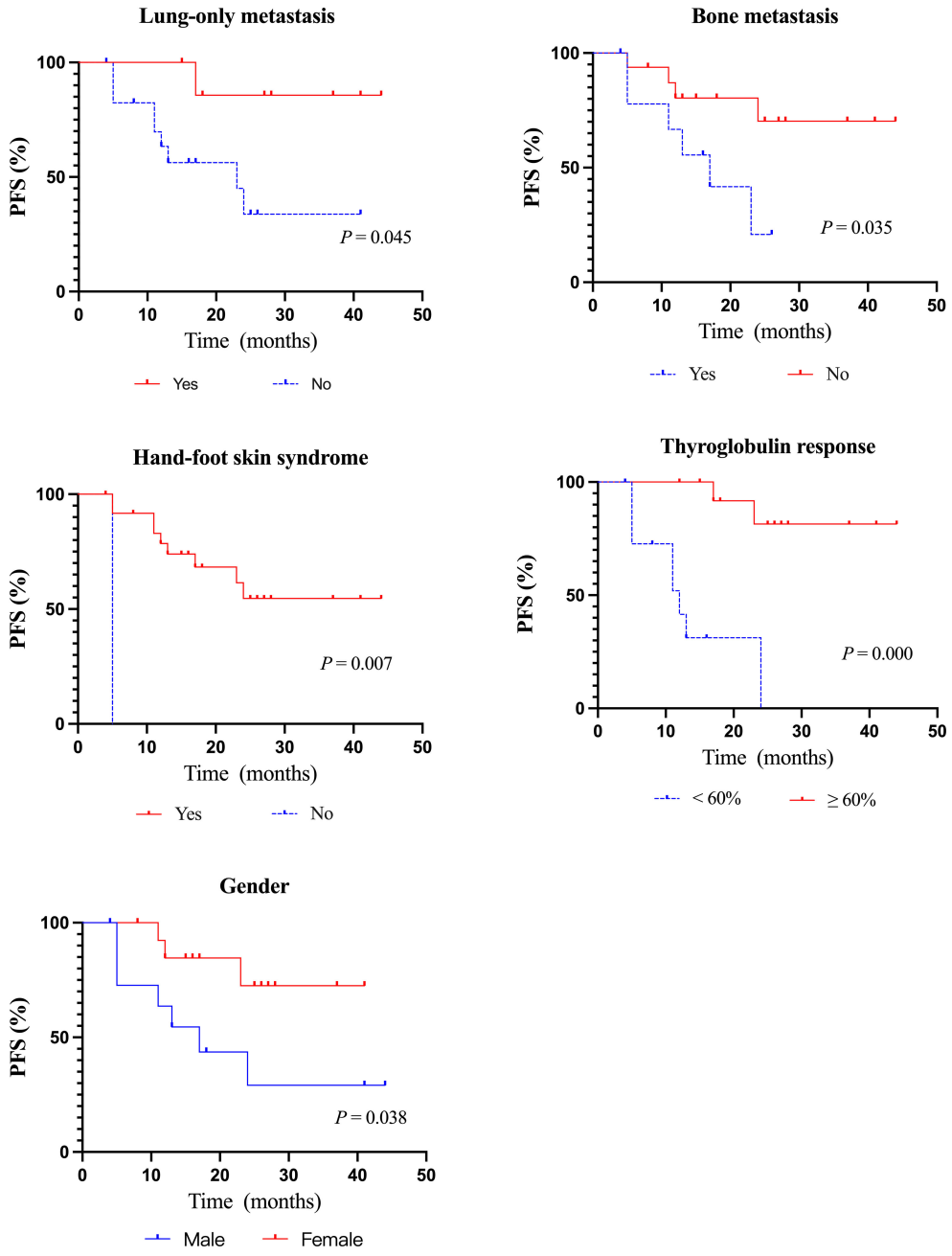


FIGURE 3
Kaplan–Meier curves of PFS according to clinicopathological features. PFS, progression-free survival.

TABLE 5 Adverse events occurring in patients treated with sorafenib.

	Grades 1–2	Grades 3–4	Any grade
Adverse events	*N* (%)	*N* (%)	*N* (%)
Hand-foot skin syndrome	26 (83.9)	3 (9.7)	29 (93.5)
Diarrhea	21 (67.7)	5 (16.1)	26 (83.9)
Alopecia	23 (74.2)	0	23 (74.2)
Abnormal liver function	15(48.4)	2 (6.5)	17 (54.8)

(Continued)

TABLE 5 Continued

Adverse events	Grades 1–2 *N* (%)	Grades 3–4 *N* (%)	Any grade *N* (%)
Generalized weakness	17 (54.8)	0	17 (54.8)
Hypertension	13 (41.9)	2 (6.5)	15 (48.4)
Proteinuria	10 (32.3)	2 (6.5)	12 (38.7)
Hypocalcemia	9 (29.0)	1 (3.2)	10 (32.3)
Mucositis	8 (25.8)	0	8 (25.8)
Nausea and vomiting	6 (19.4)	1 (3.2)	7 (22.6)
Thrombocytopenia	4 (12.9)	0	4 (12.9)
Myodynia	4 (12.9)	0	4 (12.9)
Weight loss	1 (3.2)	0	1 (3.2)

Serum Tg is a well-recognized prognostic indicator for the presence of metastases or occult lesions after total thyroidectomy (6, 30, 31). However, RR-DTC lesions have various degrees of dedifferentiation, resulting in the loss of the ability to produce Tg; thus, it is controversial to use Tg to evaluate curative effects (32, 33). Our subgroup analysis revealed that patients with ≥ 60% decrease in Tg had longer PFS and better DCRs. Besides, patients in PR had a significant diminishment of the best change in Tg (more than a 90% decrease in six cases and a 58.2% decrease in one case). Kim et al. also found that a ≥ 60% decrease in Tg was associated with longer PFS and DCRs (14), and a similar conclusion was expounded by Cheng et al. (16). In contrast, Benekli et al. emphasized that Tg levels had no significance in prognosis or predicting response (19), and decreased or no change in Tg was observed in patients with advanced thyroid cancer treated with other

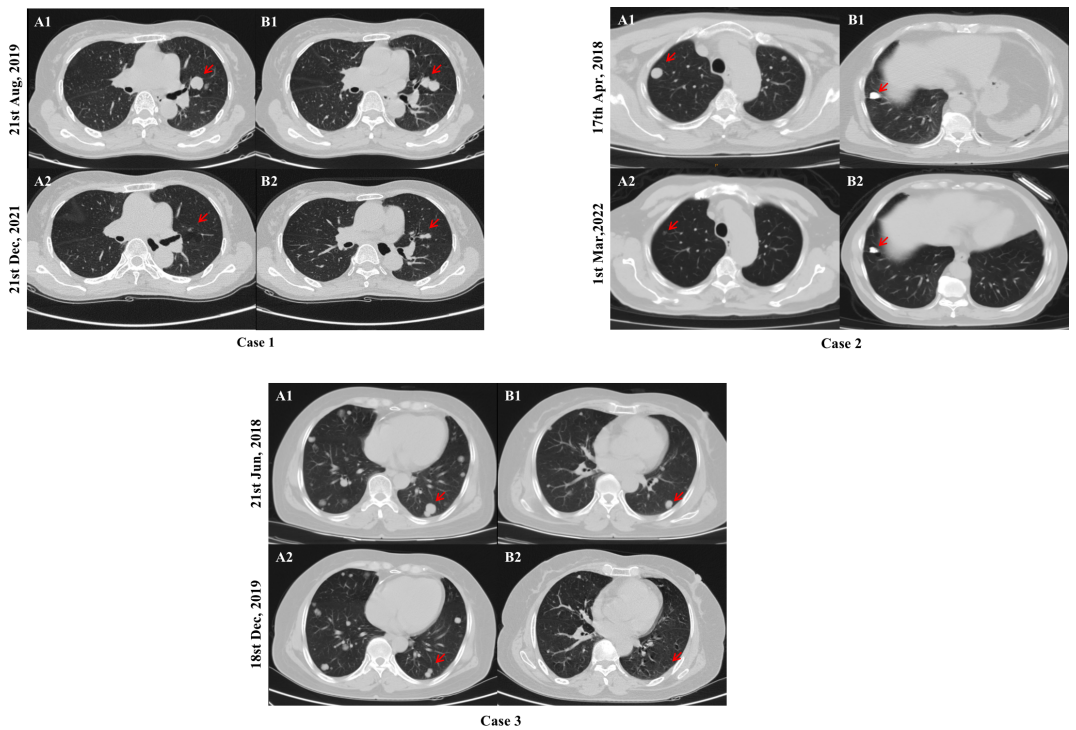


FIGURE 4
Comparison of chest computed tomography scans of typical cases. After 28, 46, and 17 months of sorafenib treatment, a significant reduction in the size of target lesions was observed in cases 1, 2, and 3, respectively. Chest computed tomography scans at baseline (A1 and B1) and the end of follow-up (A2 and B2) are shown above. The red arrows indicates the location of the target lesions at baseline and the last follow-up.

TABLE 6 Reported real-world studies and the DECISION trial.

Authors	Year	Study design	Patients included	Sample sizes	Follow-up time (months)	DCR (%)	ORR (%)	PFS (months)	AEs (%)
Brose et al.	2014	Phase III RCT multicenter	96.4% metastatic or locally advanced RR-DTC	207	16.2	54.1 (≥ 6 months)	12.2	10.8	98.6
Benekli et al.	2014	single center prospective	RR-DTC	14	21.3	56.9	14.0	21.3	NM
Massicotte et al.	2014	single center prospective	Metastatic or locally advanced RR-DTC	39	NM	92.0	15.0	7.2	82.1
Kim et al.	2019	retrospective multicenter	RR-DTC	85	9.1	67.0	10.3	9.7	95.0
Cheng et al.	2020	single center prospective	RR-DTC	72	17.6	73.3	21.7	17.6	73.6

ORR, objective response rate; DCR, disease control rate; PFS, progression-free survival; AEs, adverse events; RCT, randomized controlled trial; NM, not mentioned; DTC, differentiated thyroid cancer; RR-DTC, radioactive iodine refractory differentiated thyroid cancer.

TKIs (21, 34, 35). Whether the measurement of Tg can monitor therapeutic effects remains to be determined, but dynamic monitoring of changes in Tg such as the degree of Tg diminishment and Tg-doubling time can evaluate the efficacy of postoperative patients with thyroid cancer to a certain extent (31).

In our study, dose reduction and drug withdrawal occurred in 42.3% (n = 18) of patients due to AEs, mainly because of HFS, and a lower rate of AEs was found compared with the DECISION trial (93.1% versus 98.6%) (13), but a lower rate of AEs was found in the study of Cheng et al. (73.6%) than ours; therefore, a higher dose may result in a higher rate of AEs (16). Since common AEs were HFS, diarrhea, alopecia, abnormal liver function, generalized weakness, and hypertension, clinicians should closely monitor patients' skin condition, blood pressure, and general situation when using sorafenib to avoid dose reduction or discontinuation.

It has been confirmed that patients treated with TKIs are at high risk of HFS (36). Virtually all patients had HFS (93.5%), most of them were grades 1–2, and only three were grades 3 and above. Moreover, the occurrence of HFS was correlated with better PFS in our analysis. Many studies have confirmed that HFS is associated with a higher cumulative sorafenib dose and with anti-VEGF and anti-VEGFR activities (17, 37). Since anti-angiogenesis is one of the main mechanisms of sorafenib in treating RR-DTC, HFS could be a significant therapeutic index during the administration of TKIs (38, 39). Thus, clinicians could estimate whether TKIs are starting to work based on the appearance of HFS.

As the sample size included in our study was not large and the median follow-up was only 17.5 months, no factors affecting PFS were identified in multivariate analysis. We will continue to follow up with patients for PFS and include more patients to analyze the factors influencing PFS.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by Medical Ethics Committee of Zhujiang Hospital of Southern Medical University. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

Author contributions

YL, WO and HF designed the study. YL and XX collected and analyzed the data and wrote the manuscript. YL, XX, and JL checked and corrected the data and manuscript. QZ, PC, LP, and ML participated in measuring and collecting the data. WO and HF reviewed and edited the manuscript and approved the final draft. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Thyroid hormones and minerals in immunocorrection of disorders in autoimmune thyroid diseases

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Thyroid hormones and essential elements iodine (I), selenium (Se), iron (Fe), copper (Cu), zinc (Zn), calcium (Ca), magnesium (Mg), etc. play an important role in the work of many organs and systems of the body, including the immune system and the thyroid gland, and a violation of their supply can be the cause of pathological changes in them. In pathology, the interaction between thyroid hormones (TG), minerals and the immune system is disturbed. The review of the literature examines the immunomodulatory role of TG, minerals, their properties, and their participation in the pathogenesis of autoimmune thyroid diseases (AITD). The study of the relationship between the excess or deficiency of minerals and AITD is described. The basis of the development of AITD - Hashimoto's thyroiditis (HT), Graves' disease (GD), Graves' ophthalmopathy (GO) is the loss of immune tolerance to thyroid antigens - thyroid peroxidase (TPO), thyroglobulin (Tg) and thyroid-stimulating hormone receptor (TSH-R). Immune-mediated mechanisms - production of autoantibodies to thyroid antigens and lymphocytic thyroid infiltration - are involved in the pathogenesis of AITD. Insufficiency of regulatory T cells (Treg) and regulatory B cells (Breg), imbalance between Th17-lymphocytes and Treg-lymphocytes, abnormal production of pro-inflammatory cytokines has a significant influence on the progression of AITD. With AITD, the balance between oxidants and antioxidants is disturbed and oxidative stress (OS) occurs. The lack of modern effective pharmacological therapy of AITD prompted us to consider the mechanisms of influence, possibilities of immunocorrection of pathogenetic factors using TG, micro/macronutrients. In order to develop a more effective treatment strategy, as well as approaches to prevention, a critical analysis of the ways of immunotherapeutic use of dietary supplements of I, Se, Zn, Mg and other minerals in AITD was carried out.

KEYWORDS

autoimmune thyroid diseases, thyroid hormones, iodine, selenium, zinc, iron, magnesium, immunomodulating effect

Introduction

Autoimmune thyroid diseases (AITD), namely autoimmune thyroiditis (AIT)/Hashimoto's thyroiditis (HT), Graves' disease (GD), Graves' ophthalmopathy (GO), are complex, polygenic lesions with both similarities and differences that largely programmed by genetic factors (1, 2). The implementation of these factors depends on various environmental influences, such as stress, smoking, bacterial and viral infections, chemical pollutants, as well as dietary iodine (1, 3). AITD are one of the most common pathologies in the structure of endocrine diseases and are the most frequent cause of hypothyroidism and thyrotoxicosis. AIT begins asymptomatically, is combined with damage to thyrocytes, and the synthesis of autoantibodies is the final stage of the immune response to autoantigens TPO (thyroid peroxidase) and Tg (thyroglobulin). The prevalence of TPOAb (11.3%) and TgAb (2.0%) in euthyroid subjects was observed in 15.3% of individuals (4). The odds of TPOAb is higher in females (5). Over time, latent AIT turns into subclinical and overt thyroiditis with hypothyroidism. The incidence of manifest AIT in different countries was diagnosed from 27 to 273 cases per 100,000/year of the population (6). Among children and adolescents with HT, euthyroidism was detected in 52.1% of patients, overt or subclinical hypothyroidism in 41.4%, overt or subclinical hyperthyroidism in 6.5% (7). The prevalence of thyroid dysfunction in pregnant women is high (11%) and is 5.6% with subclinical hypothyroidism, 3.5% with overt hypothyroidism, and 1.5% with subclinical hyperthyroidism in patients observed in the 3rd trimester of pregnancy (8). The postpartum period is characterized by an exacerbation of the disease (9). AITD can occur both in isolation and in combination with other autoimmune disorders, such as type 1 diabetes, celiac disease, vitiligo, etc. (10, 11).

The prevalence of hyperthyroidism is 1.2-1.6%, of which 0.5-0.6 are obvious and 0.7-1% are subclinical (12). The most common cause of this disease is GD. The age-adjusted incidence of GD in adults in Sheffield, UK is 24.8 per 100,000/year (13). In patients with GD binding of antibodies to the thyroid-stimulating hormone receptor (TSH-R) increases intracellular cAMP production, resulting in thyroid hormone (TG) release, thyrotoxicosis, and thyrocyte hyperplasia. Graves' Ophthalmopathy associated with GD (GO) occurs in 25-30% of cases and is its extrathyroidal manifestation (14, 15). The frequency of GO in women is higher (2.67–3.3 cases/100,000/year) than in men (0.54–0.9 cases/100,000/year) (16).

Treatment of TG of patients with HT is not effective enough. Patients with HT who received replacement with levothyroxine (L-T4) did not always achieve clinically significant results, which requires additional therapeutic agents (17). After discontinuation of antithyroid drugs, 73.6% of GD patients went into remission, but 13.3% had treatment failure and 36.7% relapsed (13). The benefit of intravenous glucocorticoids in mild GO is limited and does not justify the possible side effects. However, treatment of mild GO is necessary to prevent progression to more severe forms (16).

The significant prevalence of AITD, a long latent period and the involvement of autoimmune processes in their occurrence, development of concomitant diseases, insufficient effectiveness of the main pharmacotherapy, and a decrease in the quality of life of patients require the development of adequate approaches for the correction of immune processes in order to prevent and treat diseases. Until recently, there are no clear established effective recommendations for the treatment of AITD, and the separation of their certain manifestations from an obvious disease. The problem of treatment and prevention of AITD is of particular social importance in connection with environmental contamination, unbalanced iodine prophylaxis, the use of some drugs that act as factors of damage to the immune system, and the realization of the effect at the level of target organs, in particular the thyroid. The need for diagnosis, treatment and prevention of AITD is also due to the need to prevent concomitant diseases, including disorders of the cardiovascular system, hypercholesterolemia, atrial fibrillation, encephalopathy, others autoimmune diseases (1–3, 10, 11, 18).

Cellular and humoral immunity in the pathogenesis of AITD

In addition to genetic, cellular and humoral immune mechanisms, loss of immune tolerance to thyroid autoantigens is the basis for the development of AITD. In patients with AITD, autoreactive lymphocytes infiltrate the thyroid parenchyma, where they recognize thyroid antigens: Tg, TPO, TSH-R, and the sodium iodide symporter (19, 20). Thyroid antigens are presented by dendritic cells (DCs), macrophages (MF) and B-lymphocytes to both maturing $CD4^+$ lymphocytes and cytotoxic $CD8^+$ lymphocytes (21).

In the pathogenesis of AITD, cells of both adaptive (specific) immunity - $CD4^+$-, $CD8^+$-lymphocytes, peripheral regulatory T-cells (pTreg), etc. and innate immunity - natural killer cells (NK-cells), neutrophils, natural Treg (nTreg), which interact and are mutually regulated, play a significant role (22–25).

The clinical manifestation of the autoimmune phenotype towards HT or GD largely depends on the balance of the immune response induced by T-helper cells (Th1 or Th2), antigen-presenting cells (APCs) and the cytokine profile that dominates at that moment in the thyroid parenchyma (26). In the pathogenesis of AITD, considerable attention is focused on the role of subpopulations of T cells - Treg and Th17 lymphocytes, as well as B lymphocytes and APCs, especially DCs (27). Cytokines that produce Th2 cause excessive stimulation of B lymphocytes and plasma cells, as a result of which the production of thyroid autoantibodies begins (22, 26).

In HT patients, primary hypothyroidism occurs after the destruction of $CD8^+$ and MF directly or with the help of released cytokines by NK-cells in ADCC (antibody-dependent cell-mediated cytotoxicity) of a sufficient number of follicular cells that produce TG (19, 20, 28). Accumulation of reactive oxygen species (ROS) in thyroid tissues leads to apoptosis of thyroid cells during HT (29). In HT patients, the induction of neutrophil extracellular traps (NET) increases, which depends on the accumulation of ROS through the activation of nicotinamide adenine dinucleotide phosphate

(NADPH) oxidase and the production of interleukin (IL) IL-6 (30). Oxidative stress (OS) is closely related to the autoimmune process in thyroid disease (GD and GO, as well as HT) - a process that damages cellular structures, including lipids and membranes, proteins and DNA (31–33). The main points of action of TG, minerals on innate and acquired immunity and their targets are presented in Figure 1.

In the following text, we considered these questions in more detail.

Thyroid status is one of the components of immunomodulating effect in AITD

In physiological and pathological conditions, the interaction between TG - 3,3',5,5'-tetraiodo-L-thyroxine (T4) and 3,3',5-triiodo-L-thyronine (T3) and the immune system has been established. Immunocytes can be considered as important target cells of TG, which, if necessary, can produce and deposit TSH and T3, have receptors for these hormones and related genes, which makes them capable of forming new endocrine glands (34). TG metabolism and thyroid status are related to various aspects of the immune response. TG play an important role in both innate immunity and the adaptive immune response. For the most part, a hyperthyroid state activates the immune system, while hypothyroidism leads to its suppression. The effect of TG is mediated by various factors, including nuclear factor-kappa B (NF-κB), protein kinase C and β-adrenergic receptor signaling pathways (35).

Mechanisms underlying the action of TG include both genomic (via nuclear receptors) and non-genomic (membrane) effects. Mammalian target of rapamycin (mTOR) can be an important target of the non-genomic TG pathway. With the help of the membrane receptor for TG integrin αvβ3 on neutrophils, TG induces ROS production (36). An increase in the level of thyronine leads to an increase in the pro-inflammatory reaction of neutrophils, MF and DCs (37).

Thyroid hormones play an important role both in innate immunity (neutrophils, MF, NK-cells and DCs) and in the adaptive immune response (MF, DCs, $CD4^+$, $CD8^+$). Triiodothyronine promotes the phenotypic maturation of DCs by regulating main histocompatibility complex-II (MHC-II) and costimulatory molecules. Functional activation of DCs stimulates NK-cells, promotes an increase in proinflammatory cytokines (IL-12, IL-6, IL-23, IL-1β) and a decrease in antiinflammatory transforming growth factor-β (TGF-β1), which control adaptive reactions, which contributes development of Th1 and Th17, IL-17-producing γδT cells and cytotoxic T cells. At the same time, the function of the Treg subpopulation decreases. Activated T3 DCs also increase the expression of the chemokine receptor CCR7, which promotes their migration to the lymph nodes, where they present absorbed and processed antigens in a complex with MHC-II to specific T cell receptors (TCR) of naïve T cells (38). A high level of serum T3 causes inhibition of Treg function, reducing the expression of suppressive membrane proteins programmed cell death protein-1 (PD-1) in patients with GD (39).

The role of minerals in the pathogenesis of AITD. Prerequisites for their immunotherapeutic use

Iodine

Iodine deficiency is a global problem that affects all strata of the population and exists in many countries of the world. More than 2 billion of the world's population are at risk of insufficient iodine supply (40). The consequences of I deficiency are endemic goiter, irreversible brain damage, cretinism, hypothyroidism, and cancer of the thyroid, breast, and stomach. The main biological role of I is in the biosynthesis of TG, since I is a necessary structural component of TG (41).

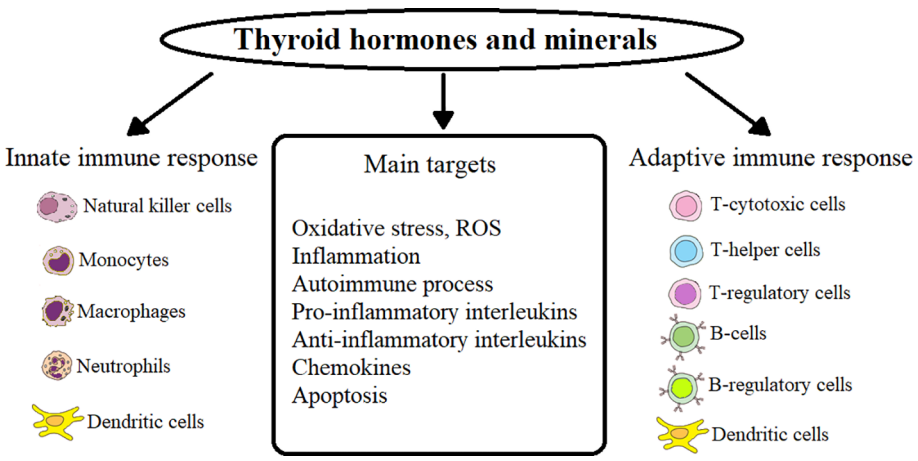


FIGURE 1
Influence of thyroid hormones and minerals on innate and adaptive immunity in AITD.

In the body, I exhibits immunomodulating effect. Oxidation of I to hypoiodite (IO^-) has significant bactericidal, antiviral, antifungal and anticancer activity. A significantly high content of I is found in leukocytes that participate in immunity and produce iodine-containing radicals. In addition, in the production of ROS by leukocytes, as in thyrocytes, the key enzyme is peroxidase. Iodine stimulates the myeloperoxidase activity of neutrophils, MF, increasing their bactericidal potential and inflammation, reduces the autophagy process. On the other hand, I also performs an antioxidant function in the body, as it is an absorber of ROS (42).

Chronic high intake of I is associated with an increased incidence of AIT. An excess of I can increase the antigenicity of Tg, stimulate the production of cytokines and chemokines, which can increase the accumulation of immunocompetent cells in the thyroid gland, increase the level of OS in thyrocytes, stimulate lipid peroxidation and damage to thyroid tissue (43). In mouse, a NOD.H-2h4 strain prone to AIT and susceptible individuals, excess I increases the number of intrathyroidal infiltrating Th17 and inhibits the development of Treg, causes abnormal expression of tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) in thyrocytes, leading to their apoptosis (44, 45). The effect of excess I on thyroid follicular cells (TFCs) obtained from HT patients resulted in inhibition of autophagy, autophagy-associated protein LC3B-II (lain chain 3B-II), TGF-β1, and increased ROS production, caspase-3 activation and apoptosis of TFC, the Akt/mTOR signaling pathway (46).

In regions with excessive consumption of I, there is an increased frequency of AIT characterized by high titers of TPOAb and TgAb (47). Deficiency or excess consumption of I may serve as a risk factor for thyroid diseases. In iodine-deficient countries, iodine-deficiency diseases predominate in the structure of thyroid disease, and in regions with normal or greater iodine supply, there is an increase in thyroid disease (48). More than adequate consumption of I in the population due to a poorly controlled iodine prophylaxis program can cause euthyroid or subclinical hypothyroid AIT (49). The initiation of a 20-year (1997-2016) salt iodization program in Denmark resulted in a steady increase in the incidence of hypothyroidism among individuals living in regions of moderate and mild iodine deficiency, but only among young and middle-aged participants (50). After universal salt iodization (USI) over 16 years in 15,008 adults from 10 cities in eastern and central China, the prevalence of clinical hypothyroidism, subclinical hypothyroidism, clinical hyperthyroidism, GD, and the presence of thyroid antibodies were found to be significantly higher in cities with more than adequate consumption of I. Compared with a five-year prospective study conducted in 1999, the prevalence of goiter decreased significantly, but there was a significant increase in thyroid nodules. The prevalence of subclinical hypothyroidism, the level of TPOAb and TgAb increased significantly (51). After two decades of USI in 78,470 individuals from 31 provinces in Mainland China, the prevalence of clinical hyperthyroidism and GD was higher in women than in men, and significantly higher in those aged 30-39 compared to those aged 60 and over, and stabilized in older individuals (48).

However, in HT patients from an iodine-deficient region of Turkey, no association was found between HT and urinary I levels, which were not elevated compared to the general population. Also, no significant difference was found between the levels of fT3, fT4, TPOAb, TgAb and thyroid gland volume in individuals with urinary I concentration above and below 100 μg/L. The difference reached a probable level only when comparing indicators with TSH (52). A national cross-sectional survey in mainland China found that after two decades of USI, the prevalence of TPOAb remained low. More than sufficient intake of I had an inverse relationship with TPOAb, and suggests that UIC from 100 to 299 μg/L is optimal and safe for the occurrence of AITD (53). Moreover, a multivariate regression analysis of a case-control study by Attard CC. et al, 2022 (5), showed that a higher intake of iodine-rich foods reduced the odds of TPOAb production (OR 0.864, 95% CI 0.761-0.981; p=0.024).

Growth in the prevalence of AITD should not limit the introduction of I supplements in the general population. Consumption of I in a concentration not exceeding 300 μg/L is safe with regard to AITD and does not increase the risk of autoimmune disorders for the population. Studies of the long-term consequences of the use of I on the occurrence and development of AITD clearly demonstrated that the early increase in thyroid antibodies is mostly transient, differs between populations under the influence of genetic and environmental factors, and does not always coincide with the presence of AITD or its further development (54). Excess I, which can disrupt thyroid function, in part, through OS, is much less harmful because it affects only a small percentage of individuals susceptible to AITD. While iodine deficiency affects the population and causes endemic consequences (55).

Today, new strategies for the diagnosis and treatment of iodine deficiency are attracting attention, focusing on the potential role of myo-inositol in combination with I (56). Myoinositol plays a crucial role in the functioning of the thyroid gland and AITD, as it regulates the organification of I and the biosynthesis of TG through the formation of hydrogen peroxide (H_2O_2) in thyrocytes (57). Combined supplementation of I and myoinositol may contribute to increased availability of I in thyrocytes, improving thyroid function (58).

Selenium

The normal function of the thyroid gland depends on a variety of trace elements for the synthesis and metabolism of TG, primarily I and Se. Selenium is a cofactor for enzymes that are present in thyroid cells, such as iodothyronine deiodinase (DIO), which catalyzes the conversion of T4 to T3, thioredoxin reductase (TR), which is involved in the regulation of the redox state, and glutathione peroxidase (GPx), which catalyzes the reduction of H_2O_2 to H_2O, using reduced glutathione (GSH) as a cosubstrate. In physiological conditions, selenoproteins are an effective defense system against a large amount of ROS, which thyrocytes constantly generate on their surface in order to accept electrons from oxidative reactions (59). In the process of iodination of tyrosyl residues and TG synthesis, H_2O_2 is a necessary substrate for TPO. Thyroid H_2O_2 production is stimulated by myoinositol, which indirectly controls the inositol-dependent signaling pathway of TSH (56, 57).

Selenoproteins are important for immunomodulating effect. In mice, a high-Se diet increased TCR signaling in $CD4^+$ T cells, increased IL-2 expression, shifted the Th1/Th2 balance toward a Th1 phenotype, higher levels of interferon-γ (IFN-γ) and CD40 ligand (60). In physiological conditions, selenoproteins participate in the activation of proliferation, differentiation and redox metabolism of T cells. At the same time, Se is involved in reducing excessive immune reactions and chronic inflammation (61). Through molecular mechanisms, selenoproteins regulate T-cell effector function related to redox signaling, calcium (Ca^{2+}) flux, and OS (62).

Selenoproteins prevent the excessive formation of ROS, which can lead to autoimmune diseases or chronic inflammation. Decreased serum Se concentration may lead to chronic OS through neutrophil ROS production (63). Selenium deficiency reduces the expression and activity of these antioxidant enzymes (64).

However, excessive production of ROS is involved in various stages of the pathogenesis of thyroid diseases and activates possible common triggers of GD and HT, differentiated thyroid cancer and even endemic goiter (31, 64). Deficiency of Se or its excess with dysfunction of mitochondria (organelles that contain selenoproteins) leads to thyroid disorders (65). Thyrocytes and thyroid fibroblasts pre-incubated with selenocompounds (selenomethionine and selenite) showed a dose-dependent increase in viability and a decrease in apoptosis indicators (caspase-3 activity, BAX mRNA levels) and an increase in the level of the inhibitor of apoptosis (BCL-2 mRNA) after incubation with H_2O_2, and also caused an increase in GPx activity (66).

Selenium together with I play a key role in the development of AITD (67). There is a relationship between insufficient intake of Se and AITD. Epidemiological data indicate the prevalence of AITD with Se deficiency (68). Although some authors did not note the relationship between the prevalence of HT and Se content (69).

In patients with GD, an inverse relationship of serum Se levels with T4 and TSH hormones and an inverse relationship with T3, T4, and TSH hormones were found in patients with GO, although there was no significant difference in Se levels in patients compared to healthy individuals (70). However, the results of other studies recorded a significant decrease in the level of Se in the serum of patients with newly diagnosed GD and autoimmune overt hypothyroidism (71). Deficiency of Se in the serum of patients with GD increased with age and was correlated with an increased titer of antibodies to the TSH receptor (TRAb). In addition, a relationship between the level of Se and ioduria was registered in patients with GD (67). A decrease in serum Se levels in HT and especially in GD and GO is associated with the pathogenesis of the disease (72).

Treatment of HT remains symptomatic and is based on taking, as needed, synthetic TG to correct hypothyroidism. Surgery is performed when the goiter is large enough. The value of Se and the presence of its deficiency in AITD argue for the need to replenish it in the body.

The formation of TPOAb and TgAb is most often associated with HT, while the activation of TRAb formation is most often associated with GD, although their overlap has been recorded. There is conflicting information regarding the effect of Se supplementation on TPOAb and TgAb levels in HT patients. Most authors reported a decrease in TPOAb and TgAb levels after using different doses of Se (selenomethionine) supplements with or without L-T4 (73, 74). TPOAb titers decreased to varying degrees in patients with different genotypes of the single nucleotide polymorphism r25191G/A, which indicates interindividual differences (75). The results of twenty-three studies on the efficacy of Se with or without L-T4 in adults with HT compared with placebo and/or L-T4 showed that populations that did not receive L-T4 but received Se had lower levels of TPOAb, TgAb for a short time. At the same time, significant changes in the TSH level were not observed (76).

Current data do not always justify the use of Se in the treatment of HT, despite the fact that it leads to a decrease in the level of TPOAb and TgAb. Perhaps this is due to the fact that autoantibodies are not a key link in the pathogenesis of HT, they begin to be produced already in response to damage to thyrocytes and are the result of an autoimmune response. There is an assumption about the indirect participation of modified autoantibodies (desialylated IgG N-glycans) in ADCC of NK cells and mononuclear lymphocytes involved in the destruction of thyrocytes (28). Despite the fact that HT is a chronic autoimmune process, and the production and circulation of autoantibodies in the peripheral blood is long-term, the therapeutic reduction of TPOAb and TgAb levels in HT is not conclusive evidence for the use of Se drugs.

The results of research on the use of selenium are conflicting. Se supplementation is necessary in Se-deficient individuals, but may have some health risks in Se-excessive individuals, including diabetogenic effect. Addition of Se to L-T4 therapy may be appropriate in patients with low Se intake and mild or early stage HT. Se supplementation is well tolerated but should not be universally recommended (59). Done by Winther K.H. et al, 2017 (17) systematic review of eleven publications covering nine controlled trials and a meta-analysis of case-effect models found no effect of Se supplementation on TSH and quality of life. Individuals receiving Se supplementation but not receiving L-T4 replacement had no changes in thyroid ultrasound findings, and individuals receiving L-T4 replacement occasionally showed clinically significant findings. Available research data do not support the use of routine Se supplementation in the treatment of patients with HT or GD. However, correction of moderate and severe Se deficiency may be an advantage in the prevention and treatment of these disorders.

The effect of Se on immunological indicators may depend on its dose and the stage of HT development (hyperthyroid, euthyroid, hypothyroid). Se concentration in serum decreases in inflammatory conditions and may vary depending on the severity and duration of the inflammatory process. The immunomodulating effect of Se on the humoral and cellular components of the autoimmune process in the thyroid gland is noted. In rats with experimental AIT, after the use of selenium yeast dietary supplement, in addition to a decrease in the levels of TgAb, TPOAb and a decrease in B-cell-activating factor (BAFF) in the blood serum, the expression of IL-10 in the

thyroid tissue increased, the number of Breg in the spleen increased, and also decreased TSH level (77). Selenium increased the percentage and enhanced the activity of Treg, suppressed the secretion of pro-inflammatory cytokines, prevented the apoptosis of follicular cells and provided protection against thyroiditis (45, 74). Treatment of patients with HT L-T4 and capsule with Se yeast for 3 months not only reduced the level of autoantibodies, but also the level of IL-2, which indicates the suppression of the inflammatory process (73).

The main proposed therapeutic mechanisms of AITD include increasing the activity of GPx and TR enzymes in blood plasma and reducing toxic concentrations of H_2O_2 and lipid hydroperoxides. As part of selenoproteins, Se takes an active part in antioxidant, redox and anti-inflammatory processes (59). Treatment of HT patients with selenium yeast tablets for 6 months significantly increased the level of Se, GPx3, and selenoprotein P1 (74). In patients with primary hypothyroidism, after treatment with L-T4 and Se, the level of the OS marker - malondialdehyde (MDA) decreased (78). Se supplementation in euthyroid, subclinical, or overt hypothyroid patients not only reduced thyroid autoantibodies, lowered or maintained TSH, decreased fT4/fT3 ratio, but also decreased OS and inflammation, and improved quality of life, structure, and volume thyroid gland (79). The use of antioxidant nutrients, including Se, reduces OS and thyroid damage in patients with HT (33).

Thyroid function in patients with HT and hypothyroidism may be favorably influenced by myoinositol, which regulates the formation of H_2O_2 in thyrocytes. Insufficiency of myoinositol or dysfunction of the inositol-dependent signaling pathway of TSH action can cause the development of hypothyroidism (56). The combined use of selenomethionine and myoinositol in euthyroid patients with HT reduced the level of TSH, antithyroid antibodies 6 and 12 months after treatment, and the level of TSH decreased earlier than in the group treated with selenomethionine alone (80). Moreover, the immunomodulating effect of the combination of selenomethionine and myoinositol in HT was confirmed by the fact that after treatment the level of the chemokine CXCL10, which is a potent chemoattractant for activated T-lymphocytes, also decreased, suggesting the possibility of reducing the risk of progression of euthyroidism to hypothyroidism (81).

Selenium supplementation can be offered to patients with GD only if Se deficiency is confirmed. Thus, in a randomized clinical trial conducted in selenium-sufficient GD patients treated with methimazole, no beneficial effect of Se on the short-term control of thyroid hyperfunction was observed (82). A randomized clinical trial by Calissendorff J. et al, 2015 (83) showed that Se has a beneficial effect in a cohort of hyperthyroid and Se-deficient patients treated with methimazole and L-T4 under a block-and-replace regimen. A meta-analysis by Zheng H. et al, 2018 (84) of ten randomized placebo-controlled trials involving 796 patients showed short-term (3 months) efficacy of Se supplementation on fT4 and fT3 levels in patients with GD. TSH levels were higher in the Se group than in the control group at 3 and 6 but not at 9 months. TRAb level decreased, thyroid function improved more often after 6, but not after 9 months.

Oxidative stress plays a significant role in the pathogenesis of GO and GD (the balance between oxidants and antioxidants is disturbed in favor of ROS), and an antioxidant approach is proposed for treatment (32, 85, 86). In a primary culture of orbital fibroblasts from GO patients and control subjects treated with H_2O_2 to induce OS, the antioxidants vitamin C, N-acetyl-L-cysteine, melatonin, retinol, β-carotene and vitamin E were found to reduce the manifestations OS (proliferation of fibroblasts, the level of hyaluronic acid, the level of tumor necrosis factor-α (TNF-α), IFN-γ and IL1-β, which provided the basis for the possible clinical use of these substances in patients with GO (87). In patients with newly diagnosed hyperthyroidism, mean serum total antioxidant capacity (TAC) was low and MDA was high. Hyperthyroid patients treated with a combination of carbimazole and an antioxidant had significantly lower MDA, than hyperthyroid patients treated with carbimazole alone (86).

Treatment for GO, which is an extrathyroidal manifestation of GD, includes intravenous glucocorticoids and orbital radiation therapy and is generally offered to patients with moderate to severe GO. In contrast, patients with mild GO are usually treated only with local methods (16). In patients with moderate GO, there is clear evidence of a beneficial antioxidant effect of Se, from a large randomized clinical trial conducted by the European GO Group – EUGOGO. Based on EUGOGO guidelines, patients with moderate GO may be offered a 6-month course of Se. The use of Se significantly reduces eye damage and slows the progression of the disease in patients with mild GO. Although, serum Se levels were not measured in the EUGOGO study (88). Se can cause toxicity if it is administered in very high doses. A dosage of up to 200 μg/day in Se-deficient patients, or 166 μg/day in Se-sufficient patients is considered quite safe (83).

In mild GO, Se supplementation is associated with reduced disease activity as well as improved quality of life. Further studies are needed to clearly understand the biological effects of Se supplementation, considering baseline Se levels, selenoprotein gene polymorphisms, comorbidities, and main clinical outcomes (89). Results of a meta-analysis conducted by Bednarczuk T. et al, 2020 (90), who included ten prospective randomized clinical trials, suggest that Se supplementation can enhance euthyroid recovery in hyperthyroidism and GO. However, the use of Se and the determination of its effect on the level of hormones or autoantibodies requires careful conclusions due to the lack of analysis of long-term clinical observations, such as the frequency of remission after treatment with antithyroid drugs.

Iron and copper

Deficiency of Fe and I remains the main health care problem, affecting about 30% of the world's population (91). Iron ions are included in large quantities in the composition of proteins - hemoglobin, myoglobin, enzymes, etc. Many enzymes of DNA replication and repair (helicases, nucleases, glycosylases, demethylases) and ribonucleotide reductase use Fe as an indispensable cofactor for functioning (92).

The bioavailability of Fe controls complex metabolic processes in immune cell homeostasis and inflammation (93). Iron in the plasma has an immunomodulating effect on innate immunity by controlling the ratio of monocytes to neutrophils and neutrophil activity. Hypoferremia alters neutrophil functions, inhibiting antibacterial mechanisms but enhancing mitochondrial ROS-dependent NETosis associated with chronic inflammation (94). Iron affects the polarization of MF (M1/M2). Iron imunomodulates the termination of the inflammatory response, as elevated Fe level increase the M2 phenotype and reduce the pro-inflammatory M1 response, reducing the IL-12/IL-10 balance. Fe loading of MF *in vitro* reduces the percentage of M1 cells expressing costimulatory molecules CD86 and MHC-II, prevents lipopolysaccharide-induced pro-inflammatory response by reducing nuclear translocation of NF-κB p65 with reduced expression of, IL-1β, IL-6, IL-12 and TNF-α (95).

In blood plasma, Cu binds to ceruloplasmin, a transport protein that is a reactive protein of the acute phase. Copper stimulates the function of innate and adaptive immunity and is required to perform protective functions of the immune system (96, 97). Deficiency of any of the minerals such as Se, I, Zn, Cu, Fe, etc. can temporarily reduce immune competence or even disrupt the regulation of systemic inflammation (61). With severe Cu deficiency, the number and ability of neutrophils to generate O_2 and kill absorbed microorganisms, T-cell proliferation and IL-2 production decrease (96).

Redox-active ions of metals such as Fe^{2+} and Cu^{+} are the most important transition metals in cells in an inert form. Oxidized forms of metals (Fe^{3+}, Cu^{2+}) are quickly restored in the cytosolic environment, fueling the vicious circle. To bind redox metals Fe^{2+} and Cu^{+} and prevent H_2O_2 from giving $^{\bullet}OH$ in the presence of ions of these metals in the body, there are highly active enzymes of antioxidant protection - catalase and superoxide dismutase (SOD) (98). Moreover, Cu serves as an important cofactor for enzymes such as CuSOD, cytochrome C oxidase (99). In addition to Cu, SOD subunits include trace elements Zn and manganese (Mn). Cu, ZnSOD protects the vascular and immune systems from the harmful effects of ROS. In the peripheral blood, Fe ions are in a bound form with transferrin, ferritin, lactoferrin, ceruloplasmin, which are Fe^{2+} chelators and perform an antioxidant function.

In healthy individuals with adequate intake of I the concentration of Cu and Se in the blood is significantly related to TSH and fT4 (100). Deficiency or excess of Se, I, Zn, as well as Fe and Cu affects the synthesis of TG and can disrupt thyroid homeostasis (101). Copper levels were associated with increased fT4 and tT4 levels in men. In women, Cu levels were associated with elevated tT3 and tT4 levels. The level of Cu was 20% lower in men than in women (102).

The ratio of Cu and Se may directly affect thyroid function in patients with HT and overt hypothyroidism, which may be relevant for lifelong replacement therapy during L-T4 reduction (103). Observational and controlled studies have shown frequent micronutrient deficiencies in patients with HT (104). Patients with or without GD and GO who had low serum Se levels showed relatively high serum Cu levels in GD patients, while patients with GD and GO conversely had low serum Cu levels (72).

Patients with AITD often suffer from Fe deficiency, as autoimmune gastritis, which reduces Fe absorption, and celiac disease, which causes Fe loss, are frequent comorbidities (11). In patients with HT in the stage of subclinical hypothyroidism, living in a densely populated area in the I region of Ankara, Turkey, the basal levels of Se and Fe were significantly lower than in the control group (105). Both hypothyroid and hyperthyroid patients had higher odds of Fe deficiency anemia (106).

Iron deficiency impairs thyroid metabolism. TPO is a glycosylated hemoenzyme that becomes active only after binding heme, a non-protein prosthetic group containing an Fe^{2+} ion. Iron deficiency, which corresponds to a serum ferritin level <15 μg/L, is associated with a higher prevalence of TPOAb but not TgAb in pregnant women during the first trimester and in nonpregnant women of childbearing age with subclinical hypothyroidism (107). TPOAb and TgAb positive patients with HT had significantly lower serum Fe and Mg concentrations (108).

Two-thirds of women with persistent symptoms of hypothyroidism, despite appropriate L-T4 therapy, show improvement in symptoms after recovery of ferritin (the main intracellular Fe^{3+} depot) in serum above 100μg/L (109). Double enrichment of salt I and microencapsulated Fe is suggested for effective prevention of Fe deficiency and thyroid dysfunction (110).

Zinc

Zinc homeostasis is tightly controlled by the coordinated activities of Zn transporters and metallothioins that regulate Zn transport, distribution, and binding. Zinc acts as a signaling molecule, facilitating the transduction of various signaling cascades in response to extracellular stimuli (111).

Zinc is necessary as a catalytic, structural and regulatory ion. Immunomodulating effect of Zn is involved in immune responses, OS, apoptosis and aging. Metallothioins are protective against OS, exposure to toxic metals, infections and insufficient Zn content in food (112). Zinc is a key component of the antioxidant defense network that protects membranes from Fe^{2+}-induced lipid oxidation due to Zn's ability to prevent Fe^{2+} from binding to the membrane (113). Experimental studies *in vitro* and *in vivo* have provided evidence that Zn acts as an antioxidant by inhibiting the oxidation of macromolecules such as DNA/RNA and proteins, as well as the inflammatory response, which leads to a decrease in ROS production (114).

Zinc is associated with thymus function, which provides a unique microenvironment for the proliferation, differentiation, maturation and release of naïve T cells. Zinc is necessary for inducing the biological activity of the thymus hormone thymulin (Zn-FTS), a peptide synthesized by thymus epithelial cells (TECs). Zinc with the help of structural "zinc finger motifs" plays a key role in cell proliferation, apoptosis and reactivation of thymulin. Through the NO pathway, Zn is necessary for the biological action of arginine (115). TECs activated by thymulin perform a positive selection of immunocompetent T cells capable of recognizing foreign antigens in the context of their own MHC

molecules. Deficiency of Zn^{2+} ions can reduce peripheral biological activation of thymulin.

Zinc participates in the process of physiological (age-related) involution of the thymus, which plays an important role in the immunosenescence of the body and the emergence of age-related diseases - susceptibility to infections, cancer, and autoimmune diseases. With age, the level of Zn in serum or blood plasma decreases. Low bioavailability of Zn ions due to an increase in metallothiones, IL-6 can provoke complete involution of the thymus during aging and the possible appearance of autoimmune diseases (115). Immunosenescence is perceived as a slow autoimmune process controlling the thymus. Predominance of Zn deficiency in the elderly can lead to impaired function of cells of the innate immune system and contribute to immunosenescence (116).

Due to the impaired of intrathymic expression of autoantigens (TSH-R, Tg, TPO), a loss of tolerance to these thyroid peptides is possible in genetically determined individuals with AITD under the influence of environmental trigger factors. Tolerance to self-antigens in the thymus depends on the expression of promiscuous gene expression (pGE) tissue-restricted antigens (TRA) TECs genes, which is controlled by autoimmune regulator (Aire) and forebrain embryonic zinc fingerlike protein 2 (Fezf2) regulators, which contains Zn (117).

Autoimmune diseases are associated with pathologically altered Zn levels, which provoke a change in signal transduction, which leads to impaired immune response, cell differentiation and function (118). Zn deficiency suppresses both innate and adaptive immune responses. Zn homeostasis is significantly influenced by the activation of T cells, as well as the polarization of Th cells into different subpopulations - Th1, Th2, Th17, Treg (116). The level of intracellular free Zn is important for the maintenance of Treg phenotype and function, as Zn deficiency promotes a pro-inflammatory immune response. The study of Th cells and Treg in an allogeneic model of the disease "graft against the host" demonstrated that Treg, unlike Th, had a significantly increased level of intracellular free Zn (118).

Zinc plays a significant role in the expression of melatonin receptor genes on the cell membrane of thymocytes (115). Biosynthesis and secretion of melatonin, which is synthesized mainly in the pineal gland, reciprocally affects thyroid function. Melatonin suppresses thyroid function depending on the change in the light regime (119). Nutraceuticals Se, 1-carnitine, myoinositol, resveratrol and melatonin may be effective preventive and therapeutic means AITD (120). Melatonin may play an important role in reducing ROS-mediated diseases, since one of the main functions of melatonin is to scavenge ROS (121). In patients with GO, it is possible to use antioxidant substances, including melatonin, to reduce the manifestations of OS, as evidenced by experimental studies of orbital fibroblast culture from patients with GO treated with H_2O_2 (87). However, there are no controlled studies that would substantiate the use of melatonin to improve thyroid function (120). Data discrepancy may be associated with melatonin receptor gene polymorphism in GD (122).

Zinc plays a key role in TG metabolism, in particular, by regulating the activity of deiodinases, thyrotropin releasing hormone (TRH) and TSH enzymes, as well as modulating the structures of the main transcription factors involved in TG synthesis (123). Cross-sectional and controlled studies showed a relationship between Zn deficiency and TG levels (124). A study by Mahmoodianfard S. et al, 2015 (125) provided some evidence for the effect of Zn alone or in combination with Se on thyroid function in hypothyroid and overweight or obese patients. Thus, mean fT3 and fT4 were significantly increased and mean serum TSH significantly decreased in the Zn+Se group (30 mg Zn as Zn gluconate and 200 μg Se as high-Se yeast) over 12 weeks, although there was no found significant changes for tT3, fT4, fT3 or TSH between groups.

In chronic diseases, including autoimmune diseases, aging, Zn deficiency can negatively affect immunological status, increase OS, and lead to the generation of pro-inflammatory cytokines (126). Zinc and vitamin D are the most promising candidates for therapeutic intervention aimed at balancing rather than suppressing the immune system (127). In HT, the use of trace elements Zn and Se is proposed to reduce oxidative damage to the thyroid gland (33).

Calcium and magnesium

In addition to microelements I, Se, Fe, Cu, Mn, Zn, macroelements such as Ca and Mg are related to thyroid function. The interaction of Ca and Mg is important in the human body. The optimal ratio of Ca to Mg is approximately 2.0, and ratios <1.7 and >2.8 may be harmful (128).

An increase in the flow of Ca^{2+} is necessary for signal transduction, the formation of immune synapses, and the implementation of specific cytotoxicity of $CD8^+$ T cells, which involves the costimulatory molecule of the cell surface LFA-1 and Mg^{2+} (129). A change in the regulation of Ca^{2+} in lymphocytes disrupts the control of metabolism, proliferation, differentiation, secretion of antibodies, cytokines and cytotoxicity, which leads to autoimmune and inflammation diseases (130). Mitochondrial intracellular Ca^{2+} is a key regulator of cell apoptosis. It has been experimentally shown that Ca uptake and mitochondrial metabolism underlie Treg survival in OS conditions (131). With the help of Ca^{2+} signaling, BAFF activates the mTOR pathway and the proliferation and survival of B-lymphocytes as producers of autoantibodies (132). The main mechanism of increasing intracellular Ca^{2+} is Mg^{2+} deficiency (133).

Magnesium exhibits immunomodulating effect. Experimental studies have shown that Mg^{2+} is a cofactor for the formation of C3 convertase, which is involved in the proteolytic activity of complement components, as well as in the binding of antigen to MF and the response to cytokines, the adhesion of Th and B cells, the synthesis of immunoglobulins, the binding of IgM with lymphocytes, ADCC (134). As a secondary signaling messenger, Mg^{2+} is involved in the activation of T cells (129, 133). Magnesium regulates the active site of IL-2-induced T cell kinase (ITK) during the $CD8^+$ T cell response to viral infection in mice (135). At the same time, Mg^{2+} regulates anti-inflammatory and antioxidant reactions. Cultivation of lipopolysaccharide- or TNF-α-stimulated mesenchymal stem cells of C3H/10T1/2 mice with 5 mM Mg^{2+}

increased the proliferation rate, decreased the levels of pro-inflammatory IL-1β and IL-6, and increased the levels of anti-inflammatory IL-10 and prostaglandin E2 (136).

Magnesium deficiency can cause immunodeficiency, increased acute inflammatory response, reduced antioxidant response and OS (133). Magnesium deficiency is associated with low-grade chronic inflammation involving the proinflammatory cytokines TNF-α and IL-1, the messenger IL-6, as well as E-selectins, intracellular adhesion molecule-1 (ICAM-1), and vascular cell adhesion molecule-1 (VCAM-1), acute-phase reactants - C-reactive protein and fibrinogen (137).

Magnesium regulates the proper functioning of other nutrients, such as vitamin D, which regulates the homeostasis of Ca and phosphorus (P) (133). Calcium, diacylglycerol, cAMP and various phosphate derivatives of myoinositol (phosphatidylinositol and others) are essential secondary messengers involved in the regulation of intracellular signaling cascades, in response to the activation of the TSHR, and are necessary for the production H_2O_2 and TG synthesis (56).

In patients with latent HT with a high titer of TPOAb and the presence of mild iodine deficiency, the content of Ca, Mg and Zn in blood serum decreases (138). Very low serum Mg is associated with an increased risk of TgAb, HT, and hypothyroidism. The risks of hypothyroidism and subclinical hypothyroidism in the lowest Mg group (≤0.55 mmol/L) were higher than in the normal Mg group (0.851-1.15 mmol/L, $p<0.01$, OR=4.482-4.971) (139). Significantly lower serum concentrations of Mg and Fe were found in HT patients with TPOAb and TgAb (108).

Thyroid diseases play an important role in mineral metabolism, in particular, they affect the mineral density of bone tissue. Thyroid hormones are involved in the metabolism of Ca and P. In hypothyroid patients mean serum Ca and Mg levels were decreased and P levels were elevated. Among cases of hypothyroidism, a statistically significant negative correlation was observed between Ca, Mg, and TSH, while a positive correlation was observed between P and TSH (140). In hypothyroidism, the level of Ca is lower and the level of P is higher in serum than in subclinical hypothyroidism (141). In blood serum of patients with hypothyroidism, the level of Ca was significantly reduced and the level of P was significantly increased (142). In hyperthyroidism, on the contrary, the serum Ca content increases. The average value of the levels of both Ca and P in the blood serum of patients with hyperthyroidism is significantly ($p<0.001$) higher, which may increase the risk of secondary osteoporosis and bone fractures. In patients with hyperthyroidism, the prevalence of hypercalcemia was 38% (143). In hyperthyroidism can be acquired impaired mitochondrial function due to deficiency of Mg, Se and antioxidant coenzyme Q10 as signs of an inflammatory process associated with changes in the musculoskeletal system (144).

For the prevention of autoimmune diseases, the reduction of excessively induced BAFF aggressive B-lymphocytes can be used to influence the intracellular level of Ca^{2+} and mTOR (132). Magnesium supplementation is a potential way to reduce inflammation and OS. Magnesium supplements reduce the levels of NF-κB, IL-6 and TNF-α, improve mitochondrial function and increase the content of antioxidant GSH (133). It is possible to improve the function and morphology of the thyroid gland after correcting exposure to stress factors together with selenomethionine and Mg citrate supplements, which showed a direct correlation between whole blood Se and serum Mg in individuals without thyroid disease and in menopausal women, whereas it was reversed in cases of hyperthyroidism (144). Supplementation of Mg, as well as Zn and vitamin A, may have beneficial effects in patients with hypothyroidism and diseases associated with hyperthyroidism. In patients with hypothyroidism, a significant increase in serum fT4, a decrease in anthropometric indices and lower levels of serum hs-CRP were found after the intervention at 10 weeks (145).

We summarized the results of the review of existing publications on the immunomodulating effect of TG and minerals on the immune system in autoimmune thyroiditis and Grave's disease, and have presented these data in Table 1, where the main effects and mechanisms have been formulated.

TABLE 1 The immunomodulating influence of thyroid hormones and minerals on immunological target cells and their function in the physiological norm and in AITD.

TG/ minerals	Normal/ AITD	Immunological targets and effects	Corresponding references
TG	Normal	> neutrophils, MF, DCs, NK-cells, $CD4^+$, $CD8^+$, Th1, Th17, ROS, IL-12, IL-6, IL-23, IL-1β, IL-17, CCR7; < Treg	(36–38)
	GD	< Treg, PD-1	(39)
I	Normal	> neutrophils, MF, ROS, inflammation; < ROS	(42)
	In vitro	> ROS, OS, apoptosis	(43, 46)
	In vivo/HT	> Th17, TRAIL, ROS, OS, apoptosis; TPOAb, TgAb; < Treg, TGF-β1	(44, 45, 47, 51, 55, 59)
		</= TPOAb	(53, 5)
Se	Normal	> $CD4^+$, Th1, IL-2, IFN-γ, GPx, TR; < ROS, OS, inflammation	(60–62)
	In vitro	> GPx, < apoptosis	(66)
	In vivo (EAT)	> IL-10, Breg; < BAFF; < TPOAb, TgAb	(77)
	HT, GD/GO	< IL-2, TPOAb, TgAb	(73–75)
	HT	> Treg, GPx, TR, SePP1; < OS, CXCL10,	(79, 33, 81)
	HT, GD	< MDA, OS	(78, 86)
	GD	< TRAb	(84, 67)

(Continued)

TABLE 1 Continued

TG/ minerals	Normal/ AITD	Immunological targets and effects	Corresponding references
Fe	Normal *In vitro* HT	> ROS NETosis, MF < MF1, IL-12/IL-10, IL-1β, IL-6, IL-12, TNF-α; > MF2 < TPOAb, TgAb	(94) (95) (107, 108)
Cu	Normal	> neutrophils, ROS, T-cells, IL-2, inflammation > CuSOD	(96, 97) (99)
Zn	Normal *In vitro, In vivo* HT	> T-cells, polarization Th - Th1, Th2, Th17, > Treg < OS, ROS < OS, hs-CRP	(116–118) (114) (33, 145)
Mg	Normal *In vitro* HT	> MF, Th, B-cells, NK-cells, $CD8^+$ T < NF-κB, IL-6, TNF-α, OS; > GSH < TNF-α, IL-1, IL-6, E-selectins, ICAM-1, VCAM-1, hs-CRP > IL-10, prostaglandin E2; < IL-1β, IL-6 < TPOAb, TgAb, hs-CRP	(129, 134, 135) (133) (137) (136) (108, 139, 145)
Ca	Normal	> $CD8^+$ T, apoptosis, inflammation < Treg > BAFF, B-cells	(129) (131) (132)

Normal – at the physiological norm, In vitro – cell culture, In vivo – animal models, > - increase in effect, < - decrease in effect, </= - decrease/no change

Conclusion

There is currently no effective pharmacological therapy for AITD. Treatment of AITD remains symptomatic and is based on taking, if necessary, synthetic TG to correct hypothyroidism or thyrostatics for thyrotoxicosis. The significant prevalence of AITD and the involvement of autoimmune processes in their occurrence require the development of adequate approaches for the correction of immune processes in order to prevent and treat diseases.

Replacement use of TG in case of subclinical and obvious hypothyroidism and inhibition of their synthesis in thyrotoxicosis is appropriate for normalizing thyroid status, but is insufficient for reducing inflammation and OS, restoring the immune system, and suppressing the autoimmune process in the target organ in AITD. Deficiency or excess consumption of I may serve as a risk factor for thyroid diseases. Careful monitoring of iodine prophylaxis is recommended to avoid the consequences of both deficiency and excess I. Addition of Se preparations to L-T4 may be appropriate in patients with low Se and mild or early stage HT. In patients with moderate GO, there is clear evidence of a beneficial effect of Se from a large randomized clinical trial conducted by the European GO Group – EUGOGO.

For the treatment of GD, GO and HT, an antioxidant approach is possible with the use of bio-supplements of elements (I, Se, Zn, Fe, Cu and Mg) as an adjunct in patients with their deficiency. The possibilities of the immunotherapeutic effect of minerals, in addition to the level of thyroid autoantibodies, can be aimed at OS markers. The main therapeutic mechanisms of AITD are the reduction of OS, the inflammatory process that damages thyrocytes. The targets of immunocorrection with the participation of minerals (I, Se, Zn, Fe, Cu, and Mg) in AITD can be the normalization of the function of neutrophils as producers of excess ROS and inflammation, reduction of the secretion of pro-inflammatory cytokines Th17 and chemokines, activation of Treg and their cytokines as the main inhibitors of inflammation and autoimmune process.

Determining the immunomodulating effect of TG, minerals, and researching the possibility of using them as means of immunocorrection, taking into account the pathogenesis of AITD, can contribute to the development of a more effective treatment strategy, as well as approaches to prevention.

Author contributions

VK and TZ conceived and designed the review. The manuscript was written by TZ, VK. All authors have read and approved the published version of the manuscript.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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The value of total tumor diameter in unilateral multifocal papillary thyroid carcinoma: a propensity score matching analysis

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Background: Tumor multifocality is frequently observed in papillary thyroid carcinoma (PTC). However, the maximum tumor diameter (MTD), currently utilized in various staging schemes, might not accurately indicate the level of aggressiveness exhibited by multifocal tumors. We aimed to investigate the relationship between total tumor diameter (TTD) and clinicopathological features of papillary thyroid carcinoma.

Methods: Retrospective data analysis was done on 1936 individuals who underwent complete thyroidectomy for PTC. Patients were classified into subgroups according to unilateral multifocality, central lymph node metastasis (CLNM) and lateral lymph node metastasis (LLNM). The relationships of clinicopathological features among these groups were analyzed.

Results: Unilateral multifocality was observed in 117 patients. The clinicopathological features of the unilateral multifocal PTC were similar to the unifocal PTC with approximate TTD. The unilateral multifocality played no independent role in CLNM and LLNM. Moreover, the efficiency of TTD in predicting CLNM and LLNM was significantly higher than that of MTD.

Conclusion: In the case of unilateral multifocal PTC, TTD is a more accurate indicator of the biological characteristics of the tumor than MTD.

KEYWORDS

papillary thyroid carcinoma, propensity score matching analysis, total tumor diameter, unilateral multifocal, lymph node metastases

Introduction

Thyroid cancer is the ninth most common cancer worldwide, and its prevalence has increased significantly over the last 40 years (1). In 2020, there were 586,202 new cases of thyroid cancer, with papillary thyroid carcinoma (PTC) accounting for more than 80% of all thyroid cancers (1). In general, PTC is regarded as a disease with a favorable prognosis. However, some PTCs with specific clinicopathological characteristics are aggressive and have poor prognoses (2). Tumor multifocality is a frequent observation in PTC, occurring in 18–87% of documented cases (3). Multifocality in thyroid cancer is the concurrent existence of multiple tumor foci within the thyroid gland (4). Depending on where the tumors are, the meaning of multifocality can be broken down into unilateral multifocality (two or more lesions in the same lobe), bilateral multifocality (two or more lesions in the same lobe and one or more lesions in the opposite lobe), or bilaterality (one lesion in each lobe). And a report showed that unilateral multifocality and bilaterality could be two different multifocal entities in patients with PTC (5).

Multiple studies have shown that multifocality is associated with high-risk characteristics of PTC, such as an aggressive histologic subtype, extrathyroidal extension, lymph node involvement, distant metastasis, and recurrence (6, 7). However, the current staging system still defines the size of multifocal tumors in the same manner as that of unifocal tumors, concentrating on the maximum tumor diameter (MTD) (8). In recent years, many studies have also reported the concept of total tumor diameter (TTD) (9–11). Some demonstrated that TTD could reflect a tumor's biological characteristics and aggressiveness more accurately than MTD (12).

However, the previous researchers took the unilateral multifocality and bilaterality as the same multifocality in their studies (5, 11). The purpose of the present study was to evaluate the association between TTD and unilateral multifocal PTC using the propensity score matching (PSM) method to provide an updated and more comprehensive assessment of the risk for unilateral multifocal PTC.

Methods

Patients

Data for pathologically confirmed PTC patients who underwent thyroid surgery in the Department of Thyroid Surgery, West China Hospital (Chengdu, China) between 2013 and 2018 were collected and reviewed. 2322 PTC Patients who were > 18 years old and underwent the first-time total thyroidectomy with central lymph nodes dissection were enrolled. After excluding PTC with bilateral multifocality (260 cases,11.2%) and bilaterality (126 cases,5.4%), 117 unilateral multifocal cases and 1819 unifocal cases were involved in the study. The collected patient data included age, gender, MTD, TTD, central lymph node metastasis (CLNM), lateral lymph node metastasis (LLNM), capsular invasion, gross extrathyroidal extension (gETE), and so on. The medical ethics committee of West China Hospital, Sichuan University approved the study. The patients in the study provided informed consent. All methods were carried out following relevant regulations and guidelines.

Definition

In the study, MTD was referred to as either the primary tumor focus for unifocal PTC or the diameter of the greatest tumor focus in multifocal PTC. TTD was defined as the sum of each tumor lesion's diameter within a lobe. Unilateral multifocality was defined as two or more lesions in the same lobe. CLNM was defined as tumor metastasis in level VI cervical lymph nodes. Based on the postoperative pathological diagnosis, LLNM was defined as tumor metastasis in lymph nodes of levels II, III, IV, and V in the neck region. Based on the postoperative pathology diagnosis, the capsular invasion was defined as a tumor involving the capsule but not penetrating it. gETE was characterized as the tumor penetrating the thyroid capsule, and invading surrounding tissues or organs, for example, esophagus, trachea and recurrent laryngeal nerve (RLN).

Study design and grouping

A total of 1936 patients were involved in the study. First, the above data was divided into two groups according to the number of tumor lesions, the unilateral multifocal PTC group and the unifocal PTC group. PSM was performed based on age, sex, body mass index (BMI), nodular goiter (NG), Hashimoto's thyroiditis (HT), capsule invasion, gETE, and TTD or MTD to minimize the effect of confounders on the outcomes between these groups. Then the data was divided into another two groups: the with CLNM group and the without CLNM group. PSM was performed based on age, sex, BMI, NG, HT, capsule invasion, gETE, TTD and MTD. Moreover, the data was divided according to have LLNM or not. And the same PSM as CLNM was performed.

Surgical strategy

All patients enrolled in the study underwent total thyroidectomy. The central lymph nodes were routinely dissected. Patients found to have cervical lymph node metastases after undergoing fine-needle aspiration biopsy or preoperative imaging were candidates for lateral lymph node dissection. This diagnosis was verified by the intraoperative frozen sections.

Statistical analysis

SPSS 26.0 software was applied to the data analysis. Continuous variables (confirmed by the Kolmogorov-Smirnov test) with non-normal distribution were presented as the median. Differences between continuous variables were examined using the Mann–Whitney U test. Categorical variables were presented as absolute value. Pearson's $\chi 2$ test or Fisher exact test was used to investigate the heterogeneity between categorical variables. Univariate and

multivariate logistic regression analyses were used to identify the risk factors for CLNM and LLNM. The receiver operating characteristic (ROC) curve was plotted and compared the area under the curve (AUC) values. R software (R Core Team, Version 4.1.2, Vienna, Austria) with the "matchit" package was used to perform the PSM. The nearest neighbor algorithm was used as the matching method, with the caliper value set to 0.02. A P value of < 0.05 was considered statistically significant.

Results

Comparison of baseline characteristics between the unilateral multifocal PTC group and the unifocal PTC group

A total of 1936 eligible patients were enrolled and categorized into the unilateral multifocal PTC group (n =117) and the unifocal PTC group (n = 1819). Demographic and clinicopathological characteristics of the patients were presented in Table 1. Before PSM, the unilateral multifocal PTC group had larger TTD (16.00 [12.50-23.00]*vs*10.00[8.00-15.00], P<0.001). CLNM (62.4%*vs*48.9%, P=0.005) and LLNM (31.6%*vs*20.2%, P=0.003) were reported more frequently in the unilateral multifocal PTC group than in the unifocal PTC group. The patients with unilateral multifocal PTC were more likely to have NG (66.7%*vs*52.9%, P=0.009).

After PSM was performed basing on age, sex, BMI, NG, HT, capsule invasion, gETE and MTD, significant differences between the unilateral multifocal PTC group and the unifocal PTC group in the rate of CLNM (62.4%*vs*43.6%, P<0.001) and LLNM (31.6%VS15.0%, P<0.001) still existed. And the unilateral multifocal PTC group had a higher proportion with AJCC stage III-IV (4.3%*vs*2.3%, P=0.049). After PSM was performed basing on age, sex, BMI, NG, HT, capsule invasion, gETE and TTD, differences between the unilateral multifocal PTC group and the unifocal PTC group disappeared in all characteristics except MTD (10.00[8.00-15.00]*vs*15.00[10.00-21.00], P<0.001). (Table 1).

TABLE 1 Comparison of baseline characteristics between the unilateral multifocal PTC group and the unifocal PTC group.

		Before PSM		After 1:4 PSM (MTD)		After 1:4 PSM (TTD)	
	Unilateral Multifocal PTC (N=117)(%)	Unifocal PTC (N=1819) (%)	P	Unifocal PTC (N=468) (%)	P	Unifocal PTC (N=468) (%)	P
Age			0.694		0.457		0.439
<55	98(83.8)	1548(85.1)		378(80.8)		405(86.5)	
≥55	19(16.2)	271(14.9)		90(19.2)		63(13.5)	
Sex			0.705	(0)	0.780	(0)	0.684
Female	84(71.8)	1335(73.4)		342(73.1)		327(69.9)	
Male	33(28.2)	484(26.6)		126(26.9)		141(30.1)	
BMI	23.32(21.10-25.39)	23.07(20.81-25.10)	0.305	23.32(21.02-25.20)	0.789	23.32(20.96-25.39)	0.885
NG	78(66.7)	963(52.9)	**0.009**	319(68.2)	0.757	(0)	0.794
HT	26(22.2)	393(21.6)	0.913	110(23.5)	0.769	131(28)	0.208
Capsule invasion	33(28.2)	561(30.8)	0.549	152(32.5)	0.374	161(34.4)	0.203
gETE							
Esophageal	2(1.7)	29(1.6)	1	6(1.3)	0.663	8(1.7)	1
Tracheal	5(4.3)	46(2.5)	0.366	9(1.9)	0.170	12(2.6)	0.325
RLN	9(7.7)	123(6.8)	0.699	28(6)	0.497	35(7.5)	0.938
MTD	10.00(7.00-15.00)	10.00(8.00-15.00)	0.050	10.00(8.00-15.00)	0.587	15.00(10.00-21.00)	0.000
TTD	16.00(12.50-23.00)	10.00(8.00-15.00)	0.000	16.00(12.50-23.00)	0.000	15.00(10.00-21.00)	0.164
CLNM	73(62.4)	890(48.9)	0.005	204(43.6)	0.000	268(57.3)	0.314
LLNM	37(31.6)	368(20.2)	0.003	70(15)	0.000	116(24.8)	0.132
AJCC stage			0.202		0.049		0.354
I-II	112(95.7)	1777(97.7)		462(98.7)		456(97.4)	
III-IV	5(4.3)	42(2.3)		6(1.3)		12(2.6)	

1:4 PSM (MTD): PSM was performed basing on age, sex, BMI, NG, HT, capsule invasion, gETE and MTD.
1:4 PSM (TTD): PSM was performed basing on age, sex, BMI, NG, HT, capsule invasion, gETE and TTD.
BMI, Body mass index; NG, nodular goiter; HT, Hashimoto's thyroiditis; RLN, recurrent laryngeal nerve; gETE, gross extrathyroidal extension; MTD, maximum tumor diameter; TTD, total tumor diameter; CLNM, central lymph node metastasis; LLNM, lateral lymph node metastasis; AJCC, American Joint Committee on Cancer.
The bold values: emphasize the p value<0.05.

Comparison of baseline characteristics between the without LNM (lymph node metastasis) group and the with LNM group

Table 2 listed the demographic and clinicopathological traits of the patients. CLNM occurred more frequently in male patients (30.9%*vs*22.5%, P<0.001), patients aged less than 55 years (87.9% *vs* 82.2%, P = 0.001), patients with lower BMI (22.89[20.70-25.00]*vs* 23.24[20.97-25.20], P<0.001). NG was reported less frequently in the With CLNM group (47.8%*vs*59.7%, P<0.001). While unilateral multifocality was reported more frequently in the With CLNM group (7.6%vs4.5%, P=0.005). There was a higher proportion of gETE in the With CLNM group (Esophageal 2.6% *vs*0.6%, P=0.001; Tracheal 3.7%*vs*1.5%, P=0.003; RLN 9.2%*vs*4.4%, P<0.001). The group with CLNM had larger MTD (13.00[10.00-20.00]*vs*10.00 [7.00-14.00], P<0.001) and TTD (14.00[10.00-20.00]*vs*10.00[7.00-15.00], P<0.001). After PSM was performed basing on age, sex, BMI, NG, HT, capsule invasion, gETE, MTD and TTD, differences between the Without CLNM group and With CLNM group in the incidence of unilateral multifocality disappeared.

Male patients (32.3%*vs*25.2%, P=0.004), patients without NG (43.7%*vs*56.4%, P<0.001), patients with gETE (Esophageal 5.2% *vs*0.7%, P<0.001; Tracheal 6.7%*vs*1.6%, P<0.001; RLN 14.1%*vs*4.9%, P<0.001), patients with larger MTD (15.00[10.00-25.00]*vs* 10.00 [7.00-15.00], P<0.001) or TTD (16.00[10.00-25.00]*vs* 10.00[8.00-15.00], P<0.001), patients with unilateral multifocal PTC (9.1% *vs*5.2%, P=0.003) were more likely to have LLNM (Table 3). After PSM was performed basing on age, sex, BMI, NG, HT, capsule invasion, gETE, MTD and TTD, differences in the incidence of unilateral multifocality between the groups With and Without LLNM vanished.

Risk factors for CLNM and LLNM

We used both univariate and multivariate analyses to obtain the risk factors for CLNM and LLNM. Univariate analysis identified 8 variables (Sex, Age, MTD, TTD, Esophageal extension, Tracheal extension, RLN extension and Unilateral multifocality) linked with CLNM, but the relationship among unilateral multifocality and CLNM was lost after PSM was performed basing on age, sex, BMI, NG, HT, capsule invasion, gETE, MTD and TTD (Table 4). Univariate regression analysis indicated that LLNM was significantly associated with sex, MTD, TTD, unilateral

TABLE 2 Comparison of baseline characteristics between the Without CLNM group and the With CLNM group.

	Before PSM		P	After 1:1PSM		P
	Without CLNM (N=973) (%)	With CLNM (N=963) (%)		Without CLNM (N=713) (%)	With CLNM (N=713) (%)	
Age			**0.001**			0.141
<55	800(82.2)	846(87.9)		637(89.3)	619(86.8)	
≥55	173(17.8)	117(12.1)		76(10.7)	94(13.2)	
Sex			**0.000**			0.904
Female	754(77.5)	665(69.1)		523(73.4)	525(73.6)	
Male	219(22.5)	298(30.9)		190(26.6)	188(26.4)	
BMI	23.24(20.97-25.20)	22.89(20.70-25.00)	**0.000**	23.19(20.81-25.11)	22.86(20.70-24.84)	0.173
NG	581(59.7)	460(47.8)	**0.000**	370(51.9)	379(53.2)	0.633
HT	199(20.5)	220(22.8)	0.215	173(24.3)	120(16.8)	0.853
Capsule invasion	284(29.2)	310(32.2)	0.152	232(32.5)	226(31.7)	0.734
gETE						
Esophageal	6(0.6)	25(2.6)	**0.001**	6(0.8)	8(1.1)	0.591
Tracheal	15(1.5)	36(3.7)	**0.003**	15(2.1)	16(2.2)	0.856
RLN	43(4.4)	89(9.2)	**0.000**	39(5.5)	37(5.2)	0.814
MTD	10.00(7.00-14.00)	13.00(10.00-20.00)	**0.000**	10.00(8.00-15.00)	10.00(8.00-15.00)	0.432
TTD	10.00(7.00-15.00)	14.00(10.00-20.00)	**0.000**	10.00(8.00-15.00)	10.00(8.50-15.00)	0.227
Unilateral Multifocality	44(4.5)	73(7.6)	**0.005**	37(5.2)	46(6.5)	0.366

1:1PSM: PSM was performed basing on age, sex, BMI, NG, HT, capsule invasion, gETE, MTD and TTD.
BMI, Body mass index; NG, nodular goiter; HT, Hashimoto's thyroiditis; RLN, recurrent laryngeal nerve; gETE, gross extrathyroidal extension; MTD, maximum tumor diameter; TTD, total tumor diameter.
The bold values: emphasize the p value<0.05.

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TABLE 3 Comparison of baseline characteristics between the Without LLNM group and the With LLNM group.

	Before PSM			After 1:1PSM		
	Without LLNM (N=1531) (%)	With LLNM (N=405) (%)	P	Without LLNM (N=367) (%)	With LLNM (N=367) (%)	P
Age			0.958			0.915
<55	1302(85)	344(84.9)		315(85.8)	316(86.1)	
≥55	229(15)	61(15.1)		52(14.2)	51(13.9)	
Sex			**0.004**			0.431
Female	1145(74.8)	274(67.7)		242(65.9)	252(68.7)	
Male	386(25.2)	131(32.3)		125(34.1)	115(31.3)	
BMI	23.12(20.81-25.10)	23.07(20.96-25.20)	0.820	23.32(20.96-25.24)	23.03(20.76-25.10)	0.161
NG	864(56.4)	177(43.7)	**0.000**	157(42.8)	165(45)	0.552
HT	337(22)	82(20.2)	0.570	68(18.5)	79(21.5)	0.310
Capsule invasion	463(30.2)	131(32.3)	0.431	136(37.1)	124(33.8)	0.354
gETE						
Esophageal	10(0.7)	21(5.2)	**0.000**	9(2.5)	9(2.5)	1.000
Tracheal	24(1.6)	27(6.7)	**0.000**	16(4.4)	18(4.9)	0.725
RLN	75(4.9)	57(14.1)	**0.000**	44(12)	37(10.1)	0.410
MTD	10.00(7.00-15.00)	15.00(10.00-25.00)	**0.000**	15.00(10.00-20.00)	15.00(10.00-21.00)	0.742
TTD	10.00(8.00-15.00)	16.00(10.00-25.00)	**0.000**	15.00(10.00-22.00)	15.00(10.00-24.00)	0.545
Unilateral Multifocality	80(5.2)	37(9.1)	**0.003**	26(7.1)	34(9.3)	0.281

1:1PSM: PSM was performed basing on age, sex, BMI, NG, HT, capsule invasion, gETE, MTD and TTD.
BMI, Body mass index; NG, nodular goiter; HT, Hashimoto's thyroiditis; RLN, recurrent laryngeal nerve; gETE, gross extrathyroidal extension; MTD, maximum tumor diameter; TTD, total tumor diameter.
The bold values: emphasize the p value<0.05.

multifocality, esophageal extension, tracheal extension and RLN extension. The correlation between unilateral multifocality and LLNM was no longer present after PSM was performed basing on age, sex, BMI, NG, HT, capsule invasion, gETE, MTD and TTD (Table 4).

Multivariate analysis found 3 variables (Sex, Age, TTD), which were risk factors of CLNM, and 3 variables (TTD, Esophageal extension, Tracheal extension), which were risk factors of LLNM (Table 5).

Comparison of prediction efficiency of TTD and MTD for CLNM and LLNM

The respective calculated AUC values of 0.660(MTD) and 0.669 (TTD) were statistically significantly different (P=0.005) (Table 6), suggesting that TTD may be a better predictor of CLNM for unilateral multifocal PTC (Figure 1). Meanwhile, the computed AUC values of 0.712(MTD) and 0.723(TTD), respectively, differed statistically(p=0.011) (Table 6), showing that for unilateral multifocal PTC, TTD may be a stronger predictor of LLNM (Figure 2).

Discussion

The impact of TTD in multifocal tumors has been reported on diseases like breast cancer and kidney cancer (13–15). Additionally, TTD's significance in thyroid cancer has been documented in prior researches. They found that TTD was associated with lymph node metastasis and may represent the tumor biological traits and aggressiveness more accurately than MTD (9, 11, 12, 16). However, the researchers treated unilateral multifocality and bilaterality as the same multifocality types. The current study used a PSM method to investigate the association between TTD and clinicopathological characteristics in unilateral multifocal PTC based on thyroid cancer patients in our center.

The AJCC TNM classification determines the primary tumor (T) stage according to the largest tumor size and ETE (17), even in the multifocal PTC. The impact of other smaller tumor foci on aggressiveness has not been well investigated. Evaluating the largest tumor size and neglecting the smaller foci in multifocal tumors may underestimate the clinicopathologic aspects of malignancies, impacting tumor staging and treatment. In our study, we did two different types of PSM, matching the MTD and TTD respectively, to explore which diameter could represent the tumor biological

TABLE 4 Univariate logistic regression analysis of the risk factors for the CLNM and LLNM.

Variables	Before PSM		P	After 1;1 PSM		P
	OR	[95%CI]		OR	[95%CI]	
CLNM						
Sex (*vs* male)	0.648	0.529-0.794	0.000			
Age (*vs*≥55)	1.564	1.213-2.015	0.001			
MTD	1.080	1.065-1.095	0.000			
TTD	1.081	1.067-1.096	0.000			
Unilateral Multifocality	1.732	1.178-2.546	0.005	1.260	0.807-1.968	0.310
gETE						
Esophageal	4.295	1.754-10.518	0.001			
Tracheal	2.48	1.349-4.560	0.003			
RLN	2.202	1.513-3.206	0.000			
LLNM						
Sex (*vs* male)	0.705	0.556-0.895	0.004			
MTD	1.082	1.068-1.095	0.000			
TTD	1.083	1.070-1.097	0.000			
Unilateral Multifocality	1.824	1.215-2.737	0.004	1.339	0.786-2.281	0.282
gETE						
Esophageal	8.318	3.885-17.810	0.000			
Tracheal	4.485	2.559-7.862	0.000			
RLN	3.18	2.210-4.575	0.000			

1:1PSM: PSM was performed basing on age, sex, BMI, NG, HT, capsule invasion, gETE, MTD and TTD.
RLN, recurrent laryngeal nerve; gETE, gross extrathyroidal extension; MTD, maximum tumor diameter; TTD, total tumor diameter; CLNM, central lymph node metastasis; LLNM, lateral lymph node metastasis.

characteristics and aggressiveness more accurately. We found that the lymph node metastasis rate had significant differences between unilateral multifocal PTC and unifocal PTC after PSM matching the MTD. However, no difference was found after PSM matching the TTD. In other words, the tumor biological traits of the unilateral multifocal PTC were similar to the unifocal PTC with approximate TTD. This result was identical to the previous study (11), which mainly focused on papillary thyroid microcarcinoma (PTMC), showing that micro-PTC tended to behave like the macro-PTC once the TTD exceeded 1.0 cm.

TABLE 5 Multivariate logistic regression analysis of the risk factors for the CLNM and LLNM.

Variables	OR	[95%CI]	P
CLNM			
Sex(*vs* male)	0.713	0.575-0.883	0.002
Age(*vs*≥55)	1.803	1.371-2.371	0.000
TTD	1.106	1.041-1.175	0.001
LLNM			
TTD	1.098	1.037-1.163	0.001
gETE			
Esophageal	2.849	1.157-7.014	0.023
Tracheal	1.997	1.006-3.962	0.048

1:1PSM: PSM was performed basing on age, sex, BMI, NG, HT, capsule invasion, gETE, MTD and TTD.
gETE, gross extrathyroidal extension; TTD, total tumor diameter; CLNM, central lymph node metastasis; LLNM, lateral lymph node metastasis.

TABLE 6 The diagnosis efficiency of LNM comparison between MTD and TTD.

Diagnostic indicators	Cutoff value	AUC	95%CI	P	Sensitivity	Specificity	Youden index
CLNM							
MTD	11.5	0.660	0.636-0.684	0.000	0.551	0.689	0.240
TTD	11.5	0.669	0.519-0.632	0.012	0.584	0.668	0.252
LLNM							
MTD	13.5	0.712	0.683-0.742	0.000	0.630	0.696	0.326
TTD	14.5	0.723	0.695-0.752	0.000	0.654	0.692	0.346

MTD, maximum tumor diameter; TTD, total tumor diameter; LNM, lymph node metastasis; CLNM, central lymph node metastasis; LLNM, lateral lymph node metastasis.

LNM was common among PTC patients. According to earlier research, LNM was present in 30% to 90% of PTC patients, particularly in the central region (18–20), which was one of the major factors that contributed to postoperative recurrence. Although reoperation could still result in a favorable prognosis, patients were more probably to suffer surgical complications (21, 22). Therefore, it was crucial for the patient's overall prognosis that the lymph nodes were managed at the initial surgery. Because the unique anatomical position limited the diagnostic ability of the current imaging techniques (23), the clinical lymph nodes negative (cN0) was unreliable. Whether prophylactic central lymph nodes dissection (PCLND) was appropriate for cN0 PTC patients was still up for debate. PCLND at the time of the initial surgery, according to Guo et al. (21, 24), may have advantages, including avoiding the significant risk of long-term hypoparathyroidism and RLN damage associated with repeat surgery. Yoon et al. (25), on the other hand, came to the conclusion that the chance of developing permanent hypocalcemia may rise and parathyroid hormone levels may be dramatically lowered following PCLND. In order to direct therapeutic decision-making, it was helpful to identify high-risk variables for LNM. In our study, TTD was significantly correlated with CLNM and LLNM. Also, the ability of TTD to predict CLNM and LLNM was much better than that of MTD in PTC, showing that TTD might be a better predictor of LNM for unilateral multifocal PTC.

In our data, 62.4% of unilateral multifocal PTC presented with CLNM and 31.6% presented with LLNM, significantly higher than unifocal PTC (48.9%&20.2%). Other researches showed the same results (26, 27). In a report by Lombardi et al., multifocal illness increased cervical lymph node recurrence 17.9-fold (28). In another study, the rate of LNM for patients with multifocal PTCs was 55.6%, while it was only 28.6% for those with unifocal illnesses (29). However, the association between LNM and multifocality remained controversial. According to certain studies, multifocality

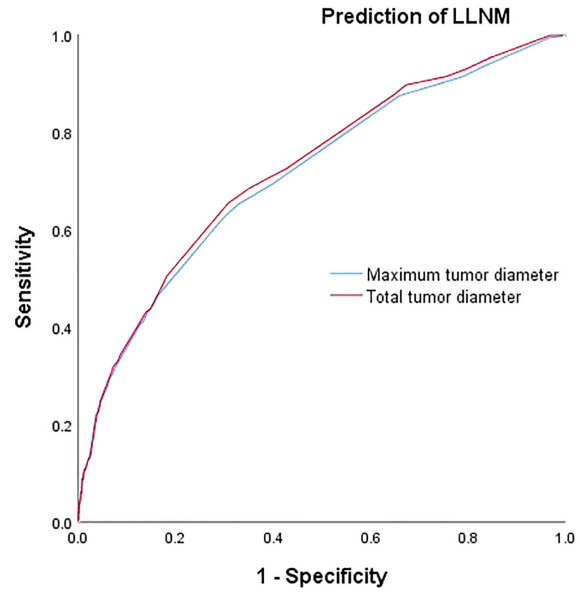


FIGURE 1
Comparison of prediction efficiency of TTD and MTD for CLNM. AUC values of 0.660(MTD) and 0.669(TTD) differed statistically (P=0.005). MTD, maximum tumor diameter; TTD, total tumor diameter; CLNM, central lymph node metastasis.

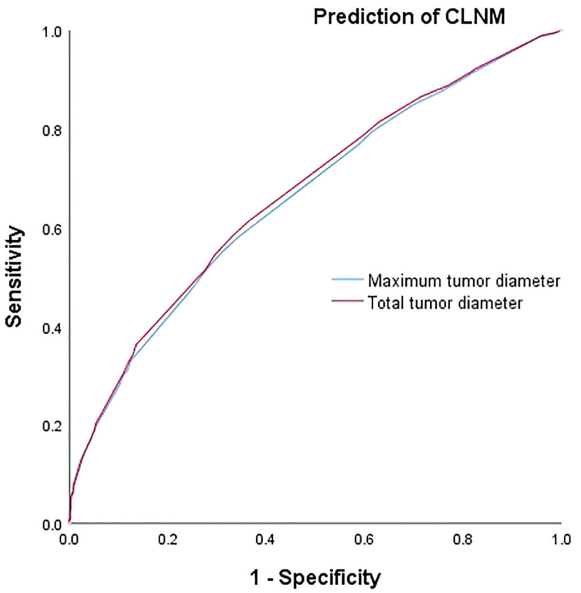


FIGURE 2
Comparison of prediction efficiency of TTD and MTD for LLNM. AUC values of 0.712(MTD) and 0.723(TTD) differed statistically (P=0.011). MTD, maximum tumor diameter; TTD, total tumor diameter; LLNM, lateral lymph node metastasis.

was not independently related to LNM (30). Many other studies have focused on the risk factors of LNM for multifocal PTC patients. For example, Zheng WH proposed that multifocality should be evaluated when selecting patients for PCLND (31). Some reports showed that multifocality was an independent risk factor for CLNM and LLNM (32, 33). As found in previous studies (33–36), our univariate analyses revealed an association between multifocality and LNM. However, on multivariate adjustment for classic clinicopathological risk factors, including MTD and TTD, the association between unilateral multifocality and LNM was lost in PTC. The result demonstrated that unilateral multifocality played no independent role in LNM.

Once, it was thought that multifocality in PTC was caused by the lymphatic spread of tumor cells from one malignant clone. This theory was backed by the fact that tumor cells were often found in lymphatic vessels and that lymph node metastases happened often. But others had the opposite ideas. Bansal et al. found that as many as 60% of multifocal PTCs may have multiple sources, and these origins were generally found in various lobes. The same mutation status was typically shared by multifocality within the same lobe, though (37, 38). A clinical research also showed that unilateral multifocality differed from bilaterality (5). Unlike earlier studies (5, 11), we only included unilateral multifocal PTC in our study to remove the distraction of different types of multifocality and used PSM method to provide an updated and more comprehensive assessment of the risk for unilateral multifocal PTC.

There were some limitations in the study. The inclusion of patients based on their characteristics resulting in a selection bias in this retrospective analysis. Despite the fact that we used PSM, the results could still be influenced by selection bias. Moreover, the participant sample of unilateral multifocal PTC was small. Our data could be hampered by the small sample size. Conducting more validation studies with a bigger cohort and long-term follow-up is necessary.

Conclusion

In conclusion, the current study offers fresh evidence of the connection between TTD and PTC. TTD can more accurately reflect the biological characteristics and aggressiveness of unilateral multifocal PTC than MTD.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving humans were approved by The medical ethics committee of West China Hospital, Sichuan University. The studies were conducted in accordance with the local legislation and institutional requirements.

Author contributions

Author Contributions (I) Conception and design: A-PS, Z-JW (II) Administrative support: A-PS (III) Provision of study materials or patients: A-PS, Z-JW (IV) Collection and assembly of data: Z-JW, Z-WC, B-YX, HG (V) Data analysis and interpretation: Z-JW, MA. All authors contributed to the article and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Survival outcomes of low-risk papillary thyroid carcinoma at different risk levels: a corollary for active surveillance

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Background: This study aims to compare the outcomes of active surveillance (AS) in low-risk papillary thyroid carcinoma (PTC) patients with different tumor sizes and lymph node metastasis status, in order to establish appropriate management strategies. By analyzing these results, this study provides valuable insights for the effective management of such patients, addressing the issues and challenges associated with AS in practical clinical practice.

Methods: The study utilized the SEER database supported by the National Cancer Institute of the United States, extracting data of PTC diagnosed between 2000 and 2015. Statistical analyses were conducted using inverse probability weighting (IPTW) and propensity score matching (PSM), including Kaplan-Meier survival curves and Cox regression models, to evaluate the impact of different tumor sizes and lymph node metastasis status on thyroid cancer-specific survival (TCSS).

Results: A total of 57,000 PTC patients were included, with most covariates having standardized mean differences below 10% after IPTW and PSM adjustments. The TCSS of PTC with a diameter smaller than 13mm is significantly better than that of tumors with a diameter larger than 13mm, regardless of the presence of lymph node metastasis. Among PTC cases with a diameter smaller than 13mm, the TCSS of patients is similar, regardless of the presence of lymph node metastasis. However, in PTC cases with a diameter larger than 13mm, the presence of lateral neck lymph node metastasis (N1b stage) significantly impacts the TCSS, although the absolute impact on TCSS rate is minimal.

Conclusion: The treatment strategy of AS is safe for patients with T1a stage papillary thyroid microcarcinoma (PTMC). However, for patients with T1b stage, if the tumor diameter exceeds 13mm or there is lymph node metastasis in the lateral neck region, the TCSS will be significantly affected. Nevertheless, the absolute impact on survival is relatively small.

KEYWORDS

papillary thyroid carcinoma, low-risk, active surveillance, IPTW, PSM

Introduction

In recent decades, the global incidence of thyroid cancer, particularly papillary thyroid microcarcinoma (PTMC), has significantly increased. This rise can be attributed to factors like widespread ultrasound use, technological advancements, and population screening programs (1–4). Although the incidence of PTMC continues to rise, mortality rates have remained relatively stable (5). Consequently, there has been a reevaluation of the traditional approach of immediate thyroid surgery for PTMC (6), leading to the emergence of active surveillance (AS) as a conservative management strategy for low-risk PTMC cases (7).

During AS, disease progression is defined by tumor enlargement or the development of lymph node or distant metastasis, with the latter being rare. Notably, long-term prospective trials conducted by Kuma Hospital and Cancer Institute Hospital, two prominent Japanese institutions, have evaluated the safety and effectiveness of AS for asymptomatic PTMC patients with T1aN0M0 status. Kuma Hospital's experience over a 10-year follow-up revealed an 8.0% incidence of tumor growth and a 3.8% incidence of cervical lymph node spread (7). Similarly, the Cancer Institute Hospital reported a 7% rate of tumor size enlargement and a 1% occurrence of new lymph node metastasis, without extrathyroidal invasion or distant metastasis (8). Based on these positive outcomes, the Japanese Clinical Guidelines for thyroid tumor treatment, published in 2010, approved AS as a management option for asymptomatic PTMC patients, making it the first country to do so (9). The American Thyroid Association (ATA) also revised its guidelines to support AS as a suitable alternative to immediate surgery for selected patients with very low-risk tumors (10).

Despite the recommendation of AS, concerns persist among healthcare providers regarding its implementation in real-world practice. Some scholars have observed aggressive central lymph node metastasis even in small-sized lesions (11, 12), which creates a paradox of "low-risk" classification alongside a high prevalence of lymph node metastasis. However, it is worth noting that tumor enlargement or lymph node metastasis may not necessarily increase the recurrence rate or mortality of the disease. Additionally, there is a growing belief that patients with T1bN0M0 status are also suitable candidates for AS (13). To establish an appropriate management strategy for cT1aN0M0 or cT1bN0M0 papillary thyroid carcinoma (PTC) patients, this study aims to compare AS outcomes among low-risk patients with varying tumor sizes and lymph node metastasis. Analyzing these outcomes will provide valuable insights for the effective management of such patients, addressing concerns and challenges associated with the implementation of AS in real-world clinical practice.

Abbreviations: AS, active surveillance; PTC, papillary thyroid carcinoma; PTMC, papillary thyroid microcarcinoma; ATA, American Thyroid Association; PSM, propensity score matching; IPTW, inverse probability of treatment weighting; RAI, radioisotopes; TCSS, thyroid cancer-specific survival; SEER, Surveillance Epidemiology and End Results; HR, hazard ratio; CI, confidence interval; SMDs, standardized mean differences.

Methods

Data source

The SEER database, supported by the National Cancer Institute, collects case data from population-based cancer registries covering approximately 34.6 percent of the U.S. population. It contains de-identified information on patient demographics, primary tumor site, tumor morphology, stage at diagnosis, and follow-up vital status. For this study, we utilized data on papillary thyroid cancer diagnosed between 2000 and 2015, obtained from the November 2022 submission of SEER. The study was reviewed and approved by the institutional review board at Shaoxing Second Hospital, and the data were deidentified, thus patient consent was not required.

Patient selection

We employed SEER*Stat 8.4.1 software to extract relevant information, including patient identification, year of diagnosis (2000-2003, 2004-2007, 2008-2011, 2012-2015), patient age (<55, ≥55), race/ethnicity (white, black, others), marital status (married, single, divorced, separated or widowed), gender (male, female), geographic region (metropolitan, nonmetropolitan), months from diagnosis to treatment (<6, 6-12, 12-24), histological type, tumor size (≤20 mm), N stage (N0, N1a, N1b), distant metastases, multifocality, surgery type (none, less than lobectomy, lobectomy, subtotal or near or total thyroidectomy), lymph node dissection (yes or none), chemotherapy (yes or none), and radiation therapy (none, radioisotopes (RAI), external radiotherapy, combined radioisotopes and external radiotherapy). All variables were categorized as outlined in Table 1. Only patients with biopsy-proven well-differentiated PTC were included. ICD-03 histologies comprised 8050, 8260, 8340–8344, 8350, and 8450–8460. To select only T1N0M0 low-risk PTC cases, patients with tumors larger than 20 mm, carcinoma in situ, extrathyroidal extension, incomplete lymph node status, distant metastases, or PTC diagnosis at autopsy were excluded. Patients who received external radiotherapy or chemotherapy or had incomplete surgical information were also excluded to enhance data accuracy and minimize potential confounding effects of subtherapeutic regimens.

Statistical analysis

We employed similar statistical analysis approaches as previous studies that examined the benefit of interventions for breast cancer subsets (14, 15). Baseline patient, tumor, and treatment characteristics were compared between different tumor size groups and N stage groups using Pearson's Chi-square test. To address missing data, we performed multiple imputation using a multivariate logistic regression model, with 10 cycles repeated to produce a final dataset. The imputation model included the following variables: year of diagnosis (2000-2003, 2004-2007, 2008-2011, 2012-2015), patient age (<55, ≥55), race/ethnicity

TABLE 1 Baseline Demographic and Tumor Characteristics of PTC Patients with Different Lymph Node Status and Tumor Sizes in the SEER Database.

Characteristics	N0						N1a						N1b					
	1-4mm N=15362	5-7mm N=9156	8-10mm N=8,630	11-13mm N=5,786	14-16mm N=5,517	17-20mm N=5,572	1-4mm N=351	5-7mm N=620	8-10mm N=999	11-13mm N=944	14-16mm N=931	17-20mm N=1,056	1-4mm N=245	5-7mm N=324	8-10mm N=396	11-13mm N=363	14-16mm N=374	17-20mm N=374
Year of diagnosis																		
2000-2003	1954 (12.7)	930 (10.2)	970 (11.2)	642 (11.1)	735 (13.3)	876 (15.7)	64 (18.2)	93 (15.0)	104 (10.4)	67 (7.1)	94 (10.1)	130 (12.3)	10 (4.1)	11 (3.4)	17 (4.3)	19 (5.2)	19 (5.1)	45 (12.0)
2004-2007	3249 (21.1)	1905 (20.8)	1760 (20.4)	1190 (20.6)	1293 (23.4)	1342 (24.1)	54 (15.4)	97 (15.6)	126 (12.6)	149 (15.8)	131 (14.1)	204 (19.3)	53 (21.6)	76 (23.5)	91 (23.0)	59 (16.3)	72 (19.3)	78 (20.9)
2008-2011	4781 (31.1)	2893 (31.6)	2731 (31.6)	1790 (30.9)	1653 (30.0)	1614 (29.0)	118 (33.6)	179 (28.9)	327 (32.7)	313 (33.2)	301 (32.3)	311 (29.5)	69 (28.2)	110 (34.0)	119 (30.1)	122 (33.6)	135 (36.1)	111 (29.7)
2012-2015	5378 (35.0)	3428 (37.4)	3169 (36.7)	2164 (37.4)	1836 (33.3)	1740 (31.2)	115 (32.8)	251 (40.5)	442 (44.2)	415 (44.0)	405 (43.5)	411 (38.9)	113 (46.1)	127 (39.2)	169 (42.7)	163 (44.9)	148 (39.6)	140 (37.4)
Age (years)																		
<55	8891 (57.9)	5832 (63.7)	5782 (67.0)	3981 (68.8)	3849 (69.8)	3977 (71.4)	250 (71.2)	480 (77.4)	785 (78.6)	786 (83.3)	745 (80.0)	865 (81.9)	157 (64.1)	246 (75.9)	311 (78.5)	290 (79.9)	303 (81.0)	294 (78.6)
≥55	6471 (42.1)	3324 (36.3)	2848 (33.0)	1805 (31.2)	1668 (30.2)	1595 (28.6)	101 (28.8)	140 (22.6)	214 (21.4)	158 (16.7)	186 (20.0)	191 (18.1)	88 (35.9)	78 (24.1)	85 (21.5)	73 (20.1)	71 (19.0)	80 (21.4)
Gender																		
Male	2421 (15.8)	1470 (16.1)	1411 (16.3)	941 (16.3)	965 (17.5)	1015 (18.2)	107 (30.5)	173 (27.9)	224 (22.4)	195 (20.7)	200 (21.5)	252 (23.9)	105 (42.9)	101 (31.2)	134 (33.8)	104 (28.7)	111 (29.7)	109 (29.1)
Female	12941 (84.2)	7686 (83.9)	7219 (83.7)	4845 (83.7)	4552 (82.5)	4557 (81.8)	244 (69.5)	447 (72.1)	775 (77.6)	749 (79.3)	731 (78.5)	804 (76.1)	140 (57.1)	223 (68.8)	262 (66.2)	259 (71.3)	263 (70.3)	265 (70.9)
Race																		
White	12797 (83.3)	7552 (82.5)	7187 (83.3)	4767 (82.4)	4573 (82.9)	4609 (82.7)	292 (83.2)	541 (87.3)	868 (86.9)	756 (80.1)	777 (83.5)	908 (86.0)	214 (87.3)	276 (85.2)	329 (83.1)	307 (84.6)	321 (85.8)	303 (81.0)
Black	1161 (7.6)	616 (6.7)	483 (5.6)	328 (5.7)	310 (5.6)	347 (6.2)	14 (4.0)	15 (2.4)	19 (1.9)	22 (2.3)	28 (3.0)	25 (2.4)	7 (2.9)	13 (4.0)	13 (3.3)	10 (2.8)	11 (2.9)	13 (3.5)
Other	1263 (8.2)	906 (9.9)	902 (10.5)	639 (11.0)	585 (10.6)	577 (10.4)	38 (10.8)	58 (9.4)	102 (10.2)	154 (16.3)	119 (12.8)	117 (11.1)	22 (9.0)	34 (10.5)	49 (12.4)	45 (12.4)	40 (10.7)	56 (15.0)
Unknown	141 (0.9)	82 (0.9)	58 (0.7)	52 (0.9)	49 (0.9)	39 (0.7)	7 (2.0)	6 (1.0)	10 (1.0)	12 (1.3)	7 (0.8)	6 (0.6)	2 (0.8)	1 (0.3)	5 (1.3)	1 (0.3)	2 (0.5)	2 (0.5)
Marital Status																		
single	2371 (15.4)	1524 (16.6)	1581 (18.3)	1093 (18.9)	1079 (19.6)	1092 (19.6)	67 (19.1)	147 (23.7)	247 (24.7)	258 (27.3)	254 (27.3)	263 (24.9)	57 (23.3)	87 (26.9)	113 (28.5)	95 (26.2)	110 (29.4)	107 (28.6)
Married	9942 (64.7)	5985 (65.4)	5658 (65.6)	3735 (64.6)	3595 (65.2)	3629 (65.1)	227 (64.7)	383 (61.8)	639 (64.0)	585 (62.0)	549 (59.0)	661 (62.6)	147 (60.0)	199 (61.4)	230 (58.1)	221 (60.9)	212 (56.7)	224 (59.9)
Widowed/divorced	2225 (14.5)	1246 (13.6)	1000 (11.6)	729 (12.6)	617 (11.2)	624 (11.2)	41 (11.7)	67 (10.8)	73 (7.3)	60 (6.4)	84 (9.0)	90 (8.5)	30 (12.2)	28 (8.6)	38 (9.6)	38 (10.5)	35 (9.4)	31 (8.3)
Unknown	824 (5.4)	401 (4.4)	391 (4.5)	229 (4.0)	226 (4.1)	227 (4.1)	16 (4.6)	23 (3.7)	40 (4.0)	41 (4.3)	44 (4.7)	42 (4.0)	11 (4.5)	10 (3.1)	15 (3.8)	9 (2.5)	17 (4.5)	12 (3.2)
Rural																		
metropolitan	13580 (88.4)	8212 (89.7)	7805 (90.4)	5257 (90.9)	4979 (90.2)	5049 (90.6)	331 (94.3)	581 (93.7)	935 (93.6)	875 (92.7)	871 (93.6)	985 (93.3)	219 (89.4)	304 (93.8)	366 (92.4)	342 (94.2)	355 (94.9)	352 (94.1)
Nonmetropolitan	1782 (11.6)	944 (10.3)	825 (9.6)	529 (9.1)	538 (9.8)	523 (9.4)	20 (5.7)	39 (6.3)	64 (6.4)	69 (7.3)	60 (6.4)	71 (6.7)	26 (10.6)	20 (6.2)	30 (7.6)	21 (5.8)	19 (5.1)	22 (5.9)
Number of lesions																		
Unifocality	12024 (78.3)	6147 (67.1)	5432 (62.9)	3543 (61.2)	3485 (63.2)	3612 (64.8)	199 (56.7)	309 (49.8)	531 (53.2)	499 (52.9)	512 (55.0)	593 (56.2)	128 (52.2)	130 (40.1)	167 (42.2)	167 (46.0)	167 (44.7)	207 (55.3)
Multifocality	3338 (21.7)	3009 (32.9)	3198 (37.1)	2243 (38.8)	2032 (36.8)	1960 (35.2)	152 (43.3)	311 (50.2)	468 (46.8)	445 (47.1)	419 (45.0)	463 (43.8)	117 (47.8)	194 (59.9)	229 (57.8)	196 (54.0)	207 (55.3)	167 (44.7)

(Continued)

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TABLE 1 Continued

Characteristics	N0						N1a						N1b					
	1-4mm N=15362	5-7mm N=9156	8-10mm N=8,630	11-13mm N=5,786	14-16mm N=5,517	17-20mm N=5,572	1-4mm N=351	5-7mm N=620	8-10mm N=999	11-13mm N=944	14-16mm N=931	17-20mm N=1,056	1-4mm N=245	5-7mm N=324	8-10mm N=396	11-13mm N=363	14-16mm N=374	17-20mm N=374
Thyroidectomy																		
None	4 (0.0)	8 (0.1)	6 (0.1)	11 (0.2)	2 (0.0)	7 (0.1)	1 (0.3)	0 (0.0)	1 (0.1)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (0.6)	1 (0.3)	0 (0.0)
Hemithyroidectomy	5479 (35.7)	2147 (23.4)	1331 (15.4)	587 (10.1)	614 (11.1)	616 (11.1)	26 (7.4)	34 (5.5)	35 (3.5)	19 (2.0)	24 (2.6)	30 (2.8)	4 (1.6)	3 (0.9)	4 (1.0)	0 (0.0)	3 (0.8)	7 (1.9)
Total thyroidectomy	9879 (64.3)	7001 (76.5)	7293 (84.5)	5188 (89.7)	4901 (88.8)	4949 (88.8)	324 (92.3)	586 (94.5)	963 (96.4)	925 (98.0)	907 (97.4)	1026 (97.2)	241 (98.4)	321 (99.1)	392 (99.0)	361 (99.4)	370 (98.9)	367 (98.1)
Lymph node dissection																		
None	10888 (70.9)	5818 (63.5)	5049 (58.5)	3203 (55.4)	3206 (58.1)	3306 (59.3)	1 (0.3)	1 (0.2)	3 (0.3)	2 (0.2)	1 (0.1)	3 (0.3)	2 (0.8)	1 (0.3)	7 (1.8)	8 (2.2)	4 (1.1)	8 (2.1)
Yes	4474 (29.1)	3338 (36.5)	3581 (41.5)	2583 (44.6)	2311 (41.9)	2266 (40.7)	350 (99.7)	619 (99.8)	996 (99.7)	942 (99.8)	930 (99.9)	1053 (99.7)	243 (99.2)	323 (99.7)	389 (98.2)	355 (97.8)	370 (98.9)	366 (97.9)
RAI																		
None	13903 (90.5)	7174 (78.4)	5590 (64.8)	2976 (51.4)	2599 (47.1)	2400 (43.1)	121 (34.5)	189 (30.5)	267 (26.7)	255 (27.0)	241 (25.9)	260 (24.6)	68 (27.8)	69 (21.3)	97 (24.5)	71 (19.6)	86 (23.0)	80 (21.4)
Yes	1459 (9.5)	1982 (21.6)	3040 (35.2)	2810 (48.6)	2918 (52.9)	3172 (56.9)	230 (65.5)	431 (69.5)	732 (73.3)	689 (73.0)	690 (74.1)	796 (75.4)	177 (72.2)	255 (78.7)	299 (75.5)	292 (80.4)	288 (77.0)	294 (78.6)
Follow-up time (mean (SD))																		
	125.97 (53.34)	123.46 (51.69)	124.76 (52.19)	124.83 (52.82)	129.80 (54.16)	134.00 (55.67)	125.52 (58.71)	125.95 (56.05)	117.13 (52.43)	115.76 (48.43)	119.07 (51.39)	125.12 (54.51)	111.41 (52.56)	116.70 (48.52)	118.15 (47.05)	114.21 (46.31)	118.99 (47.64)	127.02 (55.76)

RAI, radioactive iodine.

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(white, black, others), marital status (married, single, divorced, separated or widowed), gender (male, female), geographic region (metropolitan, nonmetropolitan), months from diagnosis to treatment (<6, 6-12, 12-24), tumor size (≤20 mm), N stage (N0, N1a, N1b), distant metastases, multifocality, surgery type (none, less than lobectomy, lobectomy, subtotal or near or total thyroidectomy), lymph node dissection (yes or none), chemotherapy (yes or none), and radiation therapy (none, radioisotopes (RAI)).

To balance the bias of confounding factors that may affect chemotherapy allocation, we utilized inverse probability of treatment weighting (IPTW) and propensity score matching (PSM) with a 1:1 ratio and a caliper of 0.01 (16, 17). Multivariable logistic regression was performed to generate propensity scores (PS) for all variables, and then weights were calculated and matching was conducted based on the PS. PSM-adjusted Kaplan-Meier curves were analyzed using log-rank tests between different tumor size groups and N stage groups. Subsequently, IPTW-adjusted multivariable Cox regression models were created, and hazard ratios (HR) for thyroid cancer-specific survival (TCSS) between different tumor size groups and N stage groups were recalculated. Subgroup analyses were also conducted following the same procedures.

Furthermore, to evaluate the stability of our findings, we performed a comprehensive set of sensitivity analyses. Firstly, we utilized a proportional subdistribution hazards model to calculate hazard ratios (HR) for different tumor size groups and N stage groups, while adjusting for competing events such as death from other causes (18). Secondly, we repeated the entire analysis employing multiple imputation techniques to address missing data, using the random survival forest methodology.

All P values were calculated from two-sided tests with a significance threshold of 0.05 to evaluate the statistical significance of survival benefit by surgery. All statistical analyses were performed using R software (version 3.6.3).

Results

Baseline characteristics of patients

A total of 57,000 patients with histologically confirmed PTC were included in our study. The patients were categorized into groups based on tumor size and N stage. The average follow-up period was 125.69 months (standard deviation: 53.14). Table 1 presents the clinicopathological characteristics of the study cohort. To address confounding factors, we applied IPTW and PSM, resulting in standardized mean differences (SMDs) below 10% for most covariates in the IPTW and PSM cohorts, indicating successful mitigation of confounding effects. Figure 1 illustrates age-related changes in mean tumor size and the average number of positive lymph nodes. The figure demonstrates a consistent decline in both tumor size and positive lymph node numbers after the age of 20, with a stable trend observed around the age of 55.

Kaplan-Meier survival curves adjusted for PSM

As shown in Figure 2, among patients with N0 stage, there were no significant differences in TCSS among patients with different tumor sizes. However, for patients with N1a stage, tumor size had minimal impact on TCSS for tumors measuring 13mm or less. Patients with tumors measuring 11-13mm had significantly better TCSS than those with tumors measuring 14-16mm, and patients with tumors measuring 14-16mm had better survival than those with tumors measuring 17-20mm. Similarly, among patients with N1b stage, apart from patients with tumors measuring 11-13mm who had better survival than those with tumors measuring 14-16mm, different tumor sizes had little impact on survival prognosis. As shown in Figure 3, among patients with tumors measuring 13mm or less, there were no significant differences in TCSS among

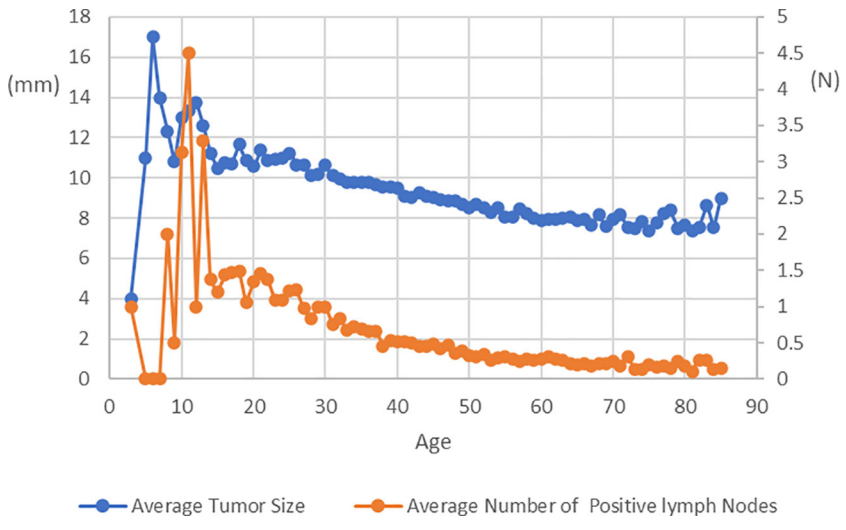


FIGURE 1
Average Tumor Size and Average Number of Positive lymph Nodes at Diagnosis in Patients with PTC of Different Age.

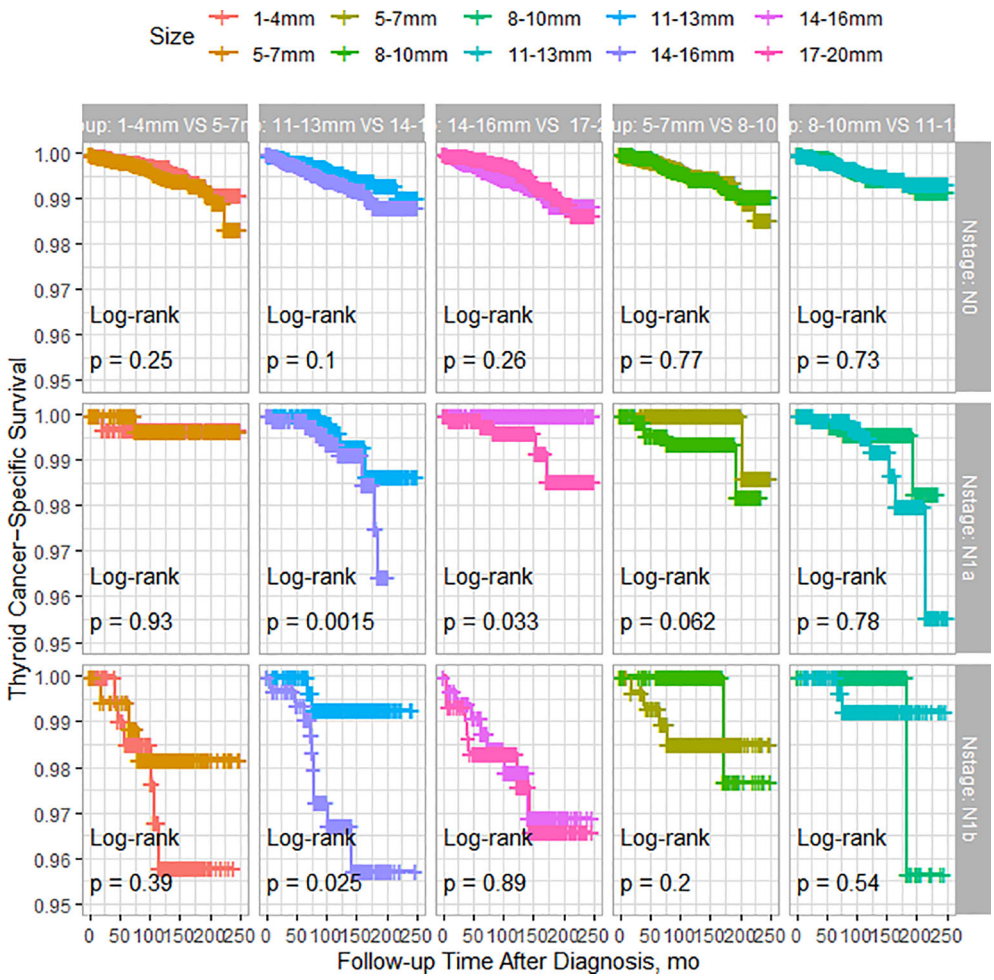


FIGURE 2
PSM-Adjusted Kaplan-Meier Curves Comparing Thyroid Cancer-Specific Survival Rates of Patients with Different Tumor Sizes: 1-4mm vs. 5-7mm, 5-7mm vs. 8-10mm, 8-10mm vs. 11-13mm, 11-13mm vs. 14-16mm, 14-16mm vs. 17-20mm, Stratified by Lymph Node Status.

patients with lymph node stages of N0, N1a, and N1b. Only among patients with tumors measuring 14mm or larger, those with N1b lymph node stage had worse prognosis compared to patients with N1a stage.

Survival benefits adjusted for IPTW

Figures 4 and 5 present the outcomes of a multivariable survival analysis adjusted using IPTW for different tumor sizes and N stages. In patients with N0 stage, a significant difference in survival was found only between the groups with tumor sizes of 11-13mm and 14-16mm. Patients with tumor sizes of 11-13mm had a significantly better prognosis compared to those with sizes of 14-16mm (HR 1.58, 95% CI 1.11-2.25). The 5-year TCSS rate for patients with tumor sizes of 11-13mm was 99.8%, with 10-year and 20-year survival rates of 99.5% and 99.1%, respectively. For patients with tumor sizes of 14-16mm, the 5-year survival rate was 99.6%, the 10-year survival rate was 99.3%, and the 20-year survival rate was 98.8%. The absolute survival differences between the two groups were 0.2% at 5 years, 0.2% at 10 years, and 0.3% at 20 years.

Among patients with N1a stage, significant differences in survival were observed between tumor sizes of 11-13mm and 14-16mm, as well as between 14-16mm and 17-20mm. Patients with tumor sizes of 11-13mm had a significantly better prognosis than those with 14-16mm tumors (HR 1.38, 95% CI 1.04-2.05). For patients with tumor sizes of 11-13mm, the 5-year TCSS rate was 99.7%, the 10-year survival rate was 99.4%, and the 20-year survival rate was 97.4%. Conversely, for patients with tumor sizes of 14-16mm, the 5-year survival rate was 99.6%, the 10-year survival rate was 99.3%, and the 20-year survival rate was 96.9%. The absolute survival differences between the two groups were 0.1% at 5 years, 0.1% at 10 years, and 0.5% at 20 years. Additionally, significant differences in survival were observed between patients with tumor sizes of 14-16mm and 17-20mm. Patients with tumors sized 14-16mm had a significantly better prognosis than those with tumors sized 17-20mm (HR 1.84, 95% CI 1.29-3.89). For patients with tumors sized 14-16mm, the 5-year TCSS rate was 99.6%, the 10-year survival rate was 99.3%, and the 20-year survival rate was 96.9%. On the other hand, for patients with tumors sized 17-20mm, the 5-year survival rate was 99.5%, the 10-year survival rate was 99.2%, and the 20-year survival rate was 96.4%. The absolute

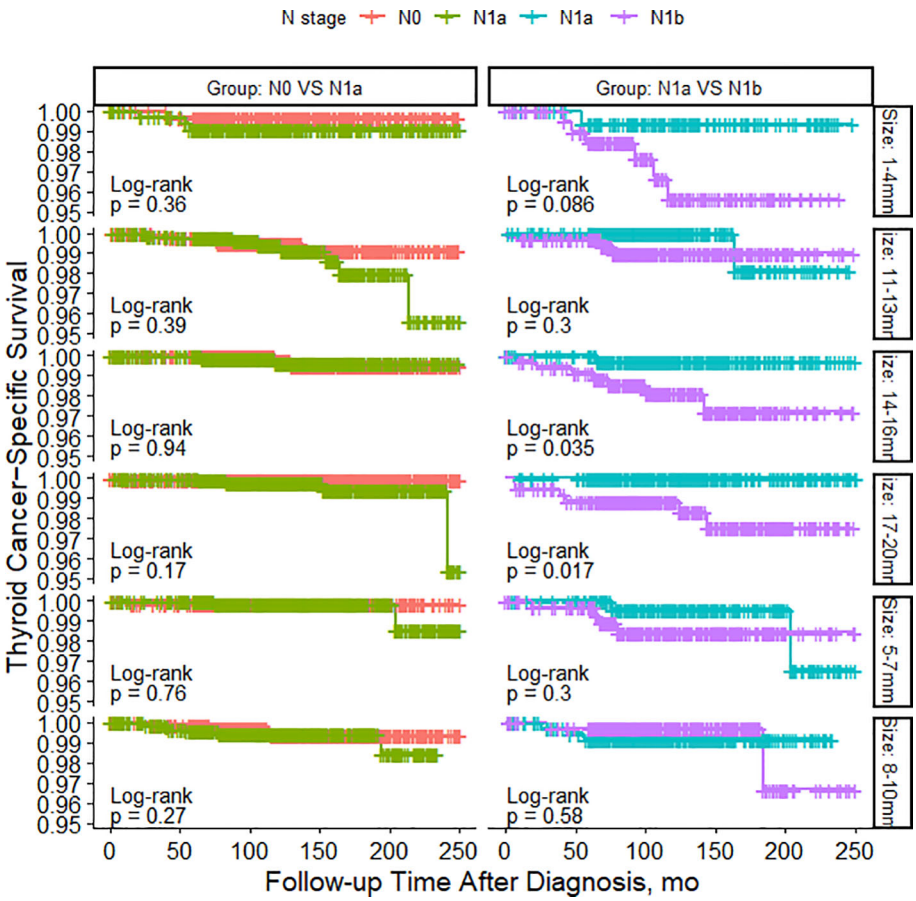


FIGURE 3
PSM-Adjusted Kaplan-Meier Curves Comparing Thyroid Cancer-Specific Survival Rates of Patients with Different Lymph Node Status: N0 to N1a and N1a to N1b, Stratified by Tumor Size.

survival differences between the two groups were 0.1% at 5 years, 0.1% at 10 years, and 0.5% at 20 years.

In patients with N1b stage, significant differences in survival were observed between patients with tumor sizes of 11-13mm and 14-16mm. Patients with tumors sized 11-13mm had a significantly better prognosis than those with tumors sized 14-16mm (HR 4.69, 95% CI 1.52-14.42). For patients with tumors sized 11-13mm, the 5-year TCSS rate was 99.7%, the 10-year survival rate was 99.1%, and the 20-year survival rate was 97.6%. Conversely, for patients with tumors sized 14-16mm, the 5-year survival rate was 99.1%, the 10-year survival rate was 98.1%, and the 20-year survival rate was 95.8%. The absolute survival differences between the two groups were 0.6% at 5 years, 1.0% at 10 years, and 1.8% at 20 years.

Among patients with tumor size less than 13mm, there were no significant differences in survival prognosis between those with N0 and N1a stage, or between N1a and N1b stage. However, in patients with tumor size between 14-16mm, although there was no significant difference between N0 and N1a stage, there was a significant difference between N1a and N1b stage. Patients with N1a stage had significantly better TCSS prognosis compared to those with N1b stage (HR 10.63, 95% CI 2.94-38.40). For patients with tumor size between 14-16mm and N1a stage, the 5-year survival rate was 99.6%, the 10-year survival rate was 99.3%, and the 20-year survival rate was 96.9%. Conversely, for patients with N1b stage, the 5-year survival rate was 99.1%, the 10-year survival rate was 98.1%, and the 20-year survival rate was 95.8%. The absolute survival differences between the two groups were 0.5% at 5 years, 1.2% at 10 years, and 1.1% at 20 years.

In patients with tumor size between 17-20mm, there were no significant differences in survival prognosis between those with N0 and N1a stage. However, patients with N1a stage had a significantly better prognosis compared to those with N1b stage (HR 8.69, 95% CI 3.82-19.80). For N1a stage patients with tumor size between 17-20mm, the 5-year survival rate was 99.5%, the 10-year survival rate was 99.2%, and the 20-year survival rate was 96.4%. Conversely, for N1b stage patients, the 5-year survival rate was 98.6%, the 10-year survival rate was 98.5%, and the 20-year survival rate was 95.2%. The absolute survival differences between the two groups were 0.9% at 5 years, 0.7% at 10 years, and 1.2% at 20 years.

To assess the reliability of our results, sensitivity analyses were conducted, yielding similar findings. These analyses employed the proportional subdistribution hazards model and utilized random survival forest methodology after multiple imputation of missing data.

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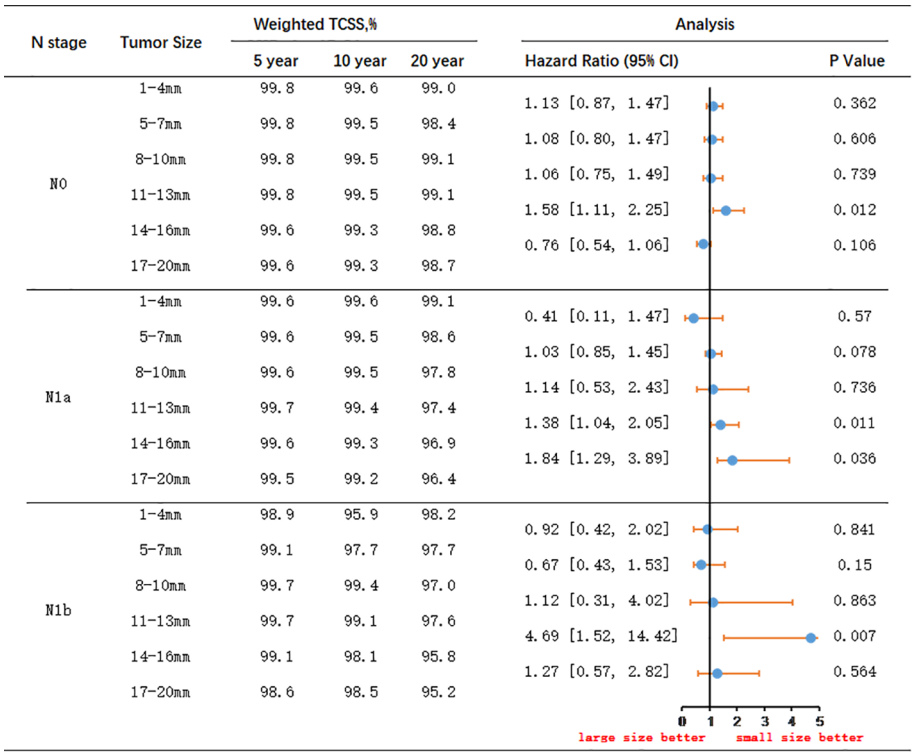


FIGURE 4
IPTW-Adjusted Hazard Ratio comparing Thyroid Cancer-Specific Survival Rates of Patients with Different Tumor Sizes: 1-4mm vs. 5-7mm, 5-7mm vs. 8-10mm, 8-10mm vs. 11-13mm, 11-13mm vs. 14-16mm, 14-16mm vs. 17-20mm, Stratified by Lymph Node Status. TCSS, thyroid cancer–specific survival.

Discussion

Recent research has highlighted concerns regarding the detection and treatment of small PTC, as it does not necessarily lead to reduced mortality rates. This issue of overdiagnosis and overtreatment in low-risk PTC cases has raised questions about patient quality of life and public health management. To address overdiagnosis, various guidelines and recommendations have been put forth. For instance, the U.S. Preventive Services Task Force advised against screening for thyroid cancer in asymptomatic adults using neck palpation or ultrasound (19). Similarly, the American Thyroid Association and the Japan Society of Ultrasonics in Medicine recommended observation rather than fine-needle aspiration for small thyroid nodules (10, 20).

While AS is an excellent strategy, it may not be suitable for all patients with PTMC, as some cases may demonstrate aggressive behavior. Identifying low-risk PTMCs that may demonstrate more aggressive behavior is important. Factors such as distant metastasis at diagnosis (very rare), vocal cord paralysis due to invasion of the recurrent laryngeal nerve, or highly malignant tumors based on cytology are generally considered unsuitable for AS (21). However, there is debate regarding the suitability of tumors located near critical structures such as trachea or recurrent laryngeal nerve. Recent studies have indicated that tumors smaller than 9mm, regardless of location, do not typically invade critical structures (22, 23).

Patients undergoing AS undergo regular ultrasound monitoring, with surgery recommended if there is an increase of 3 mm or more in the maximum diameter of PTMC or the detection of lymph node metastasis (21). In this study, patients were stratified into different risk groups based on tumor size and N stage according to criteria for disease progression, aiming to compare the survival prognosis of patients with varying levels of risk. The study findings indicate that patients in N1a stage have similar prognosis as those in N0 stage. Despite the difficulty of accurately identifying preoperative central compartment lymph node metastasis using ultrasound, delaying surgery does not significantly impact patient outcomes even in the presence of occult central compartment lymph node metastasis.

Usually, the indication for AS is limited to T1aN0M0 PTC, excluding T1bN0M0. However, studies have shown similar survival rates between T1a and T1b patients who underwent surgery (24). Limited research has focused on long-term AS for T1b tumors. The findings of this study reveal that patients with tumors smaller than 13mm had similar prognoses regardless of lymph node status. Thus, delaying surgery did not significantly affect outcomes for T1a patients, even if lateral cervical lymph node metastasis occurred or the tumor size increases to 13mm during AS. Similar results were observed in data from Kuma Hospital and the Cancer Institute Hospital, where patients with slight progression of PTMC who underwent rescue surgery did not experience life-threatening recurrence or death from thyroid carcinoma (21). In patients with T1b stage and a tumor size larger than 13mm, lateral cervical lymph node metastasis had a notable impact on TCSS, although the absolute survival difference over 20 years was only about 1%. This

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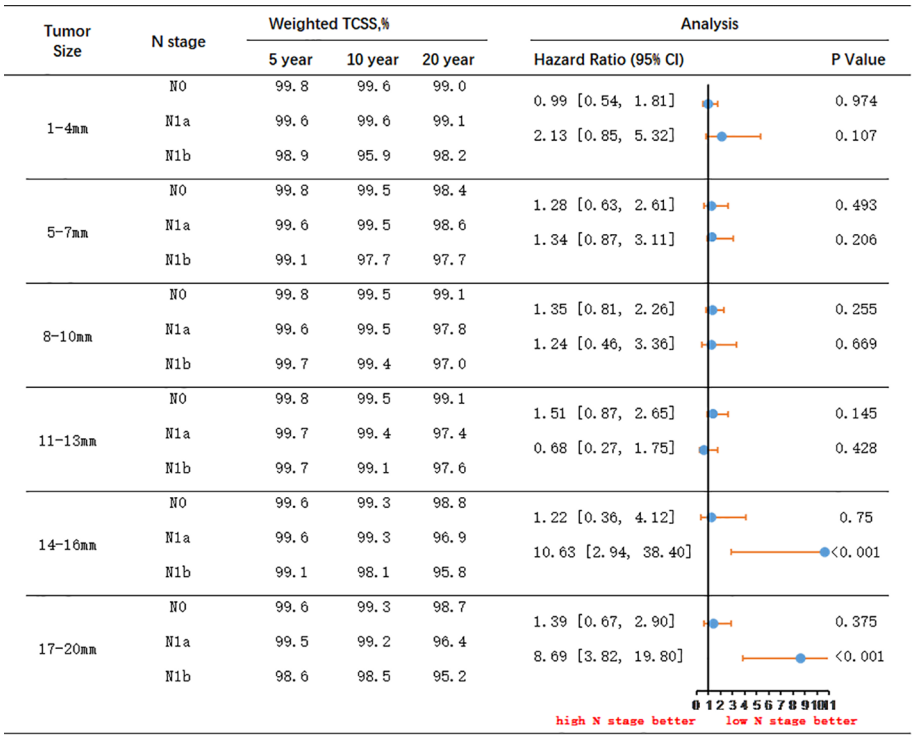


FIGURE 5
IPTW-Adjusted Hazard Ratio Comparing Thyroid Cancer-Specific Survival Rates of Patients with Different Lymph Node Status: N0 to N1a and N1a to N1b, Stratified by Tumor Size. TCSS, thyroid cancer–specific survival.

finding suggests that for patients with poorer baseline health or shorter life expectancy, AS can be considered as an alternative to immediate surgical treatment.

Age plays a significant role in the management of differentiated thyroid cancer, and it is noteworthy that age is considered a prognostic factor for this particular malignancy (25–28). Studies from Japan have indicated that younger age is associated with tumor growth during AS (7, 29). The findings of the present study were similar in that younger patients present with noticeably larger tumor sizes and a higher number of lymph node metastases, which progressively increase with advancing age. Furthermore, an additional study revealed that the estimated lifetime probabilities of disease progression for patients in their 20s, 30s, 40s, 50s, 60s, and 70s were 48.6%, 25.3%, 20.9%, 10.3%, 8.2%, and 3.5%, respectively (29). These findings suggest that approximately half of the patients in their 20s will require surgery due to disease progression, while the other half may not need surgery throughout their lifetime. Importantly, patients who underwent rescue surgery after disease progression did not experience life-threatening recurrence or death from thyroid carcinoma. Therefore, despite the higher risk of disease progression in young patients with PTMC, AS remains a viable option.

As mentioned above, there is an inverse relationship between age and the progression of low-risk PTMC. Our research confirms that in patients over the age of 30, the average tumor size gradually decreases. This could be attributed to two reasons. Firstly, PTC typically grow slowly, especially in elderly patients, where the tumor size remains stable or changes minimally. Secondly, older patients may develop new smaller tumors, thereby reducing the average tumor size. However, it is important to note that this does not mean that older patients do not require regular monitoring, as advanced age is an important factor associated with poor prognosis (30). Although rare, if PTMC progresses without early detection in older patients, it can pose a life-threatening situation. Currently, there is no available evidence indicating when and at what age AS can be discontinued.

Continuing AS throughout one's life is a reasonable option. Even after surgery, regular postoperative follow-up and thyroid hormone administration may still be necessary. Oda et al. conducted a study comparing the 10-year medical costs between immediate surgery and AS. Their findings within the Japanese medical system revealed that the costs in the immediate surgery group were approximately 4.1 times higher than those in the AS group (31). Similarly, a report from Hong Kong showed that the medical costs of the AS group remained lower for 16 years after treatment initiation, and the cost-effectiveness further improved when compared to the immediate surgery group (32).

Furthermore, AS can prevent surgery-related complications and adverse events, leading to further reduction in healthcare costs. However, it may also induce anxiety regarding disease progression. Several studies have indicated that patients with stage T1N0M0 PTMC who did not undergo surgery had significantly lower overall survival rates compared to those who did undergo surgery (33). This difference may be partly attributed to excessive psychological stress and poor medical compliance. Therefore, it is crucial to alleviate the psychological burden of PTMC patients during AS,

encourage adherence to standardized AS protocols, ensure good medical compliance, and minimize the impact of comorbidities. Providing appropriate medical education to patients can help reduce cancer-related stress and surveillance-related stress, enhance patient compliance with AS protocols, and improve follow-up rates (6).

Our study has several limitations. Firstly, the data were retrospectively obtained solely from the SEER database, lacking prospective data on patients with low-risk PTC. Additionally, our analysis focused only on the prognosis of low-risk patients based on TCSS and did not incorporate information on recurrence, which may have resulted in an overestimation of the results. Furthermore, we did not explore the impact of recurrence risk and treatment approaches on the outcomes. For patients with small PTC and N1b or N1a lymph node metastasis, selecting an appropriate surgical extent is of paramount importance. Lobectomy alone may not completely eradicate lymph node metastasis, thereby increasing the risk of recurrence. The risk of recurrence plays a pivotal role in determining the necessity of total thyroidectomy following lobectomy or when considering adjuvant RAI treatment. Another limitation is the lack of evaluation or consideration of family history, vascular invasion, other histologic findings, preoperative ultrasonographic data, and molecular mutations (such as BRAF, RAS, and TERT mutations) in our study. Furthermore, as the SEER database is based on US data, the generalizability of the findings to a global context may be limited.

Conclusion

Apart from young patients, the likelihood of disease progression during the AS of low-risk PTC is relatively low. Furthermore, even if the tumor progresses, such as an increase in size (but with a diameter ≤13mm) or the presence of central neck lymph node metastasis, delaying surgery has no significant impact on the TCSS. When the tumor size exceeds 13mm or is accompanied by lateral neck lymph node metastasis, the TCSS of the patient is affected, but the absolute impact on TCSS rate is minimal. Therefore, even if the diameter of PTC exceeds 13mm, adopting AS remains a feasible strategy in cases where patients have a shorter life expectancy or poor baseline health condition.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

Conception and design: WD, DY, JZ, GR. Administrative support: YL, ZL. Collection and assembly of data: WD, YL, ZL. Data analysis and interpretation: WD, DY, JZ. Manuscript writing: All authors. Final approval of manuscript: All authors. All authors contributed to the article and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Effectiveness and potential mechanism of Jiawei-Xiaoyao-San for hyperthyroidism: a systematic review

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Objectives: To evaluate the effectiveness and potential mechanism of traditional Chinese medicine Jiawei-Xiaoyao-San (JWXYS) as an adjunct or mono- therapy for antithyroid drugs (ATDs) in the treatment of hyperthyroidism.

Methods: Eight databases and three trial registries were searched from inception until May 2023. Randomized controlled trials (RCTs) were included and meta-analysis was conducted using RevMan 5.4 and Stata 14.0. The Cochrane risk of bias (ROB) tool 1.0 and GRADE tool was used for quality appraisal. The findings from case reports using mono-JWXYS and pharmacological studies were summarized in tables.

Results: Thirteen RCTs with 979 participants were included. The majority of the included studies were assessed as high risk of bias in one ROB domain. Compared with ATDs, JWXYS plus ATDs resulted in lower free triiodothyronine (FT3) (MD = -1.31 pmol/L, 95% CI [-1.85, -0.76]; low-certainty), lower free thyroxine (MD = -3.24 pmol/L, 95% CI [-5.06, -1.42]; low-certainty), higher thyroid stimulating hormone (MD = 0.42 mIU/L, 95% CI [0.26, 0.59]; low-certainty), higher effectiveness rate of traditional Chinese medicine syndrome (RR = 1.28, 95% CI [1.08, 1.52]; low-certainty), lower goiter score (MD = -0.66, 95% CI [-1.04, -0.29]; very low-certainty), lower thyrotrophin receptor antibody (SMD = -0.44, 95% CI [-0.73, -0.16]; low-certainty) and fewer adverse events (AEs) (RR = 0.34, 95% CI [0.18, 0.67]; moderate-certainty). Compared with regular dosage of ATDs, JWXYS plus half-dose ATDs resulted in fewer AEs (RR = 0.24, 95% CI [0.10, 0.59]; low-certainty). Compared with ATDs in 1 trial, JWXYS resulted in higher FT3, lower goiter score and fewer AEs. Three case reports showed that the reasons patients sought TCM-only treatment include severe AEs and multiple relapses. Three pharmacological studies demonstrated that JWXYS restored Th17/Treg balance, lowered deiodinases activity, regulated thyroid cell proliferation and apoptosis, and alleviated liver oxidative stress in mouse or rat models.

Conclusion: JWXYS may enhance the effectiveness of ATDs for hyperthyroidism, particularly in relieving symptoms and reducing AEs. Mono-JWXYS is not recommended except in patients intolerant to ATDs. The findings should be interpreted with caution due to overall high risk of bias. Further pharmacological studies with more reliable models are needed.

Systematic review registration: https://www.crd.york.ac.uk/prospero/, identifier CRD42023394923.

KEYWORDS

hyperthyroidism, Jiawei-Xiaoyao-San, traditional Chinese medicine, systematic review, meta-analysis, randomized controlled trials, pharmacological studies

1 Introduction

Hyperthyroidism is characterized by excessive production and secretion of thyroid hormones (THs) by the hyperactive thyroid gland (1), which is a significant public health concern worldwide. Excessive THs affect almost all tissues and organ systems, and some of the most profound effects occur within the cardiovascular system (2). Thyrotoxicosis is associated with a range of complications, including osteoporosis, atrial fibrillation, embolic events, periodic paralysis, neuropsychiatric symptoms, abnormalities in the reproductive system, and, rarely, cardiovascular collapse and death (3). Atrial fibrillation-related embolic stroke secondary to hyperthyroidism is significantly more common than from non-thyroidal causes (4). Patients with hyperthyroidism have an increased risk of all-cause mortality, with heart failure being the leading cause of increased cardiovascular mortality (5). The prevalence of hyperthyroidism ranges from 1.2% to 1.6% and is reported to be on the rise (6). The most common cause of hyperthyroidism in iodine-sufficient areas is Graves' disease (GD), accounting for 75%, followed by toxic nodular goiter (7). GD is an organ-specific autoimmune disease directly caused by thyrotrophin receptor antibody (TRAb) that binds to the thyrotropin receptor (TSHR), subsequently inducing the synthesis and release of THs, and diffuse enlargement of the thyroid gland.

The treatment of hyperthyroidism involves both symptom relief and a definitive cure (8), aiming to improve health-related quality of life (HRQoL) and reduce morbidity and mortality. Three potent classical approaches are offered to treat hyperthyroidism: blocking THs production with antithyroid drugs (ATDs), destroying the thyroid tissue with radioactive iodine (RAI), or surgical removal (thyroidectomy). However, it has been reported in patients with GD or toxic nodular goiter that severe disease-specific and generic HRQoL impairments persist up to 6 months after treatment of ATDs, RAI or thyroidectomy (9, 10), even in euthyroid patients. These impairments can last for many years after diagnosis, involving goiter symptoms, hyperthyroid symptoms, tiredness, cognitive complaints, anxiety, emotional susceptibility and impaired quality of social and daily life (9, 11). In recent decades, ATDs have been the first-line patient-preferred treatment worldwide, considering complications, financial cost, and impaired quality of life induced by RAI or thyroidectomy (11–13). ATDs are typically given for 18 - 24 months, but have an extremely high risk of relapse after withdrawal, approximately 50% at one year after discontinuation (14). In addition, ATDs may cause some side effects, with mild skin allergic reactions, agranulocytosis and hepatic dysfunction being the most common (15). Sometimes, when the side effects are too severe, ATDs need to be discontinued. The evidence-based clinical guideline for the management of thyrotoxicosis published by the American Thyroid Association in 2016 state that there is insufficient evidence to support the recommendation of alternative therapies alone for the treatment of hyperthyroidism. Therefore, there is a need to explore complementary and alternative therapies to control hyperthyroidism and relieve symptoms.

In China and some other countries, Chinese herbal medicines (CHMs) are widely accepted by patients and clinicians in the treatment of hyperthyroidism (16–18). The mechanism of CHMs in treating hyperthyroidism has also been widely explored (19). In a population-based study in Taiwan, CHMs were reported to be used in 73% of the patients with hyperthyroidism, and Jiawei-Xiaoyao-San (JWXYS) was the most commonly chosen prescription for hyperthyroidism (20). Many previous clinical studies have shown that the combination of JWXYS with ATDs in the treatment of hyperthyroidism can improve clinical effectiveness or reduce the dose and side effects of ATDs, and in particular, can significantly relieve symptoms (21, 22). Furthermore, some case reports indicate that JWXYS may be a possible alternative treatment for ATDs in the presence of contraindications (16, 23, 24). However, the clinical evidence and mechanisms have not been systematically reviewed. In order to provide decision guidance for clinicians, we conducted this systematic review and meta-analysis of existing randomized controlled trials (RCTs) of JWXYS for the treatment of hyperthyroidism, and case reports were also considered to assess the application condition. In addition, we conducted a review of the pharmacological mechanisms of JWXYS in the treatment of hyperthyroidism.

2 Methods

This systematic review was conducted referring to the Cochrane Handbook for Systematic Reviews of Interventions (25) and is reported according to the Preferred Reporting Items for Systematic Review (PRISMA) 2020 (26) (Supplementary Material S1). The protocol was registered in PROSPERO, and the registration number is CRD42023394923.

2.1 Eligibility criteria

2.1.1 Eligibility criteria of clinical trials for meta-analysis

(1) Type of Study: RCTs were selected for meta-analysis. Studies published in journals with only one author were identified as non-RCTs and excluded, even when randomization were mentioned.

(2) Participants: Participants diagnosed with hyperthyroidism by explicit diagnostic criteria referring to guidelines or textbooks were included. There were no restrictions on age, race, gender or region. Participants with co-morbidities or other common conditions that had little or no effect on thyroid function were also eligible. Trials in which participants' thyroid functions were in stable state were excluded.

(3) Interventions: The intervention groups were treated with JWXYS plus conventional therapies or alone, regardless of modification, dosage form, or administration route. Considering the large storage of thyroid hormone in thyroid follicular colloid, the intervention duration should be at least 4 weeks.

(4) Comparisons: Studies in which the control groups were treated with ATDs for hyperthyroidism and without any TCM therapies were included.

(5) Outcome measures: The primary outcomes were three thyroid functions, including free triiodothyronine (FT3), free thyroxine (FT4) and thyroid stimulating hormone (TSH). The secondary outcomes included the effectiveness rate of TCM syndrome, the TCM syndrome score, the degree of goiter, serum concentration of TRAb, relapse during follow-up, along with occurrence of adverse events (AEs, including skin allergic reactions, agranulocytosis and hepatic dysfunction). Trials that reported at least one primary outcome were included.

2.1.2 Eligibility criteria of case reports

Case reports using JWXYS alone in the treatment of hyperthyroidism were included for qualitative study.

2.1.3 Eligibility criteria of pharmacological studies

Original articles investigating the pharmacological mechanism of JWXYS in controlling hyperthyroidism were included. The disease model used should be consistent with the pathological characteristics of hyperthyroidism. If medication other than JWXYS were used, both the treatment and control groups must be administered simultaneously. THs should be measured as the primary outcome, and JWXYS should be proved effective in controlling hyperthyroidism.

2.2 Information sources and search strategy

Eight electronic databases and three clinical trial registries were searched from their inception to May 2, 2023, including PubMed, Cochrane Library, Embase, Web of Science, China National Knowledge Infrastructure (CNKI), Chongqing Chinese Science and Technology Journal Database (VIP), China BioMedical Literature Service System (SinoMed), Wanfang Database, World Health Organization International Clinical Trials Registry Platform (https://www.who.int/clinical-trials-registry-platform), ClinicalTrials.gov, and the Chinese Clinical Trial Registry (ChiCTR, http://www.chictr.org.cn/index.aspx). There were no language and publication restrictions. The references of reviews or clinical trials were searched as supplements. Search terms, such as "hyperthyroidism", "Graves' disease", "Jiawei-Xiaoyao-San", and "Danzhi-Xiaoyao-San" were identified according to previous systematic reviews, clinical practice guidelines, MeSH terms and Emtree. Detailed search strategies are presented in Supplementary Material S2 (Table 1).

2.3 Selection and data collection process

The screening was conducted independently by two authors (GW and MYX) according to the eligibility criteria. Retrieval results were imported into NoteExpress (Beijing Aegean Software company, v3.8.0.9492) and automatic duplicate removal was conducted. The first screening was conducted according to the titles and abstracts of all potential studies, and then the full texts were obtained for the second screening. Two authors (RTZ and WXM) independently extracted data from all eligible trials. A data extraction spreadsheet was designed and modified for this review. If the required data were not available in the trial, further information was sought by contacting the original researchers. Data from trials published in duplicate were extracted only once. Discussions and a third reviewer's (ZLL) proposal were required if there was any discrepancy.

2.4 Data items included

2.4.1 Data from RCTs

Basic information of study, characteristics of participants, and details of interventions were extracted as follows: the first author's name, publication year, funding source, sample size, age and gender of participants, duration of disease, diagnostic criteria, treatments with their dosage and administration route from all groups, dosage of each ingredient in JWXYS and modification, duration of intervention, and quality assessment.

The outcome measurements were extracted emphatically, including FT3, FT4, TSH, effectiveness rate of TCM syndrome, TCM syndrome score, TRAb, goier score, relapse rate and AEs. The criterion of effectiveness rate of TCM syndrome should be in compliance with the Chinese Medicine Clinical Research Guidelines (CMCRG) (27) and was defined as a significant amelioration or disappearance of clinical symptoms and signs of

TABLE 1 Summary of the characteristics in each included RCTs.

Author (Year)	Funding sources	classification	Comorbidities	Sample Size (M/F)		Age [years] (mean ± SD)		Duration of Disease [months]		Treatment Duration	Interventions			Outcome measure (s)	TCM syndrome differentiation
				I	C	I	C	I	C		I (Constituent herbs [g] and Administration)	C (Dosage [mg] and Administration)	Basic treatment		
Wang SL (2011)	NR	GD	NR	30 (8/22)	30 (9/21)	39.27 ± 11.45	39.53 ± 11.94	NR	NR	2m	Modified JWXYS (209, bid) + Halved C	Thiamazole (10*, tid)	NR	1, 2, 3, 5, 6, 8	Hyperactivity of heart-liver fire
Tang YL (2012)	NR	GD	Hashimoto's thyroiditis	29 (5/24)	26 (4/22)	38.57 ± 9.84	37.33 ± 9.19	NR	NR	4w	Modified JWXYS (183, bid)	Thiamazole (5, tid)	BT, propranolol (10mg, tid)	1, 2, 3, 4, 5, 6, 8	Liver depression with fire and spleen deficiency
Liu SY et al. (2012 + 2012 + 2016)	Government	GD	NR	36 (5/31)	36 (7/29)	30.08 ± 11.78	33.47 ± 9.56	NR	NR	12w	Modified JWXYS (226, tid) + C	Thiamazole (10*, tid)	BT	1, 2, 3, 4, 5, 6, 8	Liver depression inducing fire
Huang FX et al. (2013)	NR	NR	Hepatic dysfunction	35 (10/25)	35 (11/24)	35.4 ± 12.6	36.3 ± 14.1	57.6 ± 7.2	40.8 ± 8.4	1m	Modified JWXYS (154, bid) + Halved C	Thiamazole (10, tid)	BT, propranolol (10mg, tid)	1, 2, 3, 8	NR
Guo J (2015)	NR	GD	NR	30 (11/19)	30 (9/21)	37.97 ± 9.33	37.67 ± 9.59	8.76 ± 6.28	8.48 ± 6.38	3m	Modified JWXYS (73, bid) + C	Thiamazole (10-20/d*)	BT	1, 2, 3, 4, 8	Prosperity of liver fire
Qiu ZQ (2015)	NR	NR	Heart disease	56 (25/31)	52 (22/30)	46.29 ± 11.27	44.72 ± 10.02	56.04 ± 14.76	57.72 ± 15.72	8w	Modified JWXYS (156, bid) + C	Thiamazole (20/d) or Propylthiouracil (200-300/d); treatment for heart disease#	NR	1, 2, 3, 8	NR
Li MY (2016)	NR	NR	NR	36 (5/31)	36 (4/32)	range:18~70		<12		4w	Modified JWXYS (226, tid) + C	Thiamazole (10*, tid)	BT	1, 2, 3, 4, 5	Liver depression inducing fire
Wu MY (2017)	NR	GD	NR	30 (7/23)	30 (9/21)	31.1 ± 10.2	33.6 ± 8.98	5.53 ± 3.2	5.33 ± 2.62	6m	Modified JWXYS (205, bid) + C	Thiamazole (10*, tid)	BT	1, 2, 3, 5, 6, 8	Liver depression inducing fire
Zhang LL (2017)	NR	GD	Hepatic dysfunction	50 (26/24)	50 (28/22)	44.63 ± 16.75	43.85 ± 15.29	10.44 ± 18.72	9.72 ± 18.96	12w	Modified JWXYS (226, bid) + C	Thiamazole (10*, tid); diammonium glycyrrhizinate enteric-coated capsule (150, tid)	BT	1, 2, 3, 7	Liver depression inducing fire
Li JN (2018)	NR	GD	NR	36 (4/32)	36 (7/29)	33.89 ± 11.22	36.19 ± 13.11	12.97 ± 9.09	11.75 ± 8.84	12w	Modified JWXYS (195, tid) + C	Thiamazole (30-45/d*)	NR	1, 2, 3, 4, 5, 6, 8	Prosperity of liver fire

(Continued)

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TABLE 1 Continued

Author (Year)	Funding sources	classification	Comorbidities	Sample Size (M/F)		Age [years] (mean ± SD)		Duration of Disease [months]		Treatment Duration	Interventions			Outcome measure (s)	TCM syndrome differentiation
				I	C	I	C	I	C		I (Constituent herbs [g] and Administration)	C (Dosage [mg] and Administration)	Basic treatment		
Tian LY et al. (2020)	Government	NR	NR	49 (15/34)	49 (13/36)	39.6 ± 7.4	37.8 ± 7.2	10.4 ± 3.5	12.3 ± 2.6	4w	Modified JWXYS (189, bid) + C	Thiamazole (10-40, tid)	NR	1, 2, 3, 4, 6, 7, 8	Liver depression inducing fire
Shi LJ et al. (2020)	Government	NR	NR	45 (9/36)	45 (7/38)	36.16 ± 3.13	35.58 ± 3.23	5.36 ± 1.02	5.44 ± 0.97	3m	Modified JWXYS (122, bid) + C	Thiamazole (10*, tid)	NR	1, 2, 3, 4	Liver depression and spleen deficiency
Gao R et al. (2021)	Government	GD	NR	60 (0/60)	60 (0/60)	36.24 ± 10.32	38.35 ± 10.73	13.68 ± 5.04	14.88 ± 3.84	3m	Modified JWXYS (150, bid) + C	Thiamazole (10*, bid)	BT	1, 2, 8	Prosperity of liver fire

I: intervention group; C: control group; M: male; F: female; m: months; w: weeks; d: day; NR: not reported; *: Reduce the dosage gradually according to thyroid functions; BT: basic treatments, including low iodine diet, adequate rest and nutrition, appropriate exercise and emotion regulation et al.; #: propranolol 20-30/d for tachycardia or atrial fibrillation, diuretics, vasodilators and digoxin or low-dose propranolol for heart failure; tid: 3 times a day; bid: 2 times a day. Outcome measure (s): 1: FT3, 2: FT4, 3: TSH, 4: effectiveness rate of TCM syndrome, 5: TCM syndrome score, 6: Goiter score, 7: TRAB, 8: Adverse events.

TCM syndrome, and a reduction in syndrome score of more than 70%. In all studies included in this review, the degree of goiter was divided into three grades: Grade I: The thyroid gland is not swollen in appearance, but can be palpated; Grade II: Goiter is visible and palpable, but the goiter does not extend beyond the outer margin of the sternocleidomastoid muscle; Grade III: The goiter extends beyond the outer margin of the sternocleidomastoid muscle. The degree of goiter was denoted by goiter score, and each grade accumulates two points. Data from all post-intervention time points were extracted to find the most reported time points.

2.4.2 Data from case reports

Basic information of study, characteristics of participants, details of interventions and outcomes were included as follows: first author's name, publication year, age and gender of participants, medical history, duration of disease, diagnosis, treatments with dosage and administration route, dosage of each ingredient in JWXYS and modification, duration of intervention, and changes in clinical manifestation and laboratory tests.

2.4.3 Data from mechanism studies

The first author's name, publication year, study model, treatments of the experimental group and control group, and changes in outcomes for efficacy evaluation and mechanism research were all extracted.

2.5 Study risk of bias assessment

Two authors (RTZ and WXM) used the Cochrane Risk-of-Bias (ROB) 1.0 tool in Review Manager (RevMan) 5.4 software to evaluate the quality of the included RCTs with reference to the following aspects: (a) random sequence generation (selection bias), (b) allocation concealment (selection bias), (c) blinding of participants and personnel (performance bias), (d) blinding of outcome assessment (detection bias), (e) incomplete outcome data (attrition bias), (f) selective reporting (reporting bias), (g) other bias. Each aspect was judged as low, unclear or high. Discrepancies were resolved by discussion or involving a third author (ZLL). For case reports, we used the Joanna Briggs Institute (JBI) tool 2020 (28) for critical appraisal.

2.6 Effect measures

For RCTs, data analyses were performed using RevMan 5.4. Dichotomous variables were presented as risk ratio (RR) with 95% confidence interval (CI). Continuous variables were presented as weighted mean difference (MD) with 95% CI if the measurement units of outcomes were the same in different trials, and the standardized mean difference (SMD) was used when the measurement units of outcomes differed from one another.

2.7 Synthesis methods

Trial characteristics were summarized in tables by Microsoft excel 2021. For RCTs, the meta-analysis was performed using

RevMan 5.4. Forest plots were used to describe the results. For the comparison of different interventions and outcomes, the results were synthesized respectively. We intended to pool the collected data at similar time points, with data from one trial being synthesized only once. If the standard deviation was not reported by means, it was calculated from the information reported (29). If the required data were unavailable in the trial and we failed to contact the original researchers, we only used the available data in the analyses.

The heterogeneity test was performed by chi-square test and presented as the value of I^2 statistics. According to the Cochrane handbook, more than 25%, 50%, and 75% values were considered as mild, moderate, and severe heterogeneity, respectively (25). The fixed-effects model (FEM) was applied when $I^2 < 50\%$; otherwise, the random-effects model (REM) was applied. The Mantel-Haenszel method was used for meta-analyses of dichotomous outcomes. The inverse-variance method was used for meta-analyses of continuous outcomes. The subgroup analysis was conducted to explore any factors that might explain the heterogeneity. Overall effects with a p-value below 0.05 were considered statistically significant.

Sensitivity analysis was performed using STATA 14.0 software for the primary outcome to determine whether the conclusion was robust. When severe heterogeneity existed, sensitivity analysis was conducted for potential sources of heterogeneity.

2.8 Publication bias and certainty assessment

Funnel plot and Egger's test (30) was performed to assess potential publication bias in a single meta-analysis involving ten or more trials. Two authors (RTZ and WXM) conducted the certainty assessment of each outcome using the GRADEpro Guideline Development Tool (GRADEpro GDT) developed by the Grading of Recommendations, Assessment, Development, and Evaluation (GRADE) working team. Discrepancies were resolved by discussion or involving a third author (ZLL).

3 Results

3.1 Study selection

From 8 databases and 3 clinical trial registries, 157 records were identified. After removing 81 duplicates, 14 records irrelevant to this review were excluded, leaving 62 records for full-text screening. Of these, 60 reports were obtained in full text. For RCTs, 18, 1, 3, and 4 reports were excluded due to lack of randomization, duration of intervention less than 4 weeks, no available data, and stable thyroid function of participants, respectively. Nine case reports were excluded due to the intervention including other therapies. Three reports contained data from the same study. Finally, 25 reports (15 reports including 13 RCTs, 3 case reports including 3 cases and 7 reports including 3 experiments for mechanism research) were assessed to meet the eligibility criteria. The detailed screening process is shown in the PRISMA flow diagram (Figure 1) (26).

3.2 Study characteristics of included RCTs

In 13 RCTs from 15 reports, a total of 979 participants were enrolled, age ranged from 16 to 76 years old, with 485 in the control group and 494 in the intervention group. The data in three reports (31–33) came from the same trial. In 9 trials, hyperthyroidism in patients was explicitly described as being caused by GD, which was not reported in the other trials. Treatment duration ranged from 4 weeks to 6 months, with the most common being 12 weeks (considered equivalent to 3 months). Therefore, when outcomes at multiple time points were reported, we extracted outcomes at or closest to 12 weeks. In terms of intervention measures, 10 trials compared JWXYS plus ATDs with the same dose of ATDs (22, 24, 31–43), 2 trials compared JWXYS plus half-dose ATDs with normal dose ATDs (21, 43), and only one trial compared JWXYS with ATDs (44). Two trials reported hepatic dysfunction (21, 32), one reported heart disease (40) and one reported Hashimoto's thyroiditis (44) as comorbidity. JWXYS was administered as a modified decoction in all trials. The most common TCM syndromes are "Liver depression inducing fire" (in 5 trials), "Prosperity of liver fire" (in 3 trials), "Liver depression with fire and spleen deficiency" (in 2 trials) and "Hyperactivity of heart-liver fire" (in 1 trial). The detailed characteristics of the included trials are shown in Table 1. The

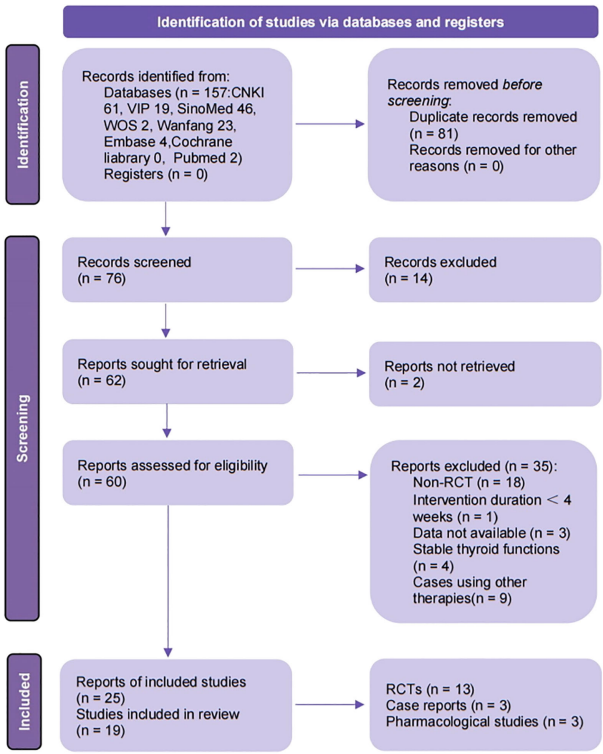


FIGURE 1
Flow diagram of study selection process. CNKI, China National Knowledge Infrastructure; VIP, Chongqing Chinese Science and Technology Journal Database; SinoMed, China BioMedical Literature Service System; ICTRP, the WHO International Clinical Trials Registry Platform; n, number; RCT, randomized controlled trails. Template for the flow diagram was provided by PRISMA.

detailed components of the prescriptions used in each study are summarized in the Supplementary Material S2 (Table 2).

3.3 Risk of bias in included RCTs

The risk of bias on primary outcomes in 13 RCTs was summarized in Figure 2. Nine trials reported the use of random number table as the random sequence generation method, which was considered to have a "low risk of bias". Five trials just mentioned "randomization" without detailed methods (21, 37, 40, 41, 44) and were considered to have an "unclear risk of bias" in random sequence generation. None of the trials mentioned the details of allocation concealment of randomization methods and implementation of blinding. All trials were considered to have an "unclear risk of bias" in allocation concealment. The majority of the trials were considered to have a "high risk of bias" in performance bias due to apparently different intervention without placebo. Four trials (21, 22, 32, 40) were considered to have an "unclear risk of bias" in performance bias and a "low risk of bias" in detection bias because they only reported physiological indicator as outcomes. In the aspect of "incomplete outcome data", two trials (31–33, 44) that did not report the cause of the missing data were considered to have an "unclear risk of bias". In two trials (32, 40), the proportion of male and female participants did not correspond to the clinical ratio of men to women, therefore, these trials were considered to have a "high risk of bias" in other bias.

3.4 Effectiveness of interventions

3.4.1 Primary outcomes

The meta-analyses showed that compared with ATDs, the combination of JWXYS and ATDs resulted in lower FT3 (MD = -1.31 pmol/L, 95% CI: -1.85 to -0.76; 10 trials, 794 participants), lower FT4 (MD = -3.24 pmol/L, 95% CI: -5.06 to -1.42; 10 trials, 794 participants) and higher TSH (MD = 0.42 mIU/L, 95% CI: 0.26 to 0.59; 8 trials, 672 participants).

For the comparison between regular-dose ATDs and JWXYS plus half-dose ATDs (2 trials, 130 participants), the meta-analyses showed no significant difference on the previous thyroid functions.

One trial showed that compared with ATDs, mono-JWXYS resulted in higher FT3 (MD = 1.53 pmol/L, 95% CI: 0.50 to 2.56; 55 participants), higher FT4 (MD = 3.07 pmol/L, 95% CI: -0.04 to 6.18; 55 participants) and similar TSH.

The heterogeneity among different comparisons was significantly high ($P < 0.00001$) in each outcome. The detailed results are summarized in Table 2 and shown as forest plots in Figure 3.

3.4.2 Secondary outcomes

3.4.2.1 TCM syndrome

The meta-analyses showed that compared with ATDs alone, the combination of JWXYS and ATDs resulted in higher effectiveness rate of TCM syndrome (RR = 1.28, 95% CI: 1.08 to 1.52; 6 trials, 464 participants) and lower TCM syndrome score (SMD = -1.62, 95% CI: -2.68 to -0.56; 5 trials, 336 participants).

One trial showed that compared with regular-dosage of ATDs, the combination of JWXYS and half-dose ATDs resulted in lower TCM syndrome score (MD = -2.87, 95% CI: -5.01 to -0.73; 60 participants).

One trial showed that compared with ATDs, mono-JWXYS resulted in higher effectiveness rate of TCM syndrome (RR = 4.50, 95% CI: 0.23 to 89.62; 55 participants), lower TCM syndrome score (MD = -3.16, 95% CI: -5.26 to -1.06; 55 participants).

The heterogeneity among different comparisons was not significant. The detailed results are summarized in Table 2 and shown as forest plots in Figure 4. It's worth noting that the symptoms and signs included in various studies were not exactly the same, mainly including irritability, intolerance to heat, palpitation, hyperphagia, tremor, emaciation, fatigue, sweating, insomnia, etc.

3.4.2.2 Goiter score

The meta-analysis showed that compared with ATDs alone, the combination of JWXYS and ATDs resulted in lower goiter score (MD = -0.66, 95% CI: -1.04 to -0.29; 5 trials, 362 participants).

One trial showed that compared with regular-dosage of ATDs, the combination of JWXYS and half-dose ATDs resulted in similar goiter score (MD = -0.07, 95% CI: -0.86 to 0.72; 60 participants).

One trial showed that compared with ATDs, mono-JWXYS resulted in lower goiter score (MD = -1.17, 95% CI: -2.17 to -0.17; 55 participants).

The detailed results are summarized in Table 2 and shown as forest plots in Figure 5.

3.4.2.3 TRAb

The meta-analyses showed that compared with ATDs alone, the combination of JWXYS and ATDs resulted in lower TRAb (SMD = -0.44, 95% CI: -0.73 to -0.16; 2 trials, 198 participants). TRAb was not reported in other comparisons. The detailed results are summarized in Table 2 and shown as forest plot in Figure 6.

3.4.2.4 Recurrence rate

No trial reported the recurrence rate at follow-up.

3.4.2.5 Adverse events

A total of 11 trials reported the AEs. Compared with ATDs, the combination of JWXYS and ATDs resulted in fewer AEs (RR = 0.34, 95% CI: 0.18 to 0.67; 7 trials, 534 participants), including less occurrence of hepatic dysfunction (14/303 versus 7/299), agranulocytosis (16/303 versus 9/299), and skin allergic reactions (6/303 versus 0/299). Compared with ATDs, the combination of JWXYS and half-dose ATDs resulted in fewer AEs (RR = 0.24, 95% CI: 0.10 to 0.59; 2 trials, 130 participants), including less occurrence of agranulocytosis (10/65 versus 3/65) and skin allergic reactions (11/65 versus 2/65). Compared with ATDs, mono-JWXYS resulted in fewer AEs (RR = 0.09, 95% CI: 0.01 to 1.58; 1 trial, 68 participants), including less occurrence of agranulocytosis (9/26 versus 0/29). The results of meta-analyses are summarized in Table 2 and shown as forest plots in Figure 7.

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TABLE 2 Effectiveness and safety of JWXYS for hyperthyroidism based on 13 randomized controlled trials.

Outcomes	No. of studies	No. of participants	I^2 (%), Effect model (R/F)	Type of effect size	Effect size [95%CI]	Quality of evidence (GRADE)
JWXYS + ATD versus ATD						
FT3 (pmol/L)	10	794	89, R	MD	-1.31 [-1.85, -0.76]	Low [a,e]
FT4 (pmol/L)	10	794	97, R	MD	-3.24 [-5.06, -1.42]	
TSH (mIU/L)	8	672	86, R	MD	0.42 [0.26, 0.59]	
Effectiveness rate of TCM syndrome	6	464	0, F	RR	1.28 [1.08, 1.52]	Low [b]
TCM syndrome score	5	336	94, R	SMD	-1.62 [-2.68, -0.56]	Very low [b,e]
Goiter score	5	382	72, R	MD	-0.66 [-1.04, -0.29]	Very low [b,e]
TRAb	2	198	0, F	SMD	-0.44 [-0.73, -0.16]	Low [a,c]
Adverse events	7	534	0, F	RR	0.34 [0.18, 0.67]	Moderate [a,d,f]
JWXYS + L-ATD versus ATD						
FT3 (pmol/L)	2	130	55, R	MD	0.47 [-0.75, 1.68]	Very low [a,c,e]
FT4 (pmol/L)	2	130	57, R	MD	-1.63 [-4.81, 1.56]	
TSH (mIU/L)	2	130	78, R	MD	-0.02 [-0.50, 0.46]	
TCM syndrome score	1	60	NA, R	MD	-2.87 [-5.01, -0.73]	Very low [b,c]
Goiter score	1	60	NA, R	MD	-0.07 [-0.86, 0.72]	Very low [b,c]
Adverse events	2	130	0, F	RR	0.24 [0.10, 0.59]	Low [a,c,d,f]
JWXYS versus ATD						
FT3 (pmol/L)	1	55	NA, R	MD	1.53 [0.50, 2.56]	Low [a,c]
FT4 (pmol/L)	1	55	NA, R	MD	3.07 [-0.04, 6.18]	
TSH (mIU/L)	1	55	NA, R	MD	-0.04 [-0.13, 0.05]	
Effectiveness rate of TCM syndrome	1	55	NA, F	RR	4.50 [0.23, 89.62]	Very low [b,c]
TCM syndrome score	1	55	NA, R	MD	-3.16 [-5.26, -1.06]	
Goiter score	1	55	NA, R	MD	-1.17 [-2.17, -0.17]	Very low [b,c]
Adverse events	1	55	NA, F	RR	0.05 [0.00, 0.78]	Low [a,c,d,f]

JWXYS: Jiawei-Xiaoyao-San; ATD: antithyroid drug; L-ATD: half-dose ATD; NA: not applicable; R: randomized-effects model; F: fixed-effects model; RR: risk ratio; SMD: standardized mean difference; MD: weighted mean difference; [a]: serious risk of bias, including a lack of description of randomization and blinding; [b]: extremely serious risk of bias, including a lack of description of randomization and blinding and the subjectivity of outcome measurement; [c]: serious imprecision due to insufficient sample size; [d]: serious imprecision due to the infrequency of events; [e]: serious inconsistency due to obvious heterogeneity; [f]: upgrade due to large effect size.

3.4.3 Sensitivity analyses

Sensitivity analyses were conducted by removing each trial, which indicated that the pooled effects of primary outcomes in the comparison between JWXYS plus ATDs and mono-ATDs were robust (Figure 8).

3.4.4 Subgroup analysis

The primary outcomes did not exhibit significant differences in subgroup analyses categorized by the course of intervention in the comparison between JWXYS plus ATDs and mono-ATDs (Figure 9), which may be attributed to the limited number of trials included in each subgroup.

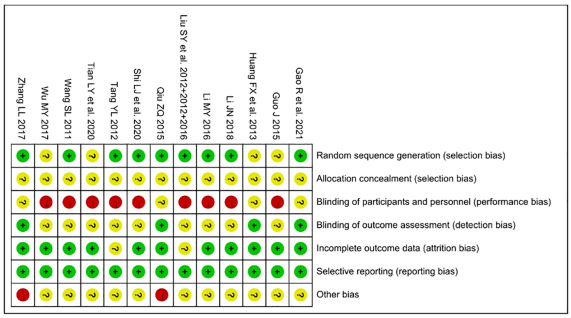


FIGURE 2
Risk of bias summary of 13 included RCTs.

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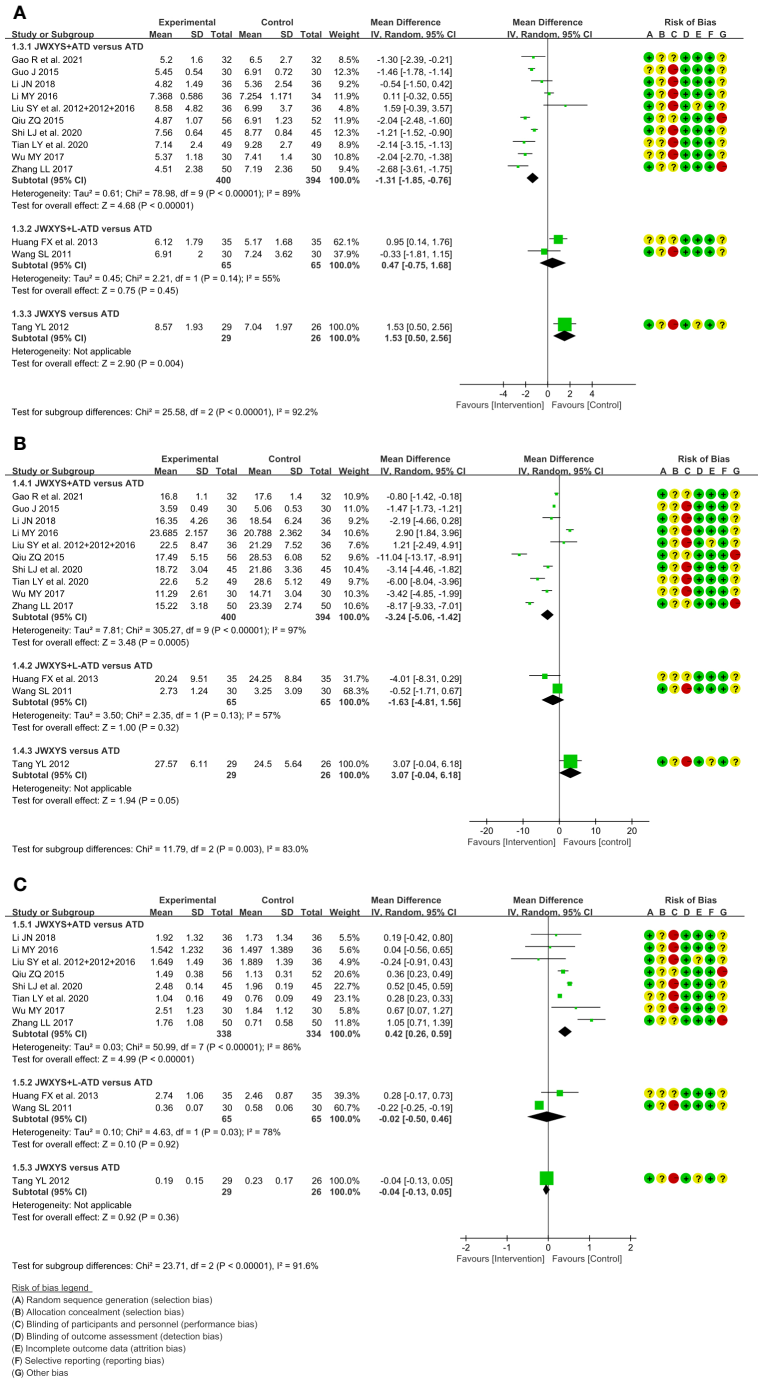


FIGURE 3
Forest plots of the effectiveness comparison on thyroid functions. **(A)**: Free triiodothyronine, **(B)**: Free thyroxine, **(C)**: Thyroid stimulating hormone. JWXYS, Jiawei-Xiaoyao-San; ATD, antithyroid drug; L-ATD, half-dose antithyroid drug.

3.5 Publication bias analysis

Funnel plots were drawn to explore the possibility of publication bias for the ten trials comparing JWSYS plus ATDs with ATDs on the primary outcomes of FT3 and FT4. The scattered points distribution in the funnel plots was basically symmetrical. Egger's tests were performed for further verification. Egger's test of funnel plot asymmetry indicated almost no publication bias in the included studies (P = 0.997, P = 0.290), suggesting reliable results (Figure 10).

3.6 Certainty of evidence

Certainty of evidence and the reasons for the upgrade and downgrade are presented in Table 2. The certainty of evidence for

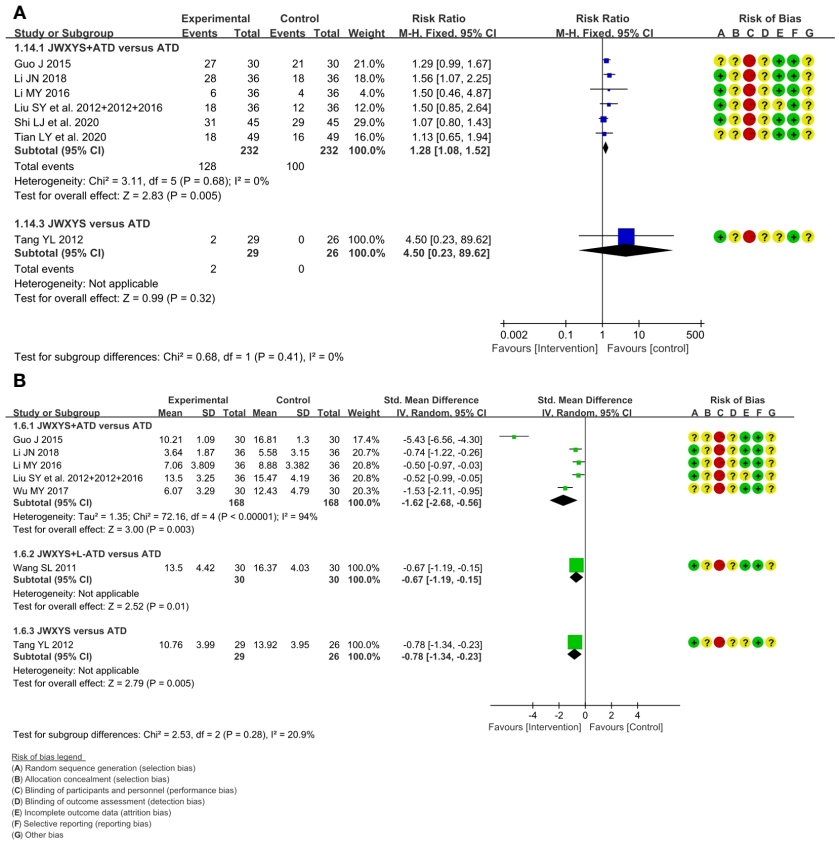


FIGURE 4
Forest plots of the effectiveness comparison on traditional Chinese medicine (TCM) syndrome. **(A)**: Effectiveness rate of TCM syndrome, **(B)**: TCM syndrome score. JWXYS, Jiawei-Xiaoyao-San; ATD, antithyroid drug; L-ATD, half-dose antithyroid drug.

each outcome was downgraded considering its risk of bias, inconsistency, indirectness, imprecision and other potential biases. The evidence for all outcomes were assessed as moderate to very low certainty. Moderate-certainty evidence showed that JWXYS plus ATDs resulted in fewer AEs. Low-certainty evidence suggests that JWXYS plus ATDs is more effective than ATDs in controlling thyroid functions (FT3, FT4, and TSH), alleviating TCM syndrome, and reducing goiter score and TRAb. The evidence of all other comparisons were assessed as low to very low certainty.

3.7 Quality of case reports

Three case reports containing three patients were included, and the quality of the reports was evaluated as high by the JBI tool (Table 3).

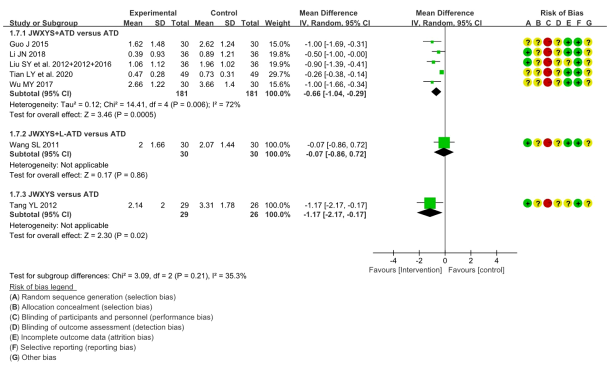


FIGURE 5
Forest plots of the effectiveness comparison on goiter score. JWXYS, Jiawei-Xiaoyao-San; ATD, antithyroid drug; L-ATD, half-dose antithyroid drug.

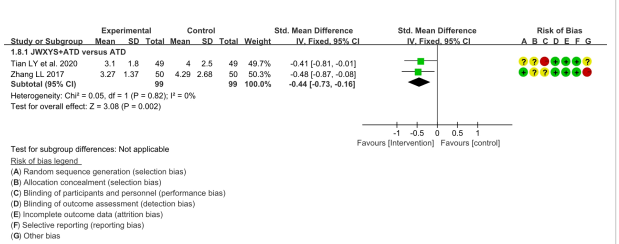


FIGURE 6
Forest plot of the effectiveness comparison on TRAb. JWXYS, Jiawei-Xiaoyao-San; ATD, antithyroid drug.

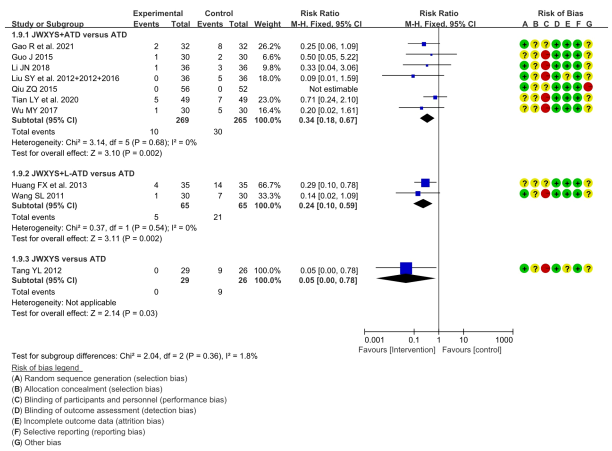


FIGURE 7
Forest plots of adverse events in different comparisons. JWXYS, Jiawei-Xiaoyao-San; ATD, antithyroid drug; L-ATD, half-dose antithyroid drug.

3.8 Main findings of case reports

Reasons for patients seeking TCM-only treatment include suffering from or being afraid of severe AEs following ATDs and multiple relapses. Complete control of hyperthyroidism can be achieved with JWXYS therapy alone for 3 months to 3 years. The detailed components of the prescriptions used in each study are summarized in Table 4.

3.9 Summary of pharmacological studies

Three experiments in seven reports were included (45–51), and the characteristics and main findings were summarized in Table 5. Wu XY's study (49) showed that JWXYS plus *Anemone flaccida Fr. Schmidt* (Diwu) regulated T cell differentiation and restored Th17/Treg cell balance by inhibiting the JAK1-STAT3 pathway of $CD4^+$ T cells. Bao CY et al. (45) found that modified JWXYS plus PTU reduced the incidence of adverse pregnancy in female mice, as well as lowered thyroid function and activity of deiodinases of offspring mice. Tan HZ et al. (46–48, 50, 51) found that modified JWXYS could regulate the proliferation and apoptosis of thyroid cells and protect liver function by alleviating oxidative stress. The detailed components of the prescriptions used in each study are summarized in the Supplementary Material.

4 Discussion

4.1 Summary of evidence

Thirteen RCTs involving 979 participants on JWXYS for hyperthyroidism were included. We found that compared with ATDs, the complementary treatment of JWXYS could help control thyroid functions, alleviate TCM syndrome and goiter, and reduce TRAb and AEs. Meanwhile, when combined with JWXYS, half-dose of ATDs could ideally control thyroid function, thus significantly reducing the occurrence of AEs, and were also effective in alleviating TCM syndrome and goiter. But the effectiveness of mono-JWXYS in controlling thyroid function was significantly inferior to that of ATDs. In cases of severe adverse reactions following ATDs, alternative therapy with JWXYS may be attempted, which may allow complete control of hyperthyroidism. Sensitivity analyses were conducted by removing each trial, which indicated that the pooled effects of primary outcomes were robust. The risk of bias was high among the studies, mainly due to the lack of description of the randomization process and the absence of blinding. Funnel plots and Egger's tests indicated no existence of publication bias. The majority of the evidence were assessed as moderate to very low certainty.

4.2 Administration of JWXYS for hyperthyroidism

JWXYS can be used to treat a variety of thyroid disorders, such as Hashimoto's thyroiditis (52), subacute thyroiditis (53), thyroid-related eye diseases (54), nodular goiter (55), etc., especially for the treatment of hyperthyroidism. In TCM theory, patients with hyperthyroidism are usually diagnosed as the syndrome of liver depression inducing fire, the syndrome of hyperactivity of fire due to yin deficiency or the syndrome of hyperactivity of heart-liver fire. As a classical CHM prescription, JWXYS is commonly used in the treatment of the syndrome of liver depression inducing fire and spleen deficiency. It is often used to relieve various symptoms, such as anxiety, depression, hot flashes, night sweating, palpitations, headache, dry eyes, sleep disturbance, rash, and irregular menstruation, all of which are just in line with the characteristics of hyperthyroidism.

JWXYS formula contains ten herbs: *Paeonia suffruticosa Andr.* (Danpi), *Gardenia jasminoides Ellis* (Zhizi), *Angelica sinensis (Oliv.) Diels* (Danggui), *Paeonia lactiflora Pall.* (Baishao), *Bupleurum chinense DC.* (Chaihu), *Poria cocos (Schw.) Wolf* (Fuling), *Atractylodes macrocephala Koidz.* (Baizhu), *Glycyrrhiza uralensis Fisch* (Gancao), *Mentha haplocalyx Briq.* (Bohe), *Zingiber officinale Rosc.* (Shengjiang). In clinical practice, JWXYS is often modified to target different symptoms of patients. In the included studies, *Prunella vulgaris* (Xiakucao), *Scutellariae Radix* (Huangqin), *Lycii Cortex* (Digupi), *Ziziphi Spinosae Semen* (Suanzaoren), and *Figwort Root* (Xuanshen) are the most commonly added herbs, while *Zingiber Officinale Roscoe* (Shengjiang) and *Menthae Herba* (Bohe) are often removed. In case of significant goiter, *Fritillariae Thunbrgii Bulbus* (Beimu), Xuanshen, *Ostrea gigas Thunberg* (Muli), and particularly Xiakucao, are often added (16, 23, 37, 43). Xiao-Luo-Wan, which consists of Beimu, Xuanshen and Muli, is also one of the classic TCM prescriptions and is mainly used to treat a variety of proliferative diseases, including goiter and thyroid nodule. Xiakucao is one of the most commonly used Chinese herbal medicines in the treatment of GD, which can be used either alone or in a Chinese herbal formula. Many clinical trials have verified their effectiveness, and several pharmacological studies have been conducted (56, 57). When the symptoms of fear of heat, dry

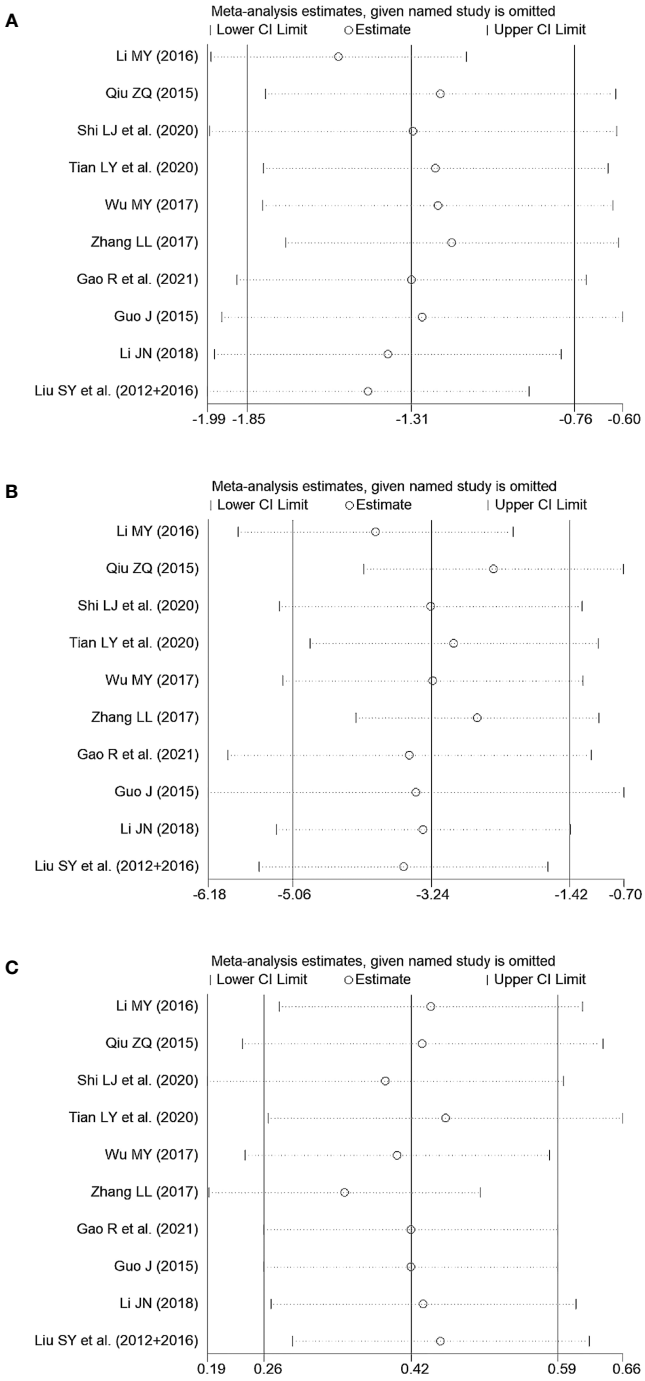


FIGURE 8
Sensitivity tests of primary outcomes in the comparison between Jiawei-Xiaoyao-San plus antithyroid drugs (ATDs) and mono-ATDs. **(A)**: Free triiodothyronine, **(B)**: Free thyroxine, **(C)**: Thyroid stimulating hormone.

mouth and hyperhidrosis are obvious, huangqin or Digupi is often added to treat TCM syndrome of fire of excessive type and deficiency type, respectively. Suanzaoren is often added to treat insomnia.

In addition to the studies and outcomes included in this systematic review, the existing literature also reported other applications and effects of JWXYS in the treatment of hyperthyroidism. Liu XX et al. (58) reported that after microwave ablation, the application of JWXYS can significantly relieve clinical symptoms such as palpitations and irritability, and help control thyroid functions. Liu HJ et al. (59) have shown that the application of JWXYS to patients with hyperthyroidism during pregnancy can

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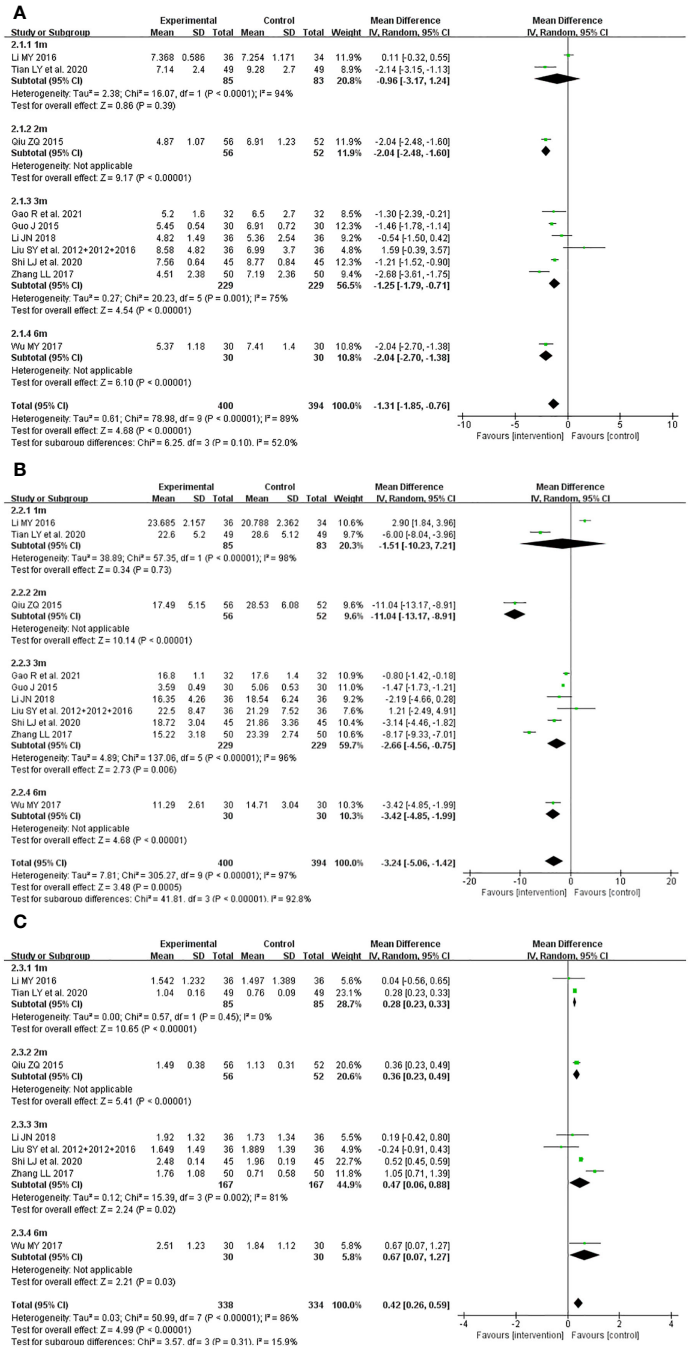


FIGURE 9
Subgroup analyses classified by course of intervention of primary outcomes in the comparison between Jiawei-Xiaoyao-San plus antithyroid drugs (ATDs) and mono-ATDs. **(A)**: Free triiodothyronine, **(B)**: Free thyroxine, **(C)**: Thyroid stimulating hormone.

improve pregnancy outcomes, such as reducing postpartum blood loss, natural labor rate, and postpartum infection rate. Furthermore, studies have shown that JWXYS can reduce the Hamilton Depression Scale score (31) and bilateral superior thyroid artery systolic peak flow velocity (60) in patients with hyperthyroidism. The above studies were not included in this systematic review for meta-analysis because they did not meet the eligibility criteria and the frequency of outcome reporting was low.

4.3 Pharmacological mechanism of JWXYS for hyperthyroidism

Hyperthyroidism is mainly caused by GD, and the core of its pathogenesis is the loss of self-tolerance to TSHR, resulting in the production of stimulating thyrotropin receptor antibodies. Three experiments explored the mechanism of JWXYS in the treatment of hyperthyroidism. All three studies were conducted in animals to

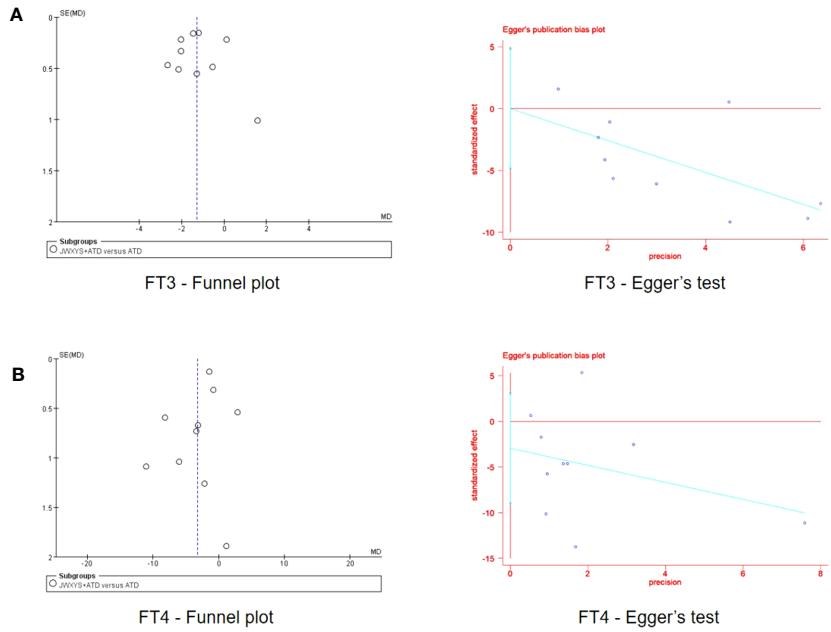


FIGURE 10
Funnel plots and Egger's tests of the effectiveness on free triiodothyronine **(A)** and free thyroxine **(B)** reported by 10 included trials in the comparison between Jiawei-Xiaoyao-San plus antithyroid drugs (ATDs) and mono-ATDs.

TABLE 3 Quality of case reports.

	Chang CC (2010)	Lin CH (2021)	Xia XG (2009)
1. Were patient's demographic characteristics clearly described?	Yes	Yes	Yes
2. Was the patient's history clearly described and presented as a timeline?	Yes	Yes	Yes
3. Was the current clinical condition of the patient on presentation clearly described?	Yes	Yes	Yes
4. Were diagnostic tests or assessment methods and the results clearly described?	Yes	Yes	Partially Yes
5. Was the intervention(s) or treatment procedure(s) clearly described?	Yes	Yes	Yes
6. Was the post-intervention clinical condition clearly described?	Yes	Yes	Yes
7. Were adverse events (harms) or unanticipated events identified and described?	Yes	Yes	No
8. Does the case report provide takeaway lessons?	Yes	Yes	Yes

simulate the onset of GD by injecting antigen (thyroid membrane protein, adenovirus or recombinant adenovirus packaging TSHR plasmid), and all used a three-dose procedure. The key to the mechanism study is the success of the disease model, and the modeling rate and the maintenance of the disease state seriously affect the results of the intervention experiment. All the studies evaluated the success of the modeling, however, none of the three studies reported the modeling rate. The recombinant adenovirus packaged with the TSHR289 plasmid in Tan HZ et al.'s study (46–48, 50, 51) was from our team. In recent years, our team has further optimized the modeling method. The improved single injection method has shortened the modeling time, and the modeling rate can reach 100% within 4 weeks after injection (61).

When GD occurs, the inhibitory function and/or proportion of regulatory T cells (Treg) is decreased, and helper T cells (Th) are abnormally activated. The increased ratio of Th1 and Th17 secretes cytokines such as interferon gama (IFN-γ) and interleukin (IL) 17, thus promoting recruitment and differentiation of B cells and plasma cells. Wu XY's study (49) shows that JWXYS derived formula can regulate T cell differentiation and restore Th17/Treg cell balance in the treatment of GD by inhibiting JAK1-STAT3 pathway of $CD4^+$T cells.

Abnormal thyroid function is an important cause of adverse pregnancy outcomes and has adverse effects on the health of offspring (62). Bao CY's study shows that modified JWXYS reduced the incidence of adverse pregnancy in female mice, and

TABLE 4 Main findings of case reports.

Case	Author (Year)	Age and gender	Diagnosis and comorbidity	Symptoms	Laboratory tests	Treatment experience	Why choose TCM	TCM administration	Course of treatment	Outcomes	Long-term effect	Adverse events
1	Chang CC (2010)	33, female	Graves' disease for 10years, Hepatitis B carrier	Palpitations, chest tightness, itching skin, string-like pulse and a thin pink tongue with thin white coating	Increased free T4, decreased TSH, increased TBII, and positive AMA	MMI (10mg, bid) for 4 months, MMI (5mg, bid to qd) for 1 year, PTU (100mg, bid)	Severe skin itching caused by ATDs and multiple relapses	Modified JWXYS as concentrated herbal extracts in powder form, orally, tid	3 years	The symptoms and thyroid functions alleviated after 3 months. T3 and T4 reached the normal range after 1 year. TSH returned to normal range another 1 year later.	No relapse in 3 years of follow-up after the discontinuance	Not occur
2	Lin CH (2021)	50, female	Graves' disease for several weeks, Chocolate cysts, myomas, and chronic depression	Chest tightness, palpitations, hand tremors, excessive sweating and body weight loss	Abnormal thyroid function (free T4: 3.11; T3: 365.39; TSH <0.01; anti-TPO: 1748.38; anti-THYG: 288.90; anti-TSHR: 3.2	Accepted both ATD and TCM therapy, initially	Afraid of side effects caused by ATDs	Modified JWXYS as concentrated herbal extracts in powder form, orally, tid	1 year	The symptoms alleviated after 2 months. After 10 months, thyroid hormone levels were within the normal range.	No relapse in 1 year of follow-up	Not occur
3	Xia XG (2009)	39, female	Hyperthyroidism for 6 months	Irritability, palpitation, insomnia, dreaminess, chest tightness, belching, fatigue, dizziness, excessive sweating, trembling hands, low menstrual volume, bitter mouth, red tongue with thin and yellow coating, stringy pulse	T3, T4 > normal range; WBC: 2.5×10^9/L; Bp: 145/92mmHg; HR: 104/min; mild goiter	Treated with methimazole, vitamin B4 and hemogenesis.	Leukocytes decreased after treatment with MMI, but did not recover after treatment with leucogen tablets. The patient feared this side effect.	Modified JWXYS decoction, Jiawei Xiaoyao Pills, orally, tid	40 days of decoction, and then a month of Jiawei Xiaoyao Pills	After 15 days, most symptoms were significantly reduced. After 40 days, all symptoms disappeared, and the thyroid function and leukocytes were in the normal range. Bp: 128/80mmHg; HR: 84/min; No goiter	No relapse in 6 years of follow-up after the discontinuance	Not reported

T3, triiodothyronine; T4, tetraiodothyronine; TSH, thyroid stimulating hormone; MMI, Methimazole; PTU, propylthiouracil; anti-TPO, thyroid peroxidase antibodies; anti-THYG, anti-thyroglobulin antibodies; AMA, antithyroid microsomal antibodies; WBC, white blood cell count; TBII, thyrotropin binding inhibiting immunoglobulin; Bp, blood pressure; HR, Heart rate; tid, 3 times a day; bid, 2 times a day; qd, 1 time a day.

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TABLE 5 Characteristics and main findings of mechanism studies.

Author (Year)	Animal	Modeling inducer	Intervention	Effects for disease	Pharmacological findings
Wu XY (2021)	SD female Rat	Rat thyroid membrane protein, i.h, three times	JWXYS + Anemone flaccida Fr. Schmidt (Diwu)	T3↓, T4↓, TRAb↓, Thyroid cell hyperplasia↓	$CD4^+/CD8^+$ on thyroid cells↓; IFN-γ, IL-10, IL-17 and IL-21 in serum↓; JAK1, STAT3, p-STAT3, RORγt, FOXP3 and RORγt/FOXP3 in spleen↓
Bao CY et al. (2019)	C57/BL6 female mice and their offspring	Ad-TSHR289/6xHis adenovirus, three times	Modified JWXYS + PTU	Incidence of adverse pregnancy in female mice↓; Body mass of offspring mice↑; FT3, FT4, TT3 and TT4 of offspring mice↓	Activity of deiodinases (type I, II, III) in the offspring mice↓
Tan HZ et al. (2017)	BALB/c female mice	pAdCMV-TSHR289/Flag (Dsredexpress2) recombinant adenovirus, i.m, three times	Modified JWXYS	T3↓, T4↓, TRAb↓, TSH↑; Food intake↓, water intake↓, body temperature↓; Thyroid cell hyperplasia↓; Pathological lesion of liver tissue	Bcl-2 in thyroid↑; Caspase-3, BAX in thyroid↓; AST, ALT, ALP in serum↓; MDA, SOD, CAT, GSH-PX, Nrf-2, HO-1, 8-OHDG in liver↓

IFN-γ, interferon gamma; IL, interleukin; JAK1, Janus kinase one; FOXP3, Forkhead box protein P3; STAT3, signal transducer and activator of transcription three; RORγt, retinoic acid related orphan receptor gamma (t); Bcl-2, B-cell lymphoma-2; BAX, BCL2-Associated X; AST, aspartate aminotransferase; ALT, alanine transaminase; ALP, alkaline phosphatase; MDA, malondialdehyde; SOD, superoxide dismutase; CAT, catalase; GSH-PX, glutathione peroxidase; Nrf-2, nuclear factor erythroid 2-related factor 2; HO-1, heme oxygenase-1; 8-OHDG, 8-hydroxy-2 deoxyguanosin.

meanwhile JWXYS reduced the secretion of thyroid hormones and activity of deiodinases (type I, II, III) in the offspring mice.

Under normal conditions, the proliferation and apoptosis of thyroid cells are balanced to maintain the morphological stability. Under the stimulation of TRAb, the proliferation and apoptosis of thyroid cells were unbalanced, resulting in hyperplasia and proliferation. Tan HZ et al.'s studies (46) showed that modified JWXYS regulated the imbalance of a pair of markers of proliferation and apoptosis (Bcl-2 and BAX). Oxidative stress is a characteristic of hyperthyroidism and can lead to hepatic damage. JWXYS reduced the concentration of aspartate aminotransferase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP) in serum (47, 48) and 8-hydroxy-2 deoxyguanosine (8-OHDG) expression in the liver (51). Meanwhile, modified JWXYS reduced the levels of malondialdehyde (MDA), superoxide dismutase (SOD) and catalase (CAT) in the liver of GD mice (46–48, 51), indicating the restoration of the balance of oxidation/antioxidant system, which is superior to methimazole. Modified JWXYS could regulate the expression of mRNA and protein of nuclear factor erythroid 2-related factor 2(Nrf2) and heme oxygenase-1(HO-1), the key factors of oxidative stress pathway (46). Therefore, modified JWXYS could protect liver function by alleviating oxidative stress in GD mice.

A network pharmacological study (49) predicted that the main active ingredients of JWXYS in GD treatment include (+)-catechin, beta-sitosterol, formononetin, kaempferol, licochalcone A, naringenin, paeoniflorin, quercetin, shinpterocarpin, sitosterol, stigmasterol and sudan III. JWXYS may intervene in inflammation, immunity, apoptosis and other mechanisms to treat Graves' disease through IL-17 signaling pathway, TNF signaling pathway, cytokine - cytokine receptor interaction and other signaling pathways. Validation is still needed *in vivo* and *in vitro*.

4.4 Strengths and limitations

To our knowledge, this is the first systematic review to evaluate the effectiveness and safety of JWXYS in the treatment of hyperthyroidism. A comprehensive search of eight databases and three trial registries was conducted to ensure that all RCTs were included, and the methodology was reported in detail and transparently to allow for replication. At the same time, we also searched for the case reports of JWXYS alone for the treatment of hyperthyroidism, and summarized the reasons for patients seeking this treatment and its effectiveness, providing a possible alternative therapy for the treatment of hyperthyroidism. In addition, we also searched and summarized the pharmacological mechanism of JWXYS in the treatment of hyperthyroidism. This systematic review provides an effective and safe TCM complementary and alternative therapy for the treatment of hyperthyroidism.

However, there are several limitations in this systematic review. The included studies were all published in Chinese and only Chinese participants were recruited, so the generalizability of the findings was limited. All of the included studies were of low quality, with small sample sizes and without rigorous experimental protocols and follow-up. None of the studies reported relapse rates. Due to the insufficient number of trials included, some comparisons could not be pooled for meta-analysis, and subgroup analyses classified by course of intervention did not show significant differences in primary outcomes. But some trials showed that the adjuvant of JWXYS achieved quicker effectiveness, especially in alleviating symptoms (41). In addition, all studies administered modified JWXYS with different drugs and dosages, and the symptoms and signs included in various studies were not exactly the same, resulting in clinical heterogeneity. Publication bias assessment was performed only for primary outcomes in the JWXYS plus ATDs and ATDs comparisons, as few studies could be included in other outcomes and comparisons.

4.5 Implications

Future studies with appropriate randomization and double-blind methods are warranted to confirm these findings. The CONSORT checklist (63) is recommended for researchers when

drafting RCTs. In the literature screening, it was found that some studies only took clinical effective rate as the primary outcome, and did not report thyroid function data. Single objective outcome is recommended as the primary outcome, rather than the compound outcome of clinical effectiveness. Thyroid volume data obtained from thyroid color ultrasound should be used to explore the improvement of goiter, rather than visual judgment of appearance. It is necessary to establish a core outcome set and promote its application to standardize the trial design, so as to reduce the clinical heterogeneity of meta-analysis. More studies are needed to explore the effect of JWXYS on TRAb, and follow-up should be conducted after treatment to obtain recurrence rates. When conducting a pharmacological mechanism study, it is essential to analyze the components of the drugs utilized. A more robust modeling approach should be employed and the success rate should be confirmed in future animal experiments to ensure the stability and reliability of the results.

5 Conclusions

The current evidence suggests that JWXYS may enhance the effectiveness of ATDs in managing hyperthyroidism, particularly with regards to symptom relief and goiter reduction. Meanwhile, JWXYS can reduce AEs caused ATDs. Mono-JWXYS is not recommended as a therapeutic alternative, except in patients who cannot tolerate ATDs. However, the findings should be interpreted with caution due to overall high risk of bias. Future studies with appropriate randomization and double-blind methods are warranted to confirm these findings. Further pharmacological studies with more reliable models are needed *via in vivo* and *in vitro* studies.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

Author contributions

W-XM and YT conceived and designed the review. W-XM drafted the manuscript. GW and M-YX conducted the data collection. R-TZ and W-XM participated in data extraction and study risk of bias assessment. P-YP and W-XM performed the statistical analysis. L-KL prepared the figures and completed the PRISMA checklist. Z-LL, J-PL, X-WZ and X-YZ were involved in critically revising the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2023.1241962/full#supplementary-material

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Global research landscape and trends of papillary thyroid cancer therapy: a bibliometric analysis

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Background: Papillary thyroid cancer (PTC) is the most common endocrine malignancy worldwide. The treatment of PTC has attracted extensive attention and discussion from the public and scholars. However, no article has systematically assessed the related literature. Therefore, we conducted a bibliometric and knowledge map analysis to reveal the dynamic scientific developments in the PTC therapy field.

Methods: We retrieved publications related to PTC therapy from the Web of Scientific Core Collection (WoSCC) on May 1, 2023. The bibliometric package in R software, VOSviewer and CiteSpace software were used to analyze countries/regions, institutions, journals, authors, references, and keywords. Then, we systematized and summarized the research landscape, global trends and hot topics of research.

Results: This bibliometric analysis spanned from 2012 to 2022 and involved 18,501 authors affiliated with 3,426 institutions across 87 countries/regions, resulting in the publication of 3,954 papers in 860 academic journals. Notably, the number of publications and citations related to PTC therapy research has exhibited a steady increase over the past decade. China and the United States have emerged as leading contributors in terms of publication count, with the United States also being the most cited country. Furthermore, among the top 10 institutions with the highest number of published papers, half were located in China. Among the journals, Thyroid is ranked first in terms of total publications and citations. The most productive individual author was Miyauchi Akira. While previous research primarily focused on surgery and radioactive iodine therapy, the increasing emphasis on health awareness and advancements in medical technology have led to the emergence of active surveillance, thermal ablation, and genomic analysis as prominent areas of research.

Conclusion: In conclusion, this comprehensive and quantitative bibliometric analysis elucidates the research trends and hotspots within PTC therapy, drawing

from a substantial body of publications. This study provides valuable insights into the historical and current landscape of PTC therapy research while also offering guidance for future research directions. This study serves as a valuable resource for researchers and practitioners seeking new avenues of exploration in the field.

KEYWORDS

papillary thyroid carcinoma, therapy, bibliometric analysis, research frontiers, hotspots

1 Introduction

Thyroid cancer (TC) has become the most common endocrine neoplasia, as its global incidence has steadily increased in the past three decades (1). Advancements in diagnostic technology have led to a year-on-year increase in the detection rate of TC, which now has the fifth highest incidence among malignant tumors (2). The increasing incidence of TC poses significant global public health concerns. Papillary thyroid carcinoma (PTC) is the most common subtype of thyroid cancer and accounts for approximately 80% of all thyroid cancer cases worldwide (3). Although most PTCs have a relatively good prognosis, approximately 10% of patients may progress to invasive disease, 5% progress to distant metastasis, and approximately 20–30% may relapse (4). The treatment of PTC has evolved over the years, and many new therapeutic options have emerged. Conventional treatments for PTC differ based on the stage of the initial diagnosis, age, and overall health of the patient. Surgery is the primary treatment for PTC, and it can be curative in most cases. The extent of surgery depends on the size of the tumor, lymph node involvement, and risk factors for recurrence. Total thyroidectomy with or without cervical lymph node dissection is recommended for patients with intermediate- and high-risk tumors (5). Radioactive iodine therapy is also recommended in some cases to ablate any remaining thyroid tissue or to treat metastatic disease. In recent years, there have been advancements in targeted therapies for thyroid papillary carcinoma. These therapies target molecular pathways that are deregulated in PTC and are associated with better outcomes in patients with refractory disease. Targeted therapies are typically used in cases where the cancer has spread or has become resistant to other types of treatments (6). Early detection and prompt treatment play a crucial role in improving the prognosis of patients with thyroid papillary carcinoma. Regular check-ups and follow-ups with healthcare providers are essential for long-term disease management. Longitudinal studies have found that active surveillance, which is a monitoring approach investigated for PTC, is a safe alternative to immediate surgery for patients confirmed to have small (<1-1.5 cm) PTCs (7, 8).

In scientific research, publication is a vital index to measure the contribution of scientific research. Bibliometric analysis is a quantitative method used to depict the knowledge structures and developmental trends of a specific field and evaluate research output, productivity, and impact (9). Unlike other major review methods, bibliometric analysis is well suited for the comprehensive evaluation of an entire academic discipline, encompassing thousands of publications. Bibliometric analysis could provide an impartial approach to evaluating the influence of scientific publications through mathematical and statistical methods, thereby aiding in the identification of research gaps and areas that require further study. In recent years, several bibliometric analyses related to thyroid cancer have been published (10–12). However, there is currently no reported information regarding the quantity and quality of research on PTC treatment. Therefore, we aimed to perform bibliometric network analysis to assess the developmental framework, current landscape, and future trends in the field of PTC treatment.

The present bibliometric analysis analyzed original articles directly related to clinical treatment for PTC published from January 1, 2012, to December 31, 2022. A total of 3954 articles were identified. Furthermore, an additional bibliometric analysis was conducted based on these publications to indicate the latest research hotspots. This study aimed to introduce a novel method for analyzing PTC research, offering an unbiased perspective on research trends, identifying influential authors and institutions, and mapping the intellectual structure of the field.

Abbreviations: PTC, Papillary Thyroid cancer; WoSCC, Web of Scientific Core Collection; TC, Thyroid cancer; WoS, Web of Science; JCR, Journal Citation Reports; J CLIN ENDOCR METAB, Journal of Clinical Endocrinology and Metabolism; LLR, log-likelihood ratio; pCND, prophylactic central neck dissection; TT, total thyroidectomy; LRR, locoregional recurrence; RAI, Radioactive iodine; RAIR-DTC, RAI-resistant differentiated thyroid cancer; TSH, thyroid-stimulating hormone; AS, active surveillance; TA, Thermal ablation; RFA, radiofrequency ablation; LA, laser ablation; MWA, microwave ablation; BRAF, B-type RAF kinase.

2 Materials and methods

2.1 Data collection

A database was built to retrieve related literature for bibliometric analysis via the Web of Science (WoS) Core Collection database. To prevent errors resulting from database upgrades, data searches and exports were conducted on May 1, 2023, encompassing articles related to PTC therapy published

between January 1, 2012, and December 31, 2022. The search strategy was designed as follows: TS= ("papillary thyroid carcinoma" or "thyroid papillary carcinoma" or "thyroid carcinoma, papillary" or "papillary thyroid cancer" or "thyroid papillary cancer" or "thyroid cancer, papillary") AND TS= (therapy OR therapies OR treatment). We initially retrieved 5503 records using this search strategy. To minimize potential bias in our analysis, the following refining criteria were applied: (1) timespan: January 1, 2012, to December 31, 2022; (2) document types included article and review; and (3) written in English. Consequently, a total of 3954 articles were obtained for in-depth analysis. Two authors independently extracted the data from the included studies. The flowchart of article inclusion is shown in Supplementary Figure S1.

2.2 Data analysis

Microsoft Office Excel 2019 software (Microsoft, Redmond, WA, USA) was used to manage data and analyze annual publications. GraphPad Prism 9 software (Dotmatics, San Diego, CA, USA) was used to generate histograms and bubble diagrams. The "bibliometrix" package of R (v4.1.1) is an open-source tool for performing comprehensive science mapping analysis (13). Additionally, the bibliometric analysis utilized two different software tools: CiteSpace [version 6.1. R2 (64-bit)] and VOSviewer (version 1.6.17). CiteSpace is a JAVA-based citation visualization software developed by Chaomei Chen that performs statistical analysis and converts the raw data into a visualization and analysis of literature networks (14). Furthermore, CiteSpace was employed to analyze keywords and references that exhibited significant citation bursts, thus enabling the creation of visualization maps depicting cocited references and keywords. VOSviewer is a powerful software tool for bibliometric mapping developed by Nees Jan van Eck and Ludo Waltman in 2009 (15). It was used to visualize the citation, collaboration, and co-occurrence relationships among journals, references, countries, and institutions. To achieve a comprehensive overview of the research field and ensure result validation, we employed a combination of both software tools. The bibliometric analysis results were subsequently analyzed with descriptive statistics, presented using tables and graphs. To visualize the number of publications in different countries and regions, we utilized Tableau v10.5.0 to create a world map.

3 Results

3.1 Annual publications

The annual trend publications associated with therapy for PTC from 2012 to 2022 are presented in Figure 1. This study identified a total of 3954 publications meeting the inclusion criteria, including 3468 articles and 486 reviews. The continuous increase in the number of publications over the past decade reflects the increase in attention toward PTC therapy research. The quantity of published documents serves as a crucial indicator of the pace of knowledge updates in this subject and helps to elucidate the trends in the field. When evaluating the number of publications per year, the year with the highest number of publications was 2021 (527, 13.3%). The bibliometric analysis revealed a consistent linear growth trend (R^2 = 0.8974) in research related to PTC therapy, indicating a growing interest in this field of study (Supplementary Figure S2).

3.2 Countries

A total of 87 countries or regions contributed to publications in the field of PTC treatment. Figure 2A visualizes the world map of publications in this field. The figure displays the top 25 countries geographically, and the minimum number of documents from a country was 13. Significant achievements were observed in North America, East Asia, and Western Europe, with Figure 2B demonstrating strong cooperation among these countries. A coauthorship network was constructed by including 43 countries/regions with a threshold of ten documents, resulting in six clustered groups. Each node in the network represented a country or region, with node size corresponding to the number of publications. The lines connecting the nodes represent cooperation between countries. In addition, Table 1 and Figure 2C present the scientific production of countries.

Among the top five countries in terms of productivity, China led with the highest number of papers published (1329, 33.59%), followed by the United States (800, 20.23%), South Korea (364, 9.21%), Italy (344, 8.70%), and Japan (170, 4.30%). Although China ranked first globally in terms of publication volume, its citation/publication rate (11.59) was comparatively low compared to other countries. This suggests that the quality of China's papers needs further improvement.

3.3 Institution analysis

The present study involved a total of 3426 institutions, with the top 10 institutions contributing 17.15% of the total papers. Table 2 lists these top 10 prolific institutions, with half of them located in

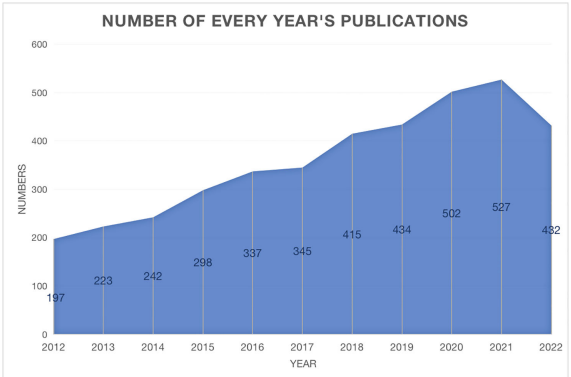


FIGURE 1
Annual trends of global publication outputs in PTC therapy research from 2012 to 2022.

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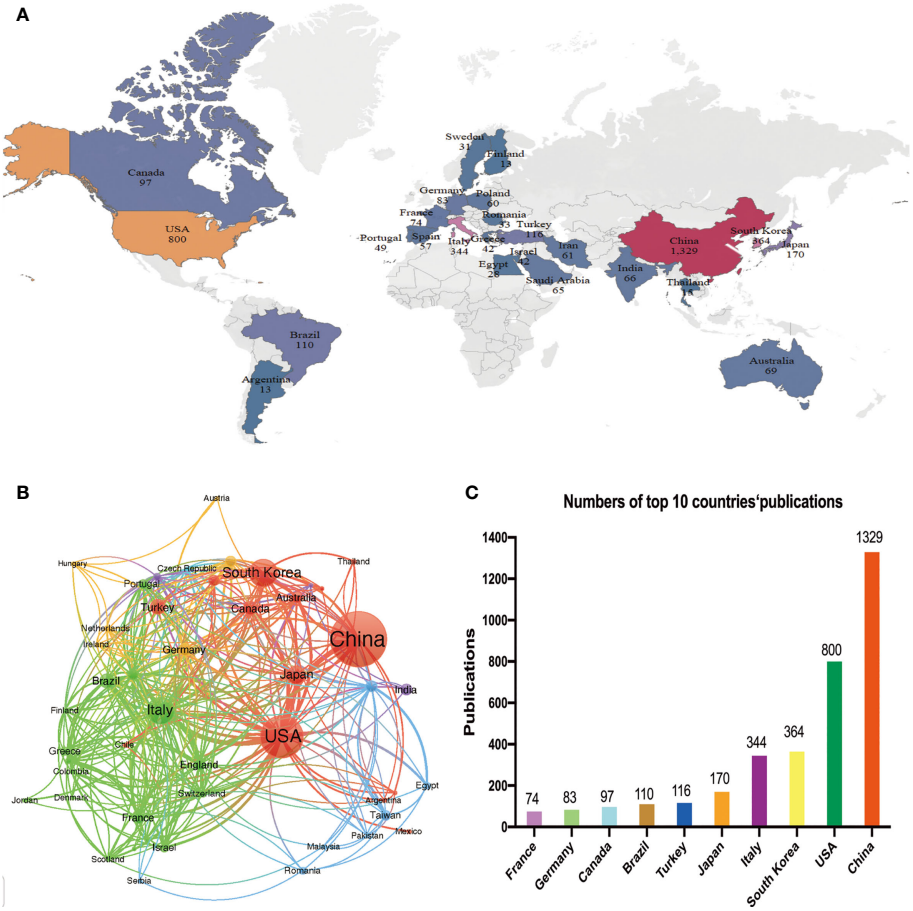


FIGURE 2
Analysis of the distribution of countries or regions. **(A)** Geographical distribution of the countries/regions in terms of publications. Country name and publication number are shown on the map. **(B)** Network mapping of international collaboration. **(C)** Histogram of the top ten countries with the number of publications.

China and the remaining institutions located in the United States, South Korea, and Italy. Shanghai Jiao Tong University made the highest contribution, with 93 papers and 1396 citations, followed by China Medical University (75 papers, 1161 citations) and Fudan University (74 papers, 947 citations). Among these top ten institutions, the University of Pisa exhibited the highest citation/publication rate (56.63). An analysis of institutional cooperation was conducted to reveal the collaborations between institutions (Figure 3). Institutions depicted in the same color demonstrated more active cooperation during the survey. Shanghai Jiao Tong

TABLE 1 The top 10 productive countries/regions in the PTC therapy research field.

Rank	Countries/regions	Publications	Proportion of publications	Citations	Citations per publication
1	China	1329	33.59%	15404	11.59
2	USA	800	20.22%	24835	31.04
3	South Korea	364	9.20%	5694	15.64
4	Italy	344	8.70%	10951	31.83
5	Japan	170	4.30%	3311	19.48
6	Turkey	116	2.93%	685	5.91
7	Brazil	110	2.78%	1657	15.06
8	Canada	97	2.45%	4160	42.89
9	Germany	83	2.10%	1737	20.93
10	France	74	1.87%	1719	23.23

TABLE 2 The top 10 productive institutions within PTC therapy research from 2012 to 2022.

Rank	Institutions	Publications	Citations	Country	Citations per publication
1	Shanghai Jiao Tong University	93	1396	China	15.01
2	China Medical University	75	1161	China	15.48
3	Fudan University	74	947	China	12.80
4	Memorial Sloan Kettering Cancer Center	70	3848	The United States	54.97
5	Yonsei University	70	890	Korea	12.71
6	University of Pisa	68	3851	Italy	56.63
7	Zhejiang University	63	571	China	9.06
8	University of Ulsan	57	930	Korea	16.32
9	The University of Texas MD Anderson Cancer Center	55	2179	The United States	39.62
10	Sichuan University	53	376	China	7.09

University, University of Pisa, and Memorial Sloan Kettering Cancer Center emerged as central partners. However, there was a notable lack of collaboration among these top research institutions within each region.

3.4 Journals and cocited journals

The 3954 included papers were published in 860 academic journals over the past decade. Table 3 presents the top 10 productive journals, offering valuable insights into the most prolific and highly cited publications. The bibliographic coupling network of journals related to PTC treatment is shown in Figure 4A. Among the 88 journals that published a minimum of 10 papers, Thyroid published the highest number of papers (186), followed by Frontiers in Endocrinology (87 papers) and Journal of Clinical Endocrinology and Metabolism (J CLIN ENDOCR METAB) (81 papers). The impact factor, quartile, and categories were retrieved from the Journal Citation Reports (JCR). Most of the listed journals were categorized under "endocrinology and metabolism" and "surgery." Except Oncotarget, which was delisted from WoS in 2017, five of the top ten journals were located in JCR quartile one, indicating their high quality. Furthermore, Figure 4B shows the results of cocitation analysis of 141 journals with a minimum citation count exceeding 200. Among the top 10 cocited journals, half of them were cited more than 1000 times. Thyroid had the

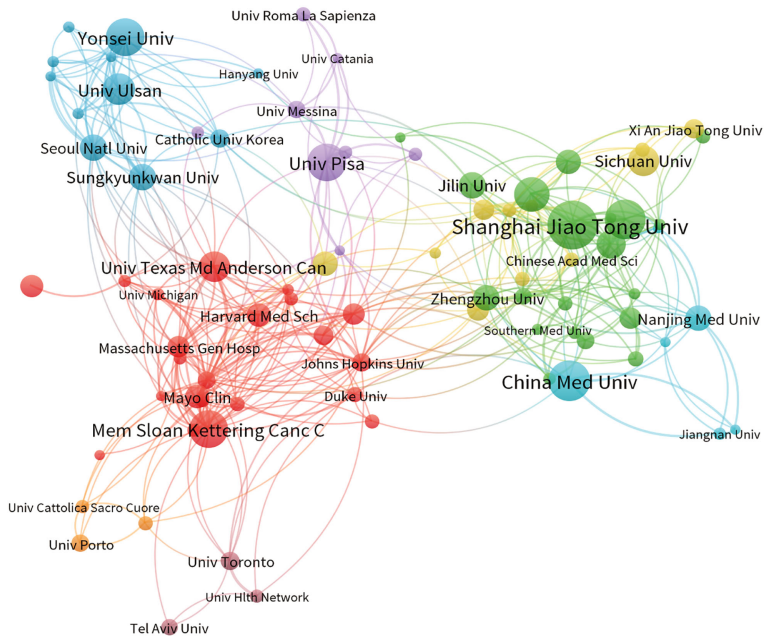


FIGURE 3
The co-occurrence map of institutions depicts the relationships between them. The size of the nodes corresponds to the number of articles associated with each institution; the thickness of the curves indicates the strength of collaboration. The colors used in the map distinguish various collaboration groups.

TABLE 3 The core journals that published publications in PTC therapy research field.

Rank	Journal	NP	TC	IF2021	SJR 2021	JCR quartile	Categories
1	THYROID	186	6034	6.506	1.673	Q1	ENDOCRINOLOGY & METABOLISM
2	FRONTIERS IN ENDOCRINOLOGY	87	416	6.055	1.375	Q1	ENDOCRINOLOGY & METABOLISM
3	J CLIN ENDOCR METAB	81	3404	6.134	1.746	Q1	ENDOCRINOLOGY & METABOLISM
4	ENDOCRINE	68	802	3.925	0.873	Q3	ENDOCRINOLOGY & METABOLISM
5	CANCERS	64	587	6.575	1.349	Q1	ONCOLOGY
6	ONCOTARGET	60	1272	NA	NA	NA	ONCOLOGY; CELL BIOLOGY
7	MEDICINE	56	619	1.817	0.470	Q3	MEDICINE, GENERAL & INTERNAL
8	SURGERY	55	1307	4.348	1.236	Q1	SURGERY
9	ONCOLOGY LETTERS	53	507	3.111	0.639	Q3	ONCOLOGY
10	WORLD JOURNAL OF SURGERY	50	1255	3.282	0.874	Q2	SURGERY

NP, Number of published; TC, Total citations.

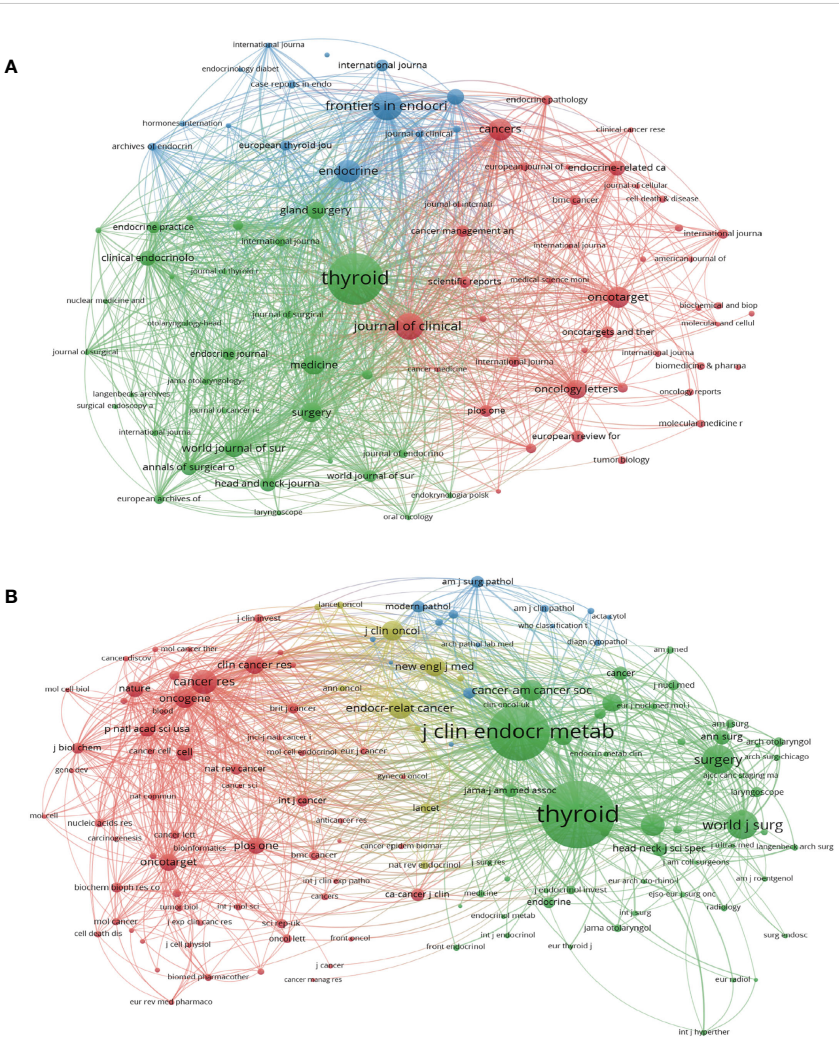


FIGURE 4
Journals and cocited journals analysis. **(A)** The bibliographic coupling network of journals related to PTC treatment. Each circle in the figure represents a journal, and the size of the circle indicates the number of publications output in that journal. **(B)** The cocitation network visualization of journals with a minimum of 200 citations. The size of the circle indicates the number of citations in that journal.

highest total number of citations (6034, IF=6.506), followed by Journal of Clinical Endocrinology and Metabolism (3404, IF=6.134) and Surgery (1307, IF=4.348). The significantly higher citation count of Thyroid compared to other journals indicates its substantial productivity and influence.

3.5 Publication distribution among author analysis

A total of 18,501 researchers and 48,186 cocited authors made contributions to the 3,954 publications in the field of PTC therapy between 2012 and 2022. Figure 5A illustrates coauthor collaboration and the publication output of each author. Collaboration among authors signifies teamwork, although there is less collaboration among teams, resulting in relatively fragmented research. Cocited authors refer to two or more authors who are simultaneously cited through another paper. As depicted in Figure 5B, the cocitation analysis revealed that Ito Yasuhiro (1,286), Xing MZ (1,093), and Haugen BR (1,145) had the highest number of cocitations. These findings indicate that the aforementioned authors displayed greater interest in PTC-related research.

Figure 6 displays the publication count and H-index of the top 10 productive authors. These ten authors collectively contributed 225 papers (5.69%). Notably, Miyauchi Akira (33), Ito Yasuhiro (29), and Tuttle R Michael (25) emerged as the top three authors in terms of publication volume. The first two authors are affiliated with Kuma Hospital in Japan, an institution specializing in thyroid

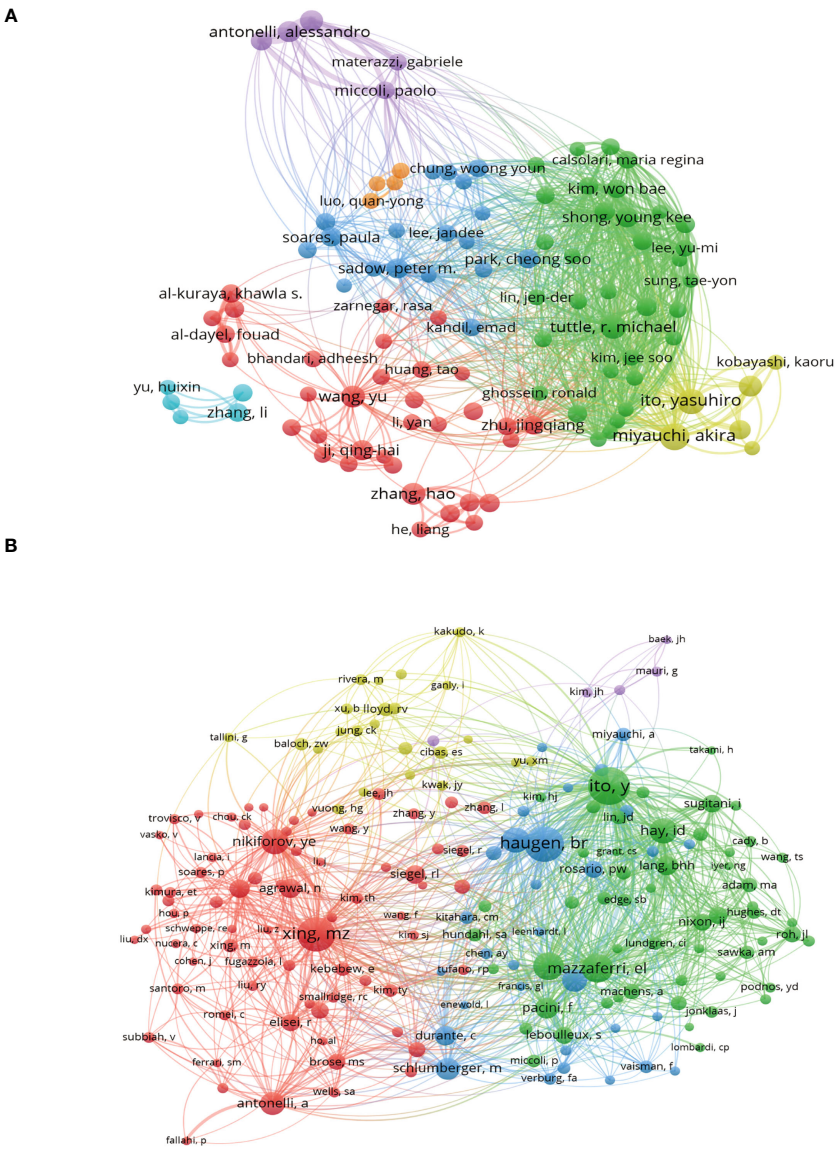


FIGURE 5
Authors and cocited authors analysis. **(A)** Co-occurrence map of authors. The size of the nodes represents the number of articles. **(B)** Cocited authors analysis map. The size of the nodes represents the number of cocitations.

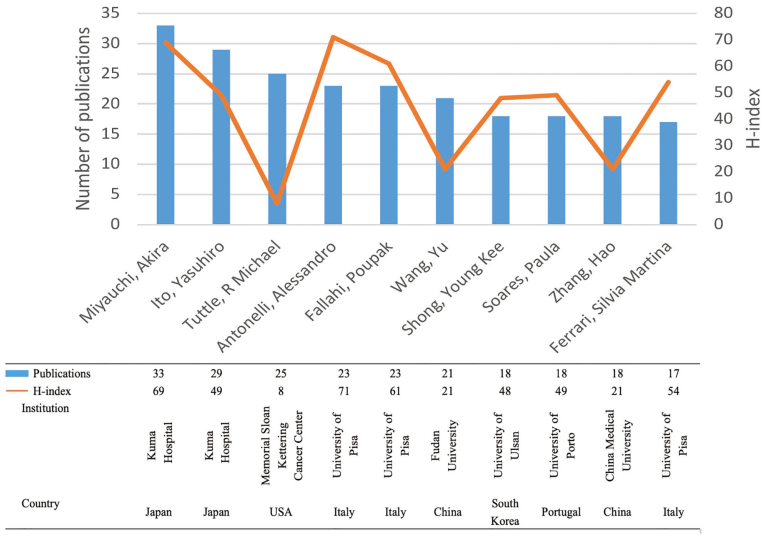


FIGURE 6
The top 10 productive authors and their H-indexes. Each bar in blue represents the number of publications of each author. Each node in yellow indicates the H-index of each author.

disorders. Among all authors, Antonelli Alessandro from the University of Pisa had the highest H-index (71), followed by Miyauchi Akira from Kuma Hospital (69) and Fallahi Poupak from the University of Pisa (61).

3.6 References

Among the 1,690 documents retrieved for this study, the top 10 most cited research articles accounted for a total of 2,012 citations, representing 19.75% of the total citations. Table 4 presents the top 10 references with the highest cocitations in descending order. Each of these references received more than 90 cocitations. Eight of the publications were from the United States, while the remaining two were from England. The most cited article, titled "2015 American Thyroid Association Management Guidelines for Adult Patients with Thyroid Nodules and Differentiated Thyroid Cancer: The American Thyroid Association Guidelines Task Force on Thyroid Nodules and Differentiated Thyroid Cancer," was published in 2016 in the journal Thyroid, which is the most cited journal in this field.

CiteSpace software was employed to conduct burst analysis and cluster analysis of cocited references. In bibliometric analysis, reference bursts can indicate research hotspots in academic fields. Figure 7A displays the top 15 cocited references with the strongest citation bursts from 2012 to 2022. The strength of these bursts ranged from 16.32 to 118.56. The blue line segment represents the time interval, while the red line segment indicates the time of frequent citations. The top three references with the most pronounced citation bursts were "Revised American Thyroid Association management guidelines for patients with thyroid nodules and differentiated thyroid cancer" (Strength: 118.56; Publication Year: 2009), "2015 American Thyroid Association Management Guidelines for Adult Patients with Thyroid Nodules and Differentiated Thyroid Cancer" (Strength: 59.16; Publication Year: 2015) and "Integrated genomic characterization of papillary thyroid carcinoma" (Strength: 40.99; Publication Year: 2016).

As depicted in Figure 7B, the research field's topic clustering map identified 10 clusters with significant modularity and silhouette scores (Q = 0.7983; S = 0.9214). These two indicators are crucial for evaluating the effectiveness of graph visualization. Q-values greater than 0.3 and S-values greater than 0.5 indicate a stable and highly convincing clustering structure. The largest cluster, labeled "prophylactic central neck dissection," was followed by clusters focused on "managing papillary thyroid carcinoma" (cluster #1), "radioactive iodine-refractory differentiated thyroid cancer" (cluster #2), and "active surveillance" (cluster #3). Other important clusters included papillary thyroid carcinoma, radioiodine therapy, Hashimoto's thyroiditis, dynamic risk stratification, and association management guidelines.

3.7 Analysis of keywords and research trends

Keywords play a crucial role in accurately depicting an article's topic and reflecting the research frontiers within a specific field of study. In this study, we collected 8,013 keywords from various authors and utilized the CiteSpace cluster function to construct a visual map clustering commonly cited keyword. Through our analysis, we categorized the research documents into nine clusters, as depicted in Figure 8A. The module's Q value was determined to be 0.9122, and the average contour S value was 0.5. The cluster nomenclature effectively represents the study frontiers in the field. In this study, the clusters were autogenerated and labeled using the log-likelihood ratio (LLR) algorithm. The largest cluster, labeled #0, was "proliferation," followed by "central neck dissection" (cluster #1), "thermal ablation" (cluster #2), and "BRAF mutation" (cluster #3). Other significant clusters included papillary

TABLE 4 The characteristics of highly cited and the most impact classic articles in PTC therapy field.

Rank	Total Citations	Article title	Journal	Published Year	Country	IF2021
1	842	2015 American Thyroid Association Management Guidelines for Adult Patients with Thyroid Nodules and Differentiated Thyroid Cancer: The American Thyroid Association Guidelines Task Force on Thyroid Nodules and Differentiated Thyroid Cancer	Thyroid	2016	USA	6.506
2	225	Revised American Thyroid Association management guidelines for patients with thyroid nodules and differentiated thyroid cancer	Thyroid	2009	USA	6.506
3	191	Trends in Thyroid Cancer Incidence and Mortality in the United States, 1974-2013	JAMA	2017	USA	157.335
4	174	Integrated genomic characterization of papillary thyroid carcinoma	Cell	2014	USA	66.850
5	106	Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries	CA: A Cancer Journal for Clinicians	2018	USA	286.130
6	98	Thyroid cancer	Lancet	2016	ENGLAND	202.731
7	98	Cancer statistics, 2022	CA: A Cancer Journal for Clinicians	2022	USA	286.130
8	95	Nomenclature Revision for Encapsulated Follicular Variant of Papillary Thyroid Carcinoma: A Paradigm Shift to Reduce Overtreatment of Indolent Tumors	JAMA Oncology	2016	USA	33.006
9	92	The changing incidence of thyroid cancer	Nature Reviews Endocrinology	2016	ENGLAND	47.564
10	91	Current thyroid cancer trends in the United States	JAMA Otolaryngology-Head & Neck Surgery	2014	USA	8.961

thyroid carcinoma, radioiodine therapy, Hashimoto's thyroiditis, dynamic risk stratification, and association management guidelines.

To further visualize the thematic map of PTC therapy, we examined the locations of keywords, which were represented by their density and centrality. This representation indicates the evolution of themes (16). The upper right quadrant (Q1) represents crucial motor themes, while the upper left quadrant (Q2) portrays well-established, albeit somewhat isolated, themes. The lower left quadrant (Q3) indicates emerging or declining themes, and the lower right quadrant (Q4) represents basic or transversal themes. As shown in Figure 8B, the terms "management" and "recurrence" are located in Q1, demonstrating that these topics are well developed and can structure the research field. The presence of "metastasis" and "mutations" in Q3 is expected, as they represent basic topics in this field. Additionally, the presence of "expression," "invasion," and "proliferation" in Q2 implies highly developed internal connections but marginal contributions to advancements in PTC therapy. The timeline viewer (Figure 8C) of keywords enables us to observe the evolution of new hotspots and explore the evolutionary trajectory of this field. The keywords were classified into nine clusters, and their evolution can be roughly divided into three periods: the early stage (2012-2015), middle stage (2016-2020), and current stage (2021-2022). Based on the cluster labels and keywords within the clusters, recent research hotspots include "tumor microenvironment," "fusion oncogene," "dynamic risk stratification," and "pediatric thyroid cancer". Supplementary Figure S3A presents the most frequently occurring keywords in the field of PTC therapy from 2012 to 2022. The size of each keyword indicates its importance and frequency of use within the field. To obtain clustering results, we utilized the FoamTrees function of Carrot2 with keywords as the data source, resulting in 34 clusters (Supplementary Figure S3B). The larger the area of the foam is, the higher the intensity of research activity. Notable keywords in the clusters include "cells in patients," "Braf in cancer," and "Expression in papillary thyroid carcinoma cells."

4 Discussion

4.1 Basic information

This study analyzed 3954 publications from 87 countries on PTC therapy over the past decade. The studies were retrieved from the WoSCC database. Bibliometric and visual analysis methods were employed to examine research trends and hotspots in this field from 2012 to 2022. The number of publications on PTC therapy has been steadily increasing each year, with 2021 having the highest volume of literature, thus indicating the growing significance of this topic. China and the United States have emerged as the leading contributors, surpassing other countries/regions in terms of publication count. The United States is the most cited country, and Canada had the highest average number of citations. However, China exhibited a low citation-to-publication ratio, suggesting a



FIGURE 7
(A) Burst analysis of the top 15 references. The blue line represents the period from 2012 to 2022, while the red line plots the periods of each burst keyword. **(B)** Reference clustering map analysis through CiteSpace. A total of 10 categories of references were obtained. The different color blocks represent different reference clusters.

need for higher-quality publications. Furthermore, half of the top 10 institutions with the most published papers were located in China, followed by the United States and South Korea.

Regarding journals and cocitations, the most productive journals were Thyroid (186 papers), Frontiers in Endocrinology (87 papers), and Journal of Clinical Endocrinology and Metabolism (81 papers). Thyroid also ranked first in terms of total citations, indicating the inclusion of a substantial number of high-quality articles. Among authors, the most productive individuals were Miyauchi Akira (33), Ito Yasuhiro (29), and Tuttle R Michael (25). The three authors with the highest H-indexes were Antonelli Alessandro (71), Miyauchi Akira (69), and Fallahi Poupak (61), highlighting the superior quality of their papers and establishing them as leading figures in the field of PTC therapy research. Among the top 10 most cited articles, in addition to the guidelines for diagnosing and managing thyroid nodules and cancers, the integrated genomic characterization (17) and the current trend of thyroid cancer (18) also gained researchers' attention. These articles are all published in JCR Q1 journals, and the quality of the articles is relatively high.

4.2 Research trends and hotspots

The analysis and discussion of the aforementioned general information reveal that influential authors and references mostly consist of review articles and clinical guidelines from internationally renowned institutions and journals. By combining co-occurrence, clustering, and burst analysis of keywords and references, we identified continuous and emerging hotspots and research trends in the field of PTC therapy. In the past, research primarily focused on surgery and radioactive iodine therapy. However, with the increasing awareness of people's health and advancements in medical technology, active surveillance, thermal ablation, and genomic analysis are becoming prominent areas of research.

4.2.1 Central neck dissection

The pros and cons of prophylactic central neck dissection (pCND) have been extensively debated in recent studies. Papillary thyroid carcinoma is a prevalent endocrine malignancy worldwide. While the prognosis for treated PTC is generally favorable, certain patients have a

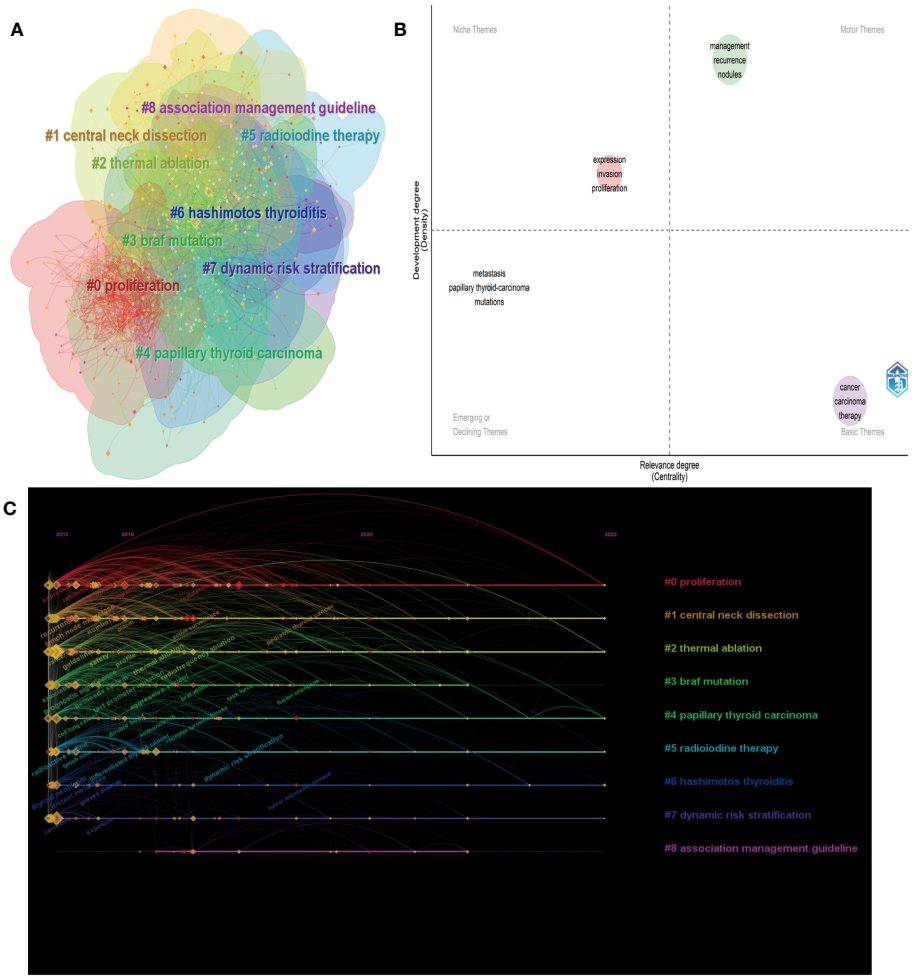


FIGURE 8
Analysis of keywords. **(A)** Keyword clustering map analysis through CiteSpace. **(B)** The timeline view for keywords related to PTC therapy. **(C)** Thematic map in the PTC therapy field.

higher risk of recurrence and lymph node metastasis. Surgery remains the most commonly used curative treatment for PTC. Based on preoperative staging and lymph node metastasis, several organizations have advocated for total thyroidectomy (TT) plus pCND as the standard procedure for PTC (5). This recommendation is supported by three primary arguments. First, lymph node metastases are frequently involved in PTC, particularly in the central compartment of the neck (level VI). Second, detecting recurrence or persistence in the paratracheal area is challenging. Last, reoperations in the central neck present an increased risk of recurrent laryngeal nerve injury and hypoparathyroidism. Over time, these three arguments have been substantiated (19, 20).

However, the value of pCND remains a topic of debate among patients without clinical evidence of nodal metastasis, primarily due to its association with an increased incidence of postoperative hypoparathyroidism and potentially permanent hypoparathyroidism (21). Several meta-analyses have reported that the risk of postoperative hypocalcemia is 2.0 to 2.7 times higher following prophylactic CND than after non-CND procedures (22). Moreover, several studies have indicated that the addition of pCND to TT does not significantly reduce the occurrence of future locoregional recurrence (LRR) compared to TT alone (23, 24). In conclusion, preventative neck dissection may be considered an option for certain high-risk patients with papillary thyroid carcinoma; however, its implementation should be carefully evaluated and discussed with a healthcare professional.

4.2.2 Radioiodine therapy

Radioactive iodine (RAI) therapy is a common treatment approach for patients with metastatic PTC (PTC), as it allows for targeted delivery of radiation to cancerous thyroid tissue. This treatment method has been shown to reduce the risk of recurrence and improve survival rates, particularly in cases of differentiated thyroid cancer with lymph node metastasis (25, 26). However, approximately 30% of PTC patients do not initially show iodine uptake for RAI therapy, and others gradually lose response to RAI during treatment. The prognosis for patients with RAI-resistant differentiated thyroid cancer (RAIR-DTC) depends on tumor burden and growth rate, with an overall 10-year survival rate of 10% and a median survival of only 3-5 years (27). The optimal dosage of RAI therapy varies based on individual patient

characteristics, considering factors such as residual thyroid tissue size, thyroid-stimulating hormone (TSH) level, and body weight (28). Several randomized prospective studies support the use of lower doses (e.g., 30 mCi) rather than higher doses (e.g., 100 mCi) for managing low-risk thyroid cancer (29). Complications resulting from RAI therapy, including radiation thyroiditis, salivary gland dysfunction, and hematological changes, can negatively impact patients' quality of life (30). Therefore, a careful assessment of the risks and benefits of RAI therapy is crucial. Potential side effects and the risk of recurrence after treatment should be considered. Close monitoring and regular follow-up care are recommended for patients undergoing radioactive iodine therapy (31).

However, not all PTC patients respond optimally to RAI therapy, and some develop resistance to its effects. Resistance to RAI therapy in PTC is a complex phenomenon with various proposed mechanisms (32). There are several key factors contributing to PTC RAI resistance. Downregulation or loss of sodium/iodide symporter (NIS) expression can limit iodine uptake, reducing the therapeutic effect (33). BRAF V600E Mutation can affect intracellular signaling pathways, including the MAPK pathway, which plays a role in NIS regulation (34). PTCs that dedifferentiate into poorly differentiated or anaplastic thyroid cancer may lose the ability to take up iodine, rendering them resistant to RAI therapy. Factors within the tumor microenvironment, such as hypoxia and inflammation, can influence RAI resistance by altering NIS expression and function (35). Elevated TSH levels can stimulate thyroid cancer cell growth and reduce the effectiveness of RAI therapy. Understanding the underlying mechanisms and implementing strategies to overcome resistance are crucial for improving treatment outcomes. Approaches aimed at increasing NIS expression, combining RAI therapy with targeted therapies and applying personalized medicine have shown promise in some cases to improve response to RAI.

A thought-provoking paper by WR Luo (36) argue that cancer represents a pathological ecosystem, emphasizing the multidimensional spatiotemporal "unity of ecology and evolution." Within this framework, cancer therapeutic resistance emerges as a dynamic and adaptive process that mirrors the principles of evolution. In the context of PTC, where therapeutic resistance remains a clinical challenge, viewing resistance as an evolutionary process within the cancer ecosystem provides a more comprehensive explanation. The tumor microenvironment, a crucial component of this ecosystem, plays a pivotal role in shaping treatment responses. Factors such as hypoxia, immune interactions, and genetic heterogeneity influence the adaptive evolution of PTC cells, leading to resistance against therapies, including tyrosine kinase inhibitors (TKIs), RAI treatment and immunotherapy. Furthermore, the concept of ecological and evolutionary dynamics in cancer underscores the importance of monitoring treatment responses over time. The emergence of resistant clones within the tumor population highlights the need for ongoing assessment and personalized treatment adjustments.

4.2.3 Thyroid hormone suppression therapy

Thyroid hormone suppression therapy is based on the understanding that thyroid-stimulating hormone (TSH), secreted by the pituitary gland, influences the growth and proliferation of thyroid cancer cells (37). Several studies have demonstrated a direct association between serum TSH concentration and the risk of differentiated thyroid cancer in patients with thyroid nodules, as well as a more aggressive course in those diagnosed with thyroid cancer (38, 39). Stimulation of thyrotropin leads to overexpression of thyroid differentiation genes, such as the sodium iodide symporter, resulting in increased uptake of RAI and enhancing the tumoricidal effect (40). The underlying hypothesis is that after adequate surgery with or without radioactive iodine ablation, long-term thyrotropin (TSH) suppression therapy should be administered to patients with PTC to decrease TSH levels and prevent the recurrence or spread of cancer cells. Studies on the effectiveness of thyroid hormone suppression therapy have produced conflicting results (41–43). There is significant discrepancy regarding the role of thyrotropin suppression in patients with intermediate- or high-risk DTC, with some studies suggesting no significant difference in recurrence rates between patients receiving the therapy and those who do not. Additionally, TSH suppression therapy can cause adverse effects such as osteoporosis and cardiac arrhythmia in certain patients, leading to controversy surrounding its use (44).

4.2.4 Active surveillance

With the widespread use of diagnostic and imaging technologies, the detection of small low-risk papillary thyroid carcinomas (PTCs) has increased, but the corresponding mortality rate has not shown a similar increase (45, 46). Considering the low risk of disease progression in incidentally discovered PTCs, surgical excision may pose more harm than benefit, leading to active discussions about alternative treatment options. The recent guidelines from the American Thyroid Association introduced active surveillance as a strategy for patients with incidentally detected PTCs, providing an alternative to immediate thyroidectomy (5). Active surveillance involves regular monitoring of the cancer through ultrasound and blood tests to assess its growth and progression. Japan was the first country to evaluate the safety of active surveillance for low-risk PTCs. In the early 1990s, two Japanese centers, the Kuma Hospital and the Cancer Institute Hospital (CIH), conducted prospective clinical studies on active surveillance for low-risk (T1aN0M0) PTCs (47, 48). These studies confirmed the safety of active surveillance for papillary thyroid microcarcinomas (<1.0 cm diameter), with 10% to 15% of patients experiencing tumor growth, usually within 5 years.

Several studies have reported promising results for active surveillance. Ito et al. (49) reported that 15.9% of PTCs <1 cm had a growth of 3 mm or more over a 10-year observation period, and new nodal metastases were detected in 3.4% over the same period. Similarly, Tuttle et al. (50) reported growth of ≥3 mm in 2.5% of PTCs up to 1.5 cm in greatest dimension at 2 years and 12.5% at 5 years with a median follow-up of 25 months. These two authors are also among the top-ranked authors in our author analysis, further highlighting the significant attention and interest in active surveillance in the field of PTC treatment. However, importantly, not all cases of thyroid papillary carcinoma are suitable for active surveillance. Patients with high-risk tumors or

aggressive forms of the disease should undergo surgical intervention as soon as possible.

4.2.5 Thermal ablations

Some patients with low-risk papillary thyroid microcarcinoma (PTMC) undergoing active surveillance may experience concerns or anxiety regarding the presence of cancer. While addressing this anxiety and unresolved cases of high-risk patients (i.e., patients younger than 40 years) in active surveillance, ultrasound-guided thermal ablation modalities offer an intermediate approach between active surveillance and surgery for patients with low-risk PTCs (51–53). Thermal ablation (TA) technologies such as radiofrequency ablation (RFA), laser ablation (LA), or microwave ablation (MWA) have recently gained interest as minimally invasive treatment modalities for PTCs, showing promising outcomes (54, 55). Importantly, patients categorized as unsuitable for active surveillance should not consider thermal ablation. However, for patients who prefer to avoid surgery, thermal ablation can be considered. The quality of the operator and treatment center plays a crucial role, as the success of the ablation procedure is operator dependent.

Numerous studies have demonstrated favorable outcomes of thermal ablation in PTC. A meta-analysis of data from 17 studies (combined n = 470) reported no instances of local tumor recurrence or distant metastasis after thermal ablation, and the rate of undergoing delayed surgery was only 1.1% (56). Zhang et al. (55) evaluated the efficacy and safety of ultrasound-guided RFA for treating low-risk PTMC. Among the 92 patients included, 10 (41.7%) achieved resolution within 6 months, and 23 (95.8%) achieved resolution within 12 months. No residual or recurrent tumor tissue was detected within the RFA area or in the residual thyroid tissue during follow-up, and no suspicious metastatic lymph nodes were found.

4.2.6 *BRAF*(V600E) mutation

In contemporary times, various molecular markers for tumors have been proposed as potential tools to identify aggressive cases of papillary thyroid carcinoma. These markers include the B-type RAF kinase (BRAF) V600E mutation, RET/PTC rearrangement, and/or RAS mutation (57). The BRAF V600E mutation serves as a prognostic genetic marker that enhances risk stratification and enables tailored management of thyroid cancer patients, even those considered to have low conventional risks (58). Approximately 40-60% of papillary carcinomas exhibit the BRAF V600E mutation, which leads to constitutive activation of the mitogen-activated protein kinase (MAPK) signaling pathway, thereby promoting cell proliferation and survival (59). Research has demonstrated that the presence of the BRAF V600E mutation is associated with aggressive clinicopathological characteristics and a worse prognosis in patients with papillary carcinoma.

A recent meta-analysis comprising 63 studies involving 20,764 patients revealed that the BRAF (V600E) mutation in PTC is associated with lymph node metastasis, extrathyroidal extension, higher TNM stage, recurrence, and reduced overall survival (60). Xing et al. (61) conducted a retrospective multicenter study involving 1849 individuals with PTC from around the world. Their findings demonstrated a significant association between the presence of the BRAF V600E mutation and increased cancer-related mortality among PTC patients. Moreover, the BRAF V600E mutation has been linked to resistance to radioiodine therapy, which is the standard care for differentiated thyroid cancer patients (62). Therefore, further investigations are necessary to assess the prognostic and therapeutic implications of BRAF V600E status in PTC.

4.2.7 Other gene mutations

Certainly, in addition to the well-documented BRAF(V600E) mutation, PTC exhibits a spectrum of genetic alterations and mutations that contribute to its pathogenesis and clinical heterogeneity. Notable genetic mutations and alterations in PTC include RAS mutations, RET/PTC rearrangements, TP53 mutations, TERT promoter mutations, PIK3CA mutations, EIF1AX mutations, and non-V600E mutations in the BRAF gene (63). Second in prevalence to BRAF mutations in thyroid cancer are RAS mutations. RAS mutations, specifically in NRAS and HRAS, have been identified in a subset of PTC cases, leading to the activation of the mitogen-activated protein kinase (MAPK) signaling pathway and facilitating tumor growth and progression (64).RET/PTC rearrangements, which result from chromosomal rearrangements involving the RET proto-oncogene, lead to constitutive activation of the RET receptor tyrosine kinase and are prevalent in PTC, particularly in radiation-induced cases (65). TP53 mutations are associated with more aggressive forms of PTC and are involved in resistance to radioiodine therapy. They are relatively rare but have been reported in a subset of cases. TERT Promoter Mutations: Mutations in the telomerase reverse transcriptase (TERT) promoter are often seen in aggressive PTC and are associated with increased tumor aggressiveness and poor prognosis (66). These genetic alterations contribute to the heterogeneity of PTC and can have implications for prognosis and treatment strategies. It's important to note that the prevalence of these mutations can vary among PTC cases and may have different clinical implications.

4.3 Limitations

To our knowledge, this study represents the first bibliometric analysis and visualization of research on therapy for PTC. However, our study does possess inherent limitations associated with bibliometric analysis. First, this study only included literature retrieved from the WoS database, and papers not included in this database were omitted. Although the WoS database is widely recognized, extensively used, and comprehensive in bibliometrics (67), the results obtained reflect the overall trends. Second, our study focused on retrieving literature related to PTC therapy published between 2012 and 2022 to capture the latest research trends and landscape in this field, thereby excluding earlier papers. Last, a certain degree of language bias was present, as our analysis only included English-language publications.

5 Conclusion

In conclusion, this study provides a comprehensive bibliometric analysis and visualization of the research area pertaining to therapy for PTC. We conducted a bibliometric analysis by retrieving literature relevant to the topic from the Web of Science (WoS) Core Collection database, spanning the years 2012 to 2022. The retrieved data were analyzed using CiteSpace and VOSviewer software, allowing us to examine the field's development across various aspects, including countries/regions, institutions, journals, authors, references, and keywords. Through this analysis, we identified current hotspots in the field and explored future directions. The most prominent research topics in the field of papillary thyroid carcinoma (PTC) therapy were found to be active surveillance, thermal ablation, and BRAF (V600E) mutation. Overall, our study provides insights into the historical and current trends in PTC therapy research, offering valuable guidance for both researchers and practitioners seeking new avenues of exploration.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

Author contributions

BS is the first author of this study. WT is the corresponding author supervising this work. BS managed the case and drafted the manuscript. ZL, CF and XZ provided major technical support. BS, ZL, CF and XZ assisted in literature review and organizing data from the literature. WT and reviewed the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2023.1252389/full#supplementary-material

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Stimulated thyroglobulin and pre-ablation antithyroglobulin antibody products can predict the response to radioiodine therapy of TgAb-positive differentiated thyroid cancer patients: a retrospective study

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Objective: We aimed to explore the predictive value of stimulated thyroglobulin (sTg) and pre-ablation antithyroglobulin (pa-TgAb) products for the effect of radioiodine therapy (RAIT) on TgAb-positive differentiated thyroid cancer (DTC) patients.

Methods: In this study, we enrolled 265 patients with TgAb-positive DTC who underwent RAIT after total thyroidectomy (TT). Based on the last follow-up result, the patients were divided into two groups: the excellent response (ER) group and the non-excellent response (NER) group. We analyzed the factors related to the effect of RAIT.

Results: The ER group consisted of 197 patients. The NER group consisted of 68 patients. For the univariate analysis, we found that the maximal tumor diameter, whether with extrathyroidal extension (ETE), bilateral or unilateral primary lesion, multifocality, preoperative TgAb (preop-TgAb), pa-TgAb, sTg × pa-TgAb, initial RAIT dose, N stage, and surgical extent (modified radical neck dissection or not), showed significant differences between the ER group and NER group (all *p*-values <0.05). The receiver operating characteristic (ROC) curves showed that the cutoff value was 724.25 IU/ml, 424.00 IU/ml, and 59.73 for preop-TgAb, pa-TgAb, and sTg × pa-TgAb, respectively. The multivariate logistic regression analysis results indicated that pa-TgAb, sTg × pa-TgAb, initial RAIT dose, and N stage were independent risk factors for NER (all *p*-values <0.05). For the Kaplan–Meier analysis of disease-free survival (DFS), the median DFS of the patients with

sTg × pa-TgAb < 59.73 and initial RAIT dose ≤ 100 mCi was significantly longer than that of the patients with sTg × pa-TgAb ≥ 59.73 (50.27 months vs. 48.59 months, p = 0.041) and initial RAIT dose >100 mCi (50.50 months vs. 38.00 months, p = 0.030).

Conclusion: We found the sTg and pa-TgAb conducts is a good predictor of the efficacy of RAIT in TgAb-positive DTC patients. It can play a very positive and important role in optimizing treatment, improving prognosis, and reducing the burden of patients.

KEYWORDS

differentiated thyroid cancer, serum antithyroglobulin antibody, radioiodine therapy, response to therapy, prognosis

Introduction

Differentiated thyroid cancer (DTC) accounts for more than 95% of thyroid cancers and is the most common endocrine tumor. Surgery, selective radioiodine therapy (RAIT) after total thyroidectomy (TT), and thyroid-stimulating hormone (TSH) suppressive therapy are the standard treatments for DTC (1). As a specific tumor marker for long-term follow-up after RAIT in DTC, serum thyroglobulin (Tg) can predict DTC persistence, distant metastasis, or recurrence (2–4). However, the Tg level can be affected by the antithyroglobulin antibody (TgAb), which makes Tg measurement unreliable.

TgAb is mainly produced by lymphocytes, which react against thyroid self-antigens (5). Almost 25% of DTC patients are positive for circulating TgAb (6–8). In such patients, the value as a tumor marker of serum Tg will be substantially weakened since positive TgAb may result in false-negative results for DTC patients when it is measured with radioimmunoassay (9, 10). Therefore, as a considerable surrogate tumor marker for evaluating dynamic risk stratification, TgAb is adopted in the American Thyroid Association (ATA) guidelines (11).

In most previous studies, researchers mainly focused on the relationship between the time trend of TgAb after RAIT and the clinical outcome of DTC and revealed that the trend of TgAb was more important for prognosis in TgAb-positive DTC patients (12–16). However, it takes time to observe the time trend of TgAb. Therefore, identifying patients with a poor prognosis early is the focus in clinical practice. Pan et al. (17) reported a negative correlation between stimulated thyroglobulin (sTg) and TgAb, and their product could be used to predict the efficacy of RAIT and prognosis of DTC patients treated with RAIT. However, the research was mainly focused on TgAb-negative patients, and the sample size of TgAb-positive patients in their study was relatively small (88 patients). There is little other literature on the combination of sTg with pre-ablation TgAb (pa-TgAb) for predicting the effect of RAIT in TgAb-positive DTC patients. Thus, the purpose of our study was to explore the value of the product of sTg and pa-TgAb for predicting the curative effect of RAIT in TgAb-positive DTC patients.

Materials and methods

Study population

In this single-center retrospective study, we enrolled DTC patients with positive serum TgAb titers before the initial thyroidectomy and initial RAIT at the Department of Nuclear Medicine in the Affiliated Hospital of Qingdao University from January 2014 to September 2020.

The inclusion criteria were as follows (1) patients who received TT with neck dissection and were diagnosed with DTC by pathology; (2) patients with available data on sTg, preoperative TgAb (preop-TgAb), and pa-TgAb concentrations; (3) positive TgAb status with preop-TgAb ≥ 115 IU/ml and pa-TgAb ≥ 40 IU/ml (18); (4) sTg and pa-TgAb with substantial values; and (5) at least 24 months of follow-up after the initial RAIT.

The exclusion criteria included the following: (1) patients with partial thyroidectomy, (2) patients with undetectable sTg or pa-TgAb (below or above the detection limit), (3) patients with other malignant tumors, and (4) patients lost to follow-up.

Clinicopathological variables and definitions

The following patients' characteristics were analyzed: sex; age at diagnosis; maximal tumor diameter; multifocality; presence or absence of Hashimoto's thyroiditis (HT); tumor with extrathyroidal extension (ETE); surgical extent (modified radical neck dissection or not) bilateral or unilateral primary lesion; tumor stage; lymph node metastasis (LNM) stage; risk stratification of recurrence; and preop-TgAb, sTg, and pa-TgAb measurements

TABLE 1 Staging guide for differentiated thyroid cancer (AJCC eighth edition) (19).

Age at diagnosis	T category	N category	M category	Stage
<55 years	Any T	Any N	M0	I
	Any T	Any N	M1	II
≥55 years	T1	N0/NX	M0	I
	T1	N1	M0	II
	T2	N0/NX	M0	I
	T2	N1	M0	II
	T3a/T3b	Any N	M0	II
	T4a	Any N	M0	III
	T4b	Any N	M0	IVA
	Any T	Any N	M1	IVB

T, tumor; N, node; M, metastasis.

before the initial RAIT. TgAb and Tg were measured by using an electrochemiluminescence immunoassay (Roche, Switzerland).

Definitions: (1) Preop-TgAb was checked within 1 week prior to surgery. (2) sTg and pa-TgAb titers were measured within 2 days prior to the initial RAIT. (3) HT was confirmed by histopathological examination after surgery. (4) Bilaterality of the tumor was defined as a tumor that was located in both thyroid lobes. (5) The TNM stage and risk stratification of recurrence followed the American Joint Committee on Cancer (AJCC) TNM staging eighth edition (19) (Table 1) and 2015 ATA guidelines (11). (6) Disease-free survival (DFS) time was defined as there being no evidence of persistent disease since the initial RAIT. The end event was DTC recurrence or metastasis (18, 20, 21). (7) For each patient, the follow-up period was defined as the length of time from the initial RAIT and the last known patient contact, which was recorded by viewing the medical history or telephoning the patient. (8) Time trends in TgAb levels were calculated based on the following definitions (22): increase: elevation of TgAb levels greater than 50%; stability: TgAb levels fluctuate, rise, or decrease by less than 50%; and decrease: TgAb levels decrease by more than 50%.

RAIT procedures and follow-up

The patients achieved the goal of TSH > 30 mU/L after thyroid hormone withdrawal and followed a low-iodine diet for 3–4 weeks. The RAIT dose was referred to as each patient's specific extent of disease based on the 2015 ATA guidelines' recommendations (11). Posttherapeutic whole-body scanning (Rx-WBS) and single-photon emission computed tomography/computed tomography (SPECT/CT) were performed 3–5 days after RAIT. After RAIT, levothyroxine treatment was maintained. The following follow-up data were recorded every 6–12 months: serum Tg, TgAb, diagnostic WBS (Dx-WBS), and neck ultrasound. In addition, the results of chest CT, ^{18}F-FDG positron emission tomography/computed tomography (PET/CT), and fine needle aspiration (FNA) biopsy were also recorded if necessary.

Responses to therapy assessments

The response to RAIT was divided into the following four categories according to serological measurements and imaging examinations (11, 22):

(1) Excellent response (ER): suppressed Tg < 0.2 ng/ml or sTg < 1 ng/ml with negative TgAb and the imaging results were negative

(2) Indeterminate response (IDR): suppressed Tg between 0.2 ng/ml and 1 ng/ml or sTg between 1 ng/ml and 10 ng/ml with negative TgAb or decreased or stable TgAb levels and the imaging results were negative

(3) Biochemical incomplete response (BIR): suppressed Tg > 1 ng/ml or sTg > 10 ng/ml with negative TgAb or increase in TgAb levels and the imaging results were negative

(4) Structural incomplete response (SIR): structural or functional evidence of disease with any Tg and TgAb levels

Based on the last follow-up result, the recruited patients were divided into two groups: the ER group, including patients with ER, and the NER group, including patients with IDR, BIR, and SIR.

Statistical analysis

Statistical analysis was performed by using SPSS 26.0 (Chicago, Illinois, USA). Categorical variables were expressed as counts with percentages, and group differences were assessed by the chi-squared test. Continuous variables were presented as medians with interquartile ranges or mean with standard deviation. The Mann–Whitney U-test was used to examine the non-normally distributed continuous variables. Spearman's correlation was used for the correlation analysis between sTg and pa-TgAb prior to the initial RAIT. Receiver operating characteristic (ROC) curve analysis was

utilized to assess the predictive value. Logistic regression was performed to obtain the independent prognostic factors for ER. Analysis of DFS was performed by using the Kaplan–Meier method, and the groups were compared by log-rank tests. The difference between the two groups was defined as statistically significant when p-values <0.05.

Results

Baseline clinical characteristics for enrolled patients

Figure 1 shows the flowchart of this study. A total of 265 TgAb-positive DTC patients were enrolled based on the inclusion and exclusion criteria. The median follow-up time was 48 months. There were 262 cases of papillary thyroid cancer (PTC) and 3 cases of follicular thyroid cancer (FTC). The PTC cases included 242 classic PTC cases, 3 tall cell variants, 3 follicular variants, 8 hobnail variants, 6 oncocytic variants, and 3 diffuse sclerosing variants. All of the cases received TT, and 112 cases (42.26%) received modified radical neck dissection (MRND). There were 187 cases with a metastatic lymph node (LN) number greater than 5 and 29 cases with an extra-nodal extension in this part of cases. For cases with a metastatic LN number less than 5 (78/265), there were totally 6 cases with an extra-nodal extension, 42 cases with a high T stage (T3–T4), and 30 cases with the maximal diameter of the metastatic LN greater than 3 cm.

Comparison between the clinicopathological features of the two groups

The ER group included 197 patients, and the NER group included 68 patients. In the NER group, there were 26 patients with IDR, 22 patients with BIR, and 20 patients with SIR (Figure 2). There were a total of 4 cases with distant metastasis, and for all of them, the distant metastasis organ was the lung. Correlation analysis showed a negative correlation between sTg and pa-TgAb (r = −0.157, p = 0.011). Maximal tumor diameter, tumor with ETE, bilateral or unilateral primary lesion, multifocality, preop-TgAb, pa-TgAb, sTg × pa-TgAb, initial RAIT dose, N stage, and surgical extent (modified radical neck dissection or not) showed significant differences between the ER group and NER group (all p-values <0.05), while sex, age at diagnosis, primary lesion, HT, tumor stage, and risk stratification of recurrence showed no statistically significant differences (p-values >0.05 for all). A comparison of clinicopathological characteristics on the efficacy of RAIT in the 265 patients is summarized in Table 2.

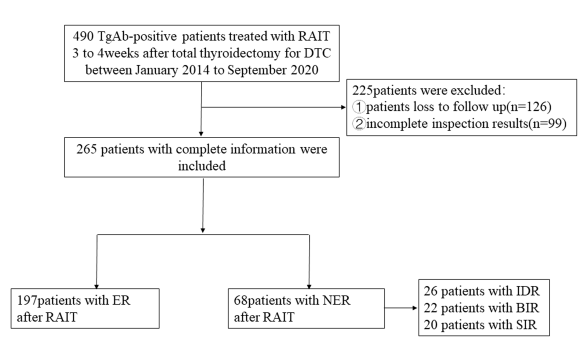


FIGURE 1
Flowchart of patient inclusions and exclusions in the study.

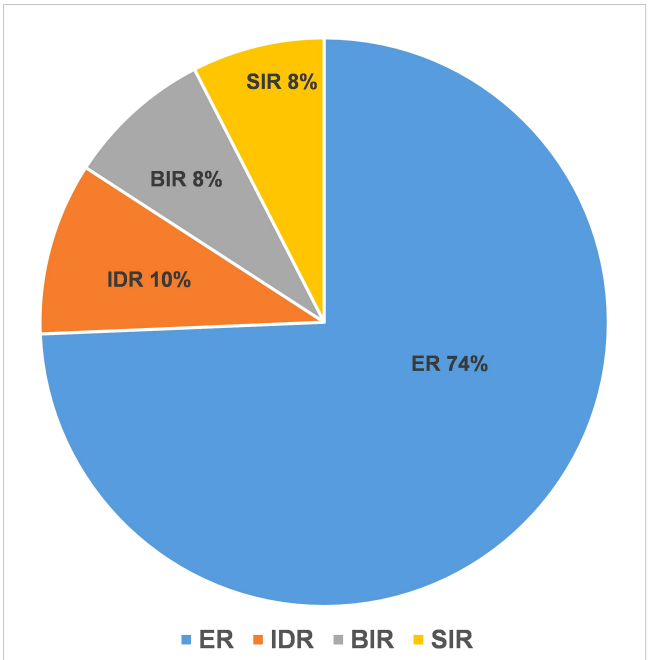


FIGURE 2
The therapeutic response to RAIT of 265 patients.

ROC curves for predicting NER

We performed an ROC curve analysis on preop-TgAb, pa-TgAb, and sTg × pa-TgAb. Figure 3 shows that the areas under the curve (AUCs) of preop-TgAb, pa-TgAb, and sTg × pa-TgAb were 0.627, 0.650, and 0.687, respectively. Table 3 shows that the corresponding cutoff values were 724.25 IU/ml, 424.00 IU/ml, and 59.73, respectively.

Univariate and multivariate logistic regression analysis

The univariate logistic regression analysis showed that maximal tumor diameter, bilateral or unilateral primary lesion, tumor with ETE, multifocality, surgical extent (modified radical neck dissection or not), preop-TgAb (≥724.25 IU/ml), pa-TgAb (≥424.00 IU/ml), sTg × pa-TgAb (≥59.73), N stage, and initial RAIT dose were significantly different between the ER group and NER group. On the

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TABLE 2 Comparison of clinicopathological characteristics on the efficacy of RAIT.

Characteristics	ER group (n = 197)	NER group (n = 68)	χ^2/Z-test	*p*-value
Gender			0.916	0.339
Men	23(11.68%)	11(16.18%)		
Women	174(88.32%)	57(83.82%)		
Age at diagnosis			1.573	0.210
<55 years	174	56		
≥55 years	23	12		
Maximal tumor diameter	1.70(1.00,2.20)	1.50(1.00,2.16)	−2.568	0.010*
Primary lesion				
Unilateral	119(60.41%)	31(45.59%)	4.519	0.034*
Bilateral	78(39.59%)	37(54.41%)		
Multifocality			3.898	0.048*
Yes	61	30		
No	133	37		
Unclear	3	1		
HT				
Yes	122(61.93%)	37(54.41%)	1.190	0.275
No	75(38.07%)	31(45.59%)		
ETE				
Yes	124(62.94%)	54(79.41%)	6.216	0.013*
No	73(37.06%)	14(20.59%)		
MRND			4.273	0.039*
Yes	76	36		
No	121	32		
Preop-TgAb (IU/ml)	402.10(251.40,762.85)	588.65(358.20,2,127.00)	−2.852	0.004*
pa-TgAb (IU/ml)	303.50(157.70,489.45)	454.00(258.38,823.13)	−3.931	0.000*
sTg × pa-TgAb	55.66(16.03,339.92)	265.82(46.61,1,441.60)	−4.270	0.000*
T stage			7.092	0.131
Tx	3(1.52%)	1(1.47%)		
T1	109(55.33%)	30(44.11%)		
T2	17(8.63%)	13(19.12%)		
T3	39(19.80%)	11(16.18%)		
T4	29(14.72%)	13(19.12%)		
N stage			19.105	0.000*
N0	31(15.74%)	1(1.47%)		
N1a	60(30.46%)	11(16.18%)		
N1b	106(53.80%)	56(82.35%)		
Risk stratification of recurrence			0.013	0.908
Intermediate risk	129(65.48%)	44(64.71%)		

(Continued)

TABLE 2 Continued

Characteristics	ER group (n = 197)	NER group (n = 68)	χ^2/Z-test	*p*-value
High risk	68(34.52%)	24(35.29%)		
Initial RAIT dose (mCi)	100(100,120)	120(100,150)	−5.136	0.000*

HT, Hashimoto's thyroiditis; ETE, extrathyroidal extension; MRND, modified radical neck dissection; preop-TgAb, preoperative TgAb; pa-TgAb, pre-ablation TgAb; RAI, radioiodine therapy; *p-value <0.05.

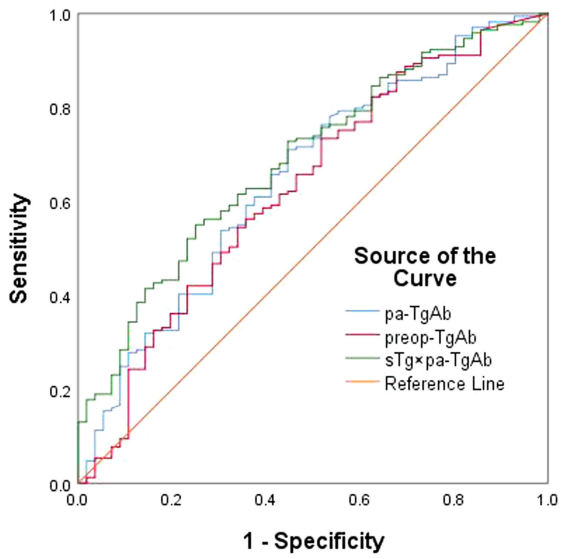


FIGURE 3
ROC curves for preop-TgAb, pa-TgAb, and sTg × pa-TgAb before initial RAIT for predicting ER.

other hand, sex, age at diagnosis, HT status, and risk stratification of recurrence were not significantly related to ER. Then, we further analyzed the significantly different factors with multivariate logistic regression analysis. We found that pa-TgAb (p = 0.034), sTg × pa-TgAb (p = 0.025), N stage (p = 0.006), and initial RAIT dose (p = 0.000) were independent risk factors for predicting NER. Table 4 shows the details of the univariate and multivariate logistic regression analysis results.

Kaplan–Meier analysis of DFS

The median DFS values of the patients with pa-TgAb ≥ 424.00 IU/ml and the patients with pa-TgAb < 424.00 IU/ml were not significantly different (48.72 months vs. 49.68 months, p = 0.427). The median DFS in the sTg × pa-TgAb < 59.73 patients and initial RAIT dose ≤100 mCi was significantly longer than that of the patients with sTg × pa-TgAb ≥ 59.73 (50.27 months vs. 48.59 months, p = 0.041) and initial RAIT dose >100 mCi (50.50 months vs. 38.00 months, p = 0.030) (Figure 4).

Discussion

In recent years, the incidence of DTC has increased globally (23, 24). As a specific tumor marker for long-term follow-up in DTC, Tg can predict DTC persistence, distant metastasis, or recurrence. However, the Tg level can be affected by TgAb, which makes Tg measurement unreliable and limits the role of Tg in prognosis in DTC patient follow-up. Therefore, TgAb has gained increasing attention as an essential indicator for DTC surveillance. Prior studies of the clinical importance of TgAb in DTC have typically focused on the sequential changes in TgAb titers (12–16). Many studies have reported that the stable or rising trend of TgAb titers after TT is an effective risk factor for predicting persistent or recurrent disease (25–27). Pan et al. indicated that TgAb affected sTg measurements even if they were negative. They reported a novel negative correlation between TgAb and Tg levels, suggesting that the sTg × TgAb product could be used to predict prognosis in patients with DTC (17). However, the majority of the patients in that study were TgAb-negative patients (321/409). Therefore, the present study explored the prognostic value of Tg × pa-TgAb in TgAb-positive DTC patients since these patients are not rare and account for almost 25% of DTC patients (6).

We enrolled 265 TgAb-positive DTC patients in the present study. The results revealed that preop-TgAb, pa-TgAb, and sTg × pa-TgAb were significantly correlated with an excellent response to RAIT. Some previous reports indicated that high preoperative TgAb was an independent predictor of nodal metastases in DTC patients (28, 29). Some studies (18, 30) indicated that pa-TgAb showed

TABLE 3 Cutoff values for predicting ER with sensitivity and specificity.

Characteristics	Cutoff value	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	AUC (95% CI)	*p*-value
Preop-TgAb (IU/ml)	724.25	73.4	48.2	38.03	81.29	0.628(0.541–0.715)	0.004
pa-TgAb (IU/ml)	424.00	71.6	54.4	39.78	81.98	0.650(0.566–0.734	0.001
sTg × pa-TgAb	59.73	51.8	73.5	34.48	85.00	0.690(0.613–0.767)	0.000

AUC, area under the curve; PPV, positive predictive value; NPV, negative predictive value; CI, confidence interval.

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TABLE 4 Univariate and multivariate logistic analysis of therapeutic effect.

Characteristics	Univariate logistic analysis		Multivariate logistic analysis	
	OR (95% CI)	*p*-value	OR (95% CI)	p-value
Gender	0.685(0.315–1.492)	0.341		
Age at diagnosis (<55 years vs. ≥55 years)	1.621(0.758–3.467)	0.213		
Maximal tumor diameter	1.426(1.029–1.976)	0.033	1.005(0.632–1.598)	0.982
Primary lesion	1.821(1.044–3.176)	0.035	1.563(0.697–3.506)	0.279
Multifocality	0.566(0.320–0.999)	0.050	0.775(0.346–1.737)	0.536
HT	0.734(0.420–1.281)	0.276		
ETE	0.481(0.253–0.914)	0.025	0.642(0.276–1.494)	0.304
MRND (yes vs. no)	0.558(0.320–0.973)	0.040	0.892(0.385–2.064)	0.789
Preop-TgAb (IU/ml) (<724.25 vs. ≥724.25)	2.388(1.277–4.467)	0.006	0.798(0.286–2.223)	0.665
pa-TgAb (IU/ml) (<424.00 vs. ≥424.00)	3.189(1.803–5.640)	0.000	2.856(1.080–7.551)	0.034*
sTg × pa-TgAb (<59.73 vs. ≥59.73)	2.922(1.593–5.362)	0.001	2.455(1.118–5.391)	0.025*
N stage	3.281(1.843–5.842)	0.000	3.526(1.433–8.676)	0.006*
Risk stratification of recurrence	1.035(0.581–1.844)	0.908		
Initial RAIT dose (mCi) (≤100 vs. >100)	4.689(2.603–8.445)	0.000	3.931(1.894–8.158)	0.000*

OR, odds ratio; CI, confidence interval. * means p-value <0.05.

significant differences between different efficacy groups. Pan et al. (17) indicated that sTg × pa-TgAb could be used to predict prognosis in patients with DTC. These results were similar to those of our present study. Our data also suggested that maximal tumor diameter, primary lesions, tumor with ETE, and N stage were significantly different between the ER group and NER group, which is consistent with previous reports (14, 31).

To confirm the predictive value of the three factors, we generated ROC curves for further analysis. The results demonstrated that the cutoff values of preop-TgAb, pa-TgAb, and sTg × pa-TgAb were 724.25 IU/ml, 424.00 IU/ml, and 59.73, respectively. According to the cutoff values, we divided the preop-TgAb, pa-TgAb, and sTg × pa-TgAb products into two groups. The multivariate logistic regression analyses indicated that pa-TgAb, sTg × pa-TgAb, and N stage were independent risk factors for NER. The risk of NER increases by 2.856-fold for each 1 kU/L increase in the level of pa-TgAb and by 2.455-fold for sTg × pa-TgAb. As mentioned before, Pan et al. (17) reported that the sTg × TgAb product cutoff value in the TgAb-positive group was 466.36, which was much higher than that in our study. One reason for the difference may be that the definition of TgAb positivity was different, which in our study was 40 IU/ml before the initial RAIT, while in Pan et al.'s study, it was 115 IU/ml. This difference led to the patients' demographic natures being different. The other reason may be the differences in sample size in the studies, in which the sample size of TgAb-positive patients in Pan et al.'s study was smaller than that in our study (88 vs. 265).

It has been reported that the RAIT dose was a vital factor affecting the therapeutic effect of RAIT ablation. Previous research studies indicated that a higher RAIT dose was an independent risk factor for unsuccessful ablation (17). The results of the present study were similar to those of previous studies. We found that unsuccessful ablation increased by 3.931-fold for every increase of 1 mCi in the RAIT dose.

In addition, we found that the median DFS of patients with sTg × pa-TgAb < 59.73 was longer than that of patients with sTg × pa-TgAb ≥ 59.73 (50.27 months vs. 48.59 months, $p = 0.041$), which confirmed the prognostic value for TgAb-positive patients. However, the results of the Kaplan–Meier analysis showed similar median DFS values for patients with pa-TgAb < 424 IU/ml and TgAb ≥ 424 IU/ml.

Nonetheless, there were still some limitations in our study. On the one hand, this study was a retrospective single-center study, and the sample size was relatively small; thus, selection bias cannot be avoided. Therefore, further prospective multicenter large-scale studies are required for validation. On the other hand, longer periods of follow-up are required to confirm the current findings.

In summary, we found that the sTg and pa-TgAb product can predict the efficacy as well as prognosis of RAIT for TgAb-positive

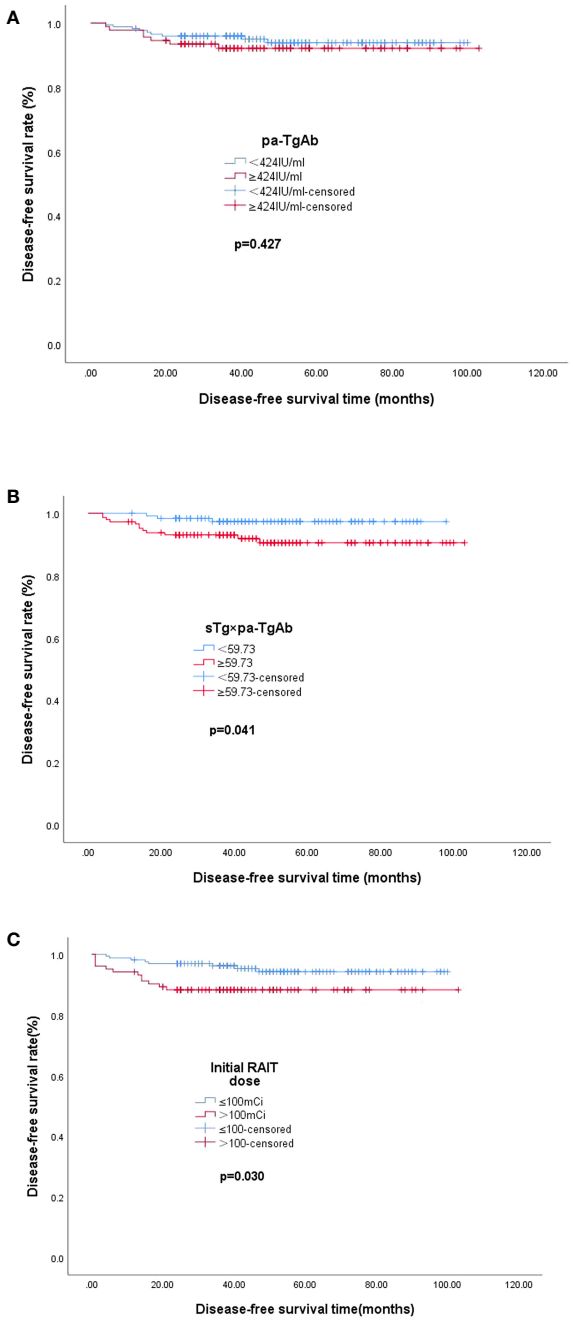


FIGURE 4
Median DFS. **(A)** Median DFS in the group of patients with pa-TgAb < 424 IU/ml (n = 173) or ≥424 IU/ml (n = 92). The median DFS was 49.68 months with pa-TgAb < 424 IU/ml and 48.72 months with pa-TgAb ≥ 424 IU/ml (p = 0.427). **(B)** Median DFS in the group of patients with sTg × pa-TgAb < 59.73 (n = 120) or ≥59.73 (n = 145). The median DFS was 50.27 months with sTg × pa-TgAb < 59.73 and 48.59 months with sTg × pa-TgAb ≥ 59.73 (p = 0.041). **(C)** Median DFS in the group of patients with initial RAIT dose ≤100 mCi (n = 162) or >100 mCi (n = 103). The median DFS was 50.50 months with initial RAIT dose ≤100 mCi and 38.00 months with initial RAIT dose >100 mCi (p = 0.030).

DTC patients. Since sTg interferes with TgAb before RAIT in these patients, the product of sTg and pa-TgAb can be an effective clinical reference indicator to recognize NER patients early, which has a very positive and important role in optimizing treatment, improving prognosis, and reducing the burden of patients.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving humans were approved by the Ethics Committee of the Affiliated Hospital of Qingdao University. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

NH wrote the main manuscript text. CL, JL, and CW helped draft the manuscript. XW helped revise the manuscript. ZZ, YZ, XL, and ZS coordinated the research and analyzed the data. GW, ZW, and FL performed the methodology and formal analysis. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Possible molecular exploration of herbal pair Haizao-Kunbu in the treatment of Graves' disease by network pharmacology, molecular docking, and molecular dynamic analysis

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Objective: To promote the development and therapeutic application of new medications, it is crucial to conduct a thorough investigation into the mechanism by which the traditional Chinese herb pair of Haizao-Kunbu (HK) treats Graves' disease (GD).

Materials and methods: Chemical ingredients of HK, putative target genes, and GD-associated genes were retrieved from online public databases. Using Cytoscape 3.9.1, a compound-gene target network was established to explore the association between prosperous ingredients and targets. STRING, Gene Ontology, and Kyoto Encyclopedia of Genes and Genomes pathway analyses visualized core targets and disease pathways. Additionally, we conducted a refined analysis of the binding interactions between active ingredients and their respective targets. To visualize these findings, we employed precise molecular docking techniques. Furthermore, we carried out molecular dynamics simulations to gain insights into the formation of more tightly bound complexes.

Results: We found that there were nine key active ingredients in HK, which mainly acted on 21 targets. These targets primarily regulated several biological processes such as cell population proliferation, protein phosphorylation, and regulation of kinase activity, and acted on PI3K-AKT and MAPK pathways to treat GD. Analysis of the molecular interaction simulation under computer technology revealed that the key targets exhibited strong binding activity to active

ingredients, and Fucosterol-AKT1 and Isofucosterol-AKT1 complexes were highly stable in humans.

Conclusion: This study demonstrates that HK exerts therapeutic effects on GD in a multi-component, multi-target, and multi-pathway manner by regulating cell proliferation, differentiation, inflammation, and immunomodulatory-related targets. This study provides a theoretical foundation for further investigation into GD.

KEYWORDS

Haizao-Kunbu, Graves' disease, network pharmacology, molecular docking, molecular dynamic analysis statements and declarations

Introduction

Graves' Disease (GD) is an autoimmune illness characterized by an enlarged and hyperactive thyroid gland (Graves' hyperthyroidism), ocular abnormalities (Graves' orbitopathy), and regional dermopathy (pretibial myxoedema). After two decades of Universal Salt Iodization (USI), GD prevalence in China has gradually decreased, with the latest epidemiological survey results at 0.53% (1). However, this implies that about seven million people still have GD. The development of GD in patients is initiated with a genetic predisposition, further influenced by some environmental factors. The complex interplay manifests through thyroid follicular cell proliferation and hypertrophy and abnormal immune cell proliferation and differentiation, which is essentially the imbalance of cell value-added apoptosis driving GD hyperthyroidism occurrence. Meanwhile, recent studies have shown that elevated levels of Th1 chemokines, such as CXCL10, are found in the serum of patients with relapsed and newly diagnosed hyperthyroidism. Th1 immune response predominates in the immunopathogenesis of GD, causing the emergence and persistence of autoimmune inflammation in the thyroid gland. The onset and treatment of GD are also closely related to cytokines such as TNF-α and IL-6 (2, 3).

Controlling hyperthyroidism by restoring standard thyroid hormone concentrations is the principal objective of GD treatment. Some therapeutic options for Graves' hyperthyroidism patients include Antithyroid Drugs (ATDs), radioiodine, and surgery. However, these treatment options have some drawbacks. Antithyroid medicines produce granulocyte count and liver enzyme abnormalities, and recurrence occurs in > 50% of cases after terminating ATDs (4). On the other hand, radioiodine or surgery may trigger hypothyroidism, necessitating lifetime levothyroxine implementation as well as clinical and laboratory supervision (5). Furthermore, treating special populations of GD patients, such as pregnant and lactating women or patients with malignant tumors, is a difficulty that cannot be overlooked.

There are records of Yingbing (goiter, 瘿病) in Traditional Chinese Medicine (TCM). The records are derived from a Chinese medicine text, *Zhu Bing Yuan Hou Lun*, which focusses on diagnosis and management of a class of diseases manifested as goiter, and GD is one of them. According to *Waike Zhengzong*, Yingbing is "not swelling of yin and yang, but stasis of blood, turbidity, and phlegm in the five organs." Consequently, doctors mostly use Chinese medications that reduce hardness and disperse stagnation for goiter treatment. From inception to the present time, the herb pair Haizao-Kunbu (HK), first appearing in *Zhou Hou Bei Ji Fang*, has been a common and effective combination therapy for goiter. According to *Shennong's Classic of the Materia Medica*, Haizao (Sargassum) can "master goiter, tumor qi, and the nucleus of the neck." Additionally, *Mingyi Bielu* reported that Kunbu (Laminaria japonica) could "treat twelve kinds of edema and goiter tumor coalescing gas." Some TCM formulas containing HK for goiter treatment include the Sihai Shuyu pill from *Yangyi Daquan*, the Haizao Yuhu decoction from *Waike Zhengzong*, and the Huaying Micro pill from *Rumen Shiqin*. Previous pharmacological research has demonstrated that besides reducing autoimmune antibody levels in rats with thyroid disease (6–8), Haizao has properties that can suppress cell proliferation and apoptosis induction (9). Kunbu, on the other hand, has enormous clinical benefits, including anti-cell proliferation, angiogenesis inhibition, apoptosis blocking, anti-inflammatory, and antioxidant properties (10, 11). Furthermore, Haizao and Kunbu are considered natural medicines with minimal side effects, given their role in protecting liver and kidney function (12, 13). However, the precise mechanism of HK in GD treatment remains unclear. For the first time, this study will elucidate the molecular mechanisms underlying this well-known combination treatment for GD.

In TCM, there is a focus on adjusting the integrity of the human body based on the balance-regulation theory. However, due to the complex character of TCM, research on its pharmacological mechanism is challenging. Network pharmacology highlights the multi-directional signaling pathway modulation, leading to enhanced therapeutic drug benefits and decreased toxic and adverse effects, increasing the potential efficacy of new pharmaceutical clinical trials, and lowering drug discovery costs. Molecular docking, on the other hand, is a simulated testing approach that models the geometry and interactions between molecules and proteins, allowing for investigations of molecular behavior at target protein binding sites (14). Finally, Molecular Dynamics (MD) modeling is a sophisticated *in silico* tool for exploring biological processes and molecular frameworks underlying linkages among macromolecules and ligands (15).

Here, we used network pharmacological analysis, molecular docking, and MD simulation technology to create a "compound-target-pathway" network. The HK chemical compounds were collected and screened for Oral Bioavailability (OB) and Drug-Likeness (DL). Subsequently, public databases were reviewed for target and GD-related genes. To predict core compositions and targets that probably participated in GD treatment, we created a network associating medicinal ingredients with target genes in HK. Additionally, Protein-Protein Interaction (PPI), Gene Ontology (GO), and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses were utilized to identify potential targets and pathways. The molecular docking and MD modeling techniques provide a foundation for further research on the HK molecular processes in GD treatment. Figure 1 depicts the study design.

Methods

Screening of active compounds

Potential compounds in Sargassum and Laminaria japonica were detected after searching the Traditional Chinese Medicine Systems Pharmacology (TCMSP) Database (http://tcmspw.com/) (16) and reassessing pertinent literature. The screening criteria included OB ≥ 30% and DL ≥ 0.18. The SwissADME online platform (www.swissadme.ch) was used to screen all ingredients (17).

Prediction of targets for the HK herb pair

The TCMSP database and Swiss Target Prediction platform (www.swisstargetprediction.ch) (18) were used to predict the gene targets, and the corresponding Sargassum and Laminaria japonica targets were collected. The PubChem Compound Identifier (CID) number of each active ingredient was obtained from TCMSP, and potential targets were updated via Swiss Target Prediction with reference to the SMILE codes and 2D images of compounds obtained from PubChem (https://pubchem.ncbi.nlm.nih.gov) (19) in SDF format. After officially annotating the potential targets on the UniProt database (https://www.uniprot.org) (20), we eventually built the "herb-compound-target" network using Cytoscape 3.9.1 software (21).

Acquisition of GD targets

To identify the target genes appertained to GD, we searched the OMIM (https://www.omim.org) (22), GeneCards (https://

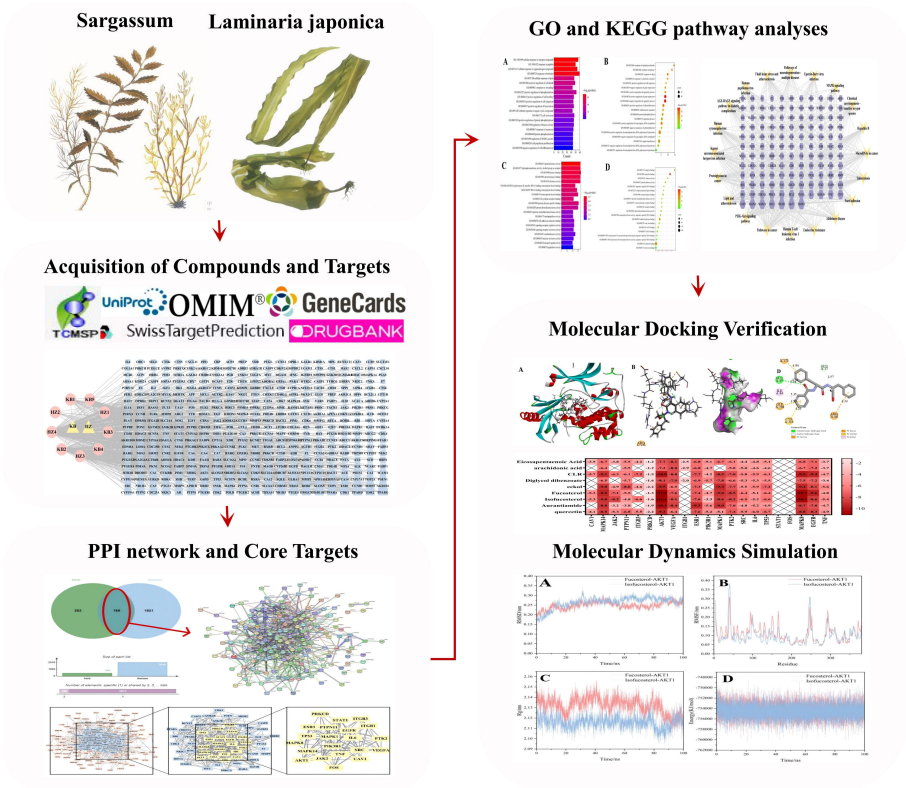


FIGURE 1
The overall workflow of the study.

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www.genecards.org) (23), Drugbank (https://go.drugbank.com) (24), and Disgenet (https://www.disgenet.org) (25) databases using "Graves' disease" as the passphrase. The online Venn mapping website (26) was eventually used to map the common HK and GD targets (26). Obvolute proteins were then regarded as immanent therapeutic targets for GD intervention.

Protein-protein interaction network building and core target identification

After importing the common targets into STRING, we built a PPI network to further explore the role of crucial targets. In our investigation, we selected interactions with the highest confidence score (≥ 0.900) for 'Homo sapiens', among which we focused on the least demanding interrelation score. The unconnected nodes were concealed and the connection network was illustrated using Cytoscape 3.9.1. Additionally, the CytoNCA (Cytoscape software add-in) (27) was installed to examine the topological characteristics of the targets. The PPI network was used to identify key targets based on Degree value (Degree), Closeness Centrality (CC), and Betweenness Centrality (BC).

Gene ontology and Kyoto encyclopedia of genes and genomes pathway enrichment analyses

To further clarify the HK gene occupation and the role of intrinsic signaling pathways in GD, Metascape (https://metascape.org) (28) and David (https://david.ncifcrf.gov) (29) were used to assess GO and KEGG pathways of 189 target genes. The Bioinformatics platform (https://www.bioinformatics.com.cn) was used to plot the bar and bubble charts with color gradients for data analysis and visualization. The FDR error control technique was used to establish whether biological processes differed significantly. After correcting the *p*-value, a significance threshold of $p < 0.05$ was used.

The molecular docking process between active ingredients and key targets

Here, we used computer-assisted technology to further confirm the intensity of the interaction between the targets and the core compound. Molecular forms of key protein targets and mol2 files of the structures of ingredients were obtained from the Protein Data Bank (PDB) (https://www.rcsb.org) (30) and TCMSP databases, respectively. The PyMOL software was used to eliminate the initial ligands and water molecules of proteins (31). The AutoDock 4.2 program (32) was used to phosphorylate and store the receptor in the PDBQT file. AutoDock Vina (33) was utilized to dock and determine a superior model. Using binding free energy, all molecules and disease targets were ranked based on their interaction strength after docking simulations. The docking was considered valid when the binding free energy was < 5.0 kcal/mol.

TABLE 1 The details of the active compounds in HK herb pair.

Serial number	The scientific name of Chinese medicine	Family	Mol ID	Molecule Name	Molecule Formula	MW	AlogP	Hdon	Hacc	OB (%)	DL
KB1	Thalluslaminariae	Laminariaceae	MOL010616	eckol	C18H12O9	372.3	3.08	6	9	87.06	0.63
KB2	Thalluslaminariae	Laminariaceae	MOL010617	Eicosapentaenoic Acid	C20H30O2	302.5	5.97	1	2	45.66	0.21
KB3	Thalluslaminariae	Laminariaceae	MOL001439	arachidonic acid	C20H32O2	304.52	6.41	1	2	45.57	0.2
KB4	Thalluslaminariae	Laminariaceae	MOL000953	CLR	C27H46O	386.73	7.38	1	1	37.87	0.68
KB5	Thalluslaminariae	Laminariaceae	MOL009622	Fucosterol	C29H48O	412.77	7.83	1	1	43.78	0.76
HZ1	Sargassum	Sargassaceae	MOL010578	Aurantiamide	C25H26N2O3	402.53	3.64	3	5	45.76	0.43
HZ2	Sargassum	Sargassaceae	MOL010580	Diglycol dibenzoate	C18H18O5	314.36	3.06	0	5	59.22	0.27
HZ3	Sargassum	Sargassaceae	MOL005440	Isofucosterol	C29H48O	412.77	7.83	1	1	43.78	0.76
HZ4	Sargassum	Sargassaceae	MOL000098	quercetin	C15H10O7	302.25	1.5	5	7	46.43	0.28

Finally, each target's highest binding energy component was visualized using Discovery Studio 2019 Client, and GraphPad Prism 8.0.2 (GraphPad, CA, USA) was used to draw the binding energy heatmap.

Molecular dynamics simulation

Ligand-receptor docked complex MD simulation was performed using GROMACS (version 2021.2) (34). Whereas the ligand topology file was produced by the ACPYPE script using the AMBER forefield, the protein topology file was created using the AMBER99SB-ILDN force field. For MD simulation, TIP3 water molecules were applied in a triclinic box, and periodic boundary conditions were employed. The system was neutralized using NaCl counter ions. Prior to MD simulation, the complex was reduced for 1000 steps and equilibrated by running NVT and NPT for 100 ps. The MD simulation for each system was run for 100 ns under periodic boundaries at 310 K and 1.0 bar of pressure. Finally, the free binding energy of a simulated target-ligand complex was computed using the gmx_mmpbsa tool from GROMACS.

Results

Screening of ingredients and selection of gene targets

We selected 11 components by searching the literature and online platforms and qualified nine ingredients through the screening threshold. Table 1 details the active ingredients. We identified 441 compound targets after deleting the duplicate and unreviewed genes. The HK "Chemical composition-target" network showing the connection between the nine components and 441 target genes was built using Cytoscape 3.9.1. The general characteristic of the network analysis was estimated to be 452 nodes and 751 edges (Figure 2).

GD targets searching

The OMIM, DrugBank, GeneCards, and Disgenet databases yielded 570, 12, 2470, and 585 GD targets, respectively. After collecting all the genes and removing duplicate data, 2,010 targets remained for further research (Supplementary Table S1). Finally, the Venn diagram displayed 189 genes as the herb pair's implicit aim for GD therapies (Figure 3).

PPI analysis

We imported 189 potential targets into STRING and obtained 165 potential targets through the screening threshold. Subsequently, we created a PPI network (165 nodes and 771 edges) after importing target genes into Cytoscape 3.9.1 software (Figure 3). Increasing quantified values were associated with the improved significance of the node. The key target topological analysis was based on Degree, CC, and BC > one-fold of the median. We first sorted 21 key gene targets using the Degree > 7, CC > 0.393, and BC > 0.003 criteria and then sorted 19 key gene targets using the Degree > 12, CC > 0.433, and BC > 0.013 criteria (Supplementary Table S2).

GO terms and KEGG pathways

We completed GO and KEGG enrichment studies to further demonstrate the proposed targets' organic functions and prospective mechanisms. The analyses involved 189 HK potential target genes underlying GD. The results were obtained after analysis of the GO and KEGG enrichment results analyzed by Metascape and David databases (Figure 4). We discovered that the common GO biological processes in the two databases were protein phosphorylation (GO: 0006468) and positive cell migration regulation (GO: 0030335), implying that phosphorylation and cell migration may be significantly involved in GD treatment by HK. Additionally, we discovered that protein homodimerization activity

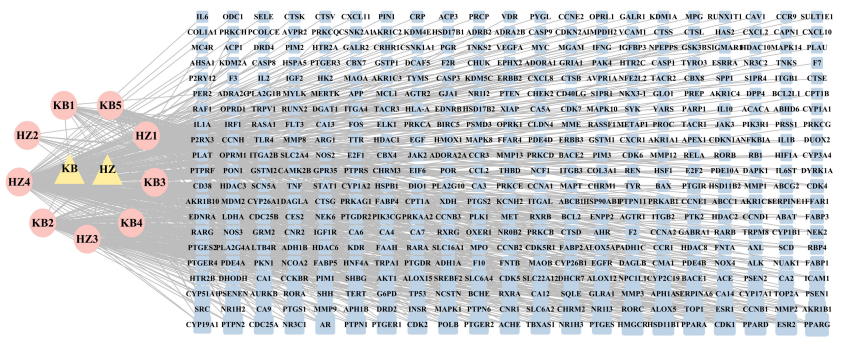


FIGURE 2
The 'Herb-Chemical composition-Target' network. The pink ellipse represents the medicinal herb of HK; the pink eclipse indicates the key components screened from HK. The blue round rectangle represents the key target points, and the edge connects the target to the active ingredient. In the network, a higher degree value is represented by a greater number of links and larger nodes, indicating that the active ingredient or target holds greater significance.

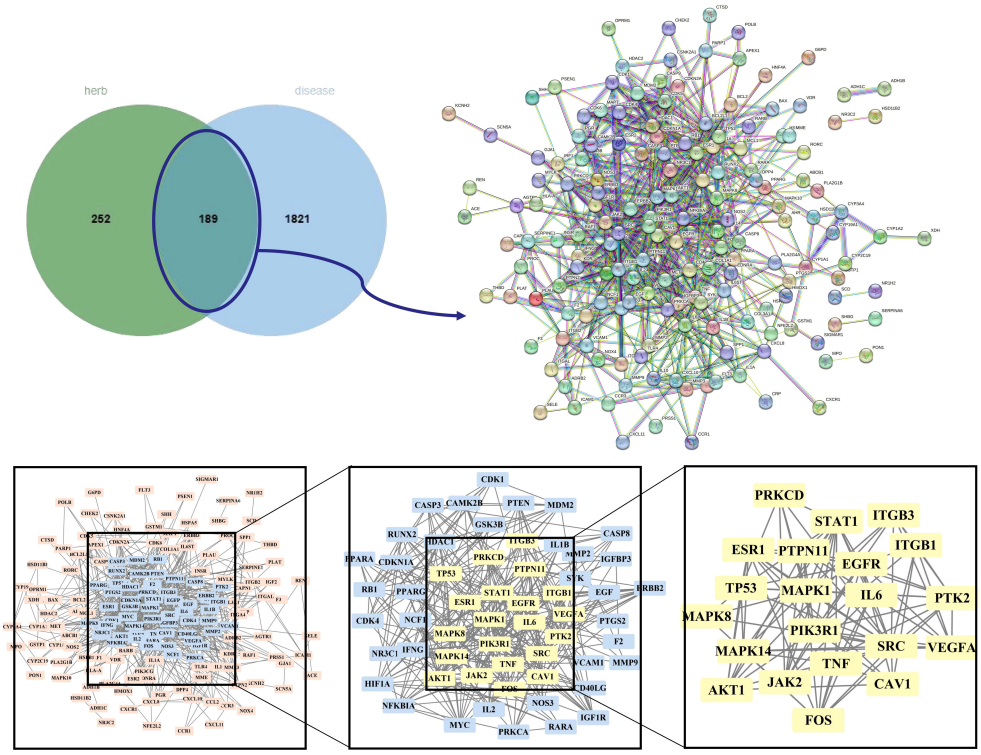


FIGURE 3
Venn plot, PPI network diagram, and screening topology of core targets of HK treatment for GD.

(GO: 0042803), protein kinase binding (GO: 0019901), protein kinase activity (GO: 0004672), kinase activity (GO: 0016301), and protein serine/threonine kinase activity (GO: 0004674) were simultaneous entries among the GO molecular functions. This finding indicates that protein kinase activity was crucial throughout the GD treatment process. Furthermore, terms such as cytoplasm perinuclear region (GO: 0048471) and membrane raft (GO: 0045121) were found to be crucial cellular components depending on the cellular composition. Cell membranes and cytoplasm are vital organelles that regulate cell signaling.

A KEGG pathway enrichment assessment was performed to further investigate the possible functions of the targets ($P<0.05$). The following 19 pathways appeared simultaneously in the top 20 critical pathways analyzed by the two databases: Cancer Pathways (hsa05200), the PI3K-AKT Signaling Pathway (hsa04151), Lipid and Atherosclerosis (hsa05417), Proteoglycans in Cancer (hsa05205), Human Cytomegalovirus (HCMV) Infection (hsa05163), Kaposi Sarcoma-associated Herpesvirus (KSHV) Infection (hsa05167), Fluid Shear Stress and Atherosclerosis (hsa05418), the AGE-RAGE Signaling Pathway in Diabetic Complications (hsa04933), Human Papillomavirus (HPV) Infection (hsa05165), Neurodegeneration-multiple Disease Pathways (hsa05022), the MAPK Signaling Pathway (hsa04010), Hepatitis B (hsa05161), Tuberculosis (hsa05152), Epstein-Barr Virus (EBV) Infection (hsa05169), Chemical Carcinogenesis-Reactive Oxygen Species (hsa05208), MicroRNAs in Cancer (hsa05206), Focal Adhesion (hsa04510), Alzheimer's Disease (hsa05010), and Endocrine Resistance (hsa01522). Based on this outcome, we finally constructed a "KEGG pathway-gene target" network (Figure 5). Detailed information is provided in Supplementary Table S3.

Molecular docking verification

We used molecular docking to identify chemicals from the PPI system based on GD-related targets. The relationships among essential active chemicals and crucial targets were examined using AutoDock4.2, Discovery Studio 2019 Client, and PyMOL software. Molecular structures of significant targets were obtained from RCSB PDB (Table 2). We did not find the PDB ID of FOS. Since the docking of CAV1, JAK2, and ITGB3 receptors to compounds was not successfully implemented in Autodock Vina, we redocked using the Genetic Algorithm in AutoDock 4.2., and the docking analysis yielded the binding scores (Figure 6). A lower value indicated a greater binding capacity. We discovered that van der Waals forces, hydrogen bonds, and aromatic stacking (Pi-Cation, Pi-Anion, Pi-Sulfur, Pi-alkyl, and alkyl interactions) were involved in the interactions between the active site residues of crucial targets and potential active substances. All active compounds exhibited an excellent binding affinity to specific core targets (only binding energies < -5 kcal/mol were demonstrated). Fucosterol-AKT1, Isofucosterol-AKT1, Isofucosterol-MAPK8, Fucosterol-MAPK8, and Aurantiamide-AKT1 were the top five binding modes (Figure 7). The Fucosterol-AKT1 complex was stabilized by 15 van der Waals forces and 5 Pi-alkyl and alkyl interactions with CYS

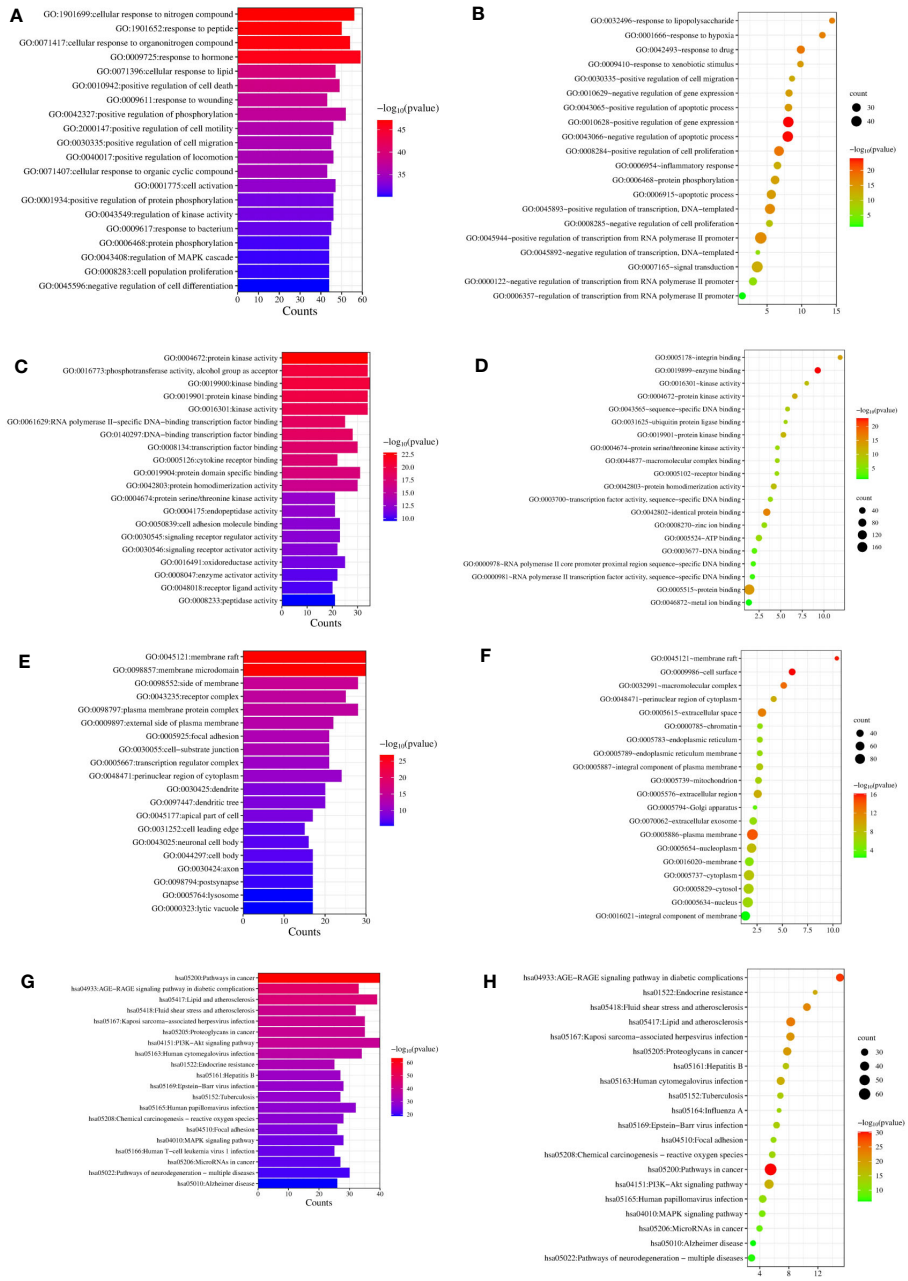


FIGURE 4
GO and KEGG analysis of HK in the treatment of GD. **(A, C, E, G)** Biological process, molecular function, cell component, and KEGG enrichment pathways from Metascape database. **(B, D, F, H)** Biological process, molecular function, cell component, and KEGG enrichment pathways from David database.

77, CYS 60, TRP 80, LEU 264, and VAL 270, respectively (Figure 7A). The Isofucosterol-AKT1 complex was stabilized by one hydrogen bond (1H-bond) with residue ALA 58, and four Pi-alkyl and alkyl interactions with LEU 210, TRP 80, LYS 268, and VAL 270 (Figure 7B). On the other hand, the Isofucosterol-MAPK8 complex was stabilized by 1H-bond with residue ASP 112 and four alkyl interactions with ILE 32, VAL 158, VAL 40, and LEU 168 (Figure 7C). Similarly, a 1H-bond with residue ASP 112 and six alkyl interactions with ILE 32, MET 108, VAL 158, VAL 40, LEU 168, and ILE 86 stabilized the Fucosterol-MAPK8 complex (Figure 7D). Finally, the Aurantiamide-AKT1 complex was stabilized by three Pi-cation and Pi-anion interactions with ARG 273, ARG 86, GLU 298, two H-bonds with residue GLU 85 and GLU 17, one Pi-alkyl bond with ILE 84, and one Pi-sulfur bond with CYS 310 (Figure 7E).

Molecular dynamics simulation of structural stability and interaction energy

We selected and implemented the top two compound-target dockings (Fucosterol-AKT1 and Isofucosterol-AKT1) for MD

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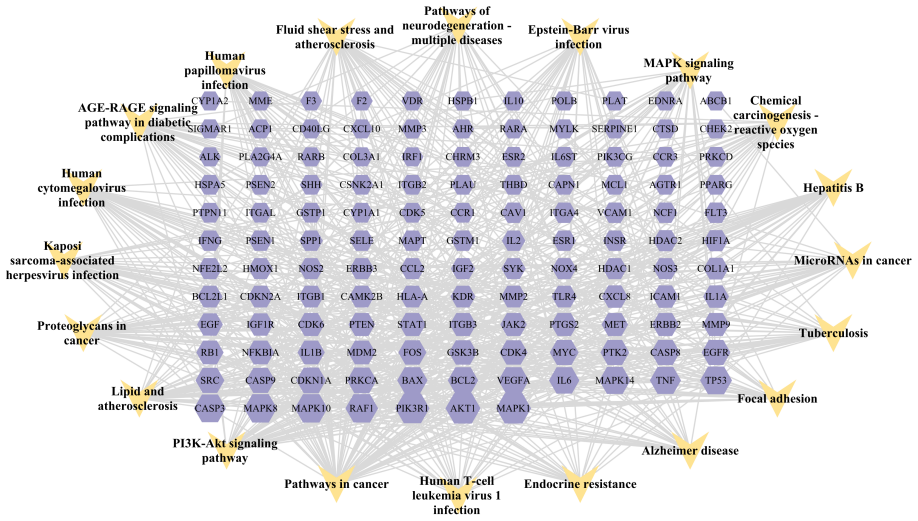


FIGURE 5
"Target gene-KEGG pathways" network: genes are indicated by the purple hexagon and pathways are presented as yellow V-shapes.

TABLE 2 Grid docking parameters in molecular docking.

Targets	PDB ID	UniProt ID	Center grid box		
			X center	Y center	Z center
CAV1	7SC0	Q03135	0	0	0
MAPK14	1OVE	Q16539	29.747	15.683	27.293
JAK2	4IVA	O60674	0.161	-11.654	-8.084
PTPN11	3B7O	Q06124	28.81	8.938	63.391
ITGB3	4G1M	P05106	-36.16	46.505	55.032
PRKCD	3UFF	Q05655	0	0	0
AKT1	4EJN	Q01314	30.889	52.185	19.493
VEGFA	4QAF	P15692	13.309	63.036	-0.942
ITGB1	7NXD	P05556	130.433	182.225	111.984
ESR1	5AAV	P03372	31.01	14.202	10.787
PIK3R1	3I5S	P27986	0	0	0
MAPK1	2OJG	P28482	-13.772	13.979	41.667
PTK2	1MP8	Q05397	36.299	-3.761	24.196
SRC	1O43	P12931	18.953	20.632	21.188
IL6	1ALU	P05231	-7.7	-12.7	0
TP53	4AGP	P04637	91.098	96.917	-46.275
STAT1	1YVL	P42224	-30.304	-13.959	146.805
MAPK8	4QTD	P45983	14.189	15.864	19.659
EGFR	5UG9	P00533	-8.371	17.712	-12.846
TNF	5UUI	P01375	41.438	43.125	1.22
FOS	–	P01100	–	–	–

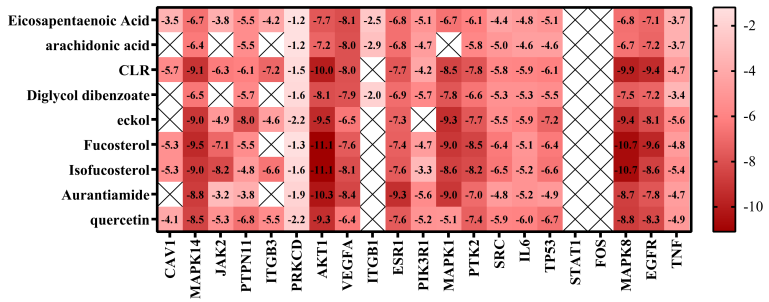


FIGURE 6
Thermographic analysis of molecular docking binding energy.

simulations. After 100 ns of MD simulations, the dynamic variations of the Fucosterol-AKT1 and Isofucosterol-AKT1 complexes were assessed. Subsequently, we computed the Root-Mean-Square Deviation (RMSD) to understand the complexes' molecular configurations and the system's stability during simulation. The RMSD curve represents the location deviations in the protein. Fucosterol-AKT1 and Isofucosterol-AKT1 had average RMSD values of 2.5 and 2.6 Å, respectively. The RMSD of the Fucosterol-AKT1 complex was less than that of the Isofucosterol-AKT1 complex between the 45-85 ns time interval, and the RMSD curves relatively stabilized after 85 ns (Figure 8A). The Root-Mean-Square Fluctuation (RMSF) graph shows the protein amino acid residue variations. According to the findings, most simulations had small alterations in amino acid structure. Furthermore, most of the residues exhibited minor structural modifications. The RMSF values of residue numbers 50-200 in AKT1 after Fucosterol binding showed greater flexibility than the same regions in AKT1 after Isofucosterol binding (Figure 8B). The gyration (Rg) radius curve represents the tightness of the protein's general configuration. The Fucosterol-AKT1 and the Isofucosterol-AKT1 complexes had stable rotation radii, although the former folded with greater force (Figure 8C). The binding free energy can be used to assess the change in the binding pattern and stability of ligands and proteins. Compared to the isofucosterol-AKT1 complex, which showed an average interaction energy and energy fluctuation of 144.536 kcal/mol and 17.43 kcal/mol, respectively, the fucosterol-AKT1 complex had an average interaction energy and energy fluctuation of 141.412 kcal/mol and13.63 kcal/mol, respectively (Figure 8D). The number of Hydrogen bonds (H-bonds) in a complex might reveal information about its binding strength. The ligands and residues of all five protein compartments created one or several hydrogen bonding connections. During the 100 ns simulations, the Fucosterol-AKT1 complex had a higher H-bond density and size (Figures 8E, F).

Discussion

The GD etiology and pathogenesis in relation to heredity, mental stimulation, environment, infection, and other factors have not been fully elucidated. Severe GD cases might result in life-threatening complications such as liver damage, heart failure, and thyroid storm. Besides being ineffective, current GD therapies have also been linked with severe limitations, such as toxic pharmacological side effects, permanent hypothyroidism, vocal cord paralysis, and other destructive damages, that all cause clinical challenges. There has been a growing demand for novel strategies that utilize traditional approaches to treat GD. In this regard, as a complement, traditional medicine has been shown to generate synergistic results with minimal toxicity. Guided by TCM theory, HK was the most common drug combination used to treat GD. However, the molecular mechanisms of HK in GD treatment have not been fully explored. Herein, we searched and selected QFP compounds and targets from multiple databases and created a "compound-gene target-disease" network. The CytoNCA software was used to perform topological analysis to estimate key protein targets involved in GD treatment and 15 essential targets including AKT1, PIK3R1, MAPK1, MAPK8, MAPK14, VEGFA, EGFR, IL6, TNF, TP53, JAK2, STAT1, ESR1, FOS, and CAV1 were identified. Subsequently, we excluded a list of irrelevant pathways in the top 20 most enriched pathways to elucidate the pathogenesis of GD and establish a prospective network model. The PI3K-AKT and MAPK pathways were identified as the most prominent penetration points in GD treatment. Furthermore, the fundamental biological processes, molecular functions, and cellular components in GO enrichment analysis helped illustrate the multi-dimensional and multi-target therapeutic pathways. Finally, the computational and validation molecular docking and MD simulation processes enabled us to further discuss the biological function of HK in the GD treatment from a micro-level perspective and to present a theoretical framework for GD therapy with TCM.

In the TCM context, HK treats GD by softening firmness and dispersing stagnation. However, Haizao and Kunbu, known as iodine-rich Chinese medicine, have been controversial in GD treatment due to their higher iodine content. Wild Laminaria japonica (0.1-1.4 g) contains approximately 150 μg of iodine, while the iodine concentration of wild Sargassum is 58-629 μg/g (35). However, in China, HK refers to specially processed TCM pieces. According to current research, the iodine content range of Haizao and Kunbu pieces are 297.67-814.59 μg/g and 855.33-5481.33 μg/g, respectively (36, 37). Consistent with an Italian study, dried pieces had higher iodine content than fresh samples (38), which can also be attributed to the different plant origins. Here, we studied processed Chinese herbal pieces. Chinese

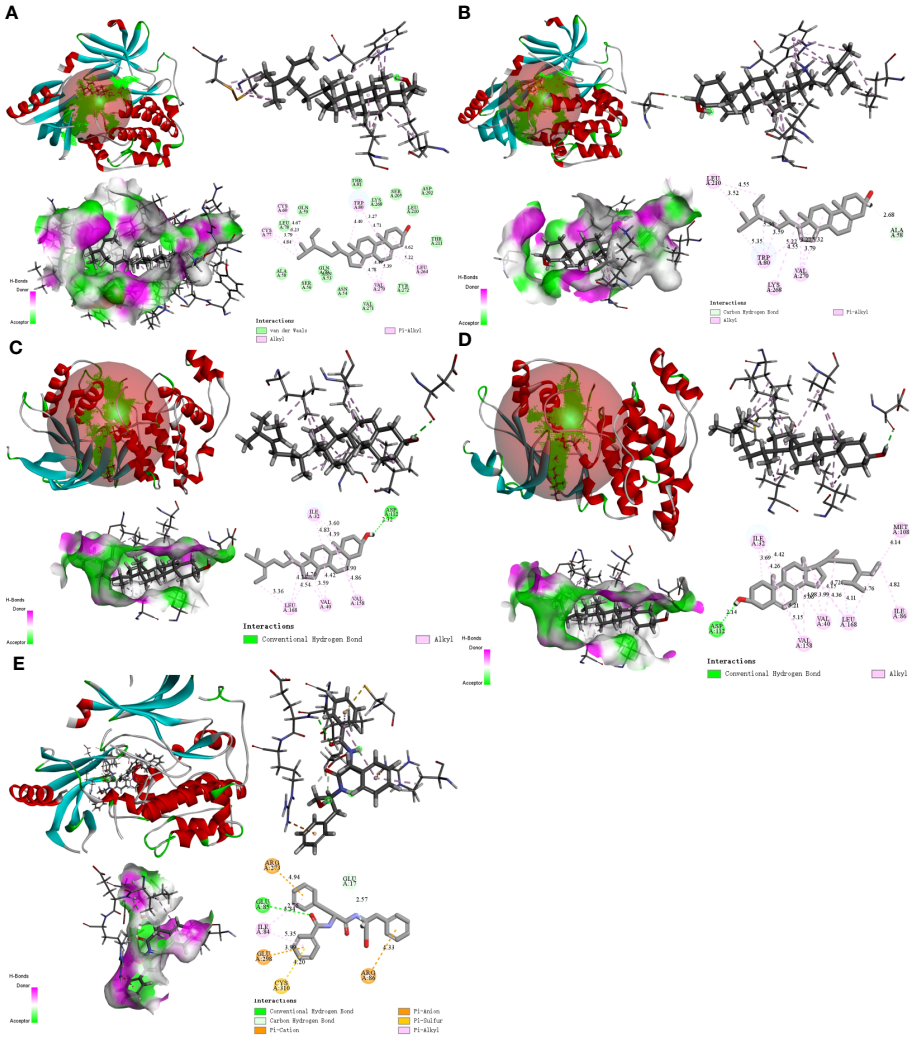


FIGURE 7
The 2D and 3D visualization plots of the top five compound–target dockings with the lowest values of docking scores. The **(A)** AKT1-Fucosterol, **(B)** AKT1-Isofucosterol, **(C)** MAPK8-Isofucosterol, **(D)** MAPK8-Fucosterol, **(E)** AKT1-Aurantiamide complexes.

herbalpieces should be decocted before use, and studies have shown that boiling in fresh water can reduce the seaweed iodine content (35). Additionally, following gastrointestinal digestion, only 49-82% of seaweed iodine appears to be available for human absorption (39). Despite its substantial iodine amounts, ingesting considerable quantities of HK does not necessarily indicate a risk of excessive iodine intake (40). There is a clinical belief that GD hyperthyroidism patients should strictly limit iodine intake. However, some new perspectives suggest that limiting iodine intake might not benefit GD patients in areas with adequate or excessive iodine intake (41). The considerable Chinese population that participated in the USI program for two decades has reached an iodine adequacy status, and the once-high hyperthyroidism prevalence has consequently been reduced to relatively stable levels (1). The latest study proves that the cumulative post-USI hyperthyroidism incidence in different iodine trophic status areas in China has not increased (42). Studies have also shown that limiting dietary iodine intake does not improve ATD effectiveness in GD treatment, nor does it increase the GD recurrence rate after ATD withdrawal, and even severe iodine intake restriction can negatively affect GD (43–45). Furthermore, some GD patients who received an acceptable excess of iodide during treatment experienced reduced hyperthyroidism and thiourea-related side effects (46). Moreover, this treatment did not affect the efficacy of radioactive iodine therapy (47). Furthermore, potassium iodide has been shown to be effective and safe in specific GD patients, such as pregnant and breastfeeding women, and patients with malignant tumors, or those undergoing radiation and chemotherapy (41, 48). An *in vivo* study from Japan recently suggested that the chronic anti-thyroid action of iodine in GD involves hormone secretion inhibition (49). In this context, researchers believe that iodine-rich TCMs such as HK can be rationally used in treating some GD patients, such as those who are intolerant to ATD or refuse surgical treatment, and patients with mild and moderate GD whose serum FT4 and Thyroid Stimulating Hormone (TSH) Receptor (TSHR) autoantibody (TRAb) levels are less than the upper limit of the detectable range in a laboratory (50–52). In our previous study, we demonstrated that an iodine-rich Chinese medicine formula could improve

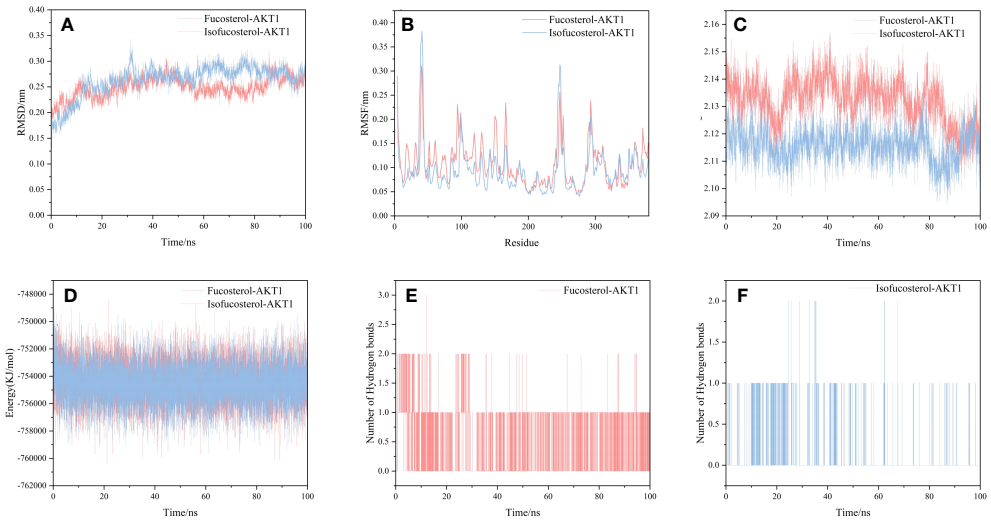


FIGURE 8
Results of molecular dynamics simulations. **(A)** The RMSD curves, **(B)** RMSF curves, **(C)** radius of rotation curves, **(D)** interaction energy curves, and number of hydrogen bonds for the **(E)** Fucosterol-AKT1 and **(F)** Isofucosterol-AKT1 complexes during the 100 ns simulations.

thyroid function and morphology in hyperthyroid rats (53). Furthermore, some previous clinical studies have proved that iodine-rich TCM for treating GD has the rapid onset, minor adverse reactions, and reduced serum TRAb advantages (54, 55). Some researchers in China have recently been conducting large-sample, multi-center, and strictly designed clinical studies on iodine-rich TCM for GD treatment (Registration numbers: ChiCTR2000032706, ChiCTR1900021572), and their findings will provide additional insights and inspiration for our subsequent research. Therefore, in future studies, we recommend a reasonable and comprehensive evaluation of the efficacy and safety of HK and further verification of its clinical effect in GD patients.

Based on our predictions, most HK active ingredients can influence biological processes, including proliferation, apoptosis, and inflammation. Aurantimide is a critical active small-molecule compound, and *in vitro* experiments have demonstrated that it could act on the PI3K/AKT signaling pathway (56) and exert anti-inflammatory effects via inhibiting the phosphorylation of the MAPK pathway (57). Eckol, a novel natural phizolian derived from marine brown algae, has been shown to downregulate EGFR, p-EGFR, JAK2, and STAT3 expression in tumor cells, indicating pro-apoptotic and anti-proliferative activities (58, 59). Eicosapentaenoic Acid (EPA), a polyunsaturated omega-3 fatty acid, has previously been reported to affect cell proliferation and inflammatory responses by inhibiting the phosphorylation of AKT (60, 61). Besides improving hormonal status in hyperthyroid rats, including T3 and TSH levels, EPA can also reduce the levels of pro-inflammatory cytokines, such as serum TNF-α (62). Fucosterol, an algae-derived unique plant sterol with various medicinal properties, has been predicted by studies to suppress the phosphorylation of Phosphatidylinositol 3-kinase/protein Kinase B (PI3K/Akt) signaling, reduce Mitogen-Activated Protein Kinase (MAPK) expression, and reduce IL-6 and TNF-α overexpression (63–65), influencing cell proliferation, apoptosis, and inflammation. On the other hand, quercetin is an excellent antioxidant that exerts sound anti-inflammatory effects and has been reported to inhibit the PI3k/Akt pathway by effectively binding to PIK3R1 (66, 67). Arachidonic acid metabolites are differentially affected by thyroid hormone status, and elevated levels of AA metabolites have been observed in the serum of patients with hyperthyroidism (68). We speculated that it is related to the Inhibitory effects of iodinated derivatives of arachidonic acid on iodine metabolism. Although the mechanism of the above active compounds in GD pathogenesis has not been fully explained, we could predict the optimal mechanism between compounds and target proteins by combining molecular docking results. Furthermore, verifying their biological feeatures in GD models is one of our future research directions.

The PPI network topology analysis revealed 21 core HK targets for treating GD. These protein targets are primarily involved in cell proliferation, apoptosis, and inflammatory processes in GD pathogenesis. The Vascular Endothelial Growth Factor (VEGF) family is required for the proliferation of blood vessels (69), and GD patients have elevated serum VEGF levels (70). According to research, iodide can decrease the expression of VEGFAs (VEGFs involved in promoting angiogenesis) upregulated by TSH (71). Besides eliminating vascular remodeling and inhibiting hair follicle hypertrophy (72), blocking VEGFA inhibits hyperthyroidism by increasing lymphatic flow in the Graves thyroid gland (73), decreasing thyroid weight during goiter development. Caveolin-1 (CAV1) is a member of the thyrosomal polyprotein complex required for thyroid hormone synthesis and thyroid cell homeostasis (74). Low CAV1 expression was observed in fat cells of GD patients (75). Pro-Epidermal Growth Factor (EGF) is an essential growth factor in thyroid tissue, and nuclear EGFR expression is elevated in GD tissue samples, implying that the EGFR-dependent modulation of thyroid cell proliferation under physiological conditions may be associated with hyperthyroidism

(76). The Tumor Protein p53 (TP53) gene is important for inducing apoptosis or cell cycle interruption, and the succession of an insufficiently effective TP53 gene substantially raises the risk of developing GD. Given that the autoimmune thyroid illness may be accompanied by DNA damage and apoptosis, the insufficiently effective TP53 gene may initiate and sustain the autoimmune GD process (77). Furthermore, some well-known cytokines (IL6 and TNF-α) are an essential part of the autoimmune response in GD patients, which can promote inflammatory cell proliferation and infiltration into thyroid tissue and affect thyroid follicular cell growth and differentiation. Furthermore, these cytokines have been proven to be related to the recalcitrant nature of the disease and the severity of clinical symptoms (78–82). Other genes have also been found to be partially responsible for the regulated proliferation and Thyroid Hormone (TH) levels in GD (83–85). These gene targets imply that we can further investigate the mechanism of HK in GD treatment in terms of cell proliferation, apoptosis, and inflammatory processes.

Additionally, KEGG enrichment analysis demonstrated that the PI3K-AKT and MAPK signaling pathways were involved in GD onset. Furthermore, some of the BP and MF items examined by GO, including protein phosphorylation, positive cell death regulation, cell activation, kinase activity regulation, MAPK cascade regulation, cell population proliferation, Transcription Factor (TF) binding, protein serine/threonine kinase activity, cytokine receptor binding, and Cell Adhesion Molecule (CAM) binding, revealed the significance of cell proliferation, differentiation, protein phosphorylation, and protein kinases in GD pathogenesis. Notably, GD patients have thicker, hypertrophied follicular cells in their thyroid glands that produce active thyroglobulin. The gland exhibits classic lymphocytic infiltrates considered to be principally connected with TSHR autoantibody secretion. Additionally, a histological examination showed occasional apoptotic cells and partial follicular wreckage (86). The presence of immune inflammation, cell proliferation, and apoptosis were all essential components in the pathological mechanism of GD. Although studies have reported that GD patients have all three types of TSHR autoantibodies, stimulating antibodies constitute the distinguishing characteristic of Grave's hyperthyroidism. When stimulated, TSHR autoantibodies can induce complex signaling cascades, mainly activating GAs and inducing cAMP/PKA pathways. The production of cAMP activates the cAMP Response Element-Binding Protein (CREB) and protein kinase A, which are directly or indirectly involved in inflammatory mediator generation, thyroid hormone synthesis, and thyroid cell proliferation (4, 87). The bioactive exertion of cAMP partially depends on the phosphorylation of the MAPK and PI3K/AKT pathways (88). Additionally, the binding of B-arrestin attracted by TSHR to the receptor can activate the MAPK pathway (89), inducing protein synthesis, cell differentiation, and angiogenesis via hemodynamic effects (4, 90). Protein Kinase B (PKB), also known as AKT, is an intracellular signaling pathway phosphorylated and activated on the plasma membrane. Once activated, AKT regulates cell survival/apoptosis, cell proliferation, and protein synthesis. Recent findings have confirmed the involvement of the PI3K-AKT pathway in GD pathogenesis (91–93). Phosphorylated AKT stimulates the activation of TFs CREB and NF-kB, as well as inflammatory gene expression (such as IL-6) (94). Previous research showed that the cAMP/PKA pathway increased IL-6 production in thyroid cells via processes influencing the stability of IL-6 mRNA, IL-6 gene promoter, and c-Fos expression (95). Increased inflammatory chemical levels further worsen thyroid follicular cell stimulation and destruction, causing the secretion of more thyroid hormones and amplifying the body's inflammatory response (96). Moreover, Janus Kinase (JAK)/Signal Transducer and Activator of Transcription (STAT) and their downstream effectors are the primary signaling cascades in TSHR activation. Our results and previous findings suggest that JAK2 and STAT1 phosphorylation may occur during GD treatment (97, 98). All the above-mentioned cascades have relevance to cellular development, survival, differentiation, cytokine and chemokine release, and apoptosis

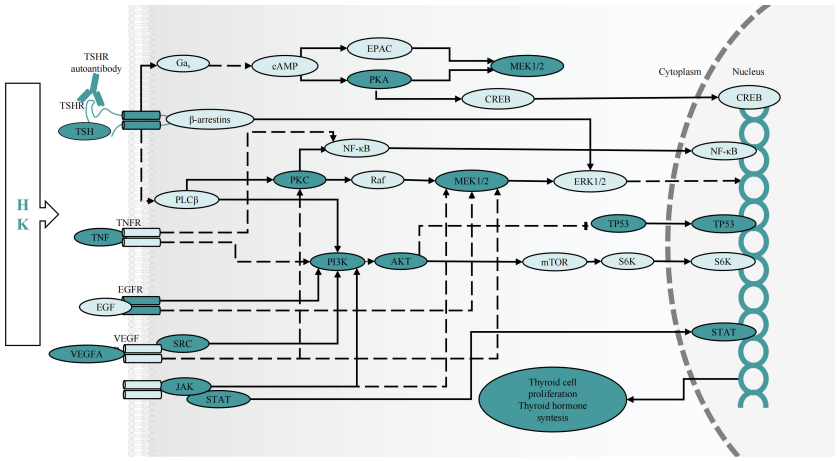


FIGURE 9
The predicted effect of HK on key targets and cascades in the treatment of GD. cAMP, cyclic adenosine monophosphate; CREB, cAMP response element-binding protein; EPAC, exchange protein activated by cAMP; MEK, mitogen-activated protein kinase kinase; mTOR, mammalian target of rapamycin; NF-κB, nuclear factor-κB; PLC, phospholipase C; Raf, RAF proto-oncogene serine/threonine protein kinase; PKA, protein kinase A; PKC, protein kinase C; S6K, ribosomal protein S6 kinase 1.

induction (99), indicating that thyroid cell activation and proliferation can be partially influenced via the regulation of protein phosphorylation within these central signaling cascades by HK (Figure 9).

MD simulation is an effective technique for examining molecular mechanisms, especially in evaluating the binding stability (100) and the selectivity of specific ligands for their target proteins (101). In this study, we conducted MD simulations to gain deeper insights into the stability of the protein-ligand complexes, specifically focusing on the Fucosterol-AKT1 and Isofucosterol-AKT1 complexes. We assessed the RMSD profiles of these complexes, which exhibited consistent patterns with RMSD values hovering around 0.27 nm. This pattern suggests that the system reached equilibrium during the simulation. We extended the MD simulation duration for these two complexes to generate a stable RMSD profile. In addition to RMSD, we evaluated several other factors, including RMSF, Rg, H-bonds, and binding free energy. These assessments provided further insights into the dynamic properties of the protein-ligand interactions. Overall, these results suggest that during MD simulation, fucosterol-AKT1 and isofucosterol-AKT1 complexes were stable and equilibrated.

Although network pharmacology, molecular docking, and molecular dynamics simulation methods were implemented to characterize the potential chemicals and targets of HK, there are some several limitations that need to be acknowledged. First, to accurately comprehend the behavior of chemical elements functioning on disease targets, network pharmacology can be utilized to predict the up-regulation and down-regulation of targets. Second, *in-vivo* and *in-vitvo* experiments should be conducted to accurately explore the therapeutic effects and associated mechanisms of the potential compound and target pairs. It is also necessary to identify the active ingredients with therapeutic effects in HK through basic experiments. These will be the focus of our future research.

Conclusion

GD is an autoimmune disease caused by genetic and environmental factors. The multifaceted nature of GD calls for a multifaceted approach to its treatment, often involving multiple targets. There is a growing interest in innovative treatment strategies that draw from the wisdom of traditional medicine and apply it to the clinical management of GD. These strategies have demonstrated favorable efficacy and safety profiles. Nine compounds including Fucosterol, Isofucosterol, Aurantiamide, Eicosapentaenoic Acid, quercetin and eckol of HK were combined to 21 core targets including AKT1, PIK3R1, MAPK1, MAPK8, MAPK14, VEGFA, EGFR, IL6, TNF, TP53, CAV1, JAK2, and STAT1, and by participating in biological processes such as cell population proliferation, protein phosphorylation, regulation of kinase activity and protein serine/threonine kinase activity and PI3K-AKT, MAPK, and other pathways play a crucial role in the treatment of GD. Molecular docking and molecular dynamics simulation demonstrated that the Fucosterol-AKT1 and Isofucosterol-AKT1 complexes exhibited the highest binding energy, indicating that HK contains key compounds and targets. While future research will necessitate further biological experiments, including additional *in vitro* and *in vivo* studies, our current findings suggest that HK holds significant promise as an effective herbal remedy for treating GD.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Materials, further inquiries can be directed to the corresponding author/s.

Author contributions

Methodology: MY, YL, XH. Software: MY, XH. Validation: MY, XH. Formal analysis: MY, XH. Visualization: MY, YL, QL, YA. Conceptualization: YL, TG. Investigation: YL, DG, QL, YW, YA. Supervision: YL, TG. Resources: DG, QL, YW, YA. Data Curation: DG, QL, YW. Writing - Original Draft: MY. Writing - Review & Editing: YL, XH, TG. Project administration: YL, TG. Funding acquisition: YL. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2023.1236549/full#supplementary-material

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Exploring blood metabolites and thyroid disorders: a bidirectional mendelian randomization study

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Background: Human blood metabolites have demonstrated close associations with thyroid disorders in observational studies. However, it's essential to determine whether these correlations imply causation. Mendelian Randomization (MR) offers a promising approach to investigate these patterns.

Aims: The primary aim of our investigation is to establish causality between blood metabolites and three thyroid disorders: TC, GD, and HT.

Methods: We employed a two-sample bidirectional MR analysis approach to assess the relationships between 452 blood metabolites and the three aforementioned thyroid disorders. Causal links were estimated using the IVW method, with sensitivity analyses conducted via MR-Egger, Weighted Median, and MR-PRESSO. We assessed potential heterogeneity and pleiotropy using MR-Egger intercept and Cochran's Q statistic. Additionally, we conducted pathway analysis to identify potential metabolic pathways.

Results: We found 46 metabolites that showed suggestive associations with thyroid disease risk, especially Aspartate (OR_{IVW}=7.41; 95%CI: 1.51-36.27; P_{IVW}=0.013) and C-glycosyltryptophan (OR_{IVW}=0.04; 95%CI: 0.00–0.29; P_{IVW}=0.001) impacted TC, Kynurenine (OR_{IVW}=2.69; 95%CI: 1.08–6.66; P_{IVW}=0.032) and 4-androsten-3beta,17beta-diol disulfate 2 (OR_{IVW}=0.78; 95% CI: 0.48–0.91; P_{IVW}=0.024) significantly impacted GD, and Alpha-ketoglutarate (OR_{IVW}=46.89; 95%CI: 4.65–473.28; P_{IVW}=0.001) and X-14189–leucylalanine (OR_{IVW}=0.31; 95%CI: 0.15–0.64 P_{IVW}=0.001) significantly impacted HT. We also detected 23 metabolites influenced by TC and GD. Multiple metabolic pathways have been found to be involved in thyroid disease.

Conclusion: Our MR findings suggest that the identified metabolites and pathways can serve as biomarkers for clinical thyroid disorder screening and prevention, while also providing new insights for future mechanistic exploration and drug target selection.

KEYWORDS

metabolites, Mendelian randomization, bidirectional, thyroid cancer, autoimmune thyroid disease

Introduction

Thyroid diseases encompass a variety of disorders involving the thyroid gland, including thyroid cancer (TC), Graves' disease (GD), and Hashimoto's thyroiditis (HT). Thyroid cancer is the most prevalent malignant thyroid tumor (1), while Graves' disease and Hashimoto's thyroiditis are common organ-specific disorders (2), with Th17 cells playing a foundational role in these conditions (3). These three diseases notably affect both incidence rates and affected individuals' quality of life. Thyroid hormones play a pivotal role in regulating numerous metabolic processes within the body, thereby influencing the overall metabolic status of individuals. The association between metabolic abnormalities and the incidence of thyroid disorders is of significance, as it directly impacts the quality of life for affected patients. Studying the relationship between metabolic issues and these thyroid diseases has garnered significant attention.

Observational research has shown a strong link between specific blood metabolites and the development and advancement of thyroid diseases. Regarding thyroid cancer, prior studies have established a correlation between blood thyroid hormone levels and thyroid cancer occurrence (4).. Additionally, some blood metabolites such as vitamin D, lipid metabolites, and carbohydrate metabolites have shown abnormal changes associated with thyroid cancer (5, 6). There is relatively less research on Graves' disease and Hashimoto's thyroiditis. Murdaca et al. discovered a certain correlation between the development of autoimmune thyroid diseases and factors such as vitamin D and the microbiome (7). In another study, there was a noticeable association between thyroid hormone levels in the blood of Graves' disease patients and lipid metabolites (8, 9).. In studies on Hashimoto's thyroiditis, the focus has been on the interaction between thyroid antibodies and immune-related metabolites in the blood (10, 11). Although prior research has explored the link between human blood metabolites and thyroid diseases, there remains a need for more extensive and systematic studies to fully determine the causal relationship between these diseases and metabolites.

Mendelian randomization (MR), an analytical approach, is vital for exploring the causal link between metabolites and thyroid diseases. Given the absence of viable randomized controlled trials or the practicality of initiating new ones, the MR method has emerged as a crucial alternative for evaluating the causal connection between metabolites and disease risk. Specifically, in the MR approach, single nucleotide polymorphisms (SNPs) are employed as instrumental variables (IVs) to represent the specific phenotype (12). The MR approach's main strength is utilizing the naturally occurring genetic variations allocated randomly during fertilization. This mimics a randomized controlled trial, effectively minimizing biases from confounding factors like gender and age in causal analysis (13). Furthermore, genotype establishment happens before the onset of disease and remains mostly uninfluenced by disease advancement, thereby enabling the effective evaluation of the causal links between metabolites and thyroid diseases (14, 15).

To identify potential candidate metabolites associated with the etiology of thyroid diseases in a more exploratory manner, we performed bidirectional two-sample MR analysis using the latest and extensive genome-wide association study (GWAS) summary data available (16). The purpose of this analysis was to explore bidirectional causal associations between human blood metabolites and both TC and AITD (including GD and HT). This research approach enhances comprehension of thyroid disease pathogenesis and metabolic pathways, while also furnishing dependable evidence for devising viable strategies in thyroid screening and prevention within clinical settings.

Abbreviations: TC, Thyroid cancer; GD, Graves' disease; HT, Hashimoto thyroiditis; AITD, Autoimmune thyroid diseases; MR, Mendelian randomization; SNPs, Single nucleotide polymorphisms; IVs, Instrumental variables; MR-PRESSO, MR pleiotropy residual sum and outlier; GWAS, Genome-wide association study; IVW, Inverse variance weighted; WM, Weighted median; LOO, Leave-one-out; KEGG, Kyoto encyclopedia of genes and genomes.

Materials and methods

MR design

We employed a bidirectional two-sample MR to evaluate the causal link between 452 human blood metabolites and TC and AITD. Figure 1 provides a schematic overview of the study design and data sources. GWAS summary statistics were obtained to extract prominent single nucleotide polymorphisms [SNPs] serving as genetic instrumental variables for 452 human blood metabolites, TC, and AITD. Initially, we designated the 452 blood metabolites as the exposure and TC, along with AITD, as the outcomes to ascertain their potential roles in either inhibiting or fostering the onset of TC and AITD. TC and AITD were then used as the exposure and blood metabolites as the outcome to investigated changes in metabolites after the occurrence of disease. The summary-level data utilized in this study can be downloaded and has been obtained with ethical approval from the respective institutions overseeing each GWAS involved.

Analyses were executed using R statistical software (version 4.2.3). MR analyses were conducted with the R-based tools "TwoSampleMR" and "MR-PRESSO," and the meta-analysis employed the "meta" package (13).

Data sources

The data for this study were sourced from two GWASs: one focusing on metabolites and the other on thyroid disease. The dataset comprising 452 metabolites for the GWAS was amalgamated from a genome-wide association scan and high-throughput metabolic analysis study conducted by Shin et al. This study enrolled 7824 participants mainly from two European population cohorts, with screening conducted for approximately 21,000 SNPs (12, 17). The participant pool comprised 1,768 individuals from the KORA F4 study in Germany and 6,056 participants from the Twin study. Both studies received approval

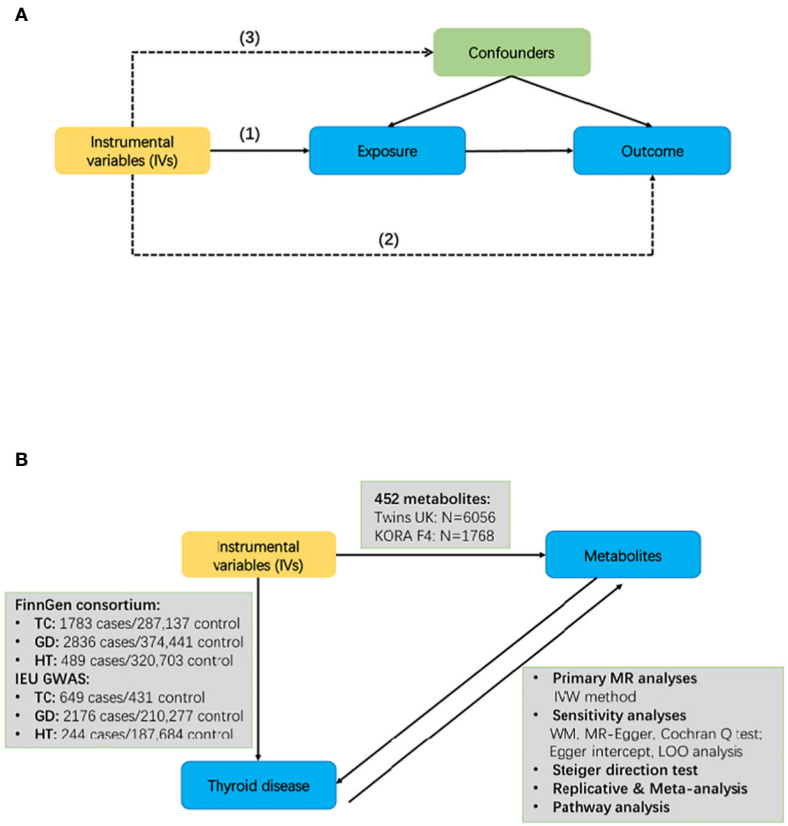


FIGURE 1
Schematic overview of the study design. **(A)** Mendelian randomization [MR] illustration. There are three principal assumptions in MR design: (1) IVs must be strongly correlated with exposure factors; (2) IVs was associated with outcomes only by exposure; (3) IVs cannot be associated with any confounding factors. **(B)** Bidirectional MR study of metabolites and thyroid disease TC, Thyroid cancer; GD, Graves' disease; HT, Hashimoto thyroiditis; IVW, Inverse variance weighted; WM, weighted median; LOO, leave-one-out.

from local ethics committees, and all participants provided informed consent. This represents the most extensive investigation to date on the genetic impact on human serum metabolism. Following rigorous quality control, a total of 452 metabolites underwent genetic analysis in both cohorts. These encompassed 275 recognized metabolites and 177 unidentified ones. As detailed in the KEGG database, the 275 established metabolites are categorized into eight major metabolic groups: amino acids, carbohydrates, cofactors and vitamins, energy, lipids, nucleotides, peptides, and allogenic metabolism (18).

The GWAS summaries for TC, GD, and HT were acquired from the FINNGEN consortium (r9.finngen.fi). The FINNGEN consortium is a Finnish national meta-analysis of GWAS that analyzed 13 cohorts and biobanks. The GWAS summary data for TC included 288,920 samples (1783 individuals with TC and 287,137 without) with a dataset of 18,707,521 SNPS. The summary data for GD included 377,277 samples (2836 GD cases and 374,441 controls), encompassing a dataset of 18,709,621 SNPs. The GWAS summary data for HT contained 321,192 samples (489 Hashimoto's thyroiditis and 320,703 controls) with a dataset of 18,708,398 SNPS. Supplementary Table S2 shows the detailed information.

Selection criteria for genetic variants

In the process of selecting instrumental variables (IVs) to signify potential exposure-outcome links, varied thresholds were established according to exposure variations. First, 452 metabolites were designated as the exposure. In this case, SNP with an association threshold of $P<1\times10^{-5}$ were extracted (19), mainly for MR Analysis, while a linkage disequilibrium parameter (r^2) of <0.001 was set in the 10,000 kb window of the European 1000 Genome reference Panel to obtain top-level independent SNP. When the chosen SNPs from the exposure dataset were not present in the outcome dataset, proxy SNPs displaying substantial association with the selected variants ($R^2>0.8$) were employed instead. Secondly, When TC and AITD were designated as exposures, IV significance adhered to the genome-wide statistical significance threshold ($p<5\times10^{-8}$) (20). Moreover, a linkage disequilibrium threshold of 0.001 and a clumping window of 10,000 kb were established. MR Steiger filters were employed to exclude SNPs with incorrect causal directions. Furthermore, R2 and F-statistic of the IVs were computed to identify potential weak IV bias (21). SNPs with F<10 were deemed weak instruments and removed to guarantee ample variance from all SNPs for the

respective exposed group (22). The design formula is shown in Table S1, adhering to the common recommendation of using a threshold of F>10 for MR analysis. Then, exposure SNPs were isolated from outcome data, while excluding SNPs associated with the outcome. Following this, alignment of allele information for exposure and outcome SNPs was performed during data harmonization. Lastly, metabolites with a minimum of 2 SNPs were retained for MR analysis.

Primary analysis

This study employed bidirectional two-sample MR to estimate causal effects between metabolites and thyroid disease using inverse variance weighting (IVW) (23). IVW is a prominent method frequently employed in MR studies, effectively aggregating Wald ratios for each SNP to yield a consolidated estimate. The random effects model of IVW is utilized in cases of heterogeneity, while the fixed effects model is applied in its absence. We further applied a multiple-testing corrected threshold of P<0.05/275 (where 275 represents the count of known metabolites) using the Bonferroni correction to elucidate statistical significance (24). P-values ranging from 1.82×10^{-4} to 0.05 were regarded as possible associations.

Sensitivity analysis and direction validation

To validate that IV influences the outcome solely through the exposure, and to improve the robustness of the findings, sensitivity analysis is also required. Therefore, Different approaches such as weighted median, MR-Egger, MR-PRESSO, and leave-one-out were employed in sensitivity analysis to validate the stability of the significant estimates found (IVW P<0.05). Among them, The weighted median method enhances causal effect detection and reduces type I errors. MR-Egger identifies IV assumption violations and offers unaffected effect estimates. Concurrently, we examined the presence of horizontal pleiotropy using MR-Egger regression and the MR-PRESSO Global test (25). Horizontal pleiotropy suggests that IVs might be linked to outcomes through non-causal pathways, possibly yielding false positives (p<0.05). The leave-one-out method assesses whether individual SNPs influence the results (26). Furthermore, the Cochran Q test was employed to identify heterogeneity, with a resulting Cochran-Q derived p-value below 0.05 indicating its presence (27). Lastly, the MR Steiger directionality test was performed to validate if our findings aligned with our hypothesis (28).

Replication analysis

We replicated the IVW analysis using GWAS data for TC, GD, and HT from the IEU Open database (29), aiming to cross-validate the reliability of our findings. (Table S2)

Metabolic pathway analysis

The chosen metabolite metabolic pathways were investigated using the web-based tool Metaconflict 5.0 (30) https://www.metaboanalyst.ca/. MetaboAnalyst 5.0 serves as a user-friendly online tool for efficient metabolomics data analysis. In this study, only metabolites exceeding the advised threshold were analyzed for metabolic pathways (P_{IVW}<0.05).

Results

Causal effects of the human blood metabolites on TC, GD, and HT

The metabolite instrumental variables ranged from 4 to 150, with a median count of 12. SNP F-statistics exceeded 10, indicating absence of weak instrumental variables. (Table S3). Based on these instrumental variables we performed the IVW MR Analysis for each pair of metabolites and TC and AITD. A total of 46 metabolites (P_{IVW}<0.05) with significant associations were identified, including 22 known metabolites and 24 unknown metabolites. Among these, 10 (Figures S1-S4), 5(Figures S5-S8) and 7(Figures S9-S12) associations of known metabolites (Table 1) and 9, 4 and 11 unknown metabolite associations were identified with increased risk of TC, GD, and HT, respectively. Genetic variants elucidating metabolite associations with three thyroid diseases were presented in Tables S5-S7. Supplementary Figures displayed scatter plots, funnel plots, and leave-one-out sensitivity analysis.

Specifically, Aspartate (OR_{IVW}=7.41; 95%CI: 1.51-36.27; P_{IVW}=0.013), Kynurenine (OR_{IVW}=2.69; 95%CI: 1.08–6.66; P_{IVW}=0.032), Alpha-ketoglutarate (OR_{IVW}=46.89; 95%CI: 4.65–473.28; P_{IVW}=0.001) were the most notably risky metabolites for thyroid cancer, Graves' disease and Hashimoto's thyroiditis. On the contrary, C-glycosyltryptophan (OR_{IVW}=0.04; 95%CI: 0.00–0.29; P_{IVW}=0.001), 4-androsten-3beta,17beta-diol disulfate 2 (OR_{IVW}=0.78; 95%CI: 0.48–0.91; P_{IVW}=0.024), X-14189–leucylalanine (OR_{IVW}=0.31; 95%CI: 0.15–0.64; P_{IVW}=0.001) were factors with highest protective value for TC, GD and HT (Table 2). Table 1 shows the characteristics of all significant pathogenic relationships between known metabolites of different types of thyroid disease. Subsequently, Bonferroni correction was employed to identify causal association characteristics ($P<1.82\times10^{-4}$). The results showed that the P-values of the selected metabolites were all between 1.82×10^{-4} and 0.05, indicating that some metabolites were potentially associated with TC and ATD, but there was no clear causal relationship. Additional research is necessary to validate their relationship.

Because IVW methods are susceptible to weak instrumental bias, sensitivity analyses were conducted to ensure the robustness of the causal assessment. The MR-Egger, weighted mode, simple mode, and weighted median approaches yield consistent causal estimates in terms of both strength and direction. Directed pleiotropy was evaluated using MR-Egger intercept and

TABLE 1 Effect of metabolites on thyroid disease.

Outcome	Exposure	N	IVW		Heterogeneity		Pleiotropy		Steiger direction	
			OR(95%CI)	P	IVW Q	P	Intercept	P	correct_causal_direction	steiger_pval
Thyroid cancer	Phenylalanine	5	258.64(2.30-29102.41)	0.021	1.260	0.868	-0.0125196	0.802	TRUE	2.923E-38
Thyroid cancer	Aspartate	4	7.41(1.51-36.27)	0.013	2.712	0.438	-0.0740803	0.364	TRUE	5.46977E-27
Thyroid cancer	C-glycosyltryptophan*	20	0.04(0.00-0.29)	0.002	11.796	0.894	0.0062277	0.854	TRUE	2.5161E-153
Thyroid cancer	Carnitine	150	0.26(0.08-0.81)	0.020	152.504	0.405	-0.013534	0.144	TRUE	0
Thyroid cancer	1-linoleoylglycerol (1-monolinolein)	13	0.54(0.30-0.98)	0.044	13.888	0.308	-0.0328429	0.363	TRUE	9.0806E-80
Thyroid cancer	Stearoylcarnitine	6	5.77(1.11-29.90)	0.037	1.651	0.895	0.0495728	0.639	TRUE	2.86814E-32
Thyroid cancer	Gamma-glutamylglutamine	19	3.54(1.15-10.90)	0.028	14.826	0.674	-0.0284283	0.115	TRUE	1.0729E-135
Thyroid cancer	Gamma-glutamylleucine	32	0.28(0.08-0.96)	0.043	31.096	0.461	-0.0184384	0.221	TRUE	3.8975E-197
Thyroid cancer	Uridine	18	0.06(0.01-0.43)	0.006	12.667	0.758	0.0165484	0.641	TRUE	3.49E-118
Thyroid cancer	Myristoleate (14:1n5)	14	0.35(0.17-0.72)	0.005	8.587	0.803	-0.0292185	0.206	TRUE	9.0905E-106
Graves' disease	Kynurenine	32	2.69(1.08-6.66)	0.033	26.860	0.679	0.0030935	0.823	TRUE	3.3375E-260
Graves' disease	Taurochenodeoxycholate	11	0.70(0.50-0.98)	0.040	6.428	0.778	-0.0282023	0.134	TRUE	3.8636E-99
Graves' disease	4-androsten-3beta,17beta-diol disulfate 2*	18	0.48(0.25-0.91)	0.024	21.012	0.226	0.0277824	0.346	TRUE	5.1913E-104
Graves' disease	Phenylalanylphenylalanine	4	3.66(1.10-12.19)	0.035	0.899	0.826	0.0211657	0.685	TRUE	5.87742E-25
Graves' disease	Phosphate	4	0.09(0.01-0.86)	0.037	2.511	0.473	-0.0348529	0.394	TRUE	4.17112E-28
Hashimoto thyroiditis	Kynurenine	32	13.00(1.15-147.44)	0.038	38.838	0.157	0.012907	0.732	TRUE	1.6446E-257
Hashimoto thyroiditis	3-methylhistidine	8	0.29(0.09-0.91)	0.034	9.720	0.205	-0.0195768	0.862	TRUE	1.55711E-71
Hashimoto thyroiditis	Phenol sulfate	10	0.21(0.05-0.85)	0.029	8.984	0.439	-0.0009382	0.993	TRUE	1.41162E-77
Hashimoto thyroiditis	2-palmitoylglycerophosphocholine*	17	9.83(1.19-81.37)	0.034	17.157	0.376	-0.0139517	0.679	TRUE	4.571E-102
Hashimoto thyroiditis	X-14189–leucylalanine	11	0.31(0.15-0.64)	0.002	7.408	0.686	-0.0208127	0.644	TRUE	6.48194E-84
Hashimoto thyroiditis	Gamma-tocopherol	11	0.21(0.06-0.71)	0.012	7.813	0.647	-0.019332	0.711	TRUE	6.5734E-104
Hashimoto thyroiditis	Alpha-ketoglutarate	11	46.89(4.65-473.28)	0.001	11.052	0.354	-0.0087717	0.957	TRUE	1.6218E-113

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TABLE 2 The most detrimental and protective factors for the three thyroid diseases.

Trait	Exposure	IVW		MR-Egger		Weighted median	
		OR (95% CI)	P value	OR (95% CI)	P value	OR (95% CI)	P value
Thyroid cancer	Aspartate	7.41(1.51-36.27)	0.013	89.24(1.01-7882.92)	0.188	6.67(0.80-55.52)	0.079
Thyroid cancer	C-glycosyltryptophan	0.04(0.00-0.29)	0.001	0.02(0.00-14.33)	0.261	0.04(0.00-0.88)	0.041
Graves' disease	Kynurenine	2.69(1.08-6.66)	0.033	2.10(0.21-21.28)	0.533	1.05(0.26-4.19)	0.945
Graves' disease	4-androsten-3beta,17beta-diol disulfate 2	0.48(0.25-0.91)	0.024	0.17(0.02-1.53)	0.133	0.40(0.17-0.94)	0.036
Hashimoto thyroiditis	Alpha-ketoglutarate	46.89(4.65-473.28)	0.001	74.02(0.00-875286845)	0.617	11.17(0.46-268.59)	0.137
Hashimoto thyroiditis	X-14189–leucylalanine	0.31(0.15-0.64)	0.001	0.40(0.11-1.50)	0.209	0.34(0.13-0.92)	0.033

IVW, inverse variance weighted.

Mendelian randomized pleiotropy residuals (MR-PRESSO), indicating no significant findings (all P > 0.05). Heterogeneity was assessed through IVW test and Cochran Q statistic in MR-Egger regression. There was also no indication of significant heterogeneity between instrument SNP effects. We further performed Steiger tests to verify the direction of effects from metabolites to TC, GD and HT. The Steiger P-value suggests no reverse causality bias in the identified causality (Table 1).

Causal effects of TC, GD, and HT on human blood metabolites

To assess any reverse causal effects, we performed a reverse MR Analysis using TC, GD and HT as exposures and 452 blood metabolites as results. Among them, the number of instrumental variables for each disease ranged from 4 to 11, and all IVs had F-statistics considerably > 10. (Table S4) Results suggested a causal effect of TC on Glutamine (OR_{IVW}=1.00, 95%CI=0.99–1.00, P_{IVW} = 0.016), Ornithine (OR_{IVW}=0.99, 95%CI=0.98–1.00, P_{IVW} = 0.036), 5-oxoproline (OR_{IVW}=0.99, 95%CI = 0.99–1.00, P_{IVW}= 0.037), X-12095–N1-methyl-3-pyridone-4-carboxamide (OR_{IVW}=1.01, 95% CI = 1.00–1.02, P_{IVW}= 0.021), and Taurolithocholate 3-sulfate (OR_{IVW}=0.98, 95%CI = 0.96–1.00, P_{IVW} = 0.009) (Figures S13-S16). GD has potential causal relationship with 13 metabolites such as Glutamine (OR_{IVW}=1.00, 95% CI = 0.99–1.00, P_{IVW}= 0.027) (Table 3; Figures S17-S20). The Q statistic of Cochran showed no heterogeneity (P>0.05). An MR-Egger intercept revealed no horizontal pleiotropy (P>0.05), while Cochran's Q statistics indicated no heterogeneity (P>0.05). By conducting MR Steiger's directionality test, SNPs with wrong directionality were excluded, and the results showed that HT had no causal relationship with metabolites. Tables S8-S9 provide the genetic variants responsible for the association between the three thyroid diseases and identified metabolites.

Replication analysis

To further validate our findings, we conducted replication analyses using GWAS data from IEU for TC, GD, and HT. The results demonstrated that the three metabolites, 1-linoleoylglycerol (1-monolinolein), Gamma-tocopherol, and 2-hydroxyisobutyrate, all passed the replication test based on IVW analysis. Subsequent combined analyses of FINNGEN and IEU Open GWAS datasets further confirmed that higher levels of 1-linoleoylglycerol (1-monolinolein) and Gamma-tocopherol were associated with reduced risk of TC and HT in individuals with genetic susceptibility. Additionally, fluctuations in 2-hydroxyisobutyrate levels were observed following the occurrence of GD (Figure 2).

Metabolic pathway analysis

We further carried out the metabolite pathway analysis using all metabolites discovered through the IVW approach (P<0.05). Through forward MR Analysis, we detected six potential metabolic pathways for thyroid cancer and four for Hashimoto's thyroiditis. (Table S7). The results show that "Aminoacyl-tRNA biosynthesis" (P=0.002), "Phenylalanine, tyrosine and tryptophan biosynthesis" (P=0.007) pathway might be participated in the genesis of TC (Figure 3A). "D-Glutamine and D-glutamate metabolism" (P=0.01) and "Butanoate metabolism" (P=0.03) pathway might be associated with HT (Figure 3B). Among them, there exists a common metabolic pathway (i.e., "Arginine biosynthesis" and "Histidine metabolism") shared by TC (P=0.02, P=0.03) and HT (P=0.03, P=0.04).

In reverse MR Analysis, we observed that TC (Figure 3C) and GD (Figure 3D) may increase or decrease the levels of related metabolites *in vivo* through four potential metabolic pathways. Among them, "arginine biosynthesis", "D-glutamine and D-glutamate metabolism", and "nitrogen metabolism" were three shared metabolic pathways present in both TC and GD (P < 0.05) (Table S8).

TABLE 3 Effect of thyroid disease on metabolites.

Outcome	Exposure	N	IVW		Heterogeneity		Pleiotropy		Steiger direction	
			OR(95%CI)	P	IVW Q	P	Intercept	P	correct_causal_direction	steiger_pval
Glutamine	Thyroid cancer	4	1.00(0.99-1.00)	0.017	0.469	0.926	-0.0007116	0.745	TRUE	0.323031496
Ornithine	Thyroid cancer	4	0.99(0.98-1.00)	0.036	1.687	0.640	0.0046533	0.375	TRUE	0.605736847
5-oxoproline	Thyroid cancer	4	0.99(0.99-1.00)	0.037	3.150	0.369	-0.0034355	0.315	TRUE	0.780286918
X-12095–N1-methyl-3-pyridone-4-carboxamide	Thyroid cancer	4	1.01(1.00-1.02)	0.022	0.171	0.982	0.0011305	0.835	TRUE	0.409721711
Taurolithocholate 3-sulfate	Thyroid cancer	4	0.98(0.96-1.00)	0.009	0.593	0.898	0.0058003	0.637	TRUE	0.777404811
Glutamine	Graves' disease	11	1.00(0.99-1.00)	0.028	5.464	0.858	-0.000132	0.896	TRUE	0.480345297
Stearate (18:0)	Graves' disease	11	1.00(1.00-1.01)	0.049	6.283	0.791	0.0019111	0.273	TRUE	0.176588404
2-hydroxystearate	Graves' disease	11	1.01(1.00-1.01)	0.047	7.492	0.678	0.0021971	0.272	TRUE	0.27966876
1-oleoylglycerol (1-monoolein)	Graves' disease	11	1.02(1.00-1.03)	0.008	7.539	0.674	0.0009299	0.832	TRUE	0.946667749
2-hydroxyisobutyrate	Graves' disease	6	1.00(0.99-1.01)	0.041	2.819	0.728	0.008937	0.268	TRUE	0.916619542
3-(4-hydroxyphenyl)lactate	Graves' disease	8	1.00(0.99-1.00)	0.043	5.290	0.382	-0.0063934	0.437	TRUE	0.379018338
Scyllo-inositol	Graves' disease	9	1.01(1.00-1.02)	0.046	4.350	0.825	-0.00558	0.149	TRUE	0.452788967
1-arachidonoylglycerophosphocholine*	Graves' disease	11	1.01(1.00-1.02)	0.007	6.976	0.728	-0.0007676	0.760	TRUE	0.598643183
1-palmitoleoylglycerophosphocholine*	Graves' disease	11	1.01(1.00-1.02)	0.003	5.263	0.873	-0.0014652	0.578	TRUE	0.523981309
1-docosahexaenoylglycerophosphocholine*	Graves' disease	11	1.01(1.00-1.02)	0.014	4.268	0.934	-0.0015551	0.592	TRUE	0.238630271
N2,N2-dimethylguanosine	Graves' disease	6	0.99(0.99-1.00)	0.049	2.120	0.832	0.00183	0.371	TRUE	0.536838388
Glutaroyl carnitine	Graves' disease	11	1.01(1.00-1.01)	0.044	10.978	0.359	-0.0020548	0.335	TRUE	0.290557508
1-myristoylglycerophosphocholine	Graves' disease	11	1.01(1.00-1.02)	0.029	3.766	0.957	-0.0009854	0.737	TRUE	0.144661835

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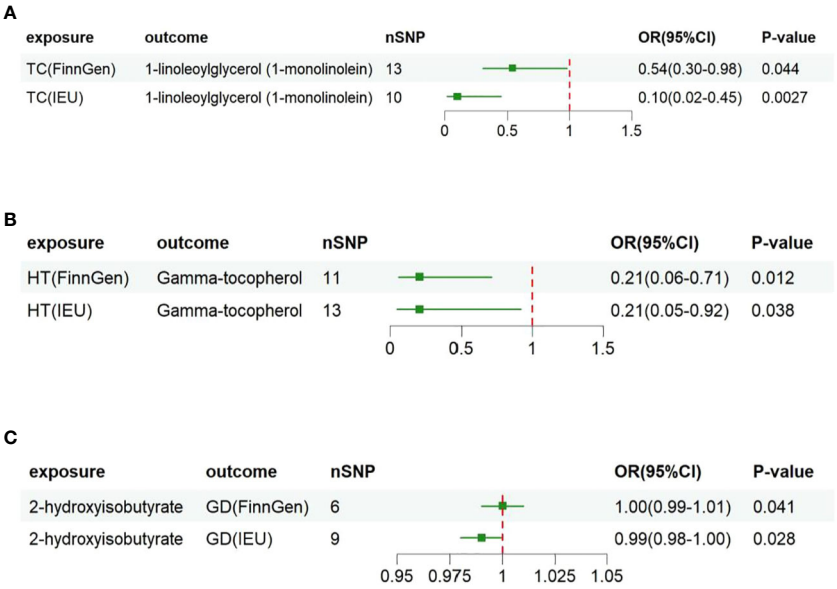


FIGURE 2
Associations of genetically predicted three metabolites with risk of thyroid disorders using IVW method with different GWAS datasets. OR, odds ratio; CI, confidence. **(A)** 1-linoleoylglycerol (1-monolinolein) shows potential causal association in two different thyroid cancer datasets (FinnGen Consortium and IEU Open GWAS); **(B)** Gamma-tocopherol shows potential causal association in two different Hashimoto's thyroiditis datasets; **(C)** Potential causal effects of 2-hydroxyisobutyrate in two different Graves' disease datasets.

Discussion

Observational studies have reported associations between metabolites and certain types of thyroid disorders. In this study, we conducted a two-sample bidirectional Mendelian Randomization (MR) analysis using publicly available GWAS summary statistics to investigate the causal relationships between human blood metabolites and various thyroid disorders, including Graves' disease, Hashimoto's thyroiditis, and thyroid cancer. Our findings revealed intricate interactions between metabolites and thyroid diseases. To the best of our knowledge, this represents the inaugural MR investigation into establishing causality between blood metabolites and thyroid disorders. In the forward MR Analysis, we found a total of 46 suggestive associations. Thyroid disease is linked to risk factors like Aspartate and protective factors like C-glycosyltryptophan. In the replication analysis, the results revealed that each of the three thyroid disorders had one overlapping metabolite in different datasets. Extensive sensitivity analyses showed that these associations were robust to the pleipotency of the MR Methods and tools used, using MR-PRESSO and leave-one analyses, and showed consistent findings.

Over the past decade, continuous discoveries of metabolic biomarkers related to thyroid disorders have emerged in the field of metabolomics research. Tissues, blood, urine, and feces are typical sample sources for metabolomic profiling. Among these, blood serves as a rich reservoir of readily accessible metabolites, making it valuable for identifying circulating biomarkers in thyroid disease screening. In metabolomic studies pertaining to plasma/serum, alterations in the metabolic profiles of three thyroid disorders have been observed, with the most common metabolic categories being amino acids, fatty acids, and lipids. For instance, in a study by Zhao et al. (31), metabolic differences were compared between thyroid cancer patients and healthy controls. This analysis revealed a significant increase in serum amino acid metabolite levels in thyroid cancer patients, while levels of lipid and choline metabolites were comparatively lower. Our research indicates that Aspartate, Stearoylcarnitine, and Gamma-glutamylglutamine are associated with a higher risk of thyroid cancer. Furthermore, after the onset of TC and GD, there is a decrease in glutamine levels. In TC patients, Taurolithocholate 3-sulfate increases, while GD patients exhibit higher levels of N2,N2-dimethylguanosine. Similar results were reported by Liu et al. (9) in their serum metabolomics pattern analysis of autoimmune thyroid disease patients, revealing differences in amino acids, fatty acids, and lipid-related metabolites among all subjects. Conversely, the occurrence of thyroid disorders also leads to fluctuations in metabolite levels. Patients with thyroid dysfunction exhibit more significant variations in metabolite levels. In our reverse MR analysis, we identified 23 metabolites with suggestive associations, suggesting that the onset of TC and GD alters the composition of metabolites. These findings may have implications for public health interventions aimed at reducing the risk of thyroid disease.

This MR analysis additionally recognized specific metabolites, some of which had been previously reported in other studies. C-glycosyltryptophan is a blood metabolite in the tryptophan pathway, and as a strong protective factor of TC, it has been shown to have a causal association with chronic kidney disease (CKD) in Cheng et al. (32). In our study, phenylalanine increased the risk of TC and kynurenine increased the risk of GD. This echoes prior discoveries. For instance, in line with metabolomics, heightened phenylalanine levels were observed in TC patients, and seven metabolites, including kynurenine, showed marked

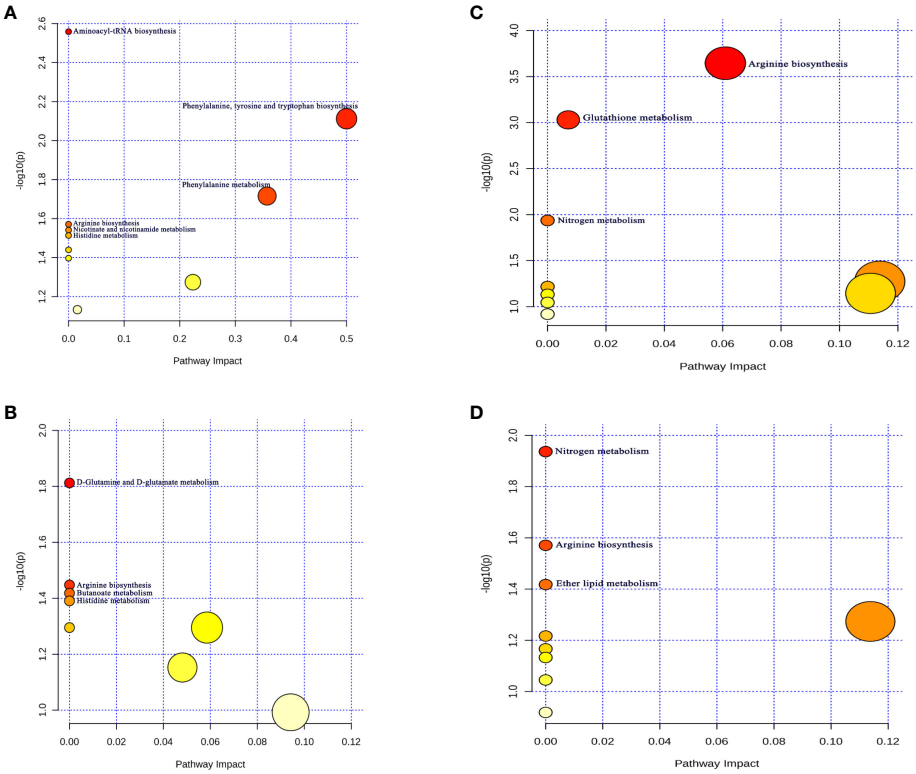


FIGURE 3
Potential metabolic pathways associated with thyroid disease. **(A)** Potential metabolic pathways involved in the pathogenesis of thyroid cancer in positive MR Analysis; **(B)** Hashimoto thyroiditis (The positive MR Analysis); related to Additional File 2 Table S7 **(C)** Thyroid cancer (The reverse MR Analysis); **(D)** Graves' disease (The reverse MR Analysis). related to Additional File 2 Table S8 Pathway analysis based on "Kyoto Encyclopedia of Genes and Genomes" (KEGG). The color and size of each circle is based on p-values (yellow: higher p-values and red: lower p-values) and pathway impact values (the larger the circle the higher the impact score) calculated from the topological analysis, respectively. Pathways were considered significantly enriched if $p < 0.05$, impact >0.1 and number of metabolite hits in the pathway >1.

elevation in GD patients when compared to healthy controls (33). Nonetheless, the sample sizes in these investigations span from dozens to hundreds, which might not adequately represent the broader population. The metabolite and thyroid disease data in this study included a larger sample size, making this study more representative. Carnitine is a quaternary ammonium compound synthesized from phosphatidylcholine, as well as the amino acids lysine and methionine. It has been studied as a therapy or protective agent for many diseases (34). Marie-Josée et al. discovered (35) that carnitine can enhance the *in vivo* bioavailability of butyrate. When combined, butyrate—a potential anticancer compound—can inhibit proliferation and induce apoptosis in human colon cancer cells. Another study (36) confirmed that carnitine reduced the effect of butyrate as an HDAC inhibitor and restrained the induction of H3 acetylation by butyrate in colorectal cancer cells. Our results confirm that carnitine is a protective factor for TC, further research is needed to explore the potential mechanisms underlying the combined impact of carnitine and butyrate in thyroid cancer. γ-tocopherol has demonstrated a protective effect against HT in two different GWAS databases. Previous analyses have reported unique antioxidant and anti-inflammatory activities associated with γ-tocopherol, suggesting its potential role in preventing thyroid disorders through the regulation of inflammation-related mechanisms (37). Future functional analysis is necessary, substantial experimental endeavors are still required in later stages to establish a more precise assessment of the initial speculation.

In this study, we identified ten significant metabolic pathways associated with TC and HT through pathway enrichment analysis. Among these pathways, "Arginine Biosynthesis" and "Histidine Metabolism" were found to be linked to both TC and HT. Medullary thyroid carcinoma is a malignant tumor originating from the C-cells of the thyroid, which synthesize and secrete calcitonin (38, 39), with arginine serving as a precursor for calcitonin synthesis (40). Patients with this condition typically require lifelong arginine supplementation to maintain normal arginine levels in their bodies. Additionally, thyroid hormone synthesis depends on both arginine and histidine (41), with tyrosine in the histidine metabolism pathway being a precursor for thyroid hormone synthesis (42). In individuals with Hashimoto's thyroiditis, the production of anti-thyroid peroxidase antibodies (anti-TPO antibodies) and anti-thyroglobulin antibodies can lead to attacks on thyroid tissue, affecting thyroid hormone synthesis and causing inflammation and damage (43). Furthermore, we also identified "Aminoacyl-tRNA Biosynthesis" as the most strongly associated pathway with TC, while "D-Glutamine and D-Glutamate Metabolism" pathway was primarily linked to GD and HT. Aminoacyl-tRNA is

synthesized through esterification of tRNA with the appropriate amino acid at its 3'-end. Oxidative stress induces the rapid translocation of TyrRS from the cytoplasm to the nucleus to prevent DNA damage (44). Inhibiting tRNA aminoacylation has proven to be an effective antibacterial strategy, impeding a critical step in protein synthesis. Furthermore, Yu et al. (45) observed microbial changes in TCs that led to alterations in aminoacyl-tRNA biosynthesis. In humans (46), autosomal recessive mutations in glutamate-pyruvate transaminase 2 (GPT2) cause a neurological syndrome characterized by intellectual disability, microcephaly, and progressive motor symptoms. Thyroid dysfunction is a major contributor to muscle weakness. Thyroid hormones (TH) serve as crucial metabolic regulators that coordinate short-term and long-term energy demands (47). Related research has shown that THs regulate glutamine and glucose metabolism through GPT2, coupling glycolysis with the TCA cycle to maintain muscle mass. In summary, these findings suggest that aminoacyl-tRNA biosynthesis and glutamine metabolism may play vital roles in the biological mechanisms of thyroid disorders. Our research findings provide a profound perspective in understanding the relationship between metabolites and thyroid disorders, revealing strong associations between various metabolites and thyroid diseases. These insights pave the way for future investigations to enhance the diagnosis, prevention, and treatment of thyroid disorders.

This study boasts several key strengths. Firstly, it stands as the most comprehensive MR investigation concerning the metabolite-thyroid disease association, boasting the largest sample size to date. Secondly, we meticulously opted for GWAS data from the FINNGEN Consortium as a primary analysis, and corroborated our findings using IEU Open GWAS datasets, markedly enhancing result reliability. Thirdly, both reverse MR Analysis and sensitivity analysis indicated the absence of pleiotropy or heterogeneity, underscoring the statistical robustness of our outcomes.

Nonetheless, certain limitations should be acknowledged in this study. Initially, owing to the constrained count of SNPs achieving genome-wide significance, we opted for a relaxed P threshold—a prevalent practice employed in similar contexts. Furthermore, the veracity of SNPs with relaxed P-values is corroborated by the genuine direction ascertained from the Steiger test. Secondly, while it's generally advisable to employ sizable GWAS sample sizes for MR Studies, our research employed a relatively modest metabolite GWAS sample size, potentially impacting the robustness of our MR findings. Another limitation pertains to the predominantly European ancestry of our participants. Caution must be exercised when generalizing these findings to other ethnic groups, necessitating further investigation. Fourthly, metabolite levels exhibit variation across diverse cell and tissue types. Our study solely examines the causal link between blood-measured metabolites and thyroid disease, failing to address the relevance of metabolite levels in more biologically pertinent tissues, such as the thyroid. Finally, post Bonferroni correction, we didn't ascertain a distinct causal connection between metabolites and thyroid disease, implying the requirement for additional research to corroborate this relationship.

Conclusion

In summary, this study establishes a bidirectional causal link between human blood metabolites and thyroid disease. Our forward MR Analysis pinpointed 46 metabolites potentially influencing thyroid disease progression, while reverse MR Analysis identified 23 metabolites possibly influenced by thyroid disease development. Additionally, potential metabolic pathways underlying metabolite-thyroid disease association were identified. Yet, comprehensive clinical investigations are required to unveil the precise metabolite-thyroid disease relationship and unravel the mechanistic underpinnings further.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/Supplementary Material.

Author contributions

ZL: Funding acquisition, Project administration, Resources, Writing – review & editing. XZ: Writing – original draft, Writing – review & editing. JZ: Conceptualization, Investigation, Software, Writing – review & editing. ZX: Data curation, Investigation, Methodology, Writing – review & editing. XL: Data curation, Formal Analysis, Project administration. JH: Writing – review & editing, Data curation, Formal Analysis, Project administration. HH: Writing – review & editing, Project administration, Validation, Visualization.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2023.1270336/full#supplementary-material

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Effect of iodine nutritional status on the recurrence of hyperthyroidism and antithyroid drug efficacy in adult patients with Graves' disease: a systemic review

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Aim: To probe the appropriate iodine nutritional status for patients with Graves'disease (GD) hyperthyroidism and on antithyroid drugs (ATD) or after drugwithdrawal.

Method: Studies were retrieved from three databases (Embase, Medline, and Cochrane Library) and were screened and evaluated using predefined criteria. The risk of bias of each trial was assessed using a tool from Cochrane. The iodine nutritional status of the subjects was redefined according to the World Health Organization (WHO) criteria and classified as insufficient/adequate/above requirements/excessive iodine intake.

Result: Two randomized controlled trials (RCTs) and 3 observational studies were selected from the 376 retrieved papers, which had different degrees of risk of bias in study design. The heterogeneity among them prevented us from further synthesizing effect indicators and subsequent statistical analyses. Two RCTs with high quality showed that insufficient or above requirements iodine intake was detrimental for ATD-treated GD patients; adequate iodine intake was associated with a lower risk of recurrence and better efficacy in controlling thyrotoxicosis. It could be speculated from three low-quality observational studies that excessive iodine intake may be associated with higher (or similar) recurrence rates and lower remission rates compared to above requirements iodine intake in these patients, but none of them could answer the question of the effect of insufficient or adequate iodine intake on this issue.

Conclusion: Although the available evidence is suboptimal, this systematic review tentatively suggests that in adult patients with GD hyperthyroidism receiving ATDs and according to WHO criteria for iodine nutritional status, adequate iodine intake is associated with a lower recurrence rate, a higher

remission rate and a better efficacy to control thyrotoxicosis than insufficient, above requirement, or excessive iodine intake. Future RCTs with large samples are expected to elucidate the actual impact of different iodine nutritional statuses on the recurrence rate of hyperthyroidism and the efficacy of ATD to control thyrotoxicosis in these patients.

Systematic review registration: identifier CRD42022359451.

KEYWORDS

Graves' disease, hyperthyroidism, antithyroid drug, iodine status, recurrence

1 Introduction

In recent years, salt iodization projects have been implemented worldwide under the call of the World Health Organization (WHO), making iodine deficiency effectively controlled (1), along with an increasing proportion of people with appropriate iodine nutritional status. Hyperthyroidism is a major endocrine disorder, of which Graves' disease (GD) is the most common cause. Iodine, an important raw material for the synthesis of thyroid hormones, is involved in its pathogenesis (2). Concerns about the relationship between iodine intake and autoimmune thyroid diseases, including GD, have emerged as the iodine nutritional status of the population has improved (3, 4).

Opinions about the relationship between the incidence or prevalence of thyroid disease and iodine intake vary around the world (5–10). Among patients with GD hyperthyroidism and on antithyroid drug (ATD) therapy, studies exploring the effect of dietary iodine intake (i.e., iodine nutritional status) on the recurrence of hyperthyroidism are scarce and with small sample sizes. To make it worse, inconsistent definitions of iodine nutritional status of different research have led to confusion and misunderstanding. In clinical practice, endocrinologists guided by traditional views often recommend a low iodine diet for patients with GD hyperthyroidism to maintain their iodine deficiency status (11); our patients are also confused as to whether they should go for iodized or non-iodized salt and whether their iodine intake should be severely restricted. Due to the lack of support from evidence-based medicine, the latest international guidelines for the management of GD hyperthyroidism seem to avoid discussion on this issue (12–14), while clinicians must answer the question of iodine intake from their patients in clinical practice.

Therefore, there is an urgent need to redefine the reported iodine nutritional status using uniform criteria to avoid confusion due to various descriptions from different authors. To this end, we have completed a systematic review to evaluate the effect of different iodine nutritional statuses on the recurrence of GD hyperthyroidism after ATD treatment using a uniform redefinition according to WHO criteria. The study will provide evidence-based conclusions on what iodine nutritional status should be maintained during the treatment of GD hyperthyroidism and guide clinicians in the management of iodine intake in these patients.

2 Method

2.1 Literature review methodology and results

We searched all the literature (to 9 September 2022) in the Medline (Ovid SP), Embase (Ovid SP) and Cochrane databases, and there was no language restriction. The 3 involved key words included "graves' disease", "antithyroid agents", and "iodine"; the detailed search strategy is accessible in Supplementary Material 1.

2.2 Inclusion and exclusion criteria for clinical intervention studies

Inclusion criteria: 1) it was an RCT, cohort study, or case –control study, 2) the disease studied was GD hyperthyroidism, 3) the study was conducted in patients with GD hyperthyroidism on ATD or after drug withdrawal, 4) there were quantitative indicators describing iodine nutritional status, 5) the outcome related to recurrence or remission of hyperthyroidism, and 6) participants had received ATD for at least 6 months and were followed up for at least 6 months after drug withdrawal.

Exclusion criteria: 1) the study did not aim to explore the influence of dietary iodine on the recurrence of GD hyperthyroidism or the efficacy of ATDs, and 2) the study population included pregnant or lactating women or adolescents.

2.3 Criteria in the analysis and evaluation of the study

1. Diagnosis of GD hyperthyroidism: GD is diagnosed by composite indicators including clinical manifestations of thyrotoxicosis, elevated thyroid hormone, decreased

thyroid stimulating hormone (TSH), and elevated thyrotropin receptor antibody (TRAb) and/or ultrasound suggesting abundant thyroid blood flow (13). Clinical diagnosis of GD is made after excluding other causes of persistent thyrotoxicosis.

2. Recurrence of hyperthyroidism: ATD treatment for more than 6 months to achieve remission criteria (remission of thyrotoxic symptoms and normalization of thyroid hormone), followed by reappearance of clinical manifestations of thyrotoxicosis, elevated thyroid hormone and decreased TSH after drug withdrawal (12–14).
3. Criteria for iodine nutritional status: according to the urinary iodine concentration (UIC) criteria from WHO: a UIC level < 100 μg/L indicates insufficient iodine intake; a level between 100 and 199 is adequate iodine intake; a level between 200 and 299 μg/L is above requirements iodine intake, and ≥ 300 μg/L indicates excessive iodine intake (15).

2.4 Clinical intervention studies included in the analysis

Two authors carried out the course of screening and data collection independently. Of the 376 articles retrieved, two RCTs and three observational studies were finally included in the analysis after removing 53 duplicates, reading the titles, abstracts, and full texts, and screening using the preset inclusion and exclusion criteria (Figure 1).

2.5 Data collection

We extracted information about the year and country in which the trial occurred, the participants' characteristics, the regime and cases of intervention and control, the evaluating method, the follow-up time, and the study design from each study. We classified the trials into two categories: 1) RCTs with outcomes about recurrence or efficacy and 2) observational studies with outcomes about recurrence.

However, given the limited trials that met our criteria and differences in the study design, outcome indicators and follow-up time, the heterogeneity became nonnegligible and stopped us from synthesizing the outcome, thus we used the original data instead.

2.6 Study evaluation and bias analysis

The purpose of this study was to evaluate the effect of different iodine nutritional statuses on the recurrence of hyperthyroidism in adult patients with GD and on ATDs. Accordingly, we set a series of evaluation criteria: 1) to uniformly rejudge the iodine nutritional status according to WHO criteria and based on the authors' descriptions, 2) to evaluate whether the definition for diagnosis, recurrence and remission of GD hyperthyroidism specified in each study met our preset criteria, and 3) to evaluate whether the duration of ATD treatment and the follow-up period after drug withdrawal met our preset standards.

Two authors worked together to evaluate the bias of each study using version 2 of the Cochrane tool for assessing risk of bias in randomized trial (RoB2) and the risk of bias in nonrandomized studies-of exposure (ROBINS-E) tool.

3 Results

3.1 RCTs

The information of the two eligible RCTs is listed in Table 1 and described separately as follows.

3.1.1 RCTs with outcomes about relapse

In 2018, an RCT from Fujian, China, examined the relationship between iodine nutritional status and recurrence rate in ATD-treated GD hyperthyroidism patients. After one month of ATD treatment with strict iodine restriction, 459 patients with newly diagnosed GD hyperthyroidism were randomly assigned to the iodine supplementation group (10 g iodized salt, approximately 200 μg iodine/day, n = 203) and the iodine restriction group (non-iodized salt + low iodine diet, n = 202). ATD treatment was withdrawn after 12 months, and follow-up was continued for 12 months (24 months in total), with fasting UIC measured every 3 months and the recurrence rate observed.

There was no significant difference in baseline UIC between the two groups (approximately 60 μg/L), that is, insufficient iodine intake by WHO criteria. After starting the dietary iodine intervention, the UIC was maintained at 155 μg/L (adequate iodine intake by WHO criteria) in the iodine supplementation group and 35 μg/L (insufficient iodine intake by WHO criteria) in the iodine restriction group. After 9 months of intervention, the TRAb in the insufficient iodine intake (iodine restriction) group began to be significantly higher than that in the adequate iodine intake (iodine supplementation) group ($P < 0.001$), which lasted until the end of follow-up (24 months). The overall recurrence rate was significantly higher in the insufficient iodine intake group than in the adequate iodine intake group within 12 months after ATD withdrawal (45.5% *vs.* 35.5%, HR = 1.381, $P = 0.04$) (16).

In this trial, after the dietary iodine intervention, the actual iodine nutritional status of the two groups was insufficient iodine intake and adequate iodine intake. During ATD treatment, although there was no significant difference in FT4 and TSH between the two groups, TRAb was significantly higher in the insufficient iodine intake group, suggesting a high risk of hyperthyroidism recurrence. The difference in the recurrence rate of hyperthyroidism after ATD withdrawal further demonstrated the relationship between insufficient iodine intake and a high recurrence rate. Compared with insufficient iodine intake, adequate iodine intake reduces the risk of recurrence of GD hyperthyroidism after ATD withdrawal.

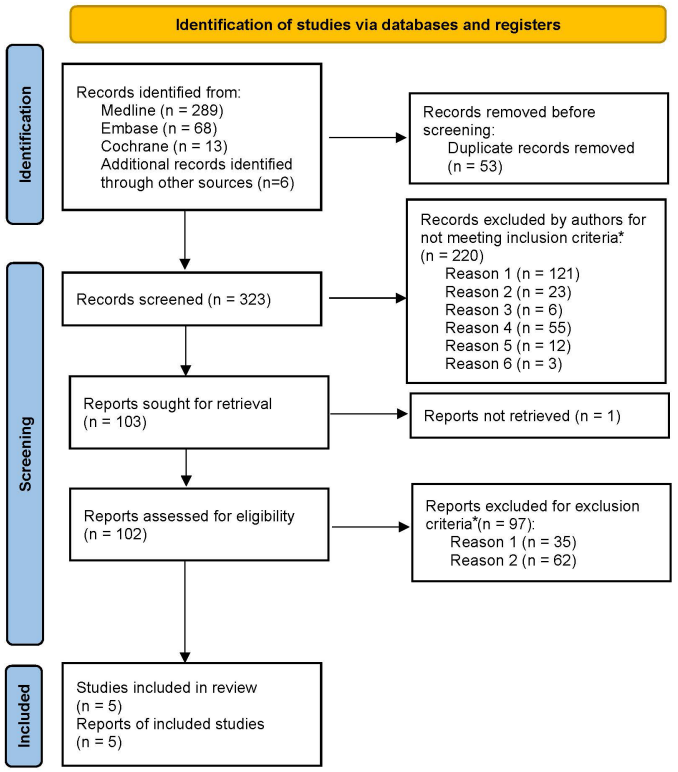


FIGURE 1
PRISMA flow diagram showing the systemic review process.

The definition of hyperthyroidism recurrence and the duration of ATD treatment in this study were in accordance with guideline recommendations. The UIC was measured every 3 months, which not only allowed for a dynamic assessment of the participants' iodine status but also suggested that the dietary intervention could successfully influence the iodine nutritional status. This trial showed a lower risk of recurrence of hyperthyroidism in ATD-treated GD patients with adequate iodine intake (compared to insufficient iodine intake) but lacked evidence about the above requirements or excessive iodine intake.

3.1.2 RCTs with outcomes about efficacy

In 2006, an RCT in Beijing, China, included 124 patients with newly diagnosed GD, all using universal salt iodization (USI) and without dietary iodine restriction prior to treatment. Patients were randomized into two groups: group A was given non-iodized salt (n= 45), and group B was given USI (35 ± 15) mg iodide/kg (n = 56). The follow-up period was 6 months after the beginning of ATD treatment, with UIC measured once before ATD treatment and once approximately 3 months after the start of treatment.

The median baseline UIC for participants in both groups was approximately 220 μg/L (above requirements iodine intake by WHO criteria). The dietary iodine intervention led to a median UIC of 148.4 μg/L (adequate iodine intake by WHO criteria) in the iodine restriction group and 242.8 μg/L (above requirements iodine intake by WHO criteria) in the USI group. Within 6 months after the beginning of ATD treatment, there was no significant difference in FT4 level and TRAb positivity between the two groups. However, from the second month onward, the ATD dose required to maintain normal thyroid function was significantly higher in the above requirements iodine intake group (using USI) than in the adequate iodine intake group (iodine restriction) ($P < 0.001$). The difference persisted until the end of the follow-up (17).

Although the trial differed from Fujian 2018 in terms of follow-up time and outcome indicators (lacking evaluation regarding recurrence rates), it was found that the ATD dose required to maintain normal thyroid function was significantly lower in the adequate iodine intake group. Since the dose of ATD for maintenance of thyroid function was associated with recurrence of GD hyperthyroidism, we hypothesized that the risk of recurrence after ATD withdrawal in the adequate iodine intake group was lower compared with the above requirements group. In addition, it was found that TRAb, an indicator associated with the risk of recurrence of GD hyperthyroidism, showed a decreasing trend in

TABLE 1 Information extracted from RCTs.

Article information			Country	Participants		Intervention	Control	Intervention period	Outcome	Reference
Author	Year	Journal		Characteristic	Age					
Huang, H.	2018	Clinical Endocrinology	China	Patients newly diagnosed with GD[a] (randomly assigned after ATD[b] therapy and dietary iodine restriction for 1 month) (N = 459)	16-65 years old	Iodine-supplemented group: 10 grams of iodized salt/day (UIC[c] about 155μg/L) (N = 203)	Iodine-restricted group: nonionized salt with low-iodine or noniodine diet (UIC about 35μg/L) (N = 202)	24 months (ATD+I[d] for 12m, I for 12 m)	The overall relapse rate within 12 months after ATD withdrawal was significantly higher in the iodine-restricted group (insufficient iodine intake) than in the iodine-supplemented group (adequate iodine intake) (45.5% *vs.* 35.5%, HR = 1.381, $P = 0.04$)	(16)
Dai, W. X.	2006	Chin Med J (Engl)	China	Patients with untreated GD (given USI[i] before treatment) (N = 124)	14-65 years old	Group A: non-iodinated salt (UIC about 148μg/L) (N = 45)	Group B: USI (35 ±15mg iodide/kg) (UIC about 242.8μg/L) (N = 56)	6 months (ATD+I)	The PTU dose required to maintain normal thyroid function within 6 months was significantly higher in group B (above requirements iodine intake) than in the group A (adequate iodine intake) ($P < 0.001$)	(17)

[a]GD, Graves' disease; [b]ATD, antithyroid drug; [c]UIC, urinary iodine concentration; [d]I, intervention or control.

the adequate iodine intake group, while no such trend was observed in the above requirement iodine intake group. Perhaps the difference would have been more pronounced if the duration of intervention and follow-up had been extended to 12 months [as recommended by a meta-analysis on the reasonable duration of ATD treatment for GD (18)] with larger sample size.

The authors originally concluded that iodine intake should be restricted in patients with GD, but after correcting for iodine nutritional status by WHO criteria, their restricted iodine intake group was actually with adequate iodine status. Thus, we think it seems more appropriate to conclude that "adequate iodine intake may be associated with a better effect of ATDs and a lower risk of recurrence compared with iodine above normal". The study measured UIC once before and after starting the intervention, indicating that dietary iodine restriction did not result in insufficient iodine intake in participants. The outcome indicator was the dose of ATD needed to maintain normal thyroid function within 6 months after starting treatment, but there was a lack of evidence on the recurrence rate of hyperthyroidism after drug withdrawal. In addition, the treatment course is short.

Although baseline and postintervention UIC level, ATD intervention time and outcome indicators were inconsistent between the two RCTs, the effect of iodine nutritional status on the recurrence of hyperthyroidism in ATD-treated GD patients tended to be the same. It can be concluded that insufficient or above required iodine intake is detrimental to ATD-treated GD patients; adequate iodine intake is associated with a lower risk of recurrence and a better efficacy to control thyrotoxicosis.

3.2 Observational studies with outcome about relapse

A cohort study from Korea in 2015 included patients who had taken ATD for at least 12 months and withdrawn drugs after achieving euthyroid function. They were followed for more than 12 months (a median of 23 months) to observe the presence of recurrence, and UIC was measured before and after ATD withdrawal. There were no significant differences in the mean UIC (412 μg/l *vs.* 408 μg/l, both excessive iodine intake) and UI/Cr (309 *vs.* 320 μg/g) between the remission and recurrence groups. Then, according to the Korean per capita UIC (358 μg/l, excessive iodine intake by WHO criteria), patients with UIC <300 μg/l (non-excessive iodine intake by WHO criteria) were defined as the average iodine intake group (n = 52), and patients with UIC ≥300 μg/l (excessive iodine intake by WHO criteria) were defined as the excess iodine intake group (n = 90), with the latter having a higher recurrence rate than the former, but no significant difference (30% *vs.* 21.2%, $P = 0.20$) (19). The authors concluded that in areas with high levels of iodine intake, it may not be necessary to restrict dietary iodine intake in ATD-treated GD patients. The definition of hyperthyroidism recurrence and the duration of ATD treatment in this trial were in accordance with guideline recommendations. UIC was tested during treatment and after drug withdrawal (without the exact time of testing), but the iodine status was estimated to be the same before and after treatment. The mean UIC of the participants during ATD treatment and after drug withdrawal was redefined as non-excessive iodine intake (the mean UIC was 358 μg/l, while the UIC of this group

was <300 μg/l, so it was presumed to belong mainly to the above requirements iodine intake) and the excessive iodine intake group according to WHO criteria. Therefore, it can only be concluded that the recurrence rate of hyperthyroidism in patients with GD after ATD withdrawal is the same when comparing the above iodine intake requirements with the excessive iodine intake. However, the effect of insufficient or adequate iodine intake compared with other iodine nutritional statuses on recurrence cannot be answered.

In 1987, a case–control study in the United States included 106 patients with GD hyperthyroidism treated with ATD for at least 6 months. Remission was defined as no relapse for at least 6 months after ATD withdrawal. From 1973 to 1985, the remission rate increased gradually (r = 0.60, $P < 0.02$), while the estimated daily iodine intake decreased (r = -0.81, $P < 0.009$) (20). Although the trial did not offer information about UIC, it provided data on daily iodine intake. We found that an estimated daily iodine intake between 200-300 μg/day (estimated above requirements iodine intake by WHO criteria) corresponded to a higher remission rate, when comparing with that between 700-800 μg/day (estimated excessive iodine intake by WHO criteria). In this study, the subjects received a short course of ATD, and the UIC was not measured, nor was the method of estimated daily iodine intake specified. Therefore, it was not possible to accurately assess the subjects' iodine nutritional status. As far as iodine intake is concerned, the WHO recommendation for adequate daily iodine intake for adults is 150 μg (21), so the subjects' iodine intake was still higher than WHO recommendation. The study did not report an explicit recurrence rate, but its definition of remission suggests its association with recurrence, so it is speculated from the data given in the study that excessive iodine intake may be associated with a lower rate of remission of hyperthyroidism (and presumably a higher rate of recurrence) for ATD-treated GD patients, but the effect of different iodine nutritional statuses on hyperthyroidism recurrence and remission cannot be answered.

In 1965, a historical control study in Britain compared the recurrence rate within 6 months after drug withdrawal in 16 GD hyperthyroidism patients who were supplemented with 200 μg potassium iodide daily (in 1965) and 41 patients who were not supplemented with iodine (in 1964). Plasma inorganic iodine (PII) was measured after ATD withdrawal. The authors concluded that the recurrence rate was higher in the iodine-supplemented group than in the noniodine-supplemented group (56% *vs.* 27%, $P < 0.05$) (22). However, there are no studies on the correspondence between PII and iodine nutritional status evaluated with UIC, preventing us from assessing them by WHO criteria. According to a previous report by this author, normal PII values of all ages were 0.04-0.57 μg/100 ml (23). The noniodine supplemented group were historical controls from the previous year, and a significant decrease in their PII was observed within 90 days after ATD withdrawal (from 0.21 ± 0.052 μg/100 ml to 0.04 ± 0.012 μg/100 ml, but still within the normal rage specified by the authors and lack of specific iodine nutritional status by WHO criteria). The iodine supplemented group, which was given 200 μg potassium iodide/day immediately after drug withdrawal, had a PII of 0.27 μg/100 ml both at the time of withdrawal and 6 months after that. Combined with the normal range given by the authors, the iodine supplemented group might not be in a state of iodine deficiency, indicating iodine overdose by daily supplementation of potassium iodide. Therefore, the high recurrence rate in this trial was actually associated with estimated excessive iodine intake (lack of specific iodine nutritional status by WHO criteria). The study did not provide a clear definition of hyperthyroidism recurrence and ATD treatment duration, nor did it measure UIC, leading to confusion about iodine nutritional status. Moreover, the study was a historical control study with confounding factors such as time span, patient compliance, and ATD treatment duration that have important effects on recurrence, so no conclusions could be drawn about the effects of different iodine statuses on the recurrence rate of GD hyperthyroidism after ATD treatment.

It was speculated from the results of the three observational studies that excessive iodine intake may be associated with high or similar recurrence and low remission rates comparing with above requirements iodine intake in ATD-treated GD patients. However, none could answer the effect of insufficient or adequate iodine intake on that issue (Table 2).

Integrating all the above findings and inferences, we conclude that the recurrence rate of hyperthyroidism is lower and the remission rate is higher with adequate iodine intake compared to insufficient or above requirements or excessive iodine intake in ATD-treated GD patients (Figure 2).

3.3 Risk of bias

The risk of bias assessments for each study are summarized in Figure 3.

Both RCTs were not double-blinded, so there were some concerns regarding the measurement of the outcome, but the outcome indicators (thyroid hormone, TRAb, and UIC) were objective quantitative indicators with little subjective influence from the participants, so the risk of bias in this area was considered to be low. In terms of missing outcome data, the first RCT had a low rate of dropout (<10%), and the survival analysis was performed taking into account the censored data, but no specific information of missed participants could be obtained. The second RCT had a high rate of dropout (approximately 16.8%) and did not include information on missed participants in the final analysis, so there were some concerns about deviations from intended interventions. Overall, the RCTs were of high quality. Although some concerns existed, the iodine status was primarily related to dietary habits rather than additional imposed interventions, so the risk of adverse effect associated with them may have been relatively low and may not be able to affect the study results.

The small sample size and bias of the three observational studies is a concern. The Korean 2015 study did consider the effect of age when analyzing the results and performed subgroup analysis. However, the study did not report any information related to dropout, and the results only included those who completed 1 year of follow-up, so there were some concerns in terms of missing data. The US 1987 and UK 1965 studies did not report any measures related to the control of confounding factors. The former had higher dropping (35%) due to incomplete data, while the latter did not report information related to dropout or no response, so there are some concerns about missing data. In

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TABLE 2 Information extracted from observational studies.

Article information			Country	Participants Characteristic	Study design (group)	Follow-up Duration	Outcome	Reference
Author	Year	Journal						
Park, S. M.	2015	European Thyroid Journal	Korea	Patients with GD[a] who had taken ATD[b] for at least 12 months to achieve euthyroid function and then stopped their ATD (N = 142)	Average iodine intake group (group 1): UIC[c]< 300 μg/L (N = 52); excessive iodine intake group (group 2): UIC ≥ 300 μg/L (N = 90)	23 months on average	No significant difference happened in the relapse rate between group 2 (excessive iodine status) and group 1 (average iodine status) about 2 years after ATD withdrawal (30% *vs.* 21.2%, $P = 0.20$)	(17)
Solomon, B. L.	1987	Annals of Internal Medicine	USA	Patients with an established diagnosis of GD (N = 69)	Investigate the remission rate and estimated iodine intake (from 700-800 μg/day to 200-300 μg/day) respectively in 1973-1985	At least 6 months	The remission rate gradually increased with year (from less than 10% to over 30%) (r = 0.60, $P < 0.02$), while the estimated daily iodine intake decreased (r = -0.81, $P < 0.009$)	(18)
Alexander, W. D.	1965	Lancet	Britain	Patients with GD after withdrawing ATD (N = 57)	Intervention group (group 1, 1965): received iodide supplements with normal PII[d] (N = 16); Historical control (group 2, 1964): did not receive any iodide supplements with low PII (N = 41)	At least 6 months	The relapse rate was higher in the group 1 (estimated excessive iodine status) than in the group 2 (estimated iodine deficiency) (56% *vs.* 27%, $P < 0.05$)	(20)

[a]GD, Graves' disease; [b]ATD, antithyroid drug; [c]UIC, urinary iodine concentration, [d]PII, plasma inorganic iodine.

addition, the 1987 USA study used data on estimated iodine intake but did not detail the estimation method and data source. The authors assessed the relationship between estimated iodine intake and year, and the remission rate and year separately to infer the relationship between estimated iodine intake and remission rate, so there were a lot of uncontrollable confounding factors, causing the final bias evaluation of high risk. The UK 1965 study used historical controls, and the baseline PII was significantly inconsistent between the two groups, resulting in a very high risk of bias considering the low comparability between subjects from different years. Overall, the risk of bias was higher in observational studies.

4 Discussion

We included two RCTs and three observational studies and concluded that, according to WHO criteria for iodine intake, ATD-treated GD patients had lower rates of recurrence and higher rates of remission when going for adequate iodine intake compared with insufficient or above requirements or excessive iodine intake.

This finding is consistent with the effect of iodine on thyroid autoimmunity. Laboratory studies have shown that the effects of excessive iodine on the thyroid gland include enhanced immunogenicity of thyroglobulin molecules (24) and increased

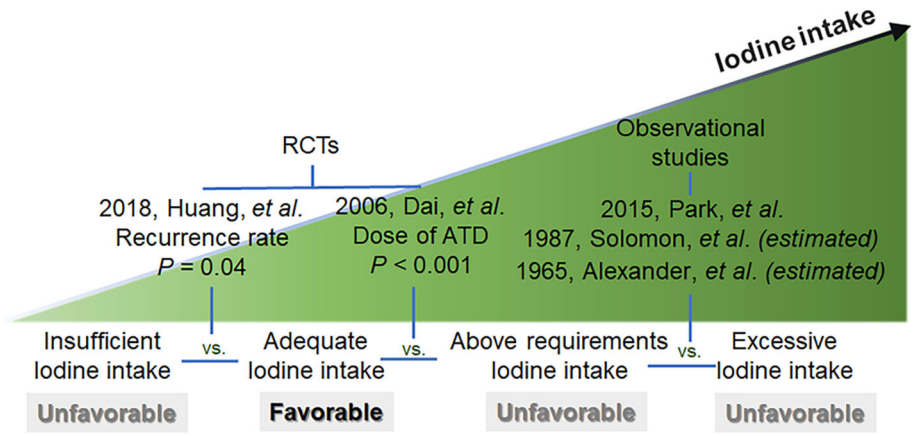


FIGURE 2
Summary Chart for trials.

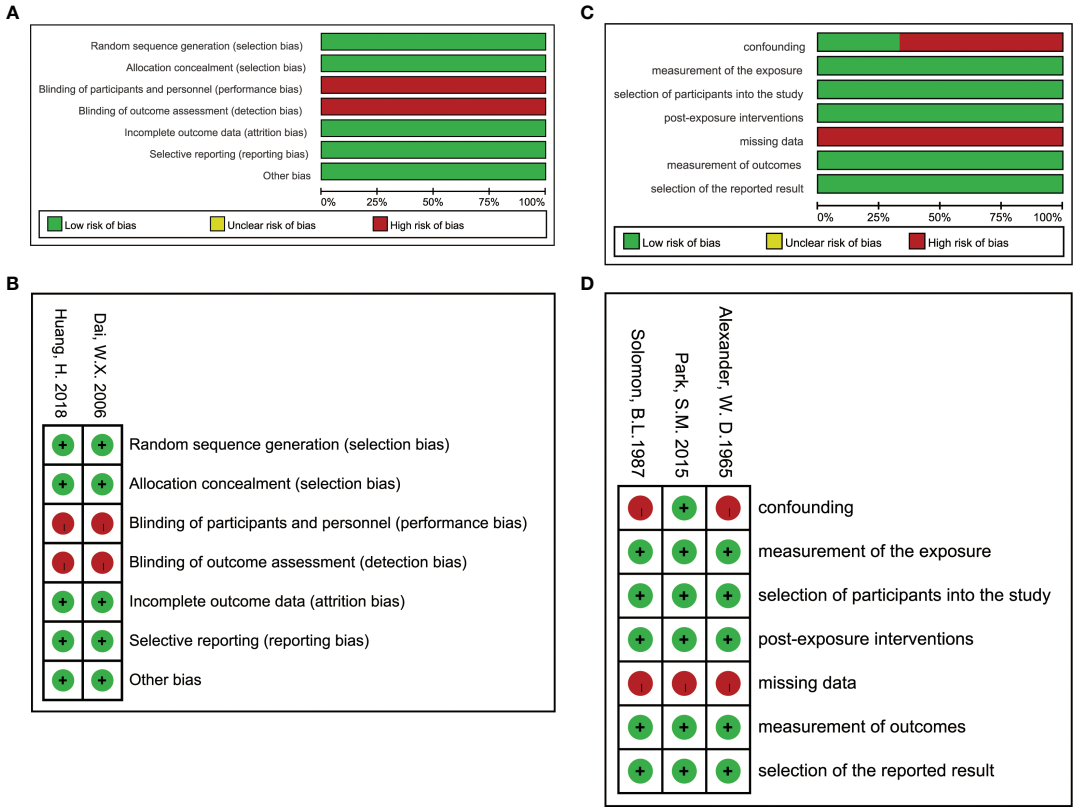


FIGURE 3
Risk of bias (using ROB2 for RCTs and ROBINS-E for observational study). **(A)** Risk of bias graph for RCTs: authors' judgements about each risk of bias item presented as percentages across all included RCTs. **(B)** Risk of bias summary for RCTs: authors' judgements about each risk of bias item for each included RCTs. **(C)** Risk of bias graph for observational studies. **(D)** Risk of bias summary for observational studies.

production of reactive oxygen species in thyroid cells (25), which exacerbates local inflammatory responses. In contrast, iodine deficiency has been shown to upregulate the expression of thyroid-specific proteins (26, 27), thereby stimulating thyroid hyperplasia and exacerbating the autoimmune response. Thyroglobulin levels were found to be significantly increased at both low and high iodine intake in infants 6-24 months of age, supporting the laboratory evidence (28).

A review detailed the effect of iodine intake on the synthesizing function of the thyroid gland. Severe iodine deficiency results in a lack of raw materials for thyroid hormones and hypothyroidism. When iodine deficiency is mild to moderate, the thyroid gland's ability to take up and recycle iodine, and synthesize thyroxine, increases as a substitute for iodine deficiency. With this chronic stimulus, the incidence of nodular toxic goiter and hyperthyroidism increases in the population (29). Epidemiological surveys from Europe also confirm this (30).

These studies on the effect of iodine nutritional status on thyroid synthetic function and thyroid autoimmune response indicating that both low and high iodine may exacerbate the thyroid autoimmune response and affect the thyroid's synthetic function, which may make the Graves' disease more difficult to control, or increase the rate of recurrence.

In addition to the five included studies, we identified other studies on iodine status and GD hyperthyroidism during the screening process, which had also been widely cited. However, after carefully checking the full text, we found that among them, some studies aimed to explore the appropriate dose of ATD (31), some aimed to explore the efficacy of iodine agents for GD hyperthyroidism (32, 33), some had too short a follow-up period to assess efficacy or recurrence (34), and some did not involve different levels of iodine intake and did not make statistical extrapolations for the comparison of recurrence rates (35). All of them did not meet the inclusion criteria and study objectives of this review, so we did not include these seemingly relevant studies, but they have contributed to some extent to the interest in this issue.

Apart from UIC, WHO also recommends the thyroid volume size (measured by ultrasound) to assess iodine deficiency (36). Previous studies found that children in iodine-deficient areas had larger thyroid volumes than those in iodine-sufficient areas (37), and goiter due to iodine deficiency persisted after correction of iodine deficiency (38). In addition to iodine intake, ethnicity, genetic factors and environmental factors all influence thyroid volume, which limits the validity of international reference ranges (39). Therefore, surveys should be conducted to determine the normal range of thyroid volume in each region and its correlation with iodine nutritional status.

Limitation: Our ideal study would have set up different iodine nutritional statuses in GD patients on ATD for at least 12 months

(18) and observed them 12 months after drug withdrawal. However, very few RCTs met these criteria, so both the duration of ATD treatment and post-withdrawal observation were adjusted to 6 months, and observational studies exploring the effect of iodine status on ATD efficacy were also included. However, this resulted in non-negligible inter-study heterogeneity, preventing us from performing statistical analyses of synthetic effect indicators:

First, the two RCTs involved populations with different iodine nutritional status and had different outcome indicators. Second, among the three observational studies, two studies did not provide UIC and could only estimate the iodine nutritional status; one of the studies did not provide specific numbers of remissions rate and total number of people; the last study involved historical controls and did not provide specific values. Finally, it was also not possible to combine the RCTs and observational studies for quantitative analysis due to differences in study design and quality of evidence. In addition, the studies we included did not provide information on thyroid volume at different iodine intakes, so we were unable to compare the effects of iodine intakes on that issue.

All three of the included observational studies were at some risk of bias, and two studies were older and reported population characteristics that may differ from the current general characteristics of patients. However, in interpreting these studies, we carefully analyzed the actual iodine nutritional status, redefined it according to WHO criteria, and corrected the conclusions of some studies that only mentioned iodine intake and ignored iodine nutritional status. The final conclusions were drawn by combining the results of the five studies, which to some extent compensates for the shortcomings of the individual studies.

In addition to this, when initially performing the literature screening, we planned to include RCTs or observational studies on populations with different etiologies of hyperthyroidism (such as nodular goiter, functional adenomas, etc.). However, during the progress, we found that there are very few studies exploring the effect of iodine nutritional status and they all focus only on the GD, the most common cause of hyperthyroidism. It is very regretful that our article cannot answer the question of the effect of different iodine intake on treatment outcome in recurrence rate for rare causes of hyperthyroidism other than GD. Hopefully, this question will be answered in the future.

Our study is the first as we know to use uniform criteria to evaluate the iodine intake status from different studies, making the results comparable. Available limited evidence may suggest that in the management of patients with GD hyperthyroidism, adequate iodine intake to achieve an appropriate iodine nutritional status may be more helpful in improving the efficacy of ATDs to control thyrotoxicosis and reducing the risk of recurrence.

Registration and protocol

The review was registered in NIHR PROSPERO, with the original title of "What iodine status should be maintained in patients with GD on ATD therapy" (CRD42022359451). Some changes have occurred in the inclusion and exclusion criteria, which were amended continuously in the process of searching.

Author contributions

Conceptualization: NT and QX; methodology: QX and XZ; software: QX and XZ; validation: JM and XL; formal analysis: QX; investigation: QX, XZ, and XL; data curation: JM; writing—original draft preparation: QX and XZ; writing—review and editing: NT and YZ; visualization: XL; supervision: NT and YZ; project administration: NT; funding acquisition: NT. All authors contributed to the article and approved the submitted version.

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Conflict of interest

NT has worked on clinical trials funded by Huadong Medicine Group Company Limited and received lecture fees from Novo Nordisk. However, these interests will not impair the justice and scientific nature of this study.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2023.1234918/full#supplementary-material

SUPPLEMENTARY MATERIAL 1
Search strategy.

SUPPLEMENTARY MATERIAL 3
The article retrieved and the reason for non-inclusion or exclusion.

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Research progress on moyamoya disease combined with thyroid diseases

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Moyamoya disease (MMD), also known as abnormal cerebral vascular network disease, is characterized by progressive occlusion or stenosis of the internal carotid and cerebral arteries, as well as the formation of an abnormal cerebral vascular network. It can occur anywhere in the world but is most common in China, Japan, and the Republic of Korea. In recent years, there have been increasing reports on the coexistence of thyroid diseases and MMD, but the mechanism of their coexistence is still unclear. For this article, we used keywords such as "moyamoya disease", "thyroid", "Grave disease", "thyrotoxicosis", and "thyroid autoimmune antibodies" to search for 52 articles that met the requirements in medical databases such as PubMed and Web of Science. This article also reviews the research on the role of thyroid hormone, the mechanism of immune antibodies, the possible correlation between thyroid diseases and MMD disease genes, and the treatment methods, and discusses the possible relationship between MMD and thyroid diseases to provide a reference for the pathogenesis and treatment of MMD with thyroid diseases.

KEYWORDS

moyamoya disease, thyroid, grave disease, thyrotoxicosis, review

Introduction

Moyamoya disease (MMD) is a rare cerebrovascular disease that was first discovered by Japanese scholars in 1957 (1). It is characterized by progressive occlusion of the superior carotid artery (ICA) and it mainly occurs within the circle of Willis. Occlusion leads to the formation of a compensatory vascular network at the bottom of the brain. The disease is called "moyamoya" ("puff of smoke" in Japanese) disease (2, 3) because it is like a stream of smoke drifting in the air during angiography. The incidence rate of MMD in East Asia is very high, with the greatest number of reported cases coming from China, Japan, and the Republic of Korea (4). Although the cause of MMD is still unclear, in the current research, it is speculated that it may be related to genes, immunity, and external factors. There are reports in the literature that the overall prevalence of autoimmune diseases in MMD patients in western China is as high as 31.0% (5). The characteristic of hyperthyroidism is

an increase in the synthesis and secretion of thyroid hormones. Elevated thyroid autoantibodies may also lead to increased hormone levels and secretion. The most common cause of hyperthyroidism is Graves' disease (6), which is an autoimmune disease. It is characterized by the presence of thyroid hormone-related antibodies (TR-Ab) that stimulate thyroid cells, resulting in the excessive secretion of thyroid hormones. Some patients with Graves' disease also show an increase in thyroid peroxidase antibodies (TPO-Ab) and anti-thyroid autoantibodies (Tg-Ab) (7, 8). In recent years, many reports have pointed out that there is a correlation between hyperthyroidism and the occurrence and development of MMD (often Graves' disease is the most common), especially when thyrotoxicosis occurs. The probability of occurrence and progression of MMD is higher than that of hyperthyroidism patients with well-controlled hormone levels (9, 10). However, the mechanism and related treatment of MMD combined with thyroid diseases are not very clear in current research. This article reviews research on the effects of thyroid hormones, immune antibody mechanisms such as TR-Ab, TPO-Ab, and Tg-Ab, the possible association between thyroid diseases and MMD disease genes, and treatment methods, and discusses the possible relationship between MMD and thyroid diseases with a view to providing a reference for the relevant mechanism and treatment of MMD with thyroid diseases (Figure 1).

Literature search

Articles in the PubMed and Web of Science databases were searched to identify articles related to MMD and abnormal thyroid function, with findings being searched up to the year 2022. The titles and abstracts of those articles were reviewed by two reviewers to confirm their quality and eligibility for further examination. The inclusion criteria were as follows: MMD and abnormal thyroid function mentioned together in title or abstract, and original studies or case reports. Other relevant articles and reviews were considered. The final bibliography is based on originality and relevance to the subject. The exclusion criteria were as follows: non-English articles and commentaries or clinical trials.

Based on the inclusion and exclusion criteria, a total of 52 eligible articles were identified and reviewed, and the year of publication ranged from 1997 to 2023. Due to space constraints, we do not look into statistical disaggregation in this report.

Thyroid hormone

MMD, as a cerebrovascular disease, often leads to serious cerebrovascular accidents due to vascular stenosis or occlusion. Some hormones in the human body can also cause lumen stenosis by contracting the blood vessels. With the continuous progress of research, it has been found that thyroid hormones are closely related to MMD (11). At present, thyroid hormones in laboratory testing usually include serum triiodothyronine (T3), thyroxine (T4), free T4, and free T3 (12). There may be differences across different reports, but the overall results show that the increase in thyroid hormone levels is important in the occurrence or development of MMD. In the study of Asian children with MMD, Hao Li and his team included 114 children with MMD and 114 healthy controls. The incidence rate of increased thyroid function was higher in the MMD group than in the control group; after adjusting for family history of cerebrovascular disease, thyroid function, homocysteine, and other variables, this result is still significant (13). This research result can also be supported by an adult case analysis. From May 2000 to December 2010 Nun Jun analyzed 12 patients from Peking Union Medical College Hospital and four patients from PLA 307 Hospital. In the study, all patients were female, they had an average age of 35.13 years ± 12.34 years (11 years–57 years), and they all had Graves' disease and MMD. Fifteen patients had cerebrovascular MMD, and the level of thyroid hormone in their bodies increased more significantly than that for patients without the disease (14). Research shows that thyroid hormone can significantly increase the risk of cerebrovascular disease In patients. Yang et al. assessed 16,808 hyperthyroidism patients with thyroid hormone and their

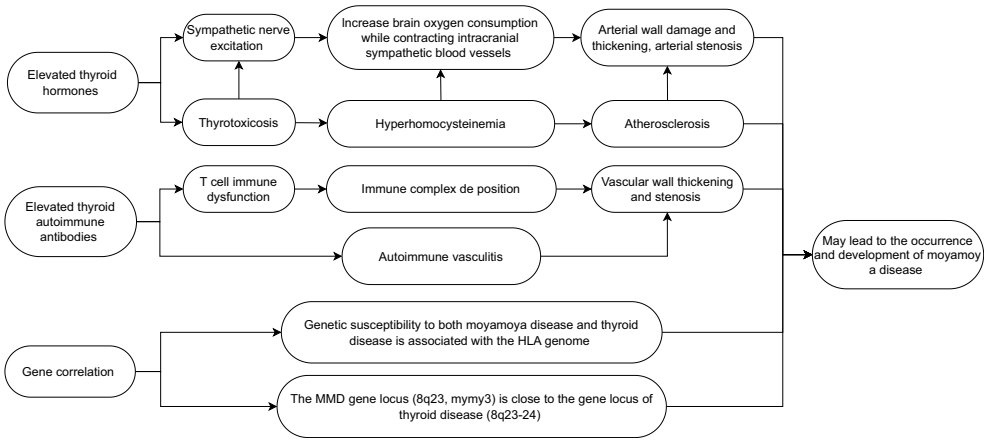


FIGURE 1
Possible factors leading to the occurrence and development of moyamoya disease caused by thyroid diseases.

probability of cerebrovascular disease. The results show that hyperthyroidism increases the risk of subsequent cerebrovascular disease by 38% (15). Other studies have put forward a similar point of view. After treatment, the blood flow of cerebral vessels in patients with hyperthyroidism will also significantly increase after the thyroid hormone level in their bodies returns to normal (16). Li evaluated the clinical characteristics and treatment effects of 21 patients with both MMD and thyroid dysfunction, and concluded that thyroid dysfunction, especially hormone level fluctuations caused by hyperthyroidism, may be an important risk factor for triggering or exacerbating symptoms in MMD patients (17). This study had a small number of data and further large-scale research may be required. In addition, the occurrence of thyrotoxicosis can further exacerbate the symptoms of patients with MMD. According to reports, a 42-year-old woman with Graves' disease developed an ischemic cerebral infarction during a thyrotoxicosis attack. The patient's MRI scan showed typical manifestations of MMD, and high thyroid hormone levels caused by thyrotoxicosis may have been related to the development of MMD in the patient (18). Therefore, when patients with Graves' disease experience worsening symptoms of cerebral ischemia during follow-up, the possibility of thyrotoxicosis should be considered. As for why the increase of thyroid hormone levels can lead to the occurrence and development of MMD, it is speculated that the following mechanisms may exist: excessive thyroid hormone levels make the sympathetic nervous system more sensitive, increase brain metabolism and oxygen consumption, and at the same time enhance the sympathetic vasoconstriction of the intracranial arteries, leading to the development of artery stenosis and posing a risk to the integrity of the arterial wall (19–21). In addition, thyrotoxicosis may lead to hyperhomocysteinemia, which may also be related to premature atherosclerosis and MMD (22). At the same time, the high level of thyroid hormone caused by the use of exogenous hormones to treat Graves' disease may also lead to the development of MMD (23). Although more and more studies indicate that an increase in thyroid hormone levels may lead to the development of MMD, the specific mechanism is not very clear, and further research is still needed. At the same time, it is still unclear whether hypothyroidism is related to MMD. More attention should be paid to this issue in the future, so as to better understand the role of thyroid hormone levels in the course of MMD.

Autoimmune antibody

Multiple reports have indicated that MMD may be related to autoimmunity (TR-Ab, TPO-Ab, Tg-Ab) (24), and the elevation of autoimmune antibodies plays an important role in the development of its course (25). With the development of technology, researchers have found that similar mechanisms may appear in different autoimmune diseases, which can lead to the coexistence both

TABLE 1 Summary of related studies on moyamoya disease combined with thyroid diseases.

STUDY AUTHOR AND YEAR	CASE NO.	AGE (YEARS)	GENDER	RESEARCH OBJECTIVE	RESEARCH CONCLUSION
Kim Suk Jae, 2010 (30)	63	35–52	M: 37 F: 26	Investigate the association between thyroid autoantibodies and MMD in patients with significantly normal thyroid function.	Thyroid autoimmune or potential immune abnormalities also play a role in the development of MMD.
Li Hao, 2011 (13)	114	6–13	M: 60 F: 54	Are thyroid function and thyroid autoantibodies associated with the risk of MMD in children?	Enhanced thyroid function and elevated thyroid autoantibodies are associated with MMD in children
Lei C, 2014 (11)	28	30–59	M: 10 F: 18	Assess the correlation between elevated thyroid function and elevated thyroid autoantibody levels and the risk of MMD.	The evidence strongly suggests that elevated thyroid autoantibodies and elevated thyroid function are independently associated with MMD.
Chen Jian Bin, 2016 (24)	384	30–50	M: 176 F: 208	Explore whether there is a difference in the prevalence of autoimmune diseases between unilateral and bilateral MMD.	Unilateral MMD may be more associated with autoimmune diseases than bilateral MMD.
Ryu Bike, 2015 (52)	8	29–51	M: 1 F: 7	Discuss the appropriate timing for optimizing thyroid hormone and Graves' disease MMD surgical treatment.	For MMD with Graves' disease, optimizing thyroid hormones and performing superficial temporal artery to middle cerebral artery double bypass surgery can successfully prevent cerebral ischemic events.
Lanterna Luigi A, 2018 (29)	37	>18	M: 9 F: 28	Explore the relationship between thyroid autoantibodies and clinical manifestations of MMD.	When the serum concentration of thyroid autoantibodies increases, patients have a higher risk of developing invasive manifestations.
Tashiro Ryosuke, 2019 (46)	136	28–48	M: 11 F: 125	Clarify the association between HLA alleles and MMD.	HLA-DRB1 * 04:10 is a risk allele for MMD, and it is also associated with thyroid diseases in MMD patients.

M represents male and F represents female.

MMD and autoimmune diseases (26). In recent years, more and more literature has shown that autoantibodies produced by thyroid-related diseases also play an important role in the occurrence and development of MMD. Thyroid autoantibodies refer to immunoglobulins produced by autoimmune disorders that target certain components of the thyroid gland. Clinically, there are two main categories: (1) antibodies targeting thyroid stimulating hormone (TSH) receptors on the surface of thyroid cells, namely TR-Ab; and (2) antibodies against thyroid cell contents, including TPO-Ab and Tg-Ab (27, 28). Currently, multiple studies have shown that thyroid autoantibodies may be important triggers for stroke and other cerebrovascular accidents in patients with MMD. After analyzing the prognosis and progression of 37 patients with MMD, Luigi found that the clinical manifestations of MMD patients may be significantly influenced by the concentration of thyroid autoantibodies in the serum, and when the concentration of thyroid autoantibodies in the serum increases, the risk of cerebrovascular accidents in patients also increases (29). At the same time, the study found that the concentration of thyroid autoimmune antibodies in patients with MMD was significantly higher than that in the normal population. In Kim's study, a total of 63 MMD patients, 71 non-MMD stroke patients, and 200 healthy controls were included. The incidence rate of elevated thyroid autoantibodies in the MMD group was significantly higher than that in other groups. Analysis suggests that thyroid autoimmunity-related or potential immune abnormalities play a role in the development of MMD (30). Leia conducted a similar study, selecting 28 patients with MMD and 28 healthy control group participants. Through a comparative analysis of their thyroid autoantibody levels, it was found that there is a correlation between MMD risk and elevated thyroid autoantibody levels (11). Numerous studies and analyses have shown that thyroid autoantibodies may play a crucial role in the progression of MMD. Although the mechanism of action is still unclear, it can be roughly summarized as the following aspects based on current research: an increase in the level of thyroid autoantibodies in patients may be a part of the reason for the immune imbalance in the MMD system (30). Consistent with this viewpoint, T-cell dysregulation is associated with the cell proliferation and vascular dysregulation observed in MMD. The deposition of autoimmune antibodies or immune complexes, as well as stimulation of blood vessels, leading to the thickening of vascular wall proliferation, may be another cause of MMD (31, 32). At the same time, the cross-reaction between TSH receptor antibodies and cerebral artery antigens can lead to autoimmune-mediated vasculitis. It may also cause the thickening and narrowing of the patient's blood vessel wall (33). Although there is a significant correlation between the two diseases, there are still some issues that need to be addressed. As MMD is already a rare disease, the number of cases coexisting with thyroid diseases is even rarer, resulting in a generally small sample size for such studies, which may lead to a lack of representativeness in experimental results. At present, literature research on this type of disease is mostly single center reports, and there are differences in sampling methods among different institutions, which may also lead to bias in experimental data. In addition, studies have shown that the increase in autoantibodies caused by Hashimoto's thyroiditis is also an independent risk factor for cerebrovascular accidents in patients (34). In addition, one of the moyamoya vascular types (MM type) is similar to the clinical manifestations of MMD. However, it is currently unclear whether MM type is an independent entity manifestation related to Hashimoto's disease or whether it is a true coincidence between MMD and Hashimoto's disease (35). These issues still need to be investigated by clinical workers.

Gene correlation

The major histocompatibility complex (MHC) encodes several key immune response genes, which are also known as human leukocyte antigens (HLA) regions in humans. It is located on chromosome 6p21 and the MHC gene, and it can be divided into three regions: MHC Class I, MHC Class II, and MHC Class III. As part of the adaptive immune response, many MHC gene products are involved in the inflammatory response and interact with natural killer cells and cytokines (36). The missense mutation of the gene in RNF213 is independently related to the occurrence of MMD, which has been reported many times (37, 38). In recent studies, it has been found that the genetic susceptibility of MMD is also related to the HLA gene. Wan et al. studied a characteristic cohort of 755 Chinese Han MMD patients and 2,031 healthy control patients. After relevant analysis, it was determined that the common variant rs3129731 within the HLA locus is the main genetic risk factor for obtaining MMD. In their study, a significant association was found between HLA class I and class II genes and MMD risk. These two genes are important components of humoral and cellular immunity, indicating that both types of immunity are involved in MMD. Their team found that the genetic polymorphisms of HLADQA2 and HLA-B may be genetic susceptibility factors for MMD in the Chinese Han population (39). In Japanese patients with MMD, there is a significant correlation between MMD and HLA-DR1 (40); HLA-DRB1 * 1501 and HLA-DQB1 * 0502 are also correlated with MMD (41). Hong concluded after studying the HLA gene in Korean patients with MMD that HLA-DRB1 * 1302 and HLA-DQB1 * 0609 are more likely to show association in familial MMD patients (42). Although there are differences in HLA among patients in Japan, the Republic of Korea, and China, the genetic susceptibility of MMD in the HLA genome has been recognized. In Graves' disease, the HLA class II DRB1, DQB1, and DQA1 genomes have been confirmed to be related to their occurrence and have demonstrated their induction of DR3 and protection against DR7 (43). At the same time, studies have also shown that the HLA genome is also associated with the occurrence of Hashimoto's thyroiditis, and HLA plays an important role in the development of thyroid autoimmune diseases (44, 45). At the same time, Tashiro conducted a case-control study on the association between HLA and MMD. Analysis shows that HLA-DRB10410 is a risk allele for MMD, and that this gene is also associated with thyroid diseases in MMD patients. Tashiro's research proves that there is a possible genetic association be"ween MMD and thyroid diseases (46). In a reported pathological analysis, a mother and daughter were diagnosed with both MMD and Graves' disease. Tokimura

believed that a gene locus (chromosome 8q23, MYMY3) of MMD is very close to autoimmune thyroid diseases (8q23–24), which may lead to the coexistence of the two (47). Although the possible pathogenic genes are also present in the HLA genome and the gene loci are very similar, the gene sequences shown in the article exhibit differences, and data from different regions show different experimental results. Therefore, to clarify the genetic correlation between autoimmune diseases more clearly, more academic research in this area is still needed.

Treatment

Patients with both MMD and thyroid diseases are currently treated differently from those with simple MMD. Some literature suggests that a patient with both MMD and Graves' disease was admitted due to acute cerebral infarction. Later, it was found that the patient was in a state of thyrotoxicosis and was discharged after receiving thyroid drug treatment. A few months later, a follow-up examination revealed a significant increase in cerebral blood flow compared with previous cerebral blood flow. It is believed that the treatment of thyroid diseases can effectively delay the development of MMD (48). At the same time, Choi also shared a case in which a patient sought medical attention for thyrotoxicosis accompanied by seizures and ischemic stroke after stopping anti-thyroid treatment for 5 weeks. A brain computed tomography scan diagnosed them as having MMD and the patient's symptoms were alleviated through surgical bypass surgery. However, after the surgery, the patient developed seizures accompanied by septic cardiogenic shock and mixed atrial fibrillation, ultimately leading to cardiac arrest and death. The author believes that it may be necessary to correct thyroid function to a normal level before undergoing revascularization surgery, which can reduce the risk of perioperative and postoperative complications (49). Endo's research also supports this conclusion (50). Due to the different clinical treatment methods for elevated thyroid hormones and elevated thyroid autoantibodies, there are also differences in the treatment plans for patients with MMD. In most cases, after controlling hyperthyroidism, the symptoms of cerebral ischemic events caused by hyperthyroidism improved. However, in the cases studied by Ohba, the symptoms of a transient ischemic attack occurred during hypothyroidism rather than hyperthyroidism. Therefore, after excluding variables, they believed that the vascular changes in the cases were caused by immune-mediated mechanisms. It is speculated that for patients with hyperthyroidism accompanied by vascular changes and exacerbation of MMD symptoms, anti-hyperthyroidism treatment may alleviate the condition. However, if the increase in antibodies caused by autoimmune diseases is believed to be mainly related to vascular changes and symptoms, simple anti-thyroid therapy does not seem to have a significant improvement in the symptoms of MMD patients. At this point, surgical treatment may be more beneficial for the patient's prognosis (31). It may be good for the treatment and prognosis of patients with MMD to use drugs or surgery to reduce the level of thyroid hormones in patients, prevent the occurrence of thyrotoxicosis, and detect and treat autoantibodies, and it needs further research in the future. For patients with severe cerebrovascular symptoms and poor efficacy in simple thyroid treatment, controlling thyroid diseases, using bypass surgery and other methods to restore cerebral blood supply in a timely manner may achieve good results in improving symptoms, quality of life, and long-term disease recovery (51, 52). For MMD patients suspected of concurrent thyroid diseases, timely screening and monitoring of their thyroid function and thyroid autoantibodies, as well as regular follow-up imaging changes, early diagnosis, and selection of appropriate treatment methods based on the situation, may help guide subsequent clinical management and improve patients' later treatment and recovery.

Discussion and limitations

With the advancement of medical technology, there are more and more reports of MMD combined with thyroid diseases, but the specific mechanisms and treatments are not very clear (Table 1). The reported literature lacks large-scale multicenter prospective research, and the number of studies on molecular mechanisms is also relatively scarce. It is still unclear whether thyroid gland disease may be involved in the occurrence and development of MMD, or whether moyamoya disease patients are more prone to thyroid diseases. In the future, more attention should be paid to this issue, and clinical workers need to increase their research in this area. This may help reveal the pathogenesis of MMD, providing reference for the treatment of thyroid diseases complicated by MMD, guide subsequent clinical management, and improve patients' treatment and recovery.

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Conflict of interest

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Identification of optimal feature genes in patients with thyroid associated ophthalmopathy and their relationship with immune infiltration: a bioinformatics analysis

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Background: Thyroid associated ophthalmopathy (TAO) is an organ-specific autoimmune disease that has a significant impact on individuals and society. The etiology of TAO is complicated and poorly understood. Thus, the goal of this study was to use bioinformatics to look into the pathogenesis of TAO and to identify the optimum feature genes (OFGs) and immune infiltration patterns of TAO.

Methods: Firstly, the GSE58331 microarray data set was utilized to find 366 differentially expressed genes (DEGs). To find important modular genes, the dataset was evaluated using weighted gene coexpression network analysis (WGCNA). Then, the overlap genes of major module genes and DEGs were further assessed by applying three machine learning techniques to find the OFGs. The CIBERSORT approach was utilized to examine immune cell infiltration in normal and TAO samples, as well as the link between optimum characteristic genes and immune cells. Finally, the related pathways of the OFGs were predicted using single gene set enrichment analysis (ssGSEA).

Results: KLB, TBC1D2B, LINC01140, SGCG, TMEM37, and LINC01697 were the six best feature genes that were employed to create a nomogram with high predictive performance. The immune cell infiltration investigation revealed that the development of TAO may include memory B cells, T cell follicular helper cells, resting NK cells, macrophages of type M0, macrophages of type M1, resting dendritic cells, active mast cells, and neutrophils. In addition, ssGSEA results found that these characteristic genes were closely associated with lipid metabolism pathways.

Conclusion: In this research, we found that KLB, TBC1D2B, LINC01140, SGCG, TMEM37, and LINC01697 are intimately associated with the development and progression of TAO, as well as with lipid metabolism pathways.

KEYWORDS

thyroid associated ophthalmopathy, optimal feature genes, pathogenesis, immune infiltration, GEO

Introduction

Thyroid associated ophthalmopathy (TAO), also known as thyroid eye disease, Graves' ophthalmopathy, or Graves' orbitopathy, is a disabling and aesthetically unpleasing condition of the orbit that is typically linked to hyperthyroidism caused by Graves' disease (1). The frequency of TAO ranges from 0.54 to 3.3 cases per 100,000 people per year, with females being more affected than males (2). TAO causes eyelid retraction, diplopia, exophthalmia, exposed keratitis, corneal opacity, and ulcers, as well as compression optic neuropathy, which affects eyesight in extreme instances (3). Patients with TAO have a higher prevalence of mild and non-progressive cases, with moderate to severe cases affecting only 5–6% of patients. Even mild TAO can have an impact on patients' quality of life (QoL) and pose a significant threat to public health (4).

Most of the signs and symptoms of TAO are brought on by increased orbital bone volume pressure caused by orbital soft tissue dilation. The pathogenesis of TAO is complex and is believed to be mainly related to environmental, genetic and immune factors. Many factors that influence TAO, including race, sex, age, smoking history (5), radiation stimulant hormone (RAI) therapy (6), Hypercholesterolemia (7), Oxidative stress and thyroid-stimulating hormone receptor antibodies, have been confirmed by studies (1, 8). It is currently believed that pathological autoimmune responses are targeted at cross-reacting autoantigens in the thyroid and retrobulbar tissues (3, 9). Cytokines and immune mechanisms are considered to play a major role in the pathogenesis of TAO. However, the underlying mechanism of TAO is still unknown, and existing therapies can only slow the disease's development. Therefore, further studies on the pathogenesis and potential therapeutic targets of TAO are urgently needed.

In this study, bioinformatics analysis, WGCNA and three machine learning methods were used to screen and identify best feature genes of TAO. For the purpose of further understanding the molecular immune mechanism during the development of TAO, the CIBERSORT method was then applied to systematically analyze the infiltrating fraction of 22 different types of immune cells in TAO and normal specimens. This analysis also examined the association between OFGs and infiltrating immune cells. Finally, ssGSEA was performed to predict the possible related pathways of OFGs.

Materials & methods

Data collecting and analyzing

We used the keyword graves ophthalmopathy to filter out the GSE58331 microarray, which contained data on TAO anterior orbit tissue and normal anterior orbit tissue, from the Gene Expression Omnibus database. The GSE58331 dataset included 175 samples (anterior orbital tissue or lacrimal gland tissue), 29 of which were from healthy individuals and the remainder from patients with inflammatory diseases such as NSOI, sarcoidosis, GPA, and TAO. The collection contained 35 TAO samples, comprising 27 samples of anterior orbital tissue and 8 samples of lacrimal glands. Forty-nine samples from GSE58331 were selected, including 27 anterior orbital tissue samples from patients with TAO and 22 anterior orbital tissue samples from normal subjects.

Differentially expressed gene analysis and WGCNA

The R (version 4.2.0) limma package was applied for examining differentially expressed genes (DEGs) from datasets. Statistical differences were defined as a P value <0.05 and a fold change (FC) ≥ 1.5 ($|\log2FC| \geq 0.584963$). Due to the interconnection of gene sets and the relationship between gene sets and phenotypes, WGCNA was used to find highly synergistic gene sets and identify biomarkers (10). The module's minimum number of genes is set to 30, and the "WGCNA" R package (11) was employed to build a coexpression network for the GSE58331. Correlation analysis was used to identify the crucial modules, and the hub genes were chosen from the genes in those modules.

DO, GO and KEGG enrichment analyses

The intersection of DEGs with hub genes is referred to as key genes. Using the "clusterProfile", "enrichplot", "ggplot2", "org.Hs.eg.db", "GOplot", and "DOSE" packages in R, we conducted a disease ontology (DO), gene ontology (GO), and Kyoto Encyclopedia of Genes and Genomes (KEGG) study to ascertain the function of the key genes.

Screening of OFGs

Three machine learning methods were utilized to select OFGs in our study. To determine the optimal penalty value with the least binomial deviation, the R package "glmnet" was utilized for least absolute shrinkage and selection operator (LASSO) (12) logistic regression analysis. The R packages "e1071", "kernlab", and "caret" were used to carry out the support vector machine recursive feature elimination (SVM-RFE) (13) method, and the result with the minimum cross validation error was studied. The RF method to determining the level with the least amount of error was developed by the R package "randomForest". By intersecting the outcomes of these three machine learning algorithms, the final OFGs will be obtained.

Validation of OFGs

The diagnostic significance of OFGs in TAO was assessed using receiver operating characteristic (ROC) curves created in R by using the "pROC" tool. The area under the ROC curve (AUC) showed the diagnostic effectiveness of OFGs.

Infiltrating immune cell evaluation and correlation analysis

A violin diagram was used to visually depict the differences in immune cells between TAO and control samples. The R software was employed to examine the relationships between OFGs and infiltrating immune cells.

Single-gene gene set enrichment analysis

To ascertain the relative enrichment of each HALLMARK pathway in the samples, ssGSEA was performed in R using the "GSVA" package. Finally, the correlation analysis between OFGs and pathway was carried out.

Results

Screening of DEGs

Figure 1 shows an overview of the present study. We performed differential gene expression analysis to investigate gene expression in TAO patients and normal samples. A total of 366 DEGs were screened from GSE58331 data set, including 69 upregulated and 297 downregulated genes (Figures 2A, B).

Screening of target modules and genes based on WGCNA

As shown in Figure 3A, clustering analysis of all samples showed that the GSM1407185 sample was poorly clustered. Therefore, this sample was excluded as an outlier in the WGCNA

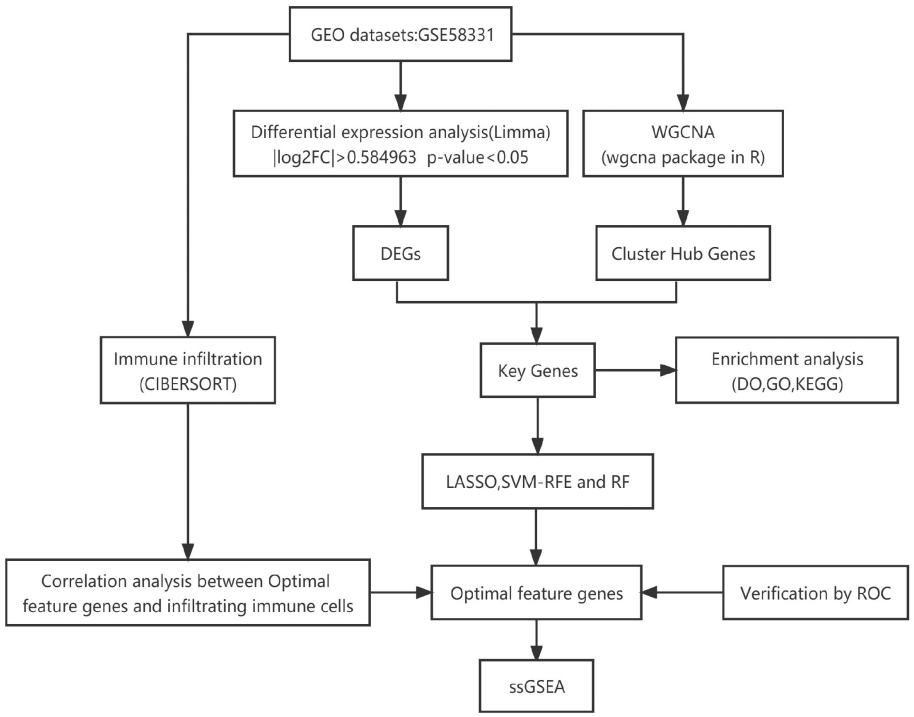


FIGURE 1
The flow chart of this investigation.

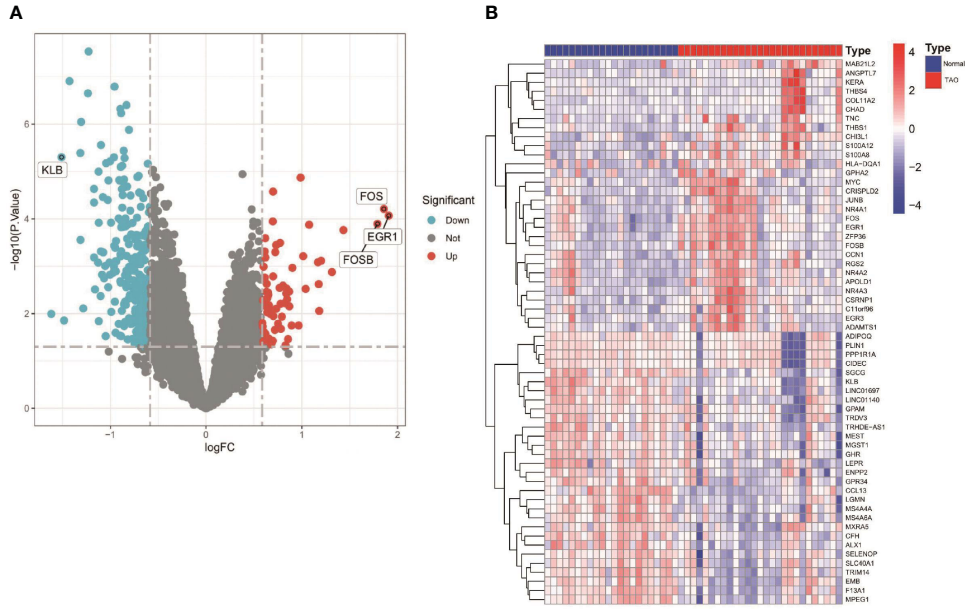


FIGURE 2
Differentially expressed genes (DEGS) in orbital adipose tissue between the TAO and Normal individuals. **(A)** Volcano plot of DEGs. Data points in red are up-regulated genes, and in blue are down-regulated genes. The top up-regulated and down-regulated genes are shown. **(B)** A Heatmap of the top 30 up-regulated DEGs and top 30 down-regulated DEGs are shown.

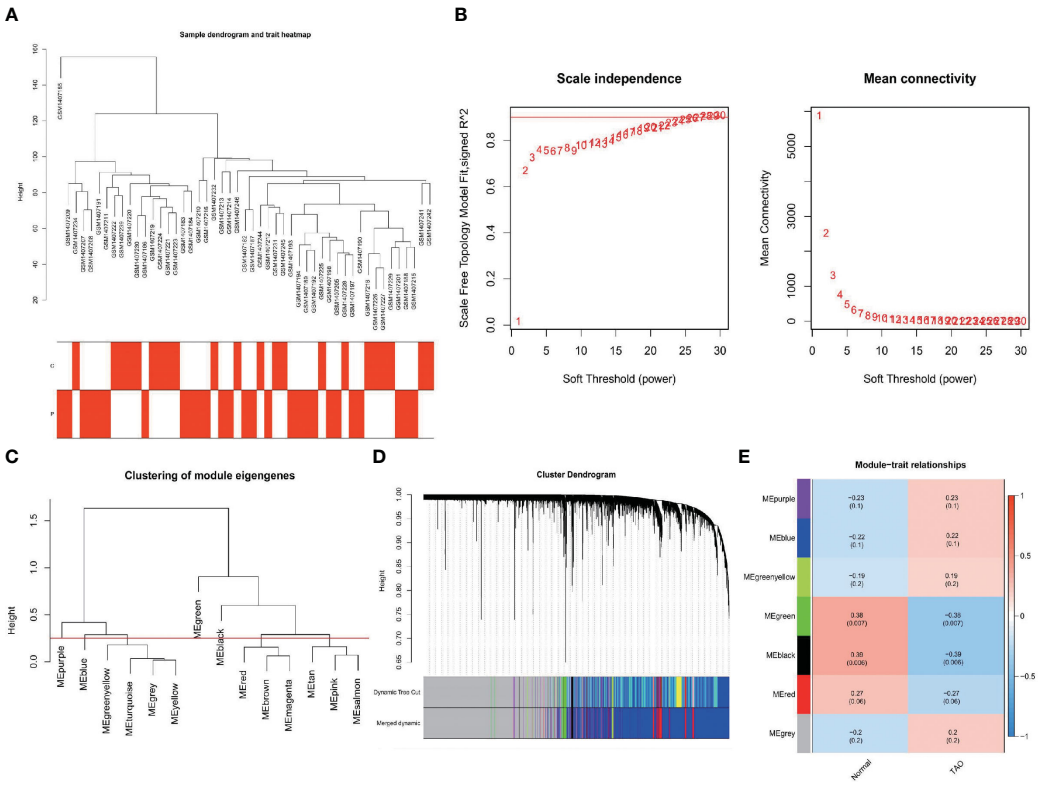


FIGURE 3
Construction of WGCNA networks. **(A)** Sample dendrogram and trait heatmap. The two traits are TAO and normal. **(B)** Scale independence and mean connectivity of various soft-thresholding values. **(C)** Clustering of module eigengenes. **(D)** Gene dendrogram and modules color. **(E)** Module-trait relationships.

analysis. When β=26, the scale-free distribution and gene connections were most consistent, according to the study of soft threshold selection (Figure 3B). By combining modules with feature factors greater than 0.75 and limiting the smallest number of genes in the module to 30 in the weighted gene coexpression network, seven coexpression modules were examined further (Figure 3C). MEgreen and MEblack are the two modules with the strongest correlation, according to our investigation into module correlation (Figure 3D). For additional investigation, 483 genes were chosen from the two modules.

Functional enrichment analyses

To obtain 63 key genes, the intersection of the EDGs and the WGCNA key module genes was employed (Figure 4A). To further understand the roles of those genes, function enrichment analysis was performed. Disease ontology (DO) enrichment analysis showed that it was mainly concentrated in coronary artery disease, skin disease, integumentary system disease, inherited metabolic disorder and nutrition disease (Figure 4B). For biological process (BP), mainly related to generation of precursor metabolites and energy, digestion, response to fatty acid and white fat cell differentiation. For cellular component (CC), it mainly contains mitochondrial outer membrane, organelle outer membrane, outer membrane and lipid droplet. Peptide binding, amide binding, growth factor binding, and STAT family protein binding were highly enriched for molecular function (MF) (Figure 4C). The key genes were predominantly abundant in the PPAR signaling pathway, histidine metabolism, tryptophan metabolism, regulation of lipolysis in adipocytes, and drug metabolism-cytochrome P450, according to the results of KEGG pathway analysis (Figure 4D).

Screening and verification of optimal feature genes

The 63 key genes were employed in LASSO, SVM-RFE, and RF analyses to select the OFGs in TAO. The LASSO logistic regression approach was used in this work to discover 8 major biomarkers from key genes (Figure 5A). The SVM-RFE method identified 34 genes as important biomarkers (Figure 5B). Furthermore, using the RF technique, 11 genes were identified as critical biomarkers (Figures 5C, D). KLB, TBC1D2B, LINC01140, SGCG, TMEM37 and LINC01697 were overlapping genes in the three algorithms

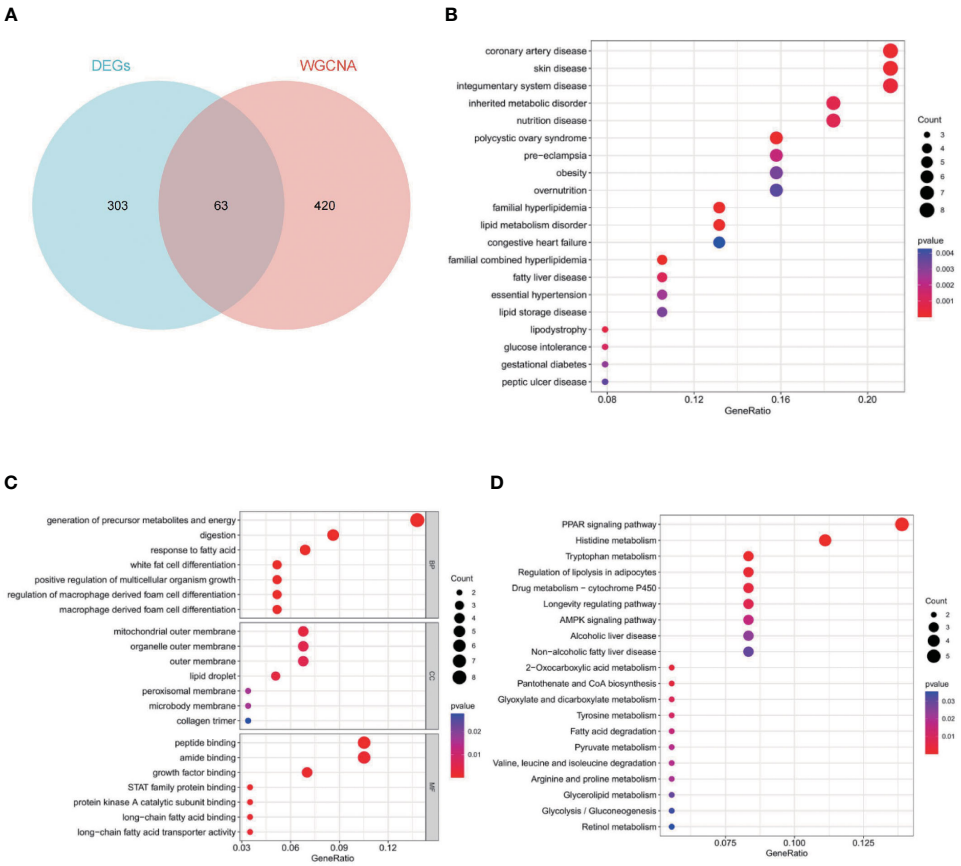


FIGURE 4
Sixty-three key genes and their functional analysis. The bubble shows significant items according to the P-value. **(A)** Venn diagram demonstrated the intersection set of WGCNA and EDGs. **(B)** Disease Ontology (DO) enrichment analysis. **(C)** Gene Ontology (GO) enrichment analysis. BP, biological processes; CC, cellular components; MF, molecular functions. **(D)** Gene Set Enrichment Analysis (GSEA).

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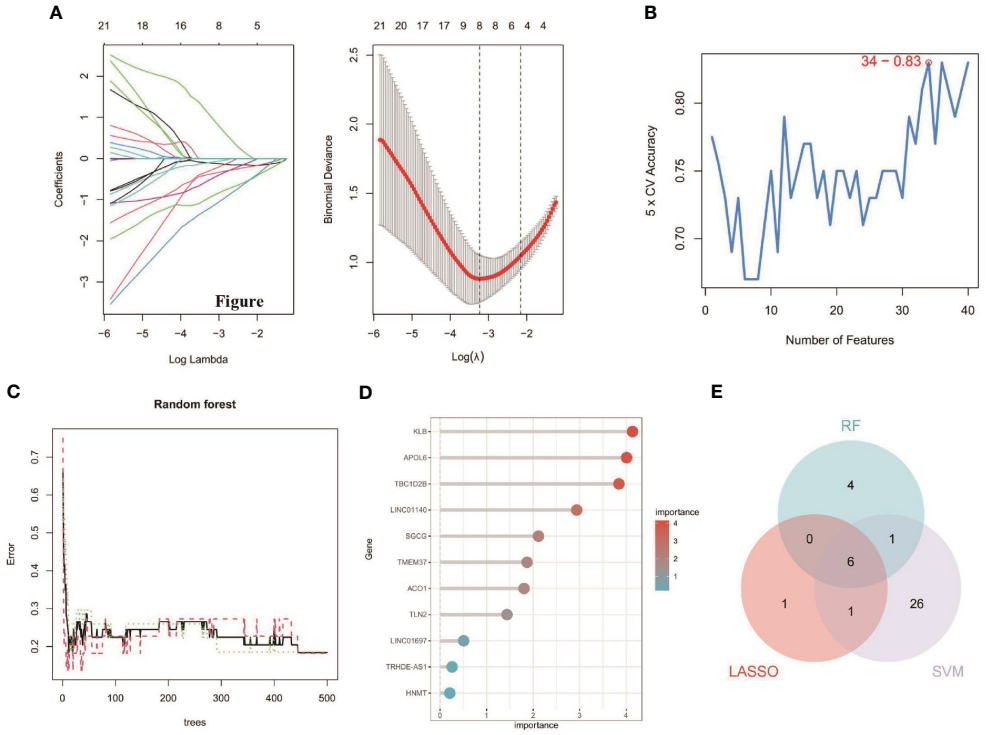


FIGURE 5
Detection of diagnostic biomarkers for TAO. **(A)** Biomarker detection using LASSO regression analysis. **(B)** biomarker detection by SVM-REF. **(C, D)** Biomarker detection by random forest. **(E)** Venn diagram shows the shared diagnostic markers between LASSO, SVM-REF, and random forest.

(Figure 5E), and these 6 genes were down-regulated genes (Figure 6A). The predictive nomogram was produced using a scoring method in which the relative expression of each gene was given a value, and the sum of all the gene scores was used to get the overall score. The nomogram's ROC analysis produced an AUC of 0.860, indicating a strong predictive value (Figure 6B). With AUCs of 0.857, 0.865, 0.830, 0.847, 0.781, and 0.859, respectively, the ROC curves of KLB, TBC1D2B, LINC01140, SGCG, TMEM37, and LINC01697 demonstrated their likelihood as important biomarkers (Figure 6C), demonstrating that the six biological indicators exhibited high prediction accuracy.

Infiltration of immune cells results

To find variations in immune infiltration between normal and TAO specimens, CIBERSORT was used (Figures 7A, B). As indicated from the correlation heatmap of the 22 types of immune cells, activated mast cells and resting NK cells, activated dendritic cells and naive B cells, T cells gamma delta and memory B cells and resting mast cells and resting T cells CD4 memory displayed a significant positive correlation, respectively. T cells regulatory (Tregs) and M2 macrophages, Tregs and resting mast cells, M0 macrophages and M2 macrophages, naive T cells CD4 and

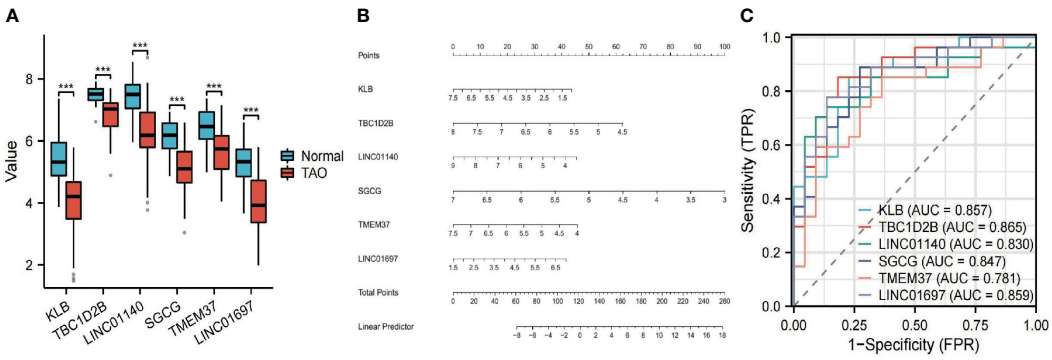


FIGURE 6
(A) The expression difference of the six genes between TAO and Normal. ***, P < 0.001. **(B)** Based on the six genes, a nomogram was constructed for TAO. **(C)** The predictive value of the six genes in TAO from the ROC curve. Each panel displayed the AUC under the curve and 95% CI.

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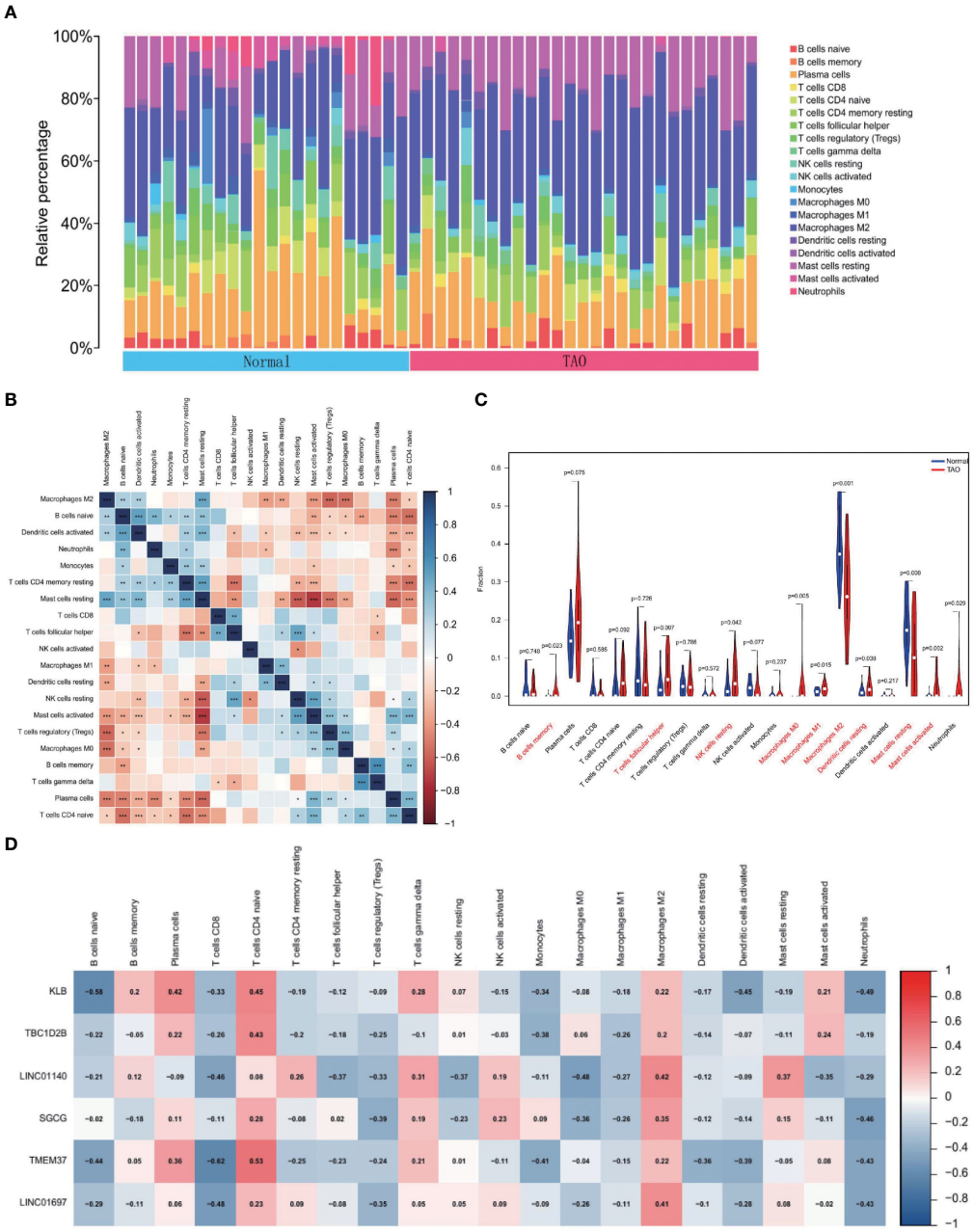


FIGURE 7
Immune cell infiltration analysis. **(A)** The bar plot shows the proportion of immune cells in different samples. **(B)** Heatmap of correlation in 22 types of immune cells. Blue represents a positive correlation, and red represents a negative correlation. Darker color implies stronger association. *P < 0.05; **P < 0.01; ***P < 0.001. **(C)** Violin diagram of the proportion of 22 types of immune cells. The red marks represent the difference in infiltration between the TAO and Normal samples. **(D)** Correlation analysis of immune cell infiltrations with six optimal feature genes. Red represents a positive correlation, and blue represents a negative correlation. Darker color implies stronger correlation.

naive B cells, naive T cells CD4 and resting T cells CD4 memory, resting NK cells and resting mast cells, activated mast cells and resting mast cells, plasma cells and resting mast cells and T cells follicular helper and resting T cells CD4 memory displayed significant negative correlations, respectively. TAO samples generally contained more memory B cells, T follicular helper cells, resting NK cells, M0 macrophages, M1 macrophages, resting dendritic cells, activated mast cells, and neutrophils compared to normal samples. However, the proportions of M2 macrophages and resting mast cells were relatively lower (Figure 7C). Correlation heat map (Figure 7D) showing correlation analysis of 6 characteristic genes with immunoinfiltrating cells. KLB revealed a positive association with plasma cells and naive T cells CD4 and a negative correlation with naive B cells, activated dendritic cells, and neutrophils based on the findings of correlation analysis. TBC1D2B and naive T cells CD4 displayed a favorable

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connection. M2 macrophages and LINC01140 exhibited a positive association, whereas T cells CD8 and M0 macrophages and LINC01140 showed a negative correlation. Neutrophils showed a negative connection with SGCG. T cells CD8, naive B cells, monocytes, and neutrophils all showed a substantial negative connection with TMEM37, but naive T cells CD4 showed a positive correlation. The findings also revealed that LINC01697 substantially linked favorably with M2 macrophages and negatively with T cell CD8 and neutrophils.

ssGSEA

According to the results of ssGSEA (Figure 8), it is not difficult to find that the six OFGs are positively correlated with heterogeneous biological metabolism, fatty acid metabolism, bile acid metabolism and fat formation to varying degrees. All OFGs except SGCG were positively correlated with cholesterol homeostasis. In addition, KLB, LINC01140, LINC01697 and SGCG were positively correlated with the peroxisome gene set.

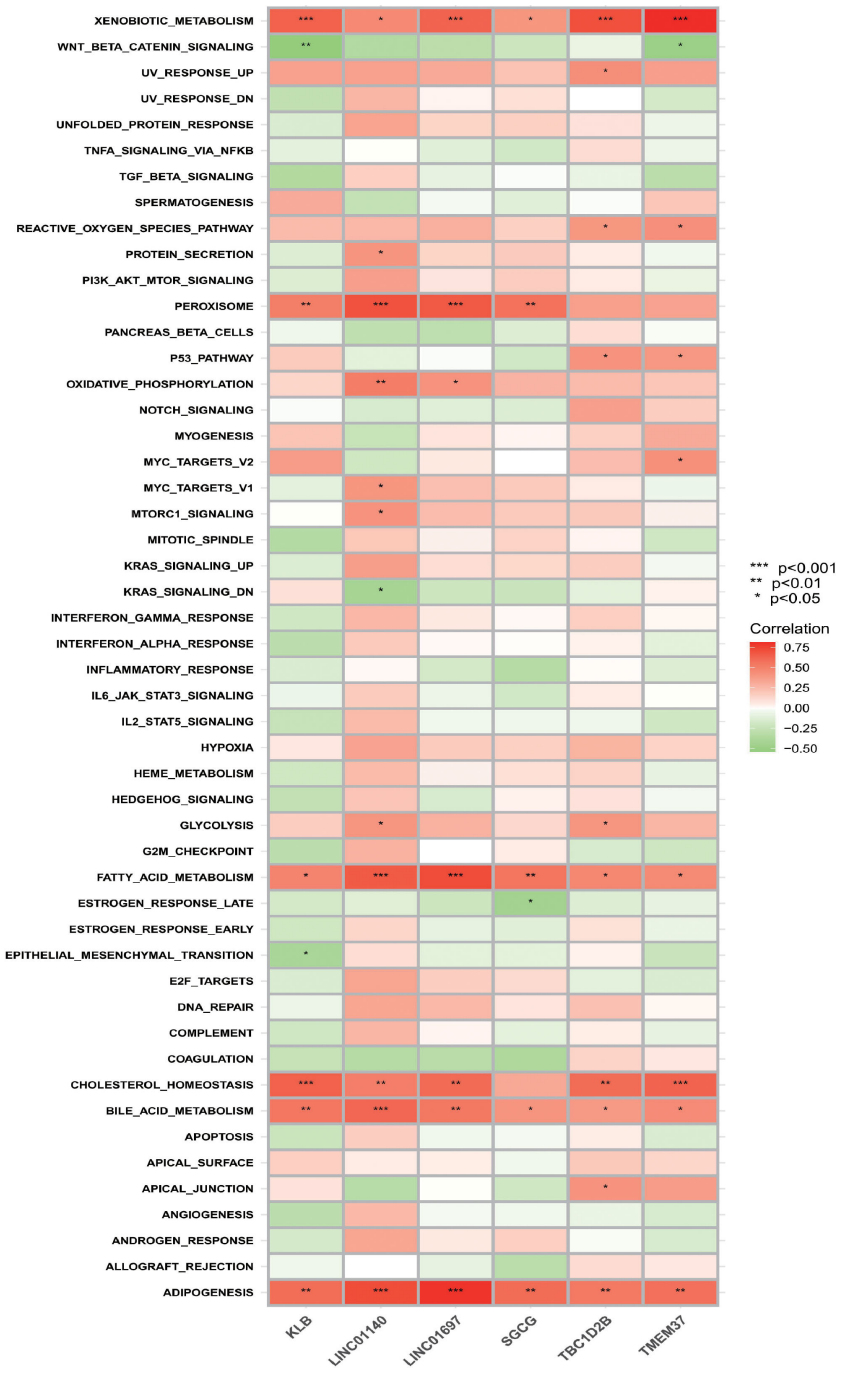


FIGURE 8
Correlation between OFGs and gene sets. Red represents a positive correlation, whereas green represents a negative correlation. The darker the color, the higher the correlation.

Discussion

Thyroid associated eye disease (TAO) is an autoimmune thyroid associated orbital disease occurring in the eye and retrobulbar tissues, and has the highest incidence among adult orbital diseases. When the symptoms are severe, the appearance will be significantly affected and the vision of the patient will be endangered. At this time, the treatment effect of drugs and surgery is poor (2). The pathogenesis of TAO is complex, with the main processes including cytokine production, inflammation, hyaluronic acid synthesis, adipogenesis, and myofibrillogenesis (14). Understanding the molecular mechanism of TAO is very important for early diagnosis and precise treatment. Therefore, the discovery of characteristic biomarkers is crucial.

In our research, 63 key genes in TAO were evaluated using DEG and WGCNA, followed by DO, GO and KEGG pathway enrichment analyses. Intriguingly, DO pathway enrichment analyses showed that the majority of DEG-enriched diseases were linked to coronary heart disease, skin disease, integumentary system disease and familial hyperlipidemia, highlighting their potential roles in the development of TAO. Based on GO and KEGG enrichment studies, we preliminarily found that these genes are closely correlated with biological processes such as adipose differentiation and metabolism (response to fatty acid, white fat cell differentiation) and their related pathways (PPAR signaling pathway, regulation of adipocyte lipolysis, fatty acid degradation). Thereafter, three machine learning algorithms were used to pick the best feature genes connected to TAO. Ultimately, ROC curves and a nomogram were created to determine the OFGs' diagnostic value. Six genes (KLB, TBC1D2B, LINC01140, SGCG, TMEM37, and LINC01697) were eventually found to have superior performance in discriminating between TAO and normal samples.

The βKlotho protein, encoded by the KLB gene, was first discovered in 2000 (15). In order to act as the physiological receptors for FGF21 and FGF19, respectively, βKlotho forms binary complexes with FGFR1c, which is expressed by adipocytes, and FGFR4, which is expressed by hepatocytes (16, 17). The liver and adipose tissue generate fibroblast growth factor-21 (FGF-21), a protein that belongs to the FGF family (18). Numerous types of physiological stress, such as hunger and inflammation, cause an increase in FGF21 levels in the blood. FGF21 interacts with the βKlotho-FGFR1c complex, causing a change in catabolic metabolism (19). Only a few tissues express βKlotho, such as the liver and both white and brown adipose tissue (20). In contrast to healthy control samples, our research found that the anterior orbital tissue of TAO patients had significantly lower levels of the KLB gene, which codes for the protein βKlotho. These results were inconsistent with the high expression of βKlotho in white adipose tissue. The significant downregulation of KLB gene in TAO orbital adipose tissue will reduce the FGF21-induced fat catabolism, which may be one of the reasons for increased orbital adipose tissue in patients with hyperthyroidism.

The TBC1D2B gene, which codes for a GTPase-activating protein involved in membrane trafficking, interacts with the early endosomal marker protein RAB5. The Rab family of small GTPases regulates internal membrane traffic by promoting the production, transport, and fusion of membrane-bound organelles and vesicles in eukaryotic cells (21). The expression of E-cadherin, which is essential for maintaining cell-cell interactions in physiology, is reduced during the epithelial-to-mesenchymal transition (EMT), which is a significant event (22). E-cadherin endocytosis has been demonstrated to be regulated by TBC1D2B, and its internalization and degradation were enhanced when TBC1D2B was downregulated (23). we hypothesize that TBC1D2B deletion may accelerate E-cadherin degradation, lead to EMT abnormalities in the anterior orbital tissue, epithelial cell differentiation into fibroblasts, and promote the development of fibrosis.

The long intergene non-coding RNAs LINC01140 and LINC01697 are able to cis-regulate transcription of nearby protein-coding genes and trans-regulate transcription of distal protein-coding genes (24). Low levels of LINC01140 have been linked to poor overall survival, poor disease-specific survival, and poor disease-free survival, according to research by Hu et al. (25) in metastatic sarcomas. Li et al.'s study (26), which found that LINC01140 expression was dramatically downregulated in breast cancer, suggests that it may be used as a biomarker to gauge a patient's prognosis. Additionally, LINC01697 has been identified as a possible prognostic biomarker for gastric cancer (27, 28)and oral squamous cell carcinoma (29, 30). According to recent research, the co-expression relationship of differentially expressed lncRNA and extracellular matrix related mRNA implies that lncRNA may be involved in the control of extracellular matrix remodeling in TAO orbital fat/connective tissue (31). Furthermore, the lncRNA-miRNA-mRNA network was built using high-throughput sequencing of orbital tissue, which was likewise linked to the etiology of TAO (32). LINC01140 has been shown in cell tests to control macrophage M2 polarization (33) and lessen their inflammatory response (34). However, no investigations on how LINC01697 operates in non-tumor cells have been published.

Sarcolemmal transmembrane glycoprotein (SGCG) is a component of the sarcoglycan complex, which connects the extracellular matrix with the F-actin cytoskeleton in muscle cells (35). Skeletal muscle fibrosis frequently occurs due to an SGCG deficiency-related vulnerability to muscle injury. After repeated cycles of degeneration, collagen and other extracellular matrix elements replace the muscle tissue, which causes the development of scar tissue and accelerates the course of the illness (36). TMEM37 is a transmembrane protein (37) involved in the transmembrane transport of calcium ions and the regulation of ion transmembrane transport. At present, there are few studies on transmembrane protein TMEM37, and its more detailed biological function is not clear. Six OFGs (KLB, TBC1D2B, LINC01140, SGCG, TMEM37, and LINC01697) are expected to affect TAO progression and serve as diagnosis indicators in light of the findings discussed above. Nevertheless, a significant number of clinically relevant articles are still needed to confirm their diagnostic value.

The current study used CIBERSORT to evaluate the immune infiltrating process within TAO in order to more precisely explore

the impacts produced by immune cell infiltration. The infiltration of memory B cells, T cells follicular helper, resting NK cells, M0 Macrophages, M1 Macrophages, resting dendritic cells, activated Mast cells and Neutrophils increased, while the infiltration of M2 Macrophages and resting Mast cells decreased, probably showing associations with TAO occurrence and progresses. Moreover, we observed that the six genes were associated with many immunocytes. T follicular helper (Tfh) cells are identified as a unique T-cell subset that aids in germinal center formation, B-cell growth and affinity maturation, and immunoglobulin class flipping as an essential component of adaptive immunity (38). Tfh cells feature the characteristic transcription factor B-cell lymphoma 6, distinct surface markers such as chemokine receptor 5, T-cell costimulatory factors, and programmed cell death 1 and distinct cytokine expression patterns (such as IL-21, IL-4, and IL-10). Serum IL-21 levels significantly increased in Graves' disease (GD) patients, decreased following therapy, and were positively correlated with the severity of GD, according to zhang et al.'s (39) research. In addition, thyroid tissues from GD patients showed higher numbers of Tfh cells and markers associated with them (CLCX13 and CXCR5) (40). Knowing how Tfh cells and IL-21 are involved in the immune system may help develop treatment strategies for autoimmune illnesses due to their active roles in the immune system. At present, there are few studies on Tfh cells in the pathogenesis of TAO, which can be further studied in the future. Depending on their various phenotypes, macrophages mediate various impacts on immunometabolism. When M1 macrophages are stimulated by lipopolysaccharide and interferon, they release tumor necrosis factor, IL-1, IL-6, and other proinflammatory cytokines, whereas M2 macrophages are stimulated by IL-4 and IL-13, and their anti-inflammatory cytokines include IL-10 and arginase-1 (41). Therefore, it is not difficult to understand that the M1 macrophages in the anterior orbital tissue of TAO are increased and the M2 macrophages are decreased compared with the normal subjects, and these OFGs (especially LINC01140 and LINC01697) have a negative association with M1 macrophages and a positive association with M2 macrophages to varying degrees. Previous studies (42, 43) have found an increased number of mast cells near fibroblasts in TAO orbital tissue, suggesting that mast cells are involved in the activation of orbital fibroblasts. Mast cells are thus thought to be orbital fibroblast activity regulators in TAO, and they may be therapeutic targets for TAO (44). Furthermore, we discovered that immune cell infiltration and OFGs had a negative connection with neutrophils. This finding may suggest that downregulating OFGs facilitates neutrophil infiltration in the anterior orbital region.

In the final results of ssGSEA, it was easy to find that the selected characteristic genes were closely related to lipid metabolism-related pathways, which also suggested that lipid metabolism was associated with the occurrence and progression of TAO.

Our research adds to our understanding of TAO pathogenesis, although it has certain limitations. First, our team only collected data from GEO databases, and sample size was small. More research studies with larger sample sizes are required to confirm the conclusions presented here. Second, while six genes have been identified as prospective TAO biomarkers, no *in vivo* or *in vitro* investigations have been undertaken, thus more research is needed to investigate the function and probable processes of these genes in the illness.

Conclusion

In this research, we found that KLB, TBC1D2B, LINC01140, SGCG, TMEM37 and LINC01697 are intimately associated with lipid metabolism pathways and the pathogenesis and progression of TAO.

Data availability statement

Publicly available datasets were analyzed in this study. This data can be found here: the GEO (https://www.ncbi.nlm.nih.gov/geo) database.

Author contributions

CX and YW: As the first author of the study, performed all the data analysis and wrote the manuscript. YL, JY SW, LW, BZ, YC and PG: Responsible for collating research results and editing pictures. HL: As the instructor of this study, participated in the whole process of the study. All authors contributed to the article and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2023.1203120/full#supplementary-material

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Exploring the role of gut microbiota in autoimmune thyroid disorders: a systematic review and meta-analysis

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Background: Autoimmune thyroid diseases (AITDs) are characterized by unique immune responses against thyroid antigens and persist over time. The most common types of AITDs are Graves' disease (GD) and Hashimoto's thyroiditis (HT). There is mounting evidence that changes in the microbiota may play a role in the onset and development of AITDs.

Objective: The purpose of this comprehensive literature study was to answer the following query: Is there a difference in microbiota in those who have AITDs?

Methods: According to the standards set out by the PRISMA statement, 16 studies met the requirements for inclusion after being screened for eligibility.

Results: The Simpson index was the only diversity measure shown to be considerably lower in patients with GD compared to healthy participants, whereas all other indices were found to be significantly greater in patients with HT. The latter group, however, showed a greater relative abundance of Bacteroidetes and Actinobacteria at the phylum level, and consequently of Prevotella and Bifidobacterium at the genus level. The strongest positive and negative relationships were seen for thyroid peroxidase antibodies and bacterial load.

Conclusion: Overall, both GD and HT patients showed significant changes in the gut microbiota's diversity and composition.

Systematic review registration: https://www.crd.york.ac.uk/PROSPERO/, identifier CRD42023432455.

KEYWORDS

graves disease, hashimoto thyroiditis, autoimmune diseases, meta - analysis, mircrobiota

1 Introduction

Hashimoto's thyroiditis (HT) and Graves' disease (GD) are the most frequent kinds of autoimmune thyroid disorders (AITDs) (1). Their aetiology may be broken down into three categories: environmental variables, genetic susceptibility, and immune system dysfunction (2). Thyroid autoantigens such thyroglobulin (TG), thyroid peroxidase (TPO), and the thyroid-stimulating hormone receptor are reactive because of immune system dysfunction. Thyroid follicle cells and immune system cells are affected by the resulting inflammatory infiltration and cytokine production (1, 3). Although they both have an autoimmune basis, the consequences of HT and GD on thyroid function are different, leading to different clinical manifestations. In contrast to GD, the most abundant reason of Hyperthyroidism in iodine-sufficient areas, which is associated with heat intolerance, weight loss, anxiety, trembling, irritability, and tachycardia (4), while HT determines hypothyroidism, is linked to fatigue, weakness, weight gain, dry skin (5), anaemia (6), and predisposition to depressive conditions (7). Graves' ophthalmopathy (GO) is a common consequence of GD that causes symptoms including discomfort, excessive weeping, swelling of the eyelids, and sensitivity to light in 25-50% of patients. Corneal collapse Vision loss, and optic nerve neuropathy do occur, but only in a subset of individuals (8, 9)

The importance of microbiota in autoimmune illnesses has come into the spotlight recently. There is no one best pattern of gut bacteria since everyone has a different mix. Nonetheless, Firmicutes and Bacteroidetes are often the most abundant phyla. Factors such as gender, lifestyle age, pharmacological regimens, level of physical activity, and food all influence the microbiome's makeup (10, 11).

Dysbiosis has been linked to the development of autoimmune illnesses such rheumatoid arthritis, atopic dermatitis, type 1 diabetes, SLE, inflammatory bowel disease, and autoimmune neurological disorders (12). Patho-mechanism interactions in these disorders may be studied at the level of bacteria and their metabolites. There are a number of proposed patho-mechanisms in the literature for AITDs. First, changes in intestinal bacterial composition may enhance intestinal permeability (13), which is linked to a higher amount of zonulin (14, 15), a protein that regulates the communication between cells. When the tight junctions between enterocytes are loosened, antigens from the microbiota may get through and trigger the immune system via molecular mimicry (16). The architecture of autoantigens and several bacterial antigens in the colon are very similar. This resemblance suggests that antigens produced on orbital fibroblasts and thyroid follicle cells in GO may stimulate plasma cells to produce antibodies (17).Increased autoantibody synthesis by posttranslational protein modification is another consequence of dysbiosis. In addition, it promotes the emergence of AITDs by reshaping the Th1 helper lymphocyte pool into the Th2 subset and activating the Toll-like receptor-4 (18, 19).

Thyroid function is also profoundly influenced by microbiome metabolites. Short-chain fatty acids (SCFAs) have been the primary focus of research because of their ability to improve enterocyte integrity, shield against the invasion of pathogenic microorganisms, affect the immune response, and suppress inflammatory response (20, 21). Furthermore, SCFAs have a pivotal role in regulating the ratio of Th17 cells to Treg cells, which is linked to the onset of autoimmune disorders (17, 22).

In addition, the microbiome has a role in thyroid hormone metabolism, which is part of the gut-thyroid axis. Deiodinases have been found in the human gut in previous research. Animal studies have shown that intestinal bacteria may absorb deconjugated iodothyronine and compete with human albumin for binding thyroid hormones (13, 23). In addition, enterohepatic metabolism of thyroid hormones is reportedly aided by intestinal bacteria (24, 25). In addition, the microbiota affects the absorption of microelements including iodine, copper, iron, selenium, and zinc that are vital to the health of the thyroid gland. Although studies in animals have demonstrated reduced iodine absorption in microbially deficient subjects, no such correlation has been shown in people with small bowel syndrome or following bariatric surgery who are parenterally fed (20, 26, 27). However, in low selenium circumstances, bioavailability decreases due to competitive bacterial absorption of selenium (26, 28, 29). Investigation of the role of the microbiome in thyroid diseases is warranted by the wide range of publications describing interactions between the thyroid gland and the microbiome as shown in Figure 1. The PICO (Population, Intervention, Comparison, and Outcome) question that guided the development of this systematic review was "Is there a difference in the microbiome of those who have autoimmune thyroid diseases?"

2 Methodology

Tis meta-analysis is reported following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guideline (30), with its protocol registered in PROSPERO CRD42023432455.

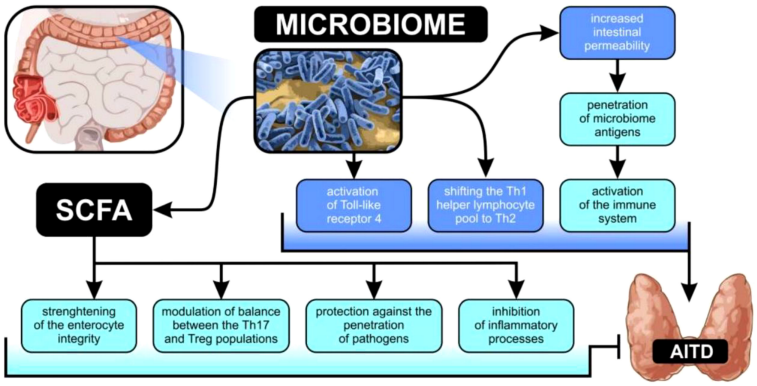


FIGURE 1
Possible connections between changes in gut microbiota and the development of autoimmune thyroid illness.

2.1 Search strategy and data extraction

A systematic literature search for relevant studies published in English was conducted in the databases Scopus, PubMed, and Web of Science from inception up to December 2022. These are some examples of queries looked up for: (ophthalmopathy OR3orbitopathy) OR (Graves OR thyroid) AND (thyroiditis OR disease] AND (Hashimoto OR Graves) AND (microbiota OR microbiome). Studies published after the year 2015 were included in the analysis. Two researchers independently reviewed the findings' titles, abstracts, and entire texts. PICO criteria was used to determine which studies would be included in this systematic review as shown in Table 1. In Figure 2, a comprehensive search process flowchart is shown.

2.2 Inclusion and exclusion criteria

The PICO parameters for this study are as follows. The inclusion criteria are patients with autoimmune thyroid diseases (AITD), including Graves' Disease (GD) and Hashimoto Thyroiditis (HT), aged 18-65 years, of both genders. The exclusion criteria are individuals with additional autoimmune disorders. Intervention and comparison are not applicable, and the outcomes are changes in the quantity, richness, and diversity of the microbiota and changes in the microbiota's composition without a clear indication of its diversity. The study design includes cohort, case-control, and cross-sectional studies, while conference reports, expert opinions, literature reviews, letters to the editor, case reports, and studies published before 2015 or not published in English are excluded.

2.3 Quality assessment

The "Study Quality Assessment Tool" developed by the National Heart, Lung, and Blood Institute at the National Institutes of Health was used to evaluate the potential for bias in each of the included studies. Both investigators filled out this form, and any discrepancies were discussed and settled. Supplementary Table 1 shows a summary of the quality ratings given to the different studies. Each possible risk criteria was assigned a point value from 1 (low) to 0.5 (uncertain) to 0 (high), and these values were added together to provide a summary of the critical evaluation. Twelve research (75%) were deemed to be of "good" quality (80% overall score), whereas four studies (25% total score) were deemed to be of "intermediate" quality. The Oxford Centre for Evidence-Based Medicine's diagnostic evidence categorization system was used to evaluate the quality of the available research. All the studies that were considered for inclusion provided either moderate or strong evidence (levels 3 and 4 on this 5-point scale).

2.4 Data synthesis and statistical analysis

Using Comprehensive Meta-analysis Software (CMA version 4) we plotted the meta-analysis findings as forest plots. The GD and HT subsets were used in the meta-analysis. The comparative richness at the phylum and genus levels was computed as a continuous variable, and the pooled standardized mean differences for diversity indices were determined.

3 Results

16 papers were included in this evaluation after the studies were screened using the exclusion and inclusion criteria. As a result, data from approximately 750 human individuals with diagnosed AITDs) and 488 controls were obtained in five different countries. Figure 1 displays the study' thorough selection process. Information on the publication year, study location, study contestants, AITD identification, exclusion and inclusion criteria, thyroid parameters measured, and supplementary drugs was gathered from each research that satisfied the inclusion criteria for this systematic review. Supplementary Table 1 lists all of the specifics, including the materials used in the lab, the microbiological procedures used, the changes in microbiota composition, and the variations in richness and diversity indices. Except for one study, which employed 16S rDNA gene sequencing, every other research looked at stool samples and studied it using 16S rRNA gene sequencing. Figures 3–6 show the plotted pooled standardized mean differences in the richness (ACE and Chao1) and diversity (Simpson and

TABLE 1 Evaluation of the study's overall quality, with special attention paid to the most serious sources of bias (risk category: green = low, yellow = uncertain, red = high; quality category: green = good, yellow = mediocre, red = bad).

	Clearly stated objective of the study	study population was well defined	Sample size justification	Study groups recruited from the same population	Inclusion and exclusion criteria clearly explained	Controls are well differentiated from cases	Randomization of participants	Clearly stated outcome measure	Participants blinded status	Appropriate statistical analysis	Quality score summarization
Ishaq et al. (2017) (31)											
Ishaq et.al., (2018) (32)											
Zhoa et al. (2018) (33)											
Shi et al. (2019) (34)											
Yang et al. (2019) (35)											
Carnejo-Pareja et al. (2020) (36)											
Liu et al. (2020) (37)											
Su et al. (2020) (38)											
Yan et al. (2020) (39)											
Cayres et al. (2021) (40)											
Chang et al. (2021) (41)											
Chen et al. (2021) (42)											
El-Zaway et al. (2021) (43)											
Jiang et al. (2021) (44)											
Shi et al. (2021) (45)											
Yang et al. (2022) (46)											

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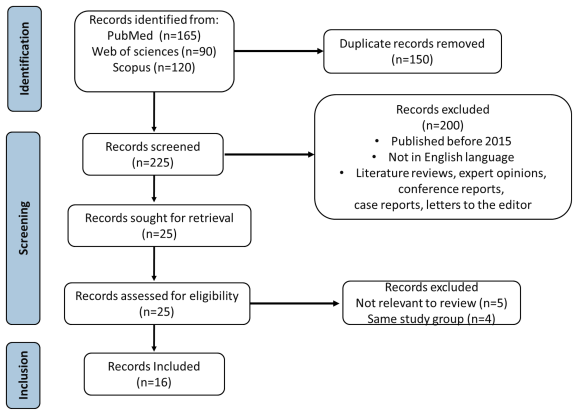


FIGURE 2
PRISMA Diagram.

Shannon) indices for GD and HT. Every value was lower in GD patients than in control groups, although ACE was exceptionally low. The ACE and Chao1 indices, however, had substantially higher mean values in HT patients than in healthy controls (p-values of 0.0059 and 0.08, respectively). Table 2 shows significant variations in relative abundance (at the genus and phylum levels) across the included studies (which provided p-values for evaluations).

4 Discussion

4.1 Alpha-diversity of the microbiota in people with AITD

Specific alpha-diversity indices are used to describe the variety of bacteria in the microbiome and the number of microbial species. Richness can be measured using indices like Chao1 and ACE, whereas diversity can be measured using indices like Simpson and Shannon (41).

Patients with GD had low levels of richness indices than healthy people in all trials included in this meta-analysis. Three studies (32, 38, 44) found statistically significant differences. Six research (34, 38, 39, 42, 44, 45) revealed considerably lower scores on the Shannon index, whereas three studies (38, 42, 45) reported significantly lower scores on the Simpson index, and one study reported significantly higher scores (44). One research showed drastically different findings, with GD patients showing higher values for all alpha-diversity indices (41). Despite these findings, the meta-analysis demonstrated a significant downward trend in all indicators in GD patients as compared to healthy controls. Hypothesized link between reduced diversity and inflammation caused by altered host immune function (44). A decline in microbial diversity has been linked to weakened ecosystem function, leaving it more vulnerable to harmful influences from the outside (47). Several clinical problems, including obesity, inflammatory bowel disease, colon cancer, diabetes, and polycystic ovarian syndrome have been linked to this condition (48–50). Interestingly, Chen et al. (42) found that methimazole therapy dramatically increased microbiome diversity after 3-5 months.

Richness indices were observed to be greater in HT participants than in healthy people in two investigations (31, 33). The Simpson and Shannon indexes were both found to have similar results in these analyses. Two studies (37, 43) found that the Shannon index was lesser in HT participants compared to healthy persons. Both richness indices and the Shannon index were shown to rise in HT patients by the current meta-analysis. This may be because hypothyroid individuals have prolonged gastrointestinal transit times due to intestinal dysmotility (51), making them more susceptible to bacterial overgrowth. An

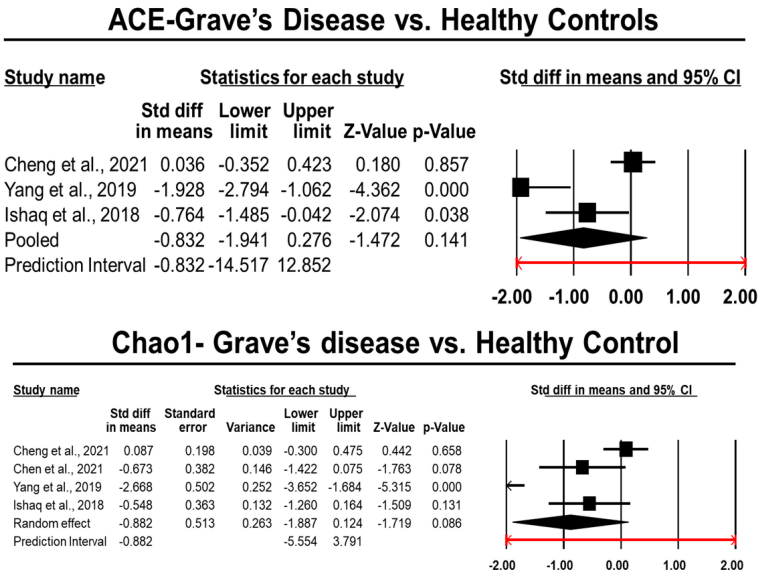


FIGURE 3
Graves' disease-specific pooled standardized mean differences in ACE and Chao1.

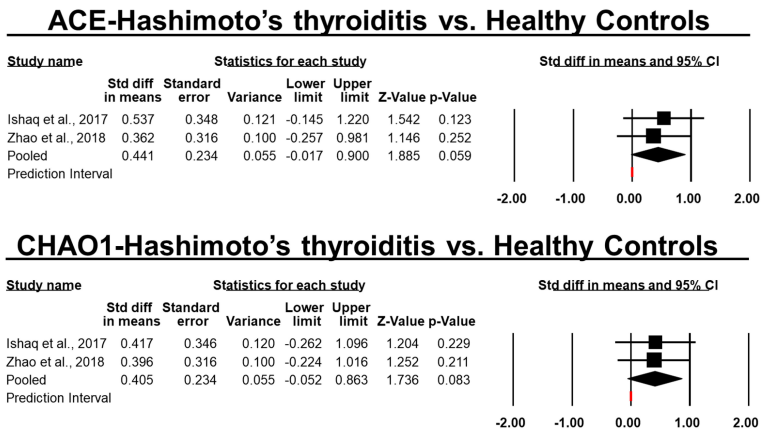


FIGURE 4
Hashimoto's thyroiditis-specific pooled standardized mean differences in ACE and Chao1.

increase in protein breakdown and a reduction in polyphenol conversion, epithelial turnover, and mucus production (18) are just some of the negative impacts that have been linked to a more diverse microbiota.

The majority of the included studies also revealed Good's coverage indices of more than 99%, indicating that the present sequencing depth correctly captured the state of fecal samples for gut microbiota. It's also important to remember that factors like food, eating habits, and origin may have a major impact on the variety of microbes living in your digestive tract (52, 53). However, only five studies (34, 41, 42, 45), and (43) eliminated strict vegetarians because they did not account for their diet. Vegetarians, according to Losasso et al. (54), have much higher Chao1 indices of richness than omnivores. Other investigations (55, 56) could not support these dietary differences between omnivores and vegans or vegetarians. Cigarette smoking, a major risk factor for both GD and GO (57), was also a possible confounding factor since it has been shown to alter the gut microbiota (57, 58).

4.2 Microbiota abundance in patients with GD and HT

The gut microbiome has been under scrutiny in recent years for its potential involvement in diversity's emergence and maintenance. Consistent with the findings of the alpha-diversity study, there were substantial differences in the phylum and genus makeup of the intestinal microflora between GD participants and healthy group. The human gut microbiota is dominated by the Firmicutes and Bacteroidetes phyla, which together account for almost 90% of the whole population. The ratio of Firmicutes to Bacteroidetes (F/B) is an important indicator of gut dysbiosis and has been linked to a wide range of clinical states (59, 60). The F/B ratio was consistently lower in GD participants compared to healthy participants, indicating a role for this index in the development of GD in the majority of studies that measured it. In patients with GO, a similar correlation was seen (34, 45). However, Yang et al. (35) found that the fraction of Firmicutes was considerably greater in GD patients compared to controls, whereas the

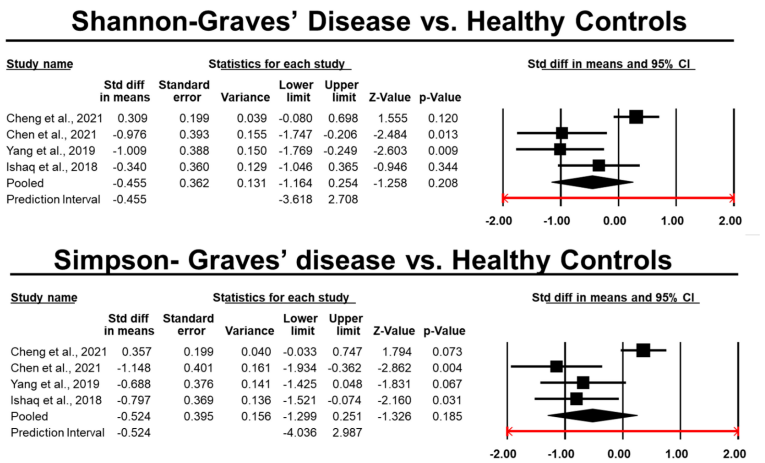


FIGURE 5
Differences in the pooled standard deviations of the Simpson and Shannon indices in Graves' disease.

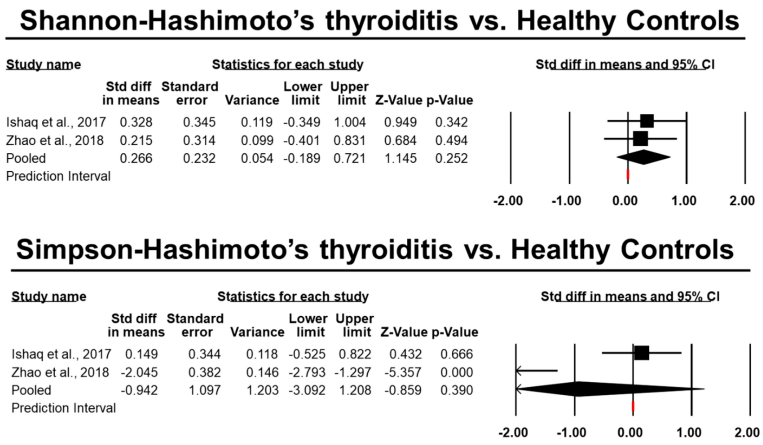


FIGURE 6
Differences in the pooled standard deviations of the Simpson and Shannon indices in Hashimoto's thyroiditis.

number of Bacteroidetes was significantly lower. Obesity is often associated with an elevated F/B ratio (48). Due to the increased basal metabolic rate and the influence of thyroid hormones on the composition and function of the gut flora, weight changes may occur (44). This meta-analysis found that compared to healthy individuals, AITD patients had a greater abundance of Bacteroidetes but no difference in the abundance of Firmicutes.

The variation in the number of the genera was substantially greater between GD patients and healthy controls at lower taxonomic levels. Two investigations (41, 44) found that GD patients had a considerably greater abundance of Bacteroides than the healthy controls. Bacteroides was shown to be considerably less common in the intestinal microbiota of GD patients compared to healthy controls, according to research published by Shi et al. (45). The INDIGO European collaboration found many disease-associated taxa, notably reduced Bacteroides, in a mouse model of GD/GO (61). Antibiotics, probiotics, and the transfer of human fecal material all influenced the gut microbiota of the GD/GO mouse model, leading to the development and modification of the illness (62). The same multinational team of researchers subsequently validated that GD and GO patients had a lesser richness of Bacteroides and a greater richness of Actinobacteria than healthy controls. Bacteroides produces leaky gut syndrome (LGS), characterized by tight junctions, reduction in mucin production, and intestinal permeability, by fermenting glucose and lactate to SCFAs other than butyrate, such as succinate, acetate, and propionate. In addition, this causes a disturbance in gut homeostasis, which may play a role in the etiology and worsening of autoimmune illnesses (63). Additionally, five studies (32, 39, 41–44) found a significantly increased prevalence of Prevotella in GD patients. In chronic inflammatory illnesses, Prevotella mediate mucosal inflammation, leading to the systemic dissemination of inflammatory mediators and bacterial products. The majority of species in this genus activate Toll-like receptor 2, which in turn induces Th17-polarizing cytokine production (IL-1, IL-6, and IL-23) and neutrophil recruitment (IL-17 synthesis) and so facilitates neutrophil recruitment (64). Prevotella may potentially influence the effectiveness of GD medications, as shown by Yan et al. (39). Similarly, Prevotella was found in greater numbers in GO patients (34).

Furthermore, it was shown that the phylum Actinobacteria was more prevalent in the afflicted than in the healthy, particularly for two genera. Bifidobacterium species may be protective or progressive in autoimmune disorders, making their involvement in immunopathogenesis unclear (65). Autoimmune illnesses are linked to Th17 polarization, which is induced by bacteria like Bifidobacterium bifidum (66). Collinsella overgrowth is also linked to increased interleukin-17 production and altered intestinal mucosal permeability (67).

However, compared to patients with GD, the comparative richness of the gut microflora in HT participants was equal to that of healthy participants, demonstrating an opposite pattern of changes. The Firmicutes genus Blautia illustrates the concept of inverse interdependence between GD and HT. Beneficial anti-inflammatory effects may be mediated by these commensal bacteria (68). Blautia abundance was also observed to be inversely associated to visceral fat deposition in both sexes (69).

In addition to diversity, the type of food also affects the comparative richness of the gut microflora. Previous research has linked the Mediterranean diet to a decrease in Streptococcus and a rise in Bifidobacterium, Prevotella, and Roseburia, all of which degrade dietary fiber (56, 70). Lower levels of Lactobacillus and Faecalibacterium were associated with a Western diet heavy in fast food (70, 71).

4.3 Thyroid functional parameters and correlations with changes in microbiota

Examining the connections between immunological and functional changes in microbiota composition and thyroid functioning parameters might provide light on the involvement of microflora in the prognosis of AITDs. Significant associations were identified for TPOAb, one of the thyroid functioning measures examined. Changes in the composition of the microbiota were also linked to elevations or decreases in TSH and TRAb. A small subset of bacteria were found to

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TABLE 2 Relative abundance of significantly altered microbiota in autoimmune thyroid disease patients.

Study	AITD Diagnosis	Bacterial Phylum	Bacterial Genus	Relative Abundance		
				Study	Control	*p*-Value
Ishaq et al., 2017 (31)	HT		*Dialister*	0.0091	0.0446	0.029
Ishaq et al., 2018 (32)	GD		*Prevotella_9*	0.497	0.1952	0.034
			Haemophilus	0.1358	0.0099	0.049
			Dialister	0.011	0.0445	0.047
			Alistipes	0.018	0.0474	0.025
			Faecalibacterium	0.0289	0.0562	0.014
Zhao et al., 2018 (33)	HT	*Firmicutes*		0.826	0.691	<0.001
		Bacteroidetes		0.099	0.227	<0.001
			Faecalibacterium	0.0987	0.151	0.004
			Bacteroides	0.0613	0.133	<0.001
			Prevotella_9	0.0183	0.0601	<0.001
			Blautia	0.0977	0.0586	<0.001
			Roseburia	0.0398	0.0312	0.01
			Lachnoclostridium	0.0241	0.0285	0.013
			Ruminococcus_torques_group	0.0306	0.02	0.002
			Romboutsia	0.0235	0.0146	0.006
			Dorea	0.02	0.0138	0.006
			Fusicatenibacter	0.0186	0.01	<0.001
			Eubacterium_hallii_group	0.0258	0.011	<0.001
Chang et al., 2021 (41)	GD	*Bacteroidetes*		0.421	0.269	<0.01
		Actinobacteria		0.0288	0.0111	<0.01
		Firmicutes		0.502	0.663	<0.01
			Collinsella	0.0052	0.0014	<0.01
			Parabacteroides	0.011	0.0009	<0.01
			Prevotella_9	0.0767	0.0017	<0.01
Chen et al., 2021 (42)	GD	*Synergistetes*		0.000012	0.0024	0.028
			Veillonella	0.0172	0.0006	0.039
El-Zawawy et al., 2021 (43)	GD and HT	*Bacteroidetes*		0.738	0.326	<0.001
		Firmicutes		0.248	0.543	<0.001
			Prevotella	0.4	0.0161	0.006
Yang et al., 2022 (46)	GD	*Actinobacteria*		0.1229	0.0175	0.003
		TM7		0.0001	0.00001	0.011
		Firmicutes		0.6088	0.7522	0.044
		Cyanobacteria		0.0002	0.00003	0.05

have any association with TGAb. However, determining the precise orientations of these relationships is challenging. Bacteroidetes linked inversely with TSH and favorably with TPOAb and TRAb at the phylum level (38, 41, 43). However, the connections between Proteobacteria and Synergistetes were very dissimilar (30, 42). In the phylum Firmicutes in particular, there was a large variation in findings at the genus level. TSH was inversely connected with Veillonella and Streptococcus, and positively correlated with TRAb and TPOAb, with the latter genera also correlating with TGAb (33, 38). Furthermore, Bifidobacterium demonstrated the similar results (46). In contrast,

Bacteroides and Faecalibacterium strains demonstrated contradictory associations (33, 36, 38, 41, 42, 44).

Collectively, our results provide credence to the hypothesis that microbiota modifications are strongly linked to the onset and progression of AITDs by confirming substantial associations between various gut bacteria and thyroid measures. The oral bacteria Veillonella and Streptococcus cause periodontitis and dental cavities (72). These two families have been shown to have metabolic crosstalk that induces cytokine production by dendritic cells, potentially disrupting thyroid autoimmunity (73). And since their amino acid sequences are similar to TG and TPO, strains of Lactobacillus and Bifidobacterium may preferentially interact with autoantibodies, setting off AITDs via molecular simulation pathways (74).

However, low levels of Faecalibacterium are associated with an increased risk of developing inflammatory bowel disease and colon cancer in the gut (75), despite the fact that this bacterium is often regarded as beneficial during autoimmune processes. In addition, it's linked to a drop in antibodies that stimulate the thyroid gland (36). Similarly, a decrease in Phascolarctobacterium levels has been linked to changes in SCFA synthesis and, by extension, a disruption of immunological homeostasis that makes the host more vulnerable to metabolic and gastrointestinal illnesses (76). The ratio of Th17 to Treg cells, which controls the production and release of autoantibodies in people with autoimmune disorders, may be influenced by the phylum-level abundance of Synergistetes (77).

Taking antithyroid medications (ATD) may also alter the bacteria that reside in your intestines. Only *in vitro* investigations involving 40 different bacterial strains have documented any effects at all from ATD, and those effects were considered to be rather small (78).

Intestinal microbial community composition may also be influenced by gender and sex hormone levels (79). Women are more likely to have and struggle to treat subclinical thyroid problems (80). Small intestine bacterial overgrowth (SIBO) has been linked to the development of subclinical hypothyroidism (SCH). TPOAb positivity was found to be more common in SIBO-positive participants than in SIBO-negative individuals, as reported by Wang et al. (81). Differences in intestinal microflora composition and metabolic function were also seen between TPOAb-positive and TPOAb-negative individuals in a separate investigation of pregnant women with SCH (82).

A similar meta-analysis was conducted in 2021 by Gong et al. and showed a significant correlation between the dynamics of gut microbiota and occurrence of Autoimmune Thyroid Disease. However, research has grown exponentially to determine this relationship since 2021, and a new meta-analysis was warranted to coalesce these findings (83).

5 Limitations

Particularly, this systematic evaluation is constrained by the shortcomings of the papers that were included. These studies sometimes had limited sample numbers, with people of different ages or sexes serving as controls. Most of the studies were done in Asia, but we also included one from Europe, one from Africa, and one from South America. Due to a lack of comprehensive data on diversity indices and relative abundance of the chosen phyla and genera, not all research could be included in the meta-analysis (and what data was available was only shown in inaccurately scaled diagrams). Despite the increasing availability of data from other, separate groups, only one research has provided an unambiguous description of GO patients. Although modifications in the gut microbiota were determined in all these investigations, the oral microbiome was not studied, which might be of relevance in future research.

6 Conclusion

This meta-analysis found that people with both GD and HT had significantly different gut microbiota in terms of diversity and composition. Patients with HT had greater diversity indices than healthy participants, whereas those with GD had lower values. Patients with GD also had a greater relative abundance of Bacteroidetes and Actinobacteria. TPOAb levels are often associated with changes in the diversity of microbiota. Additional research is needed to verify these results.

Author contributions

DA, NA, US and RS: were responsible for the conception or design of the work, drafting the work, giving final approval, and ensuring accuracy and integrity. SF, MF, FR and RW: were responsible for interpreting the data for the work, drafting the work, giving final approval, and ensuring accuracy and integrity. SG, KA, MA and DT: were responsible for interpreting the data, drafting the work, giving final approval, and ensuring accuracy and integrity. HS AD, MH and AT were responsible for interpreting the data, drafting the work, giving final approval, and ensuring accuracy and integrity. All authors contributed to the article and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2023.1238146/full#supplementary-material

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The association between serum selenium levels and pathological features of papillary thyroid cancer in 284 patients

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Objective: The relationship between serum selenium levels and papillary thyroid cancer (PTC), especially the pathological features, still remains controversial. We conducted this study to investigate the relationship between serum selenium levels and PTC in a Chinese population.

Methods: Cross-sectional data of 284 patients with PTC were collected from the First Affiliated Hospital of Shandong First Medical University. The general clinical characteristics, serum selenium levels, and tumor pathological features were described in PTC. The association between serum selenium levels and pathological features in PTC was analyzed using SPSS 26.0 statistical software.

Results: Our results showed that the median serum selenium level was 79.15 μg/L (IQR: 71.00 - 86.98 μg/L) in PTC patients. Serum selenium levels were lower in females than males (p = 0.035). Serum selenium levels were negatively correlated with the number of lymph node metastases (p = 0.048). High serum selenium (*OR* = 0.397, 95%*CI*: 0.217 - 0.725) and diastolic blood pressure (*OR* = 1.028, 95%*CI*: 1.005 - 1.051) were related factors for the incidence of bilateral tumors. High serum selenium (*OR* = 0.320, 95%*CI*: 0.166 - 0.617) and diastolic blood pressure (*OR* = 1.066, 95%*CI*: 1.031 - 1.103) were related factors for tumor multifocal incidence.

Conclusions: The serum selenium levels of PTC patients in females were lower than males. High serum selenium levels might be a protective factor in PTC patients. Further research is necessary to better understand the influence of selenium on PTC progression.

KEYWORDS

papillary thyroid cancer, pathological features, nutrition, selenium, serum

Introduction

Nowadays cancer is the second leading cause of death, severely affecting people's quality of life and longevity (1). Thyroid cancer is the most prevalent malignant tumor of the endocrine system, and its incidence has been on the rise in recent years (2, 3). Notably, papillary thyroid cancer (PTC) accounts for a vast majority of cases (4). Despite a favorable prognosis for most patients following systematic treatment, a subset experiences relapse and unfavorable outcomes (5). Previous research has identified large tumor diameters, extrathyroidal extension, and distant lymph node metastases as significant predictors of thyroid cancer recurrence and mortality (6, 7). While some risk factors for thyroid cancer have been predicted, most are uncontrollable, including advanced age, gender, race and family history. Therefore, the exploration of controllable risk factors becomes crucial for the prevention and treatment of thyroid cancer.

Selenium, an essential trace element, plays a pivotal role in maintaining homeostasis. Selenium manifests its anti-inflammatory, antioxidant, antiviral, and anticancer characteristics through its involvement in the synthesis of diverse selenoproteins (8–11). At present, a minimum of 25 selenoproteins with diverse biological functions have been identified (12). Maintaining proper selenium status is crucial for human health, as deficiency is associated with increased risks of infertility, autoimmune imbalance, inflammation, and cancer. Alternatively, excessive serum selenium levels may lead to hyperlipidemia, hyperinsulinemia, type 2 diabetes mellitus, and atherosclerosis (13).

Recently, the relationship between selenium and thyroid cancer has garnered attention. The thyroid has a significantly higher concentration of selenium than other organs (14). As oxidative stress may lead to cellular mutation and gene variation, selenoproteins have been considered to prevent the development of cancer through their antioxidant effects (15). However, studies on selenium and tumor risk have yielded inconsistent results. Most studies have found that low selenium appears to be associated with the incidence of thyroid cancer (16–19). In the study by Baltaci et al., patients with thyroid cancer demonstrated lower serum selenium levels compared to those without thyroid cancer (16). Kucharzewski et al. found that blood selenium concentrations in patients with thyroid cancer were lower than those in controls (19). In a case-control trial, serum selenium levels were inversely associated with the risk of thyroid cancer (17). Additionally, a meta-analysis involving 1291 subjects showed that blood selenium levels in thyroid cancer were lower than those in controls (18). However, a Polish study found no significant differences in serum selenium levels between patients with thyroid cancer and non-thyroid cancer patients (20). Moreover, no association between selenium intake and thyroid cancer was observed in a large prospective cohort study in the United States (14). In the study by Jonklaas et al., it was noted that serum selenium levels in patients with thyroid cancer were not significantly reduced, but they were negatively correlated with the disease stage (21).

Currently, studies focus on the relationship between serum selenium levels and the incidence of thyroid cancer, yet they lack further analysis of pathological features. Furthermore, most studies included other pathological types of thyroid cancer and did not study PTC separately. Therefore, we investigated serum selenium levels and pathological features in PTC patients to study the relationship between serum selenium levels and pathological features.

Materials and methods

Trial design and participants

This cross-sectional study was extracted from a larger prospective study. Patients with PTC who were treated at the First Affiliated Hospital of Shandong First Medical University (Qianfoshan Hospital of Shandong Province) from January 1, 2021 to December 31, 2022 were included in this study. All patients have completed the surgical pathological details, and serum selenium levels were detected during hospitalization.

Inclusion criteria

(1) Research period: January 1, 2021 to December 31, 2022.
(2) Surgical method: total thyroidectomy with or without lymph node dissection.
(3) Histopathological results: PTC was clearly diagnosed, and complete pathological details were recorded.
(4) Complete the detection of serum selenium index.

Exclusion criteria

(1) It is known that other primary tumors have metastasized to the thyroid gland.
(2) Those who have taken selenium preparations or selenium-enriched drugs in the past year.
(3) Patients who are pregnant or breastfeeding.
(4) Acute complications of diabetes (diabetic ketoacidosis, hyperglycemia and hyperosmolar state).
(5) Significant liver function abnormalities, defined as aspartate aminotransferase > 2 × ULN (upper limit of normal range) and/or alanine aminotransferase > 2 × ULN, and/or total bilirubin > 34umol/L or previous infectious hepatitis seropositive evidence.
(6) Patients with renal insufficiency (eGFR < 60ml/min/1.73m^2).

(7) Patients with severe autoimmune diseases (rheumatoid, systemic lupus erythematosus and other diseases).

Data collection

(1) General clinical data, including name, age, gender, height, weight, waist, systolic blood pressure (SBP), diastolic blood pressure (DBP) and thyroid related indicators.

(2) Serum selenium levels: Inductively coupled plasma mass spectrometry (ICP-MS), collected by the Laboratory Department of the First Affiliated Hospital of Shandong First Medical University (Qianfoshan Hospital in Shandong Province), tested and provided by Guangzhou Jinyu Medical Laboratory Institute Report.

(3) Details of thyroid surgery (total thyroidectomy, with or without lymph node dissection), histopathological results (pathological type, tumor size, number of metastatic lymph nodes, whether the lesion is unilateral or bilateral, whether the lesion is multifocal, or whether the lesion is invasive) capsule, and presence or absence of metastasis), and TNM (tumor, node, metastasis) staging based on pathological details (American Joint Committee on Cancer, AJCC 8th ed.).

TABLE 1 Demographic and clinical data observed in the cross-section study.

Subjects	Data
Onset (Year)	44.00 (35.00 - 52.00)
Gender (N, %)	
Male	85 (29.93%)
Female	199 (70.07%)
Waist (cm)	90.90 ± 10.90
BMI (kg/m^2)	25.71 (23.14 - 28.46)
Underweight (N, %)	1 (0.35%)
Normal weight (N, %)	91 (32.04%)
Overweight (N, %)	113 (39.79%)
Obese (N, %)	79 (27.82%)
SBP (mmHg)	123.00 (114.00 - 135.00)
DBP (mmHg)	80.48 (73.00 - 88.75)
FT3 (pmol/L)	4.94 (4.62 - 5.41)
FT4 (pmol/L)	17.73 (16.50 - 19.84)
TSH (uIU/mL)	1.85 (1.16 - 2.64)
TPOAB (IU/mL)	13.08 (10.04 - 20.13)
TGAB (IU/mL)	20.24 (15.72 - 49.35)

BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; FT3, free triiodothyronine; FT4, free thyroxine; TSH, thyroid stimulating hormone; TPOAB, thyroidperoxidase antibodies; TGAB, thyroglobulin antibody.

Statistical methods

All data were statistically analyzed using SPSS 26.0 statistical software. Describe patient demographics, clinical characteristics, and blood parameters using simple summary statistics. The Kolmogorov-Smirnov test was performed on the enumeration data, and the data that conformed to the normal distribution were expressed as mean ± standard deviation, and the data that did not conform to the normal distribution were expressed as the median (interquartile range). Univariate analysis was performed by t test or Wilcoxon signed rank test, and Logistic regression analysis was used for further analysis of variables with statistical differences. The independent samples Kruskal-Wallis rank sum test was used for comparison among multiple groups. Spearman or Pearson correlation analysis was used for bivariate analysis. All tests were two-sided, and $p < 0.05$ indicated a statistically significant difference.

Results

Patient characteristics

A total of 284 PTC patients were included in the study. The demographic and clinical data were shown in Table 1. Among the 284 patients, 85 were males (85/284, 29.93%) and 199 were females (199/284, 70.07%). The median age was 44 years (IQR: 35.00 - 52.00 years). The median BMI was 25.71 kg/m^2 (23.14 - 28.46 kg/m^2). According to the standards established by the China Obesity Working Group Data Summary and Analysis Collaborative Group, 1 patient (1/284, 0.35%) was underweight (<18.5 kg/m^2), 91 patients (91/284, 32.04%) were normal weight, and 113 patients (113/284, 39.79%) were overweight (24-27.9 kg/m^2), and 79 patients (79/284, 27.82%) were obese (≥28 kg/m^2). The median height was 163.00 cm (IQR: 160.00 - 170.00 cm), and the median weight was 69.5 kg (IQR: 60.25 - 80.00 kg). Waist was recorded in 282 patients, with an average of 90.90 cm. The median SBP and DBP in patients were 123.00 mmHg (IQR: 114.00 - 135.00 mmHg) and 80.48 mmHg (IQR: 73.00 - 88.75 mmHg), respectively. The clinical data of PTC patients with different genders were shown in Table S1. Females exhibited higher levels of thyroid antibodies compared to males, while males demonstrated a higher BMI and blood pressure than females.

Characteristics of serum selenium levels in PTC patients

The distribution characteristics of serum selenium levels were shown in Figure 1. The median serum selenium level was 79.15 μg/L (IQR: 71.00 - 86.98 μg/L). The serum selenium level of 232 patients (232/284, 81.69%) was below 90 μg/L. There were 199 female patients and 85 male patients. The serum selenium level was

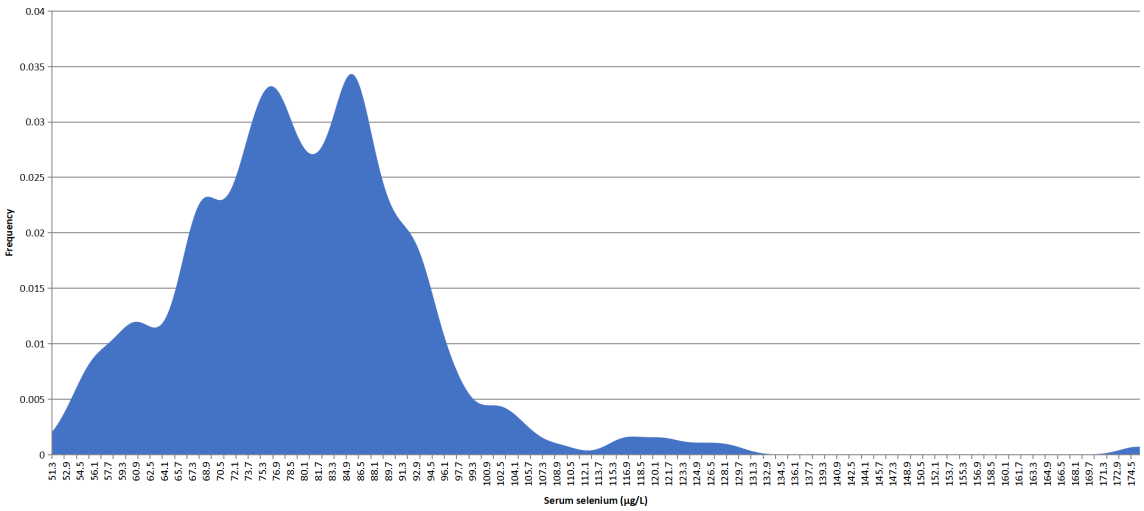


FIGURE 1
Overall characteristics of serum selenium levels in PTC patients.

78.30 μg/L (IQR: 68.80 - 86.30 μg/L) in female patients and 82.20 μg/L (IQR: 74.80 - 88.65 μg/L) in male patients. As shown in Figure 2A, the serum selenium level in female patients was lower than that in male patients (p = 0.035). Age categories for patients were classified according to the WHO criteria: 144 patients in the young group (<45 years old), 116 cases in the middle-aged group (45-59 years old), and 24 cases in the old group (≥60 years old). The serum selenium level was 79.05 μg/L (IQR: 69.65 - 86.60 μg/L) in the young group, 79.35 μg/L (IQR: 72.85 - 88.43 μg/L) in the middle-aged group, and 77.70 μg/L (IQR: 70.83 - 86.43 μg/L) in the elderly group. There was no significant difference among three groups in serum selenium levels (p = 0.944) (Figure 2B). BMI categories for patients were classified according to the China Obesity Working Group Data Summary and Analysis Collaborative Group: 91 patients were normal weight (18.5-23.9 kg/m^2), 1 patient was underweight (<18.5 kg/m^2), 113 patients were overweight (25-27.9 kg/m^2), and 79 patients were obesity (≥28 kg/m^2). Since only one patient was underweight, it was not included in the comparison. The serum selenium level in normal weight patients was 78.30 μg/L (IQR: 69.60 - 85.20 μg/L), the serum selenium level in overweight patients was 82.60 μg/L (IQR: 75.15 - 91.15 μg/L) and obesity patients was 76.10 μg/L (IQR: 68.50 - 86.20 μg/L). Serum selenium levels in overweight patients were significantly higher than those in normal weight groups (p < 0.01) (Figure 2C). The correlation between serum selenium levels and thyroid-related indicators was presented in Table S2. The results showed that there was no significant correlation between selenium and thyroid related indicators.

Pathological features in PTC patients

The detailed pathological characteristics of PTC patients were shown in Table 2. There were 142 patients (142/284, 50.00%) with

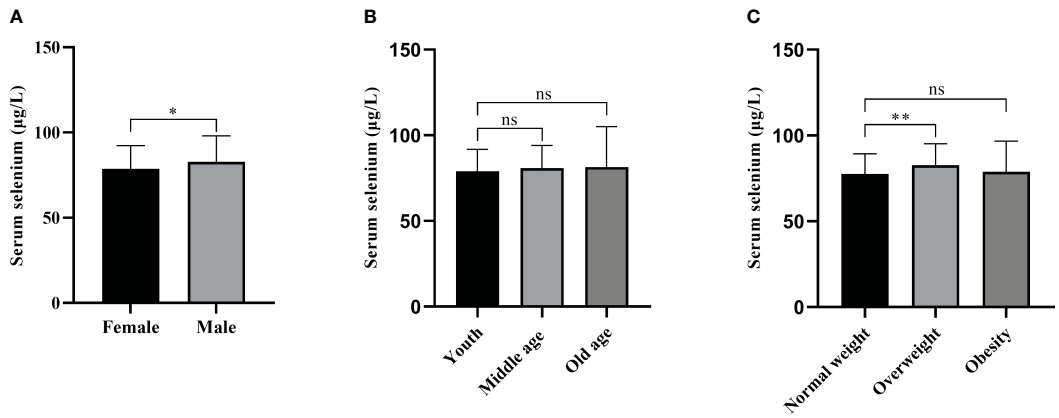


FIGURE 2
Serum selenium levels in PTC patients with different sexes, ages, and BMIs. **(A)** Serum selenium levels in PTC patients with different genders. **(B)** Serum selenium levels of PTC patients with different ages. **(C)** Serum selenium levels in PTC patients with different BMIs. ns, not significant; *, p < 0.05; **, p < 0.01.

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TABLE 2 Pathological features of enrolled patients.

Subjects	Data
Bilateral	
Unilateral lesion	142 (50.00%)
Bilateral lesion	137 (48.24%)
Not mentioned	5 (1.76%)
Multifocality	
Yes	168 (59.15%)
No	102 (35.92%)
Not mentioned	14 (4.93%)
Capsule invasion	
Yes	159 (55.99%)
No	30 (10.56%)
Not mentioned	95 (33.45%)
Tumor size (cm)	1.10 (0.70 - 1.60)
Number of metastatic lymph nodes	4.00 (1.00 - 8.00)
TNM staging (AJCC 8th edition)	
T	
Ia	127 (44.72%)
Ib	79 (27.82%)
II	28 (9.86%)
IIIa	3 (1.06%)
IIIb	11 (3.87%)
Iva	9 (3.17%)
IVb	4 (1.41%)
X	23 (8.10%)
N	
0	51 (17.96%)
Ia	159 (55.99%)
Ib	71 (25.00%)
NA	3 (1.06%)

NA, not available.

unilateral tumors and 137 patients with bilateral lesions (137/284, 48.24%). There were 168 patients (168/284, 59.15%) with multifocal tumors, and 102 patients (102/284, 35.92%) with single tumor tumors. There were 159 (159/284, 55.99%) patients with thyroid capsule invasion. The median tumor size was 1.10 cm (IQR: 0.70 - 1.60 cm). The median number of positive lymph node metastases was 4 (IQR: 1.00 - 8.00). The results of T staging showed that most of the patients were in stage I, including 127 patients in stage Ia (127/284, 44.72%) and 79 patients in stage Ib (79/284, 27.82%). N staging results showed that more than half of the patients were stage Ia.

Serum selenium levels and pathological characteristics in PTC patients

The association between serum selenium levels and pathological features in PTC patients was analyzed by Spearman correlation analysis (Table 3). Serum selenium levels were negatively correlated with the number of lymph node metastasis (r = -0.121, p = 0.048). There was no significant correlation with tumor T stage (p = 0.436), N stage (p = 0.811) and tumor maximum diameter (p = 0.795).

The serum selenium levels of different pathological subtypes in PTC patients were shown in Figure 3. There were 270 patients with classical subtype PTC, 7 patients with follicular subtype PTC, and 7 patients with hypercellular subtype PTC. There was no significant difference in serum selenium levels among patients with different pathological subtypes of PTC (p = 0.860).

The comparison of serum selenium levels in PTC patients with different pathological features was shown in Figure 4. Serum selenium levels in patients with bilateral tumors were significantly lower than those in patients with unilateral tumors (Z = -2.871, p = 0.004). Serum selenium levels in patients with multifocal tumors were significantly lower than those in patients with unifocal tumors (Z = -4.120, p < 0.001). There was no statistical difference in serum selenium levels of patients regardless of membrane invasion (t = -0.172, p = 0.864) and lymph node metastasis (t = 0.472, p = 0.637). The comparison of serum selenium levels in common papillary thyroid cancer patients with different pathological features was shown in Figure S1.

Factors related to unilateral and bilateral tumors in PTC patients

The status of unilateral or bilateral tumors in PTC patients was presented in Table 4. Univariate analysis showed that there were statistical differences in serum selenium level (p = 0.004), BMI (p = 0.033) and DBP (p = 0.018), while age (p = 0.647), height (p = 0.971), Waist (p = 0.051) and SBP (p = 0.188) were not statistically different.

Statistically significant variables in univariate analysis (serum selenium level, BMI, DBP) were included in multivariate analysis (Table 5). High serum selenium (OR = 0.387, 95%CI: 0.212 - 0.706) and DBP (OR = 1.029, 95%CI: 1.006 - 1.053) were the relevant factors for the incidence of bilateral tumors.

Factors related to single and multifocal tumors in PTC patients

The status of single or multifocal tumors in PTC patients was presented in Table 6. Univariate analysis showed that there were statistical differences in serum selenium level (p = 0.004), BMI (p = 0.033) and DBP (p = 0.018), while age (p = 0.647), height (p = 0.971), Waist circumference (p = 0.051) and SBP (p = 0.188) were not statistically different.

TABLE 3 Spearman correlation analysis of serum selenium levels and pathological features in PTC patients.

Subjects	Serum selenium	
	r	p
TNM staging-T	-0.048	0.436
TNM staging-N	-0.015	0.811
Tumor size (cm)	0.016	0.795
Metastatic lymph nodes	-0.121	0.048*

Statistically significant variables in univariate analysis (serum selenium level, BMI, SBP and DBP) were included in multivariate analysis (Table 7). High serum selenium (*OR* = 0.320, 95%*CI*: 0.166 - 0.617) and DBP (*OR* = 1.066, 95%*CI*: 1.031 - 1.103) were related factors for the onset of multifocal tumors.

Discussion

In this study, the relationship between serum selenium levels and the pathological features of PTC was analyzed. The results showed: (1) Serum selenium levels of female PTC patients were lower than those of male PTC patients. (2) Serum selenium levels of PTC patients were negatively correlated with the number of lymph node metastasis, but there was no significant correlation between serum selenium levels and the stage and pathological subtype of PTC. (3) High serum selenium levels might be a protective factor in PTC patients.

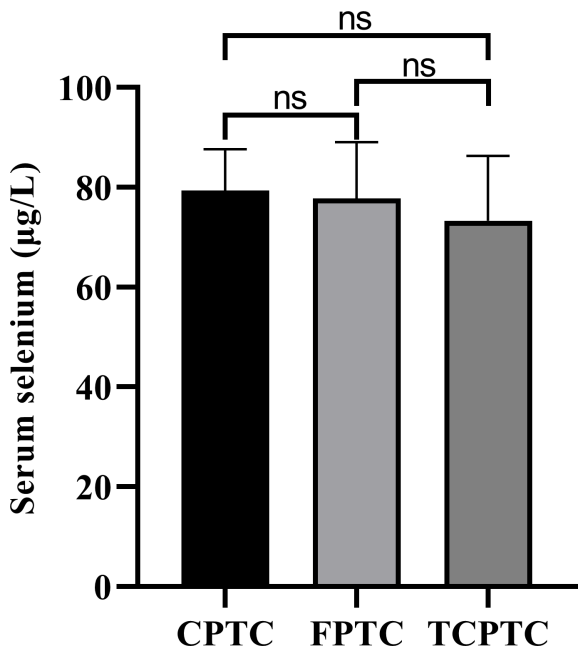


FIGURE 3
Serum selenium levels of different pathological subtypes in PTC patients. CPTC, common papillary thyroid cancer; FPTC, follicular papillary thyroid cancer; TCPTC, tall cell papillary thyroid cancer. ns, not significant.

Serum selenium levels were negatively correlated with the positive number of lymph node metastasis. First, the effects of selenium on cancer cells are highly concentration-dependent (22). Cancer cell growth was promoted by low to moderate serum selenium levels, while high serum selenium levels were cytotoxic and inhibited cancer cell growth (23). Second, as a crucial component of deiodinase, selenium plays a pivotal role in the conversion of T4 to T3 (24, 25). When there is a deficiency of selenium, it may lead to a reduction in the synthesis of thyroid hormones, potentially resulting in an elevation of thyroid stimulating hormone (TSH) levels (25). Thyroid cell growth can be promoted by TSH, and long-term high levels of TSH might induce and promote the occurrence and development of thyroid cancer. Therefore, low selenium might indirectly promote the increase of TSH and lead to the occurrence and development of tumors. However, the effect of selenium on TSH in humans has not been fully established. Rostami et al. observed a significant negative correlation ($p < 0.001$) between serum selenium levels and TSH in a low selenium population (26). Similarly, Tong et al. reported a significant negative correlation ($p < 0.001$) between serum selenium levels and TSH in healthy individuals (27). Our study did not find the association between serum selenium levels and TSH. Similarly, studies based on the NHANSE database in the United States have not found any significant association between serum selenium levels and TSH (28). Third, glutathione peroxidase 3 (GPX3), a selenoprotein, suppressed the metastasis of thyroid cancer by inhibiting the Wnt/β-catenin signaling pathway (29). Insufficient selenium levels might contribute to a reduction in GPX3 levels, thereby facilitating the metastasis of thyroid cancer. Notably, 81.69% of patients in our study demonstrated serum selenium levels below 90 ng/L. When the serum selenium concentration exceeds 90 ng/L, selenoproteins, including selenoprotein P and GPX, could be optimized; whereas, levels below this threshold are insufficient to achieve the optimal states of selenium proteins (30, 31).

High serum selenium levels might be a protective factor in PTC patients. At high selenium levels, serum selenium levels were negatively correlated to bilateral and multifocal tumors. First, hypermetabolism in tumor cells leads to increased reactive oxygen species (ROS), and these cells appear to be susceptible to oxidative stress (8, 9). The supplementation of selenium may exert an anti-tumor effect by inhibiting the ROS-mediated Akt/mTOR pathway (11). Furthermore, selenium induces cell death and apoptosis through superoxide generation in mitochondria and activation of the mitochondrial apoptotic pathway (32). Selenium could also affect tumor proliferation by affecting gene expression. Selenium inhibits tumor cell proliferation through DNA repair by p53-dependent effectors (13). In drug intervention experiments, it was shown that sodium selenite can significantly reduce cell viability and induce thyroid cancer cell G0/G1 cell cycle arrest and apoptosis in a dose-dependent manner (11). At the same time, the activities of T lymphocytes and NK cells were significantly enhanced after Se supplementation, and tumor proliferation could be suppressed (33). Notably, tumor cells have a higher metabolism than resting cells compared to healthy cells (34). In order to maintain the balance of the oxidation-antioxidation system, more

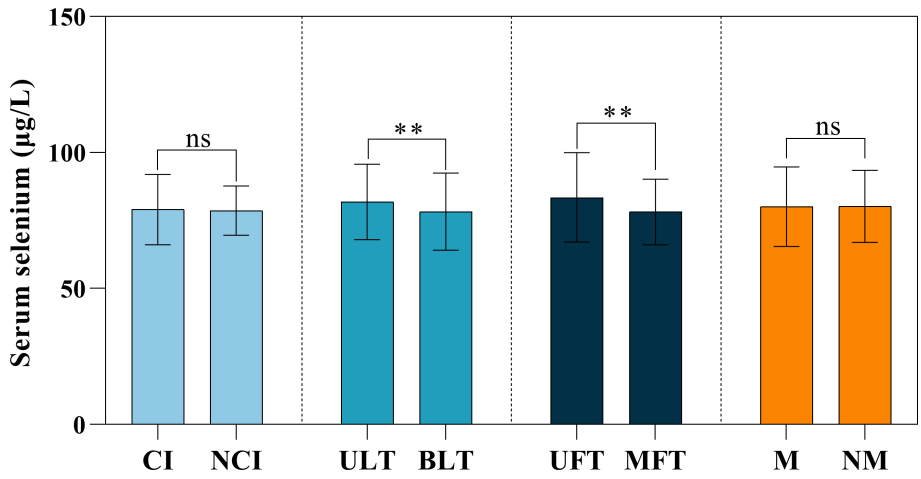


FIGURE 4
Serum selenium levels among different pathological features in PTC patients. CI, capsule invasion; NCI, no capsule invasion; ULT, unilateral tumor; BLT, bilateral tumor; UFT, unifocal tumor; MFT, multifocal tumor; M, metastasis; NM, no metastasis. ns, not significant; **, $p < 0.01$.

antioxidant molecules such as selenoproteins need to be consumed. Therefore, the reduction of serum selenium levels might also be caused by the depletion process of tumorigenesis and development.

There was no significant association between serum selenium levels and PTC stage. This notion was also supported by a study of Korean women with thyroid cancer (35). However, the study by Jonklaas et al. showed that serum selenium levels might be negatively correlated with thyroid cancer stage (21). This discrepancy might be due to a variety of factors. First, the study population was limited to patients with PTC in our study, whereas in Jonklaas et al.'s study it was patients with differentiated thyroid cancer. Results might have been influenced by incorporation of other types of thyroid cancer. Second, despite serum selenium levels might be associated with thyroid cancer risk, randomized controlled trials on the effect of selenium supplementation on cancer have yielded inconsistent results (36). Serum selenium levels might be associated with some features of PTC, but not with tumor stage.

There was no significant difference in serum selenium levels among patients with different pathological subtypes of PTC. This is a preliminary exploration of serum selenium levels in patients with different subtypes of PTC. Similarly, no correlation was found between selenium and the tumor pathological subtype in the study of breast cancer (31). However, care needs to be taken that, due to the rarity of some subtypes, a sufficiently large sample size

TABLE 4 Comparison of data between unilateral tumor group and bilateral tumor group in PTC patients.

Subject	Unilateral tumor	Bilateral tumors	t/Z	*p*
Number (%)	142 (50.90)	137 (49.10)	-	-
Age (years)	45.00 (35.00 - 52.00)	43.00 (35.00 - 52.50)	-0.458	0.647
SSL (μg/L)	82.70 (72.55 - 89.43)	76.90 (70.05 - 85.05)	-2.871	0.004
BMI (kg/m^2)	25.52 ± 3.91	26.66 ± 3.90	-2.436	0.015
Height (cm)	163.00 (160.00 - 170.00)	164.00 (160.00 - 170.00)	-0.036	0.971
Weight (kg)	66.50 (59.00 - 78.50)	70.00 (62.00 - 81.00)	-2.129	0.033
Waist (cm)	88.00 (83.00 - 97.00)	92.00 (84.25 - 98.75)	-1.949	0.051
SBP (mmHg)	122.00 (112.75 - 132.25)	124.00 (114.50 - 138.00)	-1.316	0.188
DBP (mmHg)	78.00 (71.75 - 86.00)	80.00 (74.00 - 92.00)	-2.364	0.018
FT3 (pmol/L)	4.92 (4.56 - 5.19)	5.07 (4.69 - 5.87)	-0.107	0.915
FT4 (pmol/L)	17.59 (15.40 - 20.12)	18.06 (16.97 - 19.65)	-0.721	0.471
TSH (uIU/mL)	2.04 (1.14 - 3.00)	1.49 (1.26 - 2.42)	-1.477	0.140
TPOAB (IU/mL)	13.08 (10.01 - 20.00)	12.67 (10.07 - 20.49)	-0.398	0.690
TGAB (IU/mL)	19.79 (15.06 - 44.08)	21.30 (16.03 - 55.02)	-0.820	0.412

SSL, serum selenium level; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; FT3, free triiodothyronine; FT4, free thyroxine; TSH, thyroid stimulating hormone; TPOAB, thyroidperoxidase antibodies; TGAB, thyroglobulin antibody.

TABLE 5 Multivariate logistic retrospective analysis of factors related to unilateral and bilateral tumor incidence in PTC patients.

Subject	B	S.E.	Wald	*p*	*OR*	95%*CI*
SSL (μg/L)						
Low	-	-	-	-	1	-
Middle	-0.205	0.304	0.455	0.500	0.815	0.449 - 1.478
High	-0.924	0.307	9.027	0.003	0.397	0.217 - 0.725
BMI (kg/m^2)	0.048	0.034	2.042	0.153	1.049	0.982 - 1.121
DBP (mmHg)	0.028	0.012	5.690	0.017	1.028	1.005 - 1.051

Serum selenium levels were categorized into three grades: Low: 51.3 - 74.4 μg/L (n = 93), Middle: 74.5 - 85.3 μg/L (n = 93), and High : 85.4 - 175.3 μg/L (n = 93); BMI, body mass index; DBP, diastolic blood pressure. SSL, serum selenium level.

TABLE 6 Comparison of data between single-focal tumor group and multi-focal tumor group in PTC patients.

Subject	Unilateral tumor	Bilateral tumors	t/Z	*p*
Number (%)	102 (37.78)	168 (62.22)	-	-
Age (years)	43.00 (33.75 - 52.00)	45.00 (36.00 - 53.00)	-1.120	0.263
SSL (μg/L)	85.05 (74.88 - 92.15)	76.90 (69.43 - 85.25)	-4.120	<0.001
BMI (kg/m^2)	25.43 ± 4.10	26.44 ± 3.89	-2.017	0.045
Height (cm)	163.00 (159.75 - 170.00)	164.00 (160.00 - 170.00)	-0.256	0.798
Weight (kg)	66.00 (57.38 - 79.63)	70.00 (62.00 - 80.00)	-1.940	0.052
Waist (cm)	90.19 ± 11.41	91.43 ± 10.58	-0.906	0.366
SBP (mmHg)	122.16 ± 15.85	126.64 ± 17.43	-2.120	0.035
DBP (mmHg)	77.00 (69.00 - 84.25)	80.50 (75.00 - 91.75)	-3.985	<0.001
FT3 (pmol/L)	4.96 (4.63 - 5.19)	4.94 (4.61 - 5.46)	-1.368	0.171
FT4 (pmol/L)	17.43 (15.96 - 19.19)	17.80 (16.50 - 19.90)	-0.681	0.496
TSH (uIU/mL)	2.28 (1.45 - 2.81)	1.54 (1.10 - 2.64)	-1.007	0.314
TPOAB (IU/mL)	14.44 (10.30 - 19.37)	12.55 (9.99 - 20.57)	-0.673	0.501
TGAB (IU/mL)	19.62 (15.81 - 56.31)	20.76 (15.42 - 48.62)	-0.256	0.798

SSL, serum selenium level; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure yronine; FT4, free thyroxine; TSH, thyroid stimulating hormone; TPOAB, thyroidperoxidase antibodies; TGAB, thyroglobulin antibody.

TABLE 7 Multivariate logistic retrospective analysis of factors related to unilateral and bilateral tumor incidence in PTC patients.

Subject	B	S.E.	Wald	*p*	*OR*	95%*CI*
SSL (μg/L)						
Low	-	-	-	-	1	-
Middle	-0.283	0.343	0.680	0.409	0.754	0.385 - 1.476
High	-1.139	0.335	11.586	0.001	0.320	0.166 - 0.617
BMI (kg/m^2)	0.027	0.037	0.542	0.461	1.028	0.956 - 1.105
SBP (mmHg)	-0.011	0.011	0.905	0.341	0.989	0.967 - 1.012
DBP (mmHg)	0.064	0.017	13.689	<0.001	1.066	1.031 - 1.103

Serum selenium levels were categorized into three grades: Low: 51.3 - 74.5 μg/L (n = 90), Middle: 74.5 - 85.3 μg/L (n = 90), and High : 85.3 - 175.3 μg/L (n = 90). SSL, serum selenium level; BMI, body mass index; DBP, diastolic blood pressure.

could not be obtained to improve the reliability of the evidence. Therefore, future studies with larger sample sizes need to be implemented.

The serum selenium levels of PTC patients were lower in females than males. Previous study found that there was no gender difference in serum selenium levels in Austrian (37). Similarly, the study in Western Romanesia revealed slightly lower selenium levels in young females compared to young males (38). However, some studies have found that females have lower serum selenium levels than males (39, 40). Notably, previous studies paid little attention to the differences in serum selenium levels between genders in PTC populations. The multi-pathway anti-tumor effects of selenium suggest that low selenium levels might serve as a susceptibility factor for PTC in female patients. In the future, studies on the relationship between diet and *in vivo* selenium metabolism need to be conducted in the PTC population, and gender differences should be taken into account.

This study has several limitations. First, as a cross-sectional study, a causal relationship between serum selenium levels and pathological features in PTC patients could not be established. Second, this study did not include a healthy control group, and there might be an insufficient incorporation of covariates. Third, patients positive for thyroid antibodies were not excluded during the inclusion process. Fourth, this study might have selective bias, because female PTC patients account for a relatively high proportion. Fifth, given the rarity of some subtypes of PTC, future studies with larger sample sizes are necessary to validate the reliability of the conclusions. Sixth, the relationship between selenium and pathological features in PTC patients has only been investigated at the serum level, thus further exploration of the protein molecular mechanism is warranted for a more comprehensive understanding.

Conclusions

In conclusion, the serum selenium levels of PTC patients in females were lower than males. High serum selenium levels might be a protective factor in PTC patients. Therefore, further research is necessary in this aspect to better understand the influence of selenium on PTC progression.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding authors.

Ethics statement

The study was conducted in accordance with the Declaration of Helsinki, and approved by the Ethics Committee of the First Affiliated Hospital of Shandong First Medical University (Qianfoshan Hospital in Shandong Province) under the number YXLL-KY-2019031.

Author contributions

SG: Document Retrieval, Data Extraction, Data analysis, Essay writing, and Paper submission. JZ, JY, HF and YT: Data analysis. YS, MS and JF: Data Extraction. JD and LL: Article innovation and Paper submission. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2023.1242250/full#supplementary-material

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Mapping the path towards novel treatment strategies: a bibliometric analysis of Hashimoto's thyroiditis research from 1990 to 2023

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Background: Hashimoto's thyroiditis (HT), a common form of thyroid autoimmunity, is strongly associated with deteriorating clinical status and impaired quality of life. The escalating global prevalence, coupled with the complexity of disease mechanisms, necessitates a comprehensive, bibliometric analysis to elucidate the trajectory, hotspots, and future trends in HT research.

Objective: This study aims to illuminate the development, hotspots, and future directions in HT research through systematic analysis of publications, institutions, authors, journals, references, and keywords. Particular emphasis is placed on novel treatment strategies for HT and its complications, highlighting the potential role of genetic profiling and immunomodulatory therapies.

Methods: We retrieved 8,726 relevant documents from the Web of Science Core Collection database spanning from 1 January 1990 to 7 March 2023. Following the selection of document type, 7,624 articles were included for bibliometric analysis using CiteSpace, VOSviewer, and R software.

Results: The temporal evolution of HT research is categorized into three distinct phases: exploration (1990-1999), rapid development (1999-2000), and steady growth (2000-present). Notably, the United States, China, Italy, and Japan collectively contributed over half (54.77%) of global publications. Among the top 10 research institutions, four were from Italy (4/10), followed by China (2/10) and the United States (2/10). Recent hotspots, such as the roles of gut microbiota, genetic profiling, and nutritional factors in HT management, the diagnostic dilemmas between HT and Grave's disease, as well as the challenges in managing HT complicated by papillary thyroid carcinoma and type 1 diabetes mellitus, are discussed.

Conclusion: Although North America and Europe have a considerable academic impact, institutions from emerging countries like China are demonstrating promising potential in HT research. Future studies are anticipated to delve deeper into the differential diagnosis of HT and Grave's disease, the intricate relationship between gut microbiota and HT pathogenesis, clinical management of HT with papillary thyroid carcinoma or type 1 diabetes, and the beneficial effects of dietary modifications and micronutrients supplementation in HT. Furthermore, the advent of genetic profiling and advanced immunotherapies for managing HT offers promising avenues for future research.

KEYWORDS

Hashimoto's thyroiditis, bibliometric analysis, autoimmune disease, papillary thyroid carcinoma, type 1 diabetes mellitus, thyroid autoimmunity, nutritional factors, treatment strategies

1 Introduction

Hashimoto's thyroiditis (HT), also known as chronic lymphocytic thyroiditis, is a form of autoimmune thyroiditis characterized by a large number of lymphocytes in its pathology (1). It is named after the Japanese surgeon Hakaru Hashimoto, who first reported four cases of the disease in 1912. Several studies have shown that HT is currently the most common autoimmune disease (2) and a major cause of endocrine disorders (3). The incidence of this disease is higher in women between the ages of 30 and 50, who are almost four times more likely to be affected by HT than men are (4). The prevalence rate in women ranges from 4.8% to 25.8%, compared with 0.9% to 7.9% in men (5), with the incidence of the condition increasing with age. The prevalence of HT varies between regions and socio-economic levels. A recent meta-analysis showed that the prevalence is highest in low- and middle-income countries, particularly in Africa (6). In addition, HT is more commonly observed in individuals diagnosed with other autoimmune diseases, such as type 1 diabetes mellitus (T1DM), Addison's disease, systemic lupus erythematosus, or rheumatoid arthritis. These are classified under autoimmune polyglandular syndrome (7).

HT is the leading cause of primary hypothyroidism and affects a significant number of people worldwide (8). The most serious consequence of HT is the development of hypothyroidism requiring lifelong oral levothyroxine sodium tablets. Pregnant women with TPOAb-positive status are particularly susceptible to postpartum thyroiditis and potentially permanent hypothyroidism. In addition, pregnant and perinatal women diagnosed with HT have an increased risk of miscarriage, preterm birth and fetal mental retardation (9). In particular, HT creates a pro-tumorigenic microenvironment in which gene rearrangements favor the development of thyroid cancer and inflammation further contributes to cancer progression. Recent studies indicate a higher incidence of papillary thyroid cancer in HT patients compared to the average population, suggesting the role of HT as a tangible risk factor and precursor for papillary thyroid cancer (10, 11). Therefore, early detection of HT and prompt intervention are of paramount clinical importance.

The pathogenesis of HT remains unclear (7). Current scientific research broadly implicates a number of factors including autoimmunity, genetics, environmental exposures, infections and oxidative stress (7). Clinically, HT is characterized by goiter, lymphocyte infiltration and elevated serum autoimmune antibody levels. However, there is no clear evidence of a relationship between antibody titres and the risk of HT-induced (subclinical) hypothyroidism. Given the challenges in understanding the pathogenesis of HT, no definitive and effective treatments have yet been developed, and thyroid hormone replacement therapy is restricted to symptomatic or hypothyroid patients. Before the onset of hypothyroidism, the standard of care is observation, watchful waiting and regular follow-up, and medical intervention is generally unnecessary. Given these uncertainties, the etiology, diagnosis and treatment of HT warrant continued investigation. Despite the increasing number of HT-related research studies in recent years, an objective evaluation and synthesis of the research landscape are still lacking.

Bibliometrics emerged as an independent discipline in the late 1970s, integrating statistics, philology and mathematics. It has found extensive applications in document analysis (12). Using modern computer technology, bibliometric analysis can generate clear and well-visualized knowledge graphs, providing quantitative analysis methods for studying existing documents within specific fields. CiteSpace and VOSviewer are two commonly used information visualization software tools, each with its own advantages in terms of plotting. When used in combination, they can complement each other. CiteSpace uses set theory for data standardization to measure the similarity of knowledge units. It can effectively describe the knowledge evolution process and the historical time span of documents in a given cluster (13). On the other hand, VOSviewer uses a data standardization method based on the theory of probability and provides a refined and concise representation of visual knowledge maps for keywords, institutions, authors, etc. (14).

The Web of Science Core Collection (WoSCC) database is widely recognized as a high quality digital document resource, particularly suitable for bibliometric analysis, with extensive applications in fields as diverse as geology, medicine and ecology (15). This database can competently identify and summarize the state of research and emerging trends in related disciplines. The present study is based on bibliometric analysis of the WoSCC database to identify and analyze HT-related documents, with the specific objectives of (i) identifying challenging issues and research hotspots in HT; (ii) examining the research trajectory and trend in HT; (iii) constructing knowledge maps in HT; and (iv) providing valuable insights and directions for future HT research.

2 Materials and methods

2.1 Data source and retrieval strategies

A comprehensive search of peer-reviewed publications on HT was conducted in the WoSCC database (https://www.webofscience.com/). The search was independently conducted by two authors (MG and YW). The main retrieval keywords included Hashimoto disease/Hashimoto's disease, Hashimoto struma/Hashimoto's struma, Hashimoto thyroiditis/Hashimoto's thyroiditis, Hashimoto syndrome/Hashimoto's syndrome, chronic lymphocytic thyroiditis, and autoimmune thyroiditis. Inclusion criteria for eligible documents were as follows: (i) publications from the WoSCC database, (ii) articles and reviews, (iii) publications from 1 January 1990 to 7 March 2023, and (iv) no language restrictions. The detailed search strategy using the WoSCC database is summarized in Supplementary Table 1. A total of 7,624 eligible publications (including 6,596 articles and 1,028 reviews) were included in the analysis, as shown in Figure 1A.

2.2 Data analysis

The exported data files were analyzed and visualized using CiteSpace 6.1.R3, VOSviewer 1.6.18, Bibliometrix R package and Scimago Graphica. CiteSpace 6.1.R3 was used to perform visualization analysis of institutions, references, and prediction of possible hot spot burst keywords. VOSviewer 1.6.18 was used to visualize countries, journals, authors, and cluster keywords. The Bibliometrix R Package was used to display source dynamics and

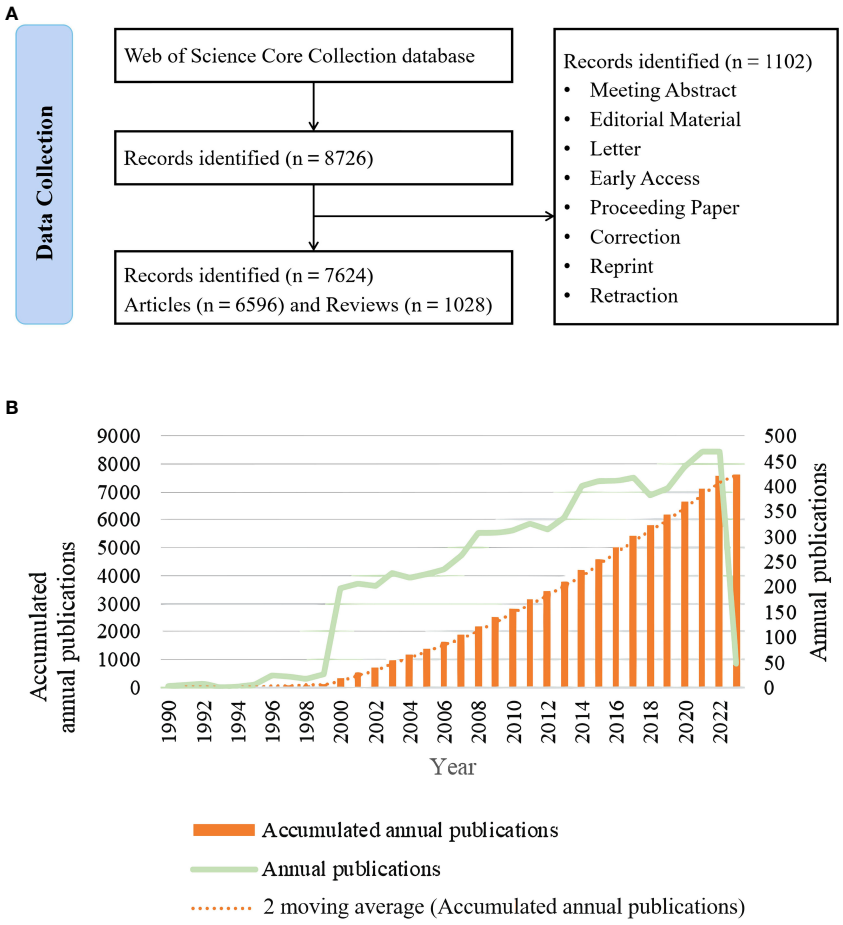


FIGURE 1
(A) Flowchart of literature selection; **(B)** Trends in the growth of publications worldwide from 1990 to 2023.

topic dynamics. Scimago Graphica was used to visualize the global distribution of publications.

3 Results

3.1 Development phases and publication trends

The number of documents in a particular field and its change trend can reflect the development stage of the research field, and even evaluate and predict the development status of the research. According to the retrieval process (Figure 1A), 7624 publications on HT from 1 January 1990 to 7 March 2023 were included, of which 6596 articles accounted for 86.52% of the total, followed by 1028 reviews (13.48%). The number of publications and the publication time of these documents were counted, as shown in Figure 1B. According to their temporal characteristics, they can be divided into three phases. The first stage was the exploration stage, and although researchers explored HT between 1990 and 1999, the number of publications was relatively small; the second stage was the rapid development stage, which took place in 1999-2000, and the number of published articles increased explosively from 27 in 1999 to 196 in 2000; the third stage was the steady development stage, which began in 2000 and continues to the present. While the number of HT-related research publications has experienced fluctuations, the overall trend has shown steady growth. 2022 saw the highest number of articles published, reaching 489. However, this record number wasn't surpassed during the entire year. However, based on the current state of research and progress, it is speculated that the field is in a stage of steady development, and there are still prospects and popularity in the future.

3.2 Global contributions and collaborations

Over the past 33 years, HT-related research has been carried out in 98 countries/regions. Using Scimago Graphica software, we created a world map of information from published articles that could reflect the collaboration between different countries on the subject of this study (Figure 2A). Countries were ranked based on the number of articles published, with only the top 10 listed in Table 1. The United States in North America topped the list with 1405 global publications, followed by China in Asia, Italy in Europe and Japan in Asia with 1089, 1079, and 603 articles, respectively. These four countries accounted for more than half (54.77%) of global publications. Turkey in Asia and Germany in Europe also made important contributions in this field, with more than 400 articles each. Nevertheless, research in this field has been absent in some regions since 1990 (Figure 2B). Meanwhile, Figure 2C shows a map illustrating cooperation between countries in HT research.

Meanwhile, a map of cooperation between countries in HT research is shown in Figure 2C. The results show that the United States cooperates with other countries, especially Italy, Japan, the United Kingdom, Germany, and China. Additionally, this study suggests that scientific research should not be constrained by geographical boundaries Therefore, the next step of international cooperation in conducting research in this field will be crucial.



FIGURE 2
(A) The network map of collaboration relations between countries generated with Scimago Graphica software; **(B)** The world map of publications of countries generated with R software; **(C)** Cooperative relationships between different countries generated with Scimago Graphica software.

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TABLE 1 Top 10 productive countries regarding the research on Hashimoto's thyroiditis.

Rank	Country	Documents(n)	Percentage (n/7624)	Citations	Average citations
1	USA	1405	18.43%	58390	41.56
2	China	1089	14.28%	13919	12.78
3	Italy	1079	14.15%	33557	31.10
4	Japan	603	7.91%	18365	30.46
5	Turkey	494	6.48%	6235	12.62
6	Germany	488	6.40%	15493	31.75
7	Poland	371	4.87%	5281	14.23
8	UK	314	4.12%	13854	44.12
9	France	277	3.63%	9651	34.84
10	South Korea	254	3.33%	5964	23.48

3.3 Institutional contributions and collaborations

CiteSpace was used to analyze and visualize 6181 institutions that have contributed to this field (Figure 3). The top 10 institutions with the highest number of publications are listed in Table 2. Over the last 33 years, four of the top 10 research institutions were located in Italy (4/10), including the top two, followed by China (2/10) and the United States (2/10). In terms of collaboration between institutions, we find that the institutions with a high degree of centrality (purple circle) belong to three countries: Italy, China and the United States, namely the University of Pisa, Shanghai Jiao Tong University and Harvard University. In addition, the University of Pisa ranked first with 214 publications, and the University of Messina (n = 148) and China Medical University (n = 103) also made significant contributions to HT-related research.

Notably, among the top 10 institutions by number of publications, Johns Hopkins University in the United States ranked tenth (n = 63), but had the highest average citation value (value = 65.16). In addition, the lack of inter-institutional collaboration is indicative of low institutional connectivity, which further underlines the importance of collaboration.

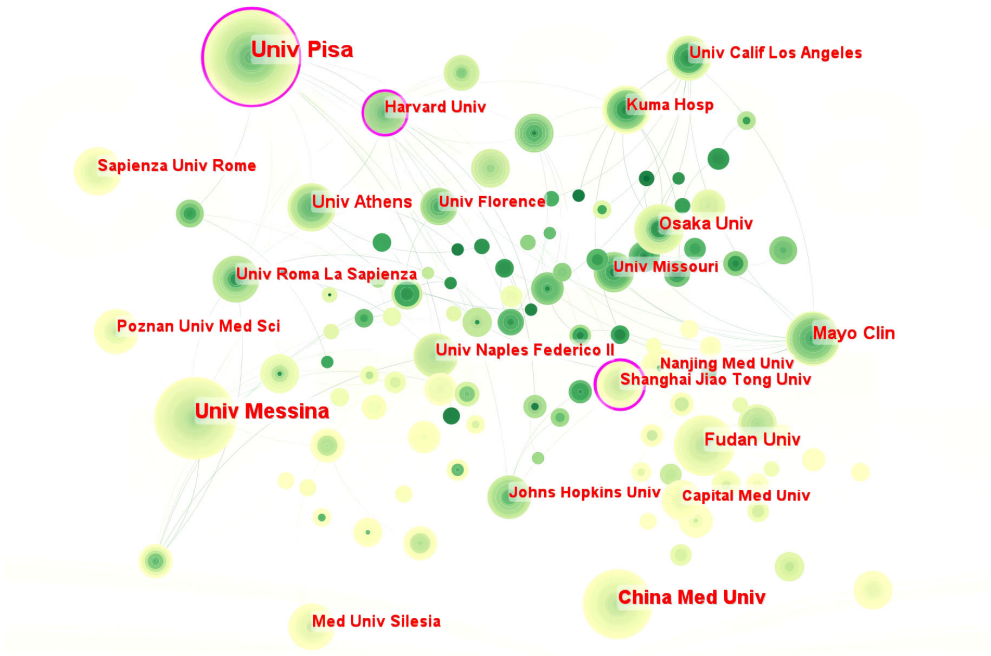


FIGURE 3
The collaboration of institutions in the field of HT.

TABLE 2 Top 10 productive institutions regarding the research on Hashimoto's thyroiditis.

Rank	Institution	Documents (n)	Percentage (n/7624)	Citations	Average citations
1	University of Pisa (Italy)	214	2.81%	8780	41.03
2	University of Messina (Italy)	148	1.94%	3430	23.18
3	China Medical University (China)	105	1.38%	2335	22.24
4	Fudan University (China)	88	1.15%	1544	17.55
5	Mayo Clinic (USA)	86	1.13%	3253	37.83
6	Osaka University (Japan)	79	1.04%	2114	26.76
7	University of Naples Federico II (Italy)	72	0.94%	2456	34.11
8	University of Athens (The Hellenic Republic)	68	0.89%	2197	32.31
9	University of Rome La Sapienza (Italy)	65	0.85%	2222	34.18
10	Johns Hopkins University (USA)	63	0.83%	4105	65.16

3.4 Leading authors and their contributions

Over the past 33 years, a total of 33,652 authors participated in the research of HT. The top 10 most productive authors are listed in Table 3 and include Antonelli A (n = 82), Fallahi P (n = 80), Ferrari SM (n = 72), Shan ZY (n = 58), Teng WP (n = 55), Benvenga S (n = 51), Zhang JA (n = 48), Tomer Y (n = 42), Iwatani Y (n = 33), Watanabe M (n = 33), of whom 5 are from Asia, 4 from Europe, and 1 from North America. The contribution of authors with minimal productivity of 10 publications (n=184) was visualized using VOSviewer software and shown in Figure 4A that their collaboration was divided into 8 clusters. When combined with the superimposed visualization of the year (Figure 4B), it reflects that the yellow, orange, and purple clusters were mainly active before 2016, and the clusters of other colors were still active in recent years.

The top 10 co-cited authors are listed in Table 4, and the top three of them are all from the University of Pisa in Italy: Antonelli A (n = 3446), Fallahi P (n = 3409), and Ferrari SM (n = 3147). Among them, Antonelli A is the author with the most published articles and the highest average citation, indicating that he has made outstanding contributions and has a high academic impact in the field. The co-citation relationship is shown in the cluster plot (Figure 4C) and the influence of co-citation authors in the field is displayed in the density plot (Figure 4D).

3.5 Journal distribution, impact, and citation analysis

A total of 1547 journals have reported on HT studies, with the top 10 publications shown in Table 5. *Thyroid* was the most prolific journal (n = 339, 22.06%), followed by *Journal of Clinical Endocrinology & Metabolism* (n = 202, 13.14%) and *Journal of Endocrinological Investigation* (n = 156, 10.15%). Of the top 10 journals, 4 are from the United Kingdom and 3 are from the United States. There are 5 journals with an impact factor > 5, namely *Thyroid* (6.506), *Journal of Clinical Endocrinology & Metabolism* (6.134), *Journal of Endocrinological Investigation* (5.467), *Frontiers in Endocrinology* (6.055), and *European Journal of Endocrinology* (6.558). In addition, based on the information provided by the Journal Citation Reports (JCR) evaluation system, we also found

TABLE 3 Top 10 productive authors in the field of Hashimoto's thyroiditis.

Rank	Author	Institution (Country)	Documents	H-index
1	Antonelli, Alessandro	University of Pisa (Italy)	82	30
2	Fallahi, Poupak	University of Pisa (Italy)	80	29
3	Ferrari, Silvia Martina	University of Pisa (Italy)	72	28
4	Shan, Zhongyan	China Medical University (China)	58	17
5	Teng, Weiping	China Medical University (China)	55	16
6	Benvenga, Salvatore	University of Messina (Italy)	51	20
7	Zhang, Jin-an	Shanghai University of Traditional Chinese Medicine (China)	48	17
8	Tomer, Yaron	Albert Einstein College of Medicine (USA)	42	29
9	Iwatani, Yoshinori	Osaka University (Japan)	33	13

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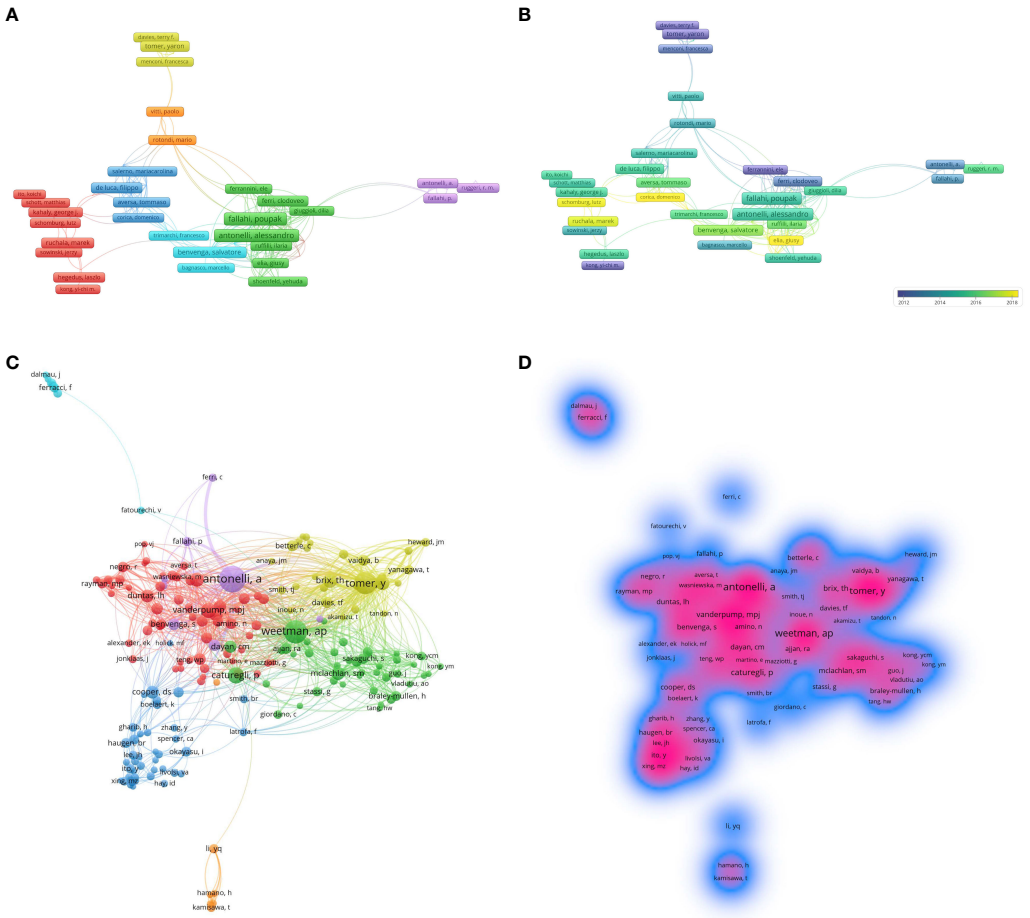


FIGURE 4
The collaboration The collaboration of authors and co-cited authors in the field of HT. **(A)** Cooperation network among the authors. **(B)** The time-overlay map of the cooperation network among the authors. **(C)** Cooperation network among the co-cited authors. **(D)** VOSviewer density visualization of the co-cited authors of institutions in the field of HT.

that the top 10 journals are mainly concentrated in the JCR of Q1 (40%) and Q4 (30%). The journals with minimum productivity of 40 publications (n = 21) were visualized using VOSviewer software, and as shown in Figure 5A, their collaboration was divided into 4 clusters. Combined with the superimposed visualization of the year (Figure 5B), it shows that the yellow and red clusters were mainly active after 2016, while the blue and green clusters were primarily active before 2016.

TABLE 4 Top 10 productive co-cited authors in the field of Hashimoto's thyroiditis.

Rank	Author	Institution (Country)	Citations	H-index
1	Antonelli, Alessandro	University of Pisa (Italy)	3446	30
2	Fallahi, Poupak	University of Pisa (Italy)	3409	29
3	Ferrari, Silvia Martina	University of Pisa (Italy)	3147	28
4	Tomer, Yaron	Albert Einstein College of Medicine (USA)	2584	29
5	Shan, Zhongyan	China Medical University (China)	1659	17
6	Teng, Weiping	China Medical University (China)	1612	16
7	Ferrannini, Ele	CNR Institute of Clinical Physiology (Italy)	1530	15
8	Shoenfeld, Yehuda	Ariel University (Israel)	1528	15
9	Drexhage, HA	University Medical Center Rotterdam (the Netherlands)	1523	12
10	Amino, Nobuyuki	Kuma Hospital (Japan)	1459	8

TABLE 5 Top 10 productive journals in the field of Hashimoto's thyroiditis.

Rank	Journal	Publications (%)	Country	Impact factor (2021)	JCR
1	Thyroid	339 (22.06)	USA	6.506	Q1
2	Journal of Clinical Endocrinology & Metabolism	202 (13.14)	USA	6.134	Q1
3	Journal of Endocrinological Investigation	156 (10.15)	Italy	5.467	Q2
4	Frontiers in Endocrinology	151 (9.82)	Switzerland	6.055	Q1
5	European Journal of Endocrinology	117 (7.61)	England	6.558	Q1
6	Clinical Endocrinology	115 (7.48)	England	3.523	Q3
7	Endocrine	108 (7.03)	USA	3.925	Q3
8	Endocrine Journal	103 (6.70)	Japan	2.860	Q4
9	Journal of Pediatric Endocrinology & Metabolism	103 (6.70)	England	1.520	Q4
10	Autoimmunity	100 (6.51)	England	2.957	Q4

The influence of journals in a research field depends on the number of citations. The co-citation analysis was performed using VOSviewer (Figure 5C), and the density map is shown in Figure 5D. The size of the nodes represents the number of references, and the lines between the nodes indicate the co-citation relationship. The *Journal of Clinical Endocrinology & Metabolism*, sponsored by the United States, is the most influential journal with 18,252 citations. It is followed by the American journal, Thyroid, with 12,298 citations, demonstrating a high degree of authority in the field of HT. Among the top 10 co-cited journals, 9 journals have an impact factor of > 5, of which the impact factor of *Lancet* is 202.731, and the impact factor of *New England Journal of Medicine* is 176.082. Both Q1 and Q2 account for 40% of the journals, indicating high-quality HT-related articles and the academic significance of this study (Table 6). VOSviewer was combined with Pajek software to create a cited map and a citing map of the journals, as shown in Figures 5E, F. Limitations within VOSviewer prevented the creation of dual map overlays.

3.6 Key co-cited references and emerging topics

We analyzed 148,499 co-cited references and listed the top 10 references in Table 7. The article " Serum TSH, T =(4), and thyroid antibodies in the United States population (1988 to 1994): National Health and Nutrition Examination Survey (NHANES III) " published in the *Journal of Clinical Endocrinology & Metabolism* (2021 IF: 5.467) in 2002 ranked first with 438 citations. This article retrospectively analyzed the average concentrations of TSH, T4, TgAb, and TPOAb in a disease-free population (n=16533) from the NHANES III database in the United States. This population included individuals who had not reported thyroid diseases, thyroid nodules, or were not taking thyroid medication. The analysis found that approximately 18% of the disease-free population had detectable levels of TgAb or TPOAb, and a large proportion of Americans may have laboratory evidence of either hypothyroidism or hyperthyroidism without being aware of it. This finding supports the effectiveness of early screening.

The most recently published article was " 2015 American Thyroid Association Management Guidelines for Adult Patients with Thyroid Nodules and Differentiated Thyroid Cancer The American Thyroid Association Guidelines Task Force on Thyroid Nodules and Differentiated Thyroid Cancer ", which published in the journal *Thyroid* (2021 IF: 6.506) in 2016. The aim of this guideline is to inform clinicians, patients, researchers, and health policy makers about the best available evidence and its limitations regarding the diagnosis and management of thyroid nodules and differentiated thyroid cancer (DTC) in adult patients. Patients with HT often have concomitant thyroid nodules, and the clinical significance of thyroid nodules lies in the need to exclude thyroid cancer. DTC consists predominantly of papillary carcinoma and follicular carcinoma, accounting for the majority of all thyroid cancers (>90%). In addition, we performed co-citation networks and clustering analysis using CiteSpace (Figure 6), and the references were divided into 15 clusters with active co-citations. The presence of papillary thyroid carcinoma (#1) and thyroid cancer (#2) also indicates that in HT-related studies, the differential diagnosis and disease progression of HT and thyroid cancer have always been a focus of academic attention.

Among the top 10 co-cited references, 6 are reviews and 4 are articles. Among them, the *New England Journal of Medicine* (2021 IF: 176.082) contains the most highly co-cited articles, which is one of the most prestigious peer-reviewed medical journals. This article reported the first case report of Hashimoto's encephalopathy (HE) in 1966. Although the number of published case reports is limited so far, and the etiology of HE is not well understood, its clinical manifestations are similar to many neurological disorders. Therefore, it is often misdiagnosed, leading to speculation that the number of cases of HE is much higher than reported.

3.7 Keyword analysis and emerging trends

Keyword analysis can reflect the current status of research topics in the HT field, including hot spots and future development directions. We constructed a co-occurrence network

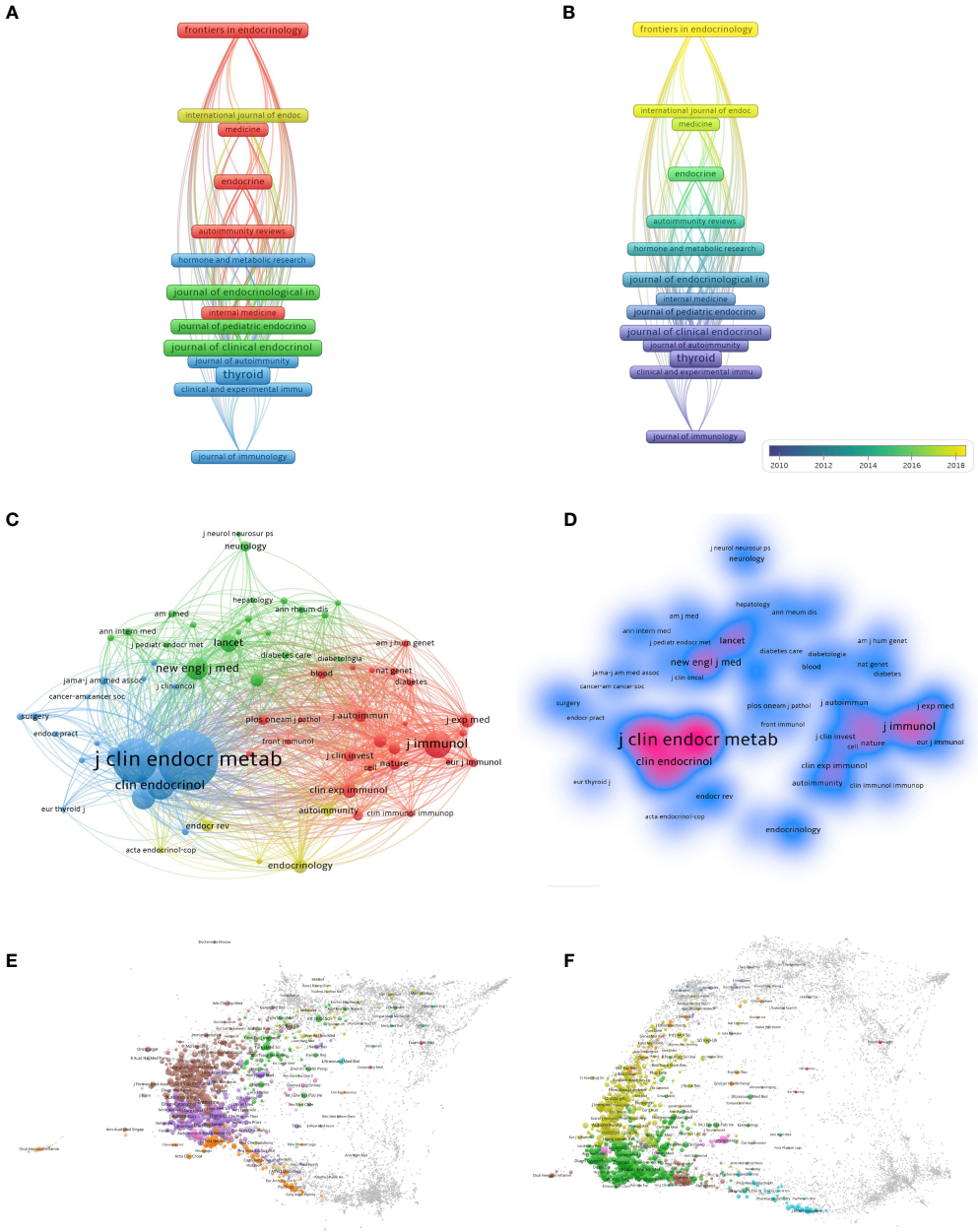


FIGURE 5
The collaboration of journals and co-cited journals in the field of HT. **(A)** Cooperation network among the journals. **(B)** The time-overlay map of the cooperation network among the journals. **(C)** Cooperation network among the co-cited journals. **(D)** VOSviewer density visualization of the co-cited journals. **(E)** The co-cited journals overlay map. **(F)** The citing journals overlay map.

for high-frequency keywords and visualized them by cluster analysis (Figure 7A). As shown in Supplementary Table 2, the keywords " hashimoto's thyroiditis ", " autoimmune thyroiditis " and " graves' disease " rank in the top three with 815, 742 and 608 occurrences, respectively. This suggests that the most commonly used name for HT is " Hashimoto's thyroiditis ". Moreover, the identification of HT and Graves' disease in the early stage, and the mutual transformation and coexistence of the two diseases in the later stage have always been the most prominent topics in HT research. In addition, the cluster was divided into three domains according to Figure 7A: (i) Clinical diagnosis and identification of HT, red cluster includes "biomarkers", " anti-thyroid antibodies", " tsh receptor ", " subacute thyroiditis ", " thyroglobulin " and " addison 's disease "; (ii) Risk factors and treatment of HT, green cluster include " risk factors ", " surgery ", " gut microbiota ", " genetic susceptibility ", " thyroid lymphoma ", " vitamin d ", and " therapy "; (iii) Complications and prevention of HT, blue cluster include " postpartum thyroiditis ", " breast cancer ", " type 2 diabetes mellitus ", " vitamin d deficiency ", " quality of life ", " depression ", " selenium " and " iodine ". Furthermore, in Figure 7B, we visualized the average year of occurrence, with yellow and blue colors representing the closer and earlier keywords, respectively. In addition, we used visualization techniques to illustrate the density of the identified keywords, as shown in Figure 7C. The color mapping

TABLE 6 Top 10 co-cited journals in the field of Hashimoto's thyroiditis.

Rank	Journal	Citations	Country	Impact factor (2021)	JCR
1	Journal of Clinical Endocrinology & Metabolism	18252	USA	5.467	Q2
2	Thyroid	12298	USA	6.506	Q1
3	Journal of Immunology	6387	USA	5.430	Q2
4	Clinical Endocrinology	6385	England	3.523	Q3
5	European Journal of Endocrinology	5037	England	6.558	Q1
6	New England Journal of Medicine	4891	USA	176.082	Q1
7	Lancet	3460	England	202.731	Q1
8	Journal of Endocrinological Investigation	2846	Italy	5.467	Q2
9	Endocrinology	2688	USA	5.051	Q2
10	Journal of Experimental Medicine	2624	Italy	5.467	Q2

of the keywords corresponds to their relative weight, with red hues indicating higher weights.

The "burst words" signify the emergence of keywords that have been heavily cited during a particular time period, thereby indicating the current focuses of academic inquiry. Through the use of CiteSpace software, the present study was able to identify keywords that exhibited citation bursts, resulting in the identification of 25 such keywords, shown in Figure 8A, with the red band indicating the burst keyword retention phase. Notably, keywords such as experimental autoimmune thyroiditis (22.42, 1997 - 2013), T cells (12.32, 2000 - 2006), gene expression (16.27, 2006 - 2010) and single nucleotide polymorphism (20.22, 2009 - 2013), showed that exploring the pathogenesis of HT through experiments has been a hot spot in HT research in the past;

TABLE 7 Top 10 co-cited references in the field of Hashimoto's thyroiditis.

Rank	Title	Citations	Year	Journal	Type	Impact factor (2021)
1	Serum TSH, T(4), and thyroid antibodies in the United States population (1988 to 1994): National Health and Nutrition Examination Survey (NHANES III)	438	2002	Journal of Clinical Endocrinology & Metabolism	Article	5.467
2	The incidence of thyroid disorders in the community: A twenty-year follow-up of the Whickham Survey	316	1995	Clinical Endocrinology	Article	3.523
3	Chronic autoimmune thyroiditis	307	1996	New England Journal of Medicine	Review	176.082
4	Hashimoto thyroiditis: Clinical and diagnostic criteria	301	2014	Autoimmunity Reviews	Review	17.390
5	Current concepts: Thyroiditis	235	2003	New England Journal of Medicine	Review	176.082
6	Autoimmune thyroid disorders	216	2015	Autoimmunity Reviews	Review	17.390
7	Autoimmune thyroid - disease: Further developments in our understanding	212	1994	Endocrine Reviews	Review	25.261
8	2015 American Thyroid Association Management Guidelines for Adult Patients with Thyroid Nodules and Differentiated Thyroid Cancer The American Thyroid Association Guidelines Task Force on Thyroid Nodules and Differentiated Thyroid Cancer	202	2016	Thyroid	Article	6.506
9	Hashimotos disease and encephalopathy	185	1966	Lancet	Article	202.731
10	Hashimoto encephalopathy: Syndrome or myth?	184	2003	Archives of Neurology	Review	7.419

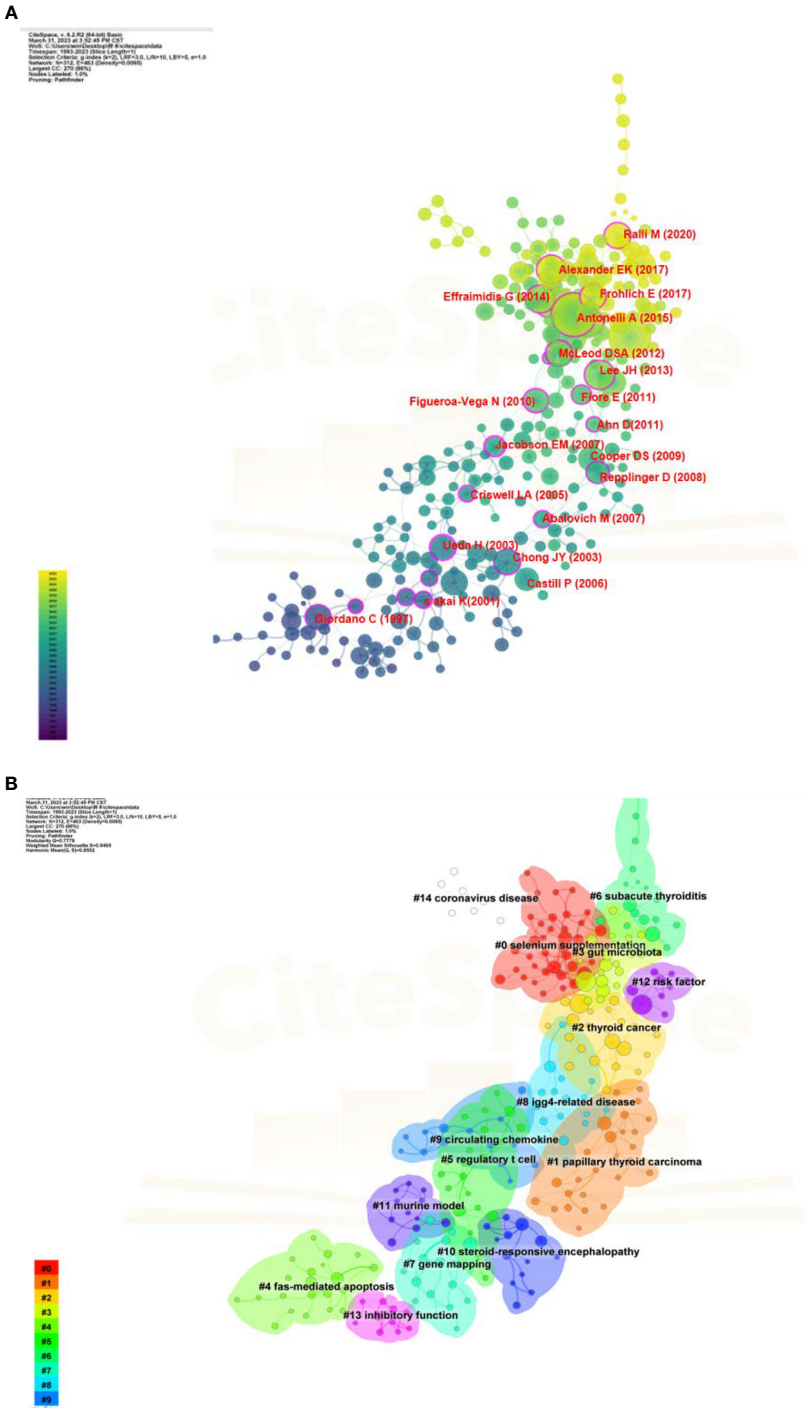


FIGURE 6
Reference analyses in the field of HT. **(A)** Co-cited networks of references. **(B)** Cluster diagrams of references.

Keywords such as papillary thyroid carcinoma (12.66, 2021-2023), papillary thyroid cancer (15.32, 2017 - 2023), and Vitamin D (18.9, 2017 - 2023) inferred novel nutritional treatment strategies supplementing trace elements and the correlation between HT and cancer have become the focus of current research in this field. In addition, we used R software to draw the annual theme dynamic map, as shown in Figure 8B.

4 Discussion

Our research used bibliometric methods to analyze trends and hotspots in HT-related research by examining publication output, growth of research interest, leading countries/regions, international collaborations, top institutions, preferred journals, and author keywords.

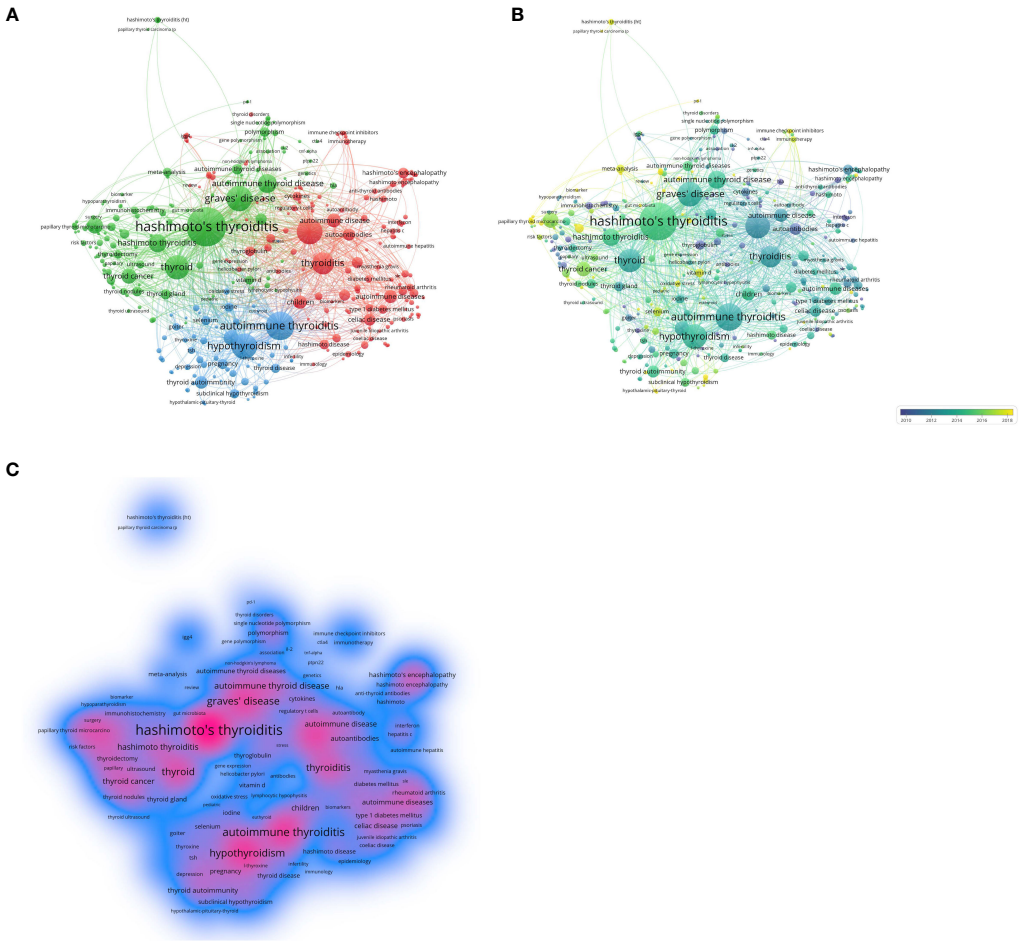


FIGURE 7
Keyword analyses in the field of HT. **(A)** VOSviewer cluster visualization of keywords. **(B)** VOSviewer density visualization of keywords. **(C)** VOSviewer overlay visualization of keywords.

4.1 Research phases of development and influencing factors

By analyzing the annual production of publications, we identified three phases in the development of the HT field over the past decades: the initial exploration stage, the rapid development phase, and the steady development stage. The number of publications in the rapid development stage increased explosively from 27 in 1999 to 196 in 2000, and this change in development speed may be attributed to the improvement of HT detection methods, the in-depth study of pathogenesis, and the development of clinical trials. Improvements in examination methods have been seen, such as the color Doppler measurement of inferior thyroid arterial blood flow in patients with autoimmune thyroid disease (AITD) (16), fine-needle aspiration of thyroid nodules (17), and the emergence of novel TSH receptor antibody assays (18). In addition, advances in the genetics, cytology, and histology of Hashimoto's disease have paved the way for a more thorough investigation of the pathogenesis (19, 20). Case reports (21) and observational clinical trials (22) have revealed the current state of research on the complex symptoms, multiple complications, and treatment limitations of Hashimoto's disease. It is noteworthy that the expansion of annual publications has plateaued since the previous year, indicating that the progress of HT has been impeded. In particular, the lack of effective remedies for HT remains a concern, and in cases where HT develops into hypothyroidism, the majority of patients require lifelong thyroid hormone replacement. Notably, the manifestation of clinical symptoms persists in many patients even after normalization of thyroid hormone replacement (23).

4.2 Dissecting the imbalance of academic resources

In terms of collaborative networks of countries, institutions, and authors, a persistent problem is the chronic imbalance in academic resources between developing and developed countries. For example, China and Turkey, as representatives of developing countries, have already been among the top countries in the world in terms of the number of publications, ranking second and fourth respectively, but their influence towards other countries in this field remains weak, as

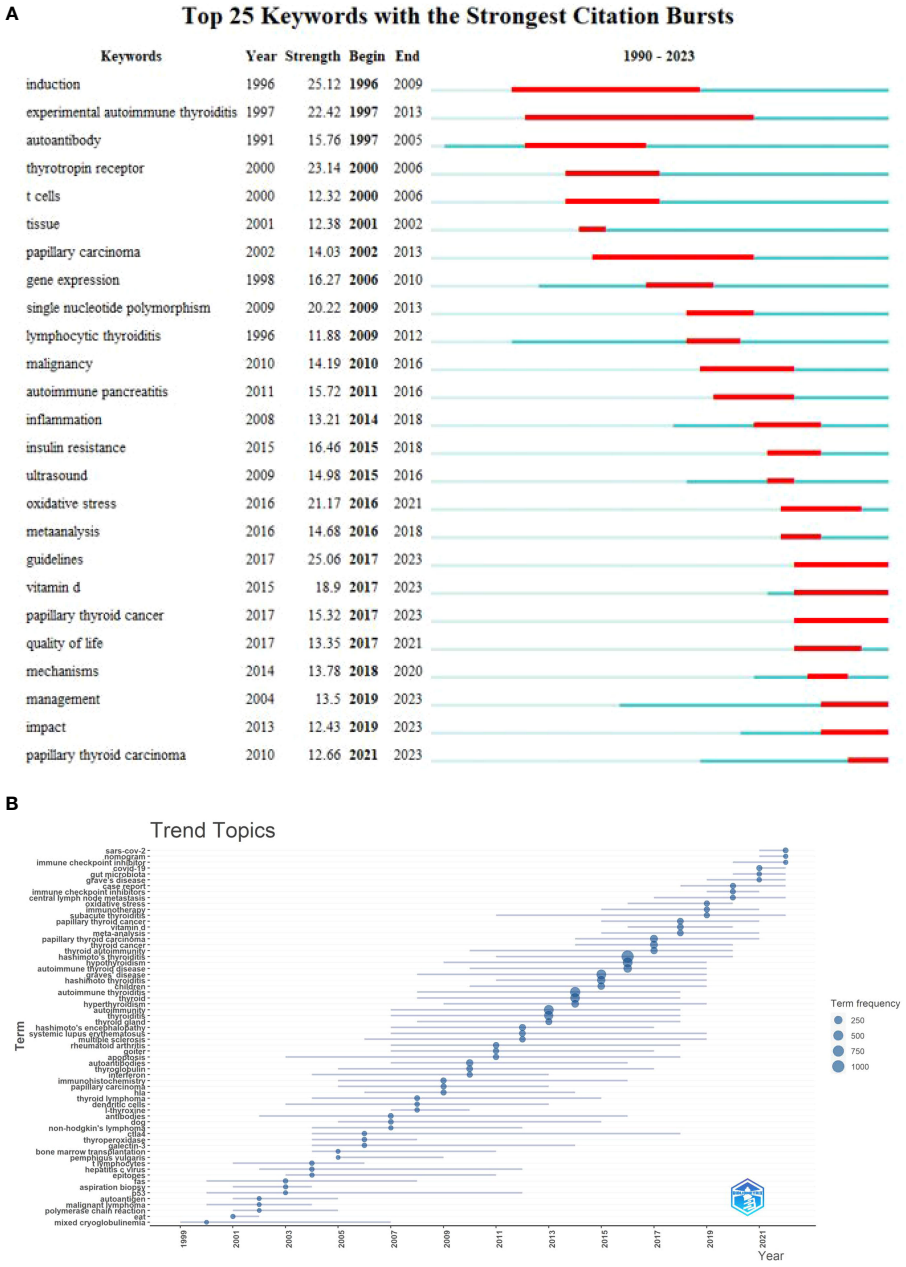


FIGURE 8
(A) Top 10 keywords with the strongest citation bursts; **(B)**. Topics dynamics in the field of HT generated by R software.

reflected in the fact that the average citations of the two countries rank first and second last among the top 10 countries in terms of the number of publications. Among the top 10 institutions for publications, 8 are located in developed countries, four of which are located in Italy. Only 2 institutions from the developing world, Fudan University and China Medical University, are in China. Notably, these two Chinese institutions rank near the bottom in terms of average citations among the top 10. This phenomenon can be explained in two ways. First, with rapidly expanding economies, scientific research has developed rapidly in growing countries. However, compared to developed countries, many of their scientific studies are characterized by replication and imitation. Second, economically developed countries and universities with higher academic status have more research resources and financial support, which encourages innovative practices. In addition, these institutions enjoy more opportunities for international exchange, which results in a notable academic gap between developed and developing regions. Nevertheless, Shanghai Jiaotong University, a Chinese research institution, has established initial partnerships with research institutions from other countries in recent years.

4.3 Journal influence and selection considerations

According to the comprehensive results of the number of publications and citations, *Thyroid* and *Journal of Clinical*

Endocrinology & Metabolism are the most influential journals in this field. *Thyroid* is the official journal of the American Thyroid Association (ATA), the only thyroid journal included in SCI, and is the most authoritative journal in the field of thyroid diseases, publishing articles covering clinical and experimental articles in the field of thyroid research. *Journal of Clinical Endocrinology & Metabolism* is also a top-level journal in the field of endocrinology. It is worth noting that the comprehensive journals *New England Journal of Medicine* (IF: 176.082) and *Lancet* (IF: 202.731) are not in the top ten in terms of the number of publications, but the number of citations ranks sixth and seventh, indicating that the quality of the publications of these two top journals is high. Analysis of journals can help researchers choose the right journals to submit papers, prioritizing high-impact journals while also considering the journal's ability to disseminate research to the right audience and contribute to the development of the field.

4.4 Research hotspots

Based on the results of keywords clustering and citations analysis, the following suggestions are provided for the future research hotspots in the field of HT.

4.4.1 Coexistence and diagnostic challenges of graves' disease in HT

The keyword "graves' disease" ranked first three with 608 occurrences, indicating that it often occurs at the same time as HT (24). Graves' disease (GD) and HT are the most common types of AITD (25). In Table 7, the article "Chronic autoimmune thyroiditis" published in the New England Journal of Medicine ranks 3rd in terms of citation frequency which extensively discusses the similarities and differences between HT and GD. GD is mainly manifested as hypermetabolic syndrome caused by hyperthyroidism, with diffuse enlargement of the thyroid gland and positive expression of TRAb. Approximately 70% of GD patients have positive TPOAb and TGAb expression (26). However, during the early stages of HT, clinical manifestations of hyperthyroidism, positive TGAb and TPOAb expression, and positive TRAb expression can also be observed in a small number of patients (27). Case studies have also shown that HT can progress to GD (28, 29). In addition, approximately 10-15% of GD patients may develop hypothyroidism following antithyroid therapy (30), strongly suggesting the coexistence of GD and HT. Thus, it is difficult for clinicians to differentiate between GD and HT, especially when presenting with atypical clinical symptoms and positive antibody test results in the early stages of either disease. Current diagnostic criteria remain unclear and can lead to serious adverse outcomes if incorrect treatment plans are pursued. Therefore, it is imperative that future research focus on developing clear and objective diagnostic criteria to ensure that patients receive the most effective and clinically appropriate treatments.

4.4.2 Gut microbiota and its implications in HT

As shown in Figure 8B, "gut microbiota" has emerged as a hot keyword over the past three years. Recent studies have shown that the gut microbiota may play an important role in the induction of HT (31). This study conducted co-citation network and clustering analysis of the co-cited references in HT-related research. It was found that gut microbiota (#3) is one of the clusters with active co-citation relationships. Several studies support the role of the gut commensal microbiota in autoimmune genesis (32, 33), but the role in HT has not received attention until recent years. Lerner A et al. (34) first proposed the concept of the "gut-thyroid axis" in their research. In the body's internal environment, only a single layer of epithelial cells separates luminal contents from effector immune cells, and disruption of the epithelium drives autoimmunity (32). 40% of HT patients suffer from lymphocytic colitis with elevated intraepithelial lymphocyte counts, further implicating the role of gut microbiota (35). Microbial metabolites have been shown to affect thyroid function and activate the immune system (36–38). Lipopolysaccharide-induced Toll-like receptor activation is associated with the development of thyroiditis or the production of anti-thyroglobulin antibodies in mice (39). Changes in the gut microbiota are clearly associated with the pathogenesis of HT, but their role in the induction or protection of thyroid autoimmunity remains to be investigated.

4.4.3 Complications and challenges: PTC and T1DM

As shown in Figure 8A, "papillary thyroid carcinoma"(12.66, 2021 - 2023) has become a prominent term in the last 3 years. Furthermore, papillary thyroid carcinoma (#1) and thyroid cancer (#2) were also identified as two important clusters with active co-citation relationships in this study. Since 1955, when Dailey et al. (40) first reported the association between HT and papillary thyroid cancer (PTC), the relationship between the two diseases has been the focus of research and controversy. Despite the controversy, many *in vitro* and *in vivo* studies have suggested that thyroid autoimmunity and thyroid cancer, mainly PTC, may coexist (41, 42). The inflammatory process of HT is considered a potential risk factor for the development of thyroid cancer (43). Lymphocytic infiltration of HT is common in the thyroid gland that has been partially removed due to tumor (44). Several recent studies have reported an increased incidence of PTC in HT patients after thyroidectomy, especially in women (45–48). On the other hand, the incidence of primary thyroid lymphoma is significantly increased in HT patients, strongly suggesting a pathogenesis link between this autoimmune disease and malignant thyroid lymphoma (49). In the future, it will be necessary to understand the relationship between thyroid autoimmunity and cancer and to develop tailored treatment options for PTC patients with AITD.

As shown in Table 7, the number of occurrences of the keyword "type 1 diabetes" ranks in the top 30. Comparatively, individuals with T1DM have an increased susceptibility to other autoimmune diseases compared to the general population, with this risk increasing over the past two decades (50). To further underscore such concerns, patients diagnosed with T1DM exhibit a greater incidence of thyroid dysfunction in contrast to both type 2 diabetes and non-diabetic controls. Specifically, patients with T1DM have an incidence of autoimmune thyroiditis ranging from 3 to 50 percent (51). A population-based twin study has provided compelling

evidence of etiologic overlap between T1DM and HT, suggesting that both disorders may share an identical origin (52). Similar to hyperthyroidism, hypothyroidism appears to affect glycemia in patients with T1DM (53), thereby increasing the risks of both diabetic retinopathy and diabetic nephropathy (54). Indeed, the clinical management of patients with both T1DM and HT poses significant challenges.

4.4.4 Nutritional factors in HTs: current understanding and controversies

As shown in Figure 8A, vitamin D (18.9, 2017 - 2023) is a burst word that has been active in the last 5 years. "Selenium" is also among one of the top 30 keywords in terms of frequency. Furthermore, the co-citation network and cluster analysis results of the co-citation references showed that selenium supplementation (#0) is one of the clusters with active co-citation relationships in this study. Nutritional deficiencies often accompany HT patients, and an appropriate diet with appropriate supplementation may be a critical aspect of the treatment process (55). However, the effects of selenium, vitamin D, iodine, iron, magnesium, and vitamin B12 on HT remain controversial. While some studies suggest a beneficial effect of vitamin D supplementation in HT patients by reducing thyroperoxidase antibody titers (56, 57), most studies show no significant associations between serum vitamin D and thyroglobulin antibody, thyroid-stimulating hormone, free triiodothyronine, or free thyroxine concentrations (5). In addition, excessive iodine intake has been shown to increase the incidence of HT (4). Further research is needed to evaluate the relationship between HT and selenium, magnesium, iron, and vitamin B12 levels. Although there are no specific dietary recommendations for patients with HT, studies support that a Mediterranean diet, rich in omega-3 fatty acids found in fatty fish (58), and an anti-inflammatory diet containing vitamins, minerals, and polyphenols may provide protective effects (59, 60). Evidence suggests that a gluten-free diet may also be beneficial for people with HT (61, 62), but further studies are needed. Medical therapy, in conjunction with appropriate diet and supplementation, is an important aspect of medical care for patients with HT. Therefore, more research is needed to determine the role of micronutrients and diet in the development and progression of HT.

4.5 Limitations

The analysis is subject to certain limitations. First, we only included English articles from the Web of Science database, possibly causing an over-representation of English-speaking countries' research. Second, taking citation count as a major factor would mean that very recent work although influential might not get featured in the study, and citation counts as an impact measure don't necessarily reflect the quality of research.

Moreover, the keyword analysis depends on the indexed keywords, potentially leading to inaccuracies in topic representation. Also, the study only covers articles published until 2023, and the research landscape may evolve with time. In addition, the software is currently unable to match and output countries with their respective published articles, which may result in the inability to conduct a comparative analysis of the quantity and types of research articles, as well as the specific mechanisms they focus on across different countries. It also hinders the exploration of potential links between variations in population proportions among different countries and differences in prevalence rates of HT. Despite these limitations, this study provides a comprehensive review of HT research and identifies key trends for future studies.

5 Conclusion

This bibliometric study has thoroughly investigated the landscape of HT research, illuminating significant trends, key contributors, and emergent hotspots. Although North America and Europe currently exert substantial influence in the academic domain, institutions in emerging nations like China are exhibiting increasing momentum in HT studies.

Research hotspots encompass areas such as the challenge of differentiating HT and Graves' disease, the complex link between gut microbiota and HT onset, and the management of HT alongside complications like papillary thyroid carcinoma or type 1 diabetes. The impact of dietary modifications and micronutrient supplementation on HT treatment is also a burgeoning research focus.

Furthermore, the emergence of sophisticated genetic profiling techniques and advanced immunotherapies brings a wave of exciting new possibilities for HT management. These trends mark out promising future research trajectories, paving the way for more comprehensive understanding and effective treatment strategies for HT. The information gleaned from this study serves as a springboard for researchers aiming to make impactful contributions to the field of HT research.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

MG: Conceptualization, Visualization, Writing – original draft. QL: Data curation, Writing – review & editing. XL: Supervision, Validation, Writing – review & editing. YW: Methodology, Writing – original draft. QY: Methodology, Writing – original draft. RL: Methodology, Writing – original draft. YZ: Formal Analysis, Writing – original draft. CL: Formal Analysis, Writing – original draft. SS: Software, Writing – original draft. HM: Software, Writing – original draft. ZL: Conceptualization, Writing – original draft. RG: Writing – review & editing, Conceptualization.

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Conflict of interest

Author MG was employed by the company Yabao Pharmaceutical Group Co., Ltd.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2023.1277739/full#supplementary-material

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Early postoperative prediction of the risk of distant metastases in medullary thyroid cancer

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Purpose: The purpose of this study was to develop and validate a nomogram for estimating the risk of distant metastases (DM) in the early postoperative phase of medullary thyroid cancer (MTC).

Patients and methods: We retrospectively reviewed cases of patients diagnosed with MTC from the Surveillance, Epidemiology, and End Results (SEER) database from 2007 to 2017. In addition, we gathered data on patients who diagnosed as MTC at Department of Thyroid Surgery in the First Hospital of Jilin University between 2009 and 2021. Four machine learning algorithms were used for modeling, including random forest classifier (RFC), gradient boosting decision tree (GBDT), logistic regression (LR), and support vector machine (SVM). The optimal model was selected based on accuracy, recall, specificity, receiver operating characteristic curve (ROC), and area under curve (AUC). After that, the Hosmer-Lemeshow goodness-of-fit test, the brier score (BS) and calibration curve were used for validation of the best model, which allowed us to measure the discrepancy between the projected value and the actual value.

Results: Through feature selection, we finally clarified that the following four features are associated with distant metastases of MTC, which are age, surgery, primary tumor (T) and nodes (N). The AUC values of the four models in the internal test set were as follows: random forest: 0.8786 (95% CI, 0.8070-0.9503), GBDT: 0.8402 (95% CI, 0.7606-0.9199), logistic regression: 0.8670(95% CI,0.7927-0.9413), and SVM: 0.8673 (95% CI, 0.7931-0.9415). As can be shown, there was no statistically significant difference in their AUC values. The highest AUC value of the four models were chosen as the best model since. The model was evaluated on the internal test set, and the accuracy was 0.84, recall was 0.76, and specificity was 0.87. The ROC curve was drawn, and the AUC was 0.8786 (95% CI, 0.8070-0.9503), which was higher than the other three models. The model was visualized using the nomogram and its net benefit was shown in both the Decision Curve Analysis (DCA) and Clinical Impact Curve (CIC).

Conclusion: Proposed model had good discrimination ability and could preliminarily screen high-risk patients for DM in the early postoperative period.

KEYWORDS

medullary thyroid carcinoma, high risk, distant metastases, predictive model, nomogram

Introduction

As of 2020, there will be 586,000 new cases of thyroid cancer worldwide, making up 3% of all cancer cases. Thyroid cancer incidence has consistently climbed over the previous 30 years (1). Medullary thyroid cancer (MTC) is a neuroendocrine tumor that develops from C cells in the thyroid gland and its incidence rate is low, accounting for just 2-4% of all thyroid cancers (2). The main treatment of MTC is surgery (3). As MTC is more aggressive and prone to recurrence and metastases compared to differentiated thyroid cancer (DTC) (4), regular postoperative follow-up is crucial for diagnosed patients. But due to differences in patient condition, tumor characteristics, and treatment strategies, individualized follow-up plans need to be tailored. When MTC patients are being followed up after surgery, some distant metastases may not be identified in a timely manner due to various reasons, resulting in poor prognosis. For example, although some typical patients may present with flushing and diarrhea when they have distant metastases (5), we have found in clinical practice that these symptoms may do not receive sufficient attention from postoperative patients because of the lack of specificity. Additionally, many atypical patients may have no specific symptoms. Moreover, calcitonin (Ctn) and carcinoembryonic antigen (CEA) are effective biomarkers to monitor tumor recurrence and distant metastases. But there are still some disadvantages about Ctn and CEA. The lengthy half-life of serum calcitonin may render early Ctn detection inefficient in accurately evaluating surgical efficacy, particularly when some patients have poor liver or kidney function or have high preoperative Ctn levels (6). Ctn doubling time requires a certain amount of time to elapse. For some non-secretory MTC, with double negative markers at the time of diagnosis and at the relapse, Ctn and CEA are not reliable biomarkers anymore (7, 8). The specificity and sensitivity of CEA are both inferior to Ctn, so we cannot just use CEA to determine whether a tumor has distant metastasis.

Given the aforementioned, we need a way to estimate individualized patient's risk of distant metastases in the early postoperative period (within 3 months after surgery). If we can make a preliminary judgment of the risk of distant metastases based on the patient's basic information, surgical strategy, and postoperative pathological report, patients will receive more accurate and personalized postoperative recommendations. Clinicians should alter their follow-up strategies for patients who have a high risk of distant metastases by decreasing the follow-up intervals and doing routine imaging tests. Follow-up intervals can be appropriately extended for individuals who have a low risk of metastases. Although relevant research on this issue has been carried out (9), we have found several shortcomings: firstly, they did not consider the impact of surgical strategy selection on distant metastases in MTC; secondly, there are many missing values in the SEER database, and they did not provide a detailed explanation of how to deal with missing values in their study; thirdly, their research lacks external validation. Therefore, based on previous research findings, we aim to further improve the research design and better address the relevant issues.

With the proposal of the concept of precision medicine (10), the idea of clinical doctors performing the same follow-up management on all patients has become outdated. There may be a possibility of over-diagnostics of low-risk patients and untimely treatment of high-risk patients. Therefore, we designed a retrospective study based on machine learning algorithms to evaluate the risk of distant metastases in the postoperative period in MTC patients.

Materials and methods

Data collection

The data for this study were collected from two sources: the Surveillance, Epidemiology, and End Results (SEER) database (n=2519) and the First Hospital of Jilin University (n=133).

The retrospective selection was carried out on case data from SEER between 2007 and 2017 based on International Classification of Diseases (ICD) code C73.9 and ICD-O-3 Hist/behave, malignant encoding of 8510, 8347, 8346, and 8345 for patients with MTC. Features related to distant metastases were preliminarily selected, including population features such as gender, age at diagnosis, and race, as well as tumor features such as "Primary Site - labeled, ICD-O-3 Hist/behav, malignant, RX Summ–Surg Prim Site (1998+), RX Summ–Scope Reg LN Sur (2003+), RX Summ–Surg Oth Reg/Dis (2003+), Reason no cancer-directed surgery, CS lymph nodes (2004-2015), Regional nodes examined (1988+), Regional nodes positive (1988+), SEER Combined Mets at DX-bone (2010+), SEER Combined Mets at DX-brain (2010+), SEER Combined Mets at DX-liver (2010+), SEER Combined Mets at DX-lung (2010+), CS tumor size (2004-2015), CS extension (2004-2015), and CS site-specific factor 1 (2004-2017 varying by schema)".

Patient data from the electronic medical records database at our hospital were screened for pathology reports from 2009 to 2021 that indicated MTC. Through telephone follow-up survey so that the patients' survival status and distant metastases were discovered.

Patients were not included in the analysis if their distant metastases status was unknown or missing. Ultimately, the SEER dataset included 1901, while the dataset from our hospital included 111. The data from SEER were divided into three sets: a training set (n=1332, 70%), an internal testing set (n=569, 30%), and the data from our department were split out as an external validation set (n=111).

Definition: Extent of surgery: no surgery (code 0): patients was diagnosed with MTC by fine-needle aspiration (FNA), and persisted in refusing surgical treatment despite being fully informed of the disease's harm by the doctor. Total thyroidectomy (code 2): removal left and right lobes and isthmus of the thyroid gland. Non-total thyroidectomy (code 1): local tumor destruction, removal of less than a lobe including local surgical excision and removal of a partial lobe only, lobectomy and/or isthmectomy including lobectomy only and isthmectomy only and lobectomy with isthmus, removal of a lobe and partial removal of the contralateral lobe, subtotal or near total thyroidectomy.

Extent of lymph node dissection: N0: there are no evidence for metastases of reginal lymph nodes. N1a: anterior compartment group including level VI and level VII. N1b: cervical lymph nodes except for central compartment.

Data preprocessing

All textual information was converted to numerical codes. For SEER data, we reclassified "ICD-O-3 Hist/behav, malignant" as follows: 8510/3: Medullary carcinoma, NOS, and 8345/3: Medullary carcinoma with amyloid stroma were coded as 0 for not merging with other types of tumors, while all other types of tumors were merged and coded as T staging was determined based on "CS tumor size (2004-2015)" and "CS extension (2004-2015)", with T1, T2, T3, and T4 stages. N staging was determined based on "CS lymph nodes (2004-2015)" "regional nodes examined (1988+)" and "regional nodes positive (1988+)", with N0, N1a, N1b, N1 stages. Metastases was determined based on "SEER Combined Mets at DX-bone (2010+)", "SEER Combined Mets at DX-brain (2010+)", "SEER Combined Mets at DX-liver (2010+)", "SEER Combined Mets at DX-lung (2010+)", and "CS mets at dx (2004-2015)", and coded as either metastases or non-metastases, with 1 and 0 respectively. Surgery was classified as none surgery, non-total thyroidectomy, or total thyroidectomy and is represented by 0, 1, and 2, respectively. Lymph nodes dissection was classified as dissection (code 1) and no dissection (code 2). To address missing data in our study, we utilized a multivariate imputation strategy implemented in Python (version 3.9) to impute missing values in both datasets obtained from the SEER database and our hospital. This method involved estimating missing values based on patterns in the available data, resulting in two complete datasets that could be used for further analysis. By utilizing this approach, we were able to maximize the amount of available data. We used the SEER dataset for model training and testing, and the hospital dataset for external model validation. The training and testing sets of the SEER dataset were split in a 7:3 ratio. Due to the scarcity of positive events in the training set, which is not conducive to algorithm learning, we balanced the training set data to roughly equalize the proportion of 0 and 1 in the target.

Statistical methods

In this study, we compared the clinical baseline characteristics of the training set and the internal test set by using SPSS, and completed the model training and validation by using Python.

Irrelevant features such as patient ID and the year of diagnosis, and features with low variances, such as primary we removed. We then used a tree-based feature selection method to determine the importance of each remaining feature and selected the most important ones, including age, surgery, T staging, N staging, and metastases.

Four models were trained using the random forest classifier (RFC), gradient boosting decision tree (GBDT), logistic regression (LR), and support vector machine (SVM). Grid search was employed to select the optimal parameters for each model. Accuracy, recall, specificity, and area under curve (AUC) with 95% confidence intervals (CI) were calculated for each model on the testing set, and the receiver operating characteristic curve (ROC) was plotted. Based on a comprehensive consideration of these indicators, the best-performing model was adopted. We used a nomogram to depict the model and related risk factors. The calibration of the prediction model was assessed using the Hosmer-Lemeshow goodness-of-fit test and calibration curve; A P value of 0.05 or higher suggests that the calibration is good. The brier score (BS) is used to assess the accuracy of the prediction model by measuring the mean squared difference between the anticipated probability and the true condition. Some studies suggest that a model with a BS greater than 0.25 may have poor predictive performance (11). The clinical efficacy of the model was then evaluated using threshold probability and net benefit following decision curve analysis (DCA) and clinical impact curve (CIC) on the model.

In both data sets, the normal distribution test and the Mann-Whitney test (U test) were used for quantitative data. The U-test was also used for ordered variables in the qualitative data. For the unordered variables, the chi-square test and Fisher's exact test were used.

Results

Demographics and patient characteristics

A summary of patient characteristics and baseline comparisons can be found in Table 1.

TABLE 1 Comparison of clinical and pathological characteristics of the training and test sets.

	Training set	Internal testing set	P value
Metastasis (yes/no)	126/1206	38/531	0.05
Sex (male/female)	561/771	232/337	0.636
age	55±16	50±12	0.633
Race (white/black/asian)	1117/120/95	477/55/37	0.913
Other_tumors (yes/no)	90/1242	29/540	0.386
Surgery (none/non-total/total)	117/110/1105	43/50/476	0.868
LND (yes/no)	929/403	404/65	0.537
T (T1/T2/T3/T4)	676/313/264/79	311/130/94/34	0.600
N (N0/N1a/N1b)	811/201/320	363/68/138	0.832
Multi focus (yes/no)	437/895	176/393	0.382

LND, lymph node dissection.

Prognosis review

In this study, we conducted regular follow-up of our hospital's patients, with an average follow-up period of 40 months, range 2-144 months. During the follow-up, we identified three patients who developed distant metastases after surgery. In contrast, the SEER database had an average follow-up period of 60 months, range 0-143 months.

Feature selection and model evaluation

Through the feature selection method described above, we obtained four highly correlated features: surgery, age, T stage, and N stage. The result of feature selection is shown in Figure 1A. For each model on the training and testing sets, model assessments are included in Tables 2, 3. These tables include the AUC with 95% CI, accuracy, recall, and specificity. The ROC curves for the four models on the training and testing sets can be seen in Figures 1B, C. The AUC and 95% CI for the four models on the testing set were 0.8670 (95% CI, 0.7927-0.9413), 0.8402 (95% CI, 0.7606-0.9199), 0.8786 (95% CI, 0.8070-0.9503), and 0.8673 (95% CI, 0.7931-0.9415). We can see that these results indicate that all four models have good discriminative ability, with no statistically significant differences in predictive performance. Therefore, we selected the RFC model with the highest AUC as the final model.

Nomogram development and validation

Based on the aforementioned findings, we determined that age, surgical approach, T stage, and N stage were risk factors for distant metastases for MTC. We then built a nomogram for estimating the probability of metastases based on these characteristics, which is shown in Figure 2. In this nomogram, we can see that older age, no surgery, small extent of surgery, higher T staging, higher N staging were related with distant metastases. From this figure, we can easily know that, the higher T staging or N staging the greater risk of distant metastases. No surgery or non-total thyroidectomy will also increase the risk of distant metastases. This figure also shows that the risk of distant metastasis shows an upward trend with the diagnosed age increasing. In the comprehensive assessment of transferred risks, T staging and surgical strategy have the greatest impact on the distant metastases, while N staging has the least impact on it. We used calibration curves to evaluate the early postoperative prediction of distant metastases risk in MTC. The results showed a good fit in both the training and internal testing sets which can be seen in Figures 3A, B. The P values for the training set and testing set in the Hosmer-Lemeshow goodness-of-fit test were both higher than 0.05, showing that the chosen model had been calibrated well. The BS score of the model was 0.102 in the training set and 0.059 in the internal testing set, both lower than 0.25, indicating good overall performance. As shown in Figures 4A–D, it can be seen that the net benefit of the model both the training and internal testing sets is

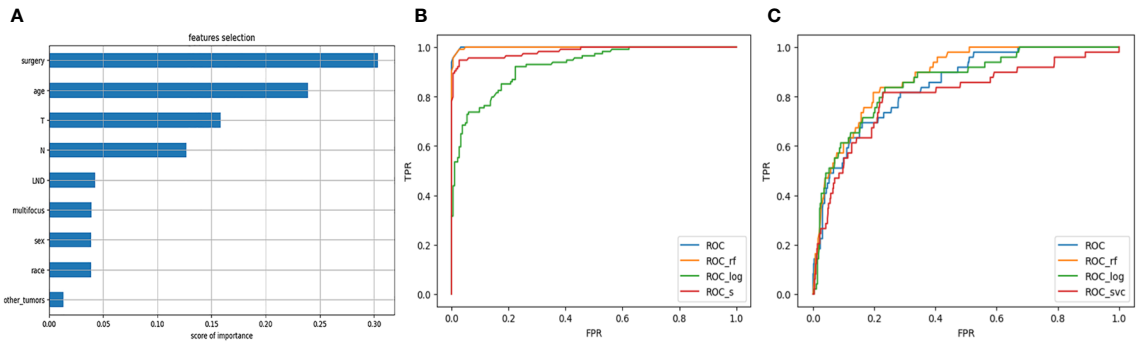


FIGURE 1
(A) Feature selection. **(B)** ROC curve of the model on the training set. **(C)** ROC curve of the model on the testing set.

TABLE 2 Evaluation of the model on the training set.

	AUC(95%CI)	Accuracy	Recall	Specificity
Random Forest	0.9961 (95%CI,0.9883-1.000)	0.97	0.94	0.98
GBDT	0.9994 (95%CI,0.9962-1.000)	0.98	0.98	0.98
Logistic Regression	0.9104 (95%CI,0.8738 -0.9470)	0.84	0.78	0.89
SVM	0.9055 (95%CI,0.8679-0.9430)	0.81	0.87	0.77

AUC, area under curve; GBDT, gradient boosting decision tree; SVM, support vector machine.

TABLE 3 Evaluation of the model on the testing set.

	AUC(95%CI)	Accuracy	Recall	Specificity
Random Forest	0.8786 (95%CI,0.8070-0.9503)	0.84	0.76	0.87
GBDT	0.8402 (95%CI,0.7606-0.9199)	0.82	0.74	0.87
Logistic Regression	0.8670 (95%CI,0.7927-0.9413)	0.87	0.61	0.89
SVC	0.8673 (95%CI,0.7931-0.9415)	0.81	0.71	0.81

AUC, area under curve; GBDT, gradient boosting decision tree; SVM, support vector machine.

higher than the extreme curve, and the range of selectable threshold values is relatively large, indicating good clinical value. Finally, we validated the final model on an external training set (clinical and pathological characteristics were shown in Table 4), with an AUC of 0.9105, accuracy of 0.84, recall of 1.0, and specificity of 0.83, demonstrating good predictive ability.

Discussion

MTC accounts for roughly 13.4% of all thyroid cancer fatalities and has a worse prognosis than DTC (12). According to prior research, the 10-year survival rate for MTC is approximately 96%, but the likelihood of distant metastases can be as high as 20%, and once it happens, the 10-year survival rate decreases dramatically to 40% (13). Parafollicular C cells are incapable of concentrating iodine, in contrast to DTC, hence radioactive iodine has no therapeutic benefit for MTC (14). It is crucial to identify DM early in order to take timely treatment. The most likely organ for MTC to metastasize is the lung (52.2%), followed by bone metastases (28.3%), mediastinal metastases (19.6%), and liver metastases (17.4%) (15). Imaging can be used to confirm metastases (16). Early imaging examinations such as ultrasound, chest CT, PET-CT, and bone scans can effectively detect metastatic lesions, which is beneficial for physicians to intervene early in patients with metastases and improve prognosis (17).

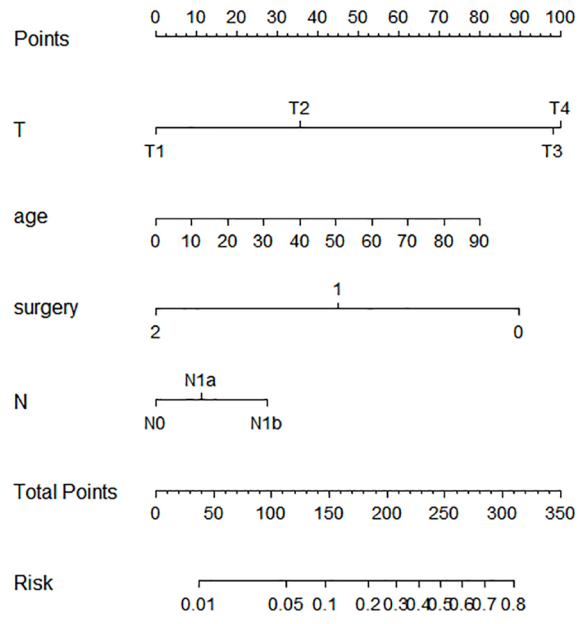


FIGURE 2
Nomogram for predicting the risk of distant metastases of medullary thyroid carcinoma. The nomogram is used by summing all points identified on the scale for each variable. The total points projected on the bottom scales indicate the probabilities of distant metastases in medullary thyroid carcinoma.

Currently, clinical guidelines recommend the concentration of Ctn and CEA in serum, as well as their doubling time, as independent risk factors for predicting disease prognosis (18). It is undeniable that the levels of Ctn and CEA and the multiplying time are highly related to disease recurrence and metastases. However, due to the characteristics of Ctn itself, there is a risk of not detecting metastases within 3 months after surgery. This research fills a void in the 3 months following surgery when there were no biochemical markers for predicting the likelihood of distant metastases in MTC patients. We included a data set of 111 MTC patients from our hospital from 2009 to 2021 as an external validation set. In the data set from the SEER, the metastases rate was approximately 8.66%, while in our hospital's data set, the metastases rate was approximately 2.268%. We conducted follow-ups on patients in the external validation set and found that the model provided high scores for patients who were found to have distant metastases during follow-up. The validated results confirm the effectiveness of the model. In practical applications, patients with high scores should receive more attention from physicians after surgery, and when developing corresponding follow-up plans, the interval between follow-ups should be shortened, and more frequent imaging examinations should be considered.

Through our research, we have found that non-surgical treatment or incomplete thyroidectomy, older age, T3, T4, and lateral cervical lymph node metastases are all high-risk factors for distant metastases of MTC after surgery. A previous study on predicting MTC distant metastases did not mention the impact of the surgical approach on postoperative metastases (9). However, in our study, we found that the choice of surgical strategy and T staging are more relevant independent hazardous factor for MTC distant metastases. In a 29-year follow-up research, it was discovered that the degree of surgery was a significant risk factor that affected the prognosis of patients, and patients who underwent total thyroidectomy had better clinical results than those who underwent no surgery or simply unilateral thyroidectomy (13). All MTC patients are advised to have total thyroidectomy according to MTC treatment guidelines (19), which indirectly confirms our research findings. Previous literature has indicated that older age is one of the independent risk factors for distant metastases (9), and the probability of distant metastases

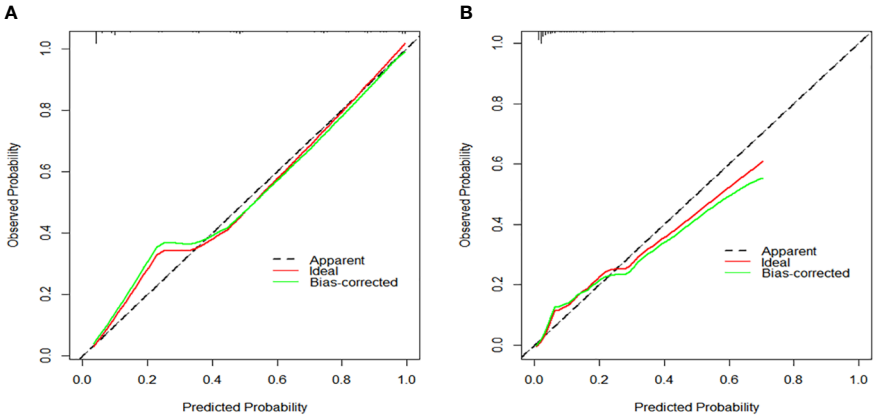


FIGURE 3
(A) Calibration curve to assess the calibration of the prediction model for the training set. **(B)** Calibration curve to assess the calibration of the prediction model for the testing set.

increases significantly in patients over 55 years old (20), which indirectly indicates that older age increases the risk of distant metastasis of tumors. Our research showed that both the T and N stages of the tumor are positively correlated with the risk of distant metastases, meaning that the likelihood of distant metastases increases with stage. Other researchers have published articles mentioning that the T stage is a significant disadvantage factor for MTC distant metastases (21). A. Machens et al. discovered that individuals with lymph node metastases were more likely to acquire distant metastases, whether familial or sporadic, than those who did

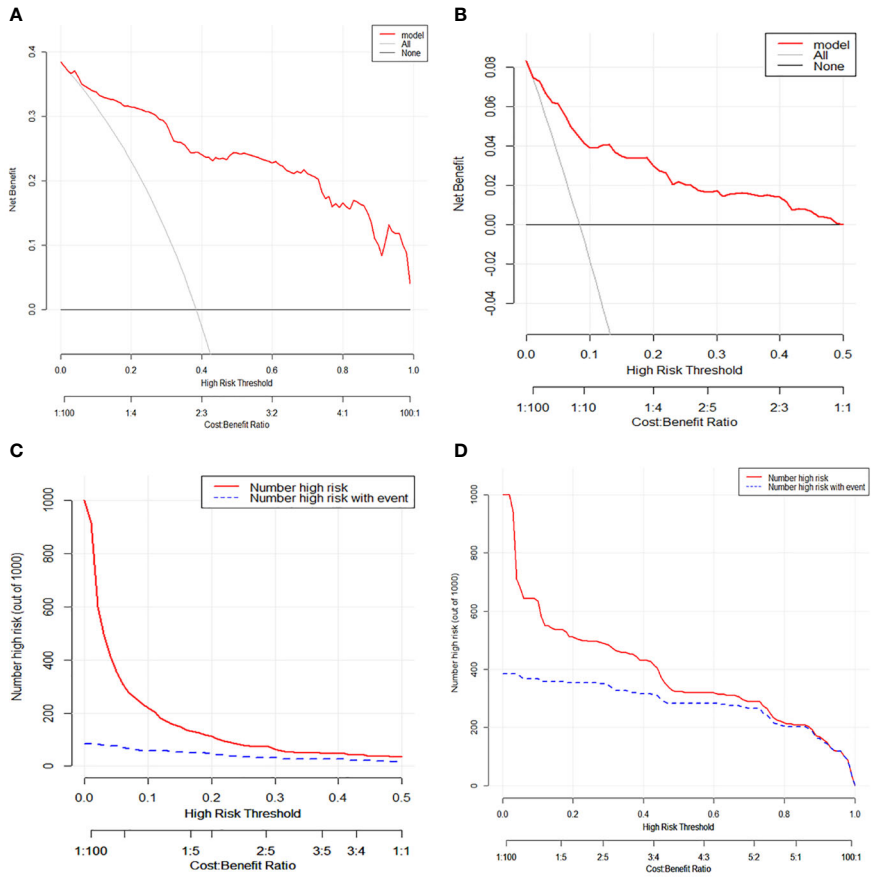


FIGURE 4
(A) Decision curve analysis and for distant metastases in medullary thyroid carcinoma patients on the training set. **(B)** Decision curve analysis for distant metastases in medullary thyroid carcinoma patients on the testing set. **(C)** Clinical impact curve for distant metastases in medullary thyroid carcinoma patients on the training set. **(D)** Clinical impact curve for distant metastases in medullary thyroid carcinoma patients on the testing set.

TABLE 4 Summary of clinical and pathological characteristics of the external validation set.

	External testing set
Metastasis (yes/no)	3/108
Sex (male/female)	37/74
age	50±11
Race (white/black/asian)	0/0/111
Other_tumors (yes/no)	72/39
Surgery (none/non-total/total)	1/31/79
LND (yes/no)	93/18
T (T1/T2/T3/T4)	54/43/12/2
N (N0/N1a/N1b)	39/31/41
Multi focus (yes/no)	19/92

not in the research involving 1115 MTC patients (22). In addition, an article from the research of Kuo. Ej et al. mentioned that disease staging is an independent risk factor for fatal distant metastases in MTC (23), which indirectly confirms our conclusions. We analyse the results that the likelihood of developing distant metastases increases with tumor size and the extent of cervical lymph node metastases because the wider the scope of the tumor invasion, the more susceptible it is to invade the small blood vessels and within the thyroid, and the greater the probability of blood dissemination. In our study, although the metastatic spread of lymph nodes is a relevant factor for distant metastases of MTC, its importance was less significant compared to the other three variables. At the same time, we found that whether or not to perform neck lymph node dissection was only weakly correlated with MTC distant metastases. Currently, there is controversy over whether regular removal of lymph nodes in the neck should be performed. A number of doctors are positive about neck lymph node dissection, believing that it can bring positive effects. A reported article mentioned that all patients who were diagnosed with MTC without distant metastases should undergo central node neck (levels VI and VII) dissection (24). But a certain proportion of doctors believe that preventive lateral neck lymph node dissection cannot significantly improve patient prognosis without evidence of lateral neck lymph node metastases (25, 26). Because it is not difficult to see from the column chart that the impact of lymph node metastases on the risk of distant metastases is relatively small, we believe that routine lateral neck lymph node dissection is unnecessary in the absence of clinical evidence of lateral neck lymph node metastases. The remaining variables in our study did not show a strong correlation with MTC distant metastases.

In this study, we utilized the SEER database to train and test our model and validated it with our own data, achieving favorable results. Our calibration curve showed a good fit between predicted and observed outcomes, indicating that our model has good discriminatory ability (27). This conclusion was further confirmed through external validation.

Certainly, like the vast majority of studies, our study has some limitations. First, the SEER database contains a large number of missing values. Although we used multiple imputation to minimize the difference from the true situation, some errors are inevitable. Second, the SEER database lacks genetic information, and it is not possible to distinguish whether the included patients have a family history. So we cannot differentiate between sporadic and familial MTC for exploration. Third, the sample size of the external validation set is small, with only three cases of distant metastases. Fourth, the extent of LND is very important but the SEER database only contains information on the extent of lymph node involvement and does not subdivide the scope of lymph node dissection. As a result, we only included the degree of regional lymph node invasion in the model. In addition, the most selected hospitalized patients had a strong willingness for surgery, as a result, cases without surgery or undergoing unilateral thyroidectomy were similarly scarce. Therefore, the results obtained from external validation lack persuasiveness.

Conclusion

Based on a large dataset of patients with MTC from SEER and the First Hospital of Jilin University, we have successfully developed a model for early postoperative prediction of distant metastases risk based on four independent risk factors, which is presented in the form of a nomogram. The predictive model performs well on the test set and can effectively screen high-risk patients within the Ctn half-life period after surgery, especially those with impaired liver or kidney function or with elevated preoperative Ctn levels leading to a prolonged Ctn half-life. Clinicians can use this model to assess the risk of distant metastases for each patient in a timely and effective manner after surgery, providing a reference for developing more accurate and personalized follow-up plans for each patient.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

This study was approved by the Institutional Ethics Committee of First Hospital of Jilin University. Informed consents were given to these patients of our medical center in the study.

Author contributions

All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Family history of malignant or benign thyroid tumors: implications for surgical procedure management and disease-free survival

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Background: Current guidelines lack a standardized management for patients with family history of thyroid carcinoma (fTC),particularly benign thyroid neoplasm (fBTN). Our objective was to investigate the influence of various family histories on the selection of surgical approaches and disease-free survival (DFS).

Methods: A cohort study was conducted involving 2261 patients diagnosed with differentiated thyroid carcinoma including those with fTC (n=224), fBTN (n=122), and individuals without a family history of thyroid carcinoma (nfTC; n=1915). Clinicopathological characteristics were collected. DFS was estimated using Kaplan-Meier analysis and factors affecting DFS were identified using Cox proportional hazard model.

Results: Compared to nfTC, small tumor size, clinically lymph node-positive, extrathyroidal extension, vascular invasion, Hashimoto's disease and nodular goiter were more common in fTC and fBTN groups. They had lower T stage and a lower rate of good response to TSH suppression therapy but received more radioiodine therapy. It is worth noting that fTC is associated with male, bilateral and multifocal tumors, as well as central lymph node metastasis and distant metastasis. Both fTC (aHR = 2.45, 95% CI=1.11-5.38; P = 0.03) and fBTN (aHR = 3.43, 95% CI=1.27-9.29; P = 0.02) were independent predictors of DFS in patients who underwent lobectomy, but not total thyroidectomy. For 1-4 cm thyroid carcinomas with clinically node-negative, fTC was identified as an independent predictor, whereas fBTN was not.

Conclusion: Our findings indicate that a family history, particularly of malignancy, is associated with a more aggressive disease. Family history does not affect the prognosis of patients who undergo total thyroidectomy, but it may

increase the risk of postoperative malignant events in those who have a lobectomy. Additionally, it may be necessary to monitor individuals with a family history of benign thyroid neoplasms.

KEYWORDS

benign thyroid neoplasms, thyroid nodules, thyroid cancer, familial nonmedullary thyroid carcinoma, family history, surgery benign thyroid neoplasms, disease-free survival

1 Introduction

Currently, the most contentious issue regarding family history of thyroid carcinoma is familial non-medullary thyroid carcinoma (FNMTC), which accounts for approximately 5-10% of all cases of thyroid carcinoma (1). FNMTC is characterized by the presence of two or more first-degree relatives affected by non-medullary thyroid carcinoma. However, current studies have not yet reached a definitive consensus on whether FNMTC tumors exhibit distinct biological behavior and prognosis compared to sporadic non-medullary thyroid carcinoma (SNMTC) in individuals (2–9).

Moreover, thyroid neoplasms can be benign or malignant, and it is unclear if individuals with a family history of benign thyroid neoplasm (BTN) should receive the same level of attention as those with FNMTC. Studies in Asian populations have shown a coaggregation of chronic thyroid diseases and multiple malignancies with non-medullary thyroid carcinoma (NMTC) (10). Other studies in Kuwait, Caledonia, and Iran found an association between a family history of benign thyroid disease (BTD) and an increased risk of thyroid cancer, indicating a familial susceptibility (11–13). However, D. Kust et al. (14) argued that a family history of BTD does not lead to the development of thyroid cancer. It is worth noting that papillary thyroid carcinoma (PTC) in children and adolescents has been linked not only to a family history of thyroid carcinoma but also to a family history of BTD (15).

In light of the current controversy surrounding family history, our study aims to analyze the characteristics as well as prognosis of patients with a family history of thyroid neoplasm. We will focus on assessing the effects of family history on the treatment and prognosis of patients with thyroid cancer, particularly those in the clinically lymph node-negative (cN0) stage with 1-4 cm tumors.

Abbreviations: fTC, Family history of thyroid carcinoma; fBTN, Family history of benign thyroid neoplasm; DFS, Disease-free survival; nfTC, non-family history of thyroid carcinoma; FNMTC, Familial non-medullary thyroid carcinoma; SNMTC, Sporadic non-medullary thyroid carcinoma; TTD, Total thyroidectomy; BTN, Benign thyroid neoplasm; BTD, Benign thyroid disease; cN0, Clinically node-negative; cN1, Clinically node-positive; TTD, Total thyroidectomy; LTD, Lobe thyroidectomy; CLND, Central lymph node dissection; LLND, Lateral lymph node dissection; TSH, Thyroid stimulating hormone; RAI, Radioiodine; RTR, Residual thyroid recurrence; LNM, Lymph node metastasis; DM, Distant metastasis; OD, Overall diameter; MaxD, Maximum diameter; CLNM, Central lymph node metastasis; LLNM, Lateral lymph node metastasis; ITM, Intraglandular dissemination; ETE, Extrathyroidal extension; VI, Vascular infiltration; AJCC, American Joint Committee on Cancer; TNM, Tumor-node-metastasis.

2 Methods

2.1 Patients and data

We obtained data from our department's clinical database, which included a total of 2261 patients from January 2010 to May 2022. Patients were directly asked about their first-degree relatives (parents, offspring, and siblings) who had been diagnosed with thyroid cancer or other thyroid diseases, and this information was documented in their medical records. First-degree relatives with suspicious nodules underwent confirmation through pathological examination. Based on hospital records, patients were classified into three groups: Patients without a family history were defined as non-familial cases (nfTC). Additionally, patients with a family history of thyroid neoplasm were categorized into two groups: fTC for those with a family history of malignancy, and fBTN for individuals with a family history of benign cases, including nodular goiter.

Patients with medullary thyroid carcinoma, undifferentiated thyroid cancer, a family history of nonthyroidal malignant tumors, or those who underwent head and neck radiotherapy were excluded from the study. Ultimately, we included 1915 nfTCs, 224 fTCs, and 112 fBTNs in this study. PTC accounts for 99% in the group without a family history, 99.1% in the group with a family history of thyroid cancer, and 100% in the group with a family history of benign thyroid tumors.

Among families with two affected first-degree relatives, we included 137 patients in the fTC group and 50 patients in the fBTN group. Additionally, there were 87 patients from families with three or more affected first-degree relatives in the fTC group and 72 patients in the fBTN group. The distribution of patients in terms of belonging to the parental generation or offspring, as well as the classification of inheritance patterns as either horizontal or vertical, is presented in Table 1.

2.2 Management and follow-up

We followed guideline-based approaches to select different surgical strategies, including total thyroidectomy (TTD) or lobe

TABLE 1 Patient characteristics[a].

Characteristics	nfTC (n=1915)	fTC (n=224)	P_1[c]	fBTN (n=122)	P_2[c]
Male	467 (24.4)	69 (30.8)	**0.04**	28 (23.0)	0.72
Age, median (IQR), y	44.0 (34.0-50.6)	43.0 (33.0-51.0)	0.43	41.0 (33.0-50.0)	0.14
≤ 55	1632 (85.2)	190 (84.8)	0.87	108 (88.5)	0.32
Family history					
The number of patients in the family with PTC					
2	NA[b]	137 (61.2)	NA	50 (41.0)	NA
≥ 3	NA	87 (38.8)		72 (59.0)	
Generation					
1st	NA	103 (46.0)	NA	58 (47.5)	NA
2nd	NA	121 (54.0)		64 (52.5)	
Genetic Mode					
sibling	NA	59 (26.3)	NA	38 (31.1)	NA
parent-offspring	NA	165 (73.7)		84 (68.9)	

nfTC, without a family history of thyroid carcinoma; fTC, family history of thyroid carcinoma; fBTN, family history of benign thyroid neoplasm; PTC, Papillary thyroid carcinoma; NA, not available.
[a]Chi-square test or Fisher's exact chi-square test or Mann-Whitney U test; Data are given as number (percentage) of patients unless otherwise specified.
[b]Data were not available due to no family history.
[c]The *P*-value that holds statistical significance is highlighted in bold.

thyroidectomy (LTD). Based on preoperative ultrasound or imaging evaluation, prophylactic unilateral/bilateral central lymph node dissection (CLND) was performed for patients with cN0 stage, while therapeutic central/lateral lymph node dissection (LLND) was conducted for those with clinical lymph nodes-positive (cN1). According to the risk stratification of patients, they received serum thyroid-stimulating hormone (TSH) suppression therapy and radioiodine (RAI) therapy.

Our department routinely performs follow-up including ultrasonography and serum thyroid function tests. Follow-up is conducted at 1, 3, and 6 months after surgery, with subsequent follow-up every 6-12 months if no suspicious lesions are detected. Additional imaging tests such as neck magnetic resonance imaging, chest computed tomography, or 18F-fluorodeoxyglucose positron emission tomography may be done if there are concerns about local recurrence or distant metastasis. Recurrence or metastasis is confirmed through fine needle aspiration cytology or core needle biopsy.

Follow-up data included disease-free survival (DFS), which was defined as the time from tumor-free status until biopsy or serial imaging confirmation of residual thyroid recurrence (RTR), lymph node metastasis (LNM), or distant metastasis (DM).

2.3 Clinicopathological features

We collected information, including basic details, family history, ultrasound evaluation, and treatment strategies. Postoperative pathological parameters included the pathological type, tumor location, number, presence of Hashimoto's disease or nodular goiter, overall diameter (OD), maximum diameter (MaxD), central lymph node metastasis (CLNM), lateral lymph node metastasis (LLNM), intraglandular dissemination (ITM), extrathyroidal extension (ETE), and vascular infiltration (VI). OD was assessed by summing the maximum diameter of each lesion. Multifocality is defined as having more than one lesion on a single-sided thyroid gland. In this study, if there was one lesion on each side (bilateral), it was not considered multifocal. Postoperative treatments encompassed TSH suppression therapy, reoperation, and radioiodine (RAI) therapy. The American Joint Committee on Cancer (AJCC) tumor-node-metastasis (TNM) staging system was used to classify thyroid carcinoma, and all parameters were evaluated according to the World Health Organization criteria.

2.4 Statistical analysis

Statistical analyses were performed using AKIBM SPSS 22.0 was used (IBM Corp., Armonk, NY, USA), and GraphPad Software 8.0.1 (GraphPad Software Inc., San Diego, CA, USA) was used to create figures. Non-normally distributed continuous variables were presented as median (interquartile range [IQR]) and compared using the Mann-Whitney U test. To compare categorical variables between two groups, we used Chi-square and Fisher's exact tests (Although this study involved multiple groups, the statistical analysis was conducted by comparing the study group to the same control group, without involving multiple comparisons), and we noted group fTC vs. nfTC as P_1, while fBTN vs. nfTC was noted as P_2. The Kaplan-Meier curve, Gehan-Breslow-Wilcoxon and log-rank test were employed to assess the impact of individual variables on prognosis. We utilized a Cox proportional hazards regression model (Cox-model) to identify independent risk factors. The model included adjusted hazard ratio (aHR), 95% confidence

interval (CI), and *P*-values for the variables. All reported *P* values in this study were two-sided, and we considered *P* values ≤ 0.05 to be statistically significant.

3 Results

3.1 Patients' characteristics

Table 1 displays the demographic characteristics of the study participants. It is worth noting the proportion of male patients was significantly higher in the family history of thyroid carcinoma group (30.8%) compared to the non-family history group (24.4%) (P = 0.04). However, no significant differences were observed among patients age ≤ 55 years.

3.2 Preoperative evaluation

Patients with a family history of malignant tumors exhibited a higher prevalence of bilateral nodules (56.3% vs. 48.0%) and multifocality(70.1% vs. 50.8%) compared to patients without a family history (all $P < 0.05$).

The MaxD/OD sizes were significantly smaller in the family histories groups (all $P < 0.001$). Moreover, cN1 was more commonly observed in both the family history of malignant tumor group (19.6%) and the benign tumor group (19.7%), (all $P < 0.001$) (Table 2).

3.3 Treatment and postoperative pathology

The selection of surgical approaches did not show significant differences between the family history groups and the non-family history group (all $P > 0.05$) according to Table 3.

In our study, PTC was the most common pathology type. The size of tumors and the presence of multifocal bilateral thyroid carcinomas demonstrated a consistent trend with preoperative ultrasound findings.

There were significant differences in the occurrence of extrathyroidal extension and vascular invasion between the familial groups and the non-familial group (all $P < 0.001$). In the familial thyroid cancer group, 62.1% of patients experienced central lymph node metastasis, which was higher compared to non-familial group (52.2%) and the family history of benign thyroid nodules group (55.7%). However,there was no significant difference in lateral lymph node metastasis. Additionally, the occurrence of Hashimoto's disease and nodular goiter was higher in both the familial thyroid cancer and familial benign thyroid nodules groups (all $P < 0.001$).

As shown in Table 4, the proportions of T stage in family history of thyroid carcinoma and benign nodule were 75.0% and 79.6%, respectively. These proportions were lower than the non-familial group (90.4%). Significant differences were also noted in the M stage ($P = 0.02$), but not in the N stage ($P > 0.05$), between the group with a family history of thyroid cancer and non-familial group.

3.4 Postoperative treatments

Significant differences were observed in the response to TSH suppression therapy (all $P < 0.001$). Specifically, a higher proportion of patients in the non-family history group (73.2%) achieved a good response compared to the family history of thyroid cancer group (67.8%) and the family history of benign thyroid nodules group (61.5%). The most notable differences were found in the category of "Uncertain response," with proportions of 0.5%, 6.7%, and 17.2% in the respective groups.

The reoperation rate was 2.3%, 8.5%, and 4.9% among patients, with a significant difference only observed between the non-family

TABLE 2 Preoperative ultrasound characteristics of the patients[a].

Preoperative ultrasound	nfTC (n=1915)	fTC (n=224)	P_1[b]	fBTN (n=122)	P_2[b]
Bilaterality	919 (48.0)	126 (56.3)	**0.02**	61 (50.0)	0.67
Isthmus nodule	197 (10.3)	27 (12.1)	0.41	13 (10.7)	0.90
Multifocality	972 (50.8)	157 (70.1)	**<0.001**	71 (58.2)	0.11
MaxD, median (IQR), cm	1.1 (0.8-1.8)	0.9 (0.5-1.5)	**<0.001**	0.5 (0.2-1.2)	**<0.001**
1 to 4	1078 (56.3)	91 (40.6)	**<0.001**	42 (34.4)	**<0.001**
OD, median (IQR), cm	1.8 (1.1-2.8)	1.4 (0.7-2.1)	**<0.001**	0.8 (0.3-1.6)	**<0.001**
1 to 4	1330 (69.5)	126 (56.2)	**<0.001**	44 (36.1)	**<0.001**
Clinical N stage					
clinically node-negative	1761 (92.0)	180 (80.4)	**<0.001**	98 (80.3)	**<0.001**
clinically node-positive	154 (8.0)	44 (19.6)		24 (19.7)	

nfTC, without a family history of thyroid carcinoma; fTC, family history of thyroid carcinoma; fBTN, family history of benign thyroid neoplasm; MaxD, maximum diameter; OD, overall diameter.

[a]Chi-square test or Fisher's exact chi-square test or Mann-Whitney U test; Data are given as number (percentage) of patients unless otherwise specified.

[b]The *P*-value that holds statistical significance is highlighted in bold.

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TABLE 3 Management of the patient's surgical approach[a].

Treatment	nfTC (n=1915)	fTC (n=224)	P_1[b]	fBTN (n=122)	P_2[b]
Total thyroidectomy	1568 (81.9)	172 (76.8)	0.06	99 (81.1)	0.83
Lobe thyroidectomy	347 (18.1)	52 (23.2)		23 (18.9)	
Central lymph node dissection	1859 (97.1)	214 (95.5)	0.21	116 (95.1)	0.33
Lateral lymph node dissection	392 (20.5)	48 (21.4)	0.74	25 (20.5)	>0.99

nfTC, without a family history of thyroid carcinoma; fTC, family history of thyroid carcinoma; fBTN, family history of benign thyroid neoplasm.
[a]Chi-square test or Fisher's exact chi-square test or Mann-Whitney U test; Data are given as number (percentage) of patients unless otherwise specified.
[b]The *P*-value that holds statistical significance is highlighted in bold.

TABLE 4 Postoperative pathological characteristics of the patients[a].

Postoperative pathology	nfTC (n=1915)	fTC (n=224)	P_1[b]	fBTN (n=122)	P_2[b]
Tumor type					
Papillary thyroid carcinoma	1896 (99.0)	222 (99.1)	> 0.99	122 (100)	0.63
Follicular thyroid carcinoma	19 (1.0)	2 (0.9)		0 (0.0)	
Multifocality	828 (43.2)	114 (50.9)	**0.03**	56 (45.9)	0.57
Bilaterality	609 (31.8)	86 (38.4)	**0.04**	40 (32.8)	0.82
MaxD, median (IQR), cm	0.7 (0.5-1.2)	0.5 (0.2-1.0)	**<0.001**	0.4 (0.2-0.9)	**<0.001**
1 to 4	712 (37.2)	61 (27.2)	**0.01**	27 (22.1)	**0.001**
OD, median (IQR), cm	1.0 (0.5-1.5)	0.7 (0.3-1.5)	**<0.001**	0.4 (0.2-1.1)	**<0.001**
1 to 4	917 (47.9)	78 (34.8)	**0.001**	33 (27.1)	**<0.001**
Intraglandular dissemination	91 (4.8)	10 (4.5)	0.85	5 (4.1)	0.74
Extrathyroidal extension	64 (3.3)	30 (13.4)	**<0.001**	20 (16.4)	**<0.001**
Vascular infiltration	6 (0.3)	6 (2.7)	**<0.001**	5 (4.1)	**<0.001**
Central lymph node metastasis	999 (52.2)	139 (62.1)	**0.005**	68 (55.7)	0.45
Lateral lymph node metastasis	305 (15.9)	42 (18.8)	0.28	20 (16.4)	0.90
Hashimoto's disease	325 (17.0)	76 (33.9)	**<0.001**	40 (32.8)	**<0.001**
Nodular goiter	328 (17.1)	66 (29.5)	**<0.001**	36 (29.5)	**<0.001**
T stage					
T:1	1731 (90.4)	168 (75.0)	**<0.001**	97 (79.6)	**<0.001**
T:2	106 (5.5)	14 (6.3)		7 (5.7)	
T:3	67 (3.5)	25 (11.2)		12 (9.8)	
T:4	11 (0.6)	17 (7.5)		6 (4.9)	
N stage					
N0	884 (46.2)	90 (40.2)	0.10	50 (41.0)	0.27
N1	1031 (53.8)	92 (59.8)		72 (59.0)	
M stage					
M0	1900 (99.2)	218 (97.3)	**0.02**	120 (98.4)	0.62
M1	15 (0.8)	6 (2.7)		2 (1.6)	
AJCC					
I	1785 (93.2)	206 (92.0)	**0.06**	117 (95.9)	0.47

(Continued)

TABLE 4 Continued

Postoperative pathology	nfTC (n=1915)	fTC (n=224)	P_1[b]	fBTN (n=122)	P_2[b]
II	126 (6.6)	15 (6.7)		5 (4.1)	
III	2 (0.1)	1 (0.4)		0 (0.0)	
IV	2 (0.1)	2 (0.9)		0 (0.0)	

nfTC, without a family history of thyroid carcinoma; fTC, family history of thyroid carcinoma; fBTN, family history of benign thyroid neoplasm; MaxD, maximum diameter; OD, overall diameter.
[a]Chi-square test or Fisher's exact chi-square test or Mann-Whitney U test; Data are given as number (percentage) of patients unless otherwise specified.
[b]The *P*-value that holds statistical significance is highlighted in bold.

history group and the family history of thyroid cancer group ($P < 0.001$). Furthermore, 32.5%, 41.1%, and 48.4% of patients received RAI 131 treatment, with significant differences between the non-family history group and family histories groups ($P_1 = 0.01$ and $P_2 < 0.001$, respectively) (Table 5).

3.5 Follow-up

The total follow-up duration (median, [IQR], months) were 49 (33–68), 51 (23–79), and 51 (42–61), respectively, with no significant difference. (All $P > 0.05$).

The rate of disease recurrence or metastasis was significantly higher in the familial thyroid cancer than in non-familial group, while there was no significant difference seen in the family history of benign thyroid nodule group ($P_1 < 0.001$ and $P_2 = 0.34$, respectively): Specifically, after primary surgery, other lobe recurrence occurred in 1.0%, 3.1%, and 1.6% of patients in the nfTC, fTC, and fBTN groups, respectively. Lymph node metastasis occurred in 4.4%, 10.7%, and 6.6% of patients in those respective groups. Distant metastasis happened in 15 (10 lung metastases, 4 bone metastases, and 1 liver metastasis.), 6 (4 lung metastases and 2 lung-to-brain metastases.), and 2 (2 lung metastases) patients, respectively (Table 5).

3.6 Effect of family history on the prognosis (DFS) for patients underwent lobectomy or total thyroidectomy

We further investigated the prognosis of different family history groups under different surgical approaches. Among patients who underwent lobectomy, the familial thyroid cancer group exhibited a survival difference compared to the group without a family history of thyroid cancer (Gehan-Breslow-Wilcoxon test, $P_1 = 0.008$; log-rank test, $P_1 = 0.02$, respectively). On the other hand, the familial benign thyroid nodules group showed a significant difference only in early stages, with no significant difference observed in long-term outcomes. (Gehan-Breslow-Wilcoxon test, $P_2 = 0.04$; log-rank test, $P_2 = 0.10$, respectively). For patients who underwent total thyroidectomy, no significant difference in survival was observed between the group with a family history (either of thyroid cancer or

TABLE 5 Postoperative follow-up management[a].

Follow-up	nfTC (n=1915)	fTC (n=224)	P_1[b]	fBTN (n=122)	P_2[b]
TSH inhibition therapy					
Good response	1403 (73.2)	152 (67.8)	**<0.001**	75 (61.5)	**<0.001**
Biochemical abnormality	63 (3.3)	12 (5.4)		6 (4.9)	
Structural abnormality	440 (23.0)	45 (20.1)		20 (16.4)	
Uncertain response	9 (0.5)	15 (6.7)		21 (17.2)	
Reoperation	45 (2.3)	19 (8.5)	**<0.001**	6 (4.9)	0.14
Radioiodine therapy	622 (32.5)	92 (41.1)	**0.01**	59 (48.4)	**<0.001**
Recurrence & Metastasis			**<0.001**		0.34
Residual thyroid recurrence	20 (1.0)	7 (3.1)		2 (1.6)	
Lymph node metastasis	85 (4.4)	24 (10.7)		8 (6.6)	
Distant metastasis	15 (0.8)	6 (2.7)		2 (1.6)	
Total follow-up period, median (IQR), months	49 (33–68)	51 (23–79)	0.81	51 (42–61)	0.89

nfTC, without a family history of thyroid carcinoma; fTC, family history of thyroid carcinoma; fBTN, family history of benign thyroid neoplasm.
[a]Chi-square test or Fisher's exact chi-square test or Mann-Whitney U test; Data are given as number (percentage) of patients unless otherwise specified.
[b]The *P*-value that holds statistical significance is highlighted in bold.

benign thyroid nodules) and the group without a family history (all $P > 0.05$) (Figure 1).

Compared to the lobectomy, the total thyroidectomy showed a survival advantage: in the lobectomy group, 89.6% of patients without a family history were in a disease-free survival state. However, the disease-free survival state was limited to a mere 67.3% within the group of malignant family history, while the benign family history group account for 78.3%. In the total thyroidectomy group, the disease-free survival rates were 94.6% (no family history), 91.3% (malignant family history), and 93.9% (benign family history) respectively.

Additionally, in the lobectomy group, the median survival time was shorter in the family history group compared to the non-family history group: 100 months (non-family history group), 79 months (malignant family history), and 83 months (benign family history). Patients with a family history of thyroid cancer had and other lobe recurrence rate of 13.5%, whereas those with a family history of benign thyroid nodules had a rate of 8.7%. In contrast, the other lobe recurrence rate was only 5.8% in patients without a family history. Furthermore, postoperative lymph node metastasis occurred in 15.4% of patients with a family history of thyroid cancer and 13.0% of patients with a family history of benign thyroid nodules in the familial group. In comparison, only 4.0% of patients without a family history experienced lymph node metastasis. Distant metastasis was observed in 3.8% of patients with a family history of thyroid cancer, while it occurred in only 0.6% of patients without a family history.

We then included all parameters in the multivariable Cox-model (Table 6). The results showed that for the lobectomy group, a family history of thyroid cancer (aHR = 2.45, 95% CI = 1.11–5.38; $P = 0.03$), a family history of benign thyroid nodules (aHR = 3.43, 95% CI = 1.27–9.29; $P = 0.02$), Multifocality (aHR = 2.58, 95% CI = 1.19–5.60; $P = 0.02$) and lateral lymph node metastasis (aHR = 5.31, 95% CI = 2.53–11.16; $P < 0.001$) were independent risk factors that affected DFS (Figure 2A). In the total thyroidectomy group, family history was not an independent factor (all $P > 0.05$). Hashimoto's disease (aHR = 0.46, 95% CI = 0.25–0.88; $P = 0.02$), central lymph node metastasis (aHR = 3.28, 95% CI = 1.79–6.01; $P < 0.001$), and lateral lymph node metastasis (aHR = 2.25, 95% CI = 1.46–3.47; $P < 0.001$) were predictive independent factors of prognosis (Figure 2B).

3.7 Effect of family history on the prognosis (DFS) for patients with cN0 stage thyroid carcinoma measuring 1–4 cm

We conducted a study on thyroid carcinomas measuring 1–4 cm, which has been controversial in cN0 stage. We used the MaxD and OD as measures of thyroid cancer size and performed survival analysis for OD = 1–4 cm (Gehan-Breslow-Wilcoxon test, $P_1 = 0.001$, $P_2 = 0.11$; log-rank test, $P_1 < 0.001$, $P_2 = 0.23$, respectively) and MaxD of 1–4 cm (Gehan-Breslow-Wilcoxon test; $P_1 = 0.08$, $P_2 = 0.002$, log-rank test, $P_1 = .004$, $P_2 = 0.01$; respectively) (see Figure 3).

According to Table 6, a family history of thyroid cancer (aHR = 2.89, 95% CI = 1.50–5.54; $P = 0.001$), undergoing lobectomy (aHR = 2.97, 95% CI = 1.53–5.75; $P = 0.001$), having pathologically confirmed central lymph node metastasis (aHR = 2.03, 95% CI = 1.07–3.85; $P = 0.03$), and having lateral lymph node metastasis (aHR = 2.38, 95% CI = 1.38–4.11; $P = 0.02$) were all identified as independent risk factors affecting DFS in patients with cN0 stage and tumor size (Overall diameter) between 1 and 4 cm (Figure 2C).

Furthermore, similar findings were observed for MaxD as for OD. A family history of thyroid cancer (aHR = 2.24, 95% CI = 1.01–4.93; $P = 0.04$), undergoing lobectomy (aHR = 2.67, 95% CI = 1.27–5.60; $P = 0.01$), having pathologically confirmed central lymph node metastasis (aHR = 2.41, 95% CI = 1.09–5.34; $P = 0.03$), and having lateral lymph node metastasis (aHR = 1.97, 95% CI = 1.08–3.58; $P = 0.03$) were identified as independent risk factors affecting prognosis (Figure 2D).

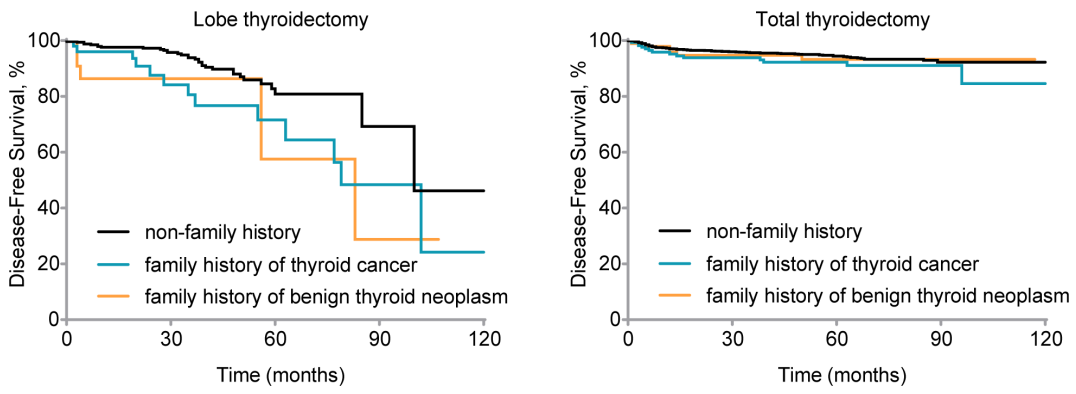


FIGURE 1
Disease-free survival (DFS) by family history for patients with different treatments. Kaplan-Meier curve displays DFS by different family histories for patients who underwent lobe thyroidectomy (Gehan-Breslow-Wilcoxon test, $P_1 = 0.008$, $P_2 = 0.04$; log-rank test, $P_1 = 0.02$, $P_2 = 0.10$) and total thyroidectomy (Gehan-Breslow-Wilcoxon test, $P_1 = 0.14$, $P_2 = 0.64$; log-rank test, $P_1 = 0.13$, $P_2 = 0.72$). nfTC, without a family history of thyroid carcinoma; fTC, family history of thyroid carcinoma; fBTN, family history of benign thyroid neoplasms.

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TABLE 6 Multivariable Cox Proportional Hazards Regression Model for Disease-Free Survival (DFS).

Predictors	Lobe thyroidectomy		Total thyroidectomy		cN0+OD 1-4 cm		cN0+MaxD 1-4 cm	
	aHR (95% CI)	*P* Value[a]	aHR (95% CI)	*P* Value[a]	aHR (95% CI)	*P* Value[a]	aHR (95% CI)	*P* Value[a]
Sex								
Male	1[Reference]		1[Reference]		1[Reference]		1[Reference]	
Female	0.81 (0.43-1.64)	0.61	1.11 (0.72-1.71)	0.64	1.21 (0.69-2.15)	0.50	1.15 (0.62-2.12)	0.66
Age								
≤55	1[Reference]		1[Reference]		1[Reference]		1[Reference]	
>55	1.18(0.51-2.76)	0.69	0.84 (0.45-1.60)	0.60	1.53 (0.77-3.04)	0.23	1.17 (0.52-2.64)	0.70
Family history								
nfTC	1[Reference]		1[Reference]		1[Reference]		1[Reference]	
fTC	2.45 (1.11-5.38)	**0.03**	1.59 (0.88-2.89)	0.13	2.89 (1.50-5.54)	**0.001**	2.24 (1.01-4.93)	**0.04**
fBTN	3.43 (1.27-9.29)	**0.02**	1.54 (0.64-3.69)	0.34	0.87 (0.12-6.40)	0.89	1.17 (0.16-8.70)	0.88
Treatment								
Total thyroidectomy	NA	NA	NA	NA	1[Reference]		1[Reference]	
Lobe thyroidectomy	NA	NA	NA	NA	2.97 (1.53-5.75)	**0.001**	2.67(1.27-5.60)	**0.01**
Multifocality								
No	1[Reference]		1[Reference]		1[Reference]		1[Reference]	
Yes	2.58 (1.19-5.60)	**0.02**	1.18 (0.61-2.28)	0.62	1.32(0.62-2.83)	0.48	1.53 (0.66-3.53)	0.32
Location								
Laterality	1[Reference]		1[Reference]		1[Reference]		1[Reference]	
Bilaterality	0.56 (0.17-1.90)	0.35	1.39 (0.75-2.60)	0.30	0.99 (0.47-2.11)	0.98	0.85 (0.36-2.01)	0.71
MaxD								
< 1	1[Reference]		1[Reference]		NA	NA	NA	NA
1 to 4	1.18 (0.30-4.71)	0.81	1.13 (0.60-2.13)	0.70	NA	NA	NA	NA
≥4	0.53 (0.02-12.0)	0.69	3.02 (0.70-13.03)	0.14	NA	NA	NA	NA
OD								
< 1	1[Reference]		1[Reference]		NA	NA	NA	NA
1 to 4	1.17 (0.31-4.44)	0.81	1.38 (0.69-2.80)	0.37	NA	NA	NA	NA
≥4	2.30 (0.15-34.26)	0.55	1.69 (0.47-6.11)	0.43	NA	NA	NA	NA
Intraglandular dissemination								
No	1[Reference]		1[Reference]		1[Reference]		1[Reference]	
Yes	0.66(0.19-2.36)	0.52	1.63 (0.92-2.90)	0.10	0.81 (0.28-2.33)	0.70	0.60(0.14-2.49)	0.48
Extrathyroidal extension								
No	1[Reference]		1[Reference]		1[Reference]		1[Reference]	
Yes	0.57 (0.15-2.19)	0.41	1.63 (0.92-2.90)	0.12	1.50 (0.70-3.20)	0.29	1.93 (0.87-4.26)	0.10

(Continued)

TABLE 6 Continued

Predictors	Lobe thyroidectomy		Total thyroidectomy		cN0+OD 1-4 cm		cN0+MaxD 1-4 cm	
	aHR (95% CI)	*P* Value[a]	aHR (95% CI)	*P* Value[a]	aHR (95% CI)	*P* Value[a]	aHR (95% CI)	*P* Value[a]
Vascular infiltration								
o	1[Reference]		1[Reference]		1[Reference]		1[Reference]	
Yes	2.27 (0.28-18.47)	0.44	0.36 (0.05-2.80)	0.33	1.31 (0.17-10.19)	0.80	0.68 (0.08-5.59)	0.72
Hashimoto's disease								
No	1[Reference]		1[Reference]		1[Reference]		1[Reference]	
Yes	0.75 (0.37-1.55)	0.44	0.46 (0.25-0.88)	**0.02**	0.58 (0.27-1.23)	0.15	0.59 (0.25-1.43)	0.59
Nodular goiter								
No	1[Reference]		1[Reference]		1[Reference]		1[Reference]	
Yes	1.85 (0.83-4.13)	0.13	0.96 (0.55-1.70)	0.90	0.84 (0.40-1.78)	0.65	1.15 (0.50-2.64)	0.75
Central lymph node metastasis								
No	1[Reference]		1[Reference]		1[Reference]		1[Reference]	
Yes	0.80 (0.42-1.53)	0.51	3.28 (1.79-6.01)	**<0.001**	2.03 (1.07-3.85)	**0.03**	2.41 (1.09-5.34)	**0.03**
Lateral lymph node metastasis								
No	1[Reference]		1[Reference]		1[Reference]		1[Reference]	
Yes	5.31 (2.53-11.16)	**<0.001**	2.25 (1.46-3.47)	**<0.001**	2.38 (1.38-4.11)	**0.02**	1.97 (1.08-3.58)	**0.03**

cN0, clinical node-negative; nfTC, without a family history of thyroid carcinoma; fTC, family history of thyroid carcinoma; fBTN, family history of benign thyroid neoplasm; MaxD, maximum diameter; NA, not available.
[a]The *P*-value that holds statistical significance is highlighted in bold.

4 Conclusion

This article primarily focuses on investigating the clinicopathological features and evaluating the influence of surgical methods on the prognosis of patients with a family history of thyroid neoplasms, encompassing both malignant and benign cases.

Some studies have shown differences in aggressive behavior, recurrence, and survival between FNMTC and SNMTC (5, 9, 16–18), while others have not (6, 7, 19–22). The controversy surrounding FNMTC may stem from different definitions and interpretations. Some argue that a significant portion of 2-hits families may represent incidental occurrences rather than genetic susceptibility (22), while others suggest that familial cases should be linked to affected generations and the number of affected individuals (23–31). Nonetheless, FNMTC has been observed to occur at an earlier age and exhibit more aggressive features in offspring, supporting its classification as a true familial disease even when only two family members are affected (32). In addition, since susceptibility genes for FNMTC have not been identified and there is genetic heterogeneity (33–35), it is considered unreliable to exclude the possibility of FNMTC when only two first-degree relatives are affected.

Current research has been discussing whether having a family history of BTD increases the likelihood of developing thyroid cancer (10–15). In clinical practice, it is not uncommon to see patients with a family history of benign thyroid nodules. From a genetic perspective (33–35), it is possible that thyroid carcinoma is a genomically incomplete epiphenomenon, meaning that family members may possess a susceptibility gene for thyroid carcinoma but exhibit only benign nodules or remain in a healthy state. Therefore, it is necessary to investigate the family history of BTN when evaluating the risk of developing thyroid cancer.

The study examined a group with a family history of benign thyroid tumors and found several clinical features compared to the non-family history group. These included smaller tumor diameter, a higher detection rate of cN1, a higher likelihood of ETE and VI, and a higher association with Hashimoto's disease and nodular goiter. The proportion of T1 stage tumors was lower. Additionally, they had a higher rate of radioiodine therapy and a lower rate of good response to TSH suppression therapy. These findings may suggest that this particular patient population could potentially have more severe disease.

These findings were similar to the characteristics observed in fTC. However, compared to the non-family history group, fTC also showed a greater tendency for male predominance, higher rates of bilateral and multifocal tumors, an elevated incidence of CLNM and distant metastasis. Additionally, fTC carried a higher risk of reoperation and recurrence after primary surgery.

The preceding discussion primarily centered around the static disease characteristics observed in patients with a family history. However, the dynamic prognosis is contingent upon two factors: the selection of the initial surgical approach and the nature of the disease itself. Our results indicate that there was no difference in prognosis

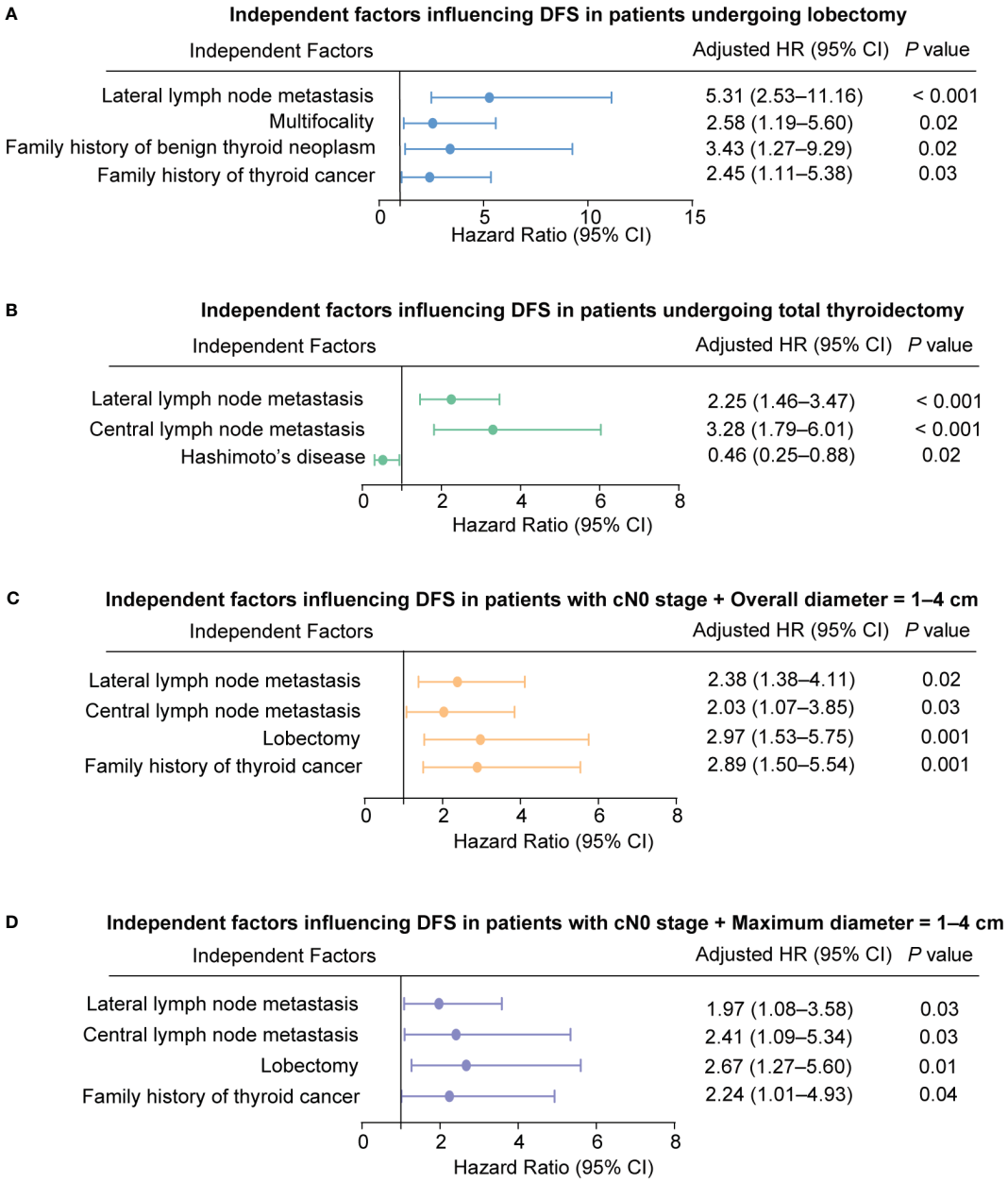


FIGURE 2
Independent factors influencing Disease-Free Survival (DFS) in Patients. **(A)** Patients undergoing lobectomy. **(B)** Patients undergoing total thyroidectomy. **(C)** Patients with cN0 stage and overall diameter of 1-4 cm. **(D)** Patients with cN0 stage and maximum diameter of 1-4 cm.

between familial and non-familial types of patients who underwent total thyroidectomy. However, among the familial thyroid cancer group, patients who underwent lobectomy exhibited a survival diaparity compared to non-familial group. As for the group with benign thyroid nodules, there was a significant difference only in the early stages, but no significant difference in long-term outcomes.

Additionally, a family history of malignant or benign thyroid tumors is an independent risk factor that affected the prognosis of patients with lobectomy but not total thyroidectomy. This, on the other hand, indicates that the prognosis of patients undergoing total thyroidectomy is not strongly associated with a family history background. Incomplete surgery may be the cause of these differences, as seen in the fBTN group. Survival analysis showed a higher occurrence of malignant events in patients with a family history of benign thyroid nodules during the early postoperative period, indicating that physicians who did not consider the patient's family history may have performed incomplete surgery.

The impact of family history on the treatment for 1-4 cm thyroid carcinoma in the cN0 stage remains unclear (36, 37). In our study, we reassessed MaxD and OD as criteria and concluded that a malignant family history was an independent risk factor for the prognosis of OD or MaxD = 1-4 cm tumors. Conversely, a benign family history was not found to be an independent risk factor for 1-4 cm tumors.

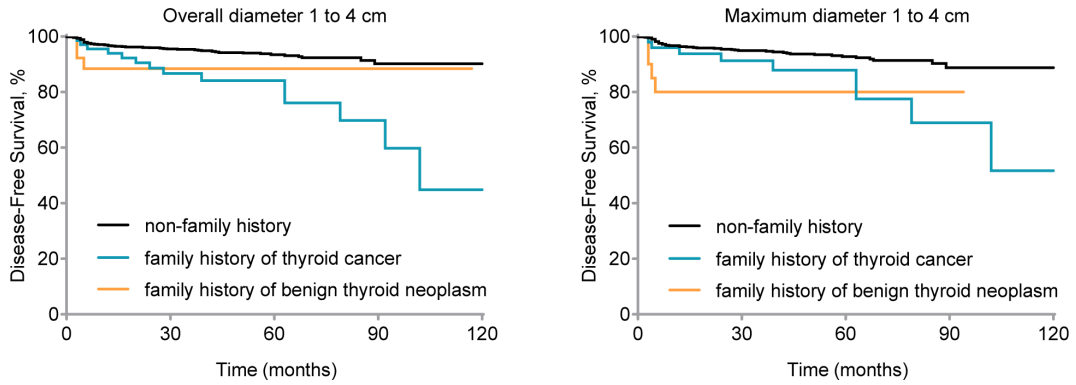


FIGURE 3
Disease-free survival (DFS) by family history for clinical node-negative (cN0) stage patients with 1 to 4 cm thyroid carcinoma. Kaplan-Meier curve displays DFS by different family histories for patients of cN0 stage and overall diameter 1 to 4 cm thyroid carcinoma (Gehan-Breslow-Wilcoxon test, $P_1 = 0.001$, $P_2 = 0.11$; log-rank test, $P_1 < 0.001$, $P_2 = 0.23$) and maximum diameter 1 to 4 cm thyroid carcinoma (Gehan-Breslow-Wilcoxon test, $P_1 = 0.08$, $P_2 = 0.002$; log-rank test, $P_1 = .004$, $P_2 = 0.01$). nfTC, without a family history of thyroid carcinoma; fTC, family history of thyroid carcinoma; fBTN, family history of benign thyroid neoplasms.

Given that patients may not always have full awareness of their own condition or the extent of the disease within their families, our aim was to provide a simple and straightforward reference value by considering only the presence of a family history, rather than relying on complex predictive models. Based on our findings, we recommend that patients with a family history (either benign thyroid nodules or thyroid carcinoma), particularly in cases of multifocality, undergo total thyroidectomy in order to avoid postoperative malignant events. Additionally, a family history of thyroid carcinoma, can serve as an independent prognostic factor for stage cN0 with 1-4 cm thyroid cancer, providing guidance in selecting the surgical approach.

The study had several limitations, including the need to expand the study population. Moreover, we plan to continue patient follow-up with an expected median follow-up of 10 years. Additionally, it is important to note that prognosis is influenced not only by clinical features but also by susceptibility genes, other cancers, environmental factors, and more. Currently, the treatment for thyroid cancer tends to be conservative, advocating for active surveillance (38, 39). However, if the presence of a family history is due to genetic alterations that contribute to disease development, such as the well-known BRAF mutation, even patients with microcarcinomas may be considered at intermediate-to-high risk (39). Therefore, when facing patients with a family history, it is unreasonable to solely rely on general characteristics of thyroid cancer to assess their actual condition. These aspects should be considered in future studies.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The study was approved by Union Hospital Tongji Medical College Huazhong University of Science and Technology Ethics Committee (2017-S062). The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required from the participants or the participants' legal guardians/next of kin because This is a retrospective study based on clinical database review.

Author contributions

Y-JJ: Conceptualization, Data curation, Formal analysis, Funding acquisition, Writing – original draft. Z-JH: Data curation, Formal analysis, Writing – original draft. Y-XH: Data curation, Writing – original draft. NZ: Methodology, Supervision, Writing – review & editing. TH: Conceptualization, Funding acquisition, Supervision, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Application of artificial intelligence in the assessment of thyroid eye disease (TED) - a scoping review

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Background: There is emerging evidence which suggests the utility of artificial intelligence (AI) in the diagnostic assessment and pre-treatment evaluation of thyroid eye disease (TED). This scoping review aims to (1) identify the extent of the available evidence (2) provide an in-depth analysis of AI research methodology of the studies included in the review (3) Identify knowledge gaps pertaining to research in this area.

Methods: This review was performed according to the 2020 Preferred Reporting Items for Systematic Reviews and Meta-Analyses statement (PRISMA). We quantify the diagnostic accuracy of AI models in the field of TED assessment and appraise the quality of these studies using the modified QUADAS-2 tool.

Results: A total of 13 studies were included in this review. The most common AI models used in these studies are convolutional neural networks (CNN). The majority of the studies compared algorithm performance against healthcare professionals. The overall risk of bias and applicability using the modified Quality Assessment of Diagnostic Accuracy Studies 2 (QUADAS-2) tool led to most of the studies being classified as low risk, although higher deficiency was noted in the risk of bias in flow and timing.

Conclusions: While the results of the review showed high diagnostic accuracy of the AI models in identifying features of TED relevant to disease assessment, deficiencies in study design causing study bias and compromising study applicability were noted. Moving forward, limitations and challenges inherent to machine learning should be addressed with improved standardized guidance around study design, reporting, and legislative framework.

KEYWORDS

Graves' ophthalmology, Graves orbitopathy, thyroid eye disease, artificial intelligence, convolutional neural networks

Introduction

Artificial intelligence (AI) is a term which refers to a branch in computer sciences that utilizes mathematical algorithms to attempt to perform tasks which usually require human cognition. In recent years, AI technology has advanced tremendously due to the enhancement of computational analytics techniques and the availability of large datasets. In healthcare, a substantial proportion of the AI literature is focused on medical imaging, where sophisticated algorithms are employed to develop models to improve diagnostic accuracy in medical image interpretation (1).

Thyroid eye disease (TED) is the main extrathyroidal manifestation of Graves' disease (GD) which develops in about 25-50% of patients with GD (2). The disease is autoimmune in etiology and is characterized by inflammation and extensive remodeling of the soft tissues surrounding the eyes (3). The pathogenesis of the disease involves loss of self-tolerance to thyrotropin receptor (TSHR) and insulin-like growth factor-1 receptor (IGF-1R), leading to activation of sub-populations of orbital fibroblasts which triggers an autoimmune cascade, causing expansion of retro-orbital fat and enlargement of extraocular muscles (4). Disease manifestations include redness and swelling of the conjunctivae and lids, forward protrusion of the globes (proptosis), ocular pain, debilitating double vision, and even sight loss due to compressive optic neuropathy or breakdown of the cornea (5). Known as the "Rundle's Curve", TED begins with an active inflammatory phase which usually lasts for 18 months to 2 years before plateauing to a fibrotic inactive phase (6). The conventional goal of management is for early detection and treatment of active TED with immunosuppressive therapy. Late complications of TED such as compressive optic neuropathy or exposure keratopathy may not respond to immunosuppression alone and may require urgent surgical orbital decompression. Rehabilitative surgeries such as orbital decompression, strabismus, and eyelid surgeries are usually carried out in a staged fashion when the disease course becomes inactive. The recent discovery of the IGF-1 inhibitor shows improvement in proptosis, strabismus, and vision in active and even inactive TED patients (7, 8). These are potential harbingers and present a new paradigm for TED management in the future. Unfortunately, the high cost and potential risk of permanent hearing loss limit its widespread use in many countries. Despite advances in treatment, a large proportion of patients remain undiagnosed before debilitating symptoms such as diplopia and exposure keratopathy occur, often leading to impairment of the quality of life despite treatment. Early diagnosis and treatment of TED thus becomes an important area of research.

The first step in the diagnosis of TED is a dedicated ophthalmological examination, then orbital imaging may be employed in selected clinical situations. Orbital imaging in TED may be performed for several reasons: 1) Diagnosis of dysthyroid optic neuropathy (DON): This is a serious complication of TED which is sight threatening. Early recognition and treatment may avoid loss of sight 2) Diagnosis of TED with an atypical presentation: Although the diagnosis of TED is generally straightforward in a hyperthyroid patient, other differentials may need to be considered when the patient is euthyroid or hypothyroid or presents with individual signs such as isolated proptosis (e.g. due to lymphoma, cavernous sinus fistula), which may occur in 20% of all TED patients (9) 3) Evaluation of disease activity for prediction of therapeutic efficacy with anti-inflammatory and monitoring of treatment response.

There is emerging evidence which suggests the utility of AI in the diagnostic assessment and pre-treatment evaluation of TED. This scoping review aims to (1) identify the extent of the available evidence (2) provide an in-depth analysis of AI research methodology of the studies included in the review (3) Identify knowledge gaps pertaining to research in this area (10).

Materials and methods

A literature search was performed by two independent investigators (CL and SW) from the earliest year of indexing until February 2023. Disagreements were resolved by consensus. This review was performed according to the 2020 Preferred Reporting Items for Systematic Reviews and Meta-Analyses statement (PRISMA) (11). A systematic literature search was performed in PubMed, Google Scholar, and Clinicaltrials.gov. The following terms were connected using Boolean operators "and", "or" "and/or", "thyroid eye disease", "thyroid orbitopathy", "thyroid-associated orbitopathy", "graves' orbitopathy", "graves' ophthalmopathy", "machine learning", "deep learning", "artificial intelligence", "convolutional neural network". The terms were searched as "Mesh terms" and as "all fields" terms.

The search generated 123 abstracts, 10 of which are repeats, and the remaining 113 were individually assessed for suitability. Only full original articles of completed studies published in peer-reviewed journals that were written in English were included in this review. 13 artificial intelligence papers met the full inclusion criteria and were included (Figure 1). 7 papers were based on radiological scan images, 4 papers were based on external photographs of patients and 2 papers were based on clinical parameters.

Tailored Quadas-2 tool for assessment of the quality of the AI studies

Given the absence of an internationally accepted AI-specific quality assessment tool for review papers, we adopted and tailored the QUADAS-2 assessment tool as recommended by the QUADAS-2 steering committee to improve its applicability in analyzing AI papers. QUADAS-2 determines the risk of bias and the applicability of each study in four main areas: patient selection, index test, reference standard, and flow and timing (12). These domains were assessed by using signaling questions with yes, no, and unclear answers. However, given the nature of the machine learning methods, we found the signaling question under "patient selection" on whether the study has avoided a case-control design to be redundant, thus this was assigned as non-applicable. Specific AI-related signaling questions were added (13, 14) to the domains of

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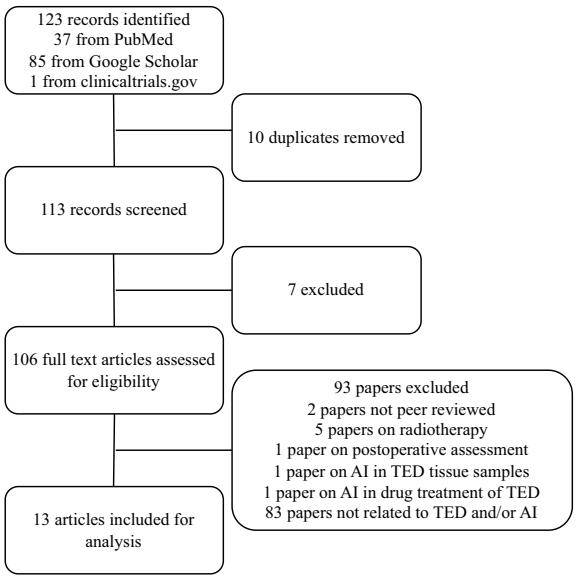


FIGURE 1
PRISMA flow diagram of included studies.

patient selection, index test(s), and reference standard (Table 1). Two reviewers independently judged the quality of each study. Disagreements were resolved by consensus with additional input from the third reviewer from the study team.

TABLE 1 Additional QUADAS-2 signalling questions tailored for this review of the AI literature.

DOMAIN 1: PATIENT SELECTION
A. Risk of Bias
Was the data in house or well curated open-source data?
Was the rationale and breakdown in the train, validate and test set described?
Is more than one institution included?
Did the study consider label imbalance if handling a classification problem?
Did the patient sample include an appropriate spectrum of patients to whom the diagnostic test will be applied in clinical practice?
DOMAIN 2: INDEX TEST(S)
A. Risk of Bias
Was the test evaluated against an external dataset?
Was overfitting avoided?
Was sufficient detail given on the algorithm to allow replication and independent validation?
Is there a specific design for end-user interpretability, e.g., saliency or probability maps?
DOMAIN 3: REFERENCE STANDARD
A. Risk of Bias
Was there a good expertise level and is there consensus amongst experts if used for performance benchmarking?

Results

A total of 13 studies related to TED assessment for diagnosis and pre-treatment evaluation were included in this review. A summary the main clinical and patient demographic features of the studies is presented in Table 2 and the full study characteristics are provided in Table 3. The number of patients recruited in the studies ranges from 108 to 2154 and main patient demographic details such as age, gender and smoking status were detailed in 8 of the 13 studies reviewed. The most common AI models used in these studies are convolutional neural networks (CNN). Three of the studies validated algorithms on external datasets (18, 23, 26). 12 of the 13 studies compared algorithm performance against healthcare professionals, whereas one study utilizes electronic medical records (EMR) phenotypes (15). Definitions of TED and threshold for diagnosis were generally based on current accepted clinical standards for all the studies included in this review. Less than half of the studies stated the method for internal validation and four studies described study design with end-user interpretability in mind (18, 19, 22, 23).

Studies based on diagnostic imaging

There were seven studies that utilizes diagnostic imaging (CT or MRI orbits) in the diagnosis and severity assessment of TED (18, 24), identification of DON (15, 25), detection of disease activity (20) or enlarged extraocular muscles (22) and prediction of therapeutic response to glucocorticoid therapy (19). The area under the curve (AUC) of these AI systems ranges from 0.81 to 0.979, sensitivity (SN) from 75.3% to 94%, specificity (SP) from 85% to 99.5% and accuracy from 82.6% to 96%.

TABLE 2 Summary of the main clinical and patient demographic features of the studies.

No	Author	Main clinical and patient demographical features
1	Chaganti et al/ 2017 (15)	788 patients in disease cohort, of which 73 had TED vs 1566 controls who had cochlear implants
2	Salvi et al/2002 (a) (16)	Inactive vs Active TAO: 246 vs 152. No differences in age between the groups but women with active TAO were older
3	Salvi et al/2002 (b) (17)	Inactive vs Active TAO vs normal: 129 vs 113 vs 103 patients. Mean age (yrs): 47.1 ± 1.3 vs 49.5 ± 1.3 vs 48.4 ± 2.1 years. M:F 1:5 vs 1:6.5 vs 1.1. Immunosuppressive (Inactive vs Active TAO): 2.1% vs 7.1%. Smokers (Inactive vs Active TAO): 37.2% vs 43.4%.
4	Song et al/ 2021 (18)	TAO vs controls: 193 vs 715
5	Hu et al/ 2022 (19)	Training cohort: Steroid responsive vs Steroid unresponsive: 44 vs 34. Mean age (yrs): 47.6 ± 11.6 49.4 ±

(Continued)

TABLE 2 Continued

No	Author	Main clinical and patient demographical features
		10.6. M/F: 15/29 vs 18/16. Disease duration (months): 5.6 ± 4.4 vs 7.6 ± 5.3. Smokers' vs Non-smokers: 14/30 vs 15/19. Euthyroid vs Non-euthyroid: 37/7 vs 28/6. CAS score: 3.6 ± 0.7 vs 3.7 ± 0.9 Validation cohort: Steroid responsive vs Steroid unresponsive: 18 vs 14. Mean age (yrs): 48.0 ± 13.5 vs 49.9 ± 11.4. M/F: 9/9 vs 6/8. Disease duration (months): 3.9 ± 2.5 vs 6.8 ± 3.4. Smokers vs Non-smokers: 5/13 vs 4/10. Euthyroid vs Non-euthyroid: 15/3 vs 12/2. CAS score: 4.2 ± 0.9 vs 3.7 ± 1.0
6	Lin et al/ 2021 (20)	108 patients with TAO. M/F: 42/66. Patients with active TAO were treated with immunosuppressives and MRI orbits of these patients were compared before and after treatment
7	Huang et al/ 2022 (21)	Active vs Quiescent vs Mild TAO: 487 vs 1073 vs 89. Moderate vs severe vs very severe TAO: 89 vs 1290 vs 181. M/F (total): 563/997. Majority of cases were below 50yo: 1055/1560
8	Hanai et al/ 2022 (22)	Enlarged extraocular muscle vs normal extraocular muscle: 199 vs 172. Mean age (yrs): 55.9 ± 13.7 vs 52.6 ± 18.4. M/F: 56/143 vs 40/132
9	Karlin et al/ 2022 (23)	TED vs controls: 829 vs 1459
10	Lee et al/ 2022 (24)	*Mild vs Moderate-to-severe GO vs controls: 99 vs 94 vs 95. Mean age (yrs): 38.4 ± 10.4 vs 47.6 ± 15.0 vs 29.3 ± 8.1. M/F: 13/86 vs 45/49 vs 37/58.
11	Wu et al/ 2022 (25)	TAO patients with DON vs TAO patients without TAO vs controls: 42 vs 49 vs 87
12	Shao et al/ 2022 (26)	TAO vs normal: 74 vs 74. Mean age (yrs): 43.76 ± 13.69 vs 43.28 ± 12.84. M/F: 17/57 vs 17/57. In the TAO group, 38 patients (51.35%) were diagnosed with bilateral TAO, and 36 patients were diagnosed (48.65%) with unilateral TAO. The TAO group consisted of 67 patients with hyperthyroidism, 4 patients with euthyroidism, 2 patients with Hashimoto thyroiditis, and 1 patient with primary hypothyroidism
13	Moon et al/ 2022 (27)	1020 patients with TAO. Mean age: 45.2 ± 15.4 years. M:F: 301/719. Mean total CAS score (available for 918/1020 patients): 2.0 ± 1.3. Active TAO (CAS ≥ 3) was observed in 272 patients (29.6%), and highly active TAO (CAS ≥ 5) in 34 (3.7%).

*Patient demographics were based on the 288 CT images used for this study. Significant differences in age and gender of the 3 patient groups.
GO, Graves' orbitopathy; TAO, Thyroid associated orbitopathy; M/F, Number of males/ Number of females; DON, dysthyroid optic neuropathy.

Song et al. reported an AUC of 0.919, with non-inferiority of the AI system demonstrated when compared to the resident group in diagnosing TED (18). The authors demonstrated higher sensitivity when they compared their 3D-Res Net (28) model to AlexNet and VGG thereby concluding its effectiveness in TED screening. The study utilizes class activation mapping (CAM) for transparency of the CNN. However, >70% of the database were patients with moderate to severe TED, and the judgment from residents rather than senior experts were used in this study. Lee et al. developed a new neural network for the diagnosis and severity assessment of TED, with a reported AUC of 0.979 for moderate to severe TED and 0.895 for mild TED (24). The performance of the new neural network was better than that of GoogLeNet, ResNet-50, Visual Geometry Group-16 (VGG-16), and even three oculoplastic surgeons, although details were not clear on the exact matrices that the experts based their decision-making on. In both studies, CT images were pre-processed via methods such as cropping, rotation or segmenting based on HU levels targeting extraocular muscles or fats.

Two studies developed AI models to identify DON (15, 25). Chaganti et al. showed improvements in the AUC of their AI model in classifying various optic nerve conditions, including DON in TED, when electronic medical records (EMR) information was incorporated into CT imaging data (15). Adding EMR phenotypes (derived from an EMR-based phenome-wide associated study (PheWAS) to imaging markers increased the AUC from 0.81 to 0.85. Wu et al. developed a deep learning hybrid model which is composed mainly of the double multiscale and multi-attention fusion module (DMs-MAFM) and a deep convolutional neural network for predicting suspected DON using CT orbits (25). The dataset was obtained from 178 patients, of which only 42 had DON. The model was trained on an augmented set of coronal views of the orbits at various distances from the interzygomatic line. The hybrid model reached a high accuracy rate of 96%, sensitivity of 94%, specificity of 99.5% and precision of 98.9%.

Lin et al. constructed DL systems based on CNN to distinguish active and inactive TED using 160 MRI orbit images (20). Network A inherited from the VGG network (29) and network B was constructed with the addition of parts of the Residual Neural Network (28). Both networks achieved high accuracy (network A 0.863 ± 0.055, network B: 0.855 ± 0.018). After modification, network B improved the sensitivity (0.750 ± 0.136 to 0.821 ± 0.021). The AUC of both networks was 0.922.

Hanai et al. developed a diagnostic software system to evaluate enlarged extraocular muscles (EEM) in TED patients using the orbital coronal CT data from 199 patients with EEM and 172 controls with normal extraocular muscles (22). The system was constructed based on a deep neural network using ResNet-50 (28) and VGG-16 (29). *Post-hoc* explainability was achieved using Score-CAM to construct heat maps for indicating where images in the convolutional neural network were focused. The system demonstrated a sensitivity of 92.5%, specificity of 88.6%, and AUC of 0.946.

Hu et al. performed radiomic analysis of MRI T2-weighted (T2w) coronal orbital images using the eight most identifiable features, all of which were related to signal intensity or heterogeneity (19). The study also found that higher minimal T2w signal intensity of the extraocular muscles, corroborated with earlier literature findings that they were more responsive to treatment, presumably due to higher water content. Integration of radiomics signature and disease duration further improved the diagnostic performance when compared to radiomics signature only (AUC of validation set improved from 0.916 to 0.952). Radiomics feature inputs with clinical value providing interpretability in these AI models. This study, however, lacked a test set to verify the model's applicability to internal or external data and it only assesses active, moderate-to-severe TED.

TABLE 3 Is a large table, submitted as supplementary materials.

Study	Author/ yr	Aim of study	No. patients	AI model	Image type	Reference standard	Internal validation	External validation	Outcome measures
1	Chaganti et al / 2017 (15)	Improve accuracy of AI model in classifying various optic nerve conditions with the addition of EMR phenotypes	2154	Boosted random forest	CT orbits CT performed for cochlear implants (controls)	NA	NA	No	AUC 0.81 With addition ofEMR phenotype: AUC 0.85
2	Salvi et al / 2002 (a) (16)	Classification and progression prediction of TED	398	3-layer neural network	Clinical ophthalmologic assessment and orbital CT or US	Expert	Hold-out	No	Classification: SN 86.2%, SP 80.2% Prediction of progression: SN 75.3% SP 52.2% Concordance between clinical assessment and neural network prediction: 67%
3	Salvi et al / 2002 (b) (17)	Classification and progression prediction of TED	345	3-layer neural network	Clinical ophthalmologic assessment and orbital CT or US	Expert	Hold-out	No	Correctly classified 78.3% of 115 eyes (87 patients) and predicted TAO progression in 69.2% of 39 eyes (28 patients)
4	Song et al / 2021 (18)	Screening of TED	908	Modified 3D-ResNet-18	CT orbit	Experts	Hold-out	Yes	AUC 0.919 Accuracy 87% SN 88% SP 85% Accuracy was 85.67% in the AI group and 84.33% in the resident group in the non- inferiority experiment
5	Hu et al / 2022 (19)	Value of T_2WI-derived radiomics for pre-treatment determination of therapeutic response to glucocorticoids	110	Logistic regression (LR), decision tree (DT), support vector machine (SVM)	MRI orbits	Experts	Hold-out	No	LR achieved the best performance; Validation set results: AUC 0.916, Accuracy 87.5%, SN 86.1%, SP 89.3%, PPV 91.2%, and NPV 83.3% Integration of radiomics signature and disease duration: AUC 0.952, Accuracy 87.5%, SN 91.7%, SP 82.1%, PPV 86.8%, NPV 88.5%
6	Lin et al / 2021 (20)	Identification of active TED	108	CNNs built with blocks from VGG and ResNet	MRI orbit	Expert	Hold-out	No	Network (A): Accuracy 86.3%, SN 75.3%, SP 89.6%, Precision 68% Network (B): Accuracy 85.5%, SN 82.1%, SP 86.5%, Precision 64% Both network (A) and (B): AUC 0.922
7	Huang et al / 2022 (21)	Detect signs of TED based on facial images	1560	Single-shot multibox detector(SSD), U-net, and ResNet-50	Facial images	Expert	Hold-out	No	The mean AUC of the seven signs of TED :0.85, mean sensitivity 80%, mean specificity of 79%

(Continued)

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TABLE 3 Continued

Study	Author/ yr	Aim of study	No. patients	AI model	Image type	Reference standard	Internal validation	External validation	Outcome measures
8	Hanai et al / 2022 (22)	Detection of enlarged EOM	371	ResNet-50, VGG-16	CT orbit	Experts	Hold-out	No	AUC: 0.946 SN 92.5%, SP 88.6%
9	Karlin et al / 2022 (23)	Detect TED using external photographs	2288	An ensemble model of five ResNet-18	Facial image	Experts	Hold-out	Yes	Accuracy 89.2%, specificity: 86.9%, recall 93.4%, precision 79.7%
10	Lee et al / 2022 (24)	Diagnosis and severity assessment of TED	300	custom built CNN	CT orbit	Experts	Hold-out	No	Moderate-severe vs normal: AUC 0.979, Accuracy 0.930 Mild TED vs normal: AUC 0.895, Accuracy 0.826 Moderate-severe vs mild vs normal: AUC 0.905, Accuracy 0.842
11	Wu et al / 2022 (25)	Prediction of DON in TED	178	Double multiscale and multi attention fusion module + EfficientNetB0	CT orbit	Experts	Hold-out	No	Accuracy 96% SN: 94% SP: 99.5%, Precision: 98.9%
12	Shao et al / 2022 (26)	Automatic measurement of eyelid morphology in TAO patients	148 (separate 30000 images (celebA) to train eye detection model and 1862 healthy volunteer images to train eye segmentation model	R2AU-Net	Facial images	Experts	Hold-out	Yes	Accuracy: 98.5%
13	Moon et al / 2022 (27)	Assess CAS and diagnose active TAO	1020	linear kernel SVM (integrated with linear kernel PCA)	Facial images	Experts	Hold-out	No	SN: 72.7% SP 83.2% (entire dataset) SN: 88.1% SP 86.9% (dataset with consistent results for the 3 ophthalmologists SN: 40% SP: 49.9% (combination of above 2 datasets)

The full study characteristics of the 13 studies in this review. SN, sensitivity; SP, specificity; AUC; area under curve.

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Studies based clinical assessments

Two studies [Salvi et al. (a) and (b)] by the same group utilized clinical assessments in combination with US or CT orbit in TED diagnosis and prediction of disease progression (16, 17) of which only one study provided the sensitivity and specificity of the AI system (16). Both studies recruited patients already known to have GD at the ophthalmology clinic in a single institution. It is unclear if there were appropriate exclusion criteria. Training set data was provided by an expert clinician (ophthalmologist and endocrinologist). However, there was no internal or external validation used for the studies. Concordant classification between AI and experts occurred in 86.2% and 78.3% and the ability to predict the progression of disease was 67% and 69.2% respectively, although the number of patients that progressed in both studies was small.

Studies based on digital facial images

Four studies utilized facial photos to detect signs of TED (21, 23, 26) or assess disease activity (27). Outcomes assessed were heterogenous amongst studies: diagnosis of TED (23) presence of severe signs of TED (21), eyelid morphology in TED (26) and prediction of CAS score (27).

In the study by Karlin et al, compared to the expert clinician, the deep learning ensemble model demonstrated higher recall (89% vs 58%) but lower specificity (84% vs 90%) than the pooled expert cohort in detecting TED using facial images (23). The study utilized Grad-CAM to perform heatmap analysis of a component neural network model within the ensemble and found pixels corresponding to the eye and periocular region most strongly activate the TED class. Shao et al. developed a fully automatic computer-based assessment system to measure eyelid morphology in patients with TED (26). Manual measurement of margin to reflex distance (MRD) 1 and 2 by experienced ophthalmologists was compared to this automatic system. The intraclass correlation coefficients (ICCs) used to assess the agreement between automatic and manual measurement of MRDs demonstrated ICC of 0.980 for MRD1 and 0.964 for MRD2 in TED eyes, and ICC of 0.967 for MRD1 and 0.932 for MRD2 in control eyes, with ICC between repeated automatic measurements of MRDs up to 0.998, reflecting a strong agreement between the two, with high repeatability demonstrated in the automatic system. The diagnostic system for TED developed by Huang et al. accurately diagnosed TED via multiple task-specific models using facial images, with the ability to detect several signs of TED (21). The diagnostic methods used in this study included modules based on eye location (Module I), ocular dyskinesis (Module II), and other TED signs (Module III). Module I had an accuracy of 0.98; Module II had an accuracy of 0.93 for corneal segmentation and 0.87 for scleral segmentation. For Module III, the area under the receiver-operating curve (AUROC) for the detection of eyelid edema was 0.90, conjunctival congestion was 0.91 and eye movement disorders were 0.93. However, the diagnostic accuracy for TED signs that require auxiliary modalities to aid evaluation, such as chemosis and corneal ulcer, were lower (AUROC 0.60 and 0.70, respectively). The mean AUROC of the seven TED signs evaluated was 0.85, with a mean sensitivity of 0.80 and specificity of 0.79. Moon et al. developed an ML-assisted system for predicting CAS and diagnosing active TED using facial images (27). The system predicted CAS within 1 point of the reference CAS in 84.6% and 89% of cases when tested using the entire dataset and in the dataset with consistent results for the three ophthalmologists, respectively. However, the system showed differences in the performance of individual inflammatory signs, which could be further improved.

Quality assessment

We performed a quality assessment of the 13 studies using a modified QUADAS-2 tool. The overall risk of bias and applicability using the modified QUADAS-2 tool led to most of the studies being classified as low risk, although higher deficiency was noted in the risk of bias in flow and timing (Figure 2). For patient selection, 9/13 (69%) studies had a low risk of bias. Most of these studies used in-house data following clinically established criteria for diagnosis, avoided inappropriate exclusions, and considered label balance in classification problems. However, for flow and timing a high or unclear risk of bias was seen in 11/13 (84.6%) of the studies. This was largely due to the unknown interval between the index test and the reference standard and whether all the patients received the same reference standard. For the reference standard domain, high or unclear risk was noted in 6/13 (46.2%) of the studies. This was mainly due to inconsistencies in the reference standard employed for the studies and concerns regarding the expertise level and level of consensus amongst experts when used for performance benchmarking. For the patient selection domain, high or unclear risk was noted in 4/13 (30.8%) of the studies. This was mainly due to a lack of description on the rationale for the breakdown of data into training, validation, and test set and whether pre-processing of data may significantly change the data set and reduce its applicability in testing on an external dataset. For the index test domain, high or unclear risk was noted in 3/11 (27.3%) of the studies, mainly due to a lack of description of the prespecified threshold settings.

Discussion

Machine learning (ML) is a subdivision of AI that constructs data analytical algorithms to extract features from data. In medical applications, input data includes medical images and patient clinical data, which includes baseline data, disease-specific data, and disease outcomes. ML algorithms can be broadly divided into two major categories: unsupervised and supervised learning. Unsupervised learning is predominantly for feature extraction, while supervised learning is suitable for predictive modelling through building some relationships between the patient characteristics (as input) and the outcome of interest (as output) (30). In general, supervised learning provides more clinically relevant results; hence AI applications in healthcare, in medical imaging analysis, supervised learning is most often used. Traditional ML techniques such as linear regression,

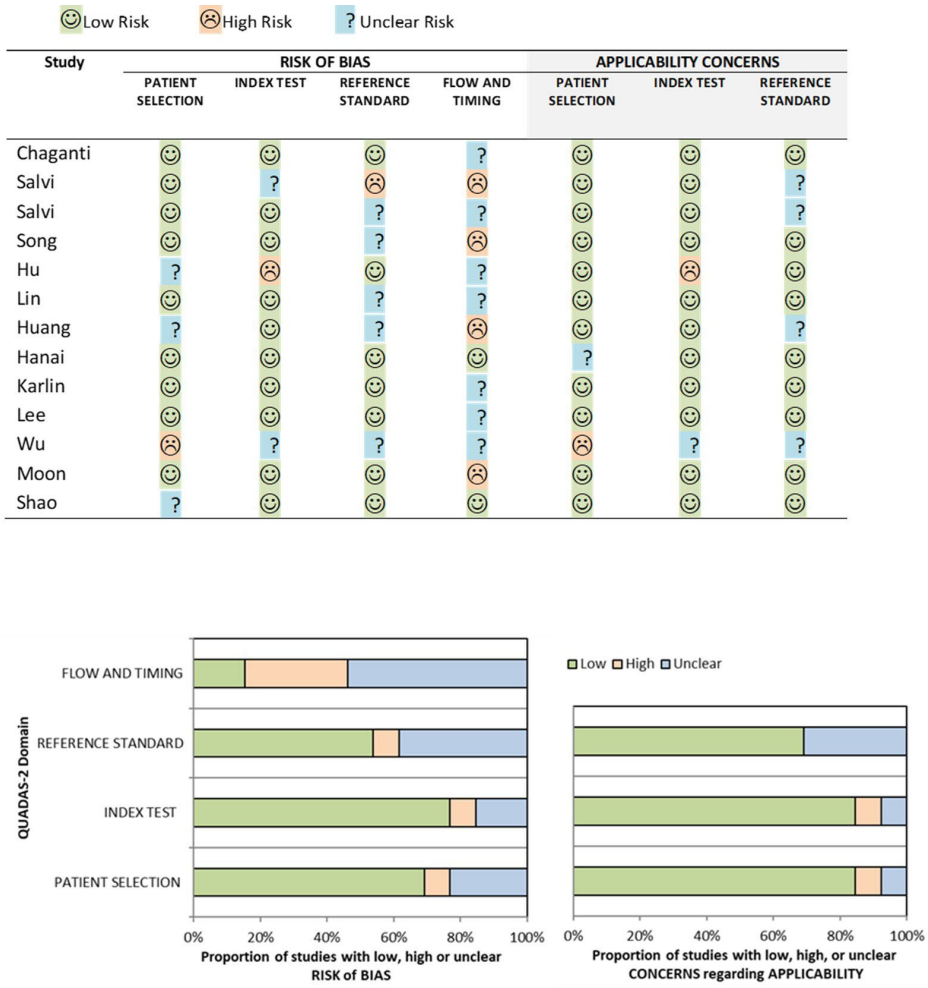


FIGURE 2
Risk of bias and applicability concerns summary about each modified QUADAS-2 domain presented as percentages for the 13 reviewed studies.

logistic regression (LR), random forest (RF), decision tree (DT), support vector machine (SVM), and neural network are feature-based supervised learning algorithms (31). For instance, Chaganti et al. employed RF classifiers comprising 100 trees to assess the diagnostic efficacy of image-derived features, phenotypes derived from electronic medical records, and clinical visual assessments (i.e., visual disability scores) in predicting optic nerve pathology (15). In another study by Hu et al, three machine learning models, namely LR, DT, and SVM were developed based on selected features to predict the response to glucocorticoid therapy in TED patients (19). Furthermore, Moon et al. proposed a submodel using linear kernel SVM integrated with linear kernel PCA. Subsequently, five such submodels, along with two consensus models (an aggregation model and a voting model), were designed for predicting the clinical activity score in TED (27). Traditional ML models offer interpretability with transparent decision-making, simplicity with well-defined theory, lower data requirements leading to computational efficiency, and robustness to noise due to explicit feature engineering. These characteristics contribute to the broad applicability of such models in the field of medical imaging analysis. However, several initial steps are necessary prior to the development of these AI algorithms, such as defining the image features to be extracted and selecting the region of interest (ROI), which needs to be done by field experts.

Deep learning (DL) is a subfield of ML and is an extension of the classical neural network technique whereby a cascade of multi-layered artificial neural networks for feature extraction and transformation. DL essentially imitates the neural connections made in the human brain. In recent years, DL has demonstrated exceptional performance across various domains, including computer vision and natural language processing (32). Leveraging the strong modelling capabilities of DL models, researchers have started to explore their application in TED-related tasks to achieve boosted performance. Some studies concentrate on utilizing Multilayer Perceptron (MLP) models. Notably, in the two studies by Salvi et al., a three-layer MLP architecture comprising an input layer, a hidden layer, and an output layer was employed to predict the progression of TED (16, 17). In both studies, the adoption of MLP models stems from their advantages over multivariate statistical analysis, as MLP models do not require explicit definitions of associations between features during modelling. Instead, they learned these associations in a data-driven learning

process. Of various DL architectures, convolutional neural networks (CNN) are commonly applied for image recognition and computer vision applications because they preserve spatial relationships in 2D data, and thus outperform other architectures on image pattern recognition Researchers employ various CNN models to facilitate TED diagnosis and pre-treatment evaluation. For instance, Song et al. proposed the 3D-ResNet model (with the original 2D convolution modified to 3D), which incorporates residual connections to mitigate performance degradation caused by larger network depth (18). Lin et al. adopted two CNN models, one inheriting the VGG network (29) with smaller filters to reduce complexity, and the other utilizing ResNet (28) to address issues such as gradient vanishing and exploding (20). Huang et al. presented a system for TED diagnosis based on facial images, consisting of three modules: (i) a single-shot multibox detector for object detection (33), (ii) U-Net for semantic segmentation (34), and (iii) ResNet50 (28) for detecting TED signs (21). In the Hanai et al. study, the focus was on leveraging AI models to automatically detect enlarged extraocular muscles in TED using CT orbits. Their model combined Residual Network-50 (28) for segmentation and VGG-16 (29) for classification (22).

In addition to utilizing existing CNN models and techniques, researchers are actively exploring the development of novel model architectures tailored to specific application requirements. They aim to go beyond using off-the-shelf CNN models and techniques, seeking to address the unique demands of their research objectives. Karlin et al. introduced a novel approach for making use of external photographs to detect TED (23). They proposed an ensemble neural network model consisting of five neural networks, each employing a ResNet18 (28). The ensemble mechanism selected the output of the neural network among the five networks, which assigned the highest prediction probability for TED. Such an ensemble had a learning strategy that aims to achieve improved predictive performance while enhancing robustness to noise and outliers. By leveraging the collective decision-making power of multiple networks, the proposed ensemble model demonstrated potential advantages in thyroid eye disease detection. In the study conducted by Lee et al, the authors focused on improving the diagnosis and severity assessment of TED by modelling clinically routine orbital CT scans using neural networks (24). To address the challenge of incorporating CT images from axial, coronal, and sagittal views, which conventional CNN models cannot directly handle, they proposed a multi-view CNN model. This model was designed to process all three views simultaneously and comprised three sets of convolutional layers, a fully connected layer, and a classifier. By leveraging multi-view learning, the model could capture a comprehensive representation of the input data, leading to enhanced analytic performance in diagnosing and assessing the severity of TED. In the work by Wu et al, an efficient and convenient method was introduced for diagnosing DON (25). The authors proposed a hybrid model that combined the double multiscale and multi attention fusion module (DMs-MAFM) with EfficientNet B0 (35). The DMs-MAFM was built on the synergy between the multiscale feature fusion module (Ms-FFM), the multiscale channel attention aggregation module (MsCAAM), and the spatial attention module (SAM). This integration enhanced the model's ability to attend to small objects and effectively extract features. By leveraging the DMs-MAFM and EfficientNet B0, the proposed hybrid model offered improved performance and convenience for diagnosing DON. Finally, in the study conducted by Shao et al, the authors focused on the image analysis of eyelid morphology in TED (26). They proposed a novel model called Attention R2U-Net (36), which combined a recurrent residual convolutional neural network with attention gate connections based on U-Net. The Attention R2U-Net aimed to achieve more accurate segmentation of eyelid morphology. The traditional convolutional block in the model was replaced with a recurrent convolutional unit to effectively capture low-layer features, resulting in improved performance and enhanced segmentation accuracy. By incorporating attention gate connections and leveraging the recurrent convolutional unit, the Attention R2U-Net model offered promising advancements in the analysis of eyelid morphology in TED. A summary of the various AI techniques used in the studies reviewed in this paper is presented in Table 4.

This review aims to quantify the diagnostic accuracy of AI models in the field of TED assessment and appraise the quality of these studies using the modified QUADAS-2 tool. The QUADAS-2 tool is the most used instrument in the quality assessment of diagnostic accuracy studies (12) and its use is recommended by current PRISMA 2020 guidance (11). The tool provides transparency in the rating of the study bias and applicability in answering its review question. However, QUADAS-2 does not accommodate for specific terminology encountered in AI-related diagnostic test accuracy studies, nor does it educate researchers on the sources of bias found within this class of study (14). As such, we tailored QUADAS-2 components to better suit the quality assessment of studies related to TED diagnosis and pre-treatment evaluation based on the framework proposed by Soundarajah et al. (14) which addresses the unique potential biases related to AI-related diagnostic studies (Table 1).

To build a robust AI model, data quality is important. which requires an appropriately curated source. The data is more reliable if the data was collected in-house or from a well-curated open-source database since a poorly curated open-source database runs the risk of data duplication and erroneous labelling (14). Label imbalance should be addressed, particularly when identifying a rare disease (such as TED), to avoid the accuracy paradox problem that can result in a model with excellent accuracy but is inapplicable clinically (37). An appropriate spectrum of patients identified and the inclusion of more than one institution is favorable in building a model that is reproducible and beneficial to a wider community. For the assessment of the index test, we took into consideration if the test was evaluated with an external dataset that would assess its reliability performance; if solutions to avoid overfitting on the testing set were mentioned; if there were sufficient details provided about the algorithm to allow for independent validation, and specific design for end-user interpretability to see if the model is assessing the target rather than potential "noise". In assessing the study's reference standard, we evaluated if widely accepted clinical criteria for diagnosis were used, such as Bartley's and Gorman (38) or EUGOGO (39) diagnostic criteria for TED. If benchmarking was performed against clinicians, we assessed if a suitable domain expert

TABLE 4 Categorization of AI models used in TED studies.

AI Model Category	AI Model Subcategory	TED Studies
Traditional Machine Learning Models	Random Forests	Chaganti et al., 2017 (15)
	Support Vector Machine, Decision Tree, Logistic Regression	Hu et al., 2022 (19) Moon et al., 2022 (26)
Deep Learning Models	Multilayer Perceptron	Salvi et al., 2002 (a) (16) Salvi et al., 2002 (b) (17)
	Convolutional Neural Networks	Song et al., 2021 (18) Lin et al., 2021 (20) Hanai et al., 2022 (22) Huang et al., 2022 (21) Karlin et al., 2022 (23) Lee et al., 2022 (24) Wu et al., 2022 (25) Shao et al., 2022 (26)

level was used. The AI models reviewed by this study have a relatively high diagnostic accuracy in identifying presence, activity and severity of TED, using either facial photographs or radiological images such as those derived from CT or MRI. The ML diagnostic systems can be used as a screening or diagnostic tool, potentially reducing barriers to accessing specialist care and contributing to earlier diagnosis and timely treatment. Clinically, radiological investigations are generally performed in patients with more severe TED, hence limiting its utility for TED screening. Radiological approach via CT or MRI may also be limited by cost, availability, and the exposure to ionizing radiation. However, based on the results of the studies included in this review, AI systems based on radiological investigations has potential use in early identification of dysthyroid optic neuropathy, active disease and disease progression, and predicting treatment response. Beyond screening and diagnosis, AI models utilizing orbital imaging may also aid in surgical planning, such as predicting appearance change with orbital decompression surgery (40).

On the other hand, digital facial images-based AI systems have potential use in TED screening and disease monitoring, which can be adapted to mobile devices and cloud services, providing automated and remote diagnostic services for patients with TED. It may also be used for screening of TED in patients with autoimmune thyroid diseases. These systems could serve as a telemedicine screening tool to identify TED in patients with diverse phenotypical characteristics, irrespective of their care location. This has important implications in the remote patient monitoring, enabling early intervention and enhancing patient care. However, a greater training set including photographs of patients with differentials of TED, such as lid retraction (e.g., previous eyelid surgery) or conjunctival chemosis (e.g., carotid-cavernous fistula) will be required to improve the specificity of such a model.

Although we remain optimistic that such AI technologies will eventually be adopted at a large scale to benefit TED patient care, ML is not without its challenges and controversies. One of the criticisms of DL models is the black box paradigm, in which the internal workings of how the output classification, in this case, TED, is determined by the model is unknown. This is the so-called black box phenomenon and could eventually lead to a reduced acceptance of this technology by clinicians (41). There are several strategies used to help people gain insight into how these models work, including the use of Class Activation Mapping (CAM) and saliency map. For example, Song et al. used CAM to highlight areas of the CT scan deemed important by its model to diagnose TED and it revealed this to be at the anterior aspect of the orbits. The prevalence of TED is much lower than other ocular diseases, such as diabetic retinopathy and cataracts. Thus, the next challenge arises from the limited small number of available training samples to build robust models without suffering from overfitting i.e., the predicting model learns exactly the training set but fails to fit new data from the test. For example, the ability to distinguish patients other than moderate-severe TED may be challenging in the proposed AI model by Song et al. due to the smaller dataset of mild TED cases (18). Similar challenges are faced in AI models developed to detect DON due to the scarcity of sample data (25). Various strategies have been described to try to mitigate this challenge (42). In the studies reviewed in this paper, techniques such as data augmentation and transfer learning, which is essentially the use of pre-trained networks (typically on natural images) to circumvent the (perceived) requirement of large datasets for deep network training (43). For example, in the study by Shao et al, they trained the eye detection model using 30,000 facial images with landmark locations of the eye extracted from the CelebFaces Attributes Dataset (26) and in the study by Karlin et al, CNN was pre-trained on ImageNet, a large, labelled collection of low-resolution color photographs (23). While such strategies reduce the chance of overfitting the model, its applicability here to an external test set and clinical setting has yet to be tested. Ethnic differences exist in TED phenotypes, related to orbital and lid anatomy, genetic background and autoimmune responses (44). Hence, AI models trained based on Caucasian data may not be applicable to Asians, and vice versa. Datasets employed in the studies in this review were relatively small and detailed patient demographics and clinical findings were not available for several studies (Table 2), potentially limiting the generalizability and reproducibility of the developed ML algorithms to other patient populations. This underscores the significance of training and assessing models using extensive and varied datasets through collaborations and data pooling from multiple institutions and publicly available datasets to enhance its performance. The majority of the studies in this review evaluate AI systems in either imaging or facial images alone. The integration of clinical assessments, serologic markers and imaging features could be used to further enhance the reliability of these AI models and

should be explored in future studies. Other limitations inherent to AI systems using digital facial images highlighted by several authors' studies include the influence of imaging environments on diagnostic accuracy, the need for larger amounts of data to further improve the performance of the AI platform and inherent limitations of using 2-dimensional photos. Lastly, ethical and legal implications need to be considered when implementing AI solutions in clinical practice. Ethical challenges include (1) informed consent to use data, (2) safety and transparency, (3) algorithmic fairness and biases, and (4) data privacy. On the other hand, legal challenges include (1) safety and effectiveness, (2) liability, (3) data protection, and privacy, (4) cybersecurity, and (5) intellectual property law (45). It is therefore crucial that the relevant stakeholders and regulatory authorities collaborate to overcome these challenges to ensure successful implementation of any proposed AI solutions to benefit a wider community.

Conclusion

The application of AI in clinical practice has enormous promise to improve the care of patients with TED. This review appraised the quality of the literature and quantified the diagnostic accuracy of AI models in the field of TED assessment. While the results of the review showed high diagnostic accuracy of the AI models in identifying features of TED relevant to disease assessment, several knowledge gaps in the current research in this area were identified through this review when the studies were objectively critiqued with our modified QUADAS-2 tool. Deficiencies in study design causing study bias such as small datasets, label imbalance and lack of external validation of the AI models, compromising study applicability were noted. Moving forward, these limitations and challenges inherent to ML should be addressed with improved standardized guidance around study design, reporting, and legislative framework for successful implementation in clinical practice.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

Author contributions

C-LC: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Project administration, Resources, Writing – original draft, Writing – review & editing. KZ: Formal Analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. AK: Investigation, Writing – original draft. M-HL: Investigation, Writing – original draft. DT: Supervision, Writing – review & editing. CW: Investigation, Writing – original draft. GH: Formal Analysis, Methodology, Writing – original draft. BO: Supervision, Writing – review & editing. SK: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing.

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Global scientific trends on thyroid disease in early 21st century: a bibliometric and visualized analysis

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Background: Bibliometrics has been used to analyze the literature in the field of thyroid disease studies in the early 21st century, indicating the changes in current international study trends.

Methods: In this study, a bibliometric analysis of data retrieved from the Web of Science (WoS) database was conducted, and the publication trends and thematic evolution in the field of thyroid disease research from January 1, 2000, to November 16, 2022, were analyzed. A total of 69283 articles related to thyroid diseases were evaluated for their characteristics, including annual publication volume, countries, journals, institutions, authors, keywords, and references. VOSviewer was utilized to perform the analysis of co-authorship, co-citation, co-occurrence and descriptive.

Results: The annual publication volume of thyroid disease research literature showed a fluctuating upward trend from 2000 to 2021, exceeding 5,000 articles for the first time in 2021. The United States (16120 counts, 678255 cities) ranks first in terms of publication volume and citation. *Thyroid* (n=3201) and *Journal of Clinical Endocrinology&Metabolism* (n=140399) are the most prolific and cited journals, respectively. The organization with the highest publication volume and citation frequency is Harvard University (1011 counts, 59429 cities), Miyauchi Akira (n=422), Schlumberger, and Martin (n=24839) possess the highest publication volume and citation frequency, respectively. Co-occurrence analysis of 307 keywords with frequencies of more than 20 resulted in 6 clusters (1): Thyroid dysfunction and diseases (2); mechanism of occurrence and development of thyroid cancer (3); autoimmune thyroiditis (4); scope and postoperative management of thyroid surgery (5); fine needle aspiration of thyroid nodules (6); radioactive iodine therapy for thyroid cancer. Active monitoring, thermal ablation, Lenvatinib, and long noncoding RNA refer to the latest keywords. Discussing the six clusters helps scholars to determine the scope and direction of studies.

Conclusion: Over the past two decades, the literature related to thyroid diseases has increased year by year, with closer collaboration between countries,

institutions, and authors. In this study, the global trends, research hotspots, emerging subjects, and basic knowledge of literature related to thyroid diseases were respectively elucidated, which will facilitate researchers in this field to seek better development.

KEYWORDS

bibliometric, thyroid disease, thyroid cancer, autoimmune thyroiditis, fine needle aspiration, radioactive iodine

1 Introduction

Thyroid diseases are most common clinical endocrine system diseases, mainly including hyperthyroidism, hypothyroidism, thyroid nodules and thyroid adenomas, etc. The incidence of thyroid disorders has increased over the past few decades. It's worth noting that the incidence of thyroid cancer has increased slightly, with PTC growth accounting for the majority of the increase. However, the mortality rate of patients with thyroid cancer remains low, which may be caused by overdiagnosis. Therefore, whether to choose immediate surgery or active surveillance for the treatment of thyroid cancer has become one of the hot topics of discussion. In addition, benign thyroid diseases (e.g., hyperthyroidism and hypothyroidism), are still the most common endocrine diseases worldwide (1, 2). With the deepening of research, research directions and hot spots have also shifted. Up to now, researchers have made significant progress in exploring the mechanisms of thyroid disease, and the diagnosis, treatment, and prevention of thyroid disorders are becoming increasingly personalization. Researches are beneficial for thyroid disease patients to receive more accurate treatment and obtain a better prognosis.

The field of bibliometrics is expanding with the increase in the publication of scientific literature. Bibliometrics is a quantitative analysis of publications. By analyzing the data in the database and using appropriate statistical methods, bibliometrics can be used to evaluate the development state of a certain field over time, which has been an essential component of research quality assessment (3). To date, several bibliometric articles have been published in the direction of thyroid diseases (4, 5), but no comprehensive bibliometric analysis has been performed for all thyroid diseases. This study is the first bibliometric analysis of the whole field of thyroid diseases based on plenty of thyroid disease literature.

By analyzing the number and citation frequency of thyroid disease publications in the early 21st century, this study aims to reveal the publishing trend in research of thyroid disease during this period, identify influential journals, countries, institutions, and authors, and explore the international cooperative network of research, research hotspots, and emerging subjects. Analysis of these data contributes to a vivid exhibitions of shifts in research hotspots and helps researchers focus on areas of interest and significance.

2 Methods

This study is based on the Web of Science (WoS) database. As the most commonly used website for obtaining specific literature citations, WoS has historically been the standard for defining citations included in the calculation (6).

As shown in the following figure, data retrieval was conducted on the WoS core collection on November 16, 2022, and the retrieved publications were required to meet the following criteria:

(1) Retrieval strategy: Title, Abstract, Author Keywords, and Keywords Plus Containing Items Related to Thyroid Disease. The retrieval strategy was as follows: TS=("Thyroid Disease$" or "Euthyroid Sick Syndrome$" or "Sick Euthyroid Syndrome$" or "Non Thyroidal Illness Syndrome$" or "Low T3 Low T4 Syndrome$" or "Low T3 and Low T4 Syndrome$" or "Low T3 High T4 Syndrome$" or "High T4 Syndrome$" or "Low T3 Syndrome$" or "Goiter$" or "Graves$ Disease*" or "Basedow* Disease$" or "Graves Ophthalmopathy" or "Thyroid Eye Disease$" or "Thyroid Associated Ophthalmopath*" or "Dysthyroid Ophthalmopath*" or "Graves Eye Disease$" or "Graves Orbitopathy" or "Myopathic Ophthalmopath*" or "Congestive Ophthalmopath*" or "Edematous Ophthalmopath*" or "Infiltrative Ophthalmopath*" or "Hyperthyroid*" or "Thyrotoxicos*" or "Thyroid Crisis" or "Thyrotoxic Storm" or "Thyrotoxic Crisis" or "Thyroid Storm" or "Hyperthyroxinemia$" or "Thyroid Hormone Resistance" or "Refetoff DeWind DeGroot Syndrome" or "Refetoff Syndrome" or "Generalized Resistance to Thyroid Hormone" or "Hypothyroidism$" or "Thyroid Stimulating Hormone Deficienc*" or "TSH Deficienc*" or "Cretinism" or "Fetal Iodine Deficiency Disorder" or "Myxedema$" or "Lingual Thyroid$" or "Thyroid Dysgenesis" or "Thyroid Hypoplasia" or "Ectopic Thyroid$" or "Thyroid Agenesis" or "Thyroid Nodule $" or "Thyroid Neoplasm$" or "Thyroid Carcinoma$" or "Cancer$ of Thyroid" or "Thyroid Cancer$" or "Cancer$ of the Thyroid" or "Thyroid Adenoma$" or "Thyroiditis" or "Thyroiditides" or "Hashimoto* Disease" or "Hashimoto* Struma" or "Hashimoto* Syndrome$"). Please refer to Figure 1 and the Appendix for details.

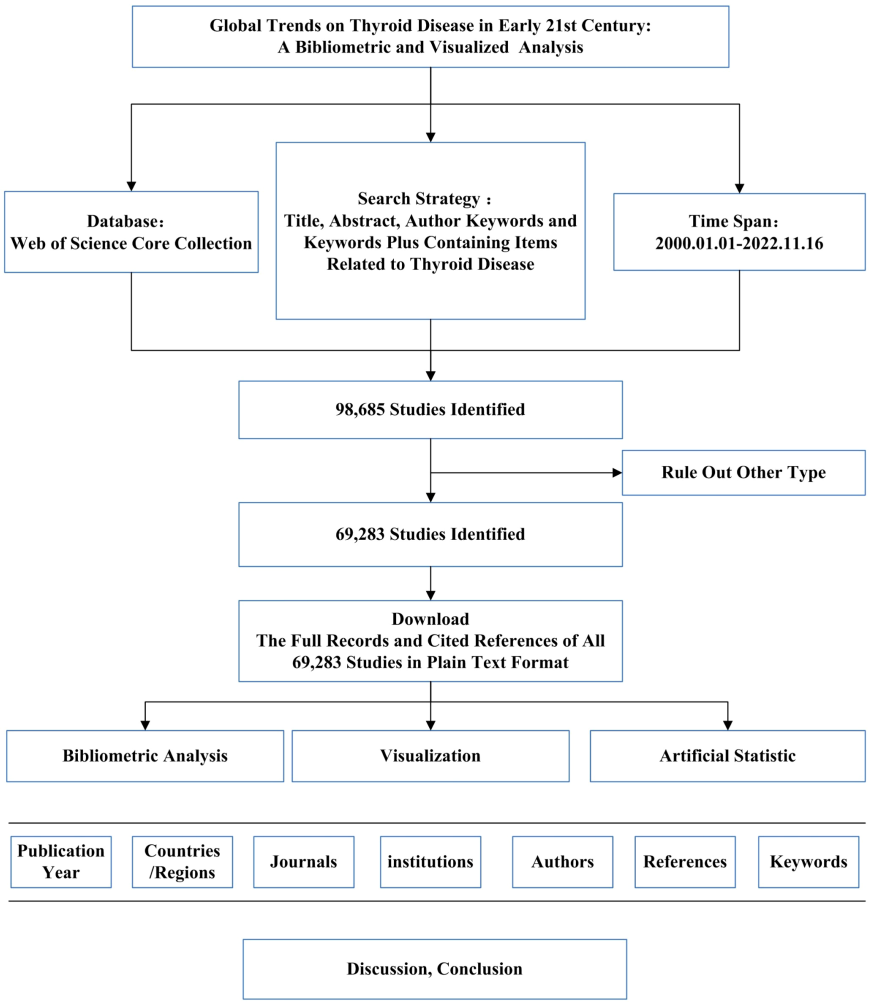


FIGURE 1
The data collection and retrieval strategy.

(2)Time frame of publication: January 1, 2000, to November 16, 2022.

(3)Only "article" was selected as the article type.

To avoid the impact of database updates, all bibliometric data were downloaded by year on November 16, 2022.

VOSviewer is a classic bibliometric analysis software which is used for building and viewing bibliometric maps. It focuses more on the mapping representation of bibliometric maps and is now widely used for bibliometric analysis and research (7). In this study, Vosviewer version 1.6.18 was used for Bibliometric and Visualized Analysis. Additionally, the citation analysis of journals, the co-authorship analysis of institutions and authors, the co-citation analysis of references, and the co-occurrence analysis of keywords were carried out. A descriptive analysis of publication year, journals, countries, institutions, authors, and references were also performed.

3 Results

3.1 Annual publication volume

A total of 69,283 relevant publications were retrieved, of which 66803 were in English, accounting for 96.42%. The trend figure of annual publication volume from 2000 to 2021 is depicted in Figure 2 (2022 is not full-year data and not shown in the figure). Linear regression analysis found that the increase in publications per year was statistically significant (P<0.001) (8). The annual publication volume of Thyroid Disease research tends to rise between 2000 and 2021. In particular, there were 1819 publications on thyroid disease research in 2000. In 2021, the number of publications reached the peak of the annual number of publications, which is 2.75 times the number of publications in 2000.

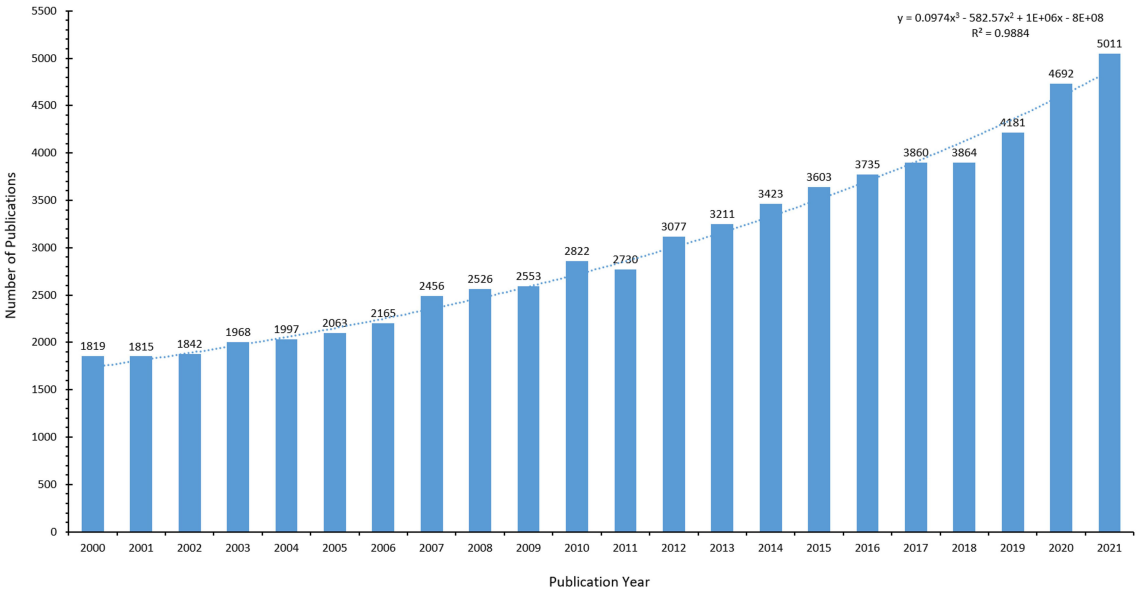


FIGURE 2
Number of articles on thyroid diseases per year from 2000 to 2021.

3.2 Countries and regions

Globally, 175 countries and regions are already involved in thyroid disease research. The threshold for the number of articles issued by countries/regions was set to 100 and 54 high-yield countries/regions in the Thyroid Release research were obtained. The distribution of high-yield countries/regions is described in Figure 3. The top ten countries ranked by publication volume are shown in Table 1. The United States (16120 counts, 678255 citations) ranks first for both publication volume and citations. This is followed by China (10,565 counts, 141,486 citations) and Italy (6,333 counts, 222,807 citations). Among the top 10 countries, France has the highest average citations (45 avg. Citations). Moreover, China has the highest average publication year, followed by South Korea.

3.3 Journals

Based on the retrieval results, a total of 4203 journals with publications about thyroid disease research was found. The threshold for journal publication volume was set at 100 articles, and 113 high-yield journals were obtained. These high-yield journals have published 31348 articles, comprising 45.25% of the total publication volume.

Furthermore, a citation analysis on 113 high-yield journals was conducted and an overlay visualization map was constructed (Figure 4). The size of the circle represents the number of publications, and the color range from blue to red denotes the average number of citations from low to high. It can be seen that *Thyroid* (n=3201) is the most prolific journal in this field, followed

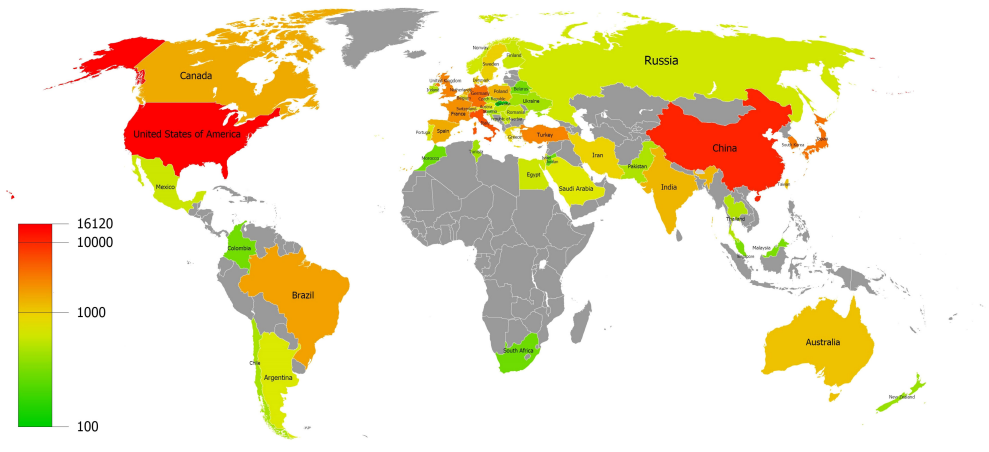


FIGURE 3
Distribution of countries and regions.

TABLE 1 Top 10 countries in terms of publication volume.

Rank	Country	Counts	Citations	Avg. Citations	Avg. Pub. Year
1	United States of America	16120	678255	42	2012.20
2	China	10565	141486	13	2017.72
3	Italy	6333	222807	35	2012.24
4	Germany	4374	128569	29	2011.45
5	Japan	4317	100976	23	2011.21
6	South Korea	3868	91837	24	2015.19
7	United Kingdom	3341	135132	40	2011.92
8	Turkey	3323	40572	12	2012.91
9	France	2744	124023	45	2011.18
10	Brazil	2131	45430	21	2012.86

by the *Journal of Clinical Endocrinology & Metabolism* (n=2285) and *Clinical Endocrinology* (n=1110). The Journal of Clinical *Endocrinology and Metabolism* (n=140399) is the most cited journal overall, followed by *Thyroid* (n=113871) and *Clinical Endocrinology* (n=35716). 14 journals (as shown in red) have an average of more than 40 citations.

3.4 Institutions

32935 institutions were involved in the publication of thyroid disease papers, and the top 20 institutions by publication volume are shown in Table 2. Harvard University (n=1011) is the institution with the highest number of publications, followed by University of Pisa (n=913) and Shanghai Jiao Tong University (n=778). Harvard University(n=59429) is the most frequently cited institution, followed by Memorial Sloan Kettering Cancer Center(n=53014) and University of Pisa(n=44829). Among the top 20 institutions, the Memorial Sloan Kettering Cancer Center and The University of Texas MD Anderson Cancer Center have the highest average citations. Besides, Fudan University has the latest average publication year, followed by Shanghai Jiao Tong University and China Medical University. The threshold for institutional publications was set at 100 and 273 high-yield institutions out of 32,935 were identified. Vosviewer was used to conduct co-authorship analysis of 273 high-yield institutions, all of which were in a co-authorship network, consisting of 8 clusters (Figure 5). The red cluster is the largest and consists of 75 institutions. Harvard University has partnerships with 222 high-yield institutions, followed by Johns Hopkins University, with 186

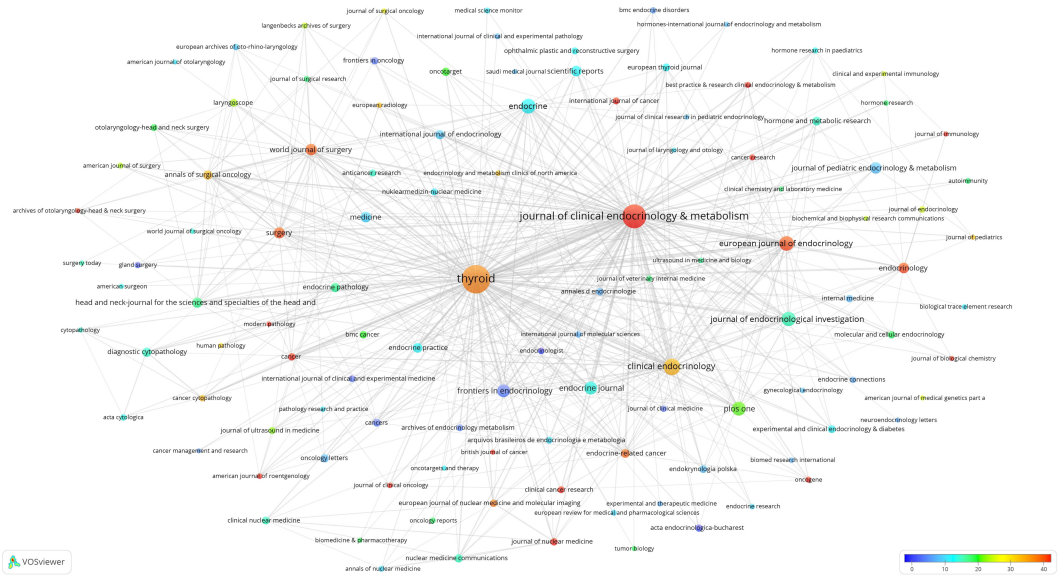


FIGURE 4
Map of 113 high-yield journals and their citation frequency.

TABLE 2 Top 20 production institutions.

Rank	Institution	Counts	Citations	Avg. Citations	Avg. Pub. Year
1	Harvard University	1011	59429	59	2013.72
2	University of Pisa	913	44829	49	2011.59
3	Shanghai Jiao Tong University	778	10480	13	2017.23
4	University of Naples Federico II	768	30346	40	2010.63
5	Yonsei University	717	18668	26	2014.86
6	Mayo Clinic	680	39744	58	2013.50
7	Johns Hopkins University	645	45392	70	2012.39
8	National Cancer Institute	640	36789	57	2012.59
9	Memorial Sloan Kettering Cancer Center	636	53014	83	2013.66
10	Seoul National University	630	17637	28	2015.48
11	Fudan University	554	7598	14	2017.47
12	Sungkyunkwan University	543	13434	25	2014.92
13	University of California, Los Angeles	529	25033	47	2010.97
14	University Of Sao Paulo	521	15444	30	2012.45
15	University of Ulsan	520	20198	39	2015.34
16	The University of Texas MD Anderson Cancer Center	510	42508	83	2015.45
17	China Medical University	506	9568	19	2016.96
18	University of California, San Francisco	503	27716	55	2011.89
19	University of Milan	486	20422	42	2013.03
20	Kuma Hospital	482	13664	28	2011.19

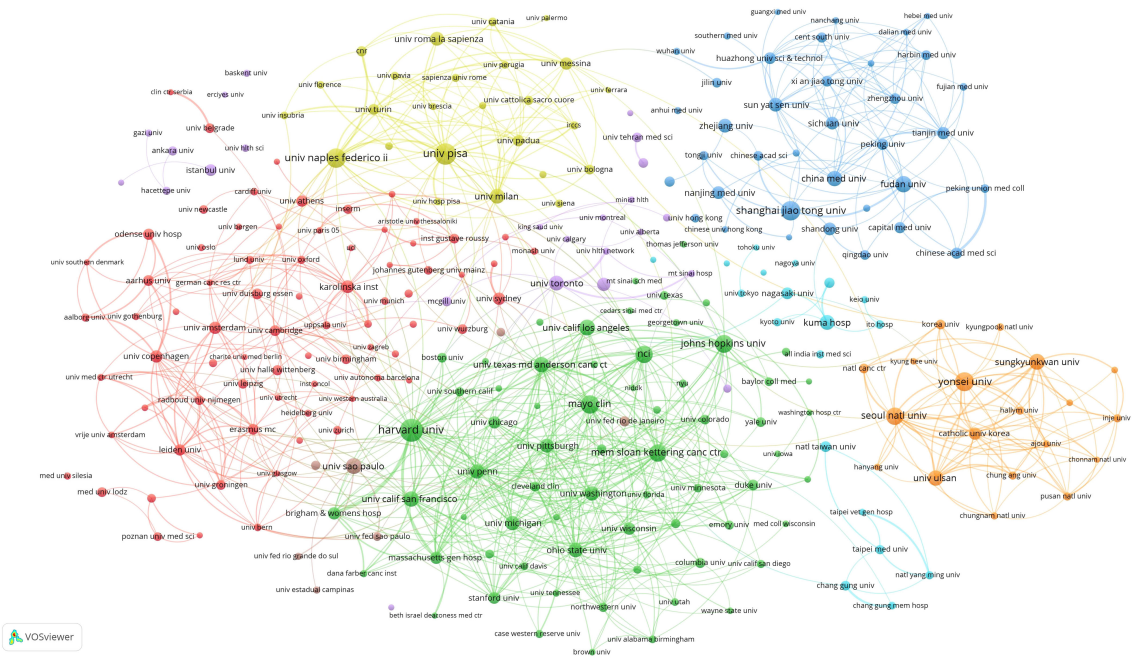


FIGURE 5
Co-authorship institutional network.

high-yield institutions. Mayo Clinic and The University of Texas MD Anderson Cancer Center both have partnerships with 173 high-yield institutions.

3.5 Authors

A total of 248011 authors participated in the publication of *Thyroid Disease*, and the top 20 authors ranked by publication volume are in Table 3. Miyauchi, Akira (n=422) is the author with the highest number of publications, followed by Tuttle, R. Michael (n=236) and Ito, Yasuhiro (n=234). Schlumberger, Martin (n=24839) is the most frequently cited author, followed by Tuttle, R. Michael (n=22632) and Elisei, Rossella (n=15474). On average, Schlumberger, Martin (n=117) is the most frequently cited, followed by Tuttle, R. Michael (n=96) and Elisei, Rossella(n=74). Among the top 20 authors, Baek, Jung Hwan has the latest average publication year, followed by Shan, Zhongyan, and Teng, Weiping.

The author publication threshold was set at 40 articles and 397 highly productive authors from 248011 authors were identified. As demonstrated in Figure 6, a co-authorship analysis on 397 high-yield authors was carried out using Vosviewer. 388 of the 397 high-producing authors formed the largest network of co-authors, consisting of 12 clusters. Tuttle, R. Michael has partnerships with 89 high-yield authors. Secondly, Elisei, Rossella has partnerships with 77 high-yield authors, while Fugazzola, Laura has partnerships with 71 high-yield authors.

3.6 Reference

A total of 852113 references were cited in 69283 publications, of which 852113 references appeared a total of 2282274 times, with an average of 33 references per publication. The threshold of reference citations was set to 300 times and 130 highly cited references from 852113 reference articles were identified. As shown in Figure 7, a co-citation analysis on 130 highly cited references was conducted using Vosviewe.

The top five articles cited are as follows: Haugen br, 2016, thyroid, v26(citation frequency:4549), Ooper ds, 2009, thyroid, v19 (citation frequency:3306), Davies l, 2006, jama-j am med assoc, v295(citation frequency:1700), Hollowell jg, 2002, j clin endocr metab, v87(citation frequency:1454),Mazzaferri el, 1994, am j med, v97(citation frequency:1253).

TABLE 3 Top 20 authors in terms of publication volume.

Rank	Author	Counts	Citations	Avg. Citations	Avg. Pub. Year
1	Miyauchi, Akira	422	11677	28	2010.97
2	Tuttle, R. Michael	236	22632	96	2012.58
3	Ito, Yasuhiro	234	8242	35	2010.60
4	Dralle, Henning	233	11436	49	2009.48
5	Schlumberger, Martin	213	24839	117	2011.16
6	Elisei, Rossella	209	15474	74	2013.62
7	Shong, Young Kee	199	7475	38	2015.11
8	Hegedus, Laszlo	198	8573	43	2012.12
9	Pinchera, A	195	12508	64	2005.84
10	Vitti, Paolo	191	7847	41	2012.37
11	Kim, Tae Yong	190	5865	31	2015.56
12	Teng, Weiping	188	4774	25	2015.94
13	Kim, Eun-Kyung	182	6636	36	2013.39
14	Miya, Akihiro	178	6111	34	2011.08
15	Kim, Won Bae	177	5619	32	2015.10
16	Basolo, Fulvio	176	10197	58	2012.63
17	Shan, Zhongyan	176	4568	26	2016.03
18	Kwak, Jin Young	175	5499	31	2014.18
19	Baek, Jung Hwan	175	6369	36	2016.32
20	Chen, Herbert	173	7220	42	2012.53

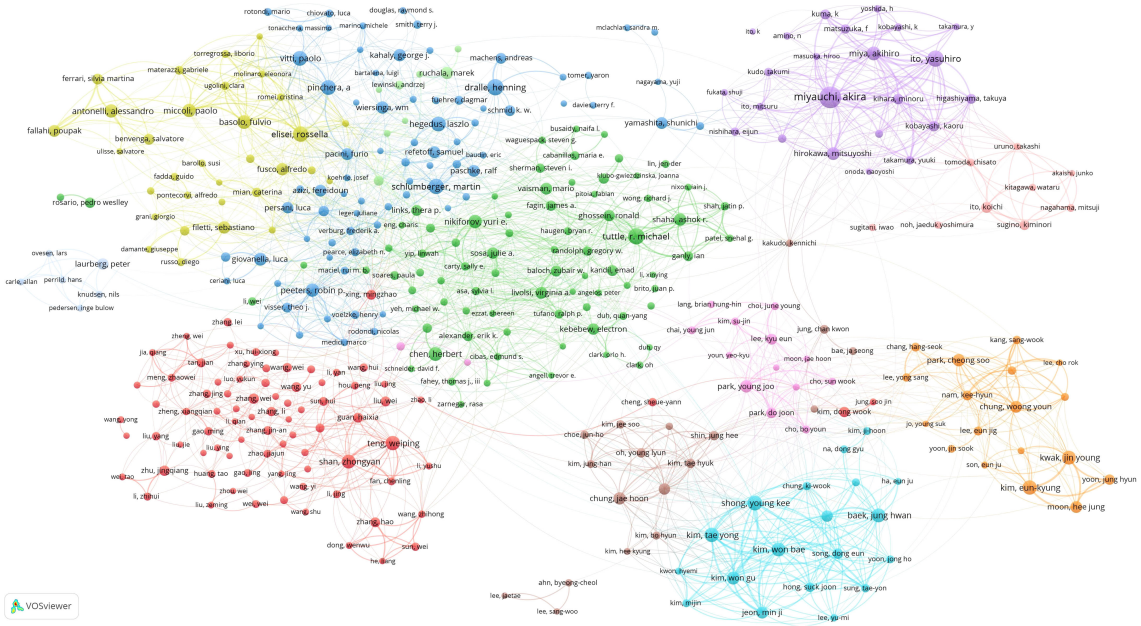


FIGURE 6
Co-authorship network.

3.7 Keywords analysis

There are 92426 keywords in 69283 articles. The threshold of high-frequency keywords was set to 20, of which 307 keywords were selected. A Co-occurrence analysis was conducted on 307 high-frequency keywords and a co-occurrence network map was constructed. At the same time, we also built heat maps for a clearer and more intuitive presentation of the results (Figure 8). The co-occurrence analysis of 307 high-frequency keywords formed 6 clusters, represented by different colors (Figure 9). The red cluster consists of 102 keywords and is the largest cluster. Cluster1(red), Cluster2(green), Cluster3(blue), Cluster4(yellow), Cluster5(purple),

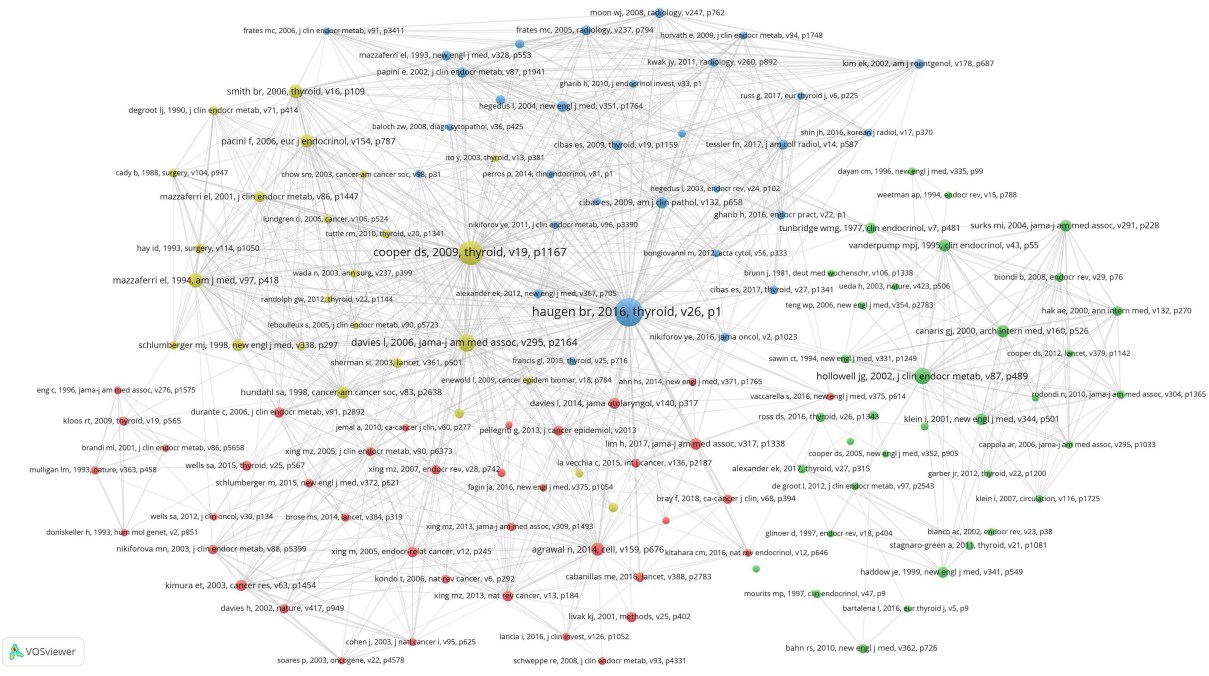


FIGURE 7
Citation analysis of references.

and Cluster6(light blue)focus on the subject respectively: 1) Thyroid dysfunction and diseases; 2) mechanism of occurrence and development of thyroid cancer; 3) autoimmune thyroiditis; 4) scope and postoperative management of thyroid surgery; 5) fine needle aspiration of thyroid nodules; and 6) radioactive iodine therapy for thyroid cancer.

An Overlay map of 307 high-frequency keywords was constructed (Figure 10). The color of each node in the graph indicates the average year of publication for that node and the year of publication changes from blue to red.

4 Discussions

4.1 Overall trend

The data shows that from 2000 to 2021, the overall annual number of publications in thyroid disease research has increased each year for the past 21 years, although the number of publications fluctuated slightly in some years. The United States ranks first in both publication volume and citation frequency, and is the leader in this field. Interestingly, in recent years, there has been a rapid increase in research on thyroid disorders in China and Korea, which may be related to the increased incidence and increased interest of researchers (9, 10). In the top 20 institutions concerning publication volume, Harvard University is the most published and most cited institution, collaborating with 222 high-yield institutions and serving as a global hub for research institutional collaboration. Of the 397 high-yield authors, 97% appeared in the collaborative network, suggesting a high level of collaboration among high-yield authors. Among them, Tuttle, R. Michael, Elisei, Rossella, and Fugazzola, Laura, et al. have particularly close collaborations, forming a close cooperative relationship.

4.2 Research hotspots

4.2.1 Cluster1(red): thyroid dysfunction and diseases

Hyperthyroidism and hypothyroidism are the most common disorders of thyroid dysfunction. Several factors have been found to contribute to thyroid dysfunction, including the environment, genetic susceptibility, micronutrients (iodine, selenium), infections, and immune system defects (11, 12). In addition, a combination of TSHR and TSHR autoantibodies, as well as amiodarone, a drug structurally similar to thyroid hormones for the treatment of arrhythmia, may also affect thyroid function (13, 14). In the past, thyroid dysfunction was thought to increase the risk of certain diseases, such as cardiovascular disease (15), Psychiatric Disorders (16), renal dysfunction (17), and changes in bone mineral density (18). However, new studies reveal that some of these diseases may not be associated with thyroid dysfunction (19, 20). Interestingly, researchers have found that early thyroid dysfunction was closely related to adrenal gene expression and hormone secretion (21). As for treatment, the cancer risk after radioactive iodine treatment for hyperthyroidism is low (22). Total thyroidectomy is a reliable method for the treatment of hyperthyroidism, without significant differences in the incidence of postoperative complications compared to patients with normal thyroid function (23). Nevertheless, children with hyperthyroidism

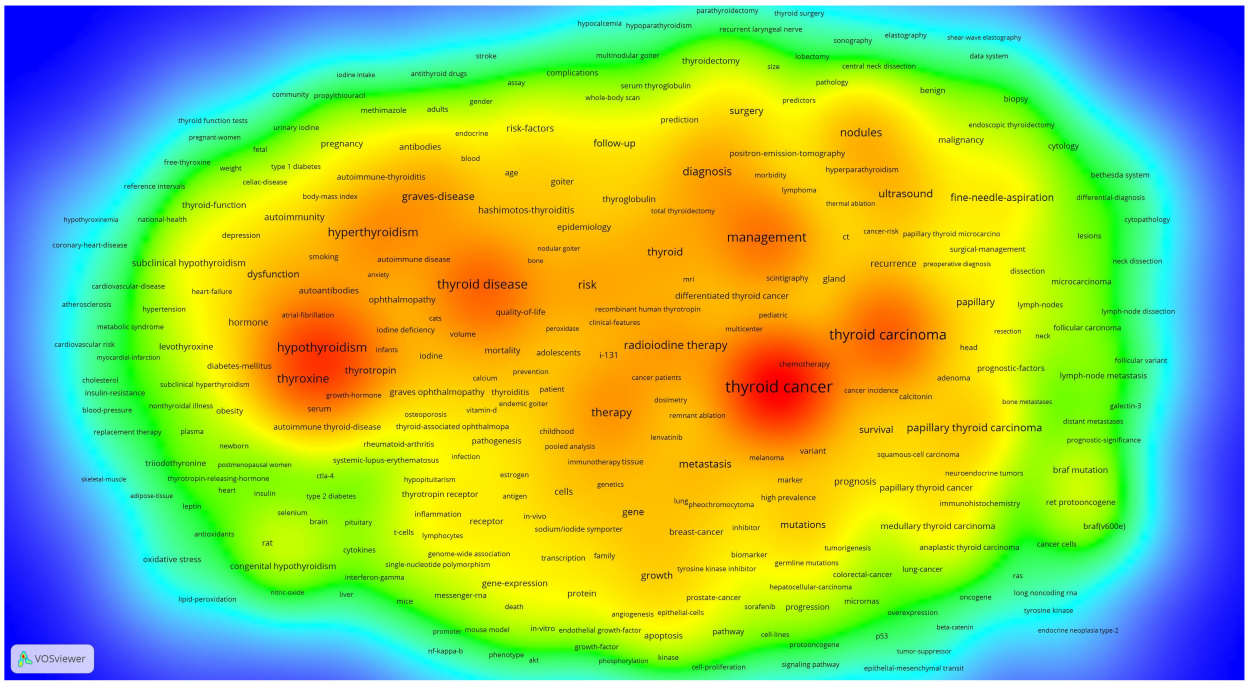


FIGURE 8
Co-occurrence heat map.

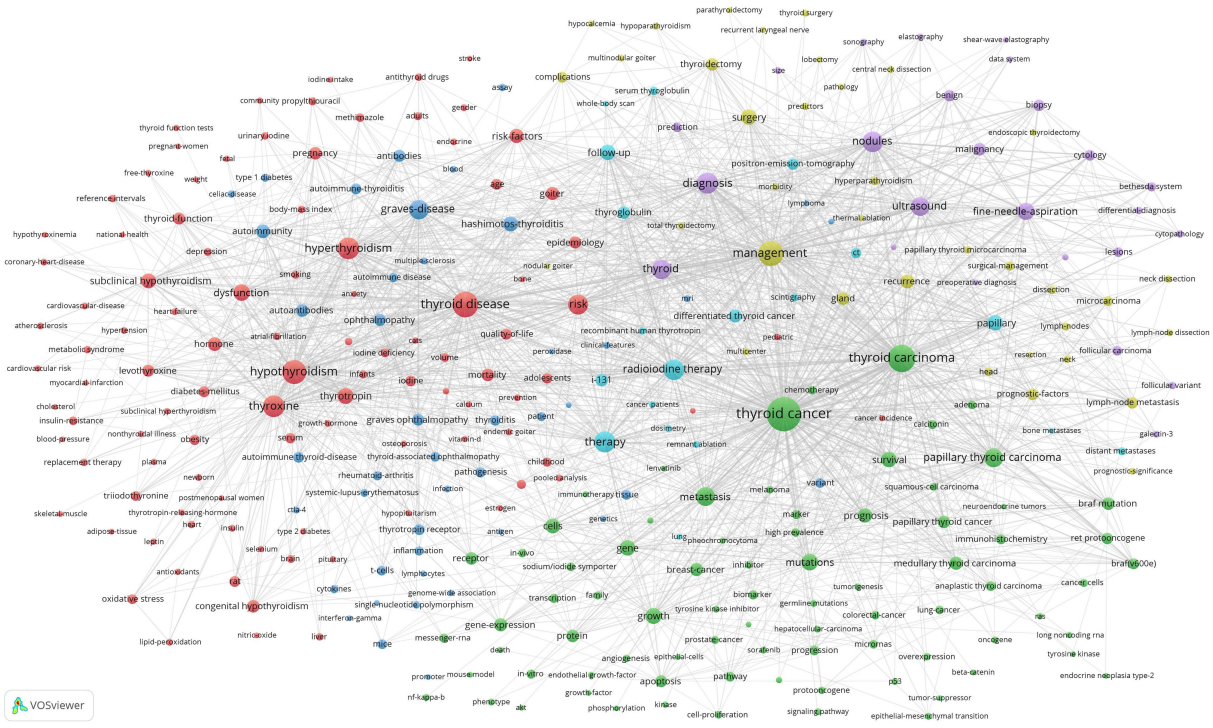


FIGURE 9
Co-occurrence network map.

are more likely to suffer from hoarseness and difficulty concentrating after surgery than adults (24). Using levothyroxine in the treatment of subclinical hypothyroidism remains controversial (25). The risks brought by long-term treatment cannot be ignored (26), and it is necessary to prevent excessive treatment from causing harm to patients as much as possible (27). Therefore, careful implementation of individualized treatment is the most beneficial option for patients, and specific treatment protocols still need to be validated by researchers through large-scale randomized trials.

4.2.2 Cluster2(green): mechanism of occurrence and development of thyroid cancer

While the mortality rate has remained relatively stable, the incidence rate of thyroid cancer has increased in recent years, which is probably due to the increase in examinations and the emergence of new technologies (28, 29). The mechanism of occurrence and development of thyroid cancer has been a focus of researches. Multiple molecular biomarkers exist in TC, among which RAF-MEK-ERK signaling levels in the MAPK pathway are involved in cancer cell proliferation, differentiation, and development. Among the three RAF subtypes, BRAF is the most common mutation type (30). When the MAPK pathway is activated in TC, cancer cells exhibit stronger invasiveness (31). As one of the most common activation pathways, the PI3K/AKT pathway, including various signaling complex proteins (32), plays an important role in the diagnosis, prognosis, and treatment of TC (33). Targeting PI3K/AKT pathway has become one of the most effective cancer treatments (34). PD-1 is a transmembrane protein expressed in immune cells (35), and its expression in tumors can lead to tumor recurrence and metastasis (36, 37). PD-L1 expression in TC is associated with lower survival rates (38), and immunotherapy targeted PD-1/PD-L1 is increasingly being elucidated (39). Epithelial-mesenchymal transition (EMT) is an important link in cancer progression, which can lead to the invasion and migration of cancer cells (40). In thyroid cancer, genetic changes in EMT-related genes and transcription factors can lead to TC proliferation, migration, and invasion (41). Non-coding RNA (ncRNA) also has an impact on the development of TC. Multiple Long non-coding RNA (lncRNA)s, miRNAs, and circRNAs have been found to play key roles in the occurrence, development, and differentiation of TC (42). The studies on these mechanisms assist in understanding the conditions of drug resistance and discovering new strategies for the treatment of TC.

4.2.3 Cluster3(blue):autoimmune thyroiditis

Autoimmune thyroid disease (AITD) mainly includes Hashimoto's thyroiditis and Graves' disease, both of which are caused by lymphocyte infiltration and autoantibody production, leading to immune system dysfunction and then attacks the thyroid gland (43). It is well known that T lymphocytes are the main infiltrating cells in AITD, causing autoimmunity through the production of inflammatory factors. Regulatory B cells have also been found to regulate AITD through inflammatory factors (44). The role of cytokines and chemokines in AITD is gradually being elucidated. Cytokines released from lymphocytes induce the activity of immune cells and contribute to the regulation of the immune response by the body. Interestingly, chemokines in AITD (e.g.,



FIGURE 10
Co-occurrence overlay map.

CXCL10), may be involved in the occurrence of tumor-related inflammation (45). TgAb and TPOAb are sensitive biomarkers of AITD, and their levels are influenced by various factors and reflect thyroid function (46). Recently, the role of microbiota in AITD has aroused researchers' interest. It was found that changes in microbiota and diversity of composition are widespread in AITD and may lead to changes in TPOAb levels (47, 48). In addition, the body also affects the development of AITD by releasing exosomes. AITD patients overexpress certain inflammatory factors through exosomes, leading to an increase in serum TPOAb and TgAb levels, thereby affecting the occurrence and development of AITD (49, 50). Myoinositol (Myo) has also been found to have beneficial effects on AITD (51). The emergence of AITD is accompanied by changes in various pathogenic factors. The mechanisms of immune system disorders leading to thyroid immune attack are still under investigation, and researchers need to carefully test more new therapeutic targets.

4.2.4 Cluster4(yellow): scope and postoperative management of thyroid surgery

The scope of thyroid surgery needs to be considered by several factors, including but not limited to tumor invasiveness, risk of recurrence, and changes in quality of life. Despite still being controversial, prophylactic central lymph node dissection is still popular among young, low-risk patients (52). The most important complications related to thyroid surgery are recurrent laryngeal nerve injury and postoperative hypoparathyroidism, both of which can affect a patient's life quality. These complications cause symptoms that may be temporary or permanent. Hence, doctors should carefully assess the potential risks before surgery. The most common modality of laryngeal return nerve injury is traction (53). Using intraoperative nerve monitoring can reduce recurrent laryngeal nerve injury (54), and continuous intraoperative nerve monitoring is most effective in preventing vocal cord paralysis (55). Permanent hypoparathyroidism is rare, with magnesium deficiency (56), gene mutation (57), and dyslipidemia (58) as potential contributing factors. The most common cause in clinical practice is surgery, and the probability of postoperative occurrence is related to the scope of surgery and professional knowledge (59, 60). Although there are different surgical options for thyroid surgery (61), an increasing number of patients express dissatisfaction with the outcome (62). Therefore, more factors should be taken into consideration when considering treatment options for patients, and new treatment methods should be actively developed.

4.2.5 Cluster5(purple): fine needle aspiration of thyroid nodules

Thyroid nodules have a high incidence in the population, and even with a slight increase in the incidence of thyroid cancer, the likelihood of being a malignant nodule remains low (63). At the same time, nodules have been found to have some characteristics of malignant tendency. For example, the risk of malignancy is higher in middle-lobe-thyroid nodules than in other sites (64). Besides, the malignant risk of spherical nodules is also relatively high (65), so the management and treatment of this kind of patients should not be underestimated. Fine needle aspiration biopsy is the most reliable

way to judge the type of thyroid nodules. This method is simple and reliable, which can be employed to improve the diagnostic accuracy of uncertain nodules through ultrasound guidance (66). Although there may be few complications, it is generally safe and effective (67). However, there is still uncertainty regarding the results of fine needle aspiration in thyroid follicular cell carcinoma, follicular thyroid tumor, and follicular variation in papillary thyroid cancer (68). Fortunately, Raman Spectroscopy and molecular diagnosis can be adopted to improve the diagnostic probability of these nodules and avoid unnecessary surgery (66, 69). In addition, the results of continuous ultrasound monitoring can also be used as a criterion to determine the timing of surgery (70). Radiofrequency ablation can be used as a means to treat uncertain thyroid nodules (71). In short, most thyroid nodules are benign, and fine needle aspiration, as the most commonly used diagnostic tool, provides the most definitive diagnostic information.

4.2.6 Cluster6(light blue): radioactive iodine therapy for thyroid cancer

DTC is the most common endocrine tumor and its incidence is increasing year by year, but the mortality is expected to remain unchanged (28). The prognosis of DTC is excellent, depending on the revision of the diagnosis and treatment strategy (72). Radioactive iodine-131 therapy is a common adjuvant treatment for patients with DTC after total thyroidectomy, mainly for the ablation of residual thyroid tissue to reduce disease recurrence and leading to specific mortality (73). Nevertheless, there are also some controversies on RAI, such as the selection of RAI dosage for low-risk thyroid cancer patients (74). It was found that the risk of developing a second primary malignancy increases when the cumulative RAI dose is greater than 150 mCi (75). For patients with DTC with tumor diameters greater than 1 cm, postoperative iodine-131 therapy remains a reasonable option (76). Furthermore, delayed treatment in patients with low-risk and lower-Intermediate risk DTC does not seem to change the final outcome (77). Therefore, additional clinical factors should be considered when using RAI in low-risk and lower-Intermediate risk patients. Unfortunately, RAI is ineffective in the treatment of some radioactive iodine-refractory DTC, such as recurrent or metastatic TC (78). Recent studies have verified that Cabozantinib and a novel molecular inhibitor [177Lu]Lu-DOTAGA.(SA.FAPi)2 may produce a safe and effective treatment for RR-DTC (79, 80). In brief, RAI therapy remains an old but mainstream approach for the treatment of patients with DTC., and higher quality studies are needed to clarify the unsolved problems.

4.3 Keywords with relatively new average publication years and emerging subjects

In recent years, there has been an increasing interest in Active Surveillance (AS), mainly due to the excellent postoperative performance of patients with papillary thyroid microcarcinoma (PTMC). AS was initially a prospective study that first suggested that patients with PTMC had a relatively low probability of clinical progression. For patients without high-risk features, regular observation is an option, and it is not too late to undergo surgery when the tumor evolve (81). Subsequently, researchers from multiple developed countries conducted experiments, indicating that this method was only effective for low-risk PTMC and that surgery remains the preferred treatment option for high-risk patients (82–84). In addition, recent studies have demonstrated that AS has a lower incidence of adverse reactions and recurrence rate compared to conversion and immediate surgery, and AS can be the first-line treatment of choice for PTMC (85). As a management strategy, it has been endorsed by several organizations, including the *Japan Association of Endocrine Surgery and the American Thyroid Association* (86–89). Nevertheless, there are also many problems in the management of AS, such as long follow-up times, the limited number of surgeries, and difficulties in specimen collection. Follow-up strategies and timing of interventions for AS require more evidence to confirm its long-term safety. In general, AS is an effective alternative strategy to surgery for low-risk PTC. Currently, concerns about the effectiveness and safety of AS are diminishing, and various versions of the guidelines recommend the implementation of AS.

Thermal Ablation (TA) is a treatment option for PTMC patients in addition to AS and immediate surgery and can significantly reduce the nodule volume, but takes a long time to achieve nodule volume reduction (90, 91). Surgery, as the standard treatment for PTC patients, is the only method to remove the primary lesion and obtain accurate staging and risk stratification. Several studies comparing the effectiveness and safety of TA and immediate surgery have shown no tumor recurrence or distant metastases with both treatments. However, the probability of complications in TA is significantly lower than that in the surgical group, and TA can reduce the probability of hypothyroidism in patients. Besides, patients will have significantly reduced surgical time, recovery time, and hospitalization time (92). The direct comparison between TA and AS currently lacks feasibility. It has been established that TA can address patient anxiety during follow-up and thus avoid surgical delays. Moreover, after five years of follow-up, patients did not develop tumor recurrence, lymph node metastasis, or distant metastases (93, 94). However, TA is not indicated for all types of thyroid nodules, and occasionally incomplete nodal response and local regeneration may occur. At this point, repeat ablation or surgical treatment may be required, which may make the procedure more difficult (95, 96). Hence, prospective studies with larger samples and longer follow-up times are needed to demonstrate its efficacy. Nevertheless, TA can serve as an alternative for low-risk PTMC patients (97).

Anaplastic thyroid carcinoma and metastatic poorly differentiated thyroid carcinoma are highly invasive malignant tumors. Furthermore, despite the availability of several treatments for iodine-refractory thyroid cancers, the overall survival rate remains unsatisfactory, especially in patients with ATC, with a

median survival of only 3-5 months (98). The treatment of such tumors with poor prognosis has always been a focus of researchers' attention. Lenvatinib is an oral, multitargeted tyrosine kinase inhibitor of VEGFR1, VEGFR2, and VEGFR3; FGFR1, FGFR2, FGFR3, and FGFR4 (99), and its role in RR-DTC has been proved in many countries (100). However, the therapeutic efficacy of ATC remains controversial (101, 102), and it leads to a decrease in quality of life (103). Recently, the combination of lenvatinib and pembrolizumab has achieved encouraging results in the efficacy of ATC/PDTC patients (104), and a larger sample is still needed to monitor the therapeutic effect of the combination of both.

Investigating the mechanisms of thyroid cancer occurrence and development may facilitate new diagnostic and therapeutic biomarkers. LncRNA has been identified as a biomarker for several cancers (105). It is highly expressed in PTC and promotes the growth and metastasis of tumor cells (106). Several lncRNAs, such as MALAT1, BANCR, and PTCSC3, have been identified to promote the proliferation and metastasis of thyroid cancers (107–109). In addition, lncRNA has also been associated with lymph node metastasis (110), and further studies are needed to elucidate the remaining unknown functions of lncRNA. This biomarker provides new evidence for individualized therapy and molecular diagnosis.

5 Conclusions

The field of thyroid diseases developed rapidly in the early 21st century. Over the past 20 years, the number of publications has been increasing year by year. The United States is the leader in this field, and China and South Korea are developing rapidly. In addition, Harvard University is the center of global research institutions, and there is close cooperation between institutions and authors around the world, especially among high-yield authors. More importantly, six major research themes were discovered by analyzing keywords. As well as active surveillance, thermal ablation and the development mechanism and treatment of thyroid cancer are current research hotspots.

6 Strengths

To our knowledge, this study is the first bibliometric analysis of the entire field of thyroid disorders based on a large body of literature on thyroid disorders. The analysis of early 21st-century publications enabled the visualization of thyroid disorders, including publication trends, global collaborations, and research hotspots. These bibliometrics can identify new subjects and frontiers for future research in the field of thyroid diseases.

7 Limitations

As with other bibliometric studies, there are certain limitations in this study.

Although there is a lot of literature available in the Web of Science database, there is still some literature that is not included in the Web of Science database, which may lead to biased results.

The statistical results may be biased due to irregularities in the writing of author and institution names.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding authors.

Author contributions

MS: Conceptualization, Data curation, Investigation, Writing – original draft. WS: Data curation, Writing – original draft. QL: Data curation, Writing – original draft. ZW: Conceptualization, Investigation, Supervision, Writing – review & editing. HZ: Conceptualization, Investigation, Supervision, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2023.1306232/full#supplementary-material

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Higher adjuvant radioactive iodine therapy dosage helps intermediate-risk papillary thyroid carcinoma patients achieve better therapeutic effect

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Objective: This retrospective study aims to evaluate the therapeutic effect of varying dosages of adjuvant radioactive iodine (RAI) therapy on intermediate-risk papillary thyroid carcinoma (PTC) patients.

Methods: This retrospective study involved a total of 427 intermediate-risk PTC patients, out of which 202 received a 3.7GBq dosage of RAI, and 225 received a 5.55GBq dosage. The evaluation involved assessing the therapeutic outcomes, number of treatment cycles, and successful remnant ablation rates in both dose groups, six months post-adjuvant RAI therapy. Univariate and multivariate logistic regression analyses were employed to identify factors linked with excellent response (ER). Following this, prognostic nomograms were constructed to provide a visual representation of the prediction models. Calibration curves, the concordance index (C-index), and the receiver operating characteristic (ROC) curve were employed to evaluate the predictive performance of these nomograms. The Hosmer-Lemeshow test was applied to assess the models' goodness-of-fit. Additionally, the clinical utility of the prognostic nomograms was appraised through decision curve analysis (DCA)

Results: The high-dose (HD) group exhibited significantly higher proportions of ER, single treatment cycles, and successful remnant ablation rates ($p<0.05$). Being male, receiving a 3.7GBq dose, having an N1b stage, an sTg level $\geq$10ng/ml, or an sTg/TSH ratio $\geq$0.11 were independent risk factors for Non-ER. Two prognostic nomograms, "sTg Nomogram" and "sTg/TSH Nomogram", were established. The ranking of factors contributing to ER, in descending order, included the sTg or sTg/TSH ratio, N stage, therapy dosage, sex, and soft tissue invasion. The "sTg/TSH Nomogram" demonstrated a higher C-index compared to the "sTg Nomogram". The calibration curves indicated excellent calibration for both nomograms. DCA demonstrated that the net benefit of the "sTg/TSH Nomogram" was higher than that of the "sTg Nomogram".

Conclusion: Higher initial RAI therapy doses can improve therapeutic efficacy for intermediate-risk PTC patients. The developed nomograms, particularly the "sTg/TSH Nomogram", could assist clinicians in optimal therapeutic decision-making.

KEYWORDS

intermediate-risk papillary thyroid carcinoma, adjuvant radioactive iodine therapy, dosages, therapy response, decision curve analysis (DCA)

Introduction

Differentiated thyroid cancer (DTC), the most common endocrine malignancy, constitutes 90% of all thyroid cancers. The primary treatment modalities for DTC include surgery, postoperative radioactive iodine (RAI) therapy, and thyrotropin (TSH) suppression. The ATA classifies DTC patients into low, intermediate, and high-risk groups based on recurrence and response to therapy. Depending on the postoperative risk stratification of the individual patients, the primary goal of RAI after total thyroidectomy includes RAI remnant ablation, RAI adjuvant therapy and RAI therapy (1). RAI therapy is routinely recommended for patients with high-risk DTC. Moreover, adjuvant RAI therapy is selectively employed for intermediate-risk patients to control persistent disease and prevent recurrence. Although these patients typically have good long-term prognoses due to the lack of distant metastases, appropriate surgical resection, and adjuvant RAI therapy, the frequency of persistent disease remains high (2). Adjuvant RAI therapy can benefit intermediate-risk DTC patients with a relatively high risk of recurrence by destroying suspected but unconfirmed residual disease (3, 4). Some studies have shown that RAI can improve overall survival or disease-specific death of intermediate-risk DTC patients (5, 6). However, consensus on the appropriate RAI dosage for adjuvant therapy in intermediate or high-risk patients is yet to be reached, particularly for those in the intermediate-risk category. Factors such as soft tissue invasion, extensive cervical lymph node metastasis, and relatively high serum thyroglobulin (Tg) levels might contribute to a higher risk of recurrence in intermediate-risk DTC patients. It remains unclear whether patients receiving a lower dosage can achieve a satisfactory response after initial RAI therapy and whether they might need additional RAI therapy due to an indeterminate response or biochemical incomplete response.

This study aims to evaluate the therapeutic impact of varying adjuvant RAI therapy dosages (3.7GBq and 5.5GBq) in intermediate-risk DTC patients, and to identify influencing factors for an excellent response (ER) to initial therapy. Prior to administering RAI, the post-operative Tg level plays a crucial role in both monitoring the disease and guiding management decisions (7–10) Notably, Tg levels can be influenced by thyroid-stimulating hormone (TSH) levels. Our previous research has indicated that the sTg/TSH ratio may serve as an additional predictor of the therapeutic effects of RAI (11). Consequently, we aim to develop two prognostic nomograms: the "sTg Nomogram" and the "sTg/TSH Nomogram, " to predict the probability of ER to treatment. The discrimination, accuracy, calibration, and clinical utility of these two nomograms will be assessed using the concordance index (C-index), receiver operating characteristic (ROC) curve, calibration curve, and decision curve analysis (DCA).

Materials and methods

Patients

We conducted a retrospective review of the clinical records of patients who underwent total thyroidectomy and postoperative RAI therapy for papillary thyroid carcinoma (PTC) at the Department of Nuclear Medicine, Tianjin Medical University General Hospital, Tianjin, China, from March 2018 to July 2021. According to the ATA guidelines (version 2015), our study included patients with intermediate-risk PTC confirmed by surgical histopathology who received an RAI dose of either 3.7GBq or 5.55GBq. Patients with a post-operative Tg level highly suggestive of distant metastases or positive anti-thyroglobulin antibodies (TgAb) were excluded. Patients were divided into two groups: a Low-Dose (LD) group where 202 patients received 3.7GBq RAI therapy, and a High-Dose (HD) group where 225 patients received 5.55GBq RAI therapy.

Postoperative radioiodine therapy and follow-up

Patients underwent RAI therapy after discontinuing levothyroxine (L-T4) for 2-4 weeks until TSH levels exceeded 30mIU/L, and adhered to a low-iodine diet for a minimum of 2 weeks. Prior to ^{131}I administration, serum stimulated thyroglobulin (sTg), TgAb, and TSH levels were measured. Neck ultrasonography (US) and chest CT were performed simultaneously. Post-ablation whole body scans (WBS) were carried out 3 to 5 days after RAI therapy. Patients resumed L-T4 immediately after RAI therapy for TSH suppression. Thyroid hormones, TSH, Tg, TgAb were typically

examined at 1-, 3-, and 6-months post RAI therapy, and neck US performed every 3 months. During this period, modulation of L-T4 dosage was undertaken as needed. A diagnostic ^{131}I scan was performed 6 months post RAI therapy. Additional RAI therapy was initiated if there were ^{131}I-avid recurrence or metastatic lesions, or rising sTg levels. In cases where clinical suspicion of recurrence and metastatic lesions existed, additional imaging studies, such as neck/chest CT or F-18-fluorodeoxyglucose positron emission tomography, were conducted.

Study design and determination of clinical outcome

We reviewed all patients' clinical records to collect data on sex, age at diagnosis, T stage, N stage, American Joint Cancer Control (AJCC) stage (8th edition), soft tissue invasion, pre-therapy TSH and sTg levels, and sTg/TSH ratio. Owing to the absence of comprehensive clinicopathological data in certain patients, some clinicopathological characteristics, including extrathyroidal extension, tumor focality, tumor laterality, and lymphovascular invasion, were not incorporated in our analysis. The therapy response to RAI was evaluated at 6 months post-RAI therapy. As per the ATA guideline (version 2015), the responses to therapy included ER (negative imaging and either sTg<1ng/mL or suppressed Tg <0.2ng/mL), Biochemical Incomplete Response (BIR) (negative imaging and suppressed Tg ≥1ng/mL or sTg ≥10ng/mL or rising TgAb levels), Structural Incomplete Response (SIR) (structural or functional evidence of disease with any Tg level with or without TgAb), and Indeterminate Response (IDR) (nonspecific findings on imaging studies, faint uptake in thyroid bed on RAI scanning, non-detectable sTg <1ng/mL, detectable sTg <10ng/mL or stable or declining TgAb in the absence of structural or functional disease) (1). BIR and SIR were collectively referred to as Incomplete Response (IR).

We compared the therapeutic effects and successful remnant ablation (where diagnostic WBS found no residual thyroid bed uptake regardless of sTg levels) rates of different dose groups in all patients at 6 months post-adjuvant RAI therapy. If ^{131}I-avid recurrence or metastatic lesions were observed, or rising sTg levels were noted, patients underwent subsequent RAI therapy. Thus, we also compared the treatment cycles across different dose groups.

We then conducted univariate and multivariate logistic regression analyses to explore the influence of various factors such as therapy dose, sex, age at diagnosis, T stage, N stage, AJCC stage, soft tissue invasion, pre-therapy TSH, sTg level, and sTg/TSH ratio on the ER probability. Given the collinearity between sTg and sTg/TSH ratio, we conducted multivariate logistic regression analysis, including sTg or sTg/TSH ratio with other variables, respectively.

Finally, we developed the prognostic nomograms "sTg nomogram" and "sTg/TSH nomogram" to predict the ER probability. The calibration curve, ROC curve and decision curve analysis (DCA) were utilized to evaluate the performance of the two prediction models.

Statistical analysis

Continuous variables, with an abnormal distribution, are represented as the median along with interquartile ranges. Categorical variables are displayed as numbers with their corresponding percentages. Differences between continuous variables in the dose groups were evaluated using the Mann-Whitney test, while a Chi-squared test was utilized to compare categorical variables. Univariate and multivariate logistic regression were applied to assess predictors of ER. Initially, univariate analysis was performed, and any variable with a p-value <0.1 in this analysis was included in the multivariate regression. A p-value <0.05 was considered statistically significant.

The discrimination of these models was evaluated using the Concordance index (C-index). Bootstrap resampling with 1000 iterations was employed for the internal validation of the predictive capabilities of these two nomograms. The calibration curve was utilized to assess the accuracy of these nomograms in estimating outcomes and to check for overfitting. A curve closely aligning with the 45-degree line indicates good calibration of the prediction model. Additionally, the Hosmer-Lemeshow test was conducted to assess the goodness-of-fit of the models. This test compares the expected event numbers from the prediction model with the observed event numbers. A p-value > 0.05 suggests a well-fitted model. The ROC curve was drawn to determine the accuracy of the prediction models. The area under the ROC curve (AUC) corresponds to the C-index in binary logistic regression analysis. DCA was used to evaluate the clinical utility of the predictive nomograms. In DCA, the x-axis represents the threshold probability for an ER, and the y-axis denotes the net benefit. The "sTg Nomogram" and "sTg/TSH Nomogram" were compared against scenarios where all or none of the patients have an ER. The nomogram demonstrating a higher net benefit is considered to have greater clinical utility.

R software (version 4.0.5, http://www.r-project.org/) was employed to perform the nomogram, Hosmer-Lemeshow test, calculate C-index, draw calibration curve, ROC curve and DCA. All other statistical analyses were conducted using SPSS (version 24.0; SPSS Inc., Chicago, Illinois, USA). The study received approval from the Institutional Review Board of the Tianjin Medical University General Hospital.

Results

No significant differences in clinical and pathologic characteristics were observed between the two groups. (Refer to Table 1).

Comparison of responses to initial RAI, treatment cycles, and successful remnant ablation rates between dose groups

As illustrated in Table 2, we identified a significant difference in the proportions of ER and Non-ER between the two groups. The HD group exhibited a significantly higher ER rate than the LD

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TABLE 1 Clinical and pathologic characteristics at baseline.

Characteristics	LD (n=202)	HD (n=225)	P
Age at diagnosis	42(34-49.25)	42(34-50.50)	0.799
Sex Male Female	 69(34.2%) 133(65.8%)	 86(38.2%) 139(61.8%)	0.383
T stage 1 2 3	 168(83.2%) 20(9.9%) 14(6.9%)	 167(74.2%) 29(12.9%) 29(12.9%)	0.059
N stage N0 1a 1b	 7(3.5%) 108(53.5%) 87(43.1%)	 3(1.3%) 105(46.7%) 117(52.0%)	0.089
AJCC stage I II	 169(83.7%) 33(16.3%)	 187(83.1%) 38(16.9%)	0.878
Soft tissue invasion No Yes	 151(74.8%) 51(29.1%)	 155(68.9%) 70(31.1%)	0.179
Pre-therapy TSH (*u*IU/mL)	76.665 (54.817-100.000)	70.332 (51.948-96.285)	0.080
sTg(ng/mL)	2.46(0.94-6.79)	2.41(0.70-6.21)	0.582
sTg/TSH	0.038(0.014-0.095)	0.036(0.011-0.100)	0.894

Dates are expressed as the median (percentiles 25-75) or frequencies.
LD, low-dose, 3.7GBq; HD, high-dose, 5.55GBq.

group (57.3% *vs* 44.1%, p<0.05). Although the proportions of BIR, SIR, and IDR in the HD group were lower than those in the LD group, these differences were not statistically significant (6.2% *vs* 10.4%, 5.3% *vs* 5.9%, 31.1% *vs* 39.6%, p>0.05, respectively). The HD group had significantly higher proportions of single treatment cycle (84.0% *vs* 75.7%, p<0.05) and a higher successful remnant ablation rate (92.4% *vs* 83.7%, p<0.05).

Univariate and multivariate logistic regression analysis for potential factors of ER to RAI

Table 3 shows that univariate logistic regression analysis found no significant association between therapeutic effects and age at diagnosis, T stage, AJCC stage, and pre-therapy TSH levels. However, sex, therapy dose, N stage, soft tissue invasion, sTg level, and sTg/TSH ratio could potentially affect the therapeutic effects (P<0.1). These factors were further evaluated using multivariate logistic regression analysis to assess their predictive value. We addressed the collinearity between sTg and the sTg/TSH ratio by conducting separate multivariate logistic regression analyses for each. The results were nearly identical, leading us to present only the sTg prediction model data in Table 3. However, the OR value and 95%CI of the sTg/TSH ratio are also provided. Results revealed that being male, receiving a 3.7GBq dose, having an N1b stage, an sTg level ≥10ng/ml, and an sTg/TSH ratio ≥0.11 were independent risk factors for Non-ER.

TABLE 2 Responses to initial RAI, treatment cycles and successful remnant ablation rates.

Characteristics	LD (n=202) N (%)	HD (n=225) N (%)	P
Response			
ER	89(44.1)	129(57.3)	0.042*
BIR	21(10.4)	14(6.2)	
SIR	12(5.9)	12(5.3)	
IDR	80(39.6)	70(31.1)	
ER Non-ER	89(44.1) 113(55.9)	129(57.3) 96(42.7)	0.006*
BIR Non-BIR	21(10.4) 181(89.6)	14(6.2) 211(93.8)	0.116
SIR Non-SIR	12(5.9) 190(94.1)	12(5.3) 213(94.7)	0.786
IDR Non-IDR	80(39.6) 122(60.4)	70(31.1) 155(68.9)	0.066
Treatment cycles Single Multiple	 153(75.7) 49(24.3)	 189(84.0) 36(16.0)	0.033*
successful remnant ablation Yes No	 169(83.7) 33(16.3)	 208(92.4) 17(7.6)	0.005*

LD, low-dose, 3.7GBq; HD, high-dose, 5.55GBq.
ER, excellent response; BIR, biochemical incomplete response; SIR, structural incomplete response; IDR, indeterminate response; IR, incomplete response.
*$p<0.05$.

Prognostic nomogram for ER probability

Using the results from logistic regression analysis, we developed two prognostic nomograms, "sTg nomogram" and "sTg/TSH nomogram", to predict ER probability (Figures 1, 2). A score was assigned to each variable by drawing a perpendicular line from the corresponding axis to the top line labeled "POINTS". The scores of all variables were then added, and a line was drawn from the axis labeled "Total Points" to estimate the ER probability. From the nomogram, we found that sTg or sTg/TSH ratio, N stage, and therapy dose were the top three contributors to ER, followed by sex and soft tissue invasion. To make the nomogram more illustrative, we developed an individual "sTg/TSH nomogram" for a female patient with N1a, no soft tissue invasion, sTg/TSH<0.11, who received 5.55GBq RAI therapy. The total points, derived from adding all variable scores, was 378, corresponding to an ER probability of 80.8% (Figure 3).

Internal validation and clinical usefulness of "sTg nomogram" and "sTg/TSH nomogram"

The C-index of "sTg/TSH nomogram" was 0.768 (95% CI: 0.724 to 0.813), which was better than that of the "sTg nomogram" 0.735

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TABLE 3 Univariate and multivariate logistic regression analysis based on all variables for ER.

Variables	Univariate analysis		Multivariate analysis	
	OR (95%CI)	P value	OR (95%CI)	P value
Age at diagnosis				
<45	Reference			
45≤Age<55	1.004(0.633-1.594)	0.986		
≥55	1.454(0.863-2.450)	0.160		
Sex				
Male	Reference		Reference	
Female	1.639(1.101-2.440)	0.015	1.653(1.050-2.603)	0.030*
Therapy dose				
3.7GBq	Reference		Reference	
5.55GBq	1.706(1.163-2.503)	0.006	2.464(1.571-3.866)	<0.001*
T stage				
1	Reference			
2	0.781(0.428-1.427)	0.422		
3	1.332(0.701-2.533)	0.382		
N stage				
N0	Reference		Reference	
1a	0.311(0.064-1.497)	0.145	0.268(0.043-1.653)	0.156
1b	0.205(0.043-0.991)	0.049	0.159(0.026-0.986)	0.048*
AJCC stage				
I	Reference			
II	1.481(0.883-2.483)	0.136		
Soft tissue invasion				
No	Reference		Reference	
Yes	0.698(0.457-1.065)	0.096	0.732(0.442-1.212)	0.225
Pre-therapy TSH(*u*IU/mL)				
30≤TSH<60	Reference			
60≤TSH<90	0.716(0.446-1.149)	0.166		
TSH≥90	1.105(0.696-1.754)	0.672		
Stimulated Tg(ng/mL)				
<10	Reference		Reference	
≥10	0.057(0.022-0.145)	<0.001	0.054(0.021-0.141)	<0.001*
sTg/TSH				
<0.11	Reference		Reference	
≥0.11	0.074(0.037-0.149)	<0.001	0.066 (0.032-0.136)	<0.001*

*p<0.05.

(95% CI: 0.688 to 0.783). Hosmer-Lemeshow test shown that two prediction models were well fitted. Chi-squared of "sTg nomogram" and "sTg/TSH nomogram" were 3.130 and 1.740, p=0.926 and 0.988 respectively. The calibration curve was shown in Figure 4A, both the two nomograms had good calibration for predicting the ER probability. ROC curve was shown in Figure 4B, the AUC of the two nomograms were same with C-index, the AUC of "sTg/TSH nomogram" is slightly higher than that of "sTg nomogram.

DCA for the "sTg nomogram" and "sTg/TSH nomogram" is presented in Figure 5. Both the "sTg nomogram" and "sTg/TSH nomogram" demonstrated greater positive net benefits across a wide range of ER threshold probabilities compared to scenarios where all or no patients achieved ER. This indicates their favorable clinical usefulness in predicting ER probability. When compared to the "sTg nomogram", the net benefits of the "sTg/TSH nomogram" were slightly higher.

Discussion

Differentiated thyroid carcinoma (DTC), being a slow-growing malignancy, often grants patients an excellent long-term prognosis when carefully managed. Clinical observations reveal that a significant number of intermediate-risk DTC patients display persistent small-volume structural disease or abnormal postoperative thyroglobulin values, indicating the persistence of disease (12). Studies suggest that adjuvant therapy can have beneficial effects in up to 30% of DTC patients by destroying potentially malignant cells or occult multifocal disease (13). Research conducted by Ruel et al. established a positive correlation between radioactive iodine (RAI) treatment and increased overall survival, noting a 29% reduced risk of death when RAI was administered in the postoperative setting (5). In a similar vein, a study by Al-Qahtani et al. presented evidence that

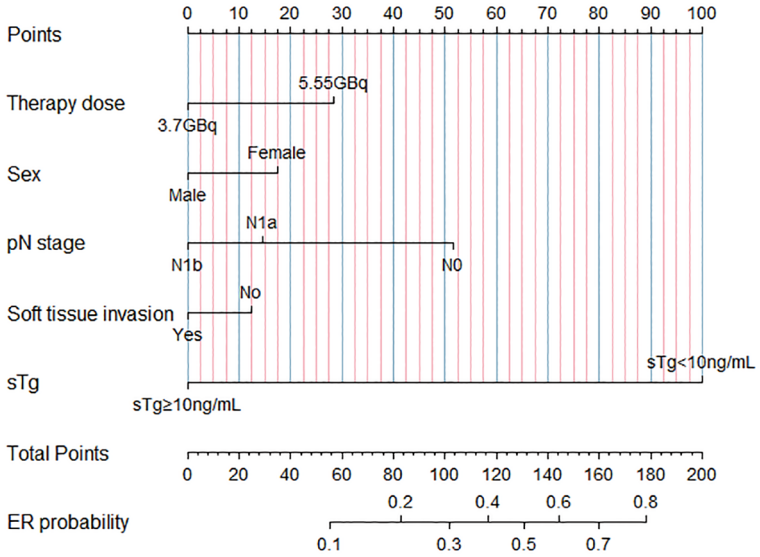


FIGURE 1
"sTg nomogram" predicting ER rates for intermediate-risk PTC patients receiving RAI.

adjuvant 131I ablation improves disease-free survival rates in papillary microcarcinoma patients, especially those with poor prognostic factors (14). Before initiating RAI therapy, it poses a significant challenge to distinguish structural or functional disease from thyroid remnants or biochemical disease based on serum thyroglobulin (sTg) levels. A study demonstrated 82.7% DTC patients with unexplained hyperthyroglobulinemia were finally attributed to biochemical, functional, or structural disease, and 81.5% of these patients yielded a non-structural or functional incomplete response 6-12 months post 5.55 GBq adjuvant RAI therapy (15). This result suggests that 5.55GBq of RAI can effectively identify and treat the lesions, especially when the overall tumor burden is relatively low (15). Despite these findings, the literature lacks sufficient information to provide guidance on the optimal administered activity for patients undergoing adjuvant therapy (12). The benefits of large RAI dosages remain uncertain to this date (16). Accordingly, the American Thyroid Association (ATA) guidelines have adjusted the dosage recommendations for adjuvant therapy, reducing the range from 3.7-7.4 GBq to 1.11-5.55 GBq (1).

In certain past studies, the therapeutic efficacy of intermediate-risk DTC patients treated with higher doses of RAI therapy (3.7GBq or 5.55GBq) has not been found to be superior to those treated with lower doses (1.11GBq or 3.7GBq) (17–21). Moreover, 3.7GBq RAI

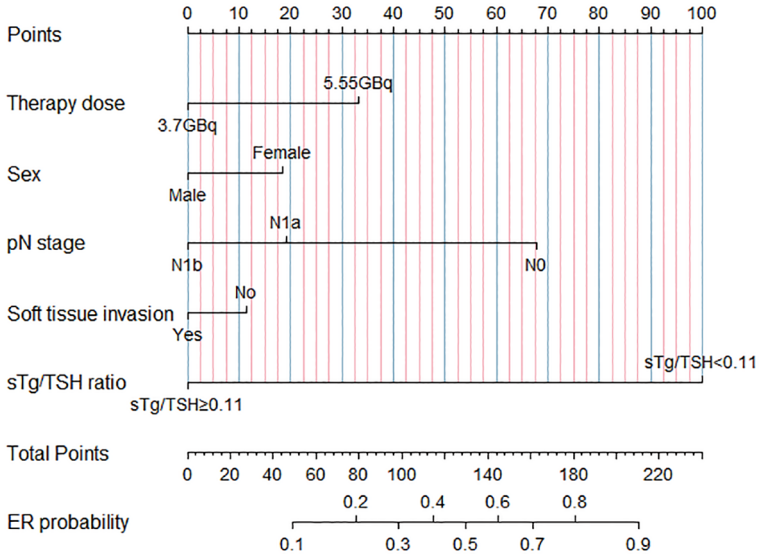


FIGURE 2
"sTg/TSH nomogram" predicting ER probability for intermediate-risk PTC patients receiving RAI.

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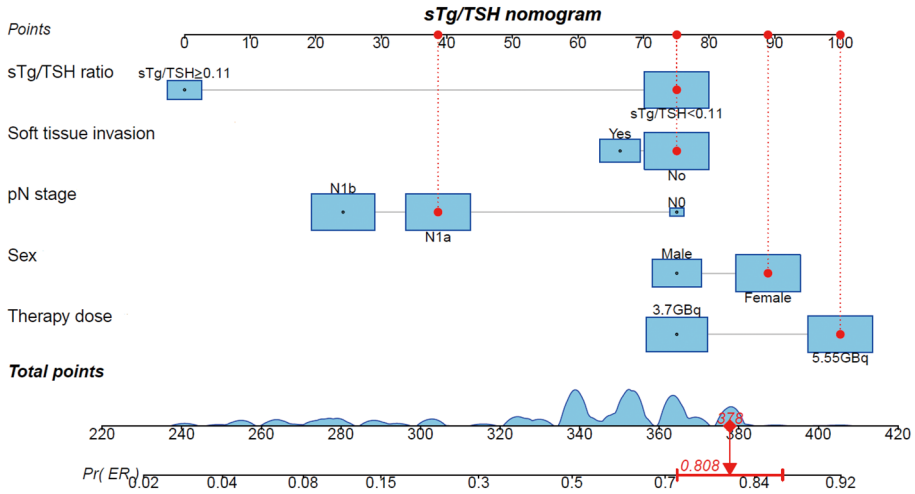


FIGURE 3
Individual "sTg/TSH nomogram" predicting ER probability for a female intermediate-risk PTC patient with N1a, no soft tissue invasion, sTg/TSH<0.11 receiving 5.55GBq RAI therapy.

therapy is often associated with a higher frequency of short-term adverse effects compared to 1.11GBq or 1.875GBq (17, 20, 21). Nevertheless, another report indicated that 5.55GBq RAI is associated with a lower recurrence rate than 3.7 GBq RAI for high-risk DTC patients (22). In the study conducted by Jeong et al., when the diagnostic whole-body scans (WBS) findings of residual thyroid bed uptake were not considered as criteria for ablation success and reliance was placed only on sTg and/or neck US, no significant difference in the ablation success rate was found between 3.7 GBq and 5.55GBq. However, when the diagnostic WBS findings were included as the assessment index, the ablation success rate of 3.7GBq was significantly lower compared to 5.55GBq (18). It is important to note that these studies involved patients with diverse clinicopathological features, recurrence risks, and RAI dosages. The subjects in our study were intermediate-risk PTC patients. We evaluated their responses to LD or HD RAI therapy six months post-initial RAI therapy. According to the 2015 ATA guidelines, the responses to initial surgery and RAI therapy were dynamically evaluated based on Tg levels and follow-up diagnostic image results. Our observations revealed differences in response to RAI therapy between the LD and HD groups. A significant difference was noted primarily in the ER rate, with the HD group exhibiting a higher ER rate than the LD group (57.3% *vs* 44.1%, p<0.05). Furthermore, the proportions of IR, BIR, SIR, IDR in HD group were lower than that in LD group, although these differences did not reach statistical significance. In 2017, a study reported that patients in the LD group demonstrated higher rates of BIR or SIR (3). Similar to our study, all patients in that research were intermediate-risk DTC patients. However, the RAI dosage in the LD group was 1.11GBq, and the patients in the HD group received 3.7GBq or 5.55GBq of RAI. The study revealed that 28.75% of patients in the LD group and 11.29% in the HD group exhibited remnant thyroid uptake. Another study suggested that insufficient RAI dosage could be one of the likely causes for frequent observations of residual thyroid bed uptake, and higher RAI dosages are more beneficial for the ablation of large normal-thyroid remnants (18). For intermediate-risk PTC patients,

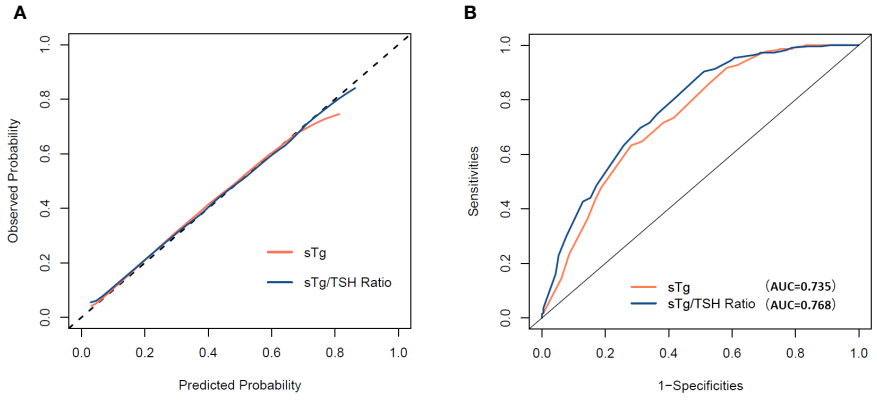


FIGURE 4
(A) Calibration curve for the "sTg nomogram" and "sTg/TSH nomogram". **(B)** ROC curve for the "sTg nomogram" and "sTg/TSH nomogram".

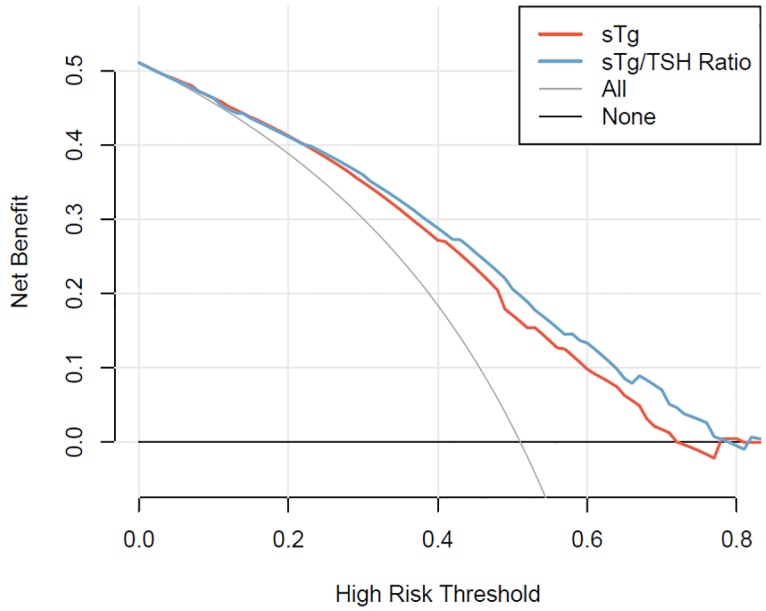


FIGURE 5
Decision curves of the "sTg nomogram" and "sTg/TSH nomogram".

our disease management team advises an adjuvant RAI therapy dosage ranging from 3.7 to 5.55 GBq, depending on the clinicopathological features. Additionally, we observed that remnant thyroid uptake was more frequently noted in the LD group than the HD group six months post-RAI therapy (16.3% *vs* 7.6%, $p<0.05$).

A previous study revealed that the number of treatment cycles and the accumulated RAI dose had differential impacts on the complete blood count of patients of different sexes and ages (23). In our comparison of treatment cycles between different dose groups, we found that the HD group had significantly higher proportions of one-time treatments. This indicates that the accumulated RAI dose of some patients in the HD group is less than those in the LD group. This result could lead to cost savings and reduced adverse effects in some patients, such as transient neck pain and edema, salivary dysfunction, nasolacrimal obstruction, and hematological toxicity.

It is well understood that certain tumor clinicopathological features have a profound impact on prognosis. The role of initial prognostic parameters is fundamental in guiding management recommendations. Undeniably, adequate treatment is essential for achieving complete or partial remission (24). In the literature, several studies have found that the size, number, and ratio of metastatic lymph nodes and extranodal extension indicate an unfavorable clinical outcome (25–27). Serum postoperative sTg and TgAb levels, which are sensitive and specific biomarkers for DTC, form part of the early postoperative disease status evaluation and impact clinical decision-making (16). Therefore, the determination of RAI dosage should take clinicopathological features into full consideration. In our study, we performed univariate and multivariate logistic regression to evaluate the predictors of ER. According to ATA guidelines, the optimal cut-off value of postoperative sTg level to guide decision-making regarding RAI administration is not known (1). A previous study by our team suggested that a pre-^{131}I treatment sTg level of less than 9.5ng/mL predicted a better therapeutic effect (11). A meta-analysis involving 3947 patients by Webb et al. demonstrated that postoperative sTg levels of less than 10ng/mL indicate a better prognosis with adjuvant RAI therapy (7). Similarly, Piccardo et al. confirmed that an sTg level of less than 10ng/mL is a strong predictor of complete remission after initial treatment, with a very high negative predictive value (93%) (24). Hence, we considered 10ng/mL as the cut-off value of sTg level for logistic regression analysis. It is widely known that sTg levels can be influenced by TSH levels. Therefore, in this study, we used the sTg/TSH ratio as another predictor of RAI therapeutic effects, using TSH to correct the predictive value of sTg. Given the collinearity between sTg and sTg/TSH, we analyzed sTg and sTg/TSH separately as influencing factors. A previous study by our team confirmed that an sTg/TSH ratio of less than 0.11 before the first RAI therapy predicted a better therapeutic effect (11). In the present study, we maintained 0.11 as the cut-off value for the sTg/TSH ratio. All the patients in our study were intermediate-risk PTC, and were divided into stages I and II by the age of 55 years according to the 8th edition of the ATCC TNM classification system. The 7th edition of the AJCC TNM classification system used the age of 45 years as a cut-off to upstage patients. Our previous study also showed that papillary thyroid microcarcinomas in patients aged under 45 years were more aggressive, especially regarding lymph node metastasis (28). Therefore, in logistic regression analysis, we set the ages of 45 and 55 years as the cut-off points to distinguish the AJCC stage.

Univariate and multivariate logistic regression analyses in our study confirmed that therapy dose is an independent risk factor for an ER. The ER rate of patients in the HD group was 2.464 times

higher than those receiving LD RAI therapy. Furthermore, both sTg and the sTg/TSH ratio showed a strong association with ER. Specifically, sTg ≥ 10ng/mL, sTg/TSH ≥ 0.11, and N1b might predict lower ER rates in intermediate-risk PTC patients receiving adjuvant RAI therapy. Interestingly, our study found male gender to be a risk factor for a non-ER. Previous studies have shown mixed results regarding whether gender impacts the efficacy of RAI treatment. However, most studies lean towards the conclusion that male gender is a risk factor for a poorer prognosis of RAI treatment (28–30), which is consistent with the results of our study. Additionally, we found no significant relationship between soft tissue invasion and ER. This result might be attributed to our research population, as we focused only on intermediate-risk PTC extending into the sternothyroid muscle or perithyroidal soft tissues, and not high-risk PTC extending beyond the capsule to invade subcutaneous soft tissues, larynx, trachea, esophagus, or the recurrent laryngeal nerve.

To better predict the therapeutic effects of initial RAI treatment in intermediate PTC patients, we constructed two nomograms - the "sTg nomogram" and the "sTg/TSH nomogram" - to predict ER probability based on logistic regression analysis. The factors contributing to ER, in descending order of impact, are sTg or sTg/TSH ratio, N stage, therapy dose, sex, and soft tissue invasion. These findings align with the results of our multivariate logistic regression analysis. In our study, the C-index and the AUC of these two nomograms were 0.735 and 0.768, respectively. This indicates that both nomograms demonstrated good discrimination ability and accuracy. Moreover, the "sTg/TSH Nomogram" outperformed the "sTg Nomogram" in terms of AUC and C-index. The calibration curves of both nomograms closely approached the ideal line, signifying excellent calibration. Additionally, the Hosmer-Lemeshow test results confirmed that both prediction models were well-fitted. Overall, these findings suggest that our prediction models exhibit exceptional discrimination, accuracy, and calibration. DCA is a tool that measures the net benefit of a diagnostic method or predictive model by jointly considering benefits and harms (31). DCA takes into account the clinical usefulness of a prediction model and guides clinical decision-making. Previous high-quality studies have used DCA to assess the net benefits of predictive models (32, 33). However, fewer studies have used DCA to evaluate predictive models for thyroid cancer. In our study, we calculated the net benefits of the "sTg nomogram" and "sTg/TSH nomogram". The results of the DCA suggested that both the "sTg nomogram" and "sTg/TSH nomogram" have clinical utility, with the "sTg/TSH nomogram" providing slightly higher net benefits, indicating superior clinical usefulness.

Our study does have some limitations. First, being a retrospective study, we did not estimate the short-term adverse effects of the two different dose groups. Second, due to a lack of long-term follow-up, we analyzed only initial therapy responses and not recurrence-free survival rates or recurrence rates. Lastly, certain other clinicopathological features, such as characteristics of metastatic lymph nodes, BRAFV600E mutation, multifocality, and so on, were not included in this study. Considering these limitations, further investigations are warranted.

Conclusion

In conclusion, different RAI doses have different therapeutic effects depending on patients' clinicopathological features. A higher initial RAI dose can help increase the probability of an ER, improve the success rate of remnant ablation, and reduce the number of treatment cycles for intermediate-risk PTC patients. Independent risk factors for Non-ER include sTg/TSH≥0.11, sTg ≥10ng/mL, N1b stage, and male gender. For intermediate-risk PTC patients with these risk factors, a higher initial RAI dose should be considered. We have developed clinically useful nomograms for calculating ER probabilities in intermediate-risk PTC patients after initial RAI therapy. The prediction models with excellent discrimination, accuracy and calibration. The "sTg/TSH nomogram" performed better than the "sTg nomogram" and could assist clinicians in making optimal clinical decisions.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving humans were approved by Ethical Committee Tianjin Medical University General Hospital Tianjin, China. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required from the participants or the participants' legal guardians/next of kin in accordance with the national legislation and institutional requirements.

Author contributions

XL: Data curation, Methodology, Writing – original draft, Writing – review & editing. HZ: Data curation, Writing – original draft. CM: Methodology, Writing – original draft. YJ: Methodology, Software, Writing – original draft. XW: Software, Writing – review & editing. DS: Data curation, Writing – review & editing. ZM: Methodology, Writing – original draft, Writing – review & editing. WZ: Methodology, Project administration, Writing – original draft, Writing – review & editing.

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Conflict of interest

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Correlation between systemic inflammatory response index and thyroid function: 2009-2012 NHANES results

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Aims: This study investigates the relationship between the Systemic Inflammatory Response Index (SIRI) and thyroid function.

Methods: Utilizing data from the National Health and Nutrition Examination Survey (NHANES) 2009-2012, we excluded participants lacking SIRI or thyroid function data, those under 20 years, and pregnant individuals. SIRI was determined using blood samples. We conducted weighted multivariate regression and subgroup analyses to discern the independent relationship between SIRI and thyroid function.

Results: The study included 1,641 subjects, with an average age of 47.26±16.77 years, including 48.65% males and 51.35% females. The population was divided into three SIRI-based groups (Q1-Q3). Q3, compared to Q1, exhibited higher age-at-onset, greater male prevalence, and increased levels of FT3, FT4, TT4, leukocytes, and triglycerides. This group also showed a higher incidence of diabetes, hypertension, and smoking. Notably, Q1 had lower LDL and HDL levels. SIRI maintained a positive association with FT4 (β = 0.01, 95% CI = 0.00-0.03, P for trend = 0.0071), TT4 (β = 0.20, 95% CI = 0.10, 0.31, *P* for trend=0.0001), and TPOAb (β = 8.0, 95% CI = 1.77-14.30, *P* for trend = 0.0120), indicating that each quartile increase in SIRI corresponded to a 0.01 ng/dL increase in FT4, a 0.2 g/dL increase in TT4, and an 8.03 IU/mL rise in TPOAb. The subgroup analysis suggested the SIRI-thyroid function correlation was influenced by hypertension.

Conclusion: Inflammation may impact the development and progression of thyroid function disorders. Proactive anti-inflammatory treatment might mitigate thyroid abnormalities.

KEYWORDS

thyroid hormone, systemic inflammatory response index, thyroid function, positive correlation, national health and nutrition examination survey

Introduction

The thyroid gland, the largest endocrine gland in adults, plays a pivotal role in regulating physiological activities (1) and is one of the most important components of the human body. Thyroid hormones (TH), primarily triiodothyronine (T3) and tetraiodothyronine (T4) are crucial for systemic metabolism, neurodevelopment, and energy metabolism, influencing protein, carbohydrate, and lipid metabolism. Recent studies indicate a rising prevalence of thyroid disorders, now second only to diabetes mellitus as a global metabolic disease. These disorders often lead to developmental and cognitive impairments, osteoporosis, and insomnia, significantly impacting patients' physical and mental health (2).

TH comprises both T3 and significant quantities of T4. The active free thyroid hormone in the body consists of free triiodothyronine (Free T3, FT3) and free thyroxine (Free T4, FT4), but when FT3 and FT4 are depleted, total triiodothyronine (Total T3, TT3) and total thyroxine (Total T4, TT4) are converted to FT3 and FT4 in the body, which continues to play the role of TH. Although TH is synthesized in the follicular epithelial cells of the thyroid gland, its synthesis, and secretion depend on the stimulation of thyroid-stimulating hormone (TSH). Thyroid peroxidase antibodies (TPOAb) are found in the microsomes of thyroid cells and are a key enzyme in the synthesis of TH. They work in concert with Thyreoglobulin to iodide L-tyrosine into TH, which is a common autoantibody in the serum of patients with autoimmune thyroid disease. It is seen in autoimmune thyroid diseases such as Hashimoto's thyroiditis (HT) (3).

Inflammation response is a defense response of the organism itself when tissues are damaged or infected by toxins or bacteria, injured by heat or other causes, they go into a normal state of self-protection and damage repair. The System Inflammation Response Index (SIRI) is a new systemic inflammation measure created in 2016 by Qi et al. (4). SIRI (1000cell/uL) = N×M/L, as the calculation method, where N, M, and L are the pretreatment peripheral neutrophil, monocyte, and lymphocyte counts, respectively. Previous studies have concluded that SIRI can be used to differentiate the immune-inflammatory response of the body's three different pathways, and to reflect the body's immune response and inflammation in a more comprehensive way. Subsequently, this index was used to measure the recurrence rate and survival rate after cancer surgery. In addition, the index has been used to measure the degree of brain damage in aneurysmal arachnoiditis (5) hemorrhage, and the development and prognosis of the Corona Virus Disease 2019 (COVID-19).

Abbreviations: NHANES, National Health and Nutritional Examination Survey; CI, Confidence interval; SIRI, System Inflammation Response Index; NLR, Neutrophil to Lymphocyte Ratio; PLR, Platelet to Lymphocyte Ratio; OR, Odds ratio; HT, Hashimoto's thyroiditis; BMI, Body Mass Index; TT3, Free Triiodothyronine; FT4, Free Thyroxine; TSH, Thyroid stimulating hormone; TPOAb, Thyroid peroxidase antibodies; TT3, Total Triiodothyronine; TT4, Total Thyroxine; WBC, White blood cell count; HEI, Healthy Eating Index; DII, Dietary Inflammatory Index.

The relationship between inflammation and thyroid function, though correlated, remains poorly understood. Therefore, our study aims to elucidate the impact of inflammation on thyroid disease development by examining the correlation between SIRI and thyroid function in participants of the National Health and Nutrition Examination Survey (NHANES). This could pave the way for novel strategies in the primary prevention of thyroid diseases. We hypothesized that elevated SIRI is associated with a higher risk of abnormal thyroid function.

Materials and methods

Study design and population

Data for this study were sourced from the National Health and Nutrition Examination Survey (NHANES), a biennial cross-sectional study conducted by the Centers for Disease Control and Prevention (U.S.) to assess the health and nutritional status of American citizens. The NHANES study protocol adhered to the Declaration of Helsinki principles and received approval from the Institutional Review Board, with all participants providing written informed consent. Data were accessed from NHANES's public domain (www.example.com; https://www.cdc.gov/nchs/nhanes/, accessed on 12 August 2023), and Figure 1 depicts the participant selection process.

This research utilized data from the 2009-2012 NHANES survey cycle, which encompassed comprehensive data on thyroid function, the System Inflammation Response Index (SIRI), and demographic information. The final study cohort, after excluding individuals with incomplete SIRI or thyroid function data, those under 20 years of age, and pregnant women, comprised 1,641 participants. All participants consented to the study, and the Ethical Review Board of the National Centre for Health Statistics authorized the research.

Measurement of peripheral blood cell counts

All blood specimens (neutrophil count, monocyte count, and lymphocyte count) were collected at Mobile Examination Centers (MECs). The procedures for quality assurance and control of these specimens adhered to the guidelines outlined in the NHANES Laboratory Procedures Manual, which can be accessed at https://wwwn.cdc.gov/nchs/data/nhanes/2009-2010/labmethods/CBC_F_met_HE.pdf.

The SIRI was defined as follows: SIRI (1000cell/uL) = N×M/L, where N, M, and L are the pretreatment peripheral neutrophil, monocyte, and lymphocyte counts, respectively (4).

Measurement of thyroid outcomes

This study involved the analysis of serum samples for thyroid function indicators including FT3, FT4, TSH, TPOAb, TT3, and TT4.

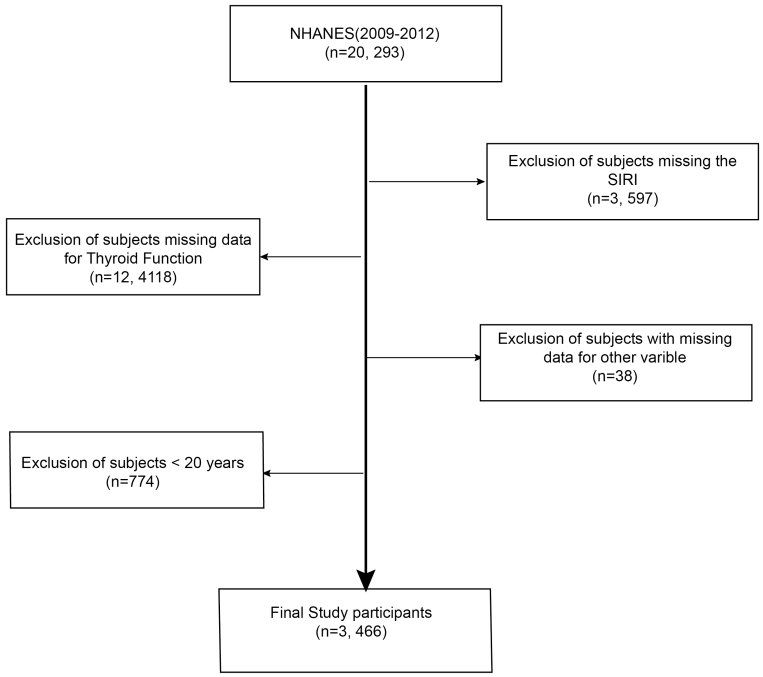


FIGURE 1
Selection process for the study sample based on the 2009-2012 National Health and Nutrition Examination Survey (NHANES).

The methods for serum specimen collection and processing are elaborately described in the National Health and Nutrition Examination Survey (NHANES) Laboratory Procedures Manual (available at: wwwn.cdc.gov/nchs/nhanes/2007-2008/THYROD_E.htm).

The covariates

The study accounted for various covariates potentially influencing thyroid function: age, gender (male, female), educational attainment (below high school, high school or equivalent, college graduate or above), marital status (married or not), and race/ethnicity (Mexican American, non-Hispanic White, non-Hispanic Black, and others). Diabetes mellitus was defined as any self-reported diagnosis of diabetes mellitus or self-reported use of insulin or antidiabetic medication or fasting blood glucose greater than 7.0 mmol/L or glycated hemoglobin greater than 6%. Hypertension was defined as any self-reported diagnosis of hypertension or self-reported use of blood pressure-lowering medication or SBP ≥ 140 mm Hg or DBP ≥ 90 mm Hg. Body Mass Index (BMI) was determined from the measured weight and height. Serum biochemistry analyses included total High-Density Lipoprotein (HDL) cholesterol, total Low-Density Lipoprotein (LDL) cholesterol, and triglycerides.

Statistical methods

Statistical analyses in this study were conducted using R packages (The R Foundation: http://www.r-project.org;version3.4.3) and Empower (R) (www.empowerstats.com, X&Y Solutions, Inc., Boston, Massachusetts). Given the complex multi-stage sampling design of the National Health and Nutrition Examination Survey (NHANES), this study utilized MEC exam weights (WTMEC4YR, WTMEC2YR) for analysis. In this study, categorical variables were expressed as weighted percentages, while continuous variables were expressed as weighted means and standard deviations. Differences in characteristics between groups were tested by chi-square test (for categorical variables) and one-way ANOVA (for continuous variables). All statistical tests were two-sided, and a P value< 0.05 was considered statistically significant.

Furthermore, the correlation between the SIRI and thyroid function was analyzed using weighted multivariate logistic regression models. SIRI was treated as a categorical variable in quartiles. Three models were employed: Model 1, without covariate adjustment; Model 2, adjusting for gender, age, and race; and Model 3, further adjusting for education level and marital status. To visually depict the relationship between SIRI and thyroid function, smoothed curve fitting was used. In these plots, the central line represents the effect size, while the surrounding area indicates the 95% CI. P value <0.05 was deemed statistically significant.

Results

Baseline characteristics of the study population

The study's baseline characteristics are detailed in Table 1, which outlines the demographic and laboratory parameters of the participants. The study encompassed 3,466 individuals, with an average age of 47.26 ± 16.77 years, comprising 48.65% males and 51.35% females. Participants were stratified into three groups

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TABLE 1 Baseline characteristics of participants in the 2009–2012 continuous NHANES, weighted.

Characteristics	Systemic inflammatory response index (SIRI (1000 cells /UL)) [a]			*P*-Value [b]
	Q1 N=1,130	Q2 N=1,150	Q3 N=1,185	
Age (years)	45.60 ± 15.68[c]	46.55 ± 16.46	49.34 ± 17.72	<0.0001
Gender (%)				<0.0001
Male	41.94[d]	47.14	55.75	
Female	58.06	52.86	44.25	
Race (%)				<0.0001
Mexican American	9.33	7.52	6.87	
Other Hispanic	6.43	5.63	5.92	
Non-Hispanic White	56.36	70.53	76.01	
Non-Hispanic Black	18.28	8.80	5.44	
Other Race - Including Multi-Racial	9.61	7.51	5.77	
Education Level (%)				0.2895
Under high school	17.01	16.29	18.86	
High school or equivalent	21.22	20.98	22.48	
College graduate or above	61.77	62.73	58.66	
Marital Status (%)				0.0723
Yes	52.10	56.91	54.22	
No	47.90	43.09	45.78	
Diabetes (%)				0.0146
Yes	33.25	29.04	34.28	
No	66.75	70.96	65.72	
Hypertension (%)				<0.0001
Yes	31.29	30.87	41.78	
No	68.71	69.13	58.22	
Smoking status (%)				<0.0001
Current smoker	13.57	14.90	21.17	
Former smoker	4.21	2.71	3.66	
Never	82.21	82.39	75.18	
BMI (kg/m^2)	28.09 ± 6.61	28.43 ± 6.22	29.34 ± 7.13	<0.0001
FT3 (pg/mL)	3.14 ± 0.38	3.18 ± 0.38	3.17 ± 0.39	0.0209
FT4 (ng/dL)	0.81 ± 0.15	0.82 ± 0.17	0.83 ± 0.16	0.0455
TSH(uIU/mL)	2.10 ± 5.29	1.87 ± 1.62	2.07 ± 3.64	0.2707
TPOAb(IU/mL)	17.03 ± 78.29	25.42 ± 101.26	24.12 ± 99.45	0.0847
TT3 (ng/dL)	113.08 ± 24.50	115.2±23.88	114.36 ±22.81	0.1049
TT4 (μg/dL)	7.82 ± 1.56	7.96 ± 1.72	8.02 ± 1.56	0.0105
WBC (1000cells/uL)	5.81 ± 1.53	6.76 ± 1.57	8.31 ± 2.36	<0.0001
Triglyceride (mg/dL)	124.21 ± 89.98	126.92±109.34	140.68±130.72	0.0348

(Continued)

TABLE 1 Continued

Characteristics	Systemic inflammatory response index (SIRI (1000 cells /UL)) [a]			*P*-Value [b]
	Q1 N=1,130	Q2 N=1,150	Q3 N=1,185	
LDL-cholesterol (mg/dL)	119.66 ± 36.53	113.68±31.63	115.44 ± 35.26	0.0110
Direct HDL-Cholesterol (mg/dL)	54.73 ± 16.50	53.67 ± 16.51	51.09 ± 15.29	<0.0001

Values in bold indicate significance at P < 0.05.
BMI, body mass index;
[a]Systemic inflammatory response index(SIRI) was divided into three groups, Q1 and Q3 are the lowest and highest quintile groups, respectively.
[b]ANOVA and chi-square tests are used to assess the significance of continuous and categorical variables, respectively.
[c]Continuous values are presented as mean and standard error.
[d]Categorical values are presented as %.
TT3, Free Triiodothyronine;
FT4, Free Thyroxine;
TSH, Thyroid stimulating hormone;
TPOAb, Thyroid peroxidase antibodies;
TT3, Total Triiodothyronine;
TT4, Total Thyroxine;
WBC, White blood cell count.

according to the tertiles of the Systemic Immune-Inflammation Index (SIRI): the first tertile group (Q1, N=1,130), the second tertile group (Q2, N=1,150), and the third tertile group (Q3, N=1,185). Notably, the Q3 group, compared to Q1, demonstrated a higher mean age, a greater male proportion, and elevated levels of FT3, FT4, TT4,WBC, and triglycerides. This group also showed a higher prevalence of diabetes, hypertension, and smoking. In contrast, lower levels of LDL and HDL were observed in the Q1 group (all $P < 0.05$).

Univariate analysis of thyroid function

Table 2 presents the results of the univariate analyses. In our study population, thyroid function showed no association with race. However, a significant positive correlation was observed between and marital status (β = 0.08, 95% CI = 0.05, 0.10), as well as WBC (β = 0.01, 95% CI = 0.00, 0.01), negatively correlated with age (β = -0.01, 95% CI = 0.01, 0.00) and gender (β = -0.09, 95% CI = -0.21, -0.16).

Regarding FT4, a significant positive correlation was noted with age (β = 0.00, 95% CI = 0.00, 0.00) and the SIRI (β = 0.01, 95% CI = 0.01, 0.02), while a negative correlation was observed with education level (β = -0.02, 95% CI = -0.03, -0.0). TSH and TPOAb both demonstrated a positive correlation with gender (TSH: β = 0.33, 95% CI = 0.08, 0.58; TPOAb: β = 18.12, 95% CI = 11.83, 24.40), with TSH also positively correlating with age (β = 0.01, 95% CI = 0.00, 0.02). TT3 showed a negative correlation with both age (β = -0.38, 95% CI = -0.42, 0.34) and gender (β = -2.07, 95% CI = -3.65, -0.49), while positively correlating with marital status (β = 3.21, 95% CI = 1.63, 4.79) and WBC (β = 0.91, 95% CI = 0.54, 1.27). Finally, TT4 exhibited a positive association with age (β = 0.01, 95% CI = 0.00, 0.01), gender (β = 0.49, 95% CI=0.39, 0.60), WBC (β = 0.08, 95% CI = 0.06, 0.11), and SIRI (β = 0.10, 95% CI = 0.03,0.17), but a negative correlation with education level (β = -0.47, 95% CI = -0.62, -0.33).

Relationship between SIRI and thyroid function

Table 3 presents the results of a weighted multivariate linear regression analysis, examining the relationship between the SIRI and thyroid function indicators. The results of the study showed that SIRI was significantly and positively correlated with FT4 and TT4 in thyroid function (FT4: Model 1, β = 0.01, 95% CI = 0.01, 0.02; Model 2, β = 0.01, 95% CI = 0.01, 0.02; Model 3, β = 0.01, 95% CI = 0.01, 0.02; TT4: Model 1, β =0.1, 95% CI = 0.03, 0.17; Model 2, β =0.16, 95% CI = 0.09, 0.23; Model 3, β =0.15, 95% CI = 0.08, 0.22), but there was no statistically significant relationship with FT3, TSH, TgAb, and TT3. Subsequently, we transformed SIRI into quartile categorical variables, and in the fully adjusted model (Model 3) we found that SIRI was associated with FT4 (β = 0.01, 95% CI = 0.00, 0.03, *P* for trend = 0.0071), TT4 (β = 0.20, 95% CI = 0.10, 0.31, *P* for trend = 0.0001), and TPOAb (β = 8.03, 95% CI = 1.77, 14.30, *P* for trend = 0.012) remained positively correlated with each other. This suggests that an increase in SIRI per quartile was associated with an increase in FT4 of 0.01ng/dL, an increase in TT4 of 0.2μg/dL, and an elevation in TPOAb of 8.03IU/mL. No statistically significant differences were found among SIRI and FT3, TSH, and TT3 even in the SIRI quartiles.

Table 4 and Figure 2 further elucidate the association between adjusted SIRI and thyroid function parameters. Utilizing a generalized additive model and restricted cubic spline (RCS) curves, which accounted for relevant confounding factors, a nonlinear association was discerned between SIRI and FT3, TT3, and TT4. Critical breakpoints (K) were determined for each hormone: 1.17 for FT3, 1.19 for TT3, and 1.18 for TT4. On the left side of these breakpoints, a positive correlation existed between SIRI and the hormones (FT3: β = 0.09, 95% CI = 0.04, 0.14, *P* for trend = 0.0003; TT3: β = -3.10, 95% CI = -4.47, -1.73, *P* for trend <0.0001; TT4: β = 0.26, 95% CI=0.05, 0.46, *P* for trend = 0.0138). Conversely, on the right side of the breakpoints, a significant negative correlation was observed between SIRI and FT3 and TT3, but not with TT4 (FT3: β = -0.04, 95% CI = -0.06,

TABLE 2 Univariate analysis of thyroid function, weighted.

	FT3 (pg/mL)	FT4 (ng/dL)	TSH(uIU/mL)	TPOAb(IU/mL)	TT3 (ng/dL)	TT4 (μg/dL)
Age, years	-0.01 (-0.01,-0.0) <0.0001	0.00 (0.00, 0.00) <0.0001	0.01 (0.00, 0.02) 0.0084	0.13 (-0.06, 0.31) 0.1871	-0.38 (-0.42,-0.3) <0.0001	0.01 (0.00, 0.01) <0.0001
Gender,n%						
Male	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.
Female	-0.19 (-0.21,-0.16) <0.0001	0.01 (-0.01,0.02) 0.3276	0.33 (0.08, 0.58) 0.0102	18.12 (11.83,24.40) <0.0001	-2.07 (-3.65,-0.49) 0.0103	0.49 (0.39, 0.60) <0.0001
Race, n%						
Mexican American	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.
Other Hispanic	0.01 (-0.06, 0.08) 0.7978	0.02 (-0.01,0.05) 0.2409	-0.00 (-0.68,0.67) 0.9977	-4.14 (-21.36,13.07) 0.6372	0.84 (-3.44,5.12) 0.6992	-0.05 (-0.34, 0.25) 0.7606
Non-Hispanic White	-0.13 (-0.18,-0.08) <0.0001	0.01 (-0.01,0.03) 0.2838	0.15 (-0.31,0.62) 0.5193	3.94 (-8.03, 15.91) 0.5189	-4.99 (-7.96,-2.01) 0.0010	-0.41 (-0.61,-0.21) <0.0001
Other Hispanic	-0.09 (-0.15,-0.03) 0.0022	0.01 (-0.02,0.03) 0.6731	-0.50 (-1.09, 0.09) 0.0939	-6.99 (-21.96, 7.98) 0.3604	-4.54 (-8.27,-0.82) 0.0169	-0.13 (-0.39, 0.12) 0.3052
Other Race	-0.09 (-0.15,-0.02) 0.0094	0.05 (0.03, 0.08) 0.0001	-0.38 (-1.01,0.26) 0.2455	-0.33 (-16.46,15.80) 0.9681	-4.07 (-8.09,-0.05) 0.0473	-0.01 (-0.29, 0.26) 0.9350
Marital Status, n%						
Yes	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.
No	0.08 (0.05, 0.10) <0.0001	0.00 (-0.01,0.01) 0.4480	0.04 (-0.21, 0.29) 0.7701	0.24 (-6.10, 6.58) 0.9405	3.21 (1.63, 4.79) <0.0001	0.06 (-0.05, 0.16) 0.3175
Education Level(%)						
Under high school	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.
High school or equivalent	-0.00 (-0.04, 0.04) 0.8685	-0.02 (-0.04,-0.0) 0.0164	0.14 (-0.26, 0.54) 0.4972	-2.00 (-12.16, 8.16) 0.6997	0.06 (-2.48,2.59) 0.9655	-0.45 (-0.62,-0.27) <0.0001
College graduate or above	-0.06 (-0.09,-0.03) 0.0007	-0.02 (-0.03,-0.0) 0.0415	0.22 (-0.11, 0.56) 0.1946	6.82 (-1.74, 15.38) 0.1185	-4.33 (-6.46,-2.19) <0.0001	-0.47 (-0.62,-0.33) <0.0001
WBC (1000cells/uL)	0.01 (0.00, 0.01) 0.0069	0.00 (-0.00,0.00) 0.8978	0.01 (-0.05, 0.06) 0.8463	-0.57 (-2.05, 0.90) 0.4450	0.91 (0.54, 1.27) <0.0001	0.08 (0.06, 0.11) <0.0001
SIRI (1000cells/uL)	-0.01 (-0.03, 0.01) 0.2162	0.01 (0.01, 0.02) <0.0001	0.01 (-0.15, 0.17) 0.9175	2.68 (-1.31, 6.67) 0.1883	-0.91 (-1.91, 0.09) 0.0743	0.10 (0.03, 0.17) 0.0046

SIRI, Systemic inflammatory response index;
TT3, Free Triiodothyronine;
FT4, Free Thyroxine;
TSH, Thyroid stimulating hormone;
TPOAb, Thyroid peroxidase antibodies;
TT3, Total Triiodothyronine;
TT4, Total Thyroxine;
CI, confidence interval.

-0.02, *P* for trend = 0.0001; TT3: β = 5.58, 95% CI = 2.62, 8.55, *P* for trend = 0.0002; TT4: β = 0.05, 95% CI = -0.05, 0.14, *P* for trend = 0.3344). The log-likelihood ratio tests yielded *P* about <0.001, <0.001, and 0.106, respectively.

Subgroup analysis of SIRI and thyroid function

Figure 3 presents subgroup analyses examining the relationship between the SIRI and thyroid function. To ascertain if SIRI's association with thyroid function is consistent across the general population and to identify distinct subgroups, we conducted analyses stratified by age, gender, BMI, and hypertension, along with interaction tests. Our findings reveal significant interactions of SIRI with FT4 in different age groups, with TT4 in the context of hypertension, and with TPOAb across age groups (all *P* for trend <0.05). However, no notable differences were observed in terms of gender and BMI. Thus, our study indicates that SIRI's correlation with thyroid function varies with hypertension status and may be relevant for individuals without hypertension. Importantly, our interaction tests did not reveal any significant differences associated with gender and BMI, indicating that

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TABLE 3 Association between SIRI and thyroid function among U.S. adults in NHANES from 2009-2012, weighted.

SIRI Tertile	FT3 (pg/mL)	FT4(ng/dL)	TSH(uIU/mL)	TPOAb(IU/mL)	TT3 (ng/dL)	TT4(μg/dL)
			β (95% CI), *P* Value[a]			
Model 1						
Continuous	-0.01 (-0.03, 0.01) 0.2162	0.01 (0.01, 0.02) <0.0001	0.01 (-0.15, 0.17) 0.9175	2.68 (-1.31, 6.67) 0.1883	-0.91 (-1.91, 0.09) 0.0743	0.10 (0.03, 0.17) 0.0046
Tertile 1	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.
Tertile 2	0.03 (-0.01, 0.06) 0.1735	0.01 (-0.01, 0.03) 0.2113	-0.29 (-0.65, 0.07) 0.1148	6.95 (-2.22, 16.11) 0.1377	-0.27 (-2.56, 2.03) 0.8206	0.10 (-0.06, 0.26) 0.2060
Tertile 3	0.05 (0.01, 0.09) 0.0070	0.02 (0.00, 0.03) 0.0251	-0.25 (-0.61, 0.10) 0.1649	10.22 (1.21, 19.23) 0.0263	3.50 (1.25, 5.75) 0.0023	0.26 (0.11, 0.41) 0.0009
Tertile 4	0.02 (-0.02, 0.06) 0.3023	0.02 (0.01, 0.04) 0.0033	-0.16 (-0.51, 0.20) 0.3885	10.52 (1.49, 19.56) 0.0225	0.04 (-2.22, 2.30) 0.9734	0.17 (0.02, 0.33) 0.0266
p for trend	0.01 (-0.02, 0.03) 0.4625	0.02 (0.01, 0.03) 0.0036	-0.05 (-0.29, 0.19) 0.6892	6.47 (0.37, 12.57) 0.0377	0.20 (-1.32, 1.73) 0.7943	0.11 (0.01, 0.22) 0.0321
Model 2						
Continuous	-0.01 (-0.02, 0.01) 0.4066	0.01 (0.01, 0.02) 0.0002	-0.03 (-0.19, 0.14) 0.7500	3.55 (-0.53, 7.62) 0.0879	-0.34 (-1.33, 0.65) 0.5005	0.16 (0.09, 0.23) <0.0001
Tertile 1	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.
Tertile 2	0.03 (-0.00,0.06) 0.0757	0.01 (-0.00, 0.03) 0.1818	-0.36 (-0.72, 0.00) 0.0521	6.23 (-2.95, 15.40) 0.1835	-0.16 (-2.37, 2.06) 0.8908	0.14 (-0.02, 0.29) 0.0838
Tertile 3	0.07 (0.03, 0.10) <0.0001	0.02 (0.00, 0.03) 0.0278	-0.36 (-0.72, -0.00) 0.0483	9.68 (0.58, 18.77) 0.0371	4.29 (2.09, 6.48) 0.0001	0.34 (0.19, 0.49) <0.0001
Tertile 4	0.03 (-0.00, 0.06) 0.0894	0.02 (0.01, 0.04) 0.0039	-0.27 (-0.63, 0.10) 0.1506	12.15 (2.90, 21.41) 0.0101	1.26 (-0.98, 3.49) 0.2713	0.32 (0.16, 0.47) <0.0001
P for trend	0.02 (-0.01, 0.04) 0.1779	0.02 (0.00, 0.03) 0.0049	-0.12 (-0.36, 0.13) 0.3551	7.86 (1.60, 14.11) 0.0139	1.10 (-0.42, 2.61) 0.1566	0.21 (0.11, 0.32) <0.0001
Model 3						
Continuous	-0.01 (-0.02, 0.01) 0.2078	0.01 (0.01, 0.02) 0.0004	-0.03 (-0.19, 0.13) 0.7357	3.68 (-0.42, 7.77) 0.0783	-0.52 (-1.51,0.46) 0.2997	0.15 (0.08, 0.22) <0.0001
Tertile 1	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.
Tertile 2	0.03 (-0.00,0.06) 0.0697	0.01 (-0.00, 0.03) 0.1607	-0.35 (-0.71, 0.01) 0.0585	6.32 (-2.86, 15.50) 0.1771	-0.18 (-2.38,2.03) 0.8735	0.14 (-0.02, 0.29) 0.0814
Tertile 3	0.06 (0.03, 0.10) 0.0001	0.02 (0.00, 0.03) 0.0287	-0.35 (-0.71, 0.01) 0.0548	9.99 (0.90, 19.09) 0.0314	4.04 (1.86, 6.23) 0.0003	0.33 (0.18, 0.48) <0.0001
Tertile 4	0.02 (-0.01, 0.06) 0.1444	0.02 (0.01, 0.04) 0.0051	-0.26 (-0.63, 0.10) 0.1585	12.40 (3.14, 21.66) 0.0087	0.99 (-1.24, 3.22) 0.3834	0.30 (0.15, 0.46) 0.0001
P for trend	0.01 (-0.01, 0.03) 0.2906	0.01 (0.00, 0.03) 0.0071	-0.12 (-0.36, 0.13) 0.3604	8.03 (1.77, 14.30) 0.0120	0.89 (-0.62, 2.40) 0.2476	0.20 (0.10, 0.31) 0.0001

[a]Model 1: no covariates were adjusted. Model 2: adjusted for gender, age, and race. Model 3: adjusted for gender, age, race, education level, and marital status;
SIRI, Systemic Immune Inflammation Index;
TT3, Free Triiodothyronine;
FT4, Free Thyroxine;
TSH, Thyroid stimulating hormone;
TPOAb, Thyroid peroxidase antibodies;
TT3, Total Triiodothyronine;
TT4, Total Thyroxine;
CI, confidence interval.

TABLE 4 Threshold effect analysis of the relationship between SIRI and thyroid function.

Outcome	FT3 (pg/mL)	FT4 (ng/dL)	TSH (uIU/mL)	TPOAb (IU/mL)	TT3 (ng/dL)	TT4(μg/dL)
Model I						
One linear effect	-0.01 (-0.03, 0.01) 0.2162	0.01 (0.01,0.02) <0.0001	0.01 (-0.15,0.17) 0.9175	2.68 (-1.31, 6.67) 0.1883	-0.91 (-1.91,0.09) 0.0743	0.10 (0.03, 0.17) 0.0046
Model II						
Breakpoint (k)	1.17	0.48	0.45	0.8	1.19	1.18
<K segment effect 1	0.09 (0.04,0.14) 0.0003	-0.06 (-0.18,0.06) 0.3453	1.63 (-1.76,5.01) 0.3466	27.91 (5.02, 50.79) 0.0169	5.58 (2.62,8.55) 0.0002	0.26 (0.05, 0.46) 0.0138
>K segment effect 2	-0.04 (-0.06, -0.02) 0.0001	0.01 (0.01, 0.02) <0.0001	-0.01 (-0.18, 0.15) 0.8763	0.02 (-4.62, 4.66) 0.9932	-3.10 (-4.47,-1.73) <0.0001	0.05 (-0.05, 0.14) 0.3344
Effect difference between 2 and 1	-0.13 (-0.19, -0.07) <0.0001	0.07 (-0.05, 0.20) 0.2461	-1.64 (-5.07, 1.79) 0.3486	-27.89 (-52.80, -2.97) 0.0283	-8.68 (-12.42,-4.95) <0.0001	-0.21 (-0.47, 0.05) 0.1066
Predicted value of equation at breakpoint	3.20 (3.18, 3.22)	0.81 (0.80, 0.82)	2.03 (1.85, 2.21)	24.77 (20.20, 29.34)	116.65 (115.35,117.94)	8.00 (7.91, 8.09)
LRT test	<0.001	0.246	0.348	0.028	<0.001	0.106

For exposure: Systemic inflammatory response index.
Model I, linear analysis; Model II, nonlinear analysis.
LRT test, logarithmic likelihood ratio test. (P < 0.05 denotes that Model II significantly differs from Model I, revealing a nonlinear relationship);
TT3, Free Triiodothyronine;
FT4, Free Thyroxine;
TSH, Thyroid stimulating hormone;
TPOAb, Thyroid peroxidase antibodies;
TT3, Total Triiodothyronine;
TT4, Total Thyroxine;
CI, confidence interval.

these factors do not influence the relationship between SIRI and thyroid function.

Discussion

The objective of this study was to evaluate the association between the SIRI and thyroid function in a population of US adults. In our cross-sectional analysis of 1,641 participants, we discovered a significant positive correlation between SIRI and both FT4) and TTT4. However, no significant correlation was found between SIRI and TSH. Adjustments for all covariates revealed elevated levels of FT4, TT4, and TPOAb in the highest quartile of SIRI (Tertile 4) compared to the lowest (Tertile 1). Additionally, we employed Generalised Additive Models and Restricted Cubic Splines to further delineate the SIRI-thyroid function relationship. Our findings indicated a nonlinear association between SIRI and FT3, TT3, and TT4. Notably, the nature of the relationship varied across the breakpoint; SIRI was positively correlated with FT3, TT3, and TT4 on the left side of the breakpoint, whereas on the right side, it was negatively correlated with FT3 and TT3. Still, the correlation with TT4 was not statistically significant. Finally, the interaction term analysis suggested that gender and BMI did not significantly influence these associations.

This study is the first to investigate the correlation between SIRI and thyroid function risk. Previous research has established connections between thyroid function and various clinicopathological factors. Chen S et al. observed a negative relationship between the Healthy Eating Index (HEI) and levels of FT3 and TT3. Additionally, they found a strong association between the Dietary Inflammatory Index (DII) with increased FT3 and TT4 levels, suggesting DII's utility in evaluating dietary impacts on inflammatory potential (6). Anti-inflammatory diets, such as DASH and Mediterranean, have been noted to lower systemic inflammation levels (7, 8). Research indicates that gut flora and dietary fiber modulate inflammatory states via inter-organ signaling, thus impacting pathophysiological aspects of obesity, diabetes, and dyslipidemia (9, 10). Leonardo et al. demonstrated that pro-inflammatory diets can lead to intestinal imbalance, bacterial overgrowth, increased intestinal permeability, and oxidative stress. This was evidenced by examining the faces of HT patients, suggesting that such an inflammatory response could accelerate HT development and support the notion of a thyroid-gut axis (6). GlycA, a glycoprotein biomarker, indicates systemic inflammation by measuring acute phase reactants and various glycosylated proteins. Research by Nilay Yukse et al. found that patients with hypothyroidism, regardless of treatment status, exhibited elevated GlycA levels, indicative of low-grade systemic inflammation. Similarly, Nizamutdinova IT identified a strong link between hyperthyroidism, inflammatory responses, apoptosis, and activation of hypertrophy-related proteins. Hyperthyroidism

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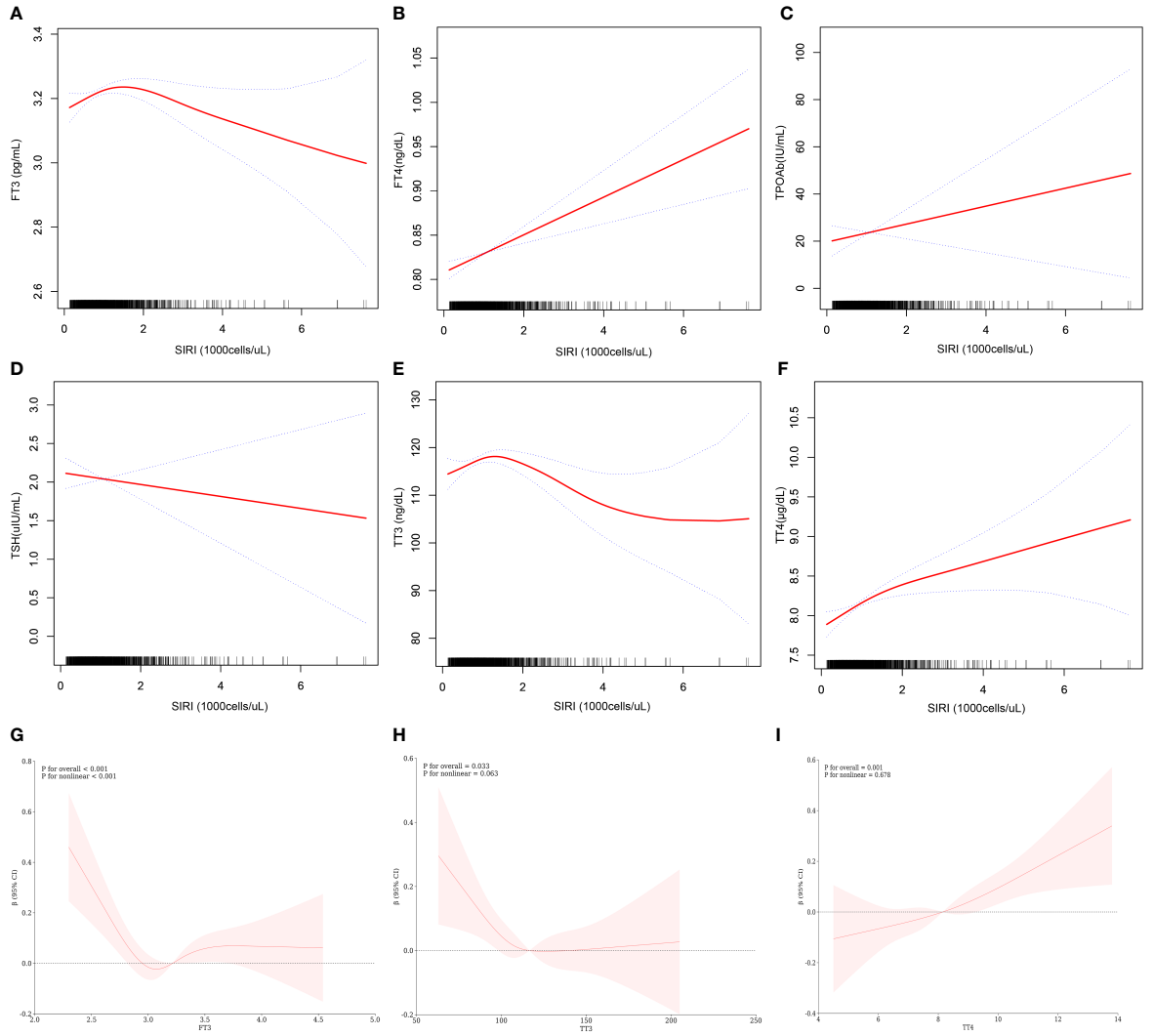


FIGURE 2
Relationship between SIRI and thyroid function. **(A–F)** is a curve-fit plot of SIRI versus thyroid function (FT3, FT4, TPOAb, TSH, TT3, and TT4). **(G–I)** is a restricted cubic spline plot of SIRI versus FT3, TT3, and TT4.

triggers NF-kB activation, escalating NO, lipid peroxidation, and free radical production. This results in a marked increase in TNF-a, IL-1b, IL-4, IL-6, and IL-10 levels (5, 11). A meta-analysis further confirmed significantly higher serum CRP levels in hypothyroid patients (12), corroborating previous findings (13). It has been demonstrated that obesity-induced adipose tissue expansion provides many internal signals that may trigger an inflammatory response (e.g. adipocyte death, hypoxia, and mechanical stress). A

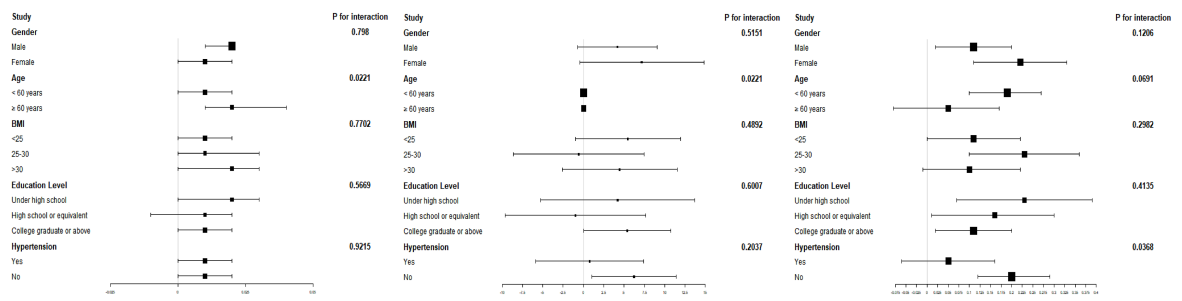


FIGURE 3
Subgroup analysis of SIRI and thyroid function.

cohort study verified that overweight and obesity are linked to elevated free T3 levels. Altered thyroid function in these patients increases energy expenditure, aiding in weight loss and preventing further weight gain. Consequently, TSH and FT3 normalization post-weight loss may contribute to the challenges in maintaining weight loss (14). Given the association between inflammation, abnormal thyroid function, and the reliability of SIRI as an inflammation indicator, a positive correlation between SIRI and thyroid function appears likely.

Prior epidemiological studies indicate that hypertension, obesity, and gender significantly impact thyroid function. Ladan Mehran et al. found that hypertension diminishes TH sensitivity (15). Research has also linked obesity to a heightened risk of hypothyroidism (16), possibly due to obesity's nature as a chronic, low-grade inflammatory state. In this state, inflammatory markers such as interleukin-1 (IL-1), IL-6, and tumor necrosis factor-alpha (TNF-α), produced by overloaded adipose tissue, increase (17). Estrogen, a key growth factor for thyroid cells, promotes growth through genomic and non-genomic pathways via the membrane-bound estrogen receptor. This receptor is associated with the MAPK and PI3K tyrosine kinase signaling pathways (18), potentially explaining the higher prevalence of thyroid diseases in women compared to men. According to our subgroup analysis, there was a significant SIRI with FT4 in age (P for interaction = 0.0221), SIRI with TT4 in hypertension (P for interaction = 0.368), and SIRI with TPOAb in age (P for interaction = 0.0221). These findings suggest that the relationship between the SIRI and thyroid function is influenced by hypertension. Hyperthyroidism has been linked to increased cardiovascular disease risk and endothelial dysfunction (19). It may also lead to secondary systolic hypertension through elevated cardiac output and increased renin, angiotensin, and aldosterone levels. Notably, a significant positive correlation between SIRI and FT4, TT4, and TPOAb persists in nonhypertensive subgroups, underscoring its relevance in this population.

The underlying mechanism driving the positive correlation between the SIRI and thyroid hormones FT4, TT4, and TPOAb remains elusive. However, existing research sheds light on this association. It's established that thyroid diseases prompt alterations in TH and related regulatory hormones. For instance, hyperthyroidism results from an excessive release of TH (T3 and T4), leading to protein, lipid, and DNA damage due to stress, increased basal metabolic rate, and oxygen consumption in body tissues (20). HT stems from an autoimmune attack on thyroid tissue, characterized by lymphocytic infiltration, fibrosis, and interstitial atrophy. RET/PTC rearrangements, implicated in HT, activate pathways that induce both HT and the expression of genes like CXCL5 and CXC chemokine receptor 2 (CXCR2), linked to inflammation and tumor invasion (21). Furthermore, Galdiero (22) et al. observed that in thyroid cancer patients, neutrophils upregulate CD 11b and CD 66b, correlating tumor size. Collectively, these findings strongly suggest a link between thyroid dysfunction and inflammation. SIRI emerges as a comprehensive marker of the body's inflammatory and immune response, surpassing traditional parameters like the neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR) in predictive capability. This extends to tumor prognosis, cardiovascular disease, chronic obstructive pulmonary disease, and COVID-19. Yuxiu Yang et al.'s study with 1527 patients with moderate coronary artery stenosis exemplifies SIRI's superior performance over conventional cardiovascular risk models (23). Multiple studies corroborate these findings, highlighting SIRI's reliability and potential applications. In our study, accounting for confounding factors, we identified a positive correlation between FT4, TT4, and TPOAb, indicating SIRI's role in the pathogenesis and progression of thyroid disorders. Thus, monitoring SIRI levels could provide essential insights for therapeutic decision-making and tracking disease progression in thyroid diseases. This study paves the way for further investigation and underscores the role of inflammation in thyroid disorders.

The robust sample size and thorough adjustment for covariates enhance the credibility and representativeness of our study. Nevertheless, it is not without limitations. The cross-sectional design precludes causal inference, necessitating future prospective studies with larger cohorts to clarify causality. Our reliance on data from a public dataset, NHANES, meant we could not modify the study design. While we accounted for several covariates, potential confounders, such as medication use (e.g., steroids), could still have influenced the outcomes. Unfortunately, NHANES did not collect data on these variables, restricting our ability to incorporate them into our analysis.

Furthermore, as NHANES is a U.S.-based study, our evaluation was confined to the association between SIRI and thyroid function in the American adult population. The limitation to a single-country sample with restricted ethnic diversity hinders the generalizability of our findings. In summary, while our study indicates a correlation between elevated SIRI levels and increased FT4, TT4, and TPOAb levels, and a nonlinear relationship among FT3, TT3, and TT4, further research is essential for validation.

Ethics Approval

The studies involving human participants were reviewed and approved by the ethics review board of the National Center for Health Statistics.

Informed consent

The patients/participants provided their written informed consent to participate in this study.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

Author contributions

YZ: Writing – original draft, Data curation, Investigation. BW: Writing – review & editing, Conceptualization, Methodology. WH: Writing – review & editing, Supervision, Validation. BY: Writing – review & editing, Formal Analysis, Investigation. JC: Writing – review & editing, Validation. FA: Writing – review & editing, Validation.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Nomogram model based on preoperative clinical characteristics of unilateral papillary thyroid carcinoma to predict contralateral medium-volume central lymph node metastasis

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Objectives: To explore the preoperative high-risk clinical factors for contralateral medium-volume central lymph node metastasis (conMVCLNM) in unilateral papillary thyroid carcinoma (uPTC) and the indications for dissection of contralateral central lymph nodes (conCLN).

Methods: Clinical and pathological data of 204 uPTC patients who underwent thyroid surgery at the Hangzhou First People's Hospital from September 2010 to October 2022 were collected. Univariate and multivariate logistic regression analyses were conducted to determine the independent risk factors for contralateral central lymph node metastasis (conCLNM) and conMVCLNM in uPTC patients based on the preoperative clinical data. Predictive models for conCLNM and conMVCLNM were constructed using logistic regression analyses and validated using receiver operating characteristic (ROC) curves, concordance index (C-index), calibration curves, and decision curve analysis (DCA).

Results: Univariate and multivariate logistic regression analyses showed that gender ($P < 0.001$), age ($P < 0.001$), tumor diameter ($P < 0.001$), and multifocality ($P = 0.008$) were independent risk factors for conCLNM in uPTC patients. Gender ($P= 0.026$), age ($P = 0.010$), platelet-to-lymphocyte ratio (PLR) ($P =0.003$), and tumor diameter ($P = 0.036$) were independent risk factors for conMVCLNM in uPTC patients. A predictive model was established to assess the risk of conCLNM and conMVCLNM, with ROC curve areas of 0.836 and 0.845, respectively. The C-index, the calibration curve, and DCA demonstrated that the model had good diagnostic value.

Conclusion: Gender, age, tumor diameter, and multifocality are high-risk factors for conCLNM in uPTC patients. Gender, age, tumor diameter, and PLR are high-risk factors for conMVCLNM in uPTC patients, and preventive conCLN dissection should be performed.

KEYWORDS

papillary thyroid carcinoma, contralateral, central lymph node metastasis, plateletto-lymphocyte ratio, nomogram

1 Introduction

Thyroid papillary carcinoma (PTC) is the most common type of differentiated thyroid cancer. Central lymph node metastasis (CLNM) is very common in PTC patients (1), and it is one of the important factors affecting recurrence and survival (2, 3). Clinical practice guidelines (4–6) recommend prophylactic central lymph node dissection for unilateral papillary thyroid carcinoma (uPTC); however, at present, guidelines recommend only routine ipsilateral central lymph node dissection (CLND) (3, 7). Patients with uPTC may present with contralateral central lymph node metastasis (conCLNM), with an incidence ranging from 3.88% to 34.6% (8, 9). For these patients, if dissection is not performed, there may be a risk of recurrence, which can affect patient prognosis; however, routine dissection significantly increases the chances of parathyroid dysfunction, with a possibility of permanent hypoparathyroidism (10, 11), and nerve damage. Therefore, it is important to rule out conCLNM.

Stratification based on the diameter of metastatic lymph nodes can effectively predict the prognosis of patients. The 2015 American Thyroid Association(ATA) guidelines define small-volume lymph node metastasis as clinically node-negative (cN0) or ≤5 metastatic lymph nodes with a diameter of <0.2 cm, whereas the guidelines define large-volume lymph node metastasis as metastatic lymph nodes with a diameter of ≥3 cm. However, there is no accepted definition for lymph node metastasis types of > 5 lesions and a diameter of <3 cm or <5 lesions with a diameter of ≤0.2 cm. It has been reported that this type of lymph node metastasis, defined as medium-volume lymph node metastasis, significantly positively impacts disease the recurrence (12, 13). However, the recommendation based on existing diagnostic and therapeutic guidelines, which only suggest ipsilateral CLND, may miss metastatic lymph nodes in these patients.

Studies have reported that preoperative blood immune indicators, such as the neutrophil-to-lymphocyte ratio (NLR), lymphocyte-to-monocyte ratio (LMR), platelet-to-lymphocyte ratio (PLR), and systemic immuno-inflammatory index (SII), can be used to evaluate immune function, characterize tumors, and predict tumor progression (14, 15). It has been reported that NLR, PLR and MLR can have good sensitivity and accuracy in predicting lymph node metastasis in differentiated thyroid cancer (16). In addition, the increase of PLR is associated with lymph node metastasis (17).However, the correlation between blood immune indicators and conCLNM or contralateral medium central volume lymph node metastasis (conMVCLNM) in uPTC remains unclear. This study seeks to explore the relationship between the preoperative clinical features of uPTC patients with conCLNM or conMVCLNM to provide a basis for accurate diagnosis and personalized treatment.

2 Materials and methods

2.1 Study patients

A retrospective analysis of clinical data was conducted on 204 patients from September 2010 to October 2022 at Hangzhou First People's Hospital. The patients were divided into two groups based on the presence of conCLNM (50 cases with conCLNM, and 154 cases without conCLNM) or conMVCLNM (26 cases with conMVCLNM, and 178 cases without conMVCLNM) (Table 1).

2.2 Inclusion and exclusion criteria

The inclusion criteria were as follows: (1) patients who were postoperatively diagnosed with unilateral papillary thyroid carcinoma, (2) patients who underwent at least total thyroidectomy and bilateral central lymph node dissection, and (3) patients with complete clinical and pathological information. The exclusion criteria were as follows: (1) patients with pathological evidence of malignant PTC which was not treated by lymph node dissection, (2) patients with

Abbreviations: ATA, American Thyroid Association; CLNM, central lymph node metastasis; conMVCLNM, contralateral medium-volume central lymph node metastasis; conCLNM, contralateral central lymph node metastasis; CLND, central lymph node dissection; CLNM, central lymph node metastasis; C-index, concordance index; DCA, decision curve analysis; ETE, extrathyroidal extension; LMR, lymphocyte-to-monocyte ratio; NLR, neutrophil-to-lymphocyte ratio; PTC, papillary thyroid cancer; PLR, platelet-to-lymphocyte ratio; ROC, receiver operating characteristic; SII, systemic immuno-inflammatory index; uPTC, unilateral papillary thyroid carcinoma.

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TABLE 1 General characteristics of PTC in conCLNM and conMVCLNM.

		Without conCLNM	With conCLNM	P value	Without conMVCLNM	With conMVCLNM	P value
All		154	50		178	26	
Gender	Male	17(11.0%)	24(48.0%)	<0.001	27(15.2%)	14(46.2%)	0.001
	Female	137(89.0%)	26(52.0%)		151(84.8%)	12(53.8%)	
Age	M(IQR)	51.000 (40.000,57.000)	35.000 (30.000,49.000)	<0.0001	49.000 (38.000,57.000)	35.000 (29.000,46.000)	<0.001
Tumor diameter(mm)	M(IQR)	9.000(5.000,15.000)	12(9.000,26.500)	<0.001	9.500 (6.000,15.000)	15.000 (9.000,30.000)	0.009
PLR	M(IQR)	129.060 (104.349,161.648)	97.772 (74.286,130.769)	0.002	127.071 (102.778,161.429)	84.526 (66.296,103.103)	<0.001
NLR	M(IQR)	1.720 (1.430,2.290)	1.570 (1.200,2.130)	0.272	1.720 (1.400,2.290)	1.340 (1.070,1.770)	0.108
MLR	M(IQR)	0.180 (0.140,0.250)	0.165 (0.130,0.230)	0.257	0.180 (0.140,0.250)	0.140 (0.130,0.250)	0.356
SII	M(IQR)	391.925 (289.710,509.440)	335.600 (248.750,476.860)	0.238	391.925 (282.180,509.440)	310.745 (239.480,388.270)	0.073
Multifocality	Yes	23(14.9%)	17(34.0%)	0.003	32(18.0%)	8(30.8%)	0.125
	No	131(85.1%)	33(66.0%)		146(82.0%)	18(69.2%)	
Capsule invasion	Yes	61(39.6%)	31(62.0%)	0.006	77(43.3%)	15(57.7%)	0.167
	No	93(60.4%)	19(38.0%)		101(56.7%)	11(42.3%)	
Extrathyroidal extension	Yes	42(27.3%)	20(40.0%)	0.089	52(29.2%)	10(38.5%)	0.338
	No	112(72.7%)	30(60.0%)		126(70.8%)	16(61.5%)	
PTMC	Yes	64(41.6%)	29(58.0%)	0.043	78(43.8%)	15(57.7%)	0.185
	No	90(58.4%)	21(42.0%)		100(56.2%)	11(42.3%)	
Lesion location	Down	45(29.2%)	20(40.0%)	0.155	51(28.7%)	14(53.8%)	0.010
	Non-down	109(70.8%)	30(60.0%)		127(71.3%)	12(46.2%)	

conCLNM, contralateral central lymph node metastasis; conMVCLNM, contralateral medium-volume central lymph node metastasis; IQR, interquartile range; LMR, lymphocyte-to-monocyte ratio; M, median; NLR, neutrophil-to-lymphocyte ratio; PTMC, papillary thyroid microcarcinoma; PLR, platelet-to-lymphocyte ratio; SII, preoperative systemic immune-inflammatory index.

hyperthyroidism or those that underwent previous history of thyroid radiotherapy, or thyroid surgery, (3) patients with previous history of other malignancies, and (4) patients with hematological disorders, autoimmune disorders, acute or chronic inflammatory diseases, or other diseases that may affect routine blood tests. This study was approved by the Ethics Committee of Hangzhou First People's Hospital, and written informed consent was obtained from all patients.

2.3 Data acquisition

Peripheral blood was obtained from the patients one week prior to thyroid surgery and processed using the automated Mindary BC-6800 blood cell analyzer (Shenzhen Mairui Biomedical Electronics Co., Ltd., Shenzhen, China) along with appropriate reagents. Peripheral blood cells were classified and counted using a combination of a sheath flow impedance method, a laser light scattering method, and flow cytometry coupled with fluorescent staining techniques. The preoperative inflammatory indicators were included preoperative platelet count, neutrophil count, lymphocyte count, macrophage count, PLR, NLR,LMR, and platelet count × neutrophil count/lymphocyte count (SII).

The diagnostic apparatuses used for thyroid examinations were the Mylab 70 XVG and MyLab Twice Color Doppler ultrasound systems, with a probe frequency of 7 to 13 MHz. The instrument settings were optimized for this purpose. Patients were placed in a supine position to allow for full neck exposure. Upon identification of a suspicious malignant nodule, the two-dimensional ultrasound image features of the nodule were interpreted and recorded. Two experienced ultrasound physicians assessed the preoperative tumor information by ultrasonography, including tumor diameter, capsule invasion, extrathyroidal extension, multifocality, and lesion location.

The ultrasound images included in this study were reviewed and preserved by two senior physicians who were part of the same team. They were also evaluated by the chief sonographer. Simultaneously, all members reading the ultrasounds blinded to the final pathology results. The diagnostic criterion for capsular invasion was the disappearance of the echogenic thyroid capsule at the site of contact with the thyroid cancer on ultrasound examination (Figure 1A). The diagnostic criterion criteria for extrathyroidal

invasion was destruction or the invasion of adjacent structures at the edge of the thyroid capsule on ultrasound examination (Figure 1B). The ultrasound evaluation criteria of lymph node metastasis (one or more of the following high-risk factors are considered lymph node metastasis) were as follows: 1)focal or diffuse hypoechogenicity; 2) ratio of the short axis to long axis ≥0.5; 3)lymphatic echogenic hilus absence; 4) cystic degeneration; 5) microcalcifications; and 6) chaotic vascular flow (18–20). The presence of lymph node metastasis and the maximum diameter of the metastatic foci in the lymph nodes were accurately determined by two pathologists with clinical research experience by microscopy (Figure 2).

2.4 Statistical analyses

Data were analyzed using SPSS software (version 25.0; IBM, Armonk, NY, USA). For measurement data, we conducted the normality test and presented the results as mean ± standard deviation (± S). Nonconforming data was expressed as the medians and interquartile ranges. To compare two independent groups, we used the Student's t-test. Categorical data were expressed as frequency and percentage, and group comparisons were performed using the chi-square (X^2) test. By utilizing univariate analyses, we identified the risk factors associated with conCLNM/conMVCLNM in PTC patients by pinpointing the influencing factors that exhibited statistically significant differences.

We utilized logistic multivariate stepwise regression analysis to examine the risk factors identified through univariate regression analysis. In addition, we analyzed the differences in preoperative blood inflammatory indexes and ultrasound features of conCLNM/conMVCLNM and identified the independent risk factors that impact conCLNM/conMVCLNM in PTC patients. We determined the odds ratios (OR) and 95% confidence intervals (95% CI).

Based on the results of logistic multivariate regression analysis, we constructed a predictive model. Receiver operator characteristic (ROC) curve analysis was employed to assess the area under the curve (AUC) and its 95% CI. We calculated the Youden index and identified the sensitivity and specificity at its highest value. To validate the predictive model, we conducted validation through bootstrap sampling. We evaluated the models using the AUC, 95% CI, sensitivity, and specificity. Additionally, we created a calibration plot. Decision curve analysis (DCA) was employed to validate the clinical net benefit rate of the predictive model. The statistical analysis was performed using R studio (version 4.0.2). We considered $P < 0.05$ as statistically significant.

3 Results

3.1 Clinical characteristics and basic information

According to the inclusion and exclusion criteria, a total of 204patients were enrolled. In the conCLNM group, among the 50 patients with conCLNM, there were 24 males (48.0%) and 26 females (52.0%), with a mean age of 39.400 ± 15.534 years. In conMVCLNM group, among the 26 patients with conMVCLNM, there were 14 males (53.8%) and 12 females (46.2%), with a mean age of 37.692 ± 17.022 years. The general characteristics of the patients are shown in Table 1.

3.2 Univariate and multivariate analyses for preoperative conCLNM and conMVCLNM variables

In the conCLNM group, univariate analysis showed that gender, age, preoperative PLR, papillary thyroid microcarcinoma (PTMC), multifocality, capsule invasion, and maximum tumor diameter were risk factors for conCLNM. Multivariable logistic regression analysis showed that gender ($P < 0.001$, OR: 7.547, 95% CI: 3.227–17.653), age ($P < 0.001$, OR: 0.952, 95% CI: 0.925–0.980), tumor diameter ($P < 0.001$, OR: 1.068, 95% CI: 1.027–1.110), and multifocality ($P = 0.008$, OR: 3.375, 95% CI: 1.383–8.232) were independent risk factors for conCLNM. In the conMVCLNM group, univariate analysis showed that gender, age, preoperative PLR, lesion location and tumor diameter were significantly correlated with conMVCLNM ($P < 0.05$). Multivariable logistic regression analysis showed that gender ($P = 0.026$, OR: 3.251, 95% CI: 1.148–9.204), age ($P = 0.010$, OR: 0.954, 95% CI: 0.921–0.982), preoperative PLR ($P = 0.003$, OR: 0.977, 95% CI: 0.962–0.992), and

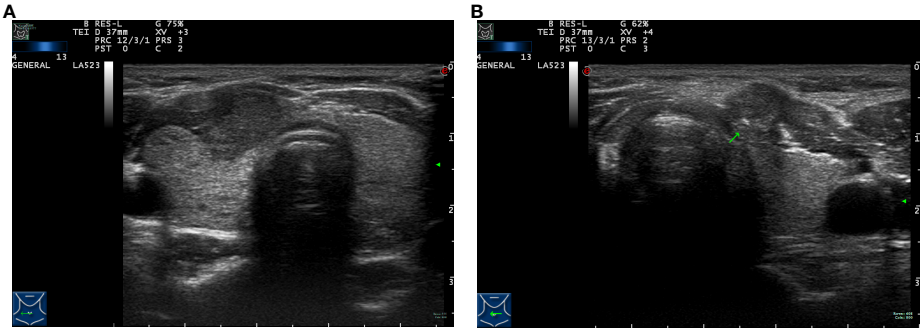


FIGURE 1
Preoperative ultrasound images of tumor lesions. **(A)** Capsule invasion. **(B)** Extrathyroidal extension (arrow).

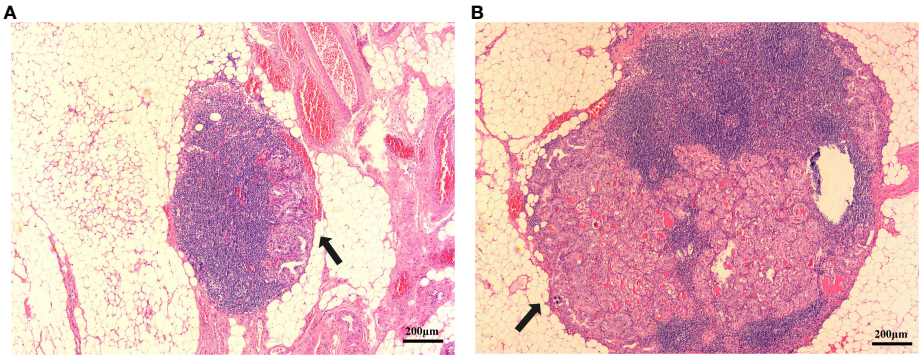


FIGURE 2
PTC metastatic lymph node lesion diameter. **(A)** Metastatic lymph node lesion diameter is <0.2 cm(arrow). **(B)** Metastatic lymph node lesion diameter is >0.2 cm. (hematoxylin and eosin; ×40) (arrow).

tumor diameter (P = 0.036, OR: 1.049, 95% CI: 1.003–1.096) were independent risk factors for conMVCLNM (Table 2).

3.3 Construction of the nomogram

The results screened by multivariable logistic regression analysis were incorporated into the prediction model for distinguishing conCLNM and conMVCLNM in uPTC patients (Figure 3). The score for each independent predictive factor was plotted and summed continuously to obtain a total score, which determined the likelihood of developing conCLNM or conMVCLNM.

3.4 Evaluation of the nomogram

The results of ROC curve analysis showed that the AUCs of the four risk factors (gender, age, tumor diameter, and multifocality) in predicting conCLNM in uPTC patients was 0.829 (P < 0.001, 95% CI: 0.756–0.902). When the Youden index was highest, the specificity was 76.0% and the sensitivity was 83.8%, indicating good discrimination (Figure 4A). The AUCs of the four risk factors (age, gender, PLR and tumor diameter) in predicting conMVLNM was 0.854 (P < 0.001, 95% CI: 0.780–0.929). When the Youden index was highest, the specificity was 65.7% and the sensitivity was 92.3% (Figure 4B).

The original dataset was resampled 1000 times using bootstrapping to establish the simulation dataset. The calibration curve demonstrated good consistency between the discrimination of the prediction model and the actual distinction between conCLNM and conMVCLNM. The mean absolute errors in the calibration curve were 0.02 and 0.055 for conCLNM and conMVCLNM, respectively, indicating good consistency between the predicted and actual values (Figure 5).

The normal range of the concordance index (C-index) is typically between 0.5 and 1, with 0.5 indicating a model with poor discrimination and 1 indicating a model with perfect discrimination. A C-index >0.7 indicates a model with strong discrimination. The C-indices for conCLNM and conMVCLNM were 0.836 and 0.845, respectively, indicating models with excellent

TABLE 2 Univariate logistic regression analysis of uPTC in conCLNM and conMVCLNM.

	B	SE	Wals	Sig.	Exp (B)	95% CI
conCLNM						
Gender	2.021	0.434	21.736	<0.001	7.547	3.227–17.653
Age	-0.049	0.015	11.729	<0.001	0.952	0.925–0.980
Tumor diameter (mm)	0.065	0.020	10.724	<0.001	1.068	1.027–1.110
Multifocality	1.216	0.455	7.146	0.008	3.375	1.383–8.232
conMVCLNM						
Gender	1.179	0.531	4.929	0.026	3.251	1.148–9.204
Age	-0.047	0.018	6.668	0.010	0.954	0.921–0.982
PLR	-0.023	0.008	8.603	0.003	0.977	0.962–0.992
Tumor diameter (mm)	0.048	0.023	4.404	0.036	1.049	1.003–1.096

B, Beta coefficient; CI, Confidence interval; conCLNM, contralateral central lymph node metastasis; conMVCLNM, contralateral medium-volume central lymph node metastasis; PLR, platelet-to-lymphocyte ratio; SE, Standard error of the mean; Sig, Statistical significance.

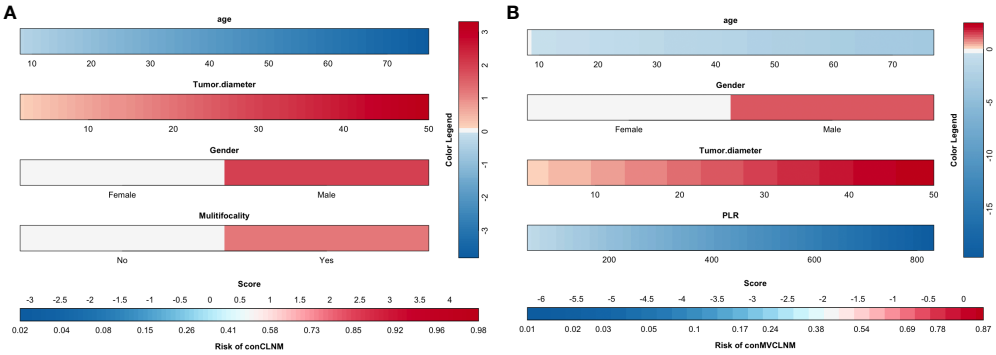


FIGURE 3
Clinical prediction models for conCLNM and conMVCLNM. Prediction model based on clinical factors **(A)** conCLNM and **(B)** conMVCLNM. conCLNM, contralateral central lymph node metastasis; conMVCLNM, contralateral medium volume central lymph node metastasis; uPTC, unilateral papillary thyroid carcinoma; PLR, platelet-to-lymphocyte ratio.

discrimination. DCA demonstrated the effectiveness of the nomograms for both groups across a wide range of threshold probabilities (Figure 6).

4 Discussion

Our study reports that age, preoperative PLR, tumor diameter, and lesion location are independent risk factors for conMVCLNM in uPTC patients. Our model effectively identifies conMVCLNM and avoids missed diagnosis or overdiagnosis to achieve the purpose of accurate diagnosis and treatment.

Based on the results of this study, we found that clinical features can effectively predict conCLNM or conMVCLNM. Other studies have reached similar conclusions; for example, a meta-analysis by Sun et al. (21) showed that gender, age, and extrathyroidal extension (ETE) are risk factors for conCLNM in uPTC. At the same time, another investigation has found that postoperative pathological features, such as multifocality and tumor diameter, are independent risk factors for conCLNM (22). However, all of these studies were retrospective in nature, and they only screened high-risk factors from postoperative pathology and failed to provide effective guidance before surgery, which needs to be improved in terms of the clinical application. In our study, the risk of conCLNM in uPTC could be predicted by preoperative indicators, and this approach is clinically practical. Additionally, in the prospective study by Chen et al. (8), bilateral CLND is recommended when preoperative imaging or intraoperative frozen pathological results suggest conCLNM. However, this approach has obvious limitations in clinical practice due to the unique structure of central lymph nodes and the difficult-to-control waiting time of frozen section pathological results during surgery. Although our study is retrospective in nature, this study not only included general clinical characteristics such as sex and age, which were easily obtained, combined with ultrasound characteristics such as tumor size and location on preoperative imaging, but also used the same

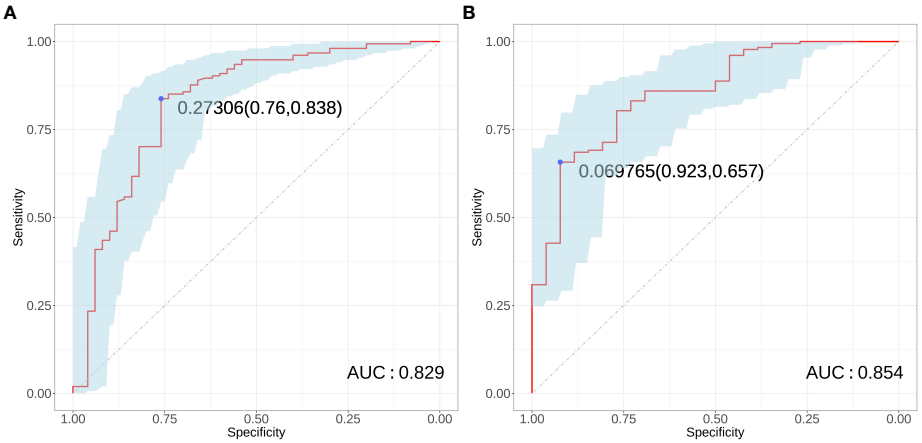


FIGURE 4
Receiver operator characteristic curve for conCLNM and conMVCLNM. Receiver operating characteristic curves **(A)** conCLNM and **(B)** conMVCLNM. conCLNM, contralateral central lymph node metastasis; conMVCLNM, contralateral medium volume central lymph node metastasis; uPTC, unilateral papillary thyroid carcinoma; ROC, receiver operator characteristic.

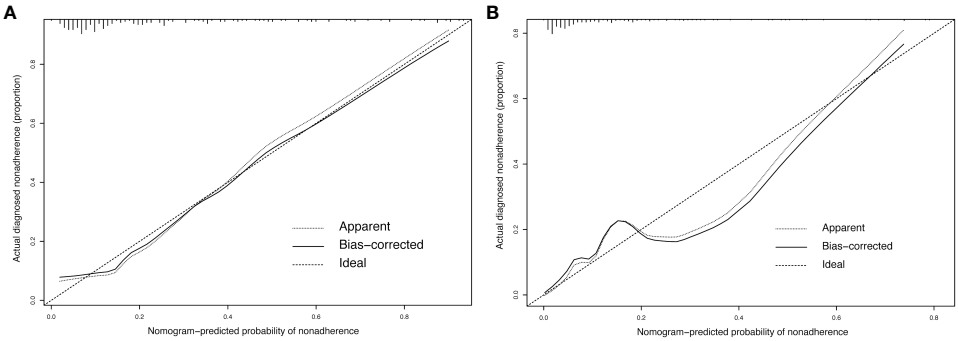


FIGURE 5
Calibration curve for conCLNM and conMVCLNM. Calibration curve **(A)** conCLNM and **(B)** conMVCLNM. The X-axis represents the predicted probability of conCLNM or conMVCLNM, whereas the Y-axis represents the actual probability of diagnosed conCLNM and conMVCLNM. The diagonal dashed line represents the ideal prediction model. The solid line represents the performance of the column chart model, with a higher fit to the diagonal dashed line indicating better predictive capability. conCLNM, contralateral central lymph node metastasis; conMVCLNM, contralateral medium volume central lymph node metastasis.

immunological indicators that are more valuable in tumors, especially thyroid tumors, that can be conveniently obtained before surgery. All of these have greatly contributed to the clinical application of this approach. In recent years, several studies have shown that immune function, inflammation, and tumorigenesis are closely related. Ceylan et al. (14) found that both NLR and PLR are related to the aggressive biological behavior of PTC and can be used as biomarkers for the risk assessment of PTC patients. Meanwhile, Zhao et al. (23) discovered that the integration of the preoperative SII index and the tumor diameter is a reliable predictor of lymph node metastasis in patients with PTC. However, despite the inclusion of numerous inflammatory indicators, this study did not observe significant predictive effects of SII, NLR, and MLR in terms of conCLNM or conMVCLNM. This limitation may be attributed to the insufficient sample size, which failed to comprehensively elucidate the issue at hand, and thus necessitates future investigations with larger sample sizes. Furthermore, Huang et al. (24) demonstrated that the reduction of PLR poses a potential risk for recurrence in patients with intermediate and high-risk PTC. Similarly, Li et al. (25) conducted a comparable investigation and revealed that combining PLR and other inflammatory indicators with clinical characteristics significantly aids in predicting the recurrence risk of PTC. The current study also identified PLR as a risk factor for conCLNM or conMVCLNM, with a higher PLR ratio correlating with an increased likelihood of conCLNM. However, further research is required to elucidate the specific underlying mechanism.

In addition, in recent years, the definition of nodal volume metastasis has been revised to refer to the number of metastatic lymph nodes. For example, the meta-analysis of Wang et al. (26) defined high-volume lymph node metastasis in PTC as involving >5 lymph nodes. At the same time, Huang et al. (27) and Zhu et al. (28) have adopted the same definition of large-volume lymph node metastasis. However, the objective of these studies was confined to the quantification of metastatic lymph nodes, with only a few reports incorporating the assessment of metastatic lymph node

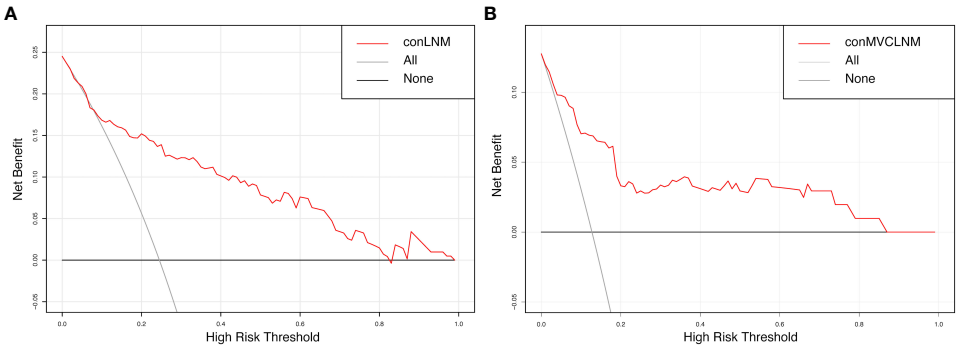


FIGURE 6
Decision curve analysis for conCLNM and conMVCLNM. Decision curve analysis **(A)** conCLNM and **(B)** conMVCLNM. The red line represents the prediction model. The grey line represents the scenario where all patients are classified as conCLNM or conMVCLNM positive. The horizontal black line represents the scenario where all patients are classified as conCLNM/conMVCLNM negative. conCLNM, contralateral central lymph node metastasis; conMVCLNM, contralateral medium volume central lymph node metastasis.

diameter. Research has demonstrated that lymph node metastases >2 mm in diameter substantially impact the likelihood of recurrence in patients diagnosed with PTC (29). According to the guidelines established by the ATA, the risk of recurrence is notably elevated in PTC patients presenting with <5 metastatic lymph nodes that are >2 mm in diameter.

The definition of medium-volume metastatic lymph nodes, as presented in this study, aligns more closely with the clinical practice guidelines. Within our study cohort of 260 patients, 34 patients were identified as having conMVCLNM. Among these patients, 27 had conCLNM with <5 metastatic lesions, yet the diameter of the metastatic lesions >2 mm. By only considering the number of metastatic lymph nodes, physicians may overlook these cases, resulting in a notable 10.4% (27/260) increase in the risk of recurrence. Therefore, in contrast to previous studies, our research carries greater significance in informing and improving practical clinical practices. This is because our approach exhibits improved effectiveness in assessing the probability of conMVCLNM in patients with uPTC and provides more effective treatment insights to enhance their prognosis.

Furthermore, previous studies have indicated that the predictive accuracy of conCLNM and conMVCLNM is relatively low. This may be attributed to the limited efficacy of the direct diagnosis of CLNM in clinical practice. For example, Kim et al. (30) found that ultrasound had a sensitivity of only 17.3% in detecting CLNM, whereas computed tomography had a sensitivity of only 23.5%. Additionally, certain clinicopathological characteristics, including gender, age, and tumor lesion information, have been shown to be effective predictors of CLNM. Feng et al. (31) developed a prediction model for conCLNM in PTC by incorporating lesion location, ETE, and other factors. The model predicted an area under the curve (AUC) of 0.754 for conCLNM. By contrast, the AUC of this study was found to be 0.837 (95% CI 0.759–0.916), with a sensitivity of 78.8% and a specificity of 79.4%, which were significantly higher than previous results. Additionally, the prediction model offers a visual representation of the results, serving as a valuable clinical tool for thyroid surgeons in formulating surgical plans. Therefore, it is recommended for widespread adoption and implementation.

Our study has the following limitations. First, the sample size of this study is not large enough. In the future, we will further expand the sample size to improve the efficiency of predicting conMVCLNM. Second, this study is a retrospective study, and there is a certain selection bias in the sample selection. We hope that prospective studies will be conducted in the future. Third, preoperative immune indicators are affected by various factors, such as inflammation, immune system diseases, and other interferences. In the future, we will add more preoperative clinical pathology information, such as genetic diagnostic information, to effectively establish a predictive model for conMVCLNM before surgery.

In conclusion, our study provides an effective method for clinically identifying uPTC patients with conMVCLNM. When uPTC patient characteristics are combined with high-risk factors, such as age, gender, tumor diameter, and PLR, contralateral central lymph node dissection is strongly recommended.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

This study was approved by Ethics Committee of Hangzhou First People's Hospital and written informed consent was obtained from all patients.

Author contributions

FW: Validation, Writing – original draft, Writing – review & editing. YT: Conceptualization, Data curation, Writing – review & editing. YZ: Investigation, Methodology, Supervision, Writing – review & editing. KH: Formal analysis, Project administration, Writing – review & editing. XH: Formal analysis, Project administration, Writing – review & editing. TP: Methodology, Supervision, Writing – original draft. JS: Data curation, Methodology, Writing – original draft. JD: Data curation, Formal analysis, Writing – review & editing. GP: Data curation, Methodology, Writing – review & editing. YP: Data curation, Formal analysis, Writing – review & editing. LZ: Investigation, Validation, Writing – review & editing. DL: Conceptualization, Data curation, Writing – original draft. YL: Validation, Writing – original draft, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Tyrosine kinase inhibitors in patients with advanced anaplastic thyroid cancer: an effective analysis based on real-world retrospective studies

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Background: Tyrosine kinase inhibitors (TKIs) contribute to the treatment of patients with anaplastic thyroid cancer (ATC). Although prospective clinical studies of TKIs exhibit limited efficacy, whether ATC patients benefit from TKI treatment in real-world clinical practice may enlighten future explorations. Therefore, we conducted this effective analysis based on real-world retrospective studies to illustrate the efficacy of TKI treatment in ATC patients.

Methods: We systematically searched the online databases on September 03, 2023. Survival curves were collected and reconstructed to summarize the pooled curves. Responses were analyzed by using the "meta" package. The primary endpoints were progression-free survival (PFS), overall survival (OS), objective response rate (ORR), and disease control rate (DCR).

Results: 12 studies involving 227 patients were enrolled in the study. Therapeutic strategies included: anlotinib, lenvatinib, dabrafenib plus trametinib, vemurafenib, pembrolizumab plus dabrafenib and trametinib, pembrolizumab plus lenvatinib, pembrolizumab plus trametinib, and sorafenib. The pooled median OS and PFS were 6.37 months (95% CI 4.19-10.33) and 5.50 months (95% CI 2.17-12.03). The integrated ORR and DCR were 32% (95% CI 23%-41%) and 40% (95% CI 12%-74%).

Conclusion: In real-world clinical practice, ATC patients could benefit from TKI therapy. In future studies, more basic experiments and clinical explorations are needed to enhance the effects of TKIs in the treatment of patients with ATC.

KEYWORDS

anaplastic thyroid cancer, tyrosine kinase inhibitor, response rates, survival outcomes, real-world retrospective analysis

Introduction

Most patients with anaplastic thyroid cancer (ATC) have an extremely poor prognosis and survive less than half a year from the disease diagnosis, with a 1-year overall survival (OS) of 20% (1, 2). Chemotherapy, surgery, and radiotherapy are the classic treatments for ATC. In recent years, targeted therapy and immunotherapy have brought novel survival benefits in patients with advanced ATC (3). However, significantly prolonging the survival time can be the biggest challenge.

Tyrosine kinase inhibitors (TKIs) have greatly improved the survival outcomes of multiple malignant tumors. In patients with radioiodine-refractory differentiated thyroid cancer, TKI therapy (lenvatinib) has been certificated to be associated with prolonged OS (HR 0.65, 95% CI 0.52-0.81) and progression-free survival (PFS) (HR 0.24, 95% CI 0.19-0.31) compared with placebo (4). Moreover, TKI therapy significantly improved the OS and PFS in different types of thyroid cancer (5). Nevertheless, a significant breakthrough of TKIs in ATC has not come (1). In the published prospective clinical trials, clinics have tried to detect the efficacy of TKIs in ATC patients. Several issues deserve our attention. Huan and Panayiotis's trials were terminated due to poor accrual, showing a 6-month survival of 36% for imatinib and a median PFS of 1.9 months for sorafenib (6, 7); Keith's trial was halted because a stopping rule was triggered, with a median PFS of 62 days and a median OS of 111 days (8); Lori's trial was halted due to the unmet response rate threshold, and reported a median PFS of 2.6 months and a median OS of 3.2 months (9). Although Takuya's trial finished, the final results were disappointing (1-year OS rate: 11.9%) (10).

Considering the above reasons, the data reported in prospective clinical trials might be incomplete, and the bias was made. Therefore, we are eager to detect the benefits of TKIs for patients with ATC in real-world clinical practice.

Similarly, owing to the low incidence rate of ATC, the effects of TKIs in ATC displayed in retrospective studies were also inconsistent. To comprehensively demonstrate the efficacy of TKIs in treating ATC patients, we reconstructed the patient-level survival data to show the survival curves directly and meta-analyzed the response rates in real-world retrospective studies.

Methods

This study was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) guideline (11).

Literature search

A systematic literature search was performed to identify real-world retrospective studies eligible for this analysis. The search was conducted in four online databases (PubMed, Web of Science, Embase, and Cochrane CENTRAL) on September 3, 2023.

The search terms were:

1. "anaplastic thyroid cancer or anaplastic thyroid carcinoma".
2. "tyrosine kinase inhibitor or sorafenib or lenvatinib or regorafenib or imatinib or cabozantinib or donafenib or apatinib".
3. "trial or study".

Inclusion and exclusion criteria

The inclusion criteria were as follows: (1) patients were diagnosed with ATC; (2) patients were treated with TKI-based therapy; (3) efficacy data were available (number of patients in a subgroup or overall population should be ≥ 10); (4) real-world retrospective studies were published in English. Meeting abstracts and case reports were excluded. The primary endpoints were PFS, OS, objective response rate (ORR), and disease control rate (DCR).

Data extraction

Two authors independently extracted the detailed data of the study design, number of patients, median age, therapeutic strategies, survival outcomes, and responses. In addition, reconstructed survival data were collected from published Kaplan-Meier curves using the Scanlt software (version 2.0.8.0).

Risk assessment

Funnel plots, sensitivity analysis, and Egger's tests were calculated to evaluate the publication bias.

Statistical analysis

R software (version 4.2.2) was applied to conduct the analyses. The pooled time-to-event survival data and response rates were analyzed by running the "metaSurvival" package and the "meta" package. A random-effects model was performed to reduce the heterogeneity.

Results

Through systematic searching, 1171 records were identified. After excluding 391 duplicate and 592 irrelevant records, 188 were available in full-text assessment. Finally, 12 published real-world retrospective studies with 227 patients were eligible for this analysis (Figure 1) (12–23).

Two of the 12 studies were multi-center studies (16, 23), while the other 10 were single-center studies. Therapeutic regimens comprised anlotinib, lenvatinib, dabrafenib plus trametinib, vemurafenib, pembrolizumab plus dabrafenib and trametinib,

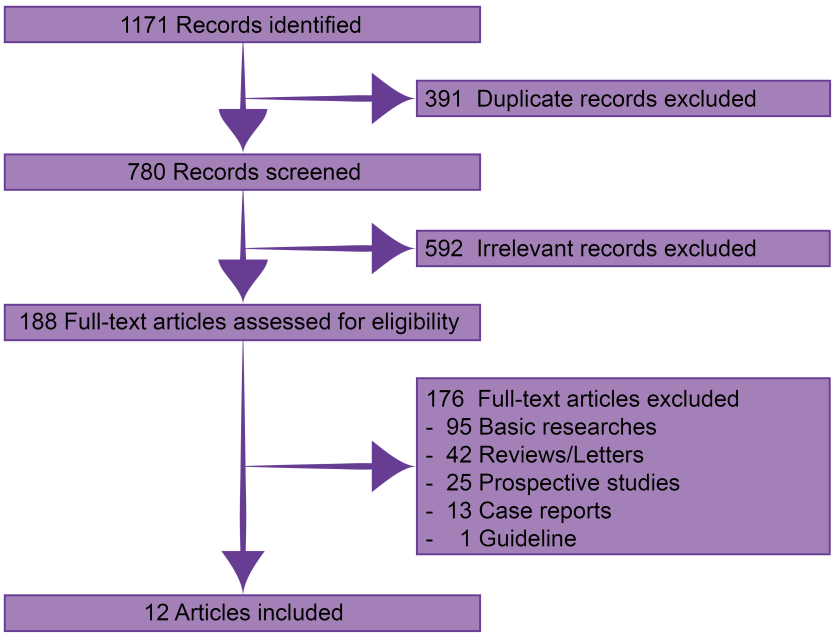


FIGURE 1
Flow diagram of the selecting process.

pembrolizumab plus lenvatinib, pembrolizumab plus trametinib, and sorafenib. The median or mean age was over 60 (Table 1).

Regarding PFS, patient-level data were extracted from four studies (12, 16, 21, 23). The pooled median PFS was 5.5 months (95% CI 2.17-12.03) (Figure 2). The 6-month, 1-year, 2-year, and 3-year PFS rates were 43.3%, 30.5%, 21.1%, and 8.0%, respectively.

Regarding OS, patient-level data were reconstructed from 10 studies (13–19, 21–23). The pooled OS was 6.37 months (95% CI 4.19-10.33) (Figure 3). The 6-month, 1-year, 2-year, and 3-year OS rates were 51.5%, 28.5%, 22.3%, and 11.8%, respectively.

For response rates, the overall ORR and DCR were 32% (95% CI 23-41) and 40% (95% CI 65-89) (Figure 4). Publication bias was not found according to the funnel plots, sensitivity analyses, and Egger's tests.

Discussion

In this analysis based on real-world clinical practice, we found that the median PFS was 5.50 months, the median OS was 6.37 months, the ORR was 32%, and the DCR was 40% in ATC patients treated with TKI-based therapy. Our results indicated that TKI treatment could be an effective therapy for ATC patients.

Chemotherapy has been playing a critical role in the treatment for ATC. Comparing TKI treatment with chemotherapy in ATC patients can be an interesting topic. In 2014, Julie A Sosa published a clinical trial and the data showed that the median OS was 4.0 months when ATC patients received paclitaxel and carboplatin (24). Another prospective study reported by Naoyoshi Onoda found that weekly paclitaxel administration in ATC patients showed a median OS of 6.7 months, with a 21% ORR (25). Moreover, in K B Ain's trial, a total response rate of 53% was achieved when patients were treated with 96-hour continuous infusion paclitaxel (26). Accordingly, the survival time of patients enrolled in our study was not superior to that of patients in the above trials.

Although our sensitivity analyses did not find any heterogeneity, patients in 10 of the 12 studies received lenvatinib-based therapy. In Priyanka C. lyer's study, ATC patients treated with dabrafenib plus trametinib showed a shorter median OS compared with patients treated with lenvatinib (7.4 months vs. 10.4 months) (21). For patients with BRAF V600E-mutant, dabrafenib plus trametinib treatment achieved a median OS of 14.5 months and a median PFS of 6.7 months (27). Therefore, confirming the accurate population of ATC patients who could benefit more from lenvatinib, dabrafenib plus trametinib, or other targeted therapy may become one of the future tasks.

By comparing our results with the data in prospective clinical trials, we noticed that the survival time reported in retrospective studies was longer than in prospective studies. In Panayiotis S's trial, patients were treated with sorafenib, and the median OS and PFS were 3.9 months and 1.9 months (6). In another trial reported by Lori W, the median OS and PFS of patients who received lenvatinib were 3.2 months and 2.6 months (9). We deduced that one of the most important reasons was that patients enrolled in prospective trials had been heavily treated with previous systemic therapy. In contrast, most patients collected in retrospective studies received targeted therapy as their first-line treatment. However, not all data reported in the prospective studies were disappointing. For example, in Shunji Takahashi's phase II study, ATC patients who received lenvatinib had a response rate of 24% and a median PFS of 7.4 months, revealing that only one patient experienced a treatment-related toxicity leading to discontinuation (28).

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TABLE 1 Basic characteristics of eligible retrospective studies.

Study	Publication year	Inclusion period	Design	No.patients	Age	Drug
Xucai Zheng (10)	2023	2017.07-2022.02	Single-center study	25	Median 65.01 (range 40-85)	Anlotinib
Hiroyuki Iwasaki (11)	2023	2011.04-2022.07	Single-center study	36	Median 72.33 (range 47-85)	Lenvatinib
Daisuke Murayama (12)	2022	2015.11-2021.05	Single-center study	26	Median 73 (IQR 65-79)	Lenvatinib
Jun Park (13)	2021	1995.11-2020.05	Single-center study	19	Mean 66.4 ± SD1.3	1. Dabrafenib plus trametinib 2. Lenvatinib 3. Vemurafenib
Mijin Kim (14)	2021	2016.08-2019.12	Multi-center study	14	Median 65.6 (IQR 59.7-72.1)	Lenvatinib
Haruhiko Yamazaki (15)	2020	2015.06-2017.08	Single-center study	12	Median 78 (range 63-89)	Lenvatinib
Hiroyuki Iwasaki (16)	2020	2011.01-2019.04	Single-center study	16	Mean 73 (range 47-89)	Lenvatinib
Soo Young Kim (17)	2020	2015.10-2018.02	Single-center study	18	Mean 64.9 (range42-86)	Lenvatinib
Hiroyuki Iwasaki (18)	2018	2015.04-2017.03	Single-center study	23	Median 77 (range 42-89)	Lenvatinib
Priyanka C. Iyer (19)	2018	2015.04-2016.05	Single-center study	16	Median 67 (range 55-82)	1. Lenvatinib 2. Dabrafenib plus trametinib
Priyanka C. Iyer (20)	2018	2016.08-2017.08	Single-center study	12	Median 60 (range 47-84)	Pembrolizumab plus 1. Dabrafenib plus trametinib 2. Lenvatinib 3. Trametinib
Yasuhiro Ito (21)	2017	2014.04-2014.09	Multi-center study	10	Median 72 (range 60-82)	Sorafenib

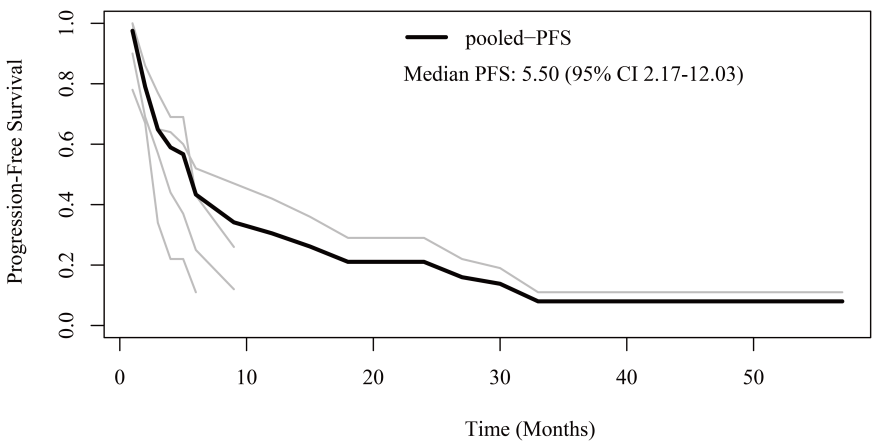


FIGURE 2
Reconstructed progression-free survival (PFS) curves of anaplastic thyroid cancer patients treated with tyrosine kinase inhibitor therapy. The dark line indicates the pooled PFS curve. The light lines are the PFS curves reconstructed from the four enrolled studies.

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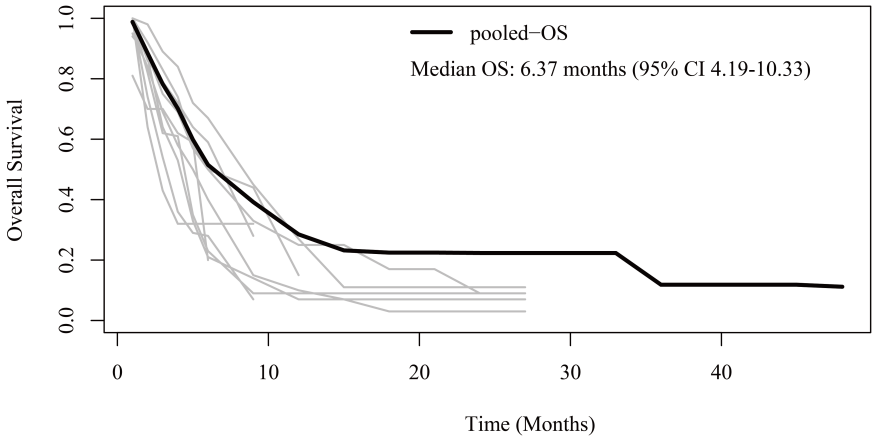


FIGURE 3
Reconstructed overall survival (OS) curves of anaplastic thyroid cancer patients treated with tyrosine kinase inhibitor therapy. The dark line indicates the pooled OS curve. The light lines are the OS curves reconstructed from the ten enrolled studies.

Immunotherapy has revolutionized the treatment of malignant tumors. In patients with ATC, anti-PD-L1 therapy showed positive results in the subset of patients with PD-L1 ≥ 50%, with a response rate of 35% (29). Combining anti-PD-L1 and anti-angiogenic targeted therapy has significantly improved the survival outcomes in cancer patients. For example, hepatocellular carcinoma, gastric cancer, and non-small cell lung cancer (30–32). Whether targeted therapy in combination with immunotherapy plays a novel efficacy in advanced ATC needs more future explorations.

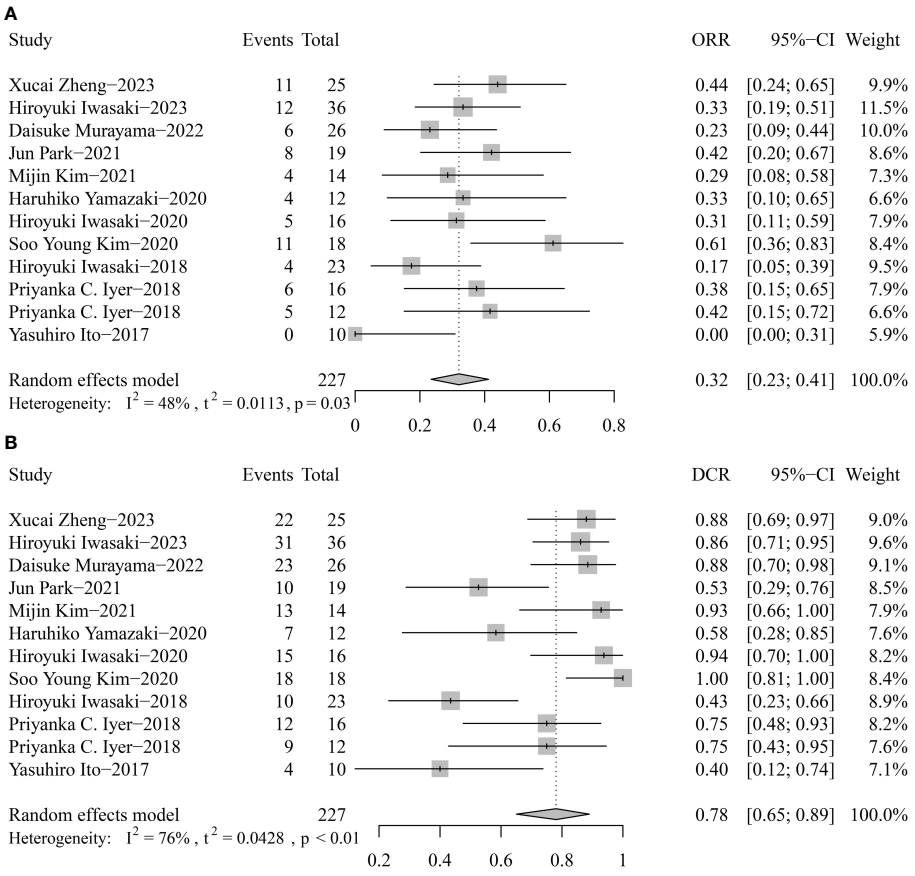


FIGURE 4
Pooled objective response rate (ORR, **A**) and disease control rate (DCR, **B**) of tyrosine kinase inhibitor therapy in anaplastic thyroid cancer patients.

In terms of treatment-related adverse events, most of the retrospective studies failed to report a comprehensive safety profile. For grade ≥ 3 adverse events, the highest incidence of TKI were hyponatremia (13% for dabrafenib and trametinib) (21), fatigue (20% for lenvatinib) (22), and hypertension (20% for lenvatinib) (23). However, we may not be sure whether the adverse events recorded are accurate because of the long inclusion period in retrospective studies. Accordingly, we reviewed the adverse events of TKIs reported in the prospective studies. The most incidence of grade ≥ 3 adverse events were loss of appetite for lenvatinib (16%) (10), hypertension for lenvatinib and pazopanib (> 20%) (8, 9, 33), rash for sorafenib (15%) (6), and lymphopenia for imatinib (45%) (7). Additionally, the tolerability of TKIs is also essential. For pazopanib, the discontinuation was mainly attributed to disease progression and treatment-related adverse events (hemorrhage, hypertension, and radiation recall tracheitis) (8). For lenvatinib, ATC patients discontinued due to treatment-related adverse events (9, 10, 33). Therefore, caution of treatment-related adverse events and tolerability of TKIs in ATC patients are warranted.

There were several limitations in this study. The baseline characteristics of the patients in each study were various. Because some patients might have received surgery, radiotherapy, or other therapeutic strategies, these elements could partially impact the final survival outcomes. In addition, owing to the long accrual time, detailed data on each patient, such as adverse events and drug discontinuation, might be incomplete. With the development of medical science, novel therapeutic strategies would contribute to improving survival time.

Conclusion

TKI therapy could be an effective therapeutic option for patients with ATC. However, there is a long way to go to prolong the survival time further and break the bottleneck.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

BK: Data curation, Investigation, Writing – original draft, Writing – review & editing. WZ: Writing – original draft, Writing – review & editing. GL: Writing – original draft, Writing – review & editing. CF: Writing – original draft, Writing – review & editing. RC: Data curation, Writing – original draft, Writing – review & editing. BW: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Causal relationship of genetically predicted gut microbiota with thyroid cancer: a bidirectional two-sample mendelian randomization study

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Background: Previous investigations have demonstrated a correlation between the composition of gut microbiota and the development of thyroid cancer (TC). Nonetheless, there was no consensus on the causal effect of gut microbiota composition on TC risk. Therefore, the present study aimed to perform a bidirectional two-sample Mendelian randomization (MR) analysis to explore potential causal associations between gut microbiota and TC risk.

Methods: Utilizing data from the MiBioGen consortium's genome-wide association studies (GWAS) meta-analysis involving a sample size of 18,340, we identified instrumental variables for 211 gut microbiota taxa. The summary statistics for TC was from relevant large-scale GWAS conducted by the FinnGen consortium. In the first stage, the Inverse-variance weighted (IVW) method was used as the primary estimate method, and the stability of estimations was tested by a battery of sensitivity analyses. In the second stage, a reverse MR analysis was applied to determine whether reverse causality existed.

Results: According to the IVW method, we identified 9 genetically predicted gut microbiota that were causally correlated with TC risk. Among them, we observed a positive causal effect of *Family Christensenellaceae* (OR = 1.664, 95% CI: 1.103–2.511, *P* = 0.015), *Family Victivallaceae* (OR = 1.268, 95% CI: 1.009–1.594, *P* = 0.042), *Genus Methanobrevibacter* (OR = 1.505, 95% CI: 1.049–2.159, *P* = 0.027), *Genus Ruminococcus2* (OR = 1.846, 95% CI: 1.261–2.704, *P* = 0.002), *Genus Subdoligranulum* (OR = 1.907, 95% CI: 1.165–3.121, *P* = 0.010), *Phylum Verrucomicrobia* (OR = 1.309, 95% CI: 1.027–1.668, *P* = 0.029) on TC risk, while *Class Betaproteobacteria* (OR = 0.522, 95% CI: 0.310–0.879, *P* = 0.015), *Family Family XI* (OR = 0.753, 95% CI: 0.577–0.983, *P* = 0.037), *Genus Sutterella* (OR = 0.596, 95% CI: 0.381–0.933, *P* = 0.024) might be correlated with a decreased risk of TC. Subsequently, various sensitivity analyses indicated no heterogeneity, directional pleiotropy or outliers. In addition, reverse analysis demonstrated a negative causal effect of TC risk on the abundance of the gut

microbiota (*Genus Ruminococcus2*, OR = 0.947, 95% CI: 0.907–0.989, *P* = 0.014).

Conclusion: Genetic evidence suggested that bidirectional causal associations of specific bacteria taxa and the risk of TC, highlighting the association of the "gut-thyroid" axis. Further exploration of the potential microbiota-related mechanisms might have profound implications for public health in terms of the early prevention and treatment of TC.

KEYWORDS

thyroid cancer, gut microbiota, mendelian randomization, causality, genetics

1 Introduction

Thyroid cancer (TC) is the most common malignant tumour of the human endocrine system and the head and neck (1). According to World Health Organization, there were an estimated 567,000 new cases and 41,000 deaths from TC worldwide in 2018 (2). The incidence of TC ranked ninth among all tumors worldwide, and the prevalence was more significant in women and people aged 50 years (3). In the United States, the overall incidence of TC has been observed to increase by 3% annually from 1975 to 2013 (4). Among the various types of TC, papillary thyroid cancer (PTC) is the most common and least aggressive histologic type, contributing to the majority of new cases (5). Over the past 30 years, the incidence of TC in the US has nearly tripled, which has been partly driven by an increase in surveillance and diagnostic testing (6). In 2019, the annual cost of TC was estimated at $1.8 billion to $2.1 billion in the United States, placing a significant clinical and economic burden on society (7). Therefore, the prevention and management of TC has been globally recognized as a crucial public health issue.

The intestinal microbiota is increasingly acknowledged as a pivotal "endocrine organ" and "metabolic regulator" within the human body (8). The microbes, genes, and gene products (proteins, enzymes) of it enable its active involvement in the regulation of metabolism, immunity, and endocrine systems (9). In recent years, there has been a growing body of research on the intestinal microbiota, which has revealed its potential influence on the secretion of the thyroid-stimulating hormone via the hypothalamus-pituitary axis, thereby playing a role in thyroid diseases (10). Additionally, a systematic review found that the gut microbiota can contribute to the accumulation of metabolites which, through specific mechanisms, induce genetic instability in the thyroid, ultimately resulting in tumorigenesis and progression (11). In the quest to elucidate these connections, Zhang et al. conducted a study comparing the gut microbiome changes in individuals with TC, thyroid nodules (TN), and healthy controls. They observed that the relative abundances of *Neisseria* and *Streptococcus* were significantly elevated in both the TC and TN groups compared to the healthy controls, while the abundances of *Butyricimonas* and *Lactobacillus* were found to be decreased (12). Similarly, Feng et al. demonstrated that TC patients exhibited a notable enrichment in 19 genera, including *Shigella*, *Clostridium*, and *Klebsiella*, and reductions in 8 genera such as, *Bacteroides*, *Prevotella*, and *Ruminococcus* when compared to the microbial community composition of healthy controls (13). Nevertheless, it was worth noting that these studies primarily relied on observational and cross-sectional analyses, leaving the question of a causal relationship between gut microbiota composition and TC risk without a consensus.

Mendelian randomization (MR) is an analytical approach that has been widely used in epidemiology to explore causal relationships between an exposure (such as a risk factor or intervention) and an outcome (such as a disease or health outcome) by leveraging genetic variants as instrumental variables (14). Recently, MR analysis has become increasingly popular in epidemiological research due to it offering a way to provide evidence for causality without conducting actual experiments on human subjects (15). The basic principle of MR method relies on the fact that genetic variants are randomly allocated during conception and are generally not influenced by confounding factors or reverse causality problems of observational epidemiological investigations (16). Unlike standard MR, the two-sample bidirectional MR involves the use of summary statistics from two independent datasets to evaluate causal relationships between exposures and outcomes, which enhances statistical power. Moreover, the bidirectional MR investigates causal relationships in both directions between two traits, providing a more comprehensive understanding of the complex interplay between variables. As far as we know, no MR analysis has been published on the causality between gut microbiota and TC risk. Therefore, we aimed to conduct a bidirectional two-sample MR analysis to explore the potential causal relationship between the gut microbiota and the risk of TC. This analysis utilized data from two independent genome-wide association study (GWAS), one for TC from the FinnGen consortium and the other for the gut microbiota composition from the MiBioGen consortium.

2 Materials and methods

2.1 Study design

This study employed a bidirectional two-sample MR design to elucidate the potential bidirectional causal relationship between gut microbiota and TC risk. The investigation involves two main stages: in the first stage, we employed single nucleotide polymorphisms (SNPs) associated with gut microbiota as instrumental variables to estimate the causal effect of gut microbiota on the TC risk. In the second stage, we used SNPs associated with TC to examine changes in gut microbiota following the development of TC. This dual approach allowed us to explore and infer the crosstalk between the gut microbiota and TC risk in a comprehensive manner. To ensure the validity of the MR analysis, this study follows the three key hypotheses outlined by Bownden et al. (17): (1) The instruments of genetic variations should be strongly correlated with exposure (gut microbiota); (b) The genetic variations should not be linked with any confounding factors related to both gut microbiota and TC; (c) The genetic variations should affect TC solely through gut microbiota, not via other pathways (Figure 1A). To uphold ethical standards, all studies included in the GWASs referenced in the analysis were approved by relevant review committees. The flowchart of our work is presented in Figure 1B.

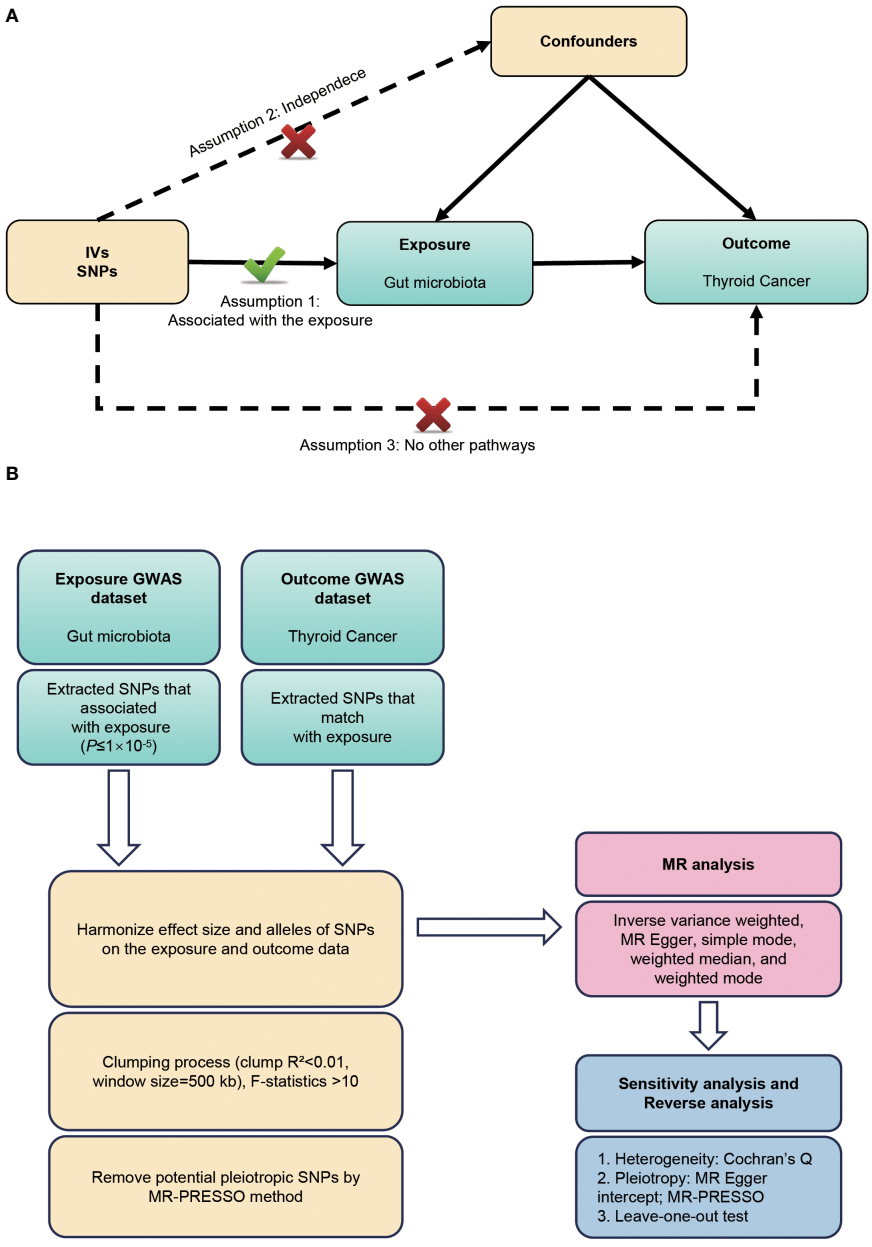


FIGURE 1
(A) Three assumptions of Mendelian randomization. **(B)** Flowchart of this Mendelian randomization study. MR, Mendelian randomization; SNP, single nucleotide polymorphism; GWAS, genome-wide association studies.

2.2 Sources of genome-wide association studies

The data on the exposure and outcome in the bi-directional MR study came from the GWAS database. The GWAS summary data of gut microbiota was extracted from a large-scale association analyses that included genome-wide genotypes and 16S fecal microbiome data from 18,340 European individuals (MiBioGen Consortium). Based on the variation of gut microbiota in different populations, the GWAS study eventually yielded 122,110 variant sites from 211 taxa (from genus to phylum level) (18).

The FinnGen research project is a large-scale, population-based initiative in Finland that aims to enhance our understanding of the genetic basis of various diseases and health-related traits. The project involves collecting and analyzing genomic data from a diverse population of Finnish individuals in order to identify genetic variants associated with various health conditions. In our study, we selected the summary data associated with malignant neoplasm of thyroid gland (all cancers excluded) from a FinnGen consortium GWAS containing a sample size of 1,525 cases and 259,583 controls. Importantly, the data employed for this study emanated from the most recent release of the FinnGen consortium (dated December 2022). This latest dataset has been instrumental in our pursuit of examining the connection between gut microbiota and TC, enhancing the accuracy and relevance of our findings (https://gwas.mrcieu.ac.uk/datasets/finn-b-C3_THYROID_GLAND_EXALLC/). Detailed characteristics of source datasets in the study were shown in Table 1.

2.3 Instrumental variable selection

All the SNPs selected as instrumental variables in the MR study should obey the three basic assumptions (19). (1) Firstly, we selected instrumental variables that were closely related to the exposure factors (the inclusion criteria of P value < 5×10^{-8}) for the MR analysis to satisfy the relevance assumption. To include sufficient instrumental variables for screening, we used a more lenient threshold (P value < 1×10^{-5}) in studies. (2) Then, we set the linkage disequilibrium (LD) threshold of $r^2 < 0.001$ and LD distance > 10,000 kb to avoid the offset caused by LD between the variables of interest in the results. (3) To prevent potential pleiotropy, PhenoScanner V2 databases were also used to further verify whether the instrumental variables mentioned above were related to other confounding factors. (4) Finally, we eliminated instrumental variables with an F-statistic < 10 to minimize potential weak instrument bias.

2.4 Statistical analysis

In this MR analysis, the inverse variance weighted (IVW) method was used as the primary approach to reveal the potential causation between gut microbiota and TC since it was deemed the most reliable method if there was no indication of pleiotropy (20). The IVW method had a strong ability to detect causality based on the fundamental premise that all genetic variants are valid instrumental variables. In addition, the weighted median, MR Egger, simple mode, and weighted mode methods were used as additional methods to estimate causal effects under different conditions. The MR-Egger method allows researchers to assess whether the causal effect estimate is biased due to pleiotropy and provides a "pleiotropy-corrected" causal estimate when pleiotropy is present. The weighted median method provides a robust estimate of causal effects by calculating the median of causal estimates from genetic instruments while considering their precision (21).

The study utilized the MR-Egger regression to assess the impact of gene pleiotropy on bias, while the MR-PRESSO method was applied to identify and correct for potential outliers resulting from horizontal polymorphisms (22). The MR-Egger intercept test was utilized to evaluate the presence of horizontal pleiotropy (23). In addition, we employed Cochran's Q test to identify heterogeneity in the included instrumental variables (24). Furthermore, we employed the leave-one-out method to examine the influence of individual SNPs on the causal association determined through MR analysis.

Ultimately, reverse-direction MR was also conducted to identify reverse causality. all MR analyses were performed with the R package of "TwoSampleMR" and "MR-PRESSO" in the R statistical software (version 4.1.3).

3 Results

3.1 Selection of instrumental variables

First of all, 14,587 SNPs correlated with the gut microbiota were identified as IVs from the MiBioGen Consortium at a relatively loose significance level ($P < 1\times10^{-5}$) (Supplementary Table 1). It contained 211 bacterial traits, including 131 genera, 35 families, 20 orders, 16 classes, and 9 phyla. After a series of quality control steps, 2,236 SNPs were finally included in the analysis. In addition, the F-value of each instrumental variable is greater than 10.

TABLE 1 Detailed characteristics of source datasets in the study.

Trait	Consortium	Population	Sex	Number of SNPs	Sample size
Exposure					
Gut microbiota	MiBioGen Consortium (PMID: 33462485)	European	Males and Females	5,708,796	14,306
Outcome					
Thyroid cancer	FINNGEN	European	Males and Females	16,380,316	1,525

GWAS, genome-wide association studies; SNP, single-nucleotide polymorphism; PMID, PubMed unique identifier.

3.2 Causal effect of gut microbiota on the risk of thyroid cancer (locus-wide significance level, $P < 1\times10^{-5}$)

According to the IVW analysis, genetically predicted *Family Christensenellaceae* (OR = 1.664, 95% CI: 1.103–2.511, $P = 0.015$), *Family Victivallaceae* (OR = 1.268, 95% CI: 1.009–1.594, $P = 0.042$), *Genus Methanobrevibacter* (OR = 1.505, 95% CI: 1.049–2.159, $P = 0.027$), *Genus Ruminococcus2* (OR = 1.846, 95% CI: 1.261–2.704, $P = 0.002$), *Genus Subdoligranulum* (OR = 1.907, 95% CI: 1.165–3.121, $P = 0.010$), *Phylum Verrucomicrobia* (OR = 1.309, 95% CI: 1.027–1.668, $P = 0.029$) were positively associated with TC risk. In contrast, genetically predicted abundance of *Class Betaproteobacteria* (OR = 0.522, 95% CI: 0.310–0.879, $P = 0.015$), *Family Family XI* (OR = 0.753, 95% CI: 0.577–0.983, $P = 0.037$), *Genus Sutterella* (OR = 0.596, 95% CI: 0.381–0.933, $P = 0.024$) was inversely correlated to TC risk (Table 2). Similarly, the MR estimates of the weighted median indicated that elevated levels of *Family Christensenellaceae* (OR = 1.893, 95% CI: 1.068–3.354, $P = 0.029$) and *Family Family XI* (OR = 0.686, 95% CI: 0.482–0.977, $P = 0.036$) were associated with elevated and reduced risk risk of TC, respectively (Table 2). The MR results of all gut microbiota on TC were presented in detail in Supplementary Table 2.

3.3 Sensitivity analysis

A series of sensitivity analyses were conducted to assess the heterogeneity and horizontal pleiotropy of the selected instrumental variables. Based on Cochran's Q test, we observed no significant heterogeneity ($P > 0.05$) (Table 3). All P values of the MR-Egger intercept tests were > 0.05, indicating no horizontal pleiotropy. Additionally, no outliers were identified through the MR-PRESSO global test (Table 3). Detailed scatter plots for each MR method analysis were shown in Figure 2. And results from a leave-one-out test suggested that no SNP exerted influential outlier effects (Figure 3).

3.4 Causal effect of gut microbiota on the risk of thyroid cancer (genome-wide statistical significance level, $P < 5\times10^{-8}$)

A total of 1,394 SNPs were identified as instrumental variables at genome-wide statistical significance level ($P < 5\times10^{-8}$) (Supplementary Table 3). When MR analysis was conducted with gut microbiome as a whole, IVW results indicated no evidence of a causal association between the gut microbiota and TC risk (Table 4). Additionally, Cochran's Q test results revealed no significant heterogeneity ($P = 0.932$) and the MR-Egger intercept shows no evidence of horizontal pleiotropy between total gut microbiome and TC risk ($P = 0.973$). Finally, we also did not discover any outliers through the MR-PRESSO global test (Table 4).

3.5 Reverse analysis of causality between thyroid cancer risk and gut microbiome

In our study, we utilized reverse causality to explore the relationship between TC incidence and gut microbiota. According to the IVW method, TC risk had a negative causal effect on the gut microbiota (*Genus Ruminococcus2*) in our study (OR = 0.947, 95% CI: 0.907–0.989, $P = 0.014$). However, the remaining 8 gut microbiota indicated no significant causal association with TC risk, including *Class Betaproteobacteria* ($P = 0.978$), *Family FamilyXI* ($P = 0.337$), *Family Victivallaceae* ($P = 0.819$), *Genus Methanobrevibacter* ($P = 0.309$), *Genus Subdoligranulum* ($P = 0.938$), *Genus Sutterella* ($P = 0.656$), *Phylum Verrucomicrobia* ($P = 0.533$) (Table 5).

4 Discussion

To our knowledge, we determined the causal influence of gut microbiota on the risk of TC by MR analysis for the first time. In our bidirectional MR analysis, we identified several gut microbiota that exhibit causal relationships with TC risk. Specifically, *Family Christensenellaceae*, *Family Victivallaceae*, *Genus Methanobrevibacter*, *Genus Ruminococcus2*, *Genus Subdoligranulum*, and *Phylum Verrucomicrobia* were correlated with an elevated risk of TC. And *Class Betaproteobacteria*, *Family Family XI*, *Genus Sutterella* exhibited a potential protective effect against TC. Moreover, our reverse analysis indicated that an increased risk of TC could potentially lead to a lower abundance of the *Genus Ruminococcus2*. This study opened up exciting new avenues for further research in the field of gut microbiota and its potential impact on TC. By shedding light on the role of specific gut microbiota in TC risk, our findings might pave the way for future investigations into targeted interventions aimed at modulating the gut microbiota to prevent or manage TC.

The gut microbiota is a large group of microbes found in the human gastrointestinal tract, and plays a pivotal role in maintaining intestinal homeostasis (25). Disruptions in gut microbiota balance can lead to intestinal epithelial barrier dysfunction, resulting in inflammation, hormone inactivation, and even tumorigenesis (26). Recently, the relationship between thyroid function and the gut microbiota has emerged as a prominent area of research. Several studies have indicated that the gut microbiota can influence the secretion of the thyroid-stimulating hormone through the hypothalamus-pituitary axis, thereby potentially impacting thyroid diseases development (10). On the one hand, modifications in the composition, structure, and metabolites of gut microbiota can directly or indirectly affect the activation and cytokine production of immune cells, potentially influencing lymphopoiesis and exerting cancer-promoting effects in TC (27). On the other hand, gut microbiota may influence the metabolism and bioavailability of certain nutrients, affecting iodine absorption and utilization by the thyroid gland. As iodine is an essential component of thyroid hormone synthesis, any interference with its availability may have implications for thyroid function and potentially even contribute to the development of TC

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TABLE 2 MR results of causal effects between gut microbiome and the risk of thyroid cancer.

Group	Bacterial traits	Nsnp	Methods	SE	OR (95% CI)	*P*-value
Class	*Betaproteobacteria*	11	MR Egger	0.888	0.850 (0.149, 4.844)	0.859
			Weighted median	0.344	0.561 (0.286, 1.101)	0.093
			Inverse variance weighted	0.266	0.522 (0.310, 0.879)	0.015
			Simple mode	0.550	0.587 (0.200, 1.726)	0.356
			Weighted mode	0.520	0.641 (0.231, 1.778)	0.413
Family	*Christensenellaceae*	11	MR Egger	0.407	1.840 (0.829, 4.084)	0.168
			Weighted median	0.292	1.893 (1.068, 3.354)	0.029
			Inverse variance weighted	0.210	1.664 (1.103, 2.511)	0.015
			Simple mode	0.465	2.131 (0.857, 5.302)	0.135
			Weighted mode	0.355	1.984 (0.990, 3.977)	0.082
Family	*Family XI*	8	MR Egger	0.872	0.366 (0.066, 2.024)	0.293
			Weighted median	0.180	0.686 (0.482, 0.977)	0.036
			Inverse variance weighted	0.136	0.753 (0.577, 0.983)	0.037
			Simple mode	0.267	0.658 (0.390, 1.111)	0.161
			Weighted mode	0.267	0.658 (0.390, 1.110)	0.161
Family	*Victivallaceae*	13	MR Egger	0.573	1.441 (0.469, 4.432)	0.537
			Weighted median	0.153	1.272 (0.942, 1.718)	0.117
			Inverse variance weighted	0.117	1.268 (1.009, 1.594)	0.042
			Simple mode	0.255	1.309 (0.794, 2.158)	0.312
			Weighted mode	0.224	1.323 (0.853, 2.053)	0.235
Genus	*Methanobrevibacter*	6	MR Egger	0.690	2.140 (0.554, 8.272)	0.332
			Weighted median	0.233	1.536 (0.973, 2.424)	0.065
			Inverse variance weighted	0.184	1.505 (1.049, 2.159)	0.027
			Simple mode	0.305	1.559 (0.858, 2.832)	0.205
			Weighted mode	0.287	1.559 (0.888, 2.736)	0.183
Genus	*Ruminococcus2*	15	MR Egger	0.469	1.894 (0.755, 4.752)	0.197
			Weighted median	0.270	1.638 (0.965, 2.780)	0.067
			Inverse variance weighted	0.195	1.846 (1.261, 2.704)	0.002
			Simple mode	0.398	1.515 (0.694, 3.304)	0.315
			Weighted mode	0.341	1.661 (0.851, 3.242)	0.159
Genus	*Subdoligranulum*	11	MR Egger	0.648	1.263 (0.355, 4.494)	0.727
			Weighted median	0.349	1.746 (0.882, 3.457)	0.110
			Inverse variance weighted	0.251	1.907 (1.165, 3.121)	0.010
			Simple mode	0.611	1.518 (0.458, 5.027)	0.510
			Weighted mode	0.561	1.533 (0.510, 4.602)	0.464
Genus	*Sutterella*	12	MR Egger	0.989	0.658 (0.095, 4.573)	0.681
			Weighted median	0.300	0.622 (0.345, 1.118)	0.113
			Inverse variance weighted	0.229	0.596 (0.381, 0.933)	0.024
			Simple mode	0.509	0.794 (0.293, 2.154)	0.659

(Continued)

TABLE 2 Continued

Group	Bacterial traits	Nsnp	Methods	SE	OR (95% CI)	*P*-value
			Weighted mode	0.514	0.725 (0.265, 1.986)	0.545
Phylum	*Verrucomicrobia*	12	MR Egger	0.546	2.241 (0.768, 6.540)	0.170
			Weighted median	0.161	1.321 (0.963, 1.812)	0.084
			Inverse variance weighted	0.124	1.309 (1.027, 1.668)	0.029
			Simple mode	0.283	1.463 (0.840, 2.549)	0.206
			Weighted mode	0.249	1.463 (0.899 2.382)	0.154

SNP, single nucleotide polymorphism; MR, mendelian randomization; SE, standard error; 95% CI, 95% confidence interval.

(28). Thus, further research in this area is essential to unravel the precise mechanisms underlying the gut-thyroid axis and its significance in thyroid health and disease prevention.

In our study, we conducted MR and sensitivity analysis on the filtered qualifying instrumental variables, and we identified a causal relationship between nine gut microbiota and TC risk. According to a multi-omics study, the abundance of *Christensenellaceae* and *Eubacterium*, both of which are closely related to lipid metabolism, were significantly reduced in the TC group compared to healthy controls (29). In addition, Ishaq et al. demonstrated that compared to healthy groups, the abundance of *Subdoligranulum*, *Verrucomicrobia*, and *Ruminococcus2* were significantly increased, while the abundance of *Prevotella9*, *Bacteroides*, and *Klebsiella* were significantly decreased in the TC group, further accentuating the microbial differences (30). *Betaproteobacteria*, one of the five Classes within the *Proteobacteria* (31). Within a harmoniously balanced gut microbiota that characterized by robust stability, the interaction with the host's immune system often curtails unregulated proliferation of *Proteobacteria*. However, as observed in this study, the surge of *Proteobacteria* indicates an unstable gut microbial community, which is associated with certain disease states (32). Similarly, a cross-sectional study found that elevated levels of *Proteobacteria* in the majority of TCs by fractional analysis, which may indicate that dysregulation of intestinal ecology is associated with the underlying pathogenesis of TC (33). In addition, we have also found a causal relationship between *Family Victivallaceae*, *Genus Methanobrevibacter*, and *Family Family XI* with the risk of TC. However, there was limited research on the role of these three groups of bacteria in TC. Thus, it was necessary to further study the possible role of *Family Victivallaceae*, *Genus Methanobrevibacter*, and *Family Family XI* in TC development.

Interestingly, our reverse MR analysis revealed that TC may induce a decrease in the of *Genus Ruminococcus2*. Among the dominant genera in our studied population, *Ruminococcus2* holds a noteworthy place as both a symbiotic bioindicator of human health

TABLE 3 Sensitivity analysis of the MR analysis results of gut microbiota and the risk of thyroid cancer.

Outcome	Bacterial traits	Cochran Q statistic	Heterogeneity *P*-value	MR-Egger Intercept	Intercept *P*-value	MR-PRESSO Global test *P*-value
Thyroid cancer	*Class Betaproteobacteria*	4.519	0.921	-0.035	0.579	0.935
	Family Christensenellaceae	6.758	0.748	-0.010	0.780	0.760
	Family FamilyXI	6.803	0.450	0.006	0.435	0.492
	Family Victivallaceae	6.716	0.876	-0.019	0.824	0.886
	Genus Methanobrevibacter	1.145	0.950	-0.052	0.624	0.736
	Genus Ruminococcus2	12.515	0.565	-0.002	0.954	0.619
	Genus Subdoligranulum	8.584	0.572	0.033	0.508	0.599
	Genus Sutterella	6.543	0.835	-0.007	0.920	0.840
	Phylum Verrucomicrobia	4.817	0.940	-0.078	0.336	0.951

MR-Egger, Mendelian randomization Egger.

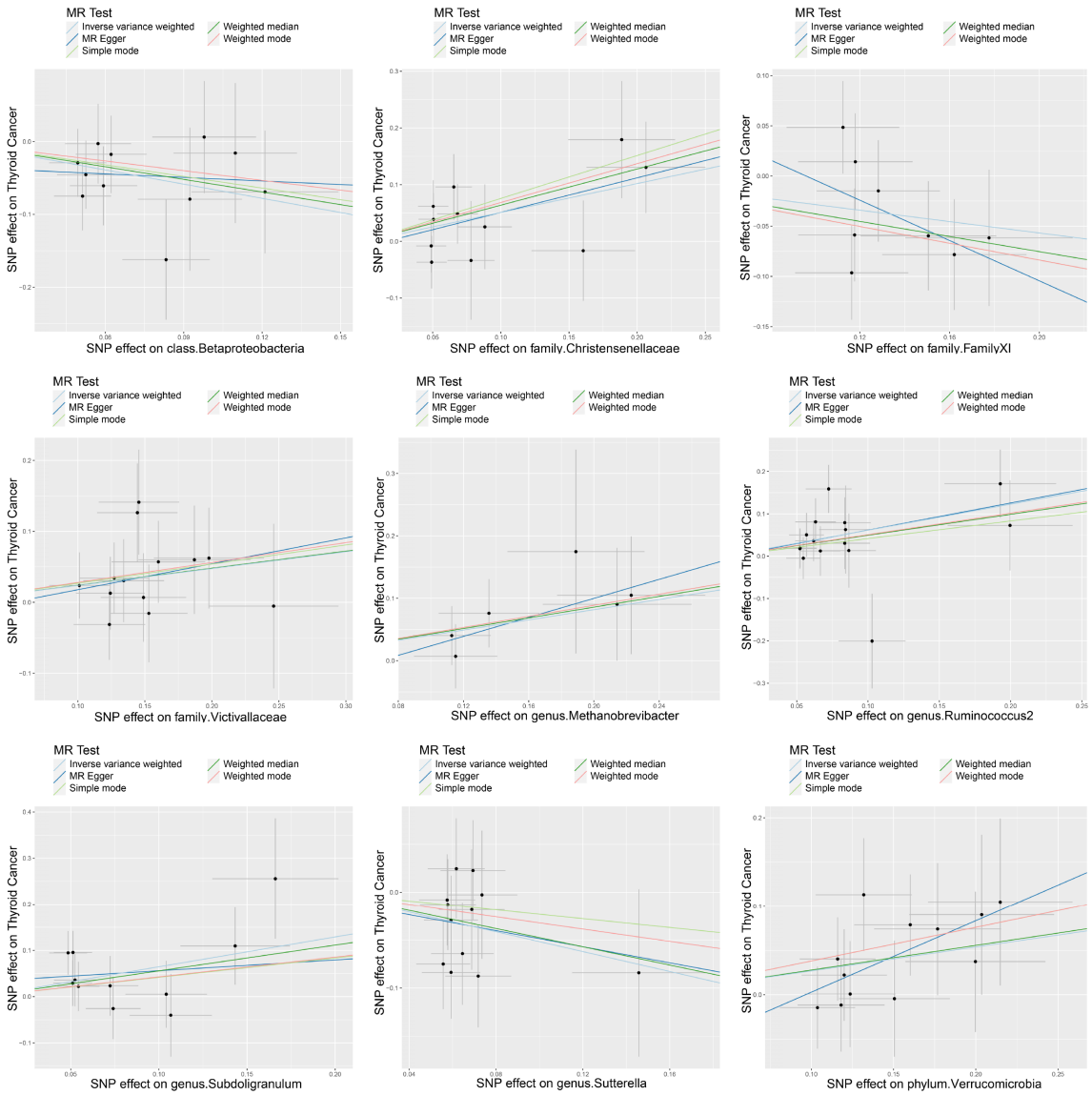


FIGURE 2
Scatter plots of significant causality of exposure (Specific gut microbiome) and outcome (thyroid cancer risk). The dots represent the effect size of each SNP on each bacterial taxon (x-axis) and thyroid cancer (y-axis), and the grey crosses represent the standard errors. Regression slopes show the estimated causal effect of each bacterial taxon on thyroid cancer. The light blue, dark blue, light green, dark green, and red regression lines represent the inverse variance weighted method, MR-Egger regression, simple mode, weighted median method, and weighted mode, respectively.

and a producer of short-chain fatty acids (SCFAs) (34). SCFA-producing genera are typically found in abundance within the human gut, benefiting the host by participating in the fermentation of carbohydrates into SCFAs, including acetate and propionate (35). It has been reported that the diminishment of SCFA-producing bacteria may promote the development of TC (33). In light of this, it was plausible to hypothesize that a reduced abundance of *Ruminococcus2* could potentially disrupt the balance of the gut microbial ecosystem, either preceding or following the development of TC. Additionally, more and more researches suggested that the abundance of *Ruminococcus2* tends to be lower in individuals with TC, which indicates *Ruminococcus2* maybe a potential regulator. Therefore, we hypothesized that there is a dynamic interaction between the incidence of TC and the level of *Ruminococcus2*.

Our research had various strengths. First of all, we employed MR analysis to deduce that the relationship between the gut microbiome and the risk of TC is less vulnerable to confounding and reverse causation in comparison to traditional observational analyses. Furthermore, we examined the causal impact of each taxon on TC risk, ranging from the genus to the phylum level, which provides guidance for the prevention and treatment of TC by targeting distinct gut microbiota during clinical practice. Nonetheless, our study also possessed certain limitations that should be acknowledged. To begin with, the identified TC risk variants only account for a small portion of the disease risk, and the

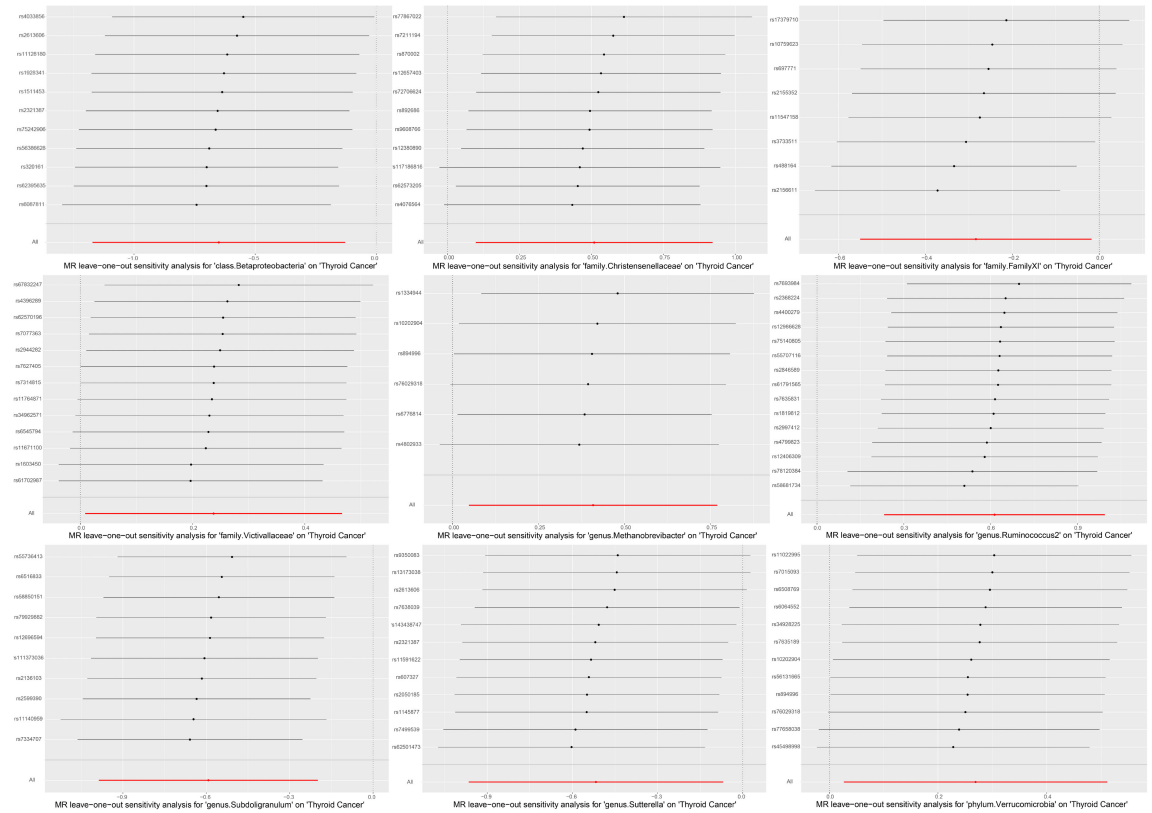


FIGURE 3
Leave-one-out stability tests causal estimates of exposure (Specific gut microbiota) on outcome (thyroid cancer risk). Circles indicate MR estimates for gut microbiota on thyroid cancer using inverse-variance weighted fixed-effect method if each SNP was omitted in turn.

intricate correlation structure among gut microbial taxa is confounded by factors such as diet and the possibility of horizontal gene transfer. Secondly, due to the absence of demographic data in the original study, we were unable to conduct additional subgroup analyses to obtain more specific correlations. Thirdly, while MR studies have been considered as excellent proxies of clinical/experimental trials in uncovering causal relationships. However, MR studies could not completely replace clinical trials, as their randomization process was subject to certain limitations such as pleiotropy, GWAS power, and representativeness

TABLE 4 MR estimates for the association between total gut microbiota and the risk of thyroid cancer.

Bacterial traits (exposure)	Traits (outcome)	Nsnp	Methods	SE	β (95% CI)	*P*-value	Cochran Q statistic	Heterogeneity *P*-value	MR-Egger Intercept	Intercept *P*-value	MR-PRESSO Global test *P*-value
Total	Thyroid cancer	12	MR Egger	0.506	0.970 (0.360, 2.614)	0.953	4.983	0.932	0.002	0.973	0.939
			Weighted median	0.183	1.019 (0.712, 1.459)	0.916					
			Inverse variance weighted	0.143	0.986 (0.744, 1.306)	0.923					
			Simple mode	0.278	1.060 (0.614, 1.829)	0.839					
			Weighted mode	0.226	1.020 (0.655, 1.589)	0.930					

SNP, single nucleotide polymorphism; MR, mendelian randomization; SE, standard error; 95% CI, 95% confidence interval.

TABLE 5 Reverse analysis of causal effects between thyroid cancer and gut microbiome.

Exposure	Outcome	Nsnp	Methods	SE	OR (95% CI)	*P*-value
Thyroid cancer	*Class Betaproteobacteria*	2	Inverse variance weighted	0.020	1.001 (0.960, 1.042)	0.978
Thyroid cancer	*Family FamilyXI*	1	Inverse variance weighted	0.056	1.055 (0.945, 1.178)	0.337
Thyroid cancer	*Family Victivallaceae*	2	Inverse variance weighted	0.043	0.990 (0.909, 1.079)	0.819
Thyroid cancer	*Genus Methanobrevibacter*	2	Inverse variance weighted	0.046	0.955 (0.873, 1.044)	0.309
Thyroid cancer	*Genus Ruminococcus2*	2	Inverse variance weighted	0.022	0.947 (0.907, 0.989)	0.014
Thyroid cancer	*Genus Subdoligranulum*	2	Inverse variance weighted	0.021	1.002 (0.962, 1.043)	0.938
Thyroid cancer	*Genus Sutterella*	2	Inverse variance weighted	0.026	1.012 (0.961, 1.065)	0.656
Thyroid cancer	*Phylum Verrucomicrobia*	2	Inverse variance weighted	0.034	71.021 (0.956, 1.091)	0.533

SNP, single nucleotide polymorphism; MR, mendelian randomization; SE, standard error; 95% CI, 95% confidence interval.

of the sample, which will inevitably lead to a degree of confounding into the results. Furthermore, the GWAS database of gut microbiota and thyroid cancer selected in this study were two independent datasets, the diversity of gut microbiota in the study population was not analyzed. In the future, further large-scale prospective studies are necessary to investigate the specific role of gut microbiota richness and diversity in TC. Last but not least, this study exclusively focused on individuals of European descent, necessitating replication in other populations to validate these findings.

5 Conclusion

Our study provided evidence of a potential causal relationship between specific gut microbiota and TC risk through the bi-directional MR analysis. These findings provided new insights into the prevention, progression, and treatment of TC through targeting specific gut microbiota. In the future, more clinical trials and mechanism studies are needed to explore the exact mechanisms underlying the interactions between the gut microbiota and the prevalence of TC.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

Author contributions

XS: Data curation, Methodology, Writing – original draft, Writing – review & editing. SC: Data curation, Methodology, Writing – original draft, Writing – review & editing. SZ: Data curation, Methodology, Writing – original draft. JW: Data curation, Formal analysis, Investigation, Writing – original draft. HC: Funding acquisition, Project administration, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2024.1284472/full#supplementary-material

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To diagnose primary and secondary squamous cell carcinoma of the thyroid with ultrasound malignancy risk stratification

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Objectives: This study aimed to investigate the clinico-ultrasound features of primary squamous cell carcinoma of the thyroid (PSCCT) and secondary SCCT (SSCCT) and evaluate the accuracy of fine needle aspiration (FNA) recommendation for SCCT with American College of Radiology-Thyroid Imaging and Reporting Data System (ACR-TIRADS) and Chinese-TIRADS (C-TIRADS).

Materials and methods: We retrieved 26 SCCT patients (11 PSCCT, 15 SSCCT) from our hospital's pathology database (5,718 patients with thyroid malignancy) over 23 years. Medical records and ultrasound data of the 26 patients with 27 SCCTs were analyzed retrospectively, and each SCCT focus was categorized based on the two TIRADSs.

Results: For 26 patients (21 males, 5 females) with an age range of 42-81 years, rapidly enlarging thyroid/neck nodules (18/26, 69.2%), dysphagia (7/26, 26.9%), hoarseness (6/26, 23.1%), dyspnea (5/26, 19.6%), cough (4/26, 15.4%), neck pain (2/26, 7.7%), B symptoms (2/26, 7.7%), and blood in sputum (1/26, 3.8%) were presented at diagnosis. Five asymptomatic patients (5/26, 19.2%) were detected by ultrasound. Hoarseness was more common in PSCCT (5/11, 45.5%) than in SSCCT (1/15, 6.7%) (P=0.032). For 27 SCCTs with a mean size of 3.7 $\pm$ 1.3 cm, the ultrasound features consisted of solid (25/27, 92.6%) or almost completely solid composition (2/27, 7.4%), hypoechoic (17/27, 63%) and very hypoechoic echogenicity (10/27, 37%), irregular/lobulated margin with extra-thyroidal extension (27/27, 100%), taller-than-wide shape (13/27, 48.1%), punctate echogenic foci (6/27, 22.2%), hypervascularity (23/27, 85.2%) and involved neck lymph (13/26, 50.0%). A total of 27 SCCTs were evaluated as high malignancy risk stratification (≥TR4 and 4B) by the two TIRADSs and recommended FNA in 96.3–100% (26/27, 27/27). Pathologically, more than half of PSCCTs (7/12, 58.3%) and a quarter of SSCCTs (4/15, 26.7%) were poorly differentiated, while moderately and well-differentiated grades were observed in 5 PSCCTs and 11 SSCCTs (P=0.007). Thirteen patients (50.0%) underwent surgery with radical operation in 5 cases (5/13, 38.5%).

Conclusion: SCCT is an extremely rare and aggressive malignancy with a male predominance. PSCCT and SSCCT had similar clinical and ultrasound features except for tumor differentiation and the symptom of hoarseness. SCCT showed a high malignancy risk stratification in ACR-TIRADS and C-TIRADS, with a high rate of FNA recommendation.

KEYWORDS

squamous cell carcinoma (SCC), thyroid, ultrasound, thyroid imaging reporting and data system (TIRADS), fine needle aspiration (FNA)

Introduction

Squamous cell carcinoma of the thyroid (SCCT) was previously considered a separate entity but is now classified as a subtype of anaplastic thyroid carcinoma (ATC) due to their analogy (1–3). SCCT comprises primary SCCT (PSCCT) and secondary SCCT (SSCCT, i.e., direct invasion from primary SCC of the adjacent soft tissue and metastasis from a distant SCC) (4). PSCCT with female predominance is most often seen in the seventh decade and more than 60% of the reported cases presented at age 60 or above. Strict adherence to the WHO definition, PSCCT without other cancer components accounts for no more than 0.5% of primary thyroid cancer (5). Thus, pure SCCT is exceptionally rare. SSCCT is about 10 times more common than PSCCT (6, 7). PSCCT is a highly aggressive disease with a poor prognosis with death within the first year in most patients, which is worse than SSCCT (4). Early diagnosis is essential for optimal management and contributes to prompt treatment to prolong life (5, 8).

Ultrasound is an optimal and initial radiological modality to detect and diagnose thyroid nodules (TN) (9). Our previous study, in which 9 PSCCTs in 8 patients were evaluated by 2017 American College of Radiology-Thyroid Imaging and Reporting Data System (ACR-TIRADS) and 2015 American Thyroid Association (ATA) guideline, proposed that PSCCT has certain ultrasonic features for malignancy. The two above guidelines could identify 88.9–100% of PSCCTs as suspicious for malignancy, and the risk stratification of TN could markedly improve diagnostic performance (10).

The 2017 ACR-TIRADS and newly published 2020 Chinese Medical Association proposed the Chinese-TIRADS (C-TIRADS) are widely used for TN evaluation in China (11, 12). We intended to use the two guidelines to assess the risk stratification of SCCT to determine whether it warrants biopsy in clinical practice. So far, the ultrasound features of PSCCT have been described almost exclusively in case reports and small case series. Although SSCCT is more common than PSCCT, its ultrasound findings are exceedingly rarely reported. Therefore, we comparatively analyze the clinic-pathology and ultrasound features of both PSCCT and SSCCT patients to improve the knowledge of SCCT and contribute to the establishment of an optimal diagnostic strategy.

Materials and methods

Patient cohort

We retrieved 29 SCCT patients from our hospital's pathology database of thyroid malignancies (n=5718) from January 1999 to November 2022. The inclusion criteria were as follows: (1) Patients with SCCT proved by pathology; (2) Patients underwent thyroid ultrasound examinations within 3 months of diagnosis. Finally, 26 patients were enrolled due to 3 patients without complete ultrasound data. There were no other primary sites in 11 patients i.e. PSCCT. Other primary sites were identified in 15 patients i.e. SSCCT. The primary sites of SSCCT were esophagus (5/15, 33%), larynx (7/15, 46. 7%), lung (2/15, 13.3%), and cervix uteri (1/15, 6.7%). One of them simultaneously developed SCC in lung and thyroid.

The study complied with the Declaration of Helsinki and was approved by the Ethics Committee of Peking University First Hospital. Individual informed consent was waived for its retrospective nature.

Thyroid ultrasound examination, 2017 ACR-TIRADS and 2020 C-TIRADS analysis

Thyroid ultrasound examinations were performed with high-resolution ultrasound instruments (GE Logiq 9, GE Logiq E9, Philips HDI 5000, Philips IU22, Philips EPIQ 7, Siemens Acuson S2000 ABVS, Aloka Prosound F75, Esaote Mylab90) equipped with 6-15 MHz linear transducers. Static images were stored in a picture archiving and communication system. All thyroid images were evaluated in a consensus manner by two ultrasound radiologists with more than 10 years of ultrasound experience and uninformed pathological results. The significant ultrasound features of TN were assigned based on ACR-TIRADS and C-TIRADS (Table 1). The risk stratification of TN was determined by adding points in the two TIRADSs. Fine-needle aspiration (FNA) was recommended based on the TN risk stratification and its maximum diameter. Extra thyroidal extension (ETE) includes extensive (frank invasion of adjacent soft tissue and/or vascular structures) and minimal ETE (presence of border abutment, contour bulging, or loss of the

echogenic thyroid border) (12). In the study, ETE was also adopted to confirm direct invasion of the thyroid from neck mass. Meantime other ultrasound features were analyzed. Enlargement of the thyroid was identified as the anteroposterior diameter of the thyroid >2 cm. Any neck lymph node with two or more of the below features or hypoechoic and enhanced posterior echo was considered suspicious for metastasis: a globular shape or diameter of short axis >5 mm, irregular blurred contours, loss of the normal echogenic hilum, heterogeneity with anechoic/cystic components, Punctate echogenic foci (PEF) and presence of peripheral rather than hilar flow or chaotic vascularity (12–14).

Statistical analysis

Descriptive data are presented as means and standard deviation (SD) for continuous variables, numbers and percentages (%) for categorical variables. Independent sample t-tests were used for the comparison of continuous data, and Fisher's exact test for the proportion comparison of categorized data. $P<0.05$ (two-tails) was considered statistically significant. SPSS 26.0 software (IBM, Armonk, NY, USA) was used for statistical analysis.

Results

The patient's clinical features and medical history

Eleven PSCCT patients (mean age 56.5 ± 10.7 years) included 9 males and 2 females, and 15 SSCCT patients (mean age 62.9 ± 9.0 years) included 12 males and 3 females. The most common presentation was rapidly enlarging neck mass, seen in 69.2% of the patients (18/26). Other symptoms include dysphagia (7/26, 26.9%), hoarseness (6/26, 23.1%), dyspnea (5/26, 19.6%), cough (4/26, 15.4%), neck pain (2/26, 7.7%), B symptoms (2/26, 7.7%), and bloody sputum (1/26, 3.8%) ensued in succession. SCCTs in 5 asymptomatic patients (5/26, 19.2%) were detected by ultrasound examination. Neither bloody sputum nor neck pain was seen in PSCCT patients. Hypocalcemia was found in 1 PSCCT patient (2 cases didn't have laboratory test). Leukocytosis occurred in 3 PSCCT patients and 6 SSCCT patients (1 case didn't have laboratory test). During follow-up, PSCCT metastasized to the lung in 27.3% (3/11) and the liver in 9.1% (1/11). In the SSCCT patients, metastasis to the lung, liver, and bone occurred in one patient each (1/15, 6.7%).

TABLE 1 Assignments of ultrasound features in 2017 ACR-TIRADS and 2020 C-TIRADS.

Ultrasound features	Assignments in ACR-TIRADS (point)	Ultrasound features	Assignments in C-TIRADS (point)
Composition			
Solid/almost completely solid	2	Solid	1
Mixed cystic and solid composition:	1		
Echogenicity			
Very hypoechoic	3	Very hypoechoic	1
Hypoechoic	2		
Hyperechoic/isoechoic	1		
Shape			
Taller-than-wide	3	Taller-than-wide	1
Wider-than-tall	0		
Margin			
Extra thyroidal extension (ETE)	3	Ill-defined/irregular/ETE	1
Irregular/lobulated	2		
Smooth/ill-defined	0		
Echogenic foci			
Punctate echogenic foci (PEF)	3	PEF/suspicious microcalcification	1
Peripheral/rim calcifications	2		
Macrocalcifications	1		
None/large comet-tail artifacts	0	Large comet-tail artifacts	−1

History of smoking was found in 8 patients (8/26, 30.8%) with 0.5-2 packs per day (6 current smoker/2 ex-smoker), alcohol consumption with 100-150g per day in 2 patients (2/26, 7.7%), pulmonary tuberculosis in 1 patient (1/26, 3.8%), and diabetes mellitus in 1 patient (1/26, 3.8%). Two PSCCT and 4 SSCCT patients (6/26, 23.1%) had documented or concomitant Hashimoto thyroiditis at diagnosis, with 2 of them with hyperthyroidism (TSH: 0.5uIU/L, 2.36uIU/L; Free T4: 23.82pmol/L, 17.62pmol/L). Hypertension, hyperlipemia, and/or hyperuricemia occurred in 1 PSCCT and 1 SSCCT patients, and chronic obstructive pulmonary disease (COPD) occurred in 2 SSCCT patients. One 81-year-old female with simultaneous lung and thyroid SCC performed radical left mastectomy for invasive ductal carcinoma 18 years ago and vocal cord polypectomy 16 years ago, and had a history of COPD and long-term smoking. One SSCCT patient, secondary to esophagus SCC, had received radiation therapy 2 years ago.

Diagnostic management and pathological features

Thirteen patients (13/26, 50.0%) with 14 SCCTs underwent ultrasound-guided core needle biopsy (CNB). Among 13 patients who underwent surgery, only 5 (5/13, 38.5%) had radical operation (2 PSCCT, 3 SSCCT patients), 6 (6/13, 46.2%) had palliative/debulking operation (3 PSCCT, 3 SSCCT patients), one had (1/13, 7.7%) incisional biopsy and one had excision operation in another hospital with unavailable surgical data. Two patients underwent tracheotomy due to trachea stenosis resulted from tumor compression and infiltration. Pathologically, 7 PSCCTs (7/12, 58.3%) and 4 SSCCTs (4/15, 26.7%) were poorly differentiated. One well-differentiated PSCCT intermingled with papillary thyroid carcinoma (PTC). Accompanied pathological necrosis was found in 4 SSCCT patients (4/15, 26.7%) and 1 PSCCT patient (1/12, 8.3%). Direct invasion of the thyroid from larynx in 1 SSCCT patient was confirmed by postoperative pathological examination.

Ultrasound-based risk stratification and recommendations for FNA

Thyroid ultrasound detected 27 SCCTs (12 PSCCTs, 15 SSCCTs) in 26 patients. The mean size was 3.7 ± 1.3 cm (range, 0.9-6.9 cm) with 11.1% (3/27)≤20 mm (1.8 cm and 0.9 cm PSCCTs, 1.0cm SSCCT). Suspicious satellite lesions with less than 5 mm were found in 3 SSCCT patients from laryngeal SCC. 85.2% (23/27) of the thyroid thickness of the disease exceeded 2 cm (2.9 ± 0.9 cm in PSCCT, 2.7 ± 1.1 cm in SSCCT). The normal size of the thyroid lobe of the disease was found in 2 PSCCT patients with 0.9 cm and 1.8 cm tumors respectively, and 1 SSCCT patient with a 2.0 cm tumor abutting the thyroid posterior border. Eight PSCCTs arose in the right lobe, 4 arose in the left lobe and 2 spread to the isthmus. Fourteen SSCCTs evenly arose in the right and left lobe, one 1.0 cm SSCCT from larynx in the isthmus of thyroid. Four SCCTs arose in the setting of nodular goiter, two concurrent with Hashimoto thyroiditis in the real-time ultrasound examination. Two SSCCTs, neck nodular foci abutting thyroid, one from neck lymph node metastasis of cervix uteri, and the other from laryngeal SCC, versus pathology, were not diagnosed by practitioners in the real-time ultrasound reports. The mean size of 5 SCCTs with radical operation was 3.3 ± 1.5 cm (range, 1.1-5.0 cm), while the mean size of 4.5 ± 1.1 cm (range, 3.0-6.0 cm) was found in 7 SCCTs with other surgery.

Twenty-seven SCCTs exhibited solid (25/27, 92.6%) or almost completely solid composition (2/27, 7.4%), hypoechoic (17/27, 63%) and very hypoechoic (10/27, 37%), irregular/lobulated margin (100%). Among the 5 SCCTs with pathological necrosis, only 1 neck nodule of SSCCT abutting and lifting thyroid showed mainly solid mixed with a small central cystic area, while the remaining 4 lesions were hypoechoic solid TNs with various internal hypoechogenicity. Taller-than wide shape was found in 48.1% of SCCTs (13/27) and PEF were found in 22.2% of SCCTs (6/27). Twenty-four SCCTs (24/27, 88.9%) had local ETE in real-time ultrasound reports, and all of them had ETE (5 minimal ETE and 22 Extensive ETE) in TIRADS evaluation. The ultrasound-based ETE showed good agreement with intraoperative observation in 13 patients with surgery (Table 2) and showed pathological agreement in 4 of 5 patients with radical operation. Various chaotic internal echoes, such as short linear, multiple sporadic cloudy hyperechoic, central flake attenuation, nodular hypoechoic, or accidental small anechoic area, were observed within the SCCT. Hypervascularity with a high resistive index (RI) was found in 23 SCCTs (85.2%), with a mean RI of 0.85 ± 0.14 (n=17) and a mean peak systolic velocity (PSV) of 23.3 ± 15.4cm/s. 50% of patients (5 PSCCT, 8 SSCCT) were accompanied by neck lymphadenopathy.

Twenty-seven SCCTs were evaluated as TR4 (2/27, 7.4%) and TR5 (25/27, 92.6%), which merited 6-14 points in ACR-TIRADS, and were classified as 4B (3/27, 11.1%), 4C (23/27, 85.2%) and 5 (1/27, 3.7%), which merited 2-5 points in C-TIRADS (Figure 1). Neither FNA nor follow-up was recommended for one 0.9cm TR4 nodule since its size in ACR-TIRADS. FNA was recommended for all SCCTs in C-TIRADS. No statistically significant differences in characteristics were found between PSCCT and SSCCT, except for tumor differentiation (P=0.007) and hoarseness (P=0.032) (Table 3). Figure 2 showed ultrasound features of poorly differentiated tumors in 2 PSCCT patients, one with and the other without hoarseness. Figure 3 revealed a moderately differentiated SSCCT with extensive ETE characterized by frank invasion of the trachea, tracheal cartilage, capsule and strap muscles, and posterior tissue.

Discussion

According to our statistical result, PSCCT was more poorly differentiated (58.3%) than SSCCT (26.7%). PSCCT comprised 0.2% (11/5718) of thyroid malignancy over 23 years, and SSCCT comprised 0.3% (18/5718), which was consistent with the proportions reported in the literature (0.12–0.5%) (5, 10, 15–17). PSCCT is rarer than SSCCT in most literature, and occasionally

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TABLE 2 Intraoperative tumor invasion in 13 SCCT patients with surgery.

Gender/age (years)	Surgery	Primary site	Grade	Location	TN size	ETE	Local invasion of intraoperative findings
Male/43	NA	Thyroid	P	Right lobe	3.1cm	Extensive	NA
Female/62	PO	Thyroid	P	Left lobe	3.0cm	Minimal	Esophagus and recurrent laryngeal nerve
Female/53	PO	Thyroid	P	Right lobe + Isthmus	3.9cm	Minimal	Esophagus, recurrent laryngeal nerve, thyroid capsule, multiple lymph node
Male/62	RO	Thyroid	W	Right lobe	5.0cm	Minimal	CCA, thyroid capsule, posterior tissue
Male/60	PO + tracheotomy	Thyroid	P	Left lobe + Isthmus	6.0cm	Both	Esophagus, trachea and muscles
Male/42	RO	Thyroid	M	Left lobe	3.6cm	Both	Surrounding fibrous adipose tissue
Male/58	PO	Esophagus	M	Right lobe	3.7cm	Both	Internal jugular vein, CCA, Trachea and Thyroid capsule, esophagus, Strap muscles
Male/60	RO	Larynx	M	Isthmus	1.1cm	Both	Strap muscles, crico thyroid membrane
Male/48	RO	Larynx	M	Right lobe + Isthmus	2.6cm	Both	Thyroid capsule, posterior muscle, cricoid cartilage
Male/59	PO	Larynx	M	Left lobe + Isthmus	4.7cm	Both	Trachea, thyroid capsule, nerve, esophagus, strap muscles
Male/74	PO + tracheotomy	Esophagus	M	Right lobe	4.5cm	Both	Surrounding muscle tissue, esophagus, trachea
Male/53	RO	Larynx	M	Left lobe	4.2cm	Minimal	Thyroid capsule, trachea
Male/60	Excisional biopsy	Thyroid	P	Left lobe + Isthmus	6.0cm	Extensive	Neck muscles, trachea, posterior soft tissue, esophagus

NA, not available; PO, palliative operation; RO, radical operation; P, poorly differentiation; W, well differentiation; M, moderately differentiation; Both, minimal + extensive; CCA, common carotid artery.

opposite report which included 17 PSCCT patients and 6 SSCCT patients (18). The rarity of SCCT may be due to the absence of squamous epithelial tissue in the thyroid gland and the rich thyroid arterial supply with fast flow, which prevents the deposition of metastatic cells of SSCCT (19, 20). The origin of PSCCT is far from conclusive (21). More widely accepted hypothesis theories in terms of the origin are that PSCCT results from squamous metaplasia/differentiation of other thyroid diseases, such as nodular goiter, Hashimoto thyroiditis, tumors (papillary, follicular, or medullary cancer), and residual squamous epithelial tissue from the thyroglossal duct or ultimobranchial body during embryonic development (15, 16, 22–24). However, the above clinical conditions only occupy a small proportion of PSCCT. PTC plus Hashimoto thyroiditis only represented approximately 27.3% (3/11) of PSCCT. PTC with a component of SCC could denote a poor prognosis with a few months' survival (25, 26). Twenty-six point seven percent of SSCCT (4/15) with a history of Hashimoto thyroiditis is higher than 13.7% (16/117) in the literature (5). Thyroid metastatic tumors are more likely to develop in the context of thyroid diseases than normal thyroid glands (27). In pace with earlier diagnosis and treatment of related thyroid disease, especially PTC, PSCCT in epidemiology has decreased in frequency from 0.5% to 0.1% over four decades (28–30). It may be the evidence to support PSCCT originating from the above hypothesis theories. Hypercalcemia-leukocytosis paraneoplastic syndrome was not found in our study, although it has been

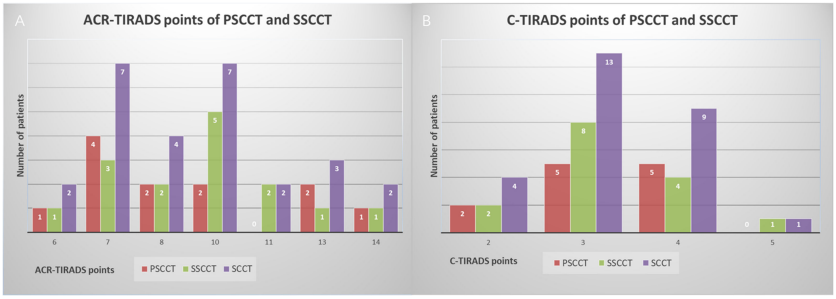


FIGURE 1
Points of SCCT (PSCCT, SSCCT) based on ultrasound in ACR-TIRADS **(A)** and C-TIRADS **(B)**.

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TABLE 3 PSCCT and SSCCT patients' clinic-pathological and ultrasound features at diagnosis.

Patients	PSCCT (*n*=11)	SSCCT (*n*=15)	SCCT (n=26)
Age, Mean ± SD (range), years	56.6 ± 10.7 (42–80)	62.9 ± 9.0 (48–81)	60.2 ± 10.1 (42–81)
Gender			
Male (%)	9 (81.8%)	12 (80.0%)	21 (80.8%)
Female (%)	2 (18.2%)	3 (20.0%)	5 (19.2%)
Chief symptoms[a]			
Rapidly increasing neck mass	8 (72.7%)	10 (66.7%)	18 (69.2%)
Dysphagia	4 (36.4%)	3 (20.0%)	7 (26.9%)
Hoarseness (p=0.032)*	5 (45.5%)	1 (6.7%)	6 (23.1%)
Dyspnea	3 (27.3%)	2 (13.3%)	5 (19.6%)
Cough, stridor and breathy	2 (18.2%)	2 (13.3%)	4 (15.4%)
Neck pain	0	2 (13.3%)	2 (7.7%)
Hemoptysis and blood in sputum	0	1 (6.7%)	1 (3.8%)
Accompanied B symptoms	1 (9.1%)	1 (6.7%)	2 (7.7%)
TNs detected by ultrasound	3 (27.3%)	2 (13.3%)	5 (19.6%)
Medical history			
Hashimoto thyroiditis	2 (18.2%)	4 (26.7%)	6 (23.1%)
Smoking	2 (18.2%)	6 (40.0%)	8 (30.8%)
Pulmonary tuberculosis	0	1 (6.7%)	1 (3.8%)
COPD	0	2 (13.3%)	2 (7.7%)
Alcohol consumption	1 (9.1%)	1 (6.7%)	2 (7.7%)
Invasive ductal carcinoma of left breast	0	1 (6.7%)	1 (3.8%)
DM	0	1 (6.7%)	1 (3.8%)
Hypertension/Hyperlipemia/ or hyperuricemia	1 (9.1%)	1 (6.7%)	2 (7.7%)
Tumor metastasis			
Neck lymph node	5 (45.5%)	8 (53.3%)	13 (50.0%)
Lung	3/11 (27.3%)	1 (6.7%)	4 (15.4%)
Bone	0	1 (6.7%)	1 (3.8%)
Liver	1 (9.1%)	1 (6.7%)	2 (7.7%)
Management at diagnosis	**Tumor foci (*n*=12)**[b]		
CNB	6 (50%)	12 (80.0%)	18 (66.7%)
Surgery	7 (58.3%)	7 (46.7%)	14 (51.9%)
Palliative/debulking surgery	3 (25%)	3 (20.0%)	6 (22.2%)
Radical operation	2 (16.7%)	3 (20.0%)	5 (18.5%)
Incisional biopsy	1 (8.3%)	0	1 (3.7%)
Not available	1 (8.3%)	0	1 (3.7%)
Pathological features			
Tumor grade (P=0.007)*			
Poorly differentiated	7 (58.3%)	4 (26.7%)	11 (40.7%)
Moderately differentiated	2 (16.7%)	11 (73.3%)	13 (48.1%)
Well differentiated	3 (25.0%)	0	3 (11.1%)
Accompanied necrosis	1 (8.3%)	4 (26.7%)	5 (18.5%)
SCCT concurrent with PTC	1 (1/11, 9.1%)	0	1 (1/26, 3.8%)
Ultrasound features			
Tumor size, Mean ± SD, cm	3.8 ± 1.5	3.5 ± 1.2	3.7 ± 1.3
Tumor size ≤20 mm >20 mm	2 (16.7%) 10 (83.3%)	1 (8.3%) 14 (93.3%)	3 (11.1%) 24 (88.9%)
Distribution of thyroid tumors			
Bilateral lobe	1 (9.1%)	0	1 (3.8%)
Left lobe (extensive to isthmus)	7 (1) (63.6%)	6 (1) (46.7%)	14 (2) (53.9%)
Right lobe (extensive to isthmus)	2 (18.2%)	6 (40.0%)	8 (30.8%)
Composition			
Solid	12 (100.0%)	15 (100.0%)	27 (100.0%)
Almost completely solid	0	2 (13.3%)	2 (7.4%)
Echogenicity			
Hypoechoic	8 (66.7%)	9 (60.0%)	17 (63.0%)
Very hypoechoic	4 (33.3%)	6 (40.0%)	10 (37.0%)
Shape			
Taller-than-wide	5 (41.7%)	8 (53.3%)	13 (48.1%)
Wider-than-tall	7 (58.3%)	7 (46.7%)	14 (51.9%)
Margin			
Irregular/spiculated/ lobulated	6 (50%)	10 (66.7%)	16 (59.2%)
Extra thyroidal extension	12 (100%)	15(100%)	27 (100%)
Echogenic foci			
No calcification	10 (83.3%)	11 (73.3%)	21 (77.8%)
PEF	2 (16.7%)	4 (26.7%)	6 (22.2%)
Posterior echoes			
Enhancement	3 (25.0%)	5 (33.3%)	8 (29.6%)
No change	4 (33.3%)	7 (46.7%)	11 (40.7%)

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TABLE 3 Continued

Patients	PSCCT (*n*=11)	SSCCT (*n*=15)	SCCT (n=26)
Attenuation	3 (25.0%)	1 (6.7%)	4 (14.8%)
Mixed	2 (16.7%)	2 (13.3%)	4 (14.8%)
Intranodular vascularity			
Absent	2 (16.7%)	2 (13.3%)	4 (14.8%)
Increased	10 (83.3%)	13 (86.7%)	23 (85.2%)
Pulsed wave imaging	(n=8)	(n=9)	(n=17)[c]
RI, mean ± SD	0.8 ± 0.2	0.8 ± 0.1	0.8 ± 0.1
PSV, mean ± SD, cm/s	18.4 ± 7.3	27.7 ± 19.5	23.3 ± 15.4
ACR-TIRADS			
Points, Mean ± SD (Median, Range)	9.2 ± 2.8 (8, 6–14)	9.5 ± 2.3 10, 6–14)	9.3 ± 2.5 (10, 6–14)
Risk levels			
TR4 for 5–20%	1 (8.3%)	1 (6.7%)	2 (7.4%)
TR5 for at least 20%	11 (91.7%)	14 (93.3%)	25 (92.6%)
Recommendation by ACR-TIRADS (%)			
FNA	11 (91.7%)	15 (100%)	26 (96.3%)
No follow-up	1 (8.3%)	0	1 (3.7%)
C-TIRADS			
Points, Mean ± SD (Median, Range)	3.3 ± 0.8 (3, 2–4)	3.3 ± 0.8 (3, 2–5)	3.3 ± 0.8 (3, 2–5)
Category for malignancy risk rate			
4B for 10–50%	2 (16.7%)	1 (6.7%)	3 (11.1%)
4C for 50–90%	10 (83.3%)	13 (86.7%)	23 (85.2%)
5 >90%	0	1 (6.7%)	1 (3.7%)
Recommendation by C-TIRADS (%)			
FNA	12 (100%)	15 (100%)	27 (100%)

[a]Various symptoms may occur in one patient, so the total exceeded 26.
[b]Two TNs respectively arose in the left and right thyroid glands in one PSCCT patient, so the total PSCCT foci was 12.
[c]Pulsed wave imaging was performed in 8 PSCCT and 9 SSCCT patients, so the total number was 17.
*P values (<0.05) of the difference were statistically significant.

reported in PSCCT (31). PSCCT is more common in females, with a female-to-male ratio of 1.2-2.4 to 1 in related English literature (5, 28, 29). However, we found reverse gender predominance, female to male ratio of 1 to 4.5 for PSCCT and 1 to 4 for SSCCT, similar to one Chinese study (18). The male is more affected in SCC of the lung, esophagus, trachea, and larynx, which may be one of the causes of male predominance of SSCCT (32). But the rationality of reverse gender predominance for PSCCT is unable to identify. The reverse gender predominance may be probably associated with geographical and ethnic differences, which is needed to investigate further. SCCT occurs in the sixth or seventh decade elderly, older than the mean age in the fifth decade of conventional PTC (5, 28, 33).

SCCT rapidly grows with accompanying symptoms related to the mass effect of cancer. Dysphagia, hoarseness, dyspnea, and cough are common symptoms of SCCT. Hoarseness was more common in PSCCT than SSCCT because the tumor's immediate vicinity, compression, and infiltration to the recurrent laryngeal nerve are inclined to cause vocal cord paralysis in PSCCT (18). The clinical presentation of metastatic SSCCT could mainly depend on the extent of the primary tumor, with only 20% of metastatic SSCCT with an enlarging neck mass and the rest being discovered at autopsy (30). For the thyroid itself, SSCCT symptoms resembled those of PSCCT at diagnosis. Rapidly enlarging neck masses along with swelling pain in 2 SSCCT patients may be associated with chronic inflammation (8).

The SCCT can be easy to pin down and be screened out by routine thyroid ultrasound examination, even in those asymptomatic patients. SCCT commonly presents as a large TN/neck mass, with only a tiny minority as multiple nodules in metastatic SSCCT, mainly involving one thyroid lobe. 88.9% of SCCTs (24/27) were >2cm at diagnosis. In some cases, oversized SCCTs extend beyond the thyroid gland, bulging the gland contour and abutting adjacent structures, resulting in 48.1% of SCCTs (13/27) with a taller-than-wide shape on the transverse sonogram. This pattern of taller-than-wide shape is different from that of PTC. However, diagnosis of PSCCT in the early stages is challenging due to its rapid growth, lack of symptoms, and absence of typical imaging findings (34). Small-size SCCTs often come across and are misdiagnosed as benign diseases such as nodular goiter or Hashimoto nodule-like change in the real-time ultrasound report.

The ultrasound features of SCCT include solid or almost completely solid composition, hypoechoic and very hypoechoic echogenicity, PEF, and irregular/lobulated margins, especially ETE, which are highly suspicious for malignancy. Very hypoechoic might reflect the assembling of resembling tumor cells. Heterogeneous echotexture with chaotic and scattered hyperechoic was seen in 60% of PSCCT and 20% of SSCCT, likely representing that the hyperplastic fibrous tissue was squeezed and deformed due to rapid proliferation of tumor cells, occasionally accompanied by necrosis, hemorrhage and more chronic inflammatory cell. Pathological necrosis is not always described as an anechoic/cystic area on the sonogram, as among the 5 SCCTs with pathological necrosis, only one from larynx showed a small central anechoic necrotic area, while the other 4 foci were solid with irregular and vertical stripe decaying bands. PEF, corresponding to the psammomatous calcifications associated with PTC, are considered a malignant sign, particularly in combination with other suspicious features. PEF with 22.2% in SCCT are lower than up to 40–50% in PTC and more common than peripheral/eggshell calcification with low specificity (7, 35–38).

The most common spread route of SCCT is local extension into adjacent structures. Ultrasound allows excellent assessment of the local infiltration. Irregular/ill-defined margin, taller-than-wide shape, and ETE are all presentations of SCCT's invasive nature. A large proportion of SCCTs (88.9–100%) were evaluated to have local ETE in real-time ultrasound reports and the two TIRADS guidelines, higher than 78.9% (15/19) in the literature (39). About 93% of PSCCT showed extensive ETE with prominent vascular and

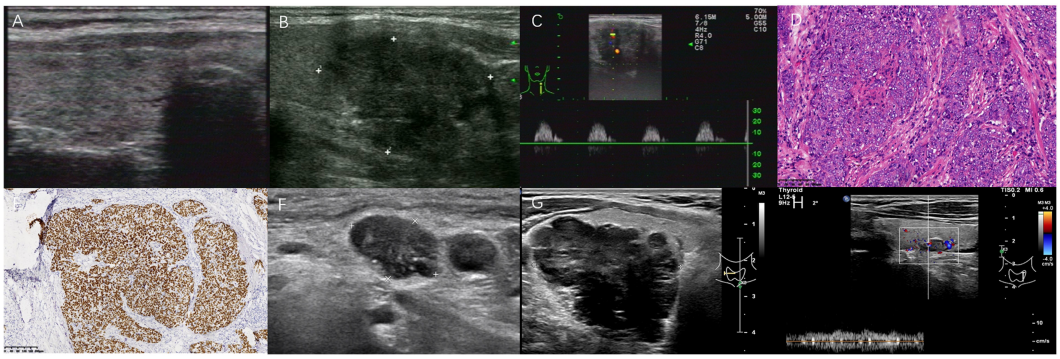


FIGURE 2
Two poorly differentiated PSCCT patients. A 62-year-old asymptomatic female underwent left lobectomy without any ETE by pathological examination **(A–F)**. Another 52-year-old male with hoarseness underwent CNB **(G, H)**. **(A)** Concomitant Hashimoto thyroiditis with multiple micronodules in right thyroid; **(B)** A 3.1×2.5 cm heterogeneous very hypoechoic TN was evaluated as TR5 with 7 points in 2017 ACR-TIRADS, 4C with 3 points in 2020 C-TIRADS. FNA was recommended; **(C)** Hypervascularity with high RI 1.0, PSV 31 cm/s; **(D)** Tumor cells presented with nest-like infiltration, mainly solid growth (H&E,×100); **(E)** Positive p63 (immunohistochemistry, ×100); **(F)** 1.4×0.9 cm hypoechoic lymph node metastasis with PEF and lobulated margin at 5.5 years after left lobectomy; **(G)** A larger TN with posterior ill-defined margin abutting tissue in right thyroid lobe; **(H)** Contralateral a small 0.9 cm lobulated TN with a central artery, was suggested for FNA recommendation in C-TIRADS, but not in 2017 ACR-TIRADS for its size.

peri-neural infiltration (5). SSCCT that directly spreads to the thyroid from adjacent structures is often incidentally diagnosed by surgical histopathology (30). The direct thyroid invasion can be clarified based on the analogy of minimal ETE, such as the presence of border abutment and lifting contour of the thyroid in 2 SSCCTs can provide clues for thyroid involvement. Posterior anatomic structures of the thyroid could not be clearly described due to limited penetration of high-frequency probe and great over-size tumor. Therefore, due to SCC's neurotropism, the ill-defined margin abutting the posterior tissue, along with hoarseness, could be reliable signs involving the recurrent laryngeal nerve, which is in line with intraoperative and pathological findings. Nodal metastases are common in neck lymph nodes, affecting 45.5% of PSCCT (5/11). Distant metastases commonly develop in lymph nodes, lungs, bone, liver, and heart (39). Only lung and liver metastases occurred in this study in 36.4% of patients (4/11), lower than the percentage of 73.7% (14/19) reported in an autopsy study (39).

Neovascularization leads to the progressive and rapid growth of SCCT. Most SCCTs were found to have hypervascularity with chaotic, malformed vessel structures. Even microcarcinomas<1.0 cm have a large central supplying artery with low velocity and resistance. Contrary to preconceived ideas, RI and PSV in SSCCT were higher than in PSCCT. Although color Doppler imaging is not included in the TIRADS guidelines due to unreliable discrimination between benign and malignant nodules (40), it can help distinguish solid tissue from echogenic debris or hemorrhage. The absence of flow within a partially attenuated area of SCCT may indicate pathological necrosis (11, 12, 41).

Both ACR-TIRADS and C-TIRADS evaluated 27 SCCTs as in high risk stratification and recommended for FNA except for a 9.7

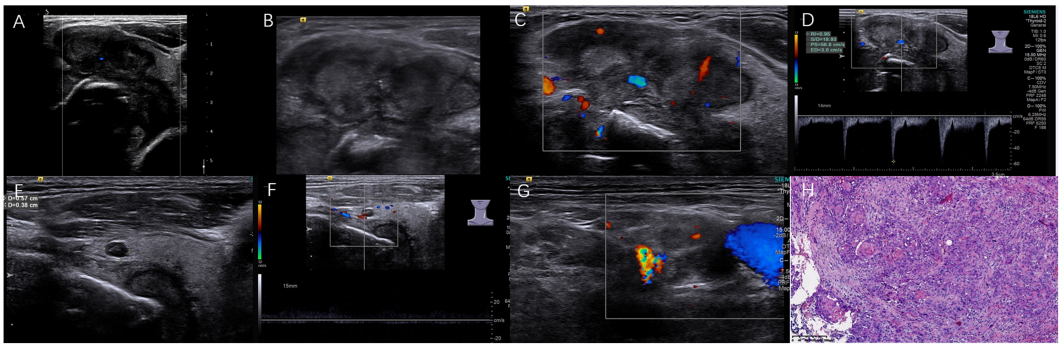


FIGURE 3
A moderately differentiated SSCCT in a 59-year-old male with the palliative operation and tracheotomy. The irregular solid TN was bestowed 14 points/TR5 in ACR-TIRADS, 5 points/5 in C-TIRADS based on preoperative ultrasound. **(A)** The tumor with extensive ETE involved thyroid left lobe and isthmus; **(B)** PEF within nodular heterogeneous hypoechoic; **(C, D)** Increased blood flow with high RI 0.95 and PSV 56.8 cm/s; **(E, F)** Another adjacent 0.4×0.6 cm small TN with central artery; **(G)** 0.9×1.5 cm neck lymph nodes with ill-defined margin; **(H)** Nodular tumor cells with incidental cornified pearl (H&E, ×100).

mm TR4 in ACR-TIRADS. Definitive diagnosis of SCCT was made by FNA in only 6.7–26% of cases (16, 42, 43), so CNB was used to obtain more abundant specimens for higher accuracy in this study. CNB was performed in the 9.7 mm 4B nodule due to its abutting posterior capsule and multifocality in C-TIRADS. However, in ACR-TIRADS, neither FNA nor follow-up was recommended since the TR4 nodule size threshold of FNA is ≧15 mm, and follow-up is ≧10 mm. Although ACR-TIRADS showed higher diagnostic performance and a lower FNA rate (44), small SCCT may be missed for FNA due to the size thresholds. C-TIRADS recommends FNA for 4B nodules >1 cm and >5 mm with minimal ETE or multifocal nodules, which have smaller size cutoffs than those advocated by ACR-TIRADS (45). Thus C-TIRADS was sensitive to recommending FNA of SCCT<1.5 cm, similar to the 2015 ATA guideline (10). The two TIRADSs can identify 1.5-2 cm SCCT foci that warrant biopsy. C-TIRADS could be used to detect newly small SCCT foci (0.5-1.5cm), and ACR-TIRADS might help reduce the unnecessary biopsies in FNA. Although SCCT is a highly aggressive malignancy, patients underwent radical operation obtained a better prognosis. Early detection and definite diagnosis could significantly increase the proportion of radical operation. The mean size of SCCTs with radical operation was found to be smaller than that of those without radical operation. Rapid treatment initiation could improve the long-term outcomes and prolong survival rates (4, 46). Radical operation with adjuvant chemoradiotherapy may obtain a favorable outcome. More than 24-34 months of survival has been reported in PSCCT patients who underwent surgery and radiation therapy with no recurrence or metastasis, longer than the 3-15 months survival commonly reported (31, 46, 47). A 62-year-old asymptomatic female (Figure 1) with minimal ETE (thyroid contour bulging) in ultrasound underwent radical operation plus radiotherapy, without ETE in pathology, and survived for 7 years to date, which is the longest survival in our study. Besides, five SSCCT patients survived for 14-72 months during the follow-up period, which is within 3-186 months in a 25-year review (4, 15). Compared to the classic thyroid cancer (predominately PTC), SCCT mainly represents as larger size and more aggressive nature (more ETE and neck lymph node metastasis). Category 5 nodule was more common in PTC (63.2–90%), 4C was more common in SCCT (85.2%) in C-TIRADS, and no difference was found in ACR-TIRADS. The details were showed in Table S1 (48–51).

The study has several limitations. Firstly, the agreement of local ETE between ultrasound and pathological examination cannot be confirmed in 22 SCCTs since palliative surgery, incisional biopsy and CNB cannot afford the pathological features of the entire tumor. Secondly, the small sample size and single-center retrospective study may lead to the absence of statistical significance between PSCCT and SSCCT in the majority of clinical and ultrasound features. Therefore, studies with large cohorts of patients and prospective research with multi-center are necessary.

In summary, SCCT is a rare malignancy that commonly presents with a rapidly enlarging neck mass with high aggressiveness. PSCCT and SSCCT have similar clinic-pathological and highly suspicious malignant ultrasound features except for hoarseness and tumor differentiation. Ultrasound is an optimal radiological modality to detect and diagnose SCCT based on the two TIRADS guidelines. Finally, the male predominance of SCCT patients in China requires further evaluation.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

XZ contributed to the conception and design of this study. Material preparation and data collection were performed by XZ, BW, LN, HZ, JZ, and YJ. Analysis was performed by XZ and BW. The first draft of the manuscript was written by XZ, WB. All authors commented on previous versions of the manuscript and read and approved the final manuscript.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2023.1238775/full#supplementary-material

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Treg cells as a protective factor for Hashimoto`s thyroiditis: a Mendelian randomization study

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Background and objectives: Hashimoto's thyroiditis (HT), a chronic autoimmune disorder impacting thyroid function, is a growing public health concern. The relationship between Treg cells and HT has been extensively studied, with Treg cells considered crucial in suppressing HT progression. However, these studies have mainly been observational, limiting our understanding of Treg cells' impact on HT risk. Leveraging large datasets, we utilized Mendelian randomization (MR) analysis to examine the causal association between Treg cell biomarkers and HT, providing additional validation for these relationships.

Methods: Comprehensive two-sample Mendelian randomization analysis was performed to determine the causal association between Treg cells signatures and HT in this study. Based on publicly available genetic data, we explored causal associations between 165 Treg cells signatures and HT risk.

Results: The European cohort study has identified five Treg cell phenotypes that causally protect against HT risk. Resting Treg %CD4 (OR = 0.975, 95% CI = 0.954~0.998, *P* = 0.030); CD4 on resting Treg (OR = 0.938, 95% CI = 0.882~0.997, *P* = 0.041; CD28- CD8dim %CD8dim (OR = 0.983, 95% CI = 0.969~0.998, *P* = 0.030); CD25 on CD39+ resting Treg (OR = 0.926, 95% CI = 0.864~0.991, *P* = 0.026); 5) CD28 on activated & secreting Treg (OR = 0.969, 95% CI = 0.942~0.996, *P* = 0.025). The Asian cohort study has identified four Treg cell phenotypes negatively correlated with the risk of HT. CD25hi %T cell (OR = 0.635, 95% CI = 0.473~852, *P* = 0.002); CD4 Treg %CD4 (OR = 0.829, 95% CI = 0.687~1.000, *P* = 0.050); CD127-CD8br %T cell (OR = 0.463, 95% CI =0.311~0.687, *P*< 0.001); CD3 on resting Treg (OR = 0.786, 95% CI = 0.621~0.994, *P* = 0.044).

Conclusion: Our study has demonstrated the close connection between Treg cells and HT by genetic means, thus providing foundational basis for future research.

KEYWORDS

Hashimoto`s thyroiditis, Treg cells, immunity, causal inference, MR analysis

1 Introduction

Hashimoto's Thyroiditis (HT) is a common chronic autoimmune disease affecting thyroid function (1, 2), named after the Japanese scientist Hashimoto, who first described it as a type of autoimmune thyroiditis typically resulting in hypothyroidism (3). The primary pathological changes include extensive enlargement of the thyroid gland, follicular hyperplasia, and the presence of plasma cells and lymphocytes (4). The incidence of HT has been rapidly increasing worldwide in recent years (4), with rates ranging between 27 and 448 per 100,000 (5). Consequently, HT has become a critical public health issue. And studies have shown that, HT is a known risk factor for thyroid cancer development (6). Therefore, Precise diagnosis and effective treatment of HT are essential for enhancing patients' quality of life and reducing their risk of thyroid cancer (7).

Despite the ongoing debate regarding the etiology and pathogenesis of HT, there is a prevailing agreement that autoimmunity maintains a crucial role in its pathogenesis (2, 8). Recently, regulatory T cells (Treg) have emerged as crucial factors in HT. Treg cells, a CD4 + T cell population, exert immunosuppressive effects on excessive immune responses (9–11). Animal experiments (12–14) indicate that the lack of Tregs could result in the spontaneous advancement of autoimmune thyroiditis. Clinical evidence (10, 15) further substantiates that Treg frequency in circulation is reduced among HT patients. These findings suggest that a decrease in Treg numbers or dysfunction leads to impaired immune tolerance towards autoantigenic stimuli, contributing to the development of Hashimoto's thyroiditis. Consequently, Treg immunomodulation has emerged as a crucial matter in recent HT immunology research. However, conflicting findings exist in other studies. Glick et al. reported no significant difference in CD4+CD25 + Tregs frequency in HT (16). Even one study found a significantly higher proportion of CD4+Foxp3+ Tregs in autoimmune thyroid disease (AITD) patients (17). The variation may be ascribed to the dual regulatory function of the immune system and the heterogeneity induced by the multiple Treg cell subtypes (18). Therefore, investigating the association between Treg cell subtypes and HT could potentially have a crucial role in precise immunotherapy for HT.

Mendelian randomization (MR) utilizes single nucleotide polymorphisms (SNPs) as instrumental variables (IVs) to infer potential causal relationships between exposure and outcomes (19, 20). follows the Mendelian gamete random allocation principle as well as the principle of free combination. This approach effectively mitigates the impact of confounding factors and reverse causation commonly encountered in conventional observational studies (21). MR is widely used in genetic epidemiology (22). With the large datasets currently available, MR analysis may be a good tool for exploring novel biomarkers in Treg cells that have a causal impact on the risk of Hashimoto's thyroiditis. Therefore, this study employs a two-sample Mendelian randomization approach, utilizing large-scale GWAS databases to explore the causal relationship between immune cell phenotypes and HT risk, To our knowledge, there haven't been previous studies using MR to assess the causal impact of Treg cell traits on the risk of HT.

2 Materials and methods

2.1 Study design

The current study applied a two-sample MR method to analyze causal relationships between 165 Treg traits and HT. Therefore, to ensure the validity of causal inference, the IVs relies on three fundamental assumptions: 1) Genetic variants have a direct connection with the exposure; 2) Genetic variants are not associated with potential confounding factors between exposure and outcome; and 3) Genetic variants do not influence the outcome through pathways unrelated to the exposure. All MR analyses were conducted using publicly available summary statistics, thus eliminating the need for additional ethical approval or informed consent.

2.2 Data source

The GWAS summary statistics for a diverse range of Treg traits were retrieved from the GWAS Catalog (23), the GWAS Catalog Accession Numbers are detailed in Supplementary Table 1. The original GWAS for immune traits utilized data from 3,757 European individuals, these datasets encompass an extensive collection of 165 Treg traits. The GWAS summary statistics for HT were sourced from the GWAS Catalog, encompassing datasets from both European (accession number GCST90018855) (24) and Asian populations (accession number GCST90018635). The initial GWAS analysis for the European cohort involved 395,640 individuals, including 15,654 cases and 379,986 controls. Likewise, the original GWAS analysis for the Asian cohort included 173,193 participants of East Asian descent, predominantly of Japanese origin, with 537 cases and 172,656 controls.

2.3 Instrumental variable selection

Genetic variants were utilized as IVs in this study. Significant SNPs that were associated with the Treg cell phenotype with genome-wide significance ($P<1\times10^{-5}$) (25) were selected based on recent research. To minimize bias due to linkage disequilibrium (LD), the following conditions were imposed: $R^2< 0.001$ and distance = 10,000 kb (26). IVs with an F< 10 were excluded to ensure strong correlation of SNPs with HT risk (Supplementary Table 2). The formula for calculating F is: $F = R^2 \times \frac{K-2}{1-R^2}$, where K is the sample size, R^2 is the proportion of variance explained by SNPs in the exposure database (27). The formula for calculating R^2 is: $R^2 = \frac{2\times\beta\times eaf\times(1-eaf)}{2\times\beta^2\times eaf\times(1-eaf)+2\times se^2\times K\times eaf\times(1-eaf)}$, where eaf is the effect allele frequency, β is the allele effect value, K is the sample size, and se is the Standard Error. The exposure and outcome datasets were merged, and incompatible alleles and palindromic SNPs were eliminated. The phenotypes associated with the remaining SNPs were identified utilizing the Human Genotype-Phenotype Association Database (http://www.phenoscanner.medschl.cam.ac.uk/) (28). The remaining SNPs were ultimately utilized as instrumental variables for exposure.

2.4 Statistical analysis

The univariable MR analysis was performed using the R packages "TwoSampleMR". Our analysis primarily utilized the inverse variance weighting (IVW) method (29). We employed four additional methods to assess the reliability and consistency of our results. These methods include MR-Egger regression (30), the weighted median estimator (WME) (31), mode-based simple estimation (32), and mode-based weighted estimation. All causal estimates were converted to odds ratios (ORs) for the outcome which was a dichotomous phenotype.

Heterogeneity among the individual estimates of genetic variation was evaluated using The Cochran Q test. No significant heterogeneity was observed when $P > 0.05$. We used the MR-Egger intercept to assess the effect of horizontal pleiotropy (33). Sensitivity analysis was conducted using the "leave-one-out" method to assess the impact of individual SNPs on the causal relationship. The Mendelian Randomization Pleiotropy RESidual Sum and Outlier (MR-PRESSO) method were utilized as indicators to evaluate and correct the level of pleiotropy (34), the MR-PRESSO was conducted using the R package "MRPRESSO" (Figure 1).

3 Results

3.1 The MR analysis of results from European cohorts

The results of the European cohorts study revealed nine Treg cell phenotypes significantly linked with HT risk. Figure 2 presents these findings. Of the identified Treg cell features, we found five that were protective against HT risk: 1) CD25 on CD39+ resting Treg (IVW OR = 0.926, 95% CI = 0.864~0.991, $P = 0.026$); 2) CD28- CD8dim % CD8dim (IVW OR = 0.983, 95% CI = 0.969~0.998, $P = 0.030$); 3) CD28 on activated & secreting Treg (IVW OR = 0.969, 95% CI = 0.942~0.996, $P = 0.025$); 4)CD4 on resting Treg (IVW OR = 0.938, 95% CI = 0.882~0.997, $P = 0.041$); 5) Resting Treg %CD4 (IVW OR = 0.975, 95% CI = 0.954~0.998, $P = 0.030$), as well as four other Treg cell subtypes (CD3 on CD39+ secreting Treg, CD3 on CD4 Treg, CD3 on CD45RA+ CD4+, CD39+ activated Treg %activated Treg). Reverse MR analysis revealed a negative causal relationship between HT and CD25 on CD39+ resting Treg (IVW OR = 0.891, 95% CI = 0.798~0.996, $P = 0.042$) (see Figure 3). The sensitivity test results are displayed in Table 1. Additionally, the leave-one-out analysis corroborated this finding (see Supplementary Figure 1). Causal effect estimates were depicted using scatter plots (see Supplementary Figure 2). Moreover, funnel plots, providing a visual appraisal, revealed no heterogeneity in the causal effect (see Supplementary Figure 3).

3.2 The MR analysis of results from East Asian cohorts

The study's findings in the East Asian cohorts indicate that seven Treg cell phenotypes are significantly linked to HT risk. Figure 4 demonstrates these associations. Four Treg cell features were identified as beneficial in mitigating HT risk: 1) CD127-CD8br %T cell (OR = 0.463, 95% CI =0.311~0.687, $P< 0.001$); 2) CD25hi % T cell (OR = 0.635, 95% CI = 0.473~852, $P = 0.002$); 3) CD3 on resting Treg (OR = 0.786, 95% CI = 0.621~0.994, $P = 0.044$); 4) CD4 Treg %CD4 (OR = 0.829, 95% CI = 0.687~1.000, $P = 0.050$). And 3 other Treg cell subtypes (CD25 on CD39+activated Treg, CD39 +CD8br %CD8br, CD39+CD8br %T cell).

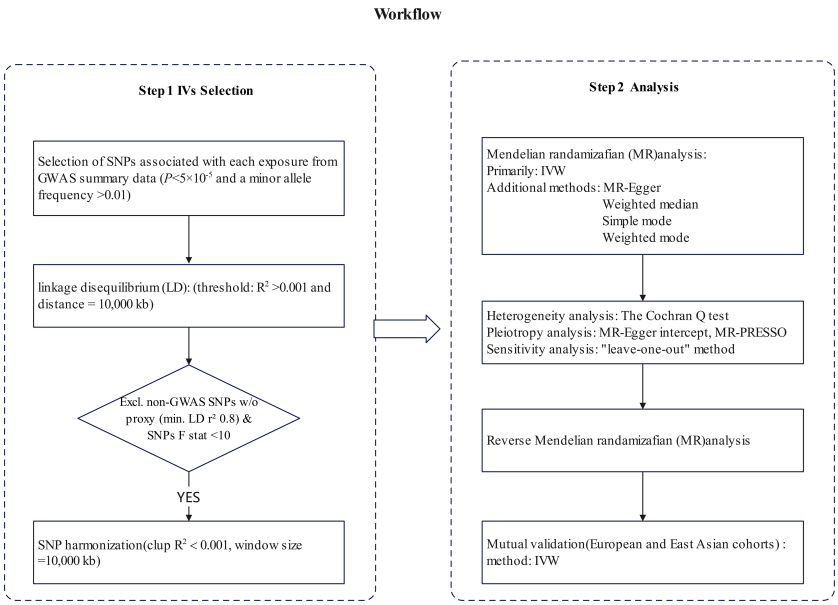


FIGURE 1
The steps of Mendelian randomization (MR)analysis. SNP, single-nucleotide polymorphism; IVW, inverse variance weighting; MR-PRESSO, Mendelian Randomization Pleiotropy RESidual Sum and Outlier.

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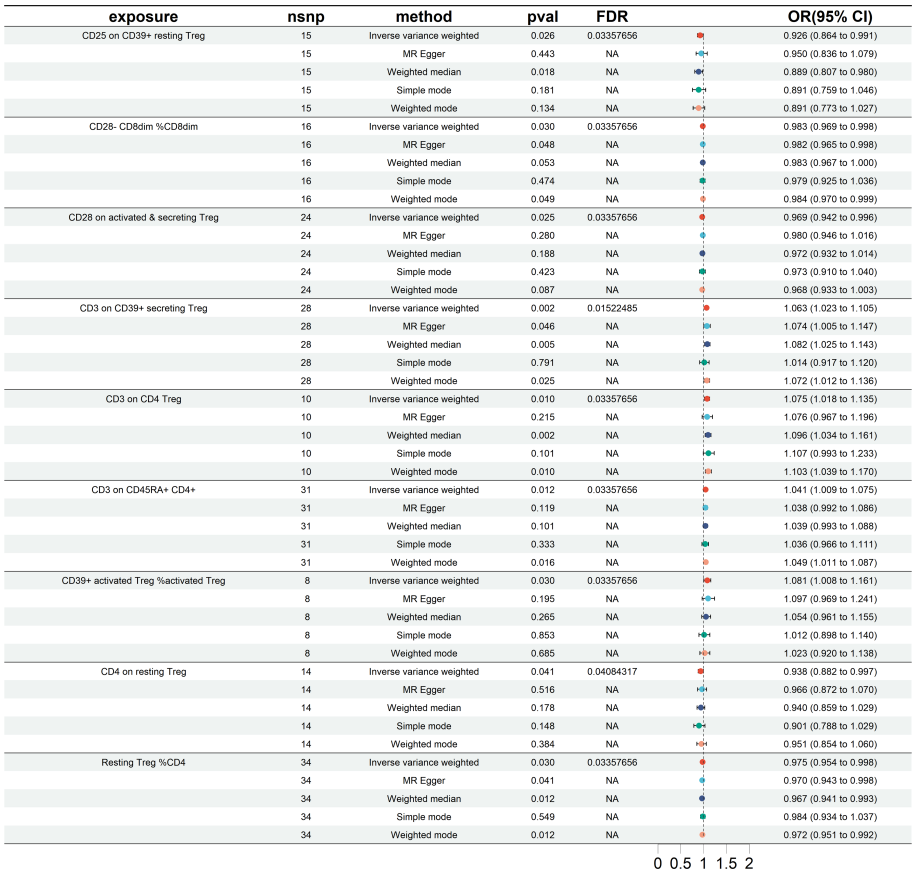


FIGURE 2
Forest plots showing significantly causal relationships between 9 Treg cell traits and HT (European cohorts). nsnp, number of single-nucleotide polymorphism; OR, odds ratio; CI, confidence interval.

Reverse MR analysis revealed no significant causal relationship between HT and Treg cell phenotype. The results of the sensitivity test are shown in Table 2. In the same vein, the leave-one-out test also supported this observation (Supplementary Figure 4). The scatter plots see Supplementary Figure 5, funnel plots see Supplementary Figure 6.

3.3 Cross-validation results comparing the European and East Asian cohorts

Mutual validation was performed between European groups and East Asian groups. The MR results showed a total of five results for both datasets that showed a similar propensity to protect as the preliminary analysis (IVW OR<1), but the significance did not reach statistical significance, as shown in Figure 5. Specifically included were CD25 on CD39+ resting Treg (East Asian OR = 0.759, and European OR = 0.926), CD28- CD8dim %CD8dim (East Asian OR = 0.991, and European OR = 0.983), CD28 on activated & secreting Treg (East Asian OR = 0.991, and European OR = 0.969), Resting Treg %CD4 (East Asian OR = 0.857, and European OR = 0.975), and CD4 Treg %CD4 (East Asian OR = 0.829, and European OR = 0.964). And 4 other Treg cell subtypes (CD4 on resting Treg, CD127-CD8br %T cell, CD25hi %T cell, and CD3 on resting Treg).

In conclusion, through MR analysis, we identified five treg cell characteristics of interest that were protective against the risk of HT. They include Resting Treg %CD4, CD4 Treg %CD4, CD28 on activated & secreting Treg, CD28- CD8dim %CD8dim, and CD25

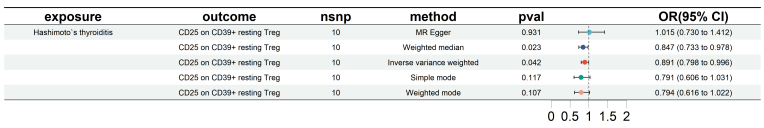


FIGURE 3
Forest plots showing reverse MR analysis results (European cohorts). nsnp, number of single-nucleotide polymorphism; OR, odds ratio; CI, confidence interval.

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TABLE 1 Result of sensitivity analysis (European cohorts).

Exposure	Outcome	Method	Cochran's Q			Pleiotropy		
			Q	Q_df	Q_pval	Egger intercept	SE	pval
CD25 on CD39+ resting Treg	Hashimoto`s thyroiditis	MR Egger	14.26	13	0.356	-0.0067	0.014	0.641
		IVW	14.51	14	0.412			
		MR-PRESSO						0.457
CD28- CD8dim %CD8dim	Hashimoto`s thyroiditis	MR Egger	18.33	14	0.192	0.0044	0.008	0.607
		IVW	18.70	15	0.228			
		MR-PRESSO						0.425
CD28 on activated & secreting Treg	Hashimoto`s thyroiditis	MR Egger	17.89	22	0.713	-0.0081	0.007	0.298
		IVW	19.02	23	0.700			
		MR-PRESSO						0.758
CD3 on CD39+ secreting Treg	Hashimoto`s thyroiditis	MR Egger	31.89	26	0.197	-0.0035	0.010	0.734
		IVW	32.04	27	0.231			
		MR-PRESSO						0.226
CD3 on CD4 Treg	Hashimoto`s thyroiditis	MR Egger	11.95	8	0.153	-0.0004	0.019	0.985
		IVW	11.95	9	0.216			
		MR-PRESSO						0.311
CD3 on CD45RA+ CD4+	Hashimoto`s thyroiditis	MR Egger	39.70	29	0.089	0.0016	0.008	0.835
		IVW	39.77	30	0.110			
		MR-PRESSO						0.104
CD39+ activated Treg %activated Treg	Hashimoto`s thyroiditis	MR Egger	7.28	6	0.296	-0.0056	0.020	0.790
		IVW	7.37	7	0.391			
		MR-PRESSO						0.452
CD4 on resting Treg	Hashimoto`s thyroiditis	MR Egger	11.91	12	0.453	-0.0081	0.012	0.499
		IVW	12.39	13	0.496			
		MR-PRESSO						0.527
Resting Treg %CD4	Hashimoto`s thyroiditis	MR Egger	47.07	32	0.042	0.0065	0.010	0.508
		IVW	47.73	33	0.047			
		MR-PRESSO						0.070
Hashimoto`s thyroiditis	CD25 on CD39+ resting Treg	MR Egger	4.74	8	0.785	-0.0221	0.027	0.435
		IVW	5.41	9	0.797			
		MR-PRESSO						0.708

IVW, Inverse variance weighted; MR-PRESSO, MR-Pleiotropy Residual Sum and Outlier.

on CD39+ resting Treg. These features exhibit a negative correlation with the risk of HT, suggesting a potential protective role against HT.

4 Discussion

HT is an autoimmune disease in which Treg cells play a crucial role in preventing the onset and progression by suppressing the production of harmful autoantibodies targeting thyroglobulin (TG) and thyroid peroxidase (TPO) (35) by autoreactive B cells, thus preventing the destruction caused by these autoreactive B cells (36, 37). Furthermore, Treg cells protect the thyroid tissue from attack by the release of immunosuppressive molecules such as IL-10 and TGF- β (38). In addition to their role in immune homeostasis, Treg cells play indispensable roles in self-tolerance (39). Numerous animal experiments (12, 13, 40) have demonstrated that the absence of Tregs can trigger the spontaneous development of autoimmune

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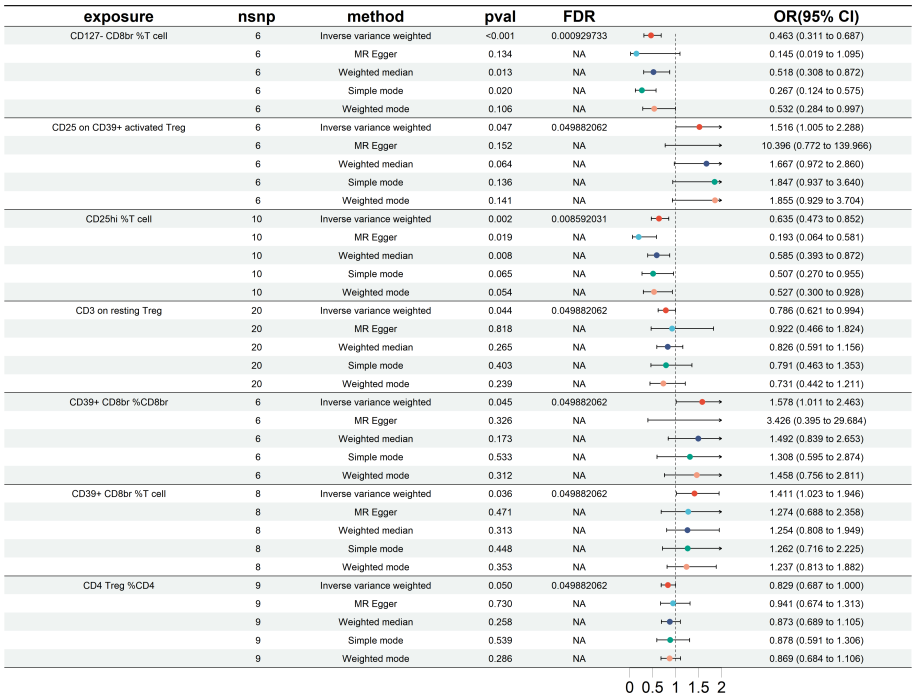


FIGURE 4
Forest plots showing significantly causal relationships between 7 Treg cell traits and HT (East Asian cohorts). nsnp, number of single-nucleotide polymorphism; OR, odds ratio; CI, confidence interval.

TABLE 2 Result of sensitivity analysis (East Asian cohorts).

Exposure	Outcome	Method	Cochran's Q			Pleiotropy		
			Q	Q_df	Q_pval	Egger intercept	SE	pval
CD127- CD8br %T cell	Hashimoto`s thyroiditis	MR Egger	4.17	4	0.38	0.19	0.165	0.32
		IVW	5.53	5	0.35			
		MR-PRESSO						0.45
CD25 on CD39+ activated Treg	Hashimoto`s thyroiditis	MR Egger	1.74	4	0.78	-0.28	0.19	0.22
		IVW	3.90	5	0.56			
		MR-PRESSO						0.6
CD25hi %T cell	Hashimoto`s thyroiditis	MR Egger	3.59	8	0.89	0.20	0.093	0.06
		IVW	8.42	9	0.49			
		MR-PRESSO						0.54
CD3 on resting Treg	Hashimoto`s thyroiditis	MR Egger	13.95	18	0.73	-0.03	0.061	0.63
		IVW	14.19	19	0.77			
		MR-PRESSO						0.78
CD39+ CD8br %T cell	Hashimoto`s thyroiditis	MR Egger	4.85	6	0.56	0.03	0.066	0.72
		IVW	5.00	7	0.66			
		MR-PRESSO						0.71
CD39+ CD8br %CD8br	Hashimoto`s thyroiditis	MR Egger	0.50	4	0.97	0.01	0.023	0.72

(Continued)

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TABLE 2 Continued

Exposure	Outcome	Method	Cochran's Q			Pleiotropy		
			Q	Q_df	Q_pval	Egger intercept	SE	pval
		IVW	1.01	5	0.96			
		MR-PRESSO						0.95
CD4 Treg %CD4	Hashimoto`s thyroiditis	MR Egger	3.71	7	0.81	-0.04	0.046	0.4
		IVW	4.51	8	0.81			
		MR-PRESSO						0.79

IVW, Inverse variance weighted; MR-PRESSO, MR-Pleiotropy Residual Sum and Outlier.

thyroiditis. Meanwhile, clinical evidence (10) suggests a decrease in the frequency of circulating Tregs among individuals diagnosed with HT. However, there are also reports indicating that Treg cells may contribute to the progression of HT (16, 17). Such discrepancies may be attributed to different detection markers or subsets with distinct phenotypes. Considering the dissection of Tregs into subpopulations and limited data reported in autoimmune thyroiditis, in order to delve deeper into the potential Treg cell phenotypes associated with reduced risk of HT, we comprehensively examined the causal relationship between 165 Treg cell phenotypes and HT utilizing public genetic data and employing Mendelian randomization methods (41). MR employs genetic variants as instrumental variables, which are fixed at conception, to perform causal inferences regarding the effects of modifiable risk factors. This approach can effectively mitigate certain types of confounding (42). Notably, this study represented a pioneering analysis utilizing Mendelian randomization to scrutinize the causal association between multiple Treg cell traits and HT. In our study, five protective factors were ultimately identified.

The first trait is Resting Treg %CD4. Previous research has demonstrated that by utilizing FoxP3 and CD45RA, three distinct subpopulations can be differentiated based on both phenotypic and functional characteristics: the CD45RA + FoxP3lo resting Treg (rTreg), the CD45RA-FoxP3hi effector Treg (eTreg), and the CD45RA-FoxP3lo cells (Fraction III, FIII). Resting Treg cells have the ability to suppress autoimmune reactions (43), which prevents the immune system from launching attacks against its own tissues. Resting-phase Treg has been demonstrated to decrease in autoimmune disease, indicating a potential role for Resting Treg in mitigating autoimmune disease (44, 45). Research on resting Tregs in HT is limited; currently, only one clinical study has reported no significant differences in the proportion of resting Tregs between HT patients and healthy volunteers, and there is no direct research establishing a link between resting Treg %CD4 and HT. However, HT is considered an autoimmune disease, and our MR analysis results also indicate a negative correlation between the percentage of resting Treg %CD4 and the risk of HT, suggesting a potential protective role of resting Treg %CD4 in HT.

The second protective trait is CD4 Treg %CD4. CD4 is a membrane protein expressed extensively on the surface of T cells, which is indispensable for recognizing most antigens in the body. The CD4 co-receptor augments the T cell sensitivities to antigens by 30- to 100-fold (46), and its primary function is to bind with MHC-II (major histocompatibility complex class II) (47) molecules that are present on the surfaces of antigen-presenting cells, mainly associating with the β-chain binding. This association allows CD4+ T cells to closely associate with antigen-presenting cells, which initiates T cell activation and guides their immune response (48, 49). This process is crucial for regulating the immune system's normal functionality and preventing excessive immune responses. Hence, the activation and execution of Treg cells' biological functions are integrally linked to CD4 molecules. Experimental *in vivo* studies have indicated a significant reduction in the proportion of CD4CD25 Treg cells in patients with HT compared to healthy controls (HCs) (50). One meta-analysis showed that newly

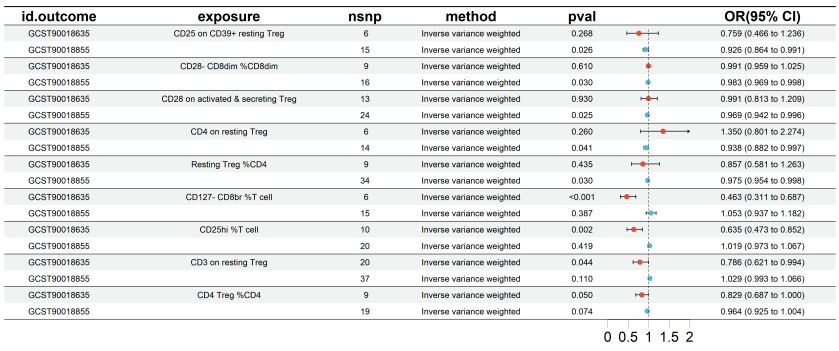


FIGURE 5
The MR analysis results of mutual validation was performed between European groups and East Asian groups. nsnp, number of SNP. CI, confidence interval.

diagnosed HT patients have lower levels of Tregs compared to HCs (51). Another meta-analysis investigated the percentage of peripheral blood Tregs among CD4+ T cells in patients with AITDs. The results revealed that the proportions of Tregs among CD4+ T cells in untreated AITDs were significantly lower than those in HCs. However, unfortunately, no significant differences were observed in untreated HT patients (52). Although our findings suggest a negative correlation between CD4 Treg %CD4 and HT, further studies are warranted to establish and validate this relationship, considering the limited research on the association between CD4 Treg %CD4 and HT.

The third and fourth involve CD28 on activated & secreting Treg, and CD28- CD8dim %CD8dim. CD28 is a type I transmembrane glycoprotein receptor typically expressed on most $CD4^+$ T cells (53). It serves as a co-stimulatory receptor expressed differently at specific stages of T cell differentiation and within specific T cell subgroups to regulate immune responses (54). T cell activation involves two signals. The first signal involves the T cell receptor (TCR) recognizing and binding specifically to the antigenic peptide-major histocompatibility complex (MHC) on antigen-presenting cells. The second signal results from the interaction between T cells and a range of co-stimulatory proteins expressed on the surface of antigen-presenting cells, generating co-stimulatory signals (55). It is worth noting that the most crucial interaction among these proteins is the binding of CD28 to CD80/CD86 (56). There is ongoing clinical development of a solitary CD28 agonist antibody, specifically TAB 08 (TGN 1412) (57, 58). However, the potential risks and benefits of this immunological agonist remain unclear and require further study (59). Additionally, CD28 superagonistic monoclonal antibodies (CD28SAB) have been found to preferentially activate and expand immuno-suppressive regulatory T (Treg) cells. In preclinical tests, CD28SABs have demonstrated the ability to alleviate the pathogenesis, progression, and clinical course of autoimmune diseases (60). Although our results revealed a negative association between CD28 on activated & secreting Treg and the risk of HT, which is consistent with previous findings, it is noteworthy that the CD28- CD8dim %CD8dim phenotype was also negatively associated with the risk of HT. Previous studies indicated that since CD28 was expressed not only on Treg but also on effector T cells, over-activation of effector T cells aggravated the progression of thyroid tissue injury or inflammation (61). Hyperreactivity of B7/CD28 signaling is closely linked with the development of autoimmune diseases (62). Cytotoxic T-Lymphocyte Antigen-4 (CTLA-4) is an inhibitory protein that competitively binds to B7 molecules (CD80 and CD86) in competition with CD28 (63), exerting immune co-suppressive responses (64), making it a highly regarded therapeutic target in the treatment of autoimmune diseases. It has been reported that using CTLA-4 fusion protein to block the interaction between CD28 and B7 can inhibit the autoimmune response in experimental rheumatoid arthritis (RA) (65). Additionally, inhibiting the CD80-CD28-mediated signaling pathway via specific RNAi and antibodies to block the interaction between CD28 and B7 reduced immune cell activation and reversed the damage in a pathological lupus nephritis model (66). Therefore, We believe that solely activating or inhibiting the CD28 molecule may not be ideal for improving HT.

The final parameter is CD25 on CD39+ resting Treg. The α-chain of the interleukin-2 receptor (IL-2R) is CD25, which is expressed mainly on immune-related cells' surfaces, including activated T cells, B cells, and NK cells. It is another Treg surface marker that interacts with the ligand IL-2 and is involved in a variety of immune responses (67, 68). In 1995, Sakaguchi et al. identified a subset of CD4+ T lymphocytes expressing abundant CD25 in thymectomized mice aged 3 days and other autoimmune models, demonstrating their capacity to impede disease progression (69). Similarly, the elevated counts of CD4+ CD25 high HLA-DR+ cells in HT patients may suggest compensatory expansion of this Treg subpopulation to suppress the immune response (70). In an *in vivo* study (71), CD4 +CD25+Foxp3+ Tregs were revealed to play a role in the pathogenesis of experimental autoimmune thyroiditis (EAT), a murine model of HT. CD25 expression is indispensable for Tregs' differentiation and adaptive proliferation. Mice lacking IL2Rα or IL2Rβ chains manifest severe systemic autoimmunity, a condition ameliorated by transferring CD4 + CD25 + T cells from wild-type mice (72). Furthermore, Mice with deficiencies in IL-2 or the IL-2 receptor exhibit diminished Treg generation, precipitating autoimmune disorders (73). Our findings indicate a negative correlation between HT risk and CD25 on CD39 + resting Treg, consistent with previous research. However, the reverse MR analysis revealed a significant causal relationship between HT risk and CD25 on CD39+ resting Treg, which may be related to unknown confounding factors. Our MR analysis attempted to exclude confounding factors, but there may still be unidentified factors that could impact the results.

Our study has inherent limitations. First, we cannot entirely exclude the potential influence of other confounding factors on this association. Considering the possible presence of additional variables, further exploration of the precise role of these Treg cell phenotypes in the pathogenesis of HT is warranted based on our study findings. Second, despite leveraging GWAS data from European and Asian populations, the generalizability of our findings to other ethnic groups remains subject to constraints. Third, the absence of individual-specific data precluded a more nuanced, stratified analysis of the population, introducing potential inaccuracies into our speculative conclusions. Finally, Our study reported on the Treg cell phenotypes that may decrease the risk of HT in both European and Asian populations. We conducted mutual validation in two study cohorts and applied a more lenient FDR correction for p-values. Although this effectively reduces the occurrence of false-negative results, it inevitably leads to an increased probability of type I errors.

5 Conclusions

In summary, we have established a causal association between five distinct Treg cell phenotypes and HT through an extensive two-way Mendelian randomization analysis, elucidating the intricate interplay between the immune system and HT. Furthermore, our research has effectively mitigated the impact of inevitable confounding factors, reverse causality, and other variables. This may serve as a foundational basis for researchers to delve into Treg cells as potential immunotherapeutic targets for HT.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

Author contributions

JG: Data curation, Methodology, Software, Visualization, Writing – original draft. GS: Investigation, Methodology, Software, Validation, Visualization, Writing – review & editing. FS: Conceptualization, Formal Analysis, Funding acquisition, Project administration, Supervision, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2024.1347695/full#supplementary-material

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The model for predicting the central lymph node metastasis in cN0 papillary thyroid microcarcinoma with Hashimoto's thyroiditis

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Background: The relationship between Hashimoto's thyroiditis (HT) and papillary thyroid microcarcinoma (PTMC) is controversial. These include central lymph node metastasis (CLNM), which affects the prognosis of PTMC patients. This study aimed to establish a predictive model combining ultrasonography and clinicopathological features to accurately evaluate latent CLNM in PTMC patients with HT at the clinical lymph node-negative (cN0) stage.

Methods: In this study, 1102 PTMC patients who received thyroidectomy and central cervical lymph node dissection (CLND) from the First Affiliated Hospital of Shandong First Medical University from January 2021 to December 2022 and the 960th Hospital of PLA from January 2021 to December 2022 were jointly collected. The clinical differences between PTMCs with HT and those without HT were compared. A total of 373 PTMCs with HT in cN0 were randomly divided into a training cohort and a validation cohort. By analyzing and screening the risk factors of CLNM, a nomogram model was established and verified. The predictive performance was measured by the receiver operating characteristic (ROC) curve, calibration curve, and clinical decision curve analysis (DCA).

Results: The ratio of central lymph node metastasis (CLNMR) in PTMCs with HT was 0.0% (0.0%, 15.0%) and 7.7% (0.0%, 40.0%) in the non-HT group (P<0.001). Multivariate logistic regression analysis showed that age, gender, calcification, adjacent to trachea or capsule, and TPOAB were predictors of CLNM in PTMCs with HT. The areas under the curve (AUC) of the prediction models in the training cohort and the validation cohort were 0.835 and 0.825, respectively, which showed good differentiation ability. DCA indicates that the prediction model also has high net benefit and clinical practical value.

Conclusion: This study found that CLN involvement was significantly reduced in PTMC patients with HT, suggesting that different methods should be used to predict CLNM in PTMC patients with HT and without HT, to more accurately assist preoperative clinical evaluation. The actual CLNM situation of PTMCs with HT in cN0 can be accurately predicted by the combination of ultrasonography and clinicopathological features.

KEYWORDS

papillary thyroid microcarcinoma, central lymph node metastasis, Hashimoto's thyroiditis, prophylactic central lymph node dissection, nomogram

Introduction

The incidence of papillary thyroid carcinoma (PTC) has significantly increased due to the widespread use of high-frequency ultrasound (1), leading to an increased detection rate of papillary thyroid microcarcinoma (PTMC) patients with a diameter ≤1cm. According to the Global Cancer Report published by WHO in 2014, more than 50% of new cases of thyroid cancer are accounted for by PTMCs (2, 3). However, current studies have shown that PTMCs are less aggressive and have a 10-year survival rate of over 91% (3, 4).

Takamura Y et al. found that the incidence of central lymph node metastasis (CLNM) in PTMC patients was as high as 30%-64% (5). CLNM of PTMC usually occurs initially in the central compartment and is an important prognostic factor associated with tumor recurrence. The 2015 American Thyroid Association (ATA) guidelines recommend therapeutic central lymph node dissection (CLND) in patients with lymph node metastasis (LNM) (6), while prophylactic central lymph node remains controversial for patients with cN0. Several studies have reported significant improvements in 10-year disease survival and local area control in PTC with bilateral pCND followed by personalized radioiodine therapy (7). Furthermore, studies have indicated that performing pCND in stage clinical lymph node-negative (cN0) patients does not significantly reduce the risk of local recurrence. However, pCND is an invasive and traumatic procedure that can lead to serious complications such as permanent hypoparathyroidism and recurrent laryngeal nerve damage (6). Nevertheless, approximately 53.5% of cN0 patients exhibit latent CLNM (8), which may impact TNM staging and treatment decisions for certain patients (9). Therefore, accurate preoperative diagnosis of CLNM is crucial.

Abbreviations: PTMC, papillary thyroid microcarcinoma; PTC, papillary thyroid carcinoma; CLNM, central lymph node metastasis; HT, Hashimoto's thyroiditis; ETE, extrathyroidal extension; CLNMR, central lymph node metastases ratio; CLN, central lymph node; A/T, anteroposterior and transverse diameter ratio; ROC, receiver operating characteristic curve; AUC, areas under the curve; DCA, decision curve analysis; pCND, prophylactic central lymph node dissection; CLND, central lymph node dissection.

Conventional ultrasound has limitations in evaluating central lymph nodes (CLNs) due to the structural characteristics of the neck and acoustic principles, resulting in a sensitivity below 50% (10). Consequently, accurately assessing CLNM becomes challenging. To address this issue, stratified management based on ultrasound features combined with prognostic factors associated with increased risk of metastasis and recurrence (e.g., age, gender, size, extrathyroidal invasion, evidence of invasive cytology) should be considered for PTMCs in cN0 patients. Those at high risk for CLNM are more likely to undergo thyroidectomy and pCND procedures.

Hashimoto thyroiditis (HT), HT is known as chronic lymphocytic thyroiditis and often coexists with PTC/PTMC (11). Some studies have suggested that PTMC patients with HT have lower clinical invasiveness and better prognosis, and are negatively correlated with lymph node metastasis (12). However, its association with PTMC remains unproven. Exploring clinical differences between PTMCs with HT versus those without HT could provide insights into lymph node involvement among these two groups. If there are distinct variations in CLNM presentation between these groups then different models can be adopted to predict the risk of CLNM for PTMCs accordingly.

The objective of this study was to establish an implicit CLNM prediction model based on independent risk factors extracted from cN0 PTMC patients with HT, which can help surgeons develop individualized treatment plans for such patients.

Materials and methods

Patients

A total of 1250 patients with PTMC pathologically diagnosed in the First Affiliated Hospital of Shandong First Medical University from January 2021 to December 2022 and 960th Hospital of PLA from January 2022 to December 2022 were jointly collected and retrospectively analyzed.

The inclusion criteria were: (1) age >18 years old; (2) performing thyroidectomy and CLND; (3) PTC confirmed by histopathology. The exclusion criteria are (1) previous history of

thyroid surgery; (2) combined with other malignant diseases; (3) Pathological findings showed thyroid carcinoma (TC) with other pathologic types except PTC, such as follicular or anaplastic thyroid carcinoma; (4) incomplete clinical data such as preoperative laboratory tests or ultrasound; (5) Preoperative clinical findings of lymph node metastasis or distant metastasis; (6) patients without CLND; (7) received TSH or other drug suppression therapy before surgery. (Figure 1. Flow diagram of the study design).

After excluding 148 patients who did not meet the criteria, 1102 PTMC patients were included. A total of 373 PTMC patients with HT were included in the study, they were randomly divided into the training cohort (261 patients) and the validation cohort (112 patients) at a ratio of 7:3.

Variable definition and evaluation

The clinicopathological features of the patients were analyzed. They included gender, age, ultrasound features (size, echogenicity, calcification, margin, shape, anteroposterior and transverse diameter ratio (AT), tumor location, presence or absence of HT ultrasound features), and thyroid function tests including free triiodothyronine (FT3), free thyroxine (FT4), thyroid stimulating hormone (TSH), thyroglobulin antibodies (TGAB) and thyroid peroxidase antibodies (TPOAB). And pathological characteristics (multifocal or unifocal, unilateral or bilateral, extrathyroidal invasion, $BRAF^{V600E}$ gene mutation, pathological type, bilateral CLNM, and pathological diagnosis of HT). The number of surgically dissected CLNs, the number of CLNM, and the CLNMR (the number of metastatic lymph nodes per patient divided by the number of CLNS removed) were recorded. The levels of TSH, TPOAB, and TGAB were grouped according to previous studies (11).

The thyroid surgery in this study followed the 2016 Chinese Expert Consensus on the Diagnosis and Treatment of Thyroid Micropapillary Carcinoma. The total thyroidectomy or thyroid lobectomy was performed for the primary tumors, and the dissection of the secondary lesions (lymph nodes) followed the principle of individualized treatment. It is generally recommended to perform the CLND, lateral cervical lymph node dissection was performed only when there was evidence of lateral cervical lymph node metastasis before or during operation.

Tumor size is the maximum tumor diameter measured by preoperative ultrasound images. Ultrasound features: lesion echogenicity is divided into two subgroups: hyperechoic/isoechoic and hypoechoic/very hypoechoic; calcification (no calcification, macrocalcification, and microcalcification); shape (regular/irregular); margin (well-defined/ill-defined). Lesions with A/T>1 showed that the anteroposterior is greater than the transverse diameter in two-dimensional ultrasound images. The tumors are classified according to the location in the upper, middle, and inferior, isthmus, and whether the tumor is adjacent to the trachea or the capsule (the tumor edge is less than 2mm from the trachea or capsule). The above ultrasonic features were determined by two physicians with more than ten years of working experience in the ultrasound department of the hospital. If the results are inconsistent, the senior physician rules.

More than one PTC lesion in the thyroid gland is defined as multifocal. The presence of more than one PTC lesion in one gland at the same time is considered to be unilateral multifocal, and the presence of more than one PTC lesion in the bilateral lobe or

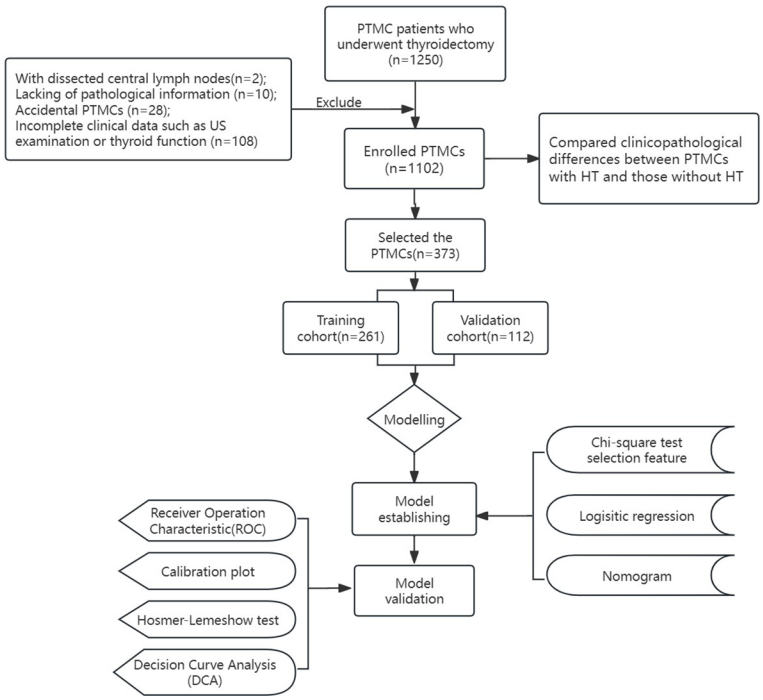


FIGURE 1
Flow diagram of the study design.

unilateral lobe plus isthmus is considered to be bilateral multifocal. In the presence of multifocal lesions, the size and location of the tumor are determined by the diameter and location of the largest dominant lesion. When the main lesion occupies 2 adjacent parts, the location of the tumor is determined by the part containing more than two-thirds of the tumor volume.

We adopted the overall definition of chronic lymphocytic thyroiditis (CLT), including (I) Elevated levels of thyroid peroxidase antibodies. (II) Diffuse heterogeneity on ultrasonography. (III) Histopathological diagnosis of diffuse lymphocytic thyroiditis. To avoid selection bias, HT was diagnosed when any of the above conditions were met in this study. The pathological types were divided into low-risk subgroup (classic, follicular subtype, encapsulation, clear-cell, eosinophilic cell, Warthin neoplastic cell) and high-risk subgroup (diffuse sclerosis type, solid growth, tall cell, columnar and Hobnail cell).

Statistical analysis

SPSS26.0 and R Studio (R.4.2.2) were used to analyze the data. When $P<0.05$, the analysis was considered statistically significant. A chi-square test was conducted for categorical variables, and an independent t-test or Mann-Whitney U test was conducted for continuous variables.

The chi-square test was used to select the characteristics of the training cohort, and then the independent risk factors for CLNM were obtained by regression analysis. A nomogram model for predicting CLNM was established, and the calibration curves were analyzed. In addition, the receiver operating characteristic (ROC) curve, C index, and Hosmer-Lemeshow good of fit test were used to evaluate the value of the predictive model, and decision curve analysis (DCA) calculated the clinical net benefit of the predictive model.

Results

Basic clinical features of HT group and non-HT group

PTMC patients were stratified based on their HT status and compared between groups (Table 1). The results revealed statistically significant differences in gender, A/T, $BRAF^{V600E}$ gene mutation, number of dissected lymph nodes, and number of CLNM and CLNMR between the two groups (all $P<0.05$). Specifically, the non-HT group had a significantly lower number of dissected lymph nodes compared to the HT group ($P<0.001$), while the non-HT group exhibited a markedly higher CLNMR than the HT group ($P<0.001$).

Baseline clinical characteristics of PTMCs with HT

The clinical characteristics of 373 PTMCs with HT in cN0 were analyzed. Among them, 139 patients presented with CLNM while 234 patients did not. All patients were randomly divided into a training cohort (261 cases) and a validation cohort (112 cases) at a ratio of 7:3 for comparison of their clinical characteristics. Table 2 demonstrates that there were no significant differences in baseline characteristics between the training and validation cohorts (all $P>0.05$), and the similarity of clinical information proves their rationality as training and validation cohorts.

TABLE 1 Clinicopathological features of PTMC patients with HT or without HT.

Variables	Without HT (n=729)	With HT (n=373)	*P*
Age (y) <45/≥45	325/404	163/210	0.780
Gender M/F	235/494	46/327	<0.001
Ultrasonic Characteristics			
Echo (hyperechoic or isoechoic/ very hypoechoic or hypoechoic)	61/668	29/344	0.734
Calcification (none/macro-/ micro-)	314/118/297	140/64/169	0.202
Margin (well-defined/ill-defined)	107/622	47/326	0.347
Shape (regular/irregular)	95/634	46/327	0.742
A/T (≤1/>1)	222/507	139/234	0.023
HT of Ultrasound (no/yes)	695/34	222/151	<0.001
Location			
(upper/middle/ inferior/isthmus)	171/313/ 159/86	76/158/90/49	0.570
(adjacent to trachea or capsule/none)	251/478	124/249	0.712
Pathology			
Multifocality (no/yes)	453/276	243/130	0.327
Bilateral (no/yes)	570/159	288/85	0.712
Extrathyroidal invasion (No/Capsule Contact/ETE)	221/452/56	122/222/29	0.703
$BRAF^{V600E}$ mutation (no/yes)	53/676	42/331	0.026
High-risk pathological type (no/yes)	724/5	366/7	0.071
CLN examined	5 (3-9)	9 (5-15)	<0.001
CLNMs	0 (0-2)	0 (0-1)	0.002
CLNMR	7.7% (0.0%-40.0%)	0.0% (0.0%-15.0%)	<0.001

Tumor size, CLN examined, and CLN positive are all expressed as the median (Q1-Q3).

Screening of risk features affecting CLNM in patients with HT PTMC

The training cohort was divided into a CLNM group (100 cases) and a non-CLNM group (161 cases) to compare variables between these two groups and identify characteristic factors influencing CLNM in PTMCs with HT. As shown in Table 3, age, gender,

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TABLE 2 Clinical baseline analysis of cN0 PTMCs with HT in the training and validation cohorts.

Variables	Subgroup	No. of patients(PTMC with HT)		*P*
		Training Cohort (n=261)	Validation Cohort (n=112)	
Age (year)		47	48	0.618
	<45	117	46	0.503
	≥45	144	66	
Gender	male	30	16	0.452
	female	231	96	
Race	Chinese	261	112	~
Tumor size (mm)		6 (5-8)	6 (5-8)	0.525
Multifocality	no	165	78	0.233
	yes	96	34	
Extrathyroidal invasion	no	87	35	0.614
	capsule contact	156	66	
	ETE	18	11	
Location	upper	55	21	0.724
	middle	112	46	
	inferior	63	27	
	isthmus	31	18	
Echogenicity	hyperechoic or isoechoic	20	11	0.618
	very hypoechoic or hypoechoic	241	101	
Calcification	none	97	43	0.922
	macro-	44	20	
	micro-	120	49	
Margin	well-defined	30	17	0.326
	ill-defined	231	95	
Shape	regular	29	17	0.273
	irregular	232	95	
A/T	≤1	93	46	0.319
	>1	168	66	
BRAF^{V600E} mutation	no	29	13	0.890
	yes	232	99	
High-Risk Pathological type	no	256	110	0.932
	yes	5	2	
TSH (μIU/mL)	≤0.35	7	3	0.999
	0.35-5	238	102	
	>5	16	7	
FT3 (pmol/L)		4.46	4.39	0.842
FT4 (pmol/L)		17.16	16.92	0.810
TGAB (IU/mL)	normal	116	53	0.517
	115-575	92	39	
	575-1150	31	8	
	≥1150	22	12	
TPOAB (IU/mL)	normal	127	56	0.925
	34-170	71	30	
	170-340	50	19	
	≥340	13	7	
CLN examined		9 (5-15)	9 (5-14)	0.907
CLN positive		0 (0-1)	0 (0-2)	0.750

A/T, aspect ratio; ETE, extrathyroidal extension.
Tumor size, CLN examined, and CLN positive are all expressed as the median (Q1-Q3). Age, FT3 and FT4 are all expressed as the median.

TABLE 3 Screening of risk features affecting CLNM in cN0 PTMCs with HT in the training cohort.

Variables	Subgroup	No. of patients(PTMC with HT)		*P*
		Without CLNM (n=161)	With CLNM (n=100)	
Age (year)	<45	62	55	**0.009**
	≥45	99	45	
Gender	male	12	18	**0.009**
	female	149	82	
Tumor size (mm)		6 (4-8)	7 (5-8)	**0.001**
Multifocality	no	110	55	**0.030**
	yes	51	45	
Bilateral	no	131	70	**0.034**
	yes	30	30	
Extrathyroidal invasion	no	60	27	0.176

(Continued)

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TABLE 3 Continued

Variables	Subgroup	No. of patients(PTMC with HT)		*P*
		Without CLNM (n=161)	With CLNM (n=100)	
	capsule contact	92	64	
	ETE	9	9	
Adjacent trachea or capsule	no	71	14	**<0.001**
	yes	90	86	
Echogenicity	hyperechoic or isoechoic	11	4	0.618
	very hypoechoic or hypoechoic	150	96	0.339
Calcification	none	81	16	**<0.001**
	macro-	36	8	
	micro-	44	76	
A/T	≤1	60	33	0.484
	>1	101	67	
BRAF^{V600E} mutation	no	21	8	0.208
	yes	140	92	
High-Risk Pathological type	no	158	98	0.938
	yes	3	2	
TSH (μIU/mL)	≤0.35	5	2	0.862
	0.35-5	146	92	
	>5	10	6	
FT3 (pmol/L)		4.83	4.81	0.556
FT4 (pmol/L)		17.16	16.87	0.134
TGAB (IU/mL)	normal	73	43	0.968
	115-575	55	37	
	575-1150	19	12	
	≥1150	14	8	
TPOAB (IU/mL)	normal	71	56	0.240
	34-170	46	25	
	170-340	34	16	
	≥340	10	3	

A/T, aspect ratio; ETE, extrathyroidal extension.
Tumor size, CLN examined, FT3, and FT4 are all expressed as the median.
The bold values indicate statistical significance.

TABLE 4 Univariate and multivariate analysis of PTMCs with HT in cN0 for CLNM.

Variables	Total (N)	Univariate analysis		Multivariate analysis		
		Odds Ratio (95% CI)	*P*	Odds Ratio (95% CI)	*B*	*P*
Age (year)	261					
≥45	144	Reference		Reference		
<45	117	1.952 (1.177 - 3.237)	**0.010**	2.540 (1.323 - 4.877)	0.842	**0.005**
Gender	261					
female	231	Reference		Reference		
male	30	2.726 (1.251 - 5.937)	**0.012**	4.353 (1.569 - 12.076)	1.314	**0.005**
Calcification	261					
no	97	Reference		Reference		
macro-	44	1.125 (0.442 - 2.866)	0.805	1.394 (0.482 - 4.029)	0.516	0.540
micro-	120	8.744 (4.555 - 16.787)	**< 0.001**	9.136 (4.238 - 19.696)	2.346	**< 0.001**
Echogenicity	261					
hyperechoic or isoechoic	15	Reference		-		-
very hypoechoic or hypoechoic	246	1.760 (0.545 - 5.686)	0.345	-		-
A/T	261					
>1	168	Reference		-		-
≤1	93	0.829 (0.490 - 1.402)	0.484	-		-
Adjacent trachea or capsule	261					
no	85	Reference		Reference		
yes	176	4.846 (2.543 - 9.235)	**< 0.001**	3.160 (1.480 - 6.749)	1.234	**0.003**
Multifocality	261					
no	165	Reference		Reference		
yes	96	1.765 (1.054 - 2.954)	**0.031**	1.841 (0.729 - 4.650)		0.197
Bilateral	261					
no	201	Reference		Reference		
yes	60	1.871 (1.044 - 3.354)	**0.035**	0.895 (0.310 - 2.585)		0.838

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TABLE 4 Continued

Variables	Total (N)	Univariate analysis		Multivariate analysis		
		Odds Ratio (95% CI)	*P*	Odds Ratio (95% CI)	*B*	*P*
Extrathyroidal invasion	261					
no extrathyroidal invasion	87	Reference		Reference		
only capsule contact	156	1.546 (0.887 - 2.693)	0.124	1.050 (0.495 - 2.226)		0.898
ETE	18	2.222 (0.794 - 6.222)	0.128	1.209 (0.308 - 4.741)		0.786
$BRAF^{V600E}$ mutation	261					
no	29	Reference				
yes	232	1.725 (0.733 - 4.059)	0.212	-		-
Tumor Size	261	1.228 (1.082 - 1.393)	**0.001**	1.084 (0.912 - 1.288)		0.360
High-Risk Pathological type	261					
no	256	Reference				
yes	5	1.075 (0.176 - 6.547)	0.938	-		-
T3	261	1.094 (0.888 - 1.347)	0.399	-		-
T4	261	1.047 (0.972 - 1.126)	0.224	-		-
TSH	261					
≤0.35	4	Reference				
0.35-5	241	1.885 (0.193 - 18.393)	0.585	-		-
>5	16	1.800 (0.151 - 21.477)	0.642	-		-
TPOAb	261					
<34	135	Reference		Reference		
34-170	68	0.658 (0.359 - 1.208)	0.177	0.576 (0.268 - 1.239)	-0.321	0.158
170-340	34	0.703 (0.322 - 1.535)	0.376	0.400 (0.149 - 1.072)	-0.907	0.068
≥340	24	0.429 (0.160 - 1.149)	0.092	0.241 (0.073 - 0.796)	-1.356	**0.020**
TGAB	261					
<115	139	Reference				
115-575	94	1.361 (0.795 - 2.327)	0.261	-		-
575-1150	18	1.169 (0.426 - 3.208)	0.762	-		-
≥1150	10	1.224 (0.330 - 4.548)	0.762	-		-

"-" representing meaninglessness.
The bold values indicate statistical significance.

calcification, multifocality, bilateral lobes, size, and adjacent to trachea or capsule all demonstrated significant correlations with CLNM development in PTMCs with HT(P<0.05).

Univariate and multivariate analyses of CLNM in cN0 PTMC with HT

The independent predictors of CLNM in PTMCs with HT in the training cohort were analyzed by logistic regression. As shown in Table 4, the results of univariate logistic regression analysis show that the factors are consistent with Table 3. Age, gender, calcification, multifocality, bilateral lobes, size, and whether the tumor is adjacent to the trachea or capsule were all predictors of CLNM(P<0.05). TPOAB (P<0.1) was also included in the multivariate logistic regression analysis. Further multivariate logistic regression analysis finally showed that five features are significantly correlated with CLNM in PTMC patients with HT, age <45 years (B=0.842, OR:2.54, P=0.005), male (B=1.314, 0R:4.35, P=0.005), microcalcification (B=2.346, OR:9.14, P<0.001) and adjacent to trachea or capsule (B=1.234, OR:3.116, P=0.003) are predictive risk factors for CLNM in PTMCs with HT, while TPOAB levels higher than 340 IU/mL (B=-1.314, OR: 0.2419.14, P=0.02) were protective factor for CLNM. With the increase in TPOAB level, the incidence of CLNM decreased (Figure 2).

Construction and validation of nomogram for predicting CLNM in PTMC with HT

Based on multivariate regression analysis results, age, gender, calcification, adjacent to the trachea or capsule, and TPOAB level higher than 340 IU/mL were identified as independent predictors of

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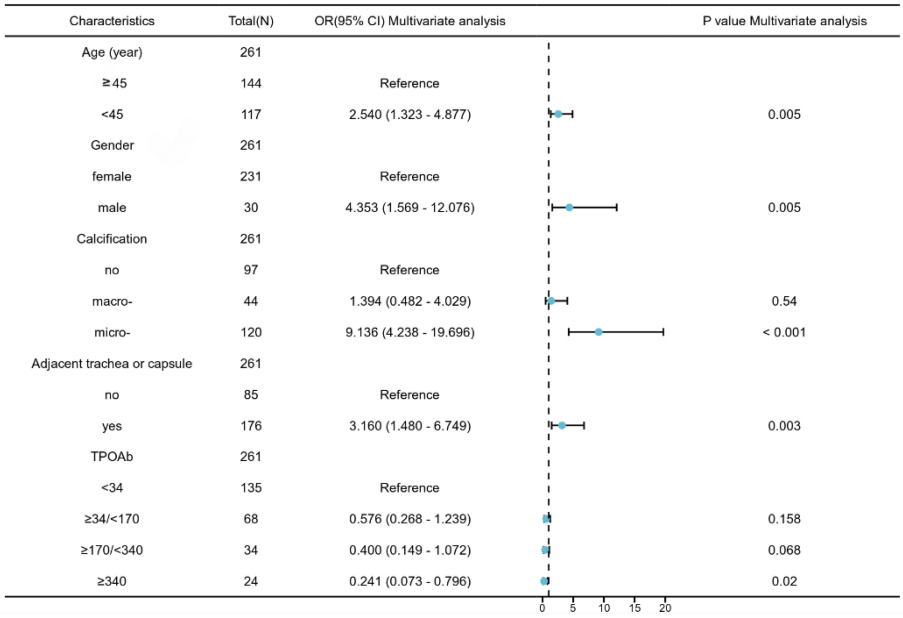


FIGURE 2
The forest plots for predicting CLNM in PTMCs with HT based on multivariate analysis.

CLNM in PTMC patients with HT. A nomogram was constructed using these variables to display corresponding CLNM risk characteristics and calculate ontogenetic CLNM risk values (Figure 3).

The ROC curve of the prediction model demonstrates that the AUC for the training cohort is 0.835 (95% CI: 0.786–0.885) (Figure 4A), while for the validation cohort, it is 0.825 (95% CI: 0.741–0.910) (Figure 4B). These results from both cohorts indicate a strong discriminatory ability of the model in predicting CLNM risk.

The verification process was conducted on the validation cohort, yielding a *P*-value of 0.8719 for the Hosmer-Lemeshow goodness-of-fit test and a *P*-value less than 0.01 for the likelihood ratio test, confirming good calibration and differentiation abilities of our model. The C-index evaluation result was found to be 0.825, further supporting its accuracy. Through bootstrap analysis with resampling performed on both cohorts, calibration curves were generated (Figure 5). The average absolute error in predicted risk was less than 0.05 in both cohorts, demonstrating excellent consistency between corrected and observed metastasizing risks. This assessment using the Brier score yielded favorable values such as slope=1, E_{90} = 0.020, and E_{50} = 0.008.

In addition to this evaluation approach, we employed DCA reassessment modeling to assess the clinical practicability of CLNM prediction in PTMCs with HT. DCA revealed that when considering risk thresholds ranging from 3.3% to 94.6% for the training cohort (Figure 6A) and from4.5% to 90.9%for the validation cohort (Figure 6B), the overall net benefit provided by the nomogram model incorporating all factors surpassed that obtained through "no intervention" or "treat-all" strategies. Furthermore, the combined predictive power of all factors outperformed individual factor predictions, suggesting enhanced accuracy in assessing CLNM risk.

The above validation demonstrated that the nomogram to evaluate CLNM risk in PTMCs with HT performed well, further confirming the robustness and accuracy of the model prediction. Two PTMC patients with HT in cN0 were selected to conduct individualized factor analysis and evaluate the risk of CLNM according to the prediction model of this study, as shown in Figures 7, 8.

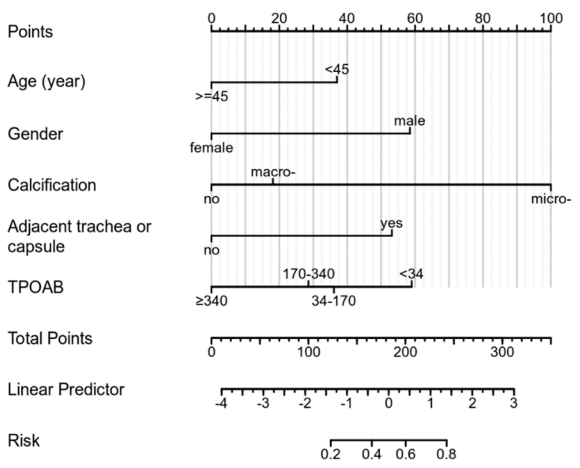


FIGURE 3
A nomogram for predicting CLNM in patients of PTMC with HT.

Discussion

With the increasing prevalence of papillary thyroid microcarcinoma (PTMC) and excessive testing worldwide,

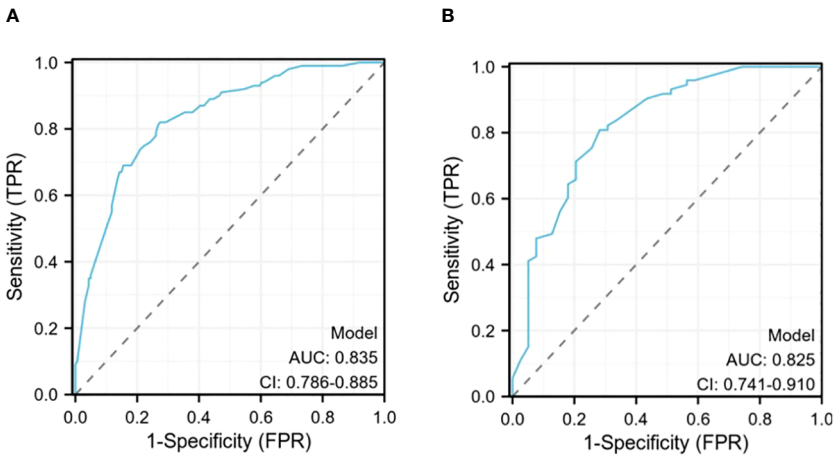


FIGURE 4
(A) The receiver operating characteristics (ROC) curve in the training cohort (AUC=0.835); **(B)** The receiver operating characteristics (ROC) curve in the validating cohort (AUC=0.825).

accounting for over 50% of new cases of thyroid cancer (13), precise management of this disease has become crucial. The 2015 ATA guidelines recommend pCND as a management strategy for PTMC patients with clinically involved CLNs or stage T3 and T4 tumors. Immediate surgical intervention is advised for PTMCs with clinical lymph node metastasis, distant metastasis, invasion of neighboring organs, or other high-risk characteristics (14). In China (15), surgical intervention remains the primary treatment method for PTMC patients. Previous studies have reported that 38% of PTMC patients had CLNM (5). However, in our study, approximately 42% of patients exhibited CLNM. Among PTMCs with HT in cN0, latent CLNM was observed in 38.3%, while among those without HT, CLNM was present in 44%. Considering the potential impact on patient outcomes, pCND should be performed during initial thyroidectomy in high-risk individuals to avoid unnecessary re-surgery and associated complications commonly seen in recurrent cases. However, preoperative imaging has limited utility in detecting lymph node metastasis (16). Thus, accurate evaluation of actual CLNM status is necessary for cN0-stage patients.

Clinical observations indicate a frequent coexistence between HT and thyroid cancer (17), moreover, HT is associated with an increased prevalence of PTMC (18, 19). Studies have demonstrated that PTMCs with concomitant HT exhibit lower invasiveness and better prognosis (12) due to chronic immune inflammation which also affects CLNs' characteristics. Additionally reported is the protective role played by HT against $BRAF^{V600E}$ gene mutation occurrence as well as extrathyroidal extension and lymph node metastasis development. Thus, indicating that PTMCs with HT are at relatively low risk. Thyroidectomy and CLND are performed in patients who already have lymph node metastasis. By targeting the risk factors for latent central compartment lymph node metastasis (CLNM) in the subgroup of PTMCs with HT in cN0 and establishing a personalized model for this specific population, we can better determine which type of patient is more likely to experience CLNM.

Some predictive models evaluate CLNM in PTMCs but overlook the impact of HT on these individuals. In this study, we found that although the number of dissected PTMCs was higher among those with HT compared to those without it, the CLNMR was significantly lower. The relationship between HT and PTC remains controversial, however, current findings suggest that certain immune cells and factors associated with HT exert protective effects on thyroid cancer by destroying cancer cells through specific antigens while infiltrating the thyroid gland lesions and their respective lymphatic drainage channels (20–22). Our results align with this perspective by demonstrating that HT is not an independent risk factor for CLNM in PTMCs; rather when comparing patients without HT to those with it there was a significantly lower CLNMR ($P<0.001$), thereby confirming our previous hypothesis. The characteristics of PTMCs with and without HT differ, thus necessitating different approaches for analyzing CLNM status and implementing predictive CLNM management strategies. For instance, Lawrence A et al. (23) found that the risk of CLNM was significantly lower in females compared to males in studies on PTC/PTMC (2). This study also observed a higher propensity for CLNM in young males with HT; however, the proportion of males in the HT group was lower than that in the non-HT group ($P<0.001$). From a gender perspective, combined HT exhibited some protective effects against CLNM.

Screening PTMCs with HT in cN0 for relevant clinical features influencing CLNM yielded results consistent with those obtained from univariate analysis. Furthermore, multivariate analysis identified four independent risk factors ($P<0.05$) for CLNM: age under 45 years old, male gender, presence of calcification adjacent to trachea or capsule, and high levels of TPOAB as a protective factor. Previous studies have highlighted tumor size and bilateral lesions as important risk factors for CLNM in PTMCs (24–26); however, these variables did not retain statistical significance after multivariate analysis when considering PTMCs with HT specifically. Future discussions may consider grouping PTMCs

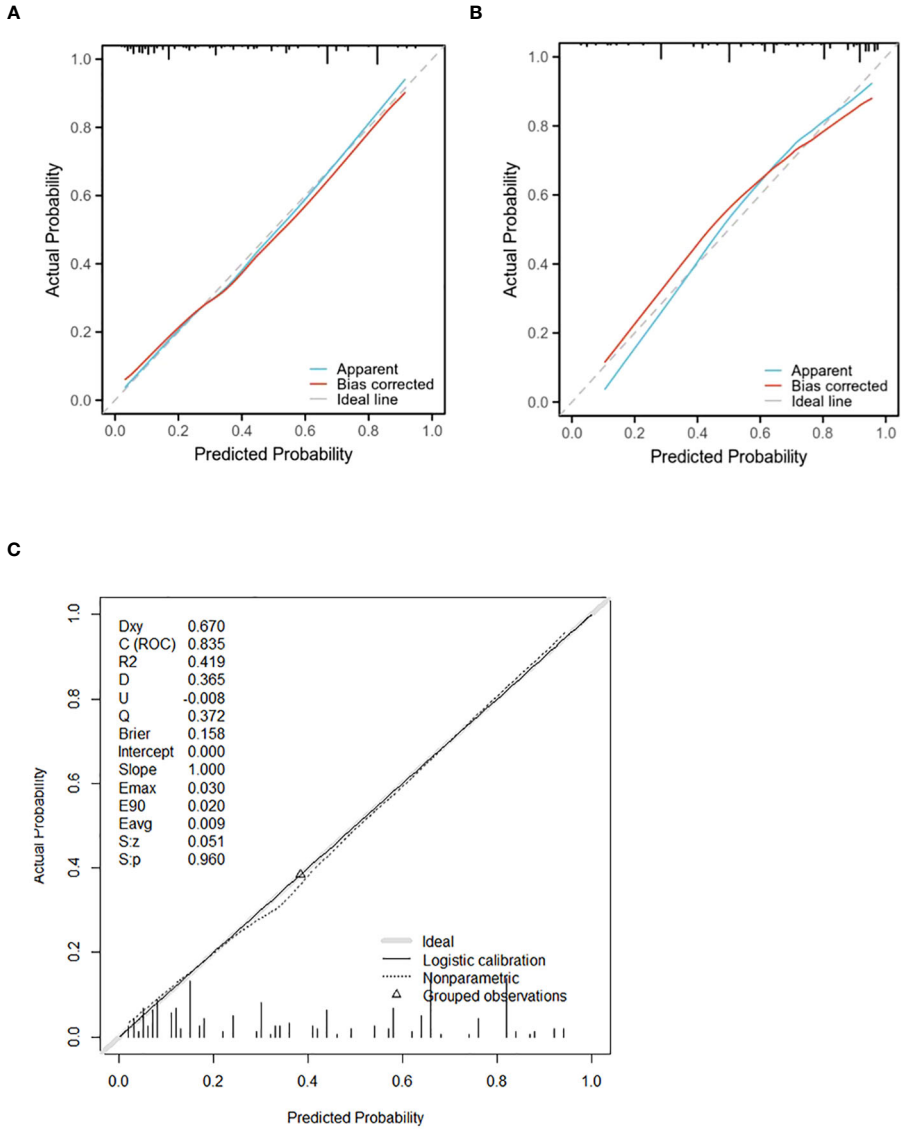


FIGURE 5
The calibration curve for evaluating the accuracy of the nomogram in the training cohort **(A)** and the validating cohort **(B)** (The mean absolute error is less than 0.05). **(C)**shows the calibration curve of the training cohort model (Brier score:0.158, slope:1.00). The calibration curve ranks all individuals from low to high probability predicted by the model, divided into 10 equal parts, with the mean of each equal predicted probability as the X-axis and the proportion of actual events occurring as the Y-axis. There are 3 lines in each graph, the blue line is the prediction curve of the model, the red line is the corrected curve, and the dashed line (45° slash) is the actual curve. A perfect prediction model's curve should be moderate to the dashed line, meaning that the model's prediction probability and the actual probability match perfectly.

based on tumor size. The role of TGAB and TPOAB in PTMC development and metastasis remains debated; nevertheless, it has been reported that elevated levels of TGAB and TPOAB are associated with HT which may contribute to thyroid cancer tumorigenesis and CLNM while also possessing prognostic value (27). High levels of TPOAB demonstrated a certain degree of protection against CLNs despite not showing significant effects during univariate analysis. Its inclusion into the multivariate analysis revealed a statistically significant association with reduced incidence of CLNM. Serum TPOAB levels were found to be higher among individuals with an underlying background of HT. These findings demonstrate the protective effect of HT on CLN, which is consistent with previous studies on CLNM (11, 28, 29). Huang et al. (30) identified microcalcification as a significant factor in CLNM among PTMCs.

Each gland lobe of the thyroid possesses its internal lymphatic system (31) and metastasis typically follows a pattern from the central ventricle to the ipsilateral cervical region before spreading to contralateral and distant lymph nodes. Our study revealed that adjacent to the trachea or capsule was associated with an increased risk of CLNM due to anatomical factors facilitating tumor cell infiltration and lymphatic drainage through deep anterior CLNs located in front and on both sides of the larynx surrounding the thyroid gland and trachea (32). Although tumor location (upper/middle/inferior) did not predict metastasis risk per se, it may determine the area affected by metastasis (33). Unlike previous

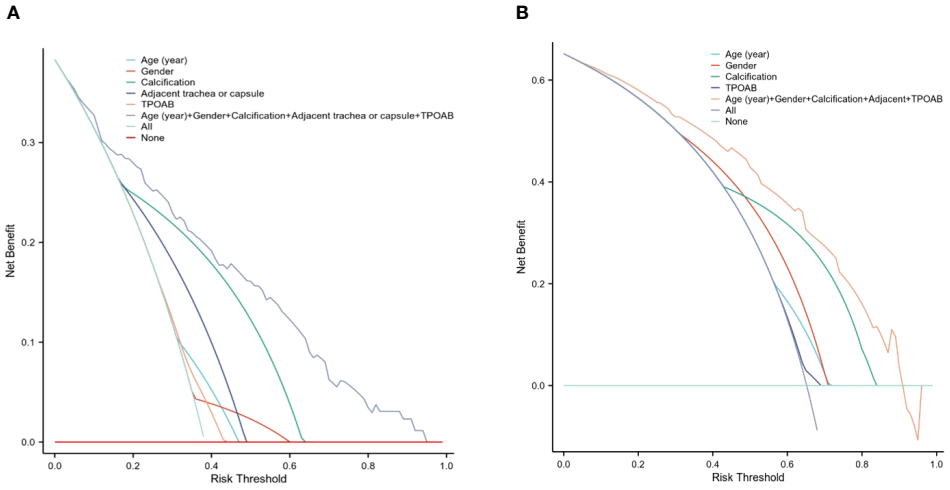


FIGURE 6
The DCA for evaluating the clinical application value of the model. The y-axis represents the net benefit different colors indicate different models. **(A)** When the threshold probability was 3.3% to 94.6% in the training cohort, the curve of the combined model was higher than that of other models, indicating a higher predictor of CLNM. **(B)** shows the DCA in the validation cohort.

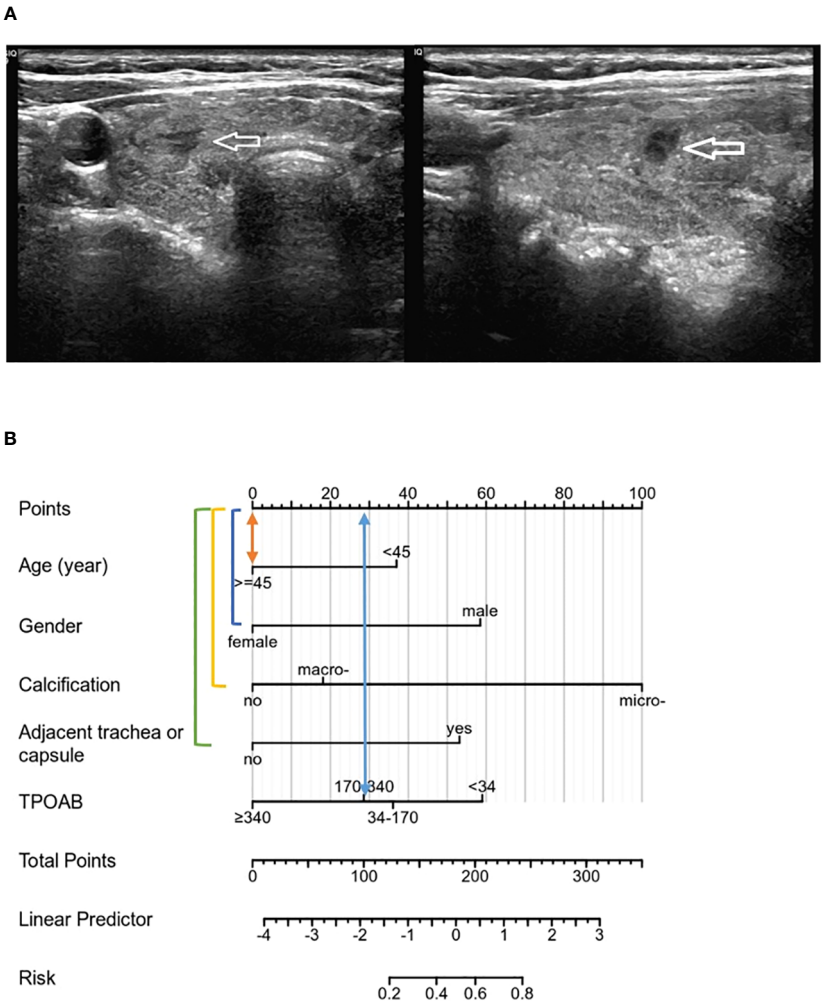


FIGURE 7
(A) PTMC lesions showed no significant calcification on ultrasound images, and were not adjacent to the trachea and capsule, and all risk factors were negative. TPOAB: 247.37 IU/mL. **(B)** According to the nomogram, the CLNM risk score was 28 points, indicating low risk (<20%), and no CLNM was confirmed by postoperative pathology.

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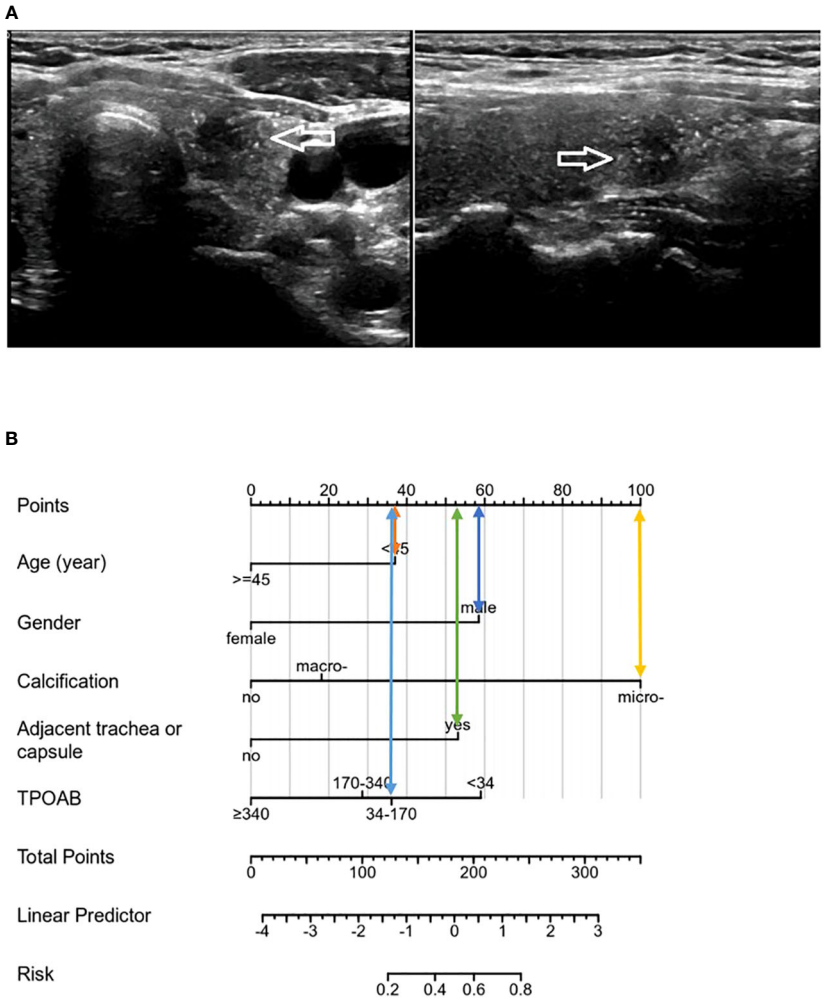


FIGURE 8
(A) The patient was a male with 35 years, and the tumor showed microcalcification on ultrasound, close to the trachea and capsule, TPOAB: 146.50 IU/mL. **(B)**. The risk score of the CLNM was 283 points, belonging to the high-risk group (>80%), and CLNM was finally confirmed by pathology.

studies that focused only on ultrasound features or perioperative laboratory results (34) for analysis, our study comprehensively considered clinical data when identifying independent risk factors (microcalcification, adjacent to trachea or capsule) predicting CLNM in PTMC patients with HT before surgery assessment.

Although TG has been partially shown to be related to the metastasis and prognosis of thyroid cancer, TGAB is elevated in most HT patients, and TGAB can affect TG levels, so this variable is not included at this stage. Unexpectedly, preoperative ultrasound assessment could determine independent risk factors (microcalcification, adjacent to the trachea or capsule) that ultimately predict CLNM in patients with PTMC and HT. Viola et al. (35) demonstrated that preoperative ultrasound assessment of CLNM (CN0/CN1) had limited sensitivity and no advantage. Some scholars have suggested that the proximity of CLNs to the trachea may interfere with ultrasound conduction due to air interference. This study addresses the limitation of accurately identifying lymph node status before surgery using ultrasound by leveraging ultrasonic characteristics for predicting CLNM risk. The model was evaluated through various validation results which consistently demonstrated good prediction consistency for metastasis risk compared to the nomogram. Additionally, the DCA from the training cohort effectively showcased its clinical practical value. This model can be flexibly applied in clinical diagnosis and treatment settings, providing more intuitive results. Based on these five identified factors, a precise and comprehensive assessment of CLNM risk was conducted for PTMCs with HT who were initially classified as cN0, enabling individualized treatment planning. Patients can be categorized into high-risk or low-risk groups based on their CLNM risk levels. For instance, PTMC patients at low risk for CLNM may benefit from active surveillance or minimally invasive ablation therapy as depicted in Figure 7 above; whereas those at high risk due to multiple factors should consider thyroidectomy and pCND (Figure 8).

However, this study has certain limitations. Firstly, the retrospective nature of the analysis prevents accurate prediction of prospective analysis results. Secondly, the limited sample size and exclusive focus on Chinese population introduce a potential selection bias. To address this limitation, future studies should

consider expanding the sample size. Thirdly, variations in surgical methods and regional anatomy among different surgeons may influence result interpretation. Nevertheless, compared to single-center non-comprehensive research studies, our study utilized samples from two centers to mitigate sample selection bias to some extent. We also considered comprehensive clinical research factors and quantitatively validated our model results, providing a relatively accurate basis for individual clinical decision-making in papillary thyroid carcinoma.

Given that nodular lymphatic systems flow widely in various directions for PTC patients (35), accurately predicting lymphatic drainage remains challenging. Therefore, further investigation into the rules governing lymph node metastasis is highly significant and holds promising prospects for development.

Conclusion

In summary, this study revealed that PTMC patients with HT had lower involvement of CLNs compared to those without HT. CLNMR was decreased in these patients indicating a protective effect against CLNM and reduced tumor invasiveness. Thus, it is recommended that a specific prediction system be employed for PTMCs with HT to more accurately evaluate CLNM risk. Additionally, we discussed and confirmed that younger age (below 45 years), male, intralesional microcalcification, and adjacence to trachea or capsule were associated with increased likelihood of CLNM occurrence; conversely, higher titers of serum TPOAB exhibited a protective effect against CLNM. For stage cN0 PTMCs with HT, a predictive model can be established by combining ultrasound and clinical characteristics to determine the risk of latent CLNM before surgery and screen out high-risk patients who are more suitable for pCND, to accurately conduct individualized treatment management.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

These studies involving humans were approved by the Ethics Committee of the First Affiliated Hospital of Shandong Province Medical University. The studies were conducted in accordance with the local legislation and institutional requirements. The ethics committee/institutional review board waived the requirement of written informed consent for participation from the participants or the participants' legal guardians/next of kin because this study is a retrospective study. Written informed consent was not required from the individual(s) for the publication of any potentially identifiable images or data included in this article because this study does not contains identifiable human images or underage patients.

Author contributions

YL: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Software, Supervision, Writing – original draft, Writing – review & editing. NC: Data curation, Writing – review & editing. FL: Methodology, Supervision, Writing – review & editing. YW: Methodology, Supervision, Writing – review & editing. BW: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Supervision, Validation, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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An age-and sex-matched postoperative therapy should be required in thyroid papillary carcinoma

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Background and purpose: Thyroid papillary carcinoma (PTC) had a high possibility of recurrence after surgery, and thyroid stimulating hormone (TSH) suppression and radioactive iodine (^{131}I) were used for postoperative therapy. This study explored the potential mechanism of lymph node metastasis (LNM) and aimed to develop differentiated treatments for PTC.

Method: This study explored the risk factors of lymph node metastasis in PTC by analyzing the clinical information of 2073 cases. The Cancer Genome Atlas Thyroid Cancer (TCGA-THCA) and the Gene Expression Omnibus (GEO) databases of gene expression were analyzed to identify the interrelationships between gene expression to phenotype.

Results: Analyzing clinical data, we found that male gender, younger age, larger tumor size, and extra-thyroidal extension (ETE) were risk significant risk factors for lymph node metastasis($P<0.05$). Conversely, thyroid function parameters such as TSH, FT3, FT4, TSH/FT3, and TSH/FT4 didn't correlate with LNM ($P>0.05$), and TSH levels were observed to be higher in females($P<0.05$). Gene expression analysis revealed that SLC5A5 was down-regulated in males, younger individuals, and those with lymph node metastasis, and a lower level of SLC5A5 was associated with a worse disease-free survival($P<0.05$). Additionally, our examination of single-cell RNA sequencing (scRNA-seq) data indicated that SLC5A5 expression was reduced in tumors and lymph node metastasis samples, correlating positively with the expression of TSHR.

Conclusion: The impact of TSH on PTC behavior remained unclear, while the capacity for absorbing ^{131}I in dependence on SLC5A5 showed variations across different genders and ages. We conclude that postoperative treatment of PTC should take into account the differences caused by gender and age.

KEYWORDS

papillary thyroid carcinoma, lymph node metastasis, TSH, ^{131}I, SLC5A5

1 Background

Papillary thyroid carcinoma (PTC) is one of the most frequently diagnosed malignancies of the endocrine system worldwide (1). Surgery is the preferred treatment for PTC, which has a satisfactory prognosis in an early stage (2). However, studies have revealed that the recurrence rate of PTC within 5 years is a high level, ranging from 2%-13% (3–6), which was difficult in the treatment of PTC. To address this issue, the use of thyroid-stimulating hormone (TSH) suppression has been advocated for postoperative patients to mitigate the growth of tumor cells and reduce the risk of recurrence (7, 8). In addition, the American thyroid association 2016 guidelines recommend radioactive iodine (131I) for patients with a high risk of recurrence, such as those with residual disease or metastasis (7, 9–11). However, patients exposed to a low level of TSH are at risk of developing subclinical hyperthyroidism including cardiovascular disease. For menopausal women, excessive TSH suppression may lead to osteoporosis and increase the risk of fractures (12–15). Patients receiving ^{131}I therapy may experience damage to the parotid gland and an increased risk of secondary tumors, which increases with the radiation dose (16, 17). Currently, decisions regarding the extent of TSH suppression and the dosage of ^{131}I are primarily based on pathological invasion assessments, which vary among physicians and surgeons. Some clinicians favor more aggressive treatments to prevent recurrences (18, 19).

Some studies have indicated that PTC exhibits varying biological behaviors across different age groups and gender demographics (20, 21). Specifically, women were considered to be more prone to developing PTC, yet they typically experienced milder behavior and a more favorable prognosis, particularly among pre-menopausal females. In addition, younger patients display a more positive prognosis but are at an increased risk of recurrence (22, 23). A study on active observation of papillary thyroid microcarcinoma(PTMC) reported that tumors are more likely to progress in younger patients (24). The diverse hormonal and immunological internal environments influenced by gender and age differences could be responsible for these variable behaviors.

Our study integrated the clinical data, preoperative examinations, and postoperative pathology of the PTC patients to delineate the clinical trends within diverse subgroups. Through analyses of the gene expression database, we investigated the gene expression levels across various subgroups to unravel the mechanism underpinning the observed phenotype. This research aimed to provide a more personalized and less harmful postoperative therapeutic regimen.

2 Materials and methods

2.1 Clinical data

A total of 2073 patients from 2014 to 2023 were selected in this study, and all of them were diagnosed with PTC and underwent surgery by the same doctor. What's more, in our center, all patients diagnosed with PTC would undergo routine prophylactic central compartment lymph node dissection, and the pathological results were obtained separately by two experienced pathologists. This study passed the ethical review based on the Declaration of Helsinki and obtained informed consent from the patients. Inclusion criteria were: 1) initial thyroid cancer surgery; 2) completed clinical and pathological data; Exclusion criteria were: 1) recurrent thyroid cancer; 2) incidental thyroid cancer without central lymph node dissection (Figure 1).

We set 45 years old as the cutoff age, which was aligned with the mean age of our entire cohort and conformed to the changes in female hormone levels. Based on the character of clinical data, all participants were divided as follows: LNM (absent and present), tumor size(less than 1 cm or more than 1 cm), extrathyroidal extension (limited in capsule or violating surrounding tissues), status of thyroid function (hypothyroidism(TSH>4.94uIU/L or FT3 ≤ 2.43pmol/L or FT4 ≤ 9.01pmol/L), normal thyroid function (TSH:0.35uIU/L-4.94uIU/L, FT3:2.43pmol/L-6.01pmol/L and FT4:9.01pmol/L-19.05) and hyperthyroidism (TSH<0.35uIU/L or FT3>6.01pmol/L or FT4>19.05pmol/L), and the level of TSH (high: TSH > 2uIU/L, low: TSH ≤ 2uIU/L). What's more, the ratio of TSH/FT3 and TSH/FT4 were used to represent the response of the hypothalamic-pituitary-thyroid axis.

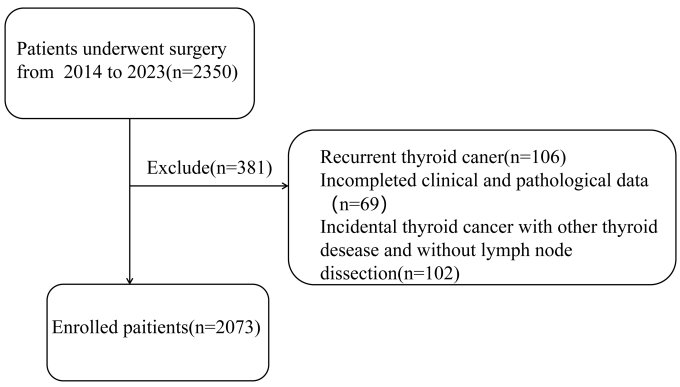


FIGURE 1
The flow chart of the patients enrolled in this study.

2.2 Public data of gene expression

We downloaded the bulk gene expression data and clinical data of PTC from the TCGA database UCSC (https://xenabrowser.net/) and deleted the cases without LNM information. Further, 497 cases with complete age and sex information were selected, and the included genes were normally detected in at least 75% of participants. scRNA-seq data were extracted from the Gene Expression Omnibus (GEO) dataset (GSE184362), and the quality control was performed according to the standard Seurat process.

2.3 Statistical analysis

The SPSS 22.0 software was used to analyze the statistical data. The continuous measurement data were expressed as $\bar{x} \pm S$ and a t-test was performed for statistical analysis. What's more, categorical variables such as sex, age, tumor size, degree of extrathyroidal extension, and status of thyroid function were counted by their frequency, and binary logistic regression analysis was performed for statistical analysis. Then, multivariate analysis was performed by multivariate logistic regression analysis. A P value <0.05 indicated a statistically significant difference. The different expressed genes (DEGs) from the TCGA database were screened using the "DESeq2" package in R 4.2.2 software. The DEGs were defined as the average gene expression ratio and two times the standard deviation, with P <0.05.

3 Results

3.1 Clinical data and single-factor analysis of LNM

According to the characteristics of clinical data, the age range was 13 to 89 years, and with 1060 patients under 45 years old,1013 were older than 45 years. All patients were divided into 556 males and 1517 females, and the ratio of males to females was 1:2.73. The average age of males was 44.2, and 45.3 for females. Among them, 942 were PTMC with a diameter less than 1cm, and 1131 carcinomas with a diameter larger than 1cm. In 1169 cases, the tumor was limited to the capsule, but in 454 cases invaded surrounding tissues. According to the definition of thyroid function, there were 121 with hypothyroidism, 1826 with normal thyroid function, and 126 patients with hyperthyroidism. Among all patients, 1101(53.1%) cases occurred lymph node metastasis, and 972(46.9%) without. The univariate analysis performed by binary logistic regression analysis showed the younger age, male gender, larger tumor size, and ETE were risk factors for LNM (P<0.05), but the factors of thyroid function weren't(P>0.05) (Table 1). Then, We showed interest in the levels of FT3, FT4, TSH, TSH/FT3, and TSH/FT4 in different lymph node metastatic status, but the results of the t-test showed no significant difference here (P>0.05) (Table 2). What's more, we conducted analyses to compare the thyroid function and the levels of FT3, FT4, TSH, TSH, TSH/FT3, and TSH/FT4 in different statuses of age, sex, tumor size, and capsule invasion (Table 3). The study's findings revealed no significant

TABLE 1 Clinicopathological characteristics and univariate analysis of the 2073 PTC patients.

Parameter	LNM(+) (1101)	LNM(-) (972)	OR (95% CI)	P
Gender			1.733(1.420–2.115)	0.000
Female	751(68.2%)	766(78.8%)		
Male	350(31.8%)	206(21.2%)		
Tumor size (cm)			2.043(1.713–2.435)	0.000
≤1	410(37.2%)	532(54.7%)		
>1	691(62.8%)	440(45.3%)		
Age			0.566(0.475–0.673)	0.000
<45 years	636(57.8%)	424(43.6%)		
≥45 years	465(42.2%)	548(56.4%)		
ETE			1.615(1.304–1.999)	0.000
Limiting in capsule	819(74.4%)	801(82.4%)		
Violating surrounding tissues	282(25.6%)	171(17.6%)		
Thyroid function				0.834
Hypothyroidism	63(5.7%)	58(6.0%)		
Normal	974(88.4%)	852(87.7%)	1.052(0.728–1.521)	0.786
Hyperthyroidism	64(5.8%)	62(6.3%)	0.950(0.577–1.566)	0.841

PTC, papillary thyroid carcinoma; ETE, extra thyroidal extension; LNM, lymph node metastasis; OR, Odds Ratio.

TABLE 2 Clinicopathological characteristics and t-test of the 2073 PTC patients.

Parameter	LNM(+) (1101)	LNM(-) (972)	Deviation (95% CI)	P
FT3	4.55 ± 1.42	4.54 ± 0.98	-0.005(-0.112–0.101)	0.926
FT4	13.22 ± 2.37	13.20 ± 2.21	-0.013(-0.212–0.185)	0.898
TSH	2.49 ± 4.73	2.35 ± 2.32	-0.133(-0.461–0.194)	0.423
TSH/FT3	0.61 ± 1.74	0.53 ± 0.53	-0.074(-0.188–0.039)	0.199
TSH/FT4	0.21 ± 0.60	0.20 ± 0.33	-0.007(-0.050–0.035)	0.729

PTC, papillary thyroid carcinoma; LNM, lymph node metastasis.

differences in thyroid function status or the levels of FT3, FT4, TSH, TSH, TSH/FT3, and TSH/FT4 when comparing different age groups and varying degrees of capsule invasion(P>0.05). However, upon conducting t-tests and binary logistic regression analyses, it was observed that within subgroups of larger sizes, there was a statistically significant increase in the levels of FT3 and FT4 (P<0.05), whereas the levels of TSH, TSH/FT3, and TSH/FT4 did not exhibit any significant differences (P>0.05). Additionally, gender-based comparisons indicated notable variations in thyroid function, as well as the levels of FT3, FT4, TSH, TSH/FT3, and TSH/FT4, between females and males (P<0.05). Women presented with a higher prevalence of hypothyroidism; the mean TSH level among

TABLE 3 Univariate analysis of risk factors to different age and gender subgroups.

Parameter	<45 years (n=1060)	≥45 years (n=1013)	P	Male (n=556)	Female (n=1517)	P
Thyroid function			0.138			0.009
Low*	52(4.9%)	69(6.8%)		18(3.3%)	103(6.8%)	
Normal	947(89.3%)	879(86.8%)	0.059	500(89.9%)	1326(87.4%)	0.003
High**	61(5.8%)	65(6.4%)	0.391	38(6.8%)	88(5.8%)	0.005
FT3(pmol/L)	4.55 ± 0.82	4.54 ± 1.55	0.860	4.45 ± 0.92	4.81 ± 1.81	0.000
FT4(pmol/L)	13.23 ± 2.17	13.19 ± 2.43	0.630	13.10 ± 2.33	13.51 ± 2.18	0.000
TSH(uIU/L)	2.45 ± 4.74	2.39 ± 2.46	0.743	2.54 ± 4.04	2.11 ± 3.01	0.022
TSH/FT3	0.59 ± 1.74	0.55 ± 0.61	0.515	0.61 ± 1.47	0.46 ± 0.73	0.024
TSH/FT4	0.21 ± 0.61	0.20 ± 0.32	0.868	0.22 ± 0.55	0.17 ± 0.27	0.039
Parameter	<1cm (n=942)	≥1cm (n=1131)	P	Limiting in capsule (n=1620)	Tissue invading (n=453)	P
Thyroid function			0.005			0.752
Low*	51(5.4%)	70(6.2%)		97(6.0%)	24(5.3%)	
Normal	851(90.3%)	975(86.2%)	0.342	1427(88.1%)	399(88.1%)	0.603
High**	40(4.3%)	86(7.6%)	0.091	96(5.9%)	30(6.6%)	0.450
FT3(pmol/L)	4.48 ± 0.75	4.60 ± 1.52	0.018	4.56 ± 1.33	4.50 ± 0.76	0.429
FT4(pmol/L)	13.07 ± 2.06	13.32 ± 2.47	0.014	13.23 ± 2.36	13.12 ± 2.07	0.381
TSH(uIU/L)	2.39 ± 3.83	2.45 ± 3.77	0.710	2.44 ± 4.16	2.34 ± 1.95	0.622
TSH/FT3	0.57 ± 1.40	0.57 ± 1.25	0.972	0.58 ± 1.46	0.54 ± 0.49	0.537
TSH/FT4	0.20 ± 0.58	0.20 ± 0.40	0.880	0.20 ± 0.52	0.36 ± 0.17	0.993

LNM, lymph node metastasis; PTC, papillary thyroid carcinoma; Low*, Hypothyroidism; High**, Hyperthyroidism.

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TABLE 4 Univariate analysis of risk factors to different age and gender subgroups.

Parameter	<45 years (n=1060)			≥45 years (n=1013)		
	LNM(+) (n=636)	LNM(-) (n=424)	P	LNM(+) (n=465)	LNM(-) (n=548)	P
Thyroid function			0.493			0.455
Low*	29(4.6%)	23(5.4%)		34(7.3%)	35(6.4%)	
Normal	574(90.3%)	373(88.0%)	0.488	400(86.0%)	479(87.4%)	0.366
High**	33(5.1%)	28(6.6%)	0.859	31(6.7%)	34(6.2%)	0.034
FT3(pmol/L)	4.52 ± 0.75	4.58 ± 0.91	0.270	4.57 ± 2.01	4.51 ± 1.03	0.510
FT4(pmol/L)	13.30 ± 2.22	13.14 ± 2.09	0.248	13.11 ± 2.56	13.25 ± 2.30	0.337
TSH(uIU/L)	2.48 ± 5.71	2.41 ± 2.68	0.809	2.49 ± 2.90	2.31 ± 2.00	0.229
TSH/FT3	0.62 ± 2.20	0.54 ± 0.60	0.446	0.58 ± 0.74	0.53 ± 0.47	0.135
TSH/FT4	0.21 ± 0.76	0.20 ± 0.28	0.736	0.20 ± 0.26	0.20 ± 0.36	0.952
Parameter	Male (n=556)			Female (n=1517)		
	LNM(+) (n=350)	LNM(-) (n=206)	P	LNM(+) (n=751)	LNM(-) (n=766)	P
Thyroid function			0.986			0.728
Low*	11(3.1%)	7(3.4%)		52(6.9%)	51(6.7%)	
Normal	315(90.0%)	185(89.8%)	0.871	659(87.7%)	667(87.1%)	0.878
High**	24(6.9%)	14(6.8%)	0.883	40(5.3%)	48(6.2%)	0.488
FT3(pmol/L)	4.85 ± 2.16	4.74 ± 0.94	0.505	4.41 ± 0.85	4.49 ± 0.98	0.086
FT4(pmol/L)	13.44 ± 2.09	13.6 ± 2.33	0.291	13.11 ± 2.49	13.09 ± 2.16	0.818
TSH(uIU/L)	2.13 ± 3.02	2.07 ± 3.00	0.832	2.65 ± 5.34	2.43 ± 2.09	0.278
TSH/FT3	0.47 ± 0.77	0.45 ± 0.66	0.755	0.67 ± 2.04	0.55 ± 0.49	0.126
TSH/FT4	0.17 ± 0.25	0.17 ± 0.31	0.992	0.23 ± 0.71	0.21 ± 0.33	0.538

LNM, lymph node metastasis; ETE, extrathyroidal extension; Low*, Hypothyroidism; High**, Hyperthyroidism.

TABLE 5 Univariate analysis of risk factors to different tumor size and ETE subgroups.

Parameter	<1cm (n=942)			≥1cm (n=1131)		
	LNM(+) (n=410)	LNM(-) (n=532)	P	LNM(+) (n=691)	LNM(-) (n=440)	P
Thyroid function			0.654			0.429
Low*	24(5.8%)	27(5.1%)		39(5.6%)	31(7.0%)	
Normal	371(90.5%)	480(90.2%)	0.628	603(87.3%)	372(84.6%)	0.310
High**	15(3.7%)	25(4.7%)	0.361	49(7.1%)	37(8.4%)	0.874
FT3	4.44 ± 0.73	4.51 ± 0.76	0.194	4.61 ± 1.70	4.59 ± 1.19	0.796
FT4	13.05 ± 2.05	13.09 ± 2.08	0.790	15.18 ± 2.54	16.28 ± 2.35	0.741
TSH	2.56 ± 5.47	2.27 ± 1.70	0.231	2.44 ± 4.23	2.46 ± 2.89	0.923

(Continued)

TABLE 5 Continued

Parameter	<1cm (n=942)			≥1cm (n=1131)		
	LNM(+) (n=410)	LNM(-) (n=532)	P	LNM(+) (n=691)	LNM(-) (n=440)	P
TSH/FT3	0.65 ± 2.06	0.51 ± 0.40	0.188	0.58 ± 1.51	0.55 ± 0.65	0.731
TSH/FT4	0.23 ± 0.86	0.18 ± 0.18	0.257	0.20 ± 0.37	0.22 ± 0.44	0.330
Parameter	Limiting in capsule (n=1620)			Tissue invading (n=453)		
	LNM(+) (n=819)	LNM(-) (n=801)	P	LNM(+) (n=282)	LNM(-) (n=171)	P
Thyroid function			0.923			0.809
Low*	48(5.9%)	49(6.1%)		15(5.3%)	9(5.3%)	
Normal	724(88.4%)	703(87.8%)	0.811	250(88.7%)	149(87.1%)	0.988
High**	47(5.7%)	49(6.1%)	0.942	17(6.0%)	13(7.6%)	0.665
FT3	4.57 ± 1.60	4.54 ± 1.00	0.604	4.47 ± 0.71	4.56 ± 0.84	0.249
FT4	14.81 ± 2.46	14.85 ± 2.24	0.985	13.18 ± 2.10	13.05 ± 2.04	0.533
TSH	2.52 ± 5.36	2.36 ± 2.41	0.449	2.38 ± 2.02	2.29 ± 1.83	0.617
TSH/FT3	0.62 ± 1.99	0.54 ± 0.56	0.231	0.56 ± 0.54	0.51 ± 0.38	0.326
TSH/FT4	0.21 ± 0.68	0.19 ± 0.27	0.499	0.19 ± 0.22	0.22 ± 0.51	0.395

LNM, lymph node metastasis; PTC, papillary thyroid carcinoma; ETE, extrathyroidal extension; Low*, Hypothyroidism; High**, Hyperthyroidism.

women was 2.54 uIU/L, compared to 2.11 uIU/L in men. Moreover, the mean TSH/FT3 ratio was significantly higher in women at 0.61, contrasting with 0.46 in men.

We conducted a further investigation to elucidate the influence of thyroid function and the concentrations of FT3, FT4, TSH, TSH/FT3, and TSH/FT4 on lymph node metastasis (LNM) across various patient subgroups (Tables 4, 5). Employing chi-square and t-test analyses within subgroups stratified by sex and age, our findings revealed no significant association between thyroid function and the levels of FT3, FT4, TSH, TSH/FT3, and TSH/FT4 with the incidence of LNM in these demographic subgroups (P>0.05). Additionally, acknowledging that larger tumor size and extrathyroidal extension are established risk factors for lymph node metastasis, we aimed to investigate the relevance of thyroid function parameters, including serum levels of FT3, FT4, TSH, TSH/FT3, and TSH/FT4, concerning LNM across various tumor sizes and invasive states, and the results indicated that these thyroid function-related factors did not exert a significant influence on the incidence of LNM (P > 0.05).

3.2 Multi-factor analysis and nomogram of LNM

Based on the above univariate analysis, factors that may have associations with LNM (P<0.05), including sex, age, tumor size, and degree of capsule invasion were included in the multivariate logistic regression model. The results showed that the larger size (OR=1.985, 95% CI 1.657–2.377; P<0.05), extrathyroidal extension (OR=1.505, 95% CI 1.206–1.877; P<0.05), male gender (OR=1.690, 95% CI 1.377–2.075; P<0.05) and younger age (OR=1.802, 95% CI 1.506–2.156; P<0.05) were independent risk factors for LNM (Table 6). Based on the results of multivariate

TABLE 6 Predictive factors of LNM in PTC patients in multiple logistic regression analysis.

Parameter	p	Adjusted OR	95%CI for Adjusted OR	
			Lower	Upper
Sex	0.000	1.690	1.377	2.075
Age	0.000	1.802	1.506	2.156
Tumor size	0.000	1.985	1.657	2.377
ETE	0.000	1.505	1.206	1.877
Constants	0.000	0.463	——	——

LNM, lymph nodes metastasis; PTC, papillary thyroid carcinoma; ETE, extrathyroidal extension.

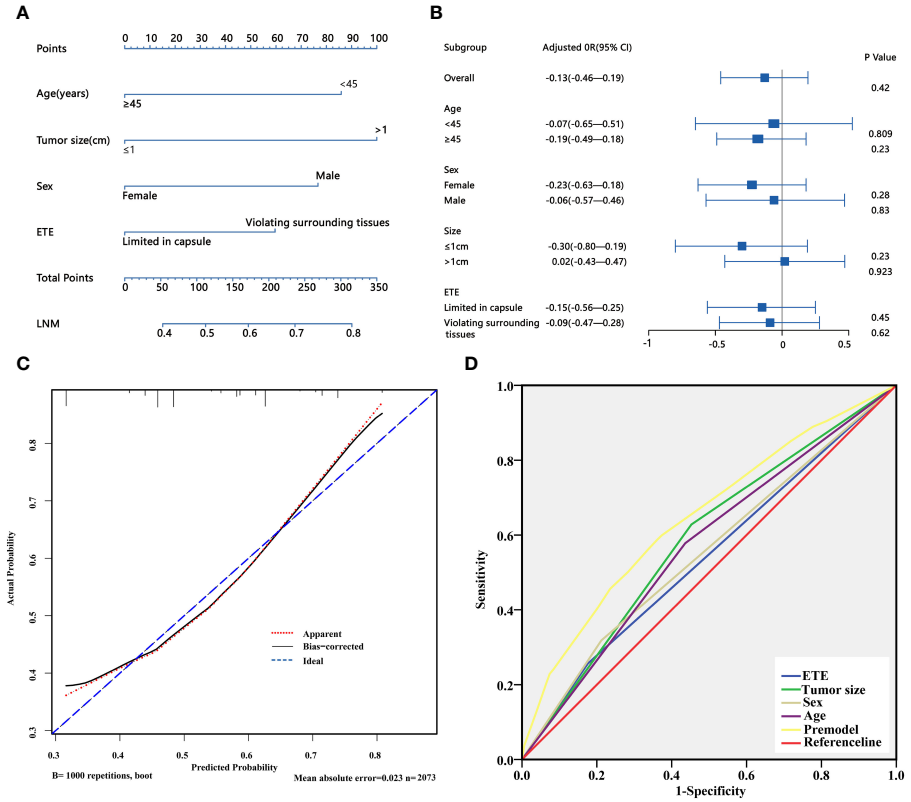


FIGURE 2
(A) Nomogram for predicting LNM in PTC patients; **(B)** The forest plot shows the impact of TSH on LNM in different subgroups. **(C)** Discrimination plot of the LNM predict mode. **(D)** ROC curve of the LNM predict model. LNM, lymph node metastasis; ETE, extrathyroidal extension.

logistic regression analysis, a nomogram predictive model was created to show the weight of each factor in this model (Figure 2A). To explore the impact of TSH on lymph node metastasis in each subgroup, a forest map was drawn, and the results showed that TSH levels had no significant effect on each subgroup(P>0.05) (Figure 2B). After 1000 internal verifications, an internal alignment curve was plotted and showed the average absolute error of the actual risk probability and the predicted risk probability of the model was 0.023 (P<0.05) (Figure 2C). The area under the receiver operating characteristic(ROC) curve was 0.647 (95% CI 0.624–0.671; P<0.05), indicating that the diagnostic efficiency of this model was superior to that of a single factor model (Figure 2D).

3.3 TCGA bulk gene expression and DEGs

Among the 497 samples, 444 were tumor tissues and 53 were para-tumor tissues. All participants were divided into subgroups according to the tissue sources, LNM status, sex, and age. The baseline was females, and older age, which conformed to the status of LNM. Genes were defined as upregulated, downregulated, and unchanged in different groups according to the definition of DEGs. The intersection DEGs of subgroups were constituted into Venn maps (Figures 3A–D). We discovered a common trend among three intersecting genes across four subgroups: SLC5A5(solute carrier family5), AVPR1A(arginine vasopressin receptor 1A), and CBLN1 (cerebellin 1 precursor protein). Specifically, both SLC5A5 and AVPR1A were downregulated in tumor and LNM tissues. Subsequently, we conducted survival analyses on these three genes and found that SLC5A5 and AVPR1A were associated with disease-free survival and that patients with a higher level of SLC5A5 and AVPR1A exhibited increased disease-free survival rates. However, at the same time, the higher level of SLC5A5 indicated poorer overall survival (Figures 4A–F).

3.4 GEO database of single-cell RNA sequencing

As members of the sodium iodide transport family, we show a strong interest in SLC5A5. By analyzing the expression of SLC5A5 across various tissues through the single-cell database, we discovered that SLC5A5 was downregulated in tumors and tissues with LNM. Furthermore, we observed a positive correlation between SLC5A5 expression and TSHR, while it appears that there is no clear association between SLC5A5 and ESR1 (Figures 5A–D).

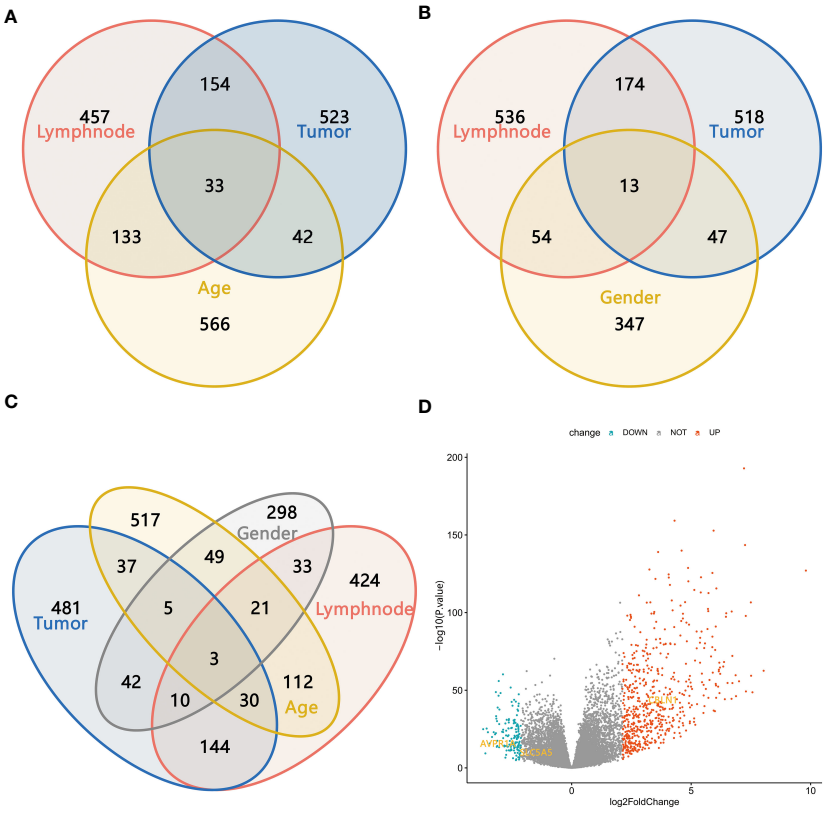


FIGURE 3
(A–C) The Venn maps based on the intersection DEGs of different subgroups; **(D)** Three intersection genes with the same trend in four subgroups.

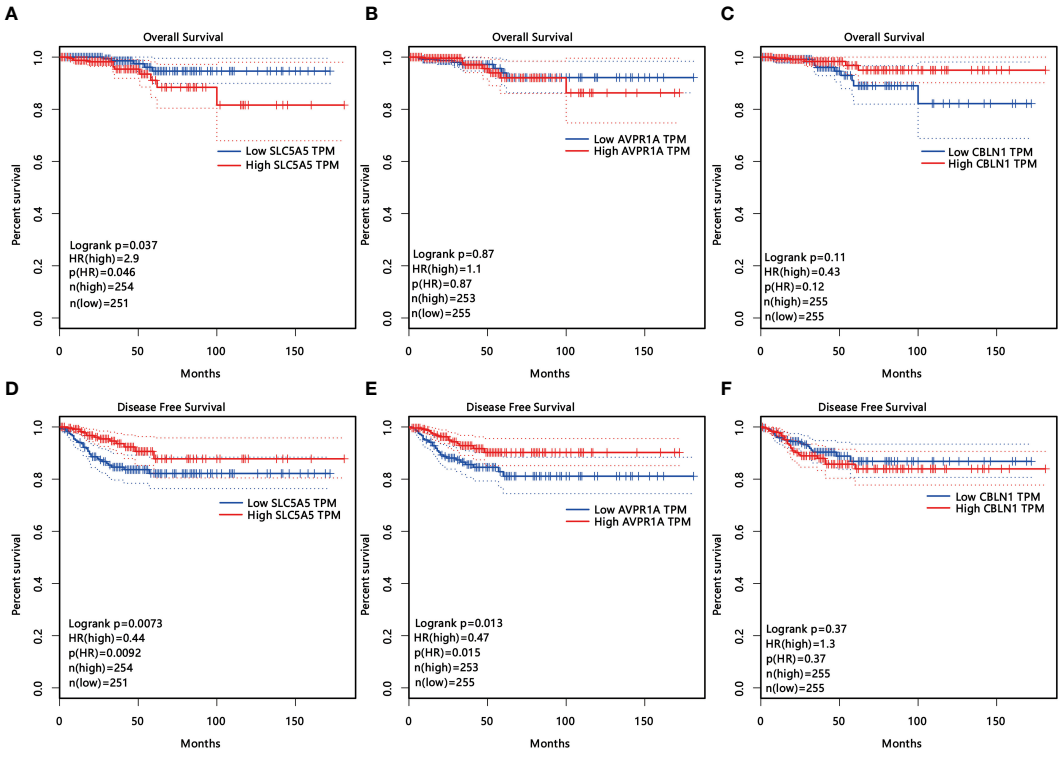


FIGURE 4
(A–F) The influence of three age- and sex-related DEGs (SLC5A5, AVPR1A, CLNB1) on overall survival **(A–C)** and disease-free survival **(D-F)**.

4 Discussion

LNM can serve as an indication of aggressive behavior and signs of recurrence potential in PTC (25, 26). In our study, women exhibited a greater propensity towards hypothyroidism and presented with elevated serum TSH levels, which may result from an increased likelihood of Hashimoto's thyroiditis in women, which may contribute to the destruction of thyroid tissue during the chronic phase. It's widely accepted that a higher TSH level promotes the proliferation of thyroid cells, leading to lymph node metastasis and invasion of surrounding tissues. Consequently, TSH suppression was theoretically an effective method to prevent the recurrence of PTC and is considered viable for low-risk PTMC patients who opt to actively monitor their condition (7). Despite some studies suggesting that TSH suppression could be beneficial for patients at high risk of recurrence, nevertheless, it was useless for low-risk patients (27). Furthermore, endogenous TSH didn't affect the outcomes of ^{131}I ablation therapy for the residual lesions (28). In our study, we found that thyroid function and the level of TSH didn't affect the status of LNM in patients with different ages, genders, tumor size, and extrathyroidal extension. On the contrary, our research found that females had a higher level of TSH but a lower risk of LNM. For these findings, we suspected that the level of TSH wasn't the exclusive factor that affects the aggressive behavior of PTC.

In addition to our findings, we discovered that in male and younger patients, the expression of SLC5A5 is downregulated, mirroring its expression in tissue with lymph node metastasis. As a sodium iodide transporter-related gene, the low expression of SLC5A5 was a manifestation of dedifferentiation in cells (29). Furthermore, our research indicated that a decreased expression of SLC5A5 correlated with a shorter disease-free survival time, which means male and younger patients are easier to recurrence. Individuals with a low level of SLC5A5 were less responsive to ^{131}I treatment and more prone to encounter LNM and postoperative recurrences (30). In addition, our study discovered a positive

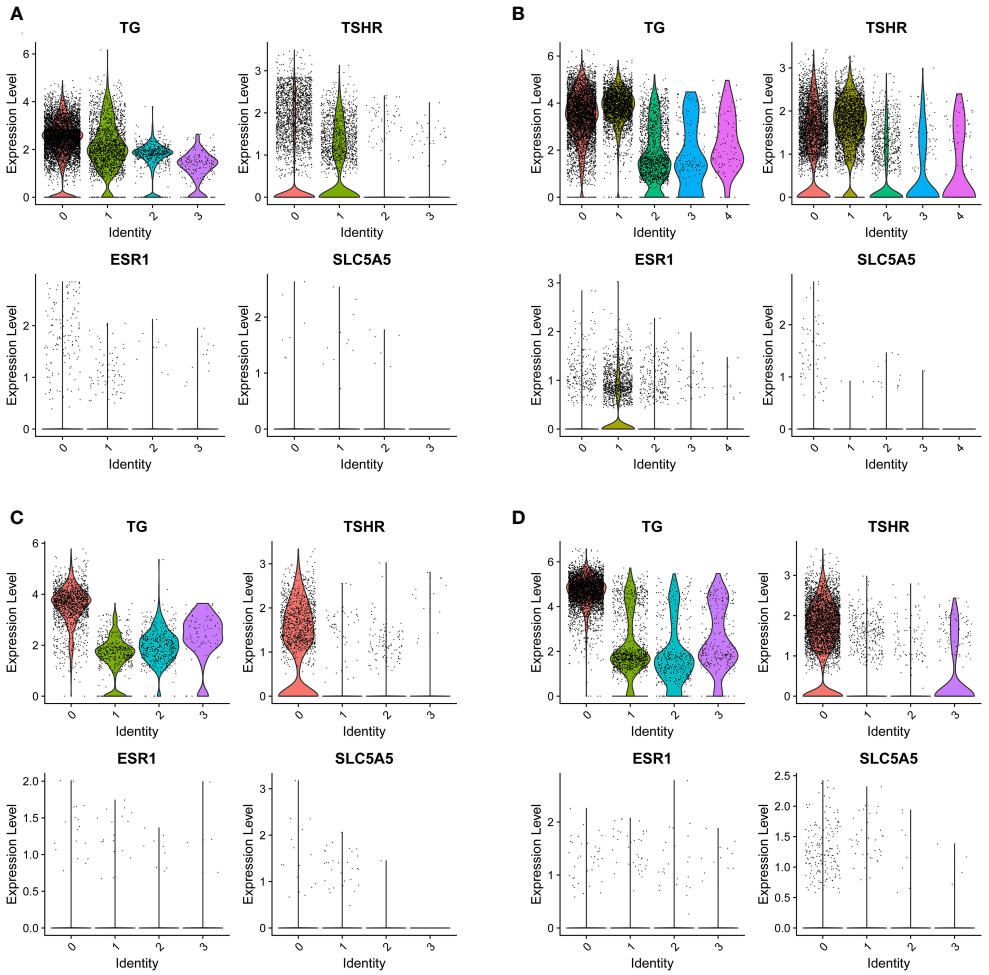


FIGURE 5
The expression of SLC5A5 and its correlation genes in four different tissues. **(A)** Tumor with lymph node metastasis; **(B)** Para-tumor tissue with lymph node metastasis **(C)** Tumor without lymph node metastasis **(D)** Para-tumor tissue without lymph node metastasis. TG, thyroglobulin; TSHR, thyrotropin receptor; SLC5A5, solute carrier family5; ESR1, estrogen receptor. The number of lateral axes represents different cell subgroups.

correction between the expression of TSHR and ESR1 to SLC5A5, which explained why the expression of SLC5A5 is higher in females. In reality, males were more likely to be resistant to the therapy of ^{131}I and experience recurrence. Consequently, we propose that patients should receive individualized doses of ^{131}I, with men requiring a higher dose. As a sodium iodide transporter-related gene, SLC5A5 was regulated by upstream gene mutations and epigenetic changes, therefore drugs targeting SLC5A5 can be studied for use in ^{131}I-resistant patients (31–33).

As a conclusion of our single-center study, it becomes imperative to collaborate with other centers to verify the reliability of the results. Additionally, our study merely captured the preoperative examinations; hence, we want to aim to accumulate more detailed insights into the postoperative follow-up to dynamically assess the intricacies of the treatment process. Lastly, we require additional evidence based on the molecular evidence to reinforce our conclusions.

5 Summary

Regarding the uncertain influence of TSH on the behavior of PTC, a flexible approach to TSH suppression indications for patients at risk of cardiovascular disease or those experiencing menopausal women was needed. Additionally, variations in ^{131}I absorption capabilities due to SLC5A5 expression levels across different genders and ages suggest that postoperative PTC therapies should take into account these demographic differences to minimize patient harm.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/Supplementary Material.

Ethics statement

The studies involving humans were approved by The Ethics Committee of the Second Hospital of Tianjin Medical University. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required from the participants or the participants' legal guardians/next of kin in accordance with the national legislation and institutional requirements.

Author contributions

CY: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Writing – original draft, Writing – review & editing. J-JS: Conceptualization, Funding acquisition, Project administration, Resources, Supervision, Visualization, Writing – review & editing. XH: Conceptualization, Funding acquisition, Project administration, Resources, Visualization, Writing – review & editing. LJ: Data curation, Formal analysis, Methodology, Software, Supervision, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2024.1339191/full#supplementary-material

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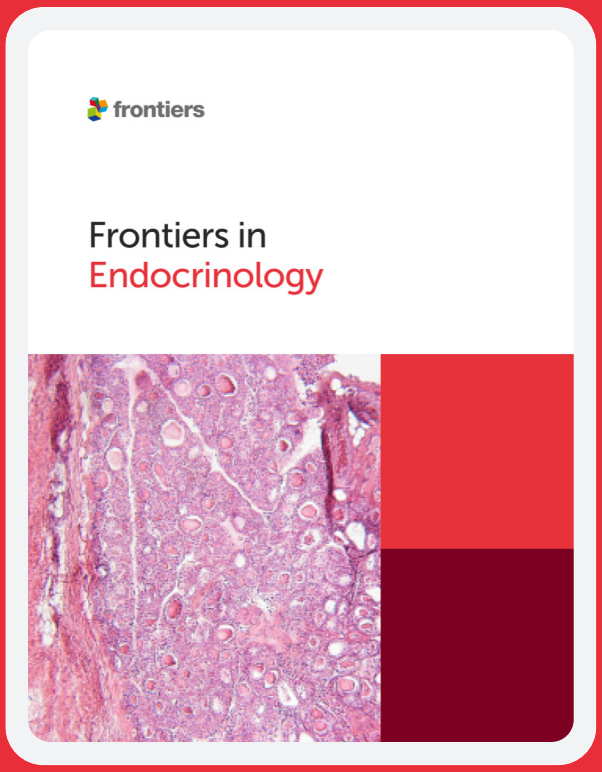
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