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Recent advances in gestational diabetes: diagnosis, treatment and prevention

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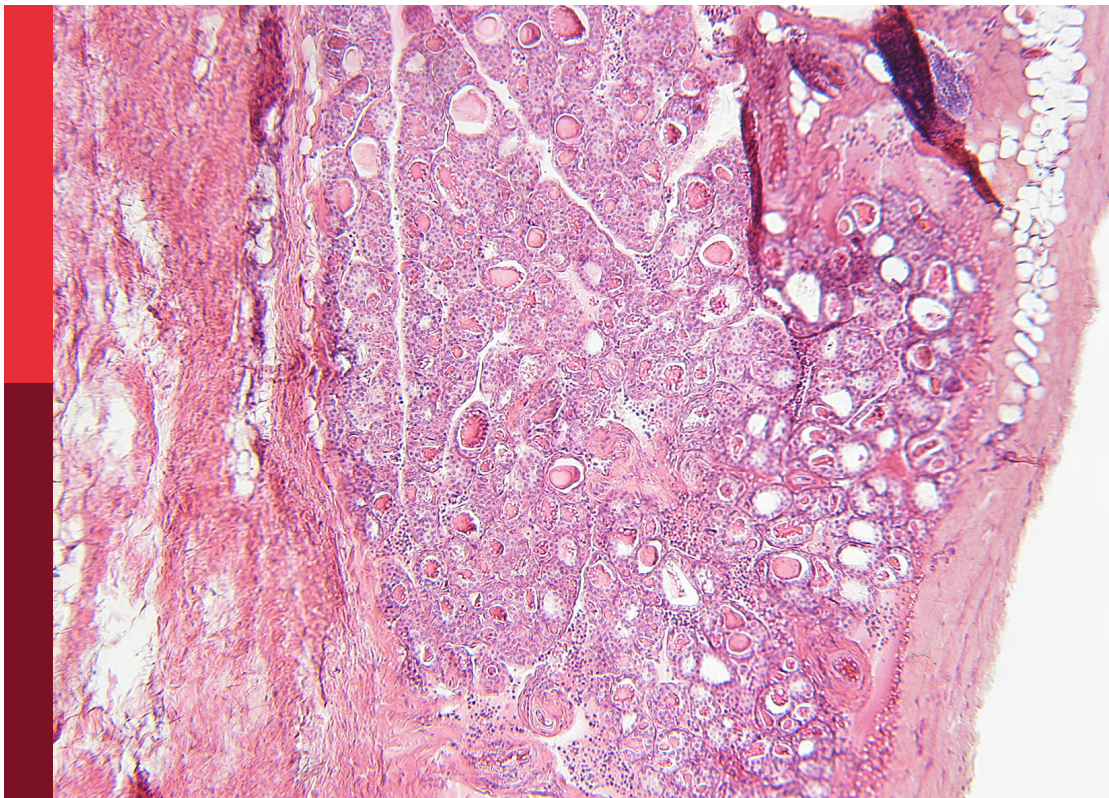

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Recent advances in gestational diabetes: diagnosis, treatment and prevention

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Editorial: Recent advances in gestational diabetes: diagnosis, treatment and prevention

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Editorial on the Research Topic
Recent advances in gestational diabetes: diagnosis, treatment and prevention

Gestational diabetes mellitus (GDM) is a common metabolic disorder in pregnancy characterized by glucose intolerance first identified in the second or third trimester (1). GDM predisposes pregnant women to several obstetric and perinatal complications and places the mother and infant at risk of long-term metabolic morbidity (2). Traditionally, GDM is diagnosed using an oral glucose tolerance test (OGTT) after 24 weeks of gestation. The current practice of GDM testing is relatively late in pregnancy, potentially limiting the opportunity for early interventions to prevent adverse pregnancy outcomes, especially among high-risk population groups. In fact, there is emerging evidence to suggest deleterious effects of 'intermediate hyperglycemia' in early pregnancy, and early therapeutic intervention could potentially reduce several serious neonatal complications (3, 4). These observations emphasize the need for a reliable test to predict or diagnose GDM in early pregnancy.

Several glycemic markers, including glycated hemoglobin (HbA1c) and fasting plasma glucose, serve as potential diagnostic markers for GDM and have been extensively studied (5). Less studied glycemic markers include 1,5-anhydroglucitol (1,5-AG), CD59 (pGCD59), second-trimester glycated albumin, and fructosamine levels (6, 7). Among these biomarkers, only HbA1c seems promising and could be an early marker for GDM. Currently, there is growing interest in identifying non-glycemic biomarkers for GDM prediction in early pregnancy. These biomarkers relate to pathogenetic events in GDM development: especially, insulin resistance and pancreatic β-cell dysfunction, caused by various factors like placental hormones, inflammation, metabolic changes, genetics, and epigenetic changes (8). The non-glycemic biomarkers under evaluation include adipokines, inflammatory and immunological markers, placenta-derived markers, thyroid function and lipid profile markers, hematological markers, and genetic markers (8).

In the present Research Topic, '*Recent Advances in Gestational Diabetes: Diagnosis, Treatment, and Prevention*, three articles focused on the association of GDM with non-

glycemic biochemical parameters in early pregnancy: serum pancreatic duodenal homeobox-1 (PDX1) gene, ferritin, and bile acids.

PDX1 is a nuclear factor that has a pivotal role in the differentiation of β-cells and is a promoter of the insulin gene expression, thereby increasing the synthesis of insulin and maintaining glucose homeostasis (9, 10). In a prospective study, Zhang et al. assessed serum PDX-1 levels at 8–12 gestational weeks among 231 pregnant Chinese women and assessed their association with GDM. PDX1 in early pregnancy was negatively correlated with fasting and 2h plasma glucose, HOMA-IR, and the triglyceride-glucose (TyG) index, and positively correlated with HOMA-β in mid-pregnancy ($P<0.05$). The adjusted analysis showed that elevated PDX1 levels in early pregnancy were associated with reduced risks of GDM (adjusted odds ratio, aOR: 0.287, 95% CI 0.130-0.636, $P = 0.002$). The area under the receiver operating characteristic (ROC) curve of PDX1 in early pregnancy for predicting the occurrence of GDM was 0.616 ($P<0.05$). The authors concluded that PDX1 has a modest predictive value for GDM, though its addition did not significantly improve the predictive value of conventional GDM risk factors.

Elevated serum ferritin (SF) levels are associated with oxidative stress (OS) and systemic inflammation in various disorders. SF is significantly increasedin early pregnancy among women with GDM and singleton pregnancies in several studies and may serve as a potential biomarker (11, 12). In the present Research Topic, Ni et al. explored the association between SF levels in early pregnancy and the risk of GDM in twin pregnancies. This retrospective cohort study included 882 twin pregnancies (700 dichorionic-diamniotic (DCDA) and 182 monochorionic-diamniotic (MCDA). In MCDA pregnancies, women with GDM had significantly higher mean SF levels compared to womenwithout GDM (101.68 ± 59.72 vs. 79.87 ± 53.11 μg/L, $p < 0.05$). In MCDA cases, SF >71.4 μg/L wasindependently associated with an increased risk of GDM (aOR = 2.775, 95% CI: 1.191–6.466; $p = 0.018$), with a significant trend across SF levels (P for trend equal to0.012). The area under the ROC curve of the prediction model of GDM in MCDA pregnancy using SF was 0.77. The authors suggest SF as a potential early biomarker for GDM prediction in MCDA pregnancies. In contrast, no significant association between SF levels and GDM was observed in DCDA pregnancies, suggesting that chorionicity is relevant in the metabolic evaluation of twin gestations.

Lu et al.'s review article suggests a potential association between GDM and bile acids. The primary focus of this review is the role of bile acids in glucose and lipid homeostasis as vital signals that regulate the Farnesoid X receptor (FXR) and Takeda G protein-coupled receptor 5 (TGR5), highlighting their potential as novel therapeutic targets for GDM management. The authors also present evidence supporting bile acids as promising biomarkers for diagnosing and assessing GDM risk. Taurocholic acid and β-muricholic acid exhibit a positive correlation with GDM risk, whereas lithocholic acid, glycodeoxycholic acid, glycoursodeoxycholic acid, and deoxycholic acid demonstrate a negative correlation.

To sum up, the three emerging non-glycemic biomarkers, PDX-1, ferritin, and bile acids, are potential predictors for GDM development in early pregnancy but lack adequate sensitivity and specificity to replace the cumbersome OGTT as a diagnostic test for GDM. Nonetheless, there remains a strong need for a reliable, simple biomarker to predict the development of GDM in early pregnancy. Early identification could reduce the period of exposure to fetal hyperglycemia through targeted prevention and therapeutic strategies, yet the heterogeneity in the aetiopathogenesis, phenotypical characteristics, and genetic architecture of GDM women remains a significant challenge (13).

Author contributions

JP: Writing – original draft, Writing – review & editing. CG: Writing – review & editing. SG: Writing – review & editing. AT: Writing – review & editing.

Conflict of interest

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Relationship between vitamin D deficiency and gestational diabetes: a narrative review

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Vitamin D, often referred to as the "sunshine vitamin," is an essential fat-soluble vitamin that plays a critical role in bone health and has been shown to improve insulin sensitivity and glucose tolerance. Vitamin D deficiency is prevalent among pregnant and pre-pregnancy women, which increases the risk of developing gestational diabetes mellitus (GDM), a common complication during pregnancy. Recent studies have explored various aspects of the relationship between vitamin D deficiency and GDM, including the mechanisms by which vitamin D affects glucose metabolism, the role of the vitamin D receptor gene, and the impact of routine vitamin D supplementation before and during pregnancy. This paper will review the current research progress in these areas.

KEYWORDS

vitamin D deficiency, vitamin D receptor gene, gestational diabetes mellitus, correlation, dose supplementation

1 Introduction

GDM refers to abnormal glucose metabolism that occurs during pregnancy, excluding pre-existing type 1 or type 2 diabetes (1, 2). The prevalence of gestational hyperglycemia in China is significant and continues to rise annually. According to the 9th edition of the International Diabetes Federation Diabetes Atlas, the estimated prevalence of GDM in China in 2019 and beyond is 14.8% (3). The 10th edition of the Global Diabetes Map indicates that the global incidence of gestational hyperglycemia in 2021 is 16.7%, of which GDM accounts for 80% (4). the average prevalence of GDM in China is 14.8% (5). Although the etiology and pathogenesis of GDM are not fully understood, several high-risk factors contribute to its increased incidence. Vitamin D is an essential nutrient obtained from sunlight, natural foods, and exogenous supplements (6). Vitamin D_3, in particular, is found in animal-based foods such as milk, deep-sea fish, cod liver oil, and egg yolk (7).The primary physiological functions of vitamin D include regulating serum calcium absorption, balancing calcium and phosphorus metabolism, promoting bone growth, and regulating cellular growth and differentiation. Vitamin D deficiency in women of childbearing age has also attracted considerable attention. It poses a major health risk not only to non-pregnant women but also to those who are pregnant. Vitamin D is crucial for women of childbearing

age and during pregnancy. Deficiency in vitamin D has been shown to affect glucose metabolism mechanisms during pregnancy, including insulin secretion and resistance. This deficiency exacerbates insulin resistance, leading to elevated blood glucose levels and increasing the risk of developing GDM. Furthermore, vitamin D deficiency significantly impacts adverse pregnancy outcomes in women with GDM. Currently, the mechanisms of glucose metabolism during pregnancy, the role of the vitamin D receptor (VDR) gene in GDM, and dose-related indicators of GDM require further research. This article reviews the correlation between vitamin D deficiency and GDM for clinical reference.

2 Overview of vitamin D deficiency

2.1 Sources of vitamin D

Vitamin D is a steroid-derived compound obtained from sunlight, natural foods, and exogenous supplements (6). It is mainly acquired through the following methods (Figure 1): Sunlight Exposure: The primary source of vitamin D for the human body is through skin exposure to sunlight. When the skin is exposed to ultraviolet B (UVB) radiation from sunlight, 7-dehydrocholesterol in the skin is converted to provitamin D_3, which then undergoes spontaneous isomerization to form vitamin D3. This process accounts for approximately 80-90% of the body's vitamin D supply. Food Intake: Vitamin D can also be ingested through dietary sources. Vitamin D_3 is predominantly found in animal-based foods, such as milk, deep-sea fish, cod liver oil, and egg yolk (7). Vitamin D_2, on the other hand, mainly comes from plant-based foods like certain mushrooms. Once consumed, vitamin D from these foods enters the lymphatic system via chylomicrons and eventually reaches the bloodstream. Supplement Intake: Due to the limited amount of vitamin D available in food and the possibility of insufficient sunlight exposure, exogenous supplements are an important alternative source of vitamin D. Vitamin D metabolism involves several key steps. In the liver, both vitamin D_3 and D_2 are converted to 25-hydroxyvitamin D, the main circulating form of vitamin D and an indicator of vitamin D levels. In the kidneys or extrarenal tissues, $25(OH)D_3$ is further converted to $1,25(OH)_2D_3$, the active form of vitamin D. This active form is crucial for maintaining calcium and phosphorus balance, bone health, and various other physiological functions.Ensuring adequate vitamin D levels is essential for overall health. In addition to sufficient sunlight exposure, increasing the intake of vitamin D-rich foods can help maintain these levels. However, because dietary sources alone are often inadequate to meet the body's needs, vitamin D is uniquely referred to as the "sunshine vitamin."

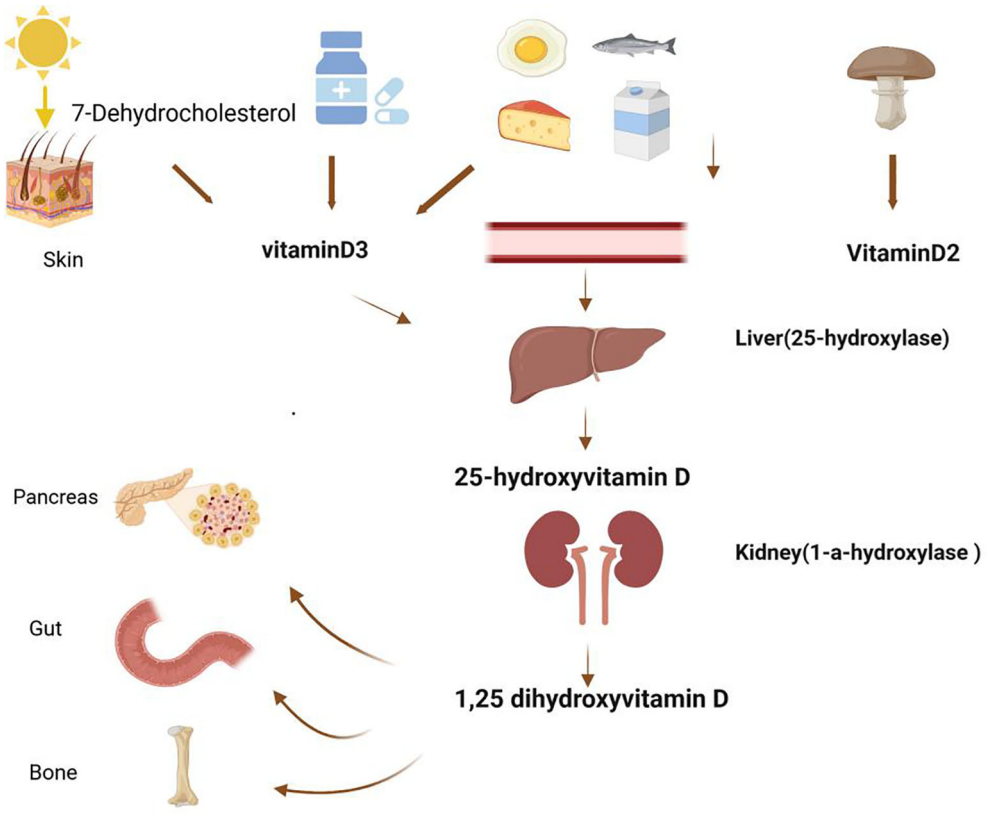

FIGURE 1
Metabolic pathways of vitamin D.

2.2 The harm of vitamin D deficiency

Vitamin D deficiency is potentially harmful to the development and progression of various diseases, with low concentrations of 25 $(OH)D_3$ serving as potential risk markers for several conditions, including cancer morbidity and mortality. The most well-recognized role of vitamin D is its impact on bone health. A deficiency in vitamin D can lead to inadequate calcium absorption, and severe deficiency may result in bone health diseases. Cancer: Vitamin D has been implicated in the development of cancers such as colon and breast cancer. A meta-analysis of prospective studies assessing the association between serum $25(OH)D_3$ levels and cancer incidence (8 studies) or cancer mortality (16 studies) found that each 20 nmol/L increase in serum $25(OH)D_3$ levels (8 μg/L) was associated with a 7% reduction in cancer risk and a 2% reduction in cancer mortality (8). Cardiovascular Disease: A meta-analysis of prospective studies found an association between reduced vitamin D status, as measured by serum $25(OH)D_3$ levels or vitamin D intake, and an increased risk of ischemic stroke and ischemic heart disease (9). Endocrine and Metabolic Diseases: Studies have indicated that vitamin D deficiency may be closely related to an increased risk of diabetes and pre-diabetes (10). Autoimmune Diseases: Research has shown that vitamin D supplementation can reduce the risk of autoimmune diseases by 22%, and long-term vitamin D supplementation can help prevent these diseases, particularly in individuals aged 50 years and older (11). Other Related Diseases: There is growing evidence that vitamin D deficiency is associated with an increased risk of acute respiratory and chronic diseases, including chronic kidney disease, neurological diseases, and metabolic syndrome. Several studies support the hypothesis that low levels of serum $25(OH)D_3$ are independently associated with the incidence and severity of respiratory tract infections in both children and adults (12, 13). Therefore, it is important to address the potential harm caused by vitamin D deficiency and reduce the incidence of systemic diseases related to it.

2.3 Potential mechanisms of vitamin D deficiency on glucose metabolism during pregnancy

2.3.1 Effect of vitamin D deficiency during pregnancy on insulin secretion

Vitamin D deficiency during pregnancy may impact insulin secretion. Over the past five years, numerous studies have highlighted vitamin D's crucial role in both insulin secretion and insulin resistance. Vitamin D can regulate insulin secretion from pancreatic β-cells by altering the expression of the proinsulin gene. Studies have shown that $1,25(OH)_2D_3$ enhances calcium influx during glucose-stimulated insulin secretion (GSIS) by up-regulating related genes, thereby modulating beta cell insulin secretion (14). Additionally, the interaction between vitamin D and the vitamin D receptor (VDR) on pancreatic β-cells can regulate extracellular calcium concentration and calcium flux through ion channels. This process facilitates calcium-dependent insulin secretion via the calcium concentration gradient across the cell membrane, promoting insulin release. L-type voltage-gated calcium channels (L-VGCC), K^+-ATP, and K^+-Ca^{2+} channels are involved in $1,25(OH)_2D_3$ signaling. Transcriptional regulation of voltage-gated calcium channels by $1,25(OH)_2D_3$ through VDR also influences GSIS (14–16). Animal studies have shown that $1,25(OH)_2D_3$ can stimulate insulin secretion in a sugar-independent manner, promoting islet insulin release (16). Bornstedt Mette Eskild found a significant increase in insulin secretion in cells treated with $1,25(OH)_2D_3$, suggesting that vitamin D enhances GSIS (17). This effect has also been observed in human islets. Conversely, vitamin D deficiency may reduce calcium ion concentration in islet cells, impairing related signaling pathways and affecting insulin synthesis and secretion, leading to elevated blood glucose levels and potentially resulting in GDM.

2.3.2 Effect of vitamin D deficiency during pregnancy on insulin resistance

2.3.2.1 Vitamin D deficiency during pregnancy reduces insulin receptor expression

Vitamin D indirectly affects insulin secretion by reducing inflammatory responses and improving insulin resistance (18). Research has verified that $1,25(OH)_2D_3$ can improve insulin resistance (IR) in trophoblast cells by inhibiting the mTOR signaling pathway, as demonstrated through the establishment of an IR BeWo cell model. $1,25(OH)_2D_3$ protects trophoblasts from high IR primarily by inhibiting mTOR signaling, which may be a potential therapeutic approach for patients with GDM (19).During pregnancy, vitamin D deficiency leads to reduced levels of $1,25(OH)_2D_3$, which diminishes the inhibition of the mTOR signaling pathway, resulting in increased insulin resistance and a higher incidence of GDM.

2.3.2.2 Vitamin D deficiency during pregnancy exacerbates inflammation and oxidative response

Vitamin D plays a crucial role in both the inflammatory response and oxidative stress. Vitamin D, by binding with its receptor, reduces pro-inflammatory cytokines in immune cells and has an immunomodulatory effect (20, 21). Studies have shown that treatment with $1,25(OH)_2D_3$ in GDM placental explants blocks the abnormal increase in leptin, tumor necrosis factor-alpha (TNF-α), and interleukin-6 (IL-6) levels, reducing both placental IR and inflammatory responses (19). This suggests that $1,25(OH)_2D_3$ is involved in maintaining normal immune inflammatory responses, especially during pregnancy when CYP27B1 is strongly expressed in the placenta, becoming an important source of $1,25(OH)_2D_3$ synthesis (22). Furthermore, low vitamin D levels not only exacerbate systemic inflammation but also promote placental inflammation (23).

2.3.2.3 Vitamin D deficiency and obesity during pregnancy increase insulin resistance

Obesity is characterized by body mass index (BMI) greater than 30, while a BMI greater than 25 shows that the individual is

overweight (24). Several studies have shown that vitamin D deficiency is strongly associated with insulin resistance, especially in obesity and in patients with metabolic syndrome (25, 26). Several studies have shown that low levels of vitamin D are strongly associated with the development of insulin resistance, especially in obese and type 2 diabetic patients (27, 28). $1,25(OH)_2D_3$ can regulate adipocyte formation and differentiation by modulating the nuclear receptor VDR and peroxisome proliferator-activated receptor γ (PPARγ) pathways. It has been reported that the serum vitamin D levels in women with GDM and those who are overweight or obese are reduced, while the expression of VDR and PPARγ mRNA in adipose tissue is up-regulated (29). This up-regulation further increases the expression in overweight or obese women with GDM and contributes to the development of GDM. Some scholars found that pregnant women with a pre-pregnancy BMI of 23.5-27.0 kg/m² could significantly reduce the risk of GDM by increasing their serum vitamin D levels, suggesting a synergistic effect between low vitamin D levels and obesity (30). Research has confirmed that vitamin D deficiency is strongly associated with obesity (25). Further studies have indicated that low serum 25OHD is positively correlated with obesity or BMI in adults and children, and vitamin D plays an important role in adipogenesis and inflammation of adipocytes and adipose tissue (31). These findings suggest that vitamin D deficiency promotes obesity by enhancing the expression of the PPARγ pathway, thereby regulating the development and differentiation of adipocytes. Vitamin D supplementation may become a nutritional intervention for GDM, with significant clinical implications for reducing the incidence of GDM, particularly in obese or overweight women.

2.4 The relationship between vitamin D level and GDM in women before pregnancy

2.4.1 Vitamin D deficiency in non-pregnant women of childbearing age

Due to lifestyle changes and environmental factors, vitamin D deficiency has become a common problem, especially for women of childbearing age. Research investigating serum $25(OH)D_3$ levels in Chinese women of gestational age from cities between 2010 and 2012 found that only 15.1% had normal vitamin D nutritional status (32). This indicates that women of childbearing age often overlook the significant health issues caused by vitamin D deficiency. A prospective cohort study showed that vitamin D deficiency in women of childbearing age can adversely affect the female reproductive system, leading to infertility (33). Furthermore, studies have demonstrated that in the polycystic ovary syndrome (PCOS) population, vitamin D deficiency has a higher prevalence of glucose intolerance than women without vitamin D deficiency (34). The study by Wehr E provides compelling evidence that women with normal ovulation have higher vitamin D levels than women with PCOS (35). A recent review by Iervolino et al. Concluded that vitamin D appears to be effective in the treatment of PCOS (36). Additionally, Di Bari noted an association between low $25(OH)D_3$ levels and obesity, hyperandrogenism, insulin resistance, and other metabolic dysfunctions associated with PCOS (37). These studies highlight the importance of vitamin D intake and supplementation for women of childbearing age. Regular examination of $25(OH)D_3$ levels should be considered a routine part of physical examinations for young women and before pregnancy. Regular assessment of $25(OH)D_3$ levels can help to monitor vitamin D status and guide the appropriate dosage of supplements. By actively maintaining adequate vitamin D levels, women of childbearing age can better protect their health.

2.5 Routine pre-pregnancy vitamin D supplementation for women of childbearing age

The increasing number of problems caused by vitamin D deficiency has gradually attracted societal attention. While the necessity of routine vitamin D supplementation before pregnancy remains a debated issue, but vitamin D supplementation is extremely necessary. Recent studies have shown that vitamin D is associated with fertility and suggest that optimal levels of 30 ng/mL or higher should be achieved with appropriate doses before and throughout pregnancy (38). It is also essential to continue vitamin D supplementation during pregnancy. Rosalyn J Singleton found that prenatal supplementation with 1000 IU of vitamin D_3 significantly increased prenatal 25(OH)D concentrations. This increase may help reduce the rate of early childhood caries and provides a reference for prenatal vitamin D supplementation in other high-risk groups for rickets (39). The benefits of routine vitamin D supplementation before pregnancy are evident, though there are currently few studies on this topic. Future research should focus on supplementing different doses of vitamin D according to varying degrees of deficiency, which requires further exploration.

2.6 The relationship between vitamin D deficiency and gestational diabetes mellitus

2.6.1 Vitamin D receptor gene and GDM

The relationship between the VDR gene and GDM has garnered significant attention in recent years. Consequently, polymorphisms in the VDR gene may be linked to an increased risk of GDM. Several studies have demonstrated that VDR gene polymorphism may play a role in the pathogenesis of GDM (Figure 2). For instance, polymorphisms at sites such as rs7975232, rs2228570, and rs1544410 have been linked to an elevated risk of GDM, providing insights into how the VDR gene influences the likelihood of developing GDM. Research has shown that the rs7975232 polymorphism in the VDR gene may be associated with GDM risk (40). A meta-analysis by Sai Liu and colleagues supported the association between the VDR rs7975232 polymorphism and GDM, and also found that the FokI (rs2228570) polymorphism was linked to increased susceptibility

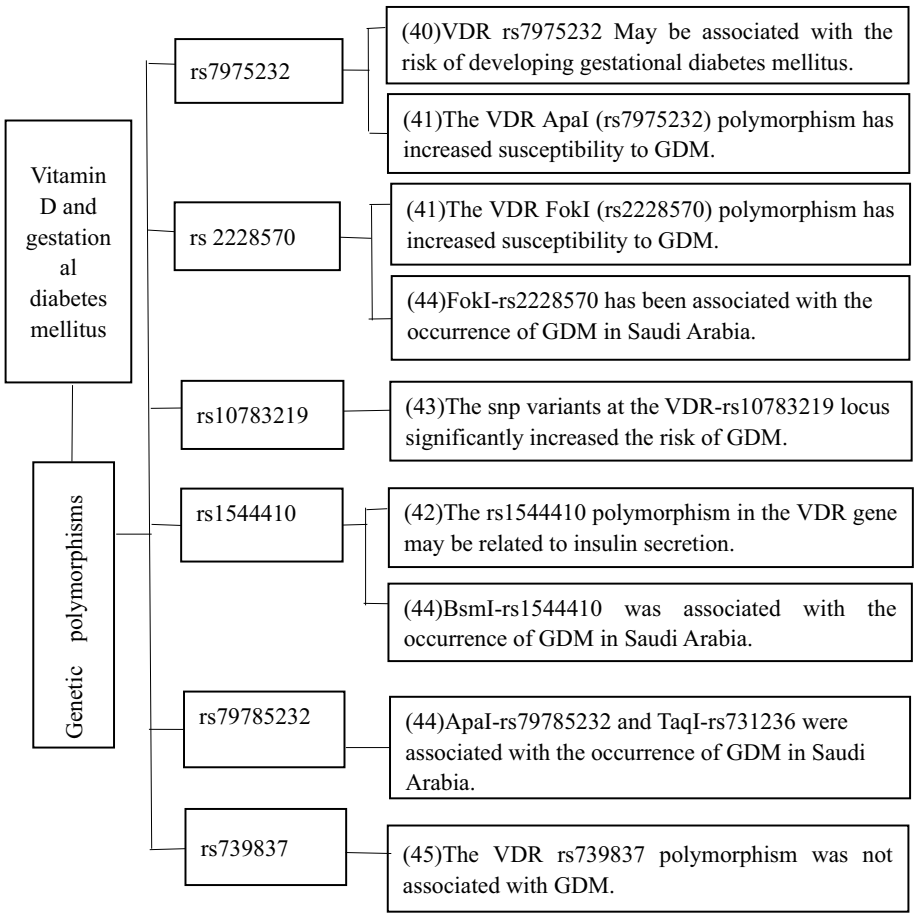

FIGURE 2
The relationship between vitamin D receptor genes and gestational diabetes mellitus.

to GDM (41). Additionally, it has been demonstrated that the rs1544410 polymorphism in the VDR gene is associated with insulin secretion in GDM patients (42). An important study confirmed that single nucleotide polymorphism (SNP) mutations at VDR-rs10783219 and MTNR1B-rs10830962 significantly increase the risk of GDM (43). Further research in Saudi Arabia found that ApaI-rs79785232, BsmI-rs1544410, FokI-rs2228570, and TaqI-rs731236 polymorphisms are related to the occurrence of GDM in the region (44). In conclusion, the VDR gene does play a role in the pathogenesis of GDM. Although most studies support the association between the VDR gene and GDM, a few have not found such a link. It has been reported that the VDR gene rs739837 polymorphism is not associated with GDM (45).

2.6.2 Relationship between vitamin D deficiency and GDM

Vitamin D deficiency is prevalent among pregnant women. A study in Switzerland found that 73.2% of pregnant women had vitamin D deficiency or insufficiency (46). Similarly, research in Boston, USA, revealed that 53.2% of 206 pregnant women had vitamin D levels below 30 ng/mL, indicating that vitamin D deficiency remains widespread and significantly increases the incidence of GDM (47). Maysa Alzaim demonstrated a 1.29-fold increase in the risk of GDM for every 12.5 nmol/L decrease in serum $25(OH)D_3$ levels (48). The Third International Conference on Vitamin D Controversy in 2020 reached an international consensus showing that about 7% of the global population suffers from severe vitamin D deficiency, with prevalence rates of 37% worldwide, 40% in Europe, and 72% in China (49). A review of 36 observational studies found that the risk of GDM in pregnant women with vitamin D deficiency increased by 18%, and serum $25(OH)D_3$ levels in women with GDM were 1.18 nmol/L lower, suggesting a link between low vitamin D concentrations and GDM (50). A retrospective cohort study by Yan Cheng showed that in the vitamin D status of pregnant women in Shanghai and its relationship with GDM, vitamin D deficiency and insufficiency were prevalent among women in Shanghai, and vitamin D levels of at least 20 ng/mL in early pregnancy was significantly associated with reduced risk of GDM (51). It is suggested that high levels of vitamin D have a protective effect on the risk of GDM. A nested case-control study by Eleonora Salakos et al. found that women with$25(OH)D_3$ levels below 20 ng/mL had a significantly higher risk of GDM compared to non-GDM patients (52). Furthermore, a prospective cohort study by Alireza Milajerdi showed that individuals with vitamin D deficiency had a 26% higher risk of developing GDM than those with normal serum vitamin D levels

(OR: 1.26; 95% CI: 1.13, 1.41). There was a significant positive association between vitamin D insufficiency and deficiency and GDM risk (OR: 1.23; 95% CI: 1.11, 1.35). The study found that the risk of GDM was lowest in individuals with serum vitamin D levels between 40 and 90 nmol/L, and a dose-response analysis revealed a U-shaped nonlinear correlation between serum vitamin D concentration and GDM risk ($P < 0.05$) (53).

2.6.3 Correlation between vitamin D dose and gestational diabetes mellitus

There are numerous reports about the controversy surrounding vitamin D supplementation for GDM, but vitamin D is generally considered an effective treatment for GDM (Table 1). The latest recommendation from the Institute of Medicine (IOM) for vitamin D supplementation during pregnancy and lactation is 600 IU per day (54). In 2011, the Endocrine Society issued guidelines on the assessment, diagnosis, and treatment of vitamin D deficiency, recommending that pregnant and lactating women should receive at least 600 IU of vitamin D per day, with a target $25(OH)D_3$ level of at least 30 ng/mL (55). The Central and Eastern European expert consensus statement recommends that women planning to become pregnant should initiate or maintain vitamin D supplementation, with healthy adults advised to take 800-2000 IU/day if they have no other risk factors. A treatment duration of entire pregnancy and lactation is recommended, with the aim to target concentrations of 30 to 50 ng/mL (59). Qingying Zhang found that high-dose and moderate-dose vitamin D supplementation reduced insulin and HOMA-IR levels in GDM patients. Randomized controlled trials indicated that high-dose vitamin D supplementation (50000 IU every two weeks) significantly reduced insulin resistance in pregnant women with GDM. It is recommended that pregnant women with GDM receive high-dose vitamin D supplementation (50000 IU every two weeks) from the 12th week of gestation until delivery (56). The AME statement from the Italian Association of Clinical Endocrinology suggests that a safe dose of vitamin D supplementation during pregnancy is 4000 IU/day, with a therapeutic target serum $25(OH)D_3$ level of > 40 ng/mL (57). The expert panel, including the Polish Association of Pediatric Endocrinology and Diabetes, recommends a dose of 2000 IU/day for pregnant and lactating women, aiming for a serum level of 30-50 ng/mL, with treatment lasting 12 weeks or until the target concentration is achieved (58). A study by Eduardo Klöppel showed that vitamin D supplementation in pregnant rats was more beneficial than no supplementation, aiding fetal development and reducing prediabetic complications (60). Another study demonstrated that GDM patients who supplemented with vitamin D and omega-3 fatty acids for six weeks experienced significant reductions in fasting blood glucose, triglycerides, high density lipoprotein, Low-density lipoprotein and total cholesterol, ultimately improving glucose and lipid metabolism (61). Therefore, vitamin D supplementation is particularly important, and further research is needed to determine optimal supplementation strategies for different baseline levels of vitamin D deficiency.

2.7 Effect of vitamin D deficiency on the outcome of pregnant women with GDM

Vitamin D plays a crucial role during pregnancy, impacting not only the health of pregnant women but also being closely related to adverse pregnancy outcomes. For instance, vitamin D deficiency has been linked to an increased rate of cesarean sections, GDM and preeclampsia. An increasing number of studies highlight the significant impact of vitamin D deficiency on pregnancy outcomes (Table 2). Anne Merewood showed that women with $25(OH)D_3$ levels below 37.5 nmol/L were four times more likely to have a cesarean section compared to those with levels of 37.5 nmol/L or higher, suggesting a negative correlation between vitamin D deficiency and

TABLE 1 Vitamin D supplementation is recommended for pregnant women.

Country or Region (Year)	Population	Size of Population	Gestational Week (GW)	Oral Vitamin D (IU)	Treatment Duration	Target Concentration (ng/mL)	First Author
Institute of Medicine (2011)	Pregnant and lactating women	/	/	600 IU/day	/	/	ACOG Committee (54)
Endocrine Society (2011) USA	Pregnant and lactating women	/	/	600 IU/day	/	30	Holick et al. (55)
Exp Ther Med (2016)	Gestational diabetes	133	24-28 GW	50000 IU/2weeks	12^{th} week to delivery	/	QINGYING ZHANG et al. (56)
Italy (2018)	Pregnant women	/	/	4000 IU/day	/	>40	Cesareo et al. (57)
Poland (2018)	Pregnant and lactating women	/	/	2000 IU/day	12 weeks	>30–50	Rusińska A et al. (58)
A Central and Eastern European (2022)	BeforePregnant 、 Pregnantand lactating women	/	/	800-2000 IU/day	throughout pregnancy and lactation	30-50	Pawel Pludowski et al. (59)

TABLE 2 Effect of vitamin D deficiency on adverse outcomes in pregnant women with GDM.

First Author (Year)	Study design	Place of study	Sample size	VitD Assay method	Outcome analyzed	Statistics (95% CI or AOR)	Sample (Serum or Plasma)
Anne Merewood, et al. (2009) (62)	Prospective cohort study	Boston, Massachusetts	253	Liquid chromatography-mass spectrometer	Primary Cesarean Section	AOR = 3.84; 95%CI (1.71-8.62)	Serum
Van Weert et al. (2016) (63)	Prospective cohort study	Netherlands	2074	Enzyme-linked immunosorbent assay	Pregnancy related hypertensive disorders	OR:1.88 (0.79-4.48)	Serum
Hanna Augustin et al. (2020) (64)	Prospective cohort study	Sweden	1832	Liquid chromatography-mass spectrometer	Emergency Caesarean Section	AOR = 2.01 p = 0.044	Serum
Bhupali Das et al. (2021) (65)	case-control study	Indian	1000cases and 1000 controls	Radioimmunoassay	preeclampsia	OR:11.308; 95%CI (7.5982-14.0097)	Serum
Shu Qin Wei, et al. (2021) (66)	Nested case–control study	/	34:65	/	pre-eclampsia	AOR=4.79; 95%CI (1.67-13.75)	plasma
Juhi Nema, et al. (2023) (67)	Longitudinal study	Pune, India.	108cases and 216controls	Enzyme-linked immunosorbent assay	preeclampsia	95% CI (0.08,0.77)	Serum
Mina Amiri, et al. (2023) (68)	Stratified randomized controlled field trial	Khuzestan	1649	Enzyme-linked immunosorbent assay method and a kit of Immunodiagnostics systems	preterm delivery、cesarean section	(95% CI: 25.69–30.02), (95% CI: 33.36–37.96)	Serum

cesarean section rates (62). Another study supported this association, finding that pregnant women in Singapore with insufficient 25(OH) D_3 levels had a higher likelihood of emergency cesarean section (OR= 1.39, 95% CI = 0.95, 2.05) (69). A prospective cohort study by Hanna Augustin found that vitamin D deficiency was associated with a two-fold increased risk of emergency cesarean section in women without epidural anesthesia (64). Similarly, Mina Amiri found that women with moderate vitamin D deficiency were more likely to undergo cesarean section. Severe vitamin D deficiency exhibited a higher probability of preterm delivery, indicating that vitamin D status at delivery can directly affect the mode of delivery (68). However, studies have been inconsistent regarding the association between vitamin D levels and pregnancy outcomes. Some research has found no association between maternal vitamin D levels and the risk of vaginal birth, instrumental delivery, primary cesarean delivery, or cesarean delivery for any other reason (70). Similarly, other studies reported that vitamin D deficiency In women with GDM at mid-pregnancy is associated with an elevated risk of postpartum glucose intolerance (71). Premature rupture of membranes (PPROM) is another adverse pregnancy outcome linked to vitamin D deficiency. A prospective study by Hyun Joo Lee measured vitamin D levels in 355 pregnant women during the first trimester and before delivery, finding that the incidence of PPROM was higher in the vitamin D deficiency group compared to the non-deficiency group. Vitamin D levels were significantly lower in the PPROM group during both the first and second trimesters, indicating a significant association between vitamin D deficiency and PPROM ($p = 0.003$) (72). A logistic regression analysis of 2074 pregnant women found that those with severe vitamin D deficiency had an increased risk of preeclampsia (OR 2.08; 95% CI, 1.05-4.13) but the association was rendered non-significant after correction (OR 1.88; 95% CI 0.79-4.48) (63). A study by Juhi Nema reported that continuous measurement of vitamin D throughout pregnancy and the risk of preeclampsia in an Indian population, suggesting that vitamin D deficiency could be an important etiological factor in the clinical diagnosis of preeclampsia (67). Another study found that vitamin D levels were inversely related to the severity of preeclampsia, and the severity of preeclampsia increased with the decrease of vitamin D levels ($p < 0.001$) (65). Additionally, Shu Qin Wei also indicated maternal vitamin D deficiency was associated with the risk of preeclampsia at 24-26 weeks of gestation (66). Therefore, it is essential to address the negative effects of vitamin D deficiency on pregnancy outcomes, particularly in women with GDM.

3 Conclusion and prospects.

Vitamin D deficiency is very common in pregnant women. With the increasing number of GDM patients worldwide, it is important to pay attention to the negative impact of vitamin D deficiency on pregnant women with GDM. Vitamin D deficiency is also associated with the occurrence of many diseases. Currently, there are numerous conclusions about the potential mechanisms of vitamin D in glucose metabolism and the relationship between the VDR gene and GDM. However, there are still varying results regarding the correlation between vitamin D deficiency and GDM, as well as the treatment and outcomes of vitamin D supplementation for GDM. Future studies should focus on vitamin D supplementation at different levels of deficiency. It is recommended to appropriately supplement vitamin D before and during pregnancy, strengthen the detection of serum 25 $(OH)D_3$ levels before pregnancy, and achieve early detection and early intervention. This approach can help reduce the impact of

vitamin D deficiency on adverse pregnancy outcomes in pregnant women with GDM.

Author contributions

CL: Conceptualization, Writing – original draft, Writing – review & editing. HL: 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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The author(s) declare that no Generative AI was used in the creation of this manuscript.

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Corrigendum: Relationship between vitamin D deficiency and gestational diabetes: a narrative review

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vitamin D deficiency, vitamin D receptor gene, gestational diabetes mellitus, correlation, dose supplementation

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In the published article, there was an error, regarding the duration of treatment.

A correction has been made to section *2.6.3 Correlation between vitamin D dose and gestational diabetes mellitus*. This sentence previously stated:

"A treatment duration of 4–12 weeks is recommended, with the aim to target concentrations of 30 to 50 ng/mL (59)."

The corrected sentence appears below:

"A treatment duration of entire pregnancy and lactation is recommended, with the aim to target concentrations of 30 to 50 ng/mL (59)."

The authors apologize for this error and state that this does not change the scientific conclusions of the article in any way. The original article has been updated.

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Gestational diabetes mellitus in previous pregnancy associated with the risk of large for gestational age and macrosomia in the second pregnancy

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Background: Since the implementation of China's new birth policy, the incidence of large for gestational age (LGA) and macrosomia associated with gestational diabetes mellitus (GDM) has increased. It remains unclear whether a history of GDM in a previous pregnancy raises the risk of LGA or macrosomia in Chinese women planning two or more pregnancies.

Aim: To analyze the association between previous GDM and the risk of LGA and macrosomia in second pregnancy.

Method: A retrospective study was conducted on a cohort of 3,131 women who had experienced two consecutive singleton births. The incidences of LGA and macrosomia in the second pregnancy were compared between women with and without previous GDM. The relationship between previous GDM and the occurrence of LGA and macrosomia was analyzed using multivariate logistic regression and stratified analysis.

Results: The incidence of LGA and macrosomia during the second pregnancy was significantly higher in women with previous GDM (22.67% and 10.25%, respectively) compared to those without prior GDM (15.34% and 5.06%, respectively) ($P < 0.05$). After adjusting for potential confounders, previous GDM was significantly associated with LGA (aOR: 1.511, 95% CI: 1.066-2.143) and macrosomia (aOR: 1.854, 95% CI: 1.118-3.076) in the second pregnancy. Stratified analysis revealed that these associations were present only in women without previous LGA, those with GDM, appropriate gestational weight gain (AGWG), non-advanced maternal age, and male newborns during the second pregnancy ($P < 0.05$). Compared to excessive GWG (EGWG), AGWG correlated with lower risks for LGA and macrosomia during the second pregnancy in women without prior GDM, an association not observed in those with previous GDM. Among women without previous GDM, if the pre-pregnancy BMI is normal, the risk of LGA and macrosomia is significant lower in AGWG

compared with EGWG ($P< 0.001$), while this difference was no significant among women with prior GDM ($P>0.05$).

Conclusion: Previous GDM is strongly linked to LGA and macrosomia in subsequent pregnancies. However, this relationship is influenced by GWG, prior LGA history, fetal sex, and maternal age. Managing weight alone may not sufficiently reduce the risk of LGA or macrosomia for women with a history of GDM.

KEYWORDS

large for gestational age, macrosomia, gestational diabetes mellitus, body mass index, gestational weight gain, multipara

1 Introduction

Large for gestational age (LGA) refers to infants whose birth weight exceeds the 90th percentile for their gestational age and sex, while macrosomia is defined as a birth weight of 4000g or more. In China, the incidence of LGA ranges from 7.4% to 16.8% (1, 2), and macrosomia affects 4.0% to 9.2% of infants (1, 3). Both LGA and macrosomia are associated with elevated risks of emergency cesarean sections, prolonged second stages of labor, shoulder dystocia, birth canal lacerations, and neonatal birth injuries (4, 5). Additionally, they pose potential long-term risks of obesity (6) and diabetes (7). Reducing the incidence of LGA and macrosomia is thus essential for maternal and child health. Known risk factors include gestational diabetes mellitus (GDM) (8), inter-pregnancy weight changes (9, 10), prolonged pregnancy intervals (11), pre-pregnancy overweight or obesity (12, 13), excessive weight gain during pregnancy (12, 14), advanced maternal age (1), multiparity (15), and fetal sex (1).

GDM is a kind of diabetes diagnosed in pregnancy, and its prevalence in China is as high as 14.8% to 16.8% (2, 16). With the increase of multipara and/or advanced pregnancies in China, the risk of GDM also rises. The association between GDM in a previous pregnancy and the risk of LGA in a second pregnancy has been suggested by a 2014 study in the United States (17). However, this particular study did not investigate the risk of macrosomia. Conversely, a recent Chinese study found no significant association between prior GDM and the risk of macrosomia in a second pregnancy, and it also did not examine the LGA risk (18). In September 2020, the growth standard curves of birth weight of Chinese newborns of different gestation was published (19), allowing for more accurate diagnosis of LGA. Thus, it is crucial to investigate the risk factors for LGA and macrosomia using these updated criteria in the Chinese population. A retrospective analysis of clinical data from our center aims to explore the relationship between GDM in a previous pregnancy and the risk of LGA and macrosomia in a subsequent pregnancy.

2 Materials and methods

2.1 Study design and population

This retrospective study comprised pregnant women who delivered two consecutive singletons at Peking University Shenzhen Hospital from January 2002 to March 2024. The inclusion criteria were: both pregnancies reached 28 weeks of gestation or later, involved singleton pregnancies, and maternal age between 18 and 50 years. The exclusion criteria included: stillbirth, fetal malformation in either pregnancy, multiple pregnancies, pregestational diabetes mellitus, and other pregnancy complications such as chronic hypertension, preeclampsia, intrahepatic cholestasis, or severe cardiac or renal disease in the second pregnancy. Cases lacking information on GDM diagnosis, pre-pregnancy body mass index (BMI), weight gain during pregnancy, and newborn birth weight were also excluded. Eligible cases that met both inclusion and exclusion criteria were selected from the hospital's medical records. Participants with two deliveries were matched by name, ID number, and delivery time. Data such as age, height, pre-pregnancy BMI, gestational weight gain, nationality, parity, delivery method, gestational age at delivery, neonatal birth weight, neonatal gender, and GDM status were collected from both the hospital's medical record system and the Shenzhen Maternal and Child Health Care System. This study received approval from the Ethics Committee of Peking University Shenzhen Hospital (No. 2023-103-1).

2.2 Diagnostic criteria and definitions of index

According to IADPSG criteria (20), GDM is diagnosed via a 75g oral glucose tolerance test if any of the following plasma glucose values are met: a fasting plasma glucose level of ≥5.1 mmol/L, or 1-h and 2-h plasma glucose levels of ≥10.0 mmol/L and ≥8.5 mmol/L,

respectively. LGA was defined as a newborn whose birth weight exceeds the 90th percentile for their corresponding gestational age and sex, according to the Growth standard curves of birth weight of Chinese newborns of different gestation (19). Macrosomia is diagnosed if a newborn's birth weight is equal to or greater than 4000g.

Body Mass Index (BMI) is calculated by dividing weight (kg) by height squared (m^2). According to the standard of Chinese population (21), a BMI of less than 18.5 kg/m^2 is classified as underweight, a BMI between 18.5 kg/m^2 and 24 kg/m^2 as normal weight, a BMI between 24 kg/m^2 and 28 kg/m^2 as overweight, and a BMI over 28 kg/m^2 as obese. The inter-pregnancy change of BMI (IPCB) is determined by subtracting the pre-pregnancy BMI of the previous pregnancy from the pre-pregnancy BMI of the subsequent pregnancy. The inter-pregnancy interval (IPI) is the period (in months) between the end of one pregnancy and the start of the next. Gestational weight gain (GWG) is calculated by subtracting pre-pregnancy weight from the weight before delivery. According to the Standard of Recommendation for Weight Gain During Pregnancy (WST801-2022) (22), appropriate GWG (AGWG) is: 11.0 to 16.0 kg for individuals with a pre-pregnancy BMI of less than 18.5 kg/m^2, 8.0 to 14.0 kg for a pre-pregnancy BMI of under 24 kg/m^2, 7.0 to 11.0 kg for a pre-pregnancy BMI of under 28 kg/m^2, and 5.0 to 9.0 kg for those with a pre-pregnancy BMI over 28 kg/m^2. GWG below these ranges is classified as insufficient (IGWG), while values above are deemed excessive (EGWG).

2.3 Statistical analysis

Data were analyzed using SPSS 26.0 statistical software. Categorical variables were presented as [n (%)], and assessed with the chi-squared test. Continuous data were expressed as mean ± SD, and normality was evaluated using the Shapiro-Wilk test. Normally distributed variables were compared using the student's *t*-test, while non-normally distributed variables were reported as median (interquartile range; IQR) and compared using the Mann-Whitney *U* test. Multivariable logistic regression models were employed to explore the association between previous GDM and the incidence of LGA and macrosomia in subsequent pregnancies. Stratified logistic multivariate analysis was conducted to examine the impact of previous GDM on LGA and macrosomia in the second pregnancy across groups divided by previous LGA, GDM, maternal age, sex of the newborn, and gestational weight gain (GWG) in the second pregnancy. A P-value of less than 0.05 was considered statistically significant.

3 Results

3.1 The characteristics of study population

This study included a total of 3,131 pregnant women (Figure 1). In their previous pregnancies, 322 cases (10.28%) had GDM, 313

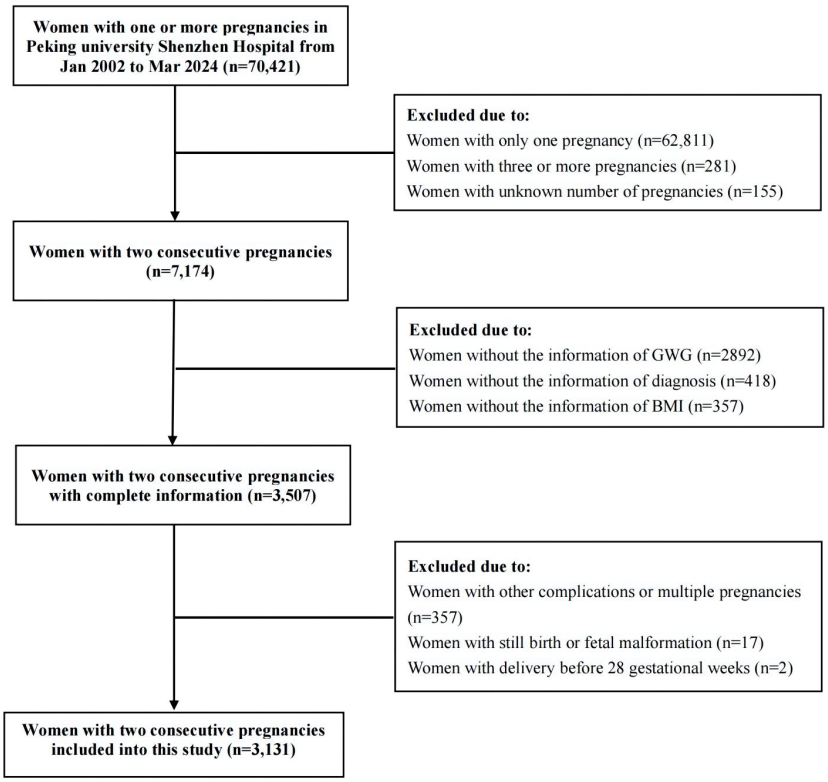

FIGURE 1
Flow chart showing inclusion and exclusion in this study. GWG: gestational weight gain; BMI: body mass index.

cases (10.00%) had LGA, and 135 cases (4.31%) had macrosomia. During their second pregnancies, 501 cases (16.00%) had GDM, 504 cases (16.10%) had LGA, and 175 cases (5.59%) had macrosomia. The average birth weight in the second pregnancy (3304.66 ± 423.57g) was significantly higher than in the previous pregnancy (3237.96±439.22g) (t=6.117, P<0.001). Additionally, the incidence of LGA was significantly higher in the second pregnancy compared to the previous one ($\chi 2$ = 51.352, P < 0.001), as was the incidence of macrosomia ($\chi 2$ = 5.430, P = 0.020). In women who experienced GDM during their first pregnancy, the likelihood of developing GDM in their second pregnancy was markedly higher compared to those who did not have GDM initially (P<0.001). No significant differences were observed in the risk of other complications and comorbidities between the groups (P>0.05) (Supplementary Table S1).

Given that GWG during the second pregnancy is a crucial confounding factor, we analyzed its association with other risk factors, including prior GDM. The GWG of the second pregnancy in women with a history of GDM (12.08 ± 4.35 kg) was significantly lower than that of women without previous GDM (13.38 ± 4.23 kg) (P < 0.001) (Supplementary Table S2). Similarly, women with GDM in the second pregnancy had a lower GWG (11.92 ± 4.19 kg) compared to those without GDM in the second pregnancy (13.50 ± 4.23 kg) (P < 0.001). However, there was no significant difference in GWG during the second pregnancy between groups categorized by previous LGA, advanced pregnancy, or male newborns in the second pregnancy (P > 0.05) (Supplementary Table S2).

The median inter-pregnancy change in BMI (IPCB) was 0.80 kg/m² (ranging from -0.04 kg/m² to 1.90 kg/m²). A total of 1319 cases (42.13%) had a stable IPCB (-1.0 kg/m² to 1.0 kg/m²), 375 cases (11.98%) had an IPCB between 2.0 kg/m² and 3.0 kg/m², and 311 cases (9.93%) had an IPCB greater than 3.0 kg/m². The pre-pregnancy BMI of the subjects with GDM in the second pregnancy was 22.34 ± 3.11 kg/m², significantly higher than that of subjects without GDM in the second pregnancy (21.19 ± 2.76 kg/m²) (t=7.708, P<0.001).

3.2 The risk of LGA and macrosomia in the second pregnancy associated with prior GDM

The incidence of LGA in the second pregnancy for women with prior GDM (22.67%, 73/322) was significantly higher than that in women without previous GDM (15.34%, 431/2809) (χ^2=11.484, P = 0.001) (Figure 2A). Similarly, the incidence of macrosomia in the second pregnancy for women with prior GDM (10.25%, 33/322) was significantly higher compared to women without previous GDM (5.06%, 142/2809) (χ^2=14.765, P<0.001) (Figure 2A). Additionally, the birth weight of babies born to women with prior GDM (3350.09 ± 474.39g) was significantly higher than those born to women without previous GDM (3299.45 ± 417.13g) (t=2.033, P=0.042) (Figure 2B).

3.3 Previous GDM independently contributed to the risk of LGA and macrosomia in the second pregnancy

In the unadjusted analysis, previous GDM, prior LGA, interpregnancy interval (IPI), maternal age, pre-pregnancy BMI, male newborn, GDM, and gestational weight gain (GWG) in the second pregnancy were all significantly associated with LGA in the second pregnancy (P<0.05) (Table 1), while nationality and IPCB were not significantly associated with LGA (Supplementary Table S3). Furthermore, previous GDM and prior LGA, IPCB, GDM, pre-pregnancy BMI, male newborn, and GWG in the second pregnancy

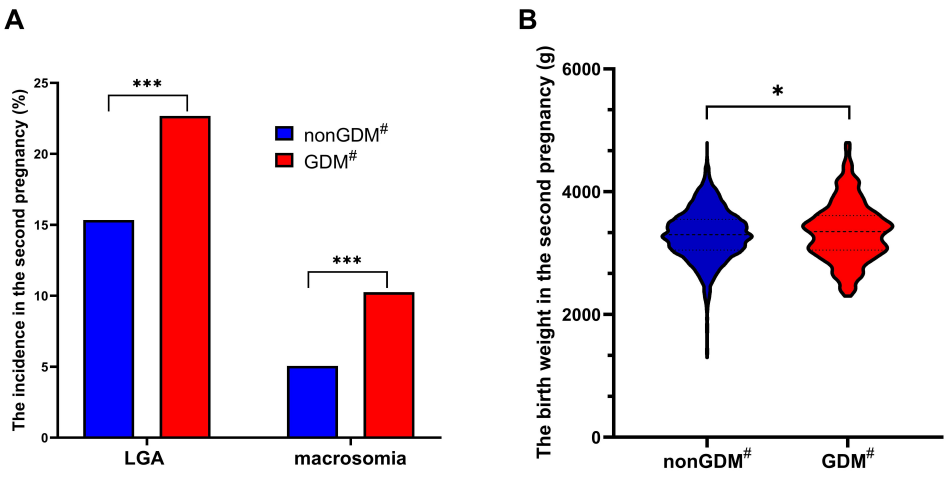

FIGURE 2
Comparison of the incidence of LGA and macrosomia and the birth weight in the second pregnancy in different groups. The incidence of LGA and macrosomia significantly increased in women with previous GDM compared with those without previous GDM **(A)**; The birth weight of second pregnancy in women with previous GDM was significantly higher than that in women without previous GDM **(B)**; GDM, gestational diabetes mellitus; LGA, large for gestational age; *P<0.05; ***P<0.001; #in previous pregnancy.

TABLE 1 Impact of previous GDM and other risk factors on LGA in subsequent pregnancy.

Risk factors	Non-adjusted			Adjusted*		
	OR	*95% CI for OR*	*P*	*OR*	*95% CI for OR*	*P*
GDM in previous pregnancy	**1.618**	**1.222-2.141**	**0.001**	**1.511**	**1.066-2.143**	**0.021**
Male newborn in the second pregnancy	**1.273**	**1.049-1.544**	**0.014**	**1.282**	**1.035-1.589**	**0.023**
LGA in previous pregnancy	**7.167**	**5.590-9.188**	**<0.001**	**6.318**	**4.818-8.285**	**<0.001**
Pre-pregnancy BMI in the second pregnancy	**1.150**	**1.114-1.187**	**<0.001**	**1.130**	**1.084-1.178**	**<0.001**
GWG in the second pregnancy	**1.067**	**1.044-1.091**	**<0.001**	**1.091**	**1.064-1.119**	**<0.001**
IPI	**1.004**	**1.001-1.007**	**0.011**	1.002	0.997-1.006	0.478
Maternal age in the second pregnancy	**1.042**	**1.016-1.070**	**0.002**	1.020	0.985-1.055	0.266
GDM in the second pregnancy	**1.374**	**1.077-1.753**	**0.010**	1.029	0.759-1.395	0.853

GDM, gestational diabetes mellitus; LGA, large for gestational age; IPI, inter-pregnancy interval; GWG, gestational weight gain; *adjusted factors: previous GDM, nationality, previous LGA, IPI, inter-pregnancy change of body mass index, maternal age in the second pregnancy, GDM in the second pregnancy, pre-pregnancy BMI in the second pregnancy, male newborn in the second pregnancy, GWG in the second pregnancy. Numbers with statistical significance were marked in bold.

were significantly linked to macrosomia in the second pregnancy ($P<0.05$) (Table 2), while nationality, IPI and maternal age were not significantly associated with macrosomia (Supplementary Table S4).

After adjusting for potential confounding factors using logistic multivariate regression, previous GDM, LGA, pre-pregnancy BMI, male newborn, and GWG in the second pregnancy were significantly associated with LGA in the second pregnancy ($P<0.05$) (Table 1). Collinearity analysis showed that there was no multicollinearity effect between these factors (Supplementary Table S5). However, the significant associations of IPI, maternal age and GDM in the second pregnancy with LGA in the second pregnancy were lost in the multivariate regression analysis (Table 1). The three-step analysis showed that maternal age in the second pregnancy was a mediator of the association between IPI and LGA (Supplementary Table S6, Supplementary Figure S1). Moreover, GDM in the first pregnancy confounded the association between GDM in the second pregnancy and LGA (Supplementary Table S7, Supplementary Figure S2).

Previous GDM, LGA, pre-pregnancy BMI, male newborn, and GWG in the second pregnancy were also significantly associated with macrosomia in the second pregnancy in logistic multivariate regression ($P<0.05$) (Table 2). However, the significant associations of IPCB and GDM in the second pregnancy with macrosomia in the second pregnancy were lost in the multivariate regression analysis (Table 2). The three-step analysis showed that pre-pregnancy BMI in the second pregnancy was a mediator of the association between IPCB and macrosomia (Supplementary Table S8, Supplementary Figure S3). Moreover, GDM in the first pregnancy confounded the association between GDM in the second pregnancy and macrosomia (Supplementary Table S9, Supplementary Figure S4).

3.4 The association between previous GDM and the occurrence of LGA and macrosomia varied in different populations

In a stratified logistic multivariate analysis, previous GDM was independently associated with an increased risk of LGA in the second pregnancy among women without prior LGA, with GDM, appropriate GWG, non-advanced pregnancy, and male newborns

TABLE 2 Impact of previous GDM and other risk factors on macrosomia in subsequent pregnancy.

Risk factors	Non-adjusted			Adjusted*		
	OR	*95% CI for OR*	*P*	*OR*	*95% CI for OR*	*P*
GDM in previous pregnancy	**2.145**	**1.441-3.192**	**<0.001**	**1.854**	**1.118-3.076**	**0.017**
LGA in previous pregnancy	**7.235**	**5.200-10.066**	**<0.001**	**5.616**	**3.857-8.177**	**<0.001**
Pre-pregnancy BMI in the second pregnancy	**1.186**	**1.134-1.241**	**<0.001**	**1.163**	**1.095-1.234**	**<0.001**
Male newborn in the second pregnancy	**2.510**	**1.779-3.541**	**<0.001**	**2.427**	**1.679-3.51**	**<0.001**
GWG in the second pregnancy	**1.112**	**1.075-1.151**	**<0.001**	**1.137**	**1.095-1.181**	**<0.001**
IPCB	**1.095**	**1.015-1.180**	**0.018**	1.022	0.936-1.117	0.627
GDM in the second pregnancy	**1.718**	**1.197-2.465**	**0.003**	1.236	0.787-1.943	0.358

GDM, gestational diabetes mellitus; LGA, large for gestational age; IPCB, inter-pregnancy change of body mass index; GWG, gestational weight gain; *adjusted factors: previous GDM, nationality, previous LGA, inter-pregnancy interval, IPCB, maternal age in the second pregnancy, GDM in the second pregnancy, pre-pregnancy BMI in the second pregnancy, male newborn in the second pregnancy, GWG in the second pregnancy. Numbers with statistical significance were marked in bold.

TABLE 3 Stratified multivariate logistic analysis of previous GDM for LGA and macrosomia in the second pregnancy.

Subgroups for analysis	Effect of previous GDM on LGA in the second pregnancy			Effect of previous GDM on macrosomia in the second pregnancy		
	aOR*	95% CI*	P for interaction	aOR*	95% CI*	P for interaction
without LGA in previous pregnancy (n=2818)	**1.738**	**1.179-2.562**	0.158	**2.299**	**1.235-4.280**	0.327
with LGA in previous pregnancy (n=313)	0.978	0.457-2.091		1.376	0.574-3.301	
without GDM in the second pregnancy (n=2630)	1.375	0.849-2.225	0.602	1.199	0.549-2.617	0.115
with GDM in the second pregnancy (n=501)	**1.789**	**1.055-3.034**		**2.769**	**1.298-5.907**	
insufficient GWG in the second pregnancy (n=316)	1.052	0.323-3.421	0.195	1.260	0.092-17.258	0.204
appropriate GWG in the second pregnancy (n=1377)	**1.926**	**1.077-3.444**		**3.198**	**1.199-8.525**	
excessive GWG in the second pregnancy (n=1438)	1.448	0.894-2.345		1.626	0.875-3.018	
maternal age less than 35 years in the second pregnancy (n=2214)	**1.799**	**1.169-2.769**	0.081	**2.067**	**1.118-3.823**	0.799
maternal age ≥ 35 years in the second pregnancy (n=917)	1.153	0.629-2.114		1.509	0.600-3.793	
male newborn in the second pregnancy (n=1689)	**1.626**	**1.026-2.578**	0.690	**2.438**	**1.347-4.413**	0.738
female newborn in the second pregnancy (n=1442)	1.409	0.818-2.425		1.122	0.402-3.134	

GDM, gestational diabetes mellitus; LGA, large for gestational age; GWG, gestational weight gain; aOR, adjusted odds ratio; *adjusted factors: previous GDM, nationality, previous LGA, IPI, IPCB, maternal age in the second pregnancy, GDM in the second pregnancy, pre-pregnancy BMI in the second pregnancy, male newborn in the second pregnancy, GWG in the second pregnancy. Numbers with statistical significance were marked in bold.

($P < 0.05$) (Table 3). The adjusted OR values for these subjects (aOR: 1.738, 1.789, 1.926, 1.799, and 1.626) were all higher than that for the overall population (aOR: 1.511). Similarly, previous GDM was independently linked to a heightened risk of macrosomia in the same cohort ($P < 0.05$) (Table 3). The adjusted ORs for these subjects (aOR: 2.299, 2.769, 3.198, 2.067, and 2.438) also exceeded those of the overall population (aOR: 1.854). However, among women with previous LGA, EGWG, advanced pregnancy, and female newborns in the second pregnancy, no significant association was found between previous GDM and LGA or macrosomia (Table 3). In women without GDM in the second pregnancy, who had significantly higher GWG compared to those GDM women (Supplementary Table S2), previous GDM was not significantly associated with the risk of LGA or macrosomia ($P > 0.05$) (Table 3). Moreover, no significant interaction between stratification factors and GDM in the first pregnancy was found in the interaction analysis ($P > 0.05$) (Table 3).

3.5 The impact of GWG on LGA and macrosomia is influenced by prior GDM

In women without prior GDM, appropriate gestational weight gain (AGWG) was linked to lower risks of LGA and macrosomia in the second pregnancy when compared to excessive gestational weight gain (EGWG) in logistic multivariate analysis (Figure 3). Further stratified analyses indicated that the risk of LGA and macrosomia was significantly reduced in AGWG compared with

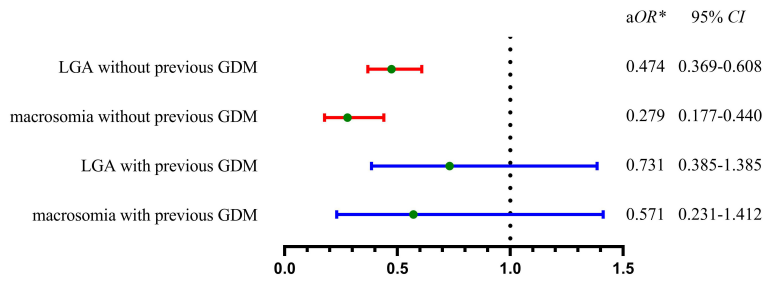

FIGURE 3
Adjusted odds ratios of AGWG versus EGWG for the risk of LGA and macrosomia in the second pregnancy. In women without previous GDM, AGWG owned significantly lower risk of LGA or macrosomia when compared with EGWG (red line). In women with previous GDM, there was no significant difference of the risk of LGA and macrosomia between AGWG and EGWG (blue line). GDM, gestational diabetes mellitus; LGA, large for gestational age; AGWG, appropriate gestational weight gain; EGWG, excessive gestational weight gain; aOR, adjusted odds ratio; *adjusted by nationality, previous LGA, IPI, IPCB, maternal age in the second pregnancy, GDM in the second pregnancy, pre-pregnancy BMI in the second pregnancy, male newborn in the second pregnancy, GWG in the second pregnancy.

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TABLE 4 Stratified multivariate logistic analysis of previous GDM for LGA and macrosomia in the second pregnancy.

	LGA			Macrosomia		
	n (%)	χ^2	*P*	n (%)	χ^2	*P*
GDM[1]+UW+AGWG[2] (n=20)	2(10.00)	–	0.437*	1(5.00)	–	–
GDM[1]+UW+EGWG[2] (n=4)	1(25.00)			0(0.00)		
GDM[1]+NW+AGWG[2] (n=92)	13(14.13)	2.007	0.157	5(5.43)	1.938	0.164
GDM[1]+NW+EGWG[2] (n=90)	20(22.22)			10(11.11)		
GDM[1]+OB+AGWG[2] (n=20)	10(50.00)	0.033	0.855	4(20.00)	0.682	0.409
GDM[1]+OB+EGWG[2] (n=40)	21(52.50)			12(30.00)		
non-GDM[1]+UW+AGWG[2] (n=194)	13(6.70)	3.150	0.076	1(0.52)	–	0.059*
non-GDM[1]+UW+EGWG[2] (n=110)	14(12.73)			4(3.64)		
non-GDM[1]+NW+AGWG[2] (n=936)	98(10.47)	**30.924**	**<0.001**	19(2.03)	**37.569**	**<0.001**
non-GDM[1]+NW+EGWG[2] (n=913)	180(19.72)			76(8.32)		
non-GDM[1]+OB+AGWG[2] (n=115)	22(19.13)	2.746	0.098	9(7.83)	0.441	0.507
non-GDM[1]+OB+EGWG[2] (n=281)	76(27.05)			28(9.96)		

GDM, gestational diabetes mellitus; LGA, large for gestational age; UW, underweight before the second pregnancy; NW, normal weight before the second pregnancy; OB, overweight or obese before the second pregnancy; AGWG, appropriate gestational weight gain; EGWG, excessive gestational weight gain; [1]in the first pregnancy; [2]in the second pregnancy; * Fisher's precision probability test. Numbers with statistical significance were marked in bold.

EGWG in the normal weight group before the second pregnancy, while this difference was not significant in the underweight, overweight, or obese groups (Table 4).

Conversely, for women with a history of GDM, the risk of LGA or macrosomia showed no significant difference whether gestational weight gain was appropriate or excessive (Figure 3). Further stratified analysis suggested that no significant difference in the risk of LGA and macrosomia between AGWG and EGWG, regardless of pre-pregnancy BMI classification (underweight, normal, overweight, or obese) (Table 4).

4 Discussion

This study indicates that previous gestational diabetes mellitus (GDM) is linked to a higher risk of subsequent large for gestational age (LGA) and macrosomia. This relationship was observed in newborns of mothers who did not previously deliver LGA babies, were younger, gained appropriate weight during pregnancy, and had male newborns. Additionally, a history of GDM may hinder a pregnant woman's ability to mitigate the risk of excessive fetal growth by controlling gestational weight gain (GWG). Over the past decade, the risk of LGA among Chinese women with GDM has remained relatively high, emphasizing the need to identify risk factors and implement effective intervention strategies (2). In the context of China's new birth policy, the findings of this study underscore the clinical importance of managing GDM in a previous pregnancy to reduce the risk of LGA and macrosomia in subsequent pregnancies.

A previous report from the United States indicated that a history of GDM increases the risk of LGA in subsequent pregnancies (17). However, a recent multicenter study in China did not find this association (18). This study suggested that the lack of association might be due to effective GDM control (18). Considering the recent reports on the birth weight curve (19) and gestational weight gain standards (22) for the Chinese population, there is a growing need to explore the relationship between GDM, LGA, macrosomia, and GWG in this demographic. The impact of gestational diabetes mellitus (GDM) in a prior pregnancy on large-for-gestational-age (LGA) infants in a subsequent pregnancy may be associated with post-pregnancy insulin resistance. Compared to women without a history of GDM, those with such a history exhibit lower insulin sensitivity and impaired β-cell function, leading to subclinical hyperglycemia in their second pregnancy (23). Insulin resistance during the second trimester is linked to an increased risk of LGA, independent of maternal obesity or blood glucose levels (24). Lin et al. (25) proposed that GDM, combined with insulin resistance, heightens the risk of LGA. Furthermore, increased insulin resistance during pregnancy has been correlated with excessive weight gain, macrosomia, and LGA in Chinese women with GDM (26).

Univariate analysis initially indicated an association between IPI, maternal age, GDM in the second pregnancy with LGA in the second pregnancy. However, these relationships were not supported by multivariate analysis. Collinearity analysis confirmed the absence of multicollinearity among these variables. Notably, IPI showed a strong positive correlation with maternal age in the second pregnancy, as revealed by the three-step method. When considering maternal age as a mediator, IPI was not independently linked to LGA in the second pregnancy. Similarly, GDM in the second pregnancy, initially significant in univariate analysis, lost its association with LGA and macrosomia in multivariate analysis, likely due to the confounding effect of GDM in the first pregnancy, which significantly influenced GDM, LGA,

and macrosomia in the second pregnancy. It is reported that the effect of pre-pregnancy overweight/obesity on the macrosomia and LGA was partly mediated by GDM (3). These findings underscore the necessity of accounting for interactions among risk factors when examining their influence on LGA in subsequent pregnancies.

In women without a history of LGA delivery, previous GDM is linked to a heightened risk of LGA and macrosomia in subsequent pregnancy. Compared to the general population, this risk is particularly higher in these women (aOR 1.738 vs. 1.511). Conversely, no such correlation is found in women with a history of LGA. This could be attributed to the fact that a history of LGA is a significant risk factor for LGA in future pregnancies (27), where the OR values for LGA and macrosomia in subsequent pregnancy are 6.318 and 5.616, respectively. The influence of GDM might be diminished by the prior LGA, rendering it non-significant. This indicates that GDM's impact may fluctuate based on the presence or absence of a history of LGA. Women without a history of LGA delivery often represent the majority and are generally perceived to have a lower risk of LGA, yet GDM can still pose significant adverse effects.

A history of GDM significantly increased the risk of LGA and macrosomia in younger women (<35 years), while this association was not observed in advanced pregnancies. According to the multivariate analysis (Tables 1, 2), the age of the second pregnancy was not an independent risk factor for LGA or macrosomia. However, studies have reported that advanced maternal age (1) or maternal age ≥30 years (28) are high risk factors for LGA and macrosomia. Another research indicates that birth weight and macrosomia increase with maternal age, with age 34 being the turning point, and the risk of low birth weight rises after age 36 (29). Animal studies suggest that placental dysfunction may cause an increased risk of fetal growth restriction in older pregnancies (30). Therefore, the effect of GDM history on excessive fetal growth may be weakened in older pregnant women. An early onset of diabetes significantly increases the risk of developing chronic complications and long-term adverse outcomes (31).

Prior GDM makes male fetuses more prone to LGA or macrosomia, unaffected by factors related to female fetuses. Since the sex of the fetus occurs randomly, it is not correlated with either GWG or LGA history. The heightened susceptibility of male fetuses to GDM-associated overgrowth compared to female fetuses may be attributed to sex differences in insulin-like growth factors (32). This is supported by the higher average birth weight of male fetuses compared to female fetuses and their greater propensity for LGA or macrosomia (33). Additionally, sex-specific extracellular miRNA have been linked to fetal growth and development (34). In female fetuses, levels of leptin (35) and the β-cell function index (HOMO-β) (36) in cord blood are higher than in male fetuses, warranting further investigation into their potential connection to LGA risk.

For women with GDM in their second pregnancy, the risk of LGA and macrosomia was significantly associated with a prior history of GDM. However, this association was not significant in women without GDM in their second pregnancy. Recurrent GDM is linked to obesity and insulin resistance (37), which explains the elevated risk of LGA and macrosomia. In women whose second pregnancy was free of GDM, metabolic disorders may have been corrected, rendering the history of GDM insignificant. Surprisingly, the overall multivariate analysis did not show a significant association between GDM in the second pregnancy and LGA or macrosomia ($P > 0.05$). We believe this outcome may be influenced by reverse causality, as women with GDM had significantly lower GWG compared to those without GDM in subsequent pregnancies (11.92 ± 4.19 kg vs 13.50 ± 4.23 kg) (Supplementary Table S2). A reduced GWG might protect pregnant women with GDM during their second pregnancy from LGA and macrosomia (38).

The results from stratified analyses suggest that the link between a history of GDM and LGA in the second pregnancy may be confined to specific subgroups. However, this association could also be influenced by the smaller sample sizes within these subgroups, as no significant interaction was found between stratification factors and GDM ($P > 0.05$). Another study from China also suggests that there was no significant interaction between GDM subtypes and pre-BMI for LGA (39). Expanding the sample size in future follow-up studies would help clarify the current study's findings. Additionally, the wider 95% confidence intervals observed in these analyses could also be a result of reduced sample sizes after stratification. The variability in the study population and insufficient adjustment for confounding factors might further explain these wide confidence intervals, potentially leading to lower statistical power that obscures significant associations. Consequently, future research should consider multi-center studies with larger samples, incorporating factors such as diet, exercise, and lipid levels, to provide a more comprehensive understanding of the risk factors involved.

Gestational weight gain (GWG) is a significant risk factor for LGA and macrosomia across all BMI categories, especially in overweight and obese women (40). Appropriate gestational weight gain is known to reduce the risk of LGA in women with GDM and obesity (41). Conversely, excessive gestational weight gain (EGWG) increased the risk of LGA (42, 43). In our stratified analyses, a history of GDM was significantly associated with the risk of LGA and macrosomia in the appropriate gestational weight gain (AGWG) group, but not in the EGWG group. The negative outcomes in women with EGWG during their second pregnancy might stem from EGWG obscuring the influence of a previous GDM history on the incidence of LGA and macrosomia.

To reduce the risk of adverse pregnancy outcomes such as LGA and macrosomia, diet (44) and exercise (45) therapy are recommended in clinical practice for controlling gestational weight. However, our study indicates that a history of GDM may influence the effectiveness of weight management. In pregnant women with prior GDM, regardless of their BMI classification before the second pregnancy, the risk of LGA or macrosomia remains significant even if GWG is within the appropriate range. Conversely, in the absence of a GDM history and with a pre-pregnancy BMI within the normal range, maintaining GWG within the recommended limits can significantly reduce the risk of LGA and macrosomia. In overweight or underweight pregnant women with AGWG, the incidence of LGA decreased significantly (from 27.05% to 19.13% and from 12.73% to 6.70%, respectively). However, this reduction is not statistically significant due to the small sample size. This finding suggests that managing GWG to

mitigate the risk of excessive fetal growth may be challenging in women with a history of GDM, while it may be more straightforward for those without GDM. A history of GDM is not only linked to an increased risk of LGA and complications in subsequent pregnancies but also affects the efficacy of weight management in mitigating these risks.

Preventing macrosomia involves the early detection of excessive fetal growth and its risk factors. Research suggests that fetal overgrowth related to GDM can be identified as early as 20 weeks of gestation (46). Additionally, blood glucose levels measured between 10 and 14 weeks show a positive correlation with estimated fetal weight from 23 weeks onward, becoming significant by 27 weeks (46). Measurements of fetal abdominal circumference and estimated fetal weight (EFW) at 19-21 weeks' gestation are considered indicative of GDM in women with specific risk factors, such as a history of gestational diabetes, a pre-pregnancy BMI of 30 kg/m² or higher, or fasting plasma glucose levels between 5.6 and 6.9 mmol/L at the initial prenatal visit (47). Even before a formal GDM diagnosis, the fetus may exhibit accelerated growth directly linked to maternal hyperglycemia (48). Italian guidelines advise GDM screening for these high-risk women between 16 and 18 weeks of gestation to enable timely intervention and risk control for macrosomia (49). Compared to high-risk pregnant women screened for GDM at 24-28 weeks, those screened earlier at 16-18 weeks show smaller fetal abdominal circumferences and estimated weights (50). Furthermore, numerous maternal biological indicators have been proposed as predictors of macrosomia; however, their efficacy in early prediction requires further investigation (51). Certain differential species of maternal gut microbiota in early pregnancy may serve as potential predictors for preventing macrosomia (52). Therefore, for women with a history of GDM, enhanced monitoring of fetal or maternal markers early in the second trimester and earlier GDM screening can aid in identifying fetal overgrowth promptly, allowing for proactive strategies to minimize the incidence of macrosomia and LGA.

There are some limitations in this study. First, this single-center retrospective study spanned over 20 years, and some early cases were excluded due to a lack of GWG or pre-pregnancy BMI data, potentially introducing selection bias. Second, information on diet, exercise, and lipid profiles of the cases was not collected, and the influence of these confounding factors cannot be ruled out. Nevertheless, over 40% of cases showed a stable weight range (± 1kg/m²) between pregnancies, and less than 10% had an IPCB of more than 3 units, suggesting minimal changes in body weight and its related factors. Third, in the stratified analysis, some subgroups had insufficient sample sizes, affecting statistical power and potentially concealing differences. Increasing the sample size is necessary for further exploration. Fourth, the impact of a history of GDM on the association between GWG and the risk of LGA and macrosomia is based solely on retrospective observational data and requires confirmation through prospective intervention studies.

In conclusion, GDM in previous pregnancy is an independent risk factor for LGA and macrosomia in subsequent pregnancies, as indicated by this study. However, this relationship is influenced by factors such as GWG, prior LGA history, fetal sex, and maternal age. Managing weight alone may not sufficiently lower the risk of LGA or macrosomia in women with a history of GDM. Following the new birth policy in China, the proportion of multipara and advanced pregnancy has increased, leading to a higher incidence of GDM, LGA, and macrosomia. The study's findings indicate a critical time window for controlling the risks of LGA and macrosomia. Mitigating the risk of GDM in a previous pregnancy can reduce the likelihood of LGA and macrosomia in subsequent pregnancies. Given the limitations of this single-center, retrospective study, a prospective multicenter study is necessary to verify these results further.

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 Research Ethics Committee of Peking University Shenzhen Hospital. 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 was a large retrospective study involving 3,131 women. Obtaining written informed consent from all participants would have prevented the study from being conducted. In addition, since this study is based on group data statistics, no individual's private information will be disclosed, so the necessity of obtaining written informed consent from the participants is small. Waiver of written informed consent was approved by the ethics committee.

Author contributions

YW: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. JY: Data curation, Formal analysis, Writing – original draft, Writing – review & editing. YL: Data curation, Writing – original draft, Software, Writing – review & editing. AY: Supervision, Writing – review & editing. YD: Supervision, Writing – review & editing. CX: Methodology, Writing – review & editing. SZ: Formal analysis, Writing – original draft, Conceptualization, Data curation, Funding acquisition, Investigation, Methodology, Project administration, Supervision, Writing – review & editing, Resources, Software, 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.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2025.1474694/full#supplementary-material

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Antagonistic effects of smoking and maternal glycemia on fetal growth: a retrospective study among 13,958 pregnant French women

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Introduction: Smoking and hyperglycemia first diagnosed during pregnancy (H1inP) have opposing effects on fetal growth. The aim of this study was to explore adverse pregnancy outcomes, particularly fetal growth, according to the smoking and H1inP status.

Methods: We included 13,958 women from a large French dataset (2012–2018). Using multivariable regression analyses, we retrospectively evaluated the risk of large-for-gestational-age (LGA) babies and other adverse outcomes according to the H1inP and smoking status in four groups: no H1inP/non-smoker (group A: n = 10,454, 88.2%), no H1inP/smoker (group B: n = 819, 5.9%), H1inP/non-smoker (group C: n = 2,570, 18.4%), and H1inP/smoker (group D: n = 115, 0.8%).

Results: The rates of LGA were 8.9%, 4.0%, 14.6%, and 8.7% in groups A, B, C, and D, respectively (global ANOVA $p < 0.0001$, factor H1inP $p = 0.0003$, factor smoking $p = 0.0002$, and interaction $p = 0.48$). After adjustment for potential confounders including age, body mass index, employment, ethnicity, parity, hypertension before pregnancy, gestational weight gain, and alcohol and drug consumption, H1inP was associated with a higher risk [odds ratio (OR) = 1.50, 95% confidence interval (95%CI) = 1.30–1.74] and smoking with a lower risk (OR = 0.35, 95%CI = 0.25–0.50) of LGA. In addition, H1inP was associated with a lower total gestational weight gain and a lower rate of small-for-gestational-age (SGA) babies, but higher rates of hypertensive disorders and more frequent caesarean sections and admissions in the neonatal

intensive care unit. Smoking was associated with higher rates of SGA, including severe SGA (<3rd centile), and this despite a higher total gestational weight gain. Smoking increased the risk of hypertensive disorders only in women with H1inP.

Discussion: Smoking among women with H1inP could mask the risk of maternal hyperglycemia for LGA babies. This could provide a false sense of security for women with H1inP who smoke, particularly when assessing for LGA alone, but these women still face other risks to their health, such as hypertensive disorders and the health of the fetus.

KEYWORDS

birthweight, cigarettes, diabetes in pregnancy, gestational diabetes mellitus, hyperglycemia in pregnancy, pregnancy outcomes, smoking, tobacco

Introduction

Tobacco use is the main preventable cause of adverse perinatal outcomes, including fetal restriction and small-for-gestational-age (SGA) babies, preterm birth, congenital malformations, and fetal loss (1). These complications are likely driven by placental dysfunction through nicotine and toxin exposure, hypoxia, oxidative stress, and epigenetic modifications (1–4).

Hyperglycemia first detected in pregnancy (H1inP) represents one of the most frequent pregnancy complications (5–8). Despite care, H1inP remains associated with several adverse neonatal and maternal outcomes (5, 6, 9). One of the main adverse outcomes is having large-for-gestational-age (LGA) babies, which in turn increases the risk of shoulder dystocia, fetal distress, and the need for urgent caesarean delivery. Fetal overgrowth during H1inP is mainly related to uncontrolled high glucose levels (5–8). Preterm delivery, neonatal hypoglycemia, and higher rates of admissions in the neonatal intensive care unit (NICU), as well as higher rates of maternal hypertensive disorders, could also reflect a poor glycemic control in the context of H1inP (5–9).

Despite careful prenatal management and smoking cessation assistance, a significant number of pregnant women with H1inP continue to smoke tobacco (10). In these women, we hypothesized that smoking and H1inP could have i) opposing effects on fetal growth, but ii) distinct and even synergistic combined effects on other adverse perinatal outcomes. Indeed, a normal fetal growth in women with H1inP who smoke could falsely reassure caregivers about the impact of glucose control and the risk of other H1inP-related adverse outcomes. Reciprocally, a normal fetal growth in smokers due to H1inP could mask fetal growth restriction and placental dysfunction. In this context, we explored these outcomes in a large French dataset according to the smoking and H1inP status.

Abbreviations: 1h-PG, plasma glucose 1 h after oral glucose tolerance test; 2h-PG, plasma glucose 2 h after oral glucose tolerance test; BMI, body mass index; FPG, fasting plasma glucose; GWG, gestational weight gain; H1inP, hyperglycemia first diagnosed in pregnancy; LGA, large-for-gestational-age; NICU, neonatal intensive care unit; OGTT, oral glucose tolerance test; SD, standard deviation; SGA, small-for-gestational-age.

Materials and methods

Our cohort

This observational cohort study was conducted at the Jean Verdier University Hospital in Bondy, a suburb of Paris, France. According to French law (31/07/1991, *programme de médicalisation des systèmes d'information*), healthcare establishments shall carry out a medical assessment and analysis of their activities. Thus, perinatal data are routinely and prospectively registered at birth for all women giving birth at the university hospital by the midwife assisting the delivery, and then checked and collected during the maternity stay by a midwife qualified in data management and storage. At our perinatal center, all patients are informed during their first prenatal visit that their medical records may be used for the assessment and improvement of our procedures, unless they oppose. Analyses were based on data from the hospital's routine electronic medical records of outcomes during pregnancy and at birth, which occurred between January 2012 and December 2018 (11–16). All data were analyzed anonymously. Our database is registered in the French Committee for computerized data (Commission Nationale de l'Informatique et des Libertés, no. 1704392v0).

Selection criteria for the present study sample

The inclusion criteria for the women comprising the present study sample were as follows: delivery between January 2012 and December 2018; age of at least 18 years; no known diabetes before pregnancy; a single fetus pregnancy; no history of bariatric surgery; a known smoking status at the beginning of prenatal care, with the exclusion of women having begun to smoke during pregnancy; and a known H1inP status (Figure 1).

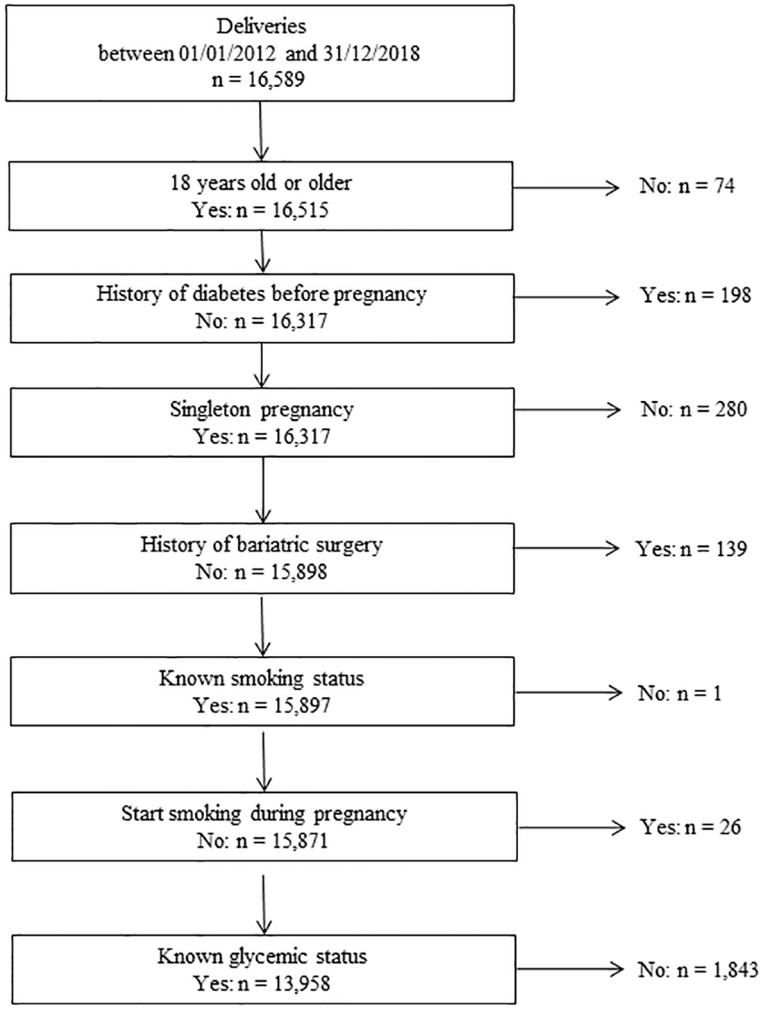

FIGURE 1
Flowchart of the study.

H1inP screening and care

The French recommendations for H1inP screening, diagnostic criteria, and care (6) were followed, except that universal screening was preferred over selective screening given the high prevalence of risk factors in our hospital population (14). Screening was performed at the beginning of pregnancy and between 24 and 28 weeks of gestation (WG) if initial screening was not performed or provided a normal result. Early screening was based on a fasting plasma glucose (FPG) measurement. Women with a FPG level ≥5.1 mmol/L were promptly provided care for H1inP. Women not diagnosed early with H1inP underwent an oral glucose tolerance test (OGTT) between 24 and 28 WG, where the FPG and the plasma glucose 1 h (1h-PG) and 2 h after OGTT (2h-PG) were measured (12). The International Association of Diabetes Pregnancy Study Group/World Health Organization (5, 7) recommendations were used to diagnose H1inP in accordance with the French regulations. H1inP was defined as a FPG ≥5.1 mmol/L and/or 1h-PG ≥10.0 mmol/L and/or 2h-PG ≥8.5 mmol/L (17).

All women diagnosed with H1inP were referred to our multidisciplinary team, which comprises a diabetologist, an obstetrician, a midwife, a dietician, and a diabetes nurse educator. Care was provided in accordance with the French recommendations. Specifically, our team provided individually tailored dietary advice and instructions to pregnant women on how to perform self-monitoring of their blood glucose levels six times a day (17). Women received insulin therapy when the pre-prandial and/or 2-h post-prandial capillary glucose levels were greater than 5.3 and 6.7 mmol/L, respectively, during follow-up. The obstetrical care provided also followed French recommendations (6).

Data collection

Smoking status was self-reported and classified into two categories: “non-smokers” were those women who did not smoke at conception and those who ceased smoking because of the current pregnancy; “smokers” were those who continued smoking during

pregnancy (10). The body mass index (BMI) was calculated according to the self-reported weight before pregnancy and the height measured during pregnancy. Ethnicity was self-reported as European, North African, Sub-Saharan African, Indian–Pakistani–Sri Lankan, Caribbean, or other. Data on the consumption of alcohol and recreation substances during pregnancy were self-reported.

Outcomes

The following sets of outcomes were considered: termed "neonatal" and "maternal" perinatal outcomes by the INSPIRED research group (8). The primary outcome was LGA (>90th percentile) infant (18). The secondary neonatal outcomes included birth weight, SGA (<10th percentile) and severe SGA (<10th percentile) and babies (18), gestational age at birth and preterm delivery (any birth occurring after 22 WG and before 37 WG), and admissions in the NICU. The following exploratory outcomes (far less frequent than the former outcomes) were also considered: shoulder dystocia (defined as the use of obstetrical maneuvers: McRoberts episiotomy after delivery of the fetal head, suprapubic pressure, posterior arm rotation to an oblique angle, rotation of the infant by 180°C, and delivery of the posterior arm) (19); neonatal hypoglycemia (at least one blood glucose measurement under 2.5 mmol/L during the first 2 days of life); fetal or neonatal death (i.e., in the first 24 h of life) or stillbirth; and any birth malformations (11–16).

The secondary maternal outcomes included gestational weight gain (GWG; i.e., the weight measured before delivery minus the self-reported pre-pregnancy weight); insulin therapy for H1inP (as this is the only pharmacological therapy permitted in France); mode of birth, including induced delivery and unscheduled (before the scheduled date or during ongoing delivery) cesarean section; and hypertensive disorders (e.g., chronic hypertension, pregnancy-induced hypertension, and/or preeclampsia). The definitions of these events have been provided in previous publications (11–16).

Statistical analyses

Continuous variables were expressed as mean ± standard deviation (SD). Categorical variables were expressed as frequencies and percentages. No data replacement procedure was used for missing data. ANOVA was used to compare continuous variables, while the chi-squared (χ^2) test or Fisher's exact test was used as appropriate to compare categorical variables.

With regard to the characteristics of the included women (Table 1), the global difference between the four groups was first examined using a global one-way ANOVA; if a significant difference was found, a two-factor ANOVA was used to analyze more specifically potential differences related to the factors H1inP status (factor H1inP), smoking status (factor smoking), and their interaction (H1inP–smoking interaction).

The rates of adverse pregnancy outcomes were compared according to the H1inP and smoking status (Figures 2, 3, Table 2).

The effects of H1inP and smoking on the primary outcome (i.e., LGA babies) were also explored using multivariable logistic regression analyses adjusted for the following confounders: age, employment, ethnicity, parity, pre-pregnancy BMI, and hypertension before pregnancy in model 1; the same variables as in model 1 + gestational weight gain in model 2; the same variables as in model 2 + alcohol and recreational substance consumption in model 3; and the same variables as in model 3 + history of macrosomic infant in model 4 (Table 3).

All tests were two-sided. Analyses were conducted using SAS 9.4 software (SAS Institute Inc., Cary, NC, USA).

Results

Study population characteristics

As shown in the flowchart in Figure 1, 13,958 women were included, of whom 2,685 (19.2%) had H1inP and 934 (6.7%) were smokers. Table 1 shows the characteristics of the study population in the four mutually exclusive groups: no H1inP/non-smoker (group A: n = 10,454, 88.2%), no H1inP/smoker (group B: n = 819, 5.9%), H1inP/non-smoker (group C: n = 2,570, 18.4%), and H1inP/smoker (group D: n = 115, 0.8%). Women with H1inP were less likely to smoke than those without H1inP (4.3% *vs.* 7.3%, $p < 0.01$). Globally, the characteristics differed between groups, such as the higher age and BMI in the case of H1inP and the lower age and BMI in smokers. There was an H1inP*smoking interaction for age and BMI. For example, age was lower in smokers than in non-smokers in women without H1inP, whereas the inverse was observed in women with H1inP.

The prevalence of smoking differed by ethnicity, with the following decreasing percentages: European, 15.3%; other, 9.8%; Caribbean, 4.5%; North African, 3.6%; and Sub-Saharan African, 0.2%; there was only one Indian–Pakistani–Sri Lankan woman who smoked ($p < 0.0001$). Smokers were more likely to consume alcohol and recreational substances during pregnancy compared with non-smokers (Table 1).

Adverse perinatal outcomes

The rates of LGA babies were 8.9%, 4.0%, 14.6%, and 8.7% in groups A, B, C, and D, respectively (global ANOVA $p < 0.0001$, factor H1inP $p = 0.0003$, factor smoking $p = 0.0002$, and interaction $p = 0.48$) (Figure 2). After adjustment for confounders, H1inP was associated with a higher risk and smoking with a lower risk of LGA infant in all four models (Table 3).

Figure 2 (neonatal outcomes) and Figure 3 (maternal outcomes) show that all adverse perinatal outcomes differed by H1inP–smoking groups (number/percentages in Table 2). H1inP was associated with a lower rate of SGA babies, more frequent NICU admissions, lower maternal GWG, and a higher rate of caesarean section and of hypertensive disorders. Smoking was associated with more severe and non-severe SGA babies and a higher GWG. Finally, the rate of hypertensive disorders was the highest (over

TABLE 1 Patient characteristics according to the glycemic and smoking status.

	Total (N = 13,958)	No H1inP		H1inP		Factor H1inP	Factor smoking	Interaction	Global ANOVA
		Non-smoker: group A (n = 10,454)	Smoker: group B (n = 819)	Non-smoker: group C (n = 2,570)	Smoker: group D (n = 115)	p-value	p-value	p-value	p-value
Characteristics of the women									
Age (years)	30.5 ± 5.6	30.2 ± 5.5	28.7 ± 5.9	32.4 ± 5.4	32.7 ± 5.8	**<0.00001**	**0.0305**	**0.0017**	**<0.00001**
Pre-pregnancy body mass index (kg/m^2)	25.1 ± 5.0	24.7 ± 4.8	23.3 ± 4.5	27.1 ± 5.5	26.7 ± 5.3	**<0.00001**	**0.0004**	**0.0441**	**<0.00001**
Pre-pregnancy obesity	2,279 (16.9%)	1,463 (14.5%)	86 (10.7%)	700 (28.0%)	30 (26.8%)	**<0.00001**	0.1038	0.2513	**<0.00001**
Family history of diabetes	3,689 (26.4%)	2,563 (24.5%)	228 (27.8%)	855 (33.3%)	43 (37.4%)	**<0.00001**	0.0980	0.9671	**<0.00001**
Employment at the beginning of pregnancy	5,322 (38.7%)	4,058 (39.4%)	361 (44.7%)	843 (33.5%)	60 (52.6%)	0.7622	**<0.00001**	**0.00052**	**<0.00001**
Hypertension before pregnancy	108 (0.8%)	62 (0.6%)	2 (0.2%)	41 (1.6%)	3 (2.6%)	**0.0003**	0.6794	0.1386	**<0.00001**
Parity	2.14 ± 1.25	2.12 ± 1.25	1.96 ± 1.09	2.31 ± 1.28	2.26 ± 1.51	**<0.00001**	0.1013	0.4066	**<0.00001**
Ethnicity						**0.0077**	**<.0001**	0.0528	**<0.00001**
Sub-Saharan African	2,818 (20.2%)	2,326 (22.3%)	46 (5.6%)	436 (17.0%)	10 (8.7%)				
North African	4,049 (29.1%)	2,976 (28.5%)	116 (14.2%)	927 (36.1%)	30 (26.1%)				
Caribbean	779 (5.6%)	636 (6.1%)	32 (3.9%)	108 (4.2%)	3 (2.6%)				
European	3,833 (27.5%)	2,762 (26.5%)	526 (64.5%)	484 (18.9%)	61 (53.0%)				
Indian–Pakistani–Sri Lankan	1,389 (10.0%)	955 (9.1%)	1 (0.1%)	433 (16.9%)	0 (0.0%)				
Other	1,068 (7.7%)	784 (7.5%)	94 (11.5%)	179 (7.0%)	11 (9.6%)				
Previous pregnancy(ies)									
History of H1inP						**<0.00001**	0.0869	0.4670	**<0.00001**[a]
First child	5,283 (37.8%)	4,063 (38.9%)	362 (44.2%)	814 (31.7%)	44 (38.3%)				
No	7,924 (56.8%)	6,074 (58.1%)	443 (54.1%)	1,350 (52.5%)	57 (49.6%)				
Yes	751 (5.4%)	317 (3.0%)	14 (1.7%)	406 (15.8%)	14 (12.2%)				
History of macrosomia						**0.0031**	**0.0468**	0.5961	**<0.00001**[a]
First child	5,283 (37.8%)	4,063 (38.9%)	362 (44.2%)	814 (31.7%)	44 (38.3%)				

(Continued)

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TABLE 1 Continued

	Total (*N* = 13,958)	No H1inP		H1inP		Factor H1inP	Factor smoking	Interaction	Global ANOVA
		Non-smoker: group A (*n* = 10,454)	Smoker: group B (*n* = 819)	Non-smoker: group C (*n* = 2,570)	Smoker: group D (*n* = 115)	*p*-value	*p*-value	*p*-value	*p*-value
Previous pregnancy(ies)									
No	8,241 (59.0%)	6,121 (58.6%)	448 (54.7%)	1,605 (62.5%)	67 (58.3%)				
Yes	434 (3.1%)	270 (2.6%)	9 (1.1%)	151 (5.9%)	4 (3.5%)				
History of renal vascular diseases in pregnancy						**0.0030**	0.5599	0.2731	**<0.00001**[a]
First pregnancy	3,427 (24.6%)	2,740 (26.2%)	170 (20.8%)	499 (19.4%)	18 (15.7%)				
No	10,214 (73.2%)	7,506 (71.8%)	638 (77.9%)	1,978 (77.0%)	92 (80.0%)				
Yes	317 (2.3%)	208 (2.0%)	11 (1.3%)	93 (3.6%)	5 (4.3%)				
History of fetal death						**0.0336**	0.7430	0.3546	**0.0345**[a]
First pregnancy	3427 (24.6%)	2,740 (26.2%)	170 (20.8%)	499 (19.4%)	18 (15.7%)				
No	10,225 (73.3%)	7,505 (71.8%)	634 (77.4%)	1,994 (77.6%)	92 (80.0%)				
Yes	306 (2.2%)	209 (2.0%)	15 (1.8%)	77 (3.0%)	5 (4.3%)				
History of fetal growth restriction						0.5905	0.0720	0.7719	**0.0173**[a]
First pregnancy	3427 (24.6%)	2,740 (26.2%)	170 (20.8%)	499 (19.4%)	18 (15.7%)				
No	10,023 (71.8%)	7,352 (70.3%)	601 (73.4%)	1,979 (77.0%)	91 (79.1%)				
Yes	508 (3.6%)	362 (3.5%)	48 (5.9%)	92 (3.6%)	6 (5.2%)				
Habits during pregnancy									
Alcohol consumption	17 (0.1%)	8 (0.1%)	6 (0.7%)	1 (0.0%)	2 (1.7%)	0.8827	**<0.0001**	0.2478	**<0.00001**
Drug consumption	70 (0.5%)	28 (0.3%)	34 (4.2%)	2 (0.1%)	6 (5.2%)	0.2468	**<0.0001**	0.0864	**<0.00001**

Data are shown as *n* (percentage) or mean ± standard deviation. Data for the study sample (13,958 women) are available. p<0.05 are written in bold.
H1inP, hyperglycemia first diagnosed in pregnancy; *OGTT*, oral glucose tolerance test; *WG*, weeks of gestation.
[a]Yes *vs.* No (no history possible if first child)

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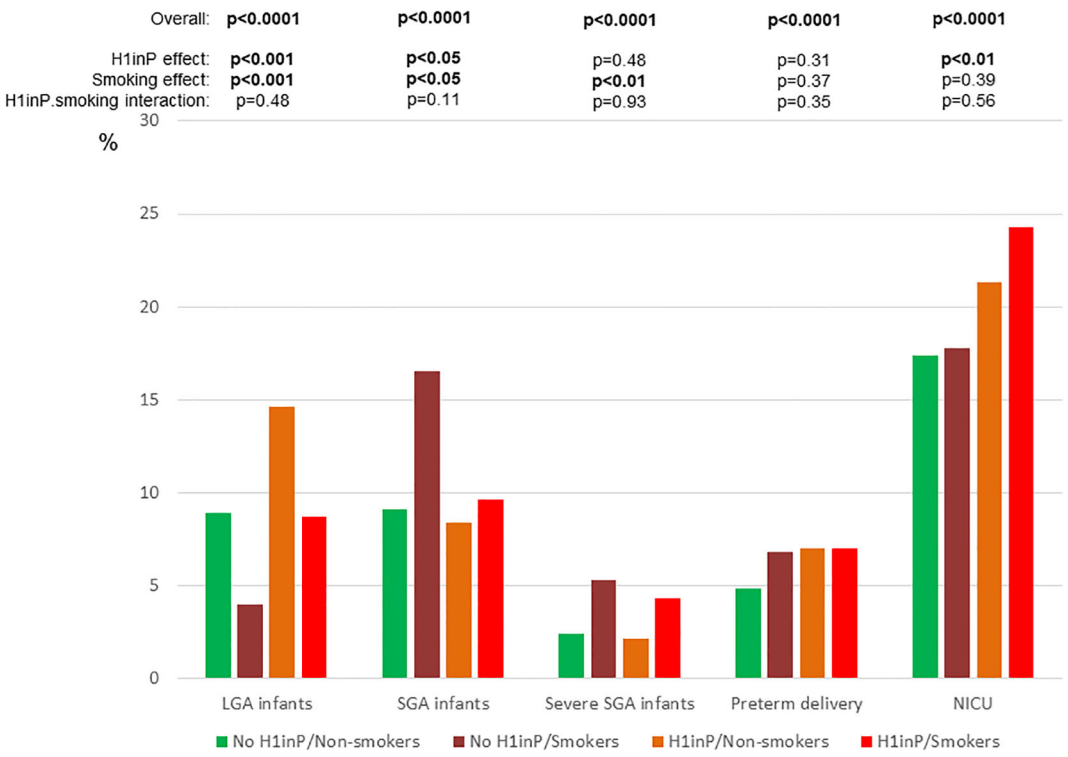

FIGURE 2
Primary and secondary neonatal outcomes according to glycemic and smoking status. *H1inP*, hyperglycemia first diagnosed in pregnancy; *LGA*, large-for-gestational-age; *NICU*, neonatal intensive care unit; *SGA*, small-for-gestational-age. p<0.05 are written in bold.

10%) in the women who had H1inP and who were smokers (H1inP*smoking interaction $p < 0.05$).

In women with H1inP, the rate of insulin therapy was similar in non-smokers and smokers (36.7% *vs.* 37.4%, $p = 0.68$), with lower insulin doses at the end of the pregnancy in the non-smokers compared with the smokers (25 ± 24 *vs.* 37 ± 35 IU, $p < 0.01$).

Table 2 also shows the results of the exploratory neonatal outcomes, with differences for neonatal hypoglycemia and any malformations according to the H1inP–smoking groups.

Discussion

Main results

In this multiethnic cohort, 6.7% of women were smokers during pregnancy. Smoking during pregnancy was associated with a reduced risk of LGA babies and H1inP with an increased risk of LGA babies, even after adjustment for confounders. Importantly, smoking was also associated with a higher GWG and, despite this, with higher rates of—especially severe—SGA babies. H1inP was associated with a lower GWG and a lower rate of SGA babies. In total, the prevalence rates of LGA and SGA babies in smokers with H1inP were similar to those in non-smokers without H1inP. Thus, the presence of H1inP and smoking might mask the respective impact and interfere with the ability to use fetal growth as a reliable marker of glycemic overload or placental dysfunction. H1inP was associated with higher rates of hypertensive disorders and of caesarean sections and more frequent admissions in the NICU. The combination of smoking and H1inP was associated with the highest risk of hypertensive disorders and NICU admissions.

Fetal growth, GWG, treatment, and complications of delivery

In this study, the birth weight and LGA rates were lower in smokers than in non-smokers, similar to that in another study (1), and were higher in women with than in those without H1inP, as previously reported (5, 6, 9). These differences remained after adjustment for confounders, including for differences in the BMI and GWG. In women with H1inP, smokers had a lower BMI compared with non-smokers, as shown in a previous study (20), but not in another cohort (20, 21). This was not found in the women with H1inP, probably due to older age and obesity being classical risk factors for H1inP (14).

The higher rate of LGA babies in women with H1inP indicates that, despite the lower GWG, current glycemic reduction is either too late or insufficient, although this was in accordance with the current guidelines regarding H1inP care (17). Thanks to our interdisciplinary care including the integration of dieticians, women with H1inP achieved lower GWG than those without. It should be noted that the women with H1inP in this cohort had a similar need for insulin treatment in both smokers and non-

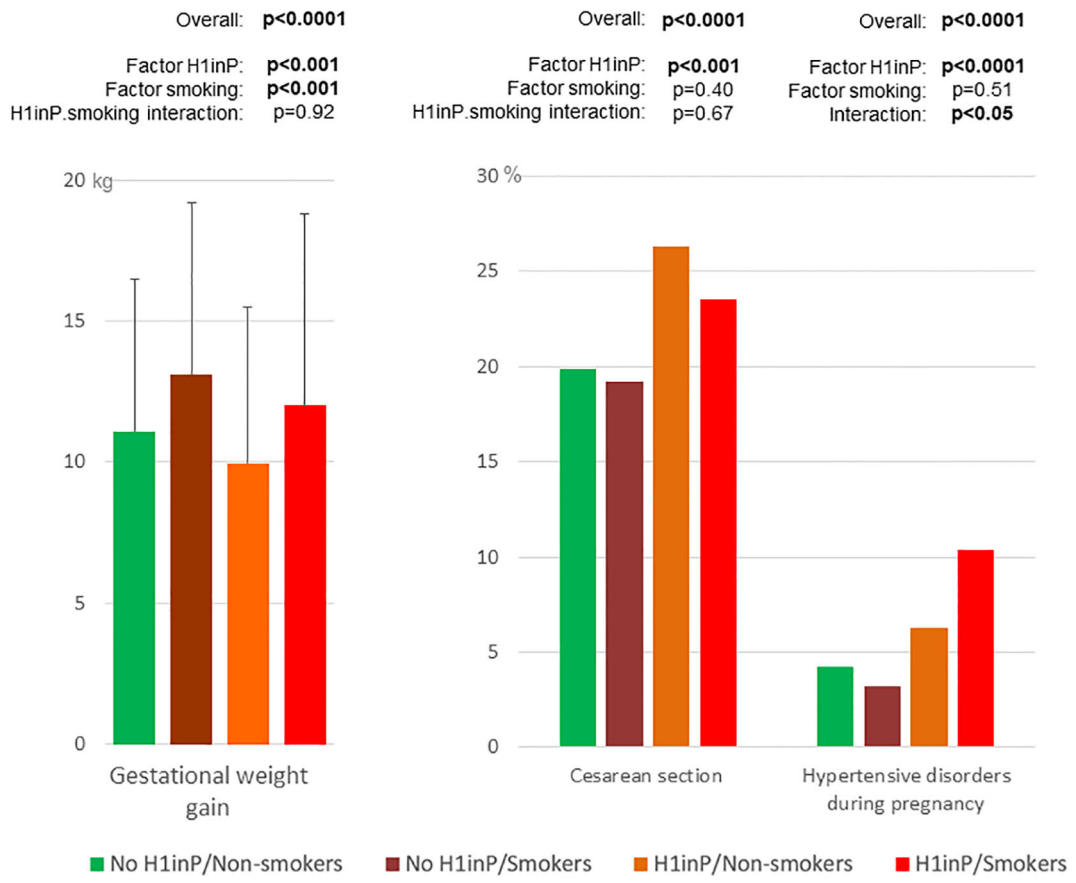

FIGURE 3
Secondary maternal outcomes according to glycemic and smoking status. *H1inP*, hyperglycemia first diagnosed in pregnancy. p < 0.05 are written in bold.

smokers. This contrasts with another study that found a higher rate of insulin therapy in smokers (21). However, the insulin dosages at the end of pregnancy were higher in smokers than in non-smokers. This might be partly driven by the higher GWG in smokers and, therefore, a higher insulin resistance (13, 22). The higher GWG observed for smokers could be linked to their unhealthy behaviors, including less frequent preventive screenings (10, 23–25) and the more frequent alcohol and recreational substance consumption observed in this study.

With regard to the combined effects of H1inP and smoking on birth weight, we only found three studies (20, 26, 27). The first study showed similar results in 400 Scandinavian women (26). The second study found in around 4,000 Finnish women that, in those without H1inP, the offspring birth weight was lowest in smokers, whereas in women with H1inP, the smoking status did not influence the offspring birth weight (20). The latter study did not explore the rate of LGA babies per se, and the changes in birth weight might have been driven by the different gestational ages at birth depending on the H1inP and smoking status. The third study, which included all Finnish primiparous women with singleton pregnancies between 2006 and 2018 (n = 290,602), found, as we did, that smoking and H1inP had opposing effects on fetal growth. Furthermore, compared with smoking after the first trimester of pregnancy, the cessation of smoking during the first trimester was associated with greater head circumference and birth weight in newborns (27).

In the present study, the rate of LGA babies in non-smokers without H1inP was similar to that in smokers with H1inP. However, we did not observe a lower rate of cesarean section or shoulder dystocia in smokers compared with non-smokers. Furthermore, the risk of severe SGA babies was increased in smokers regardless of the H1inP status, as previously reported (1, 28). This is likely due to several mechanisms (1–3), such as placental dysfunction through nicotine exposure (29), smoking-related altered endometrial maturation (30), and immune response and endothelial function (31).

Other outcomes

In this study, smoking was positively associated with hypertensive disorders, including preeclampsia, but only in women with H1inP. A systematic review and meta-analysis of prospective studies reported a negative association between smoking during pregnancy and the risk of preeclampsia, even after adjustment for several confounders including diabetes (32). However, we did not find any study investigating the impact of the combined effect of H1inP and smoking on hypertensive disorders.

TABLE 2 Neonatal and maternal adverse pregnancy outcomes in the four groups of women categorized by the presence or absence of hyperglycemia first diagnosed in pregnancy and smoking.

	Available data	No H1inP		H1inP					
		Smoking No Group A	Smoking Yes Group B	Smoking No Group C	Smoking Yes Group D	Factor H1inP	Factor Smoking	Interaction	Global ANOVA
		n=10,454	n=819	n=2,570	n=115		p	p	p p
Neonatal outcomes									
Primary									
Large-for-gestational-age infant	n=13,958	935 (8.9%)	33 (4.0%)	374 (14.6%)	10 (8.7%)		**0.0003**	**0.0002**	0.4819 **<0.0001**
Secondary									
Birthweight (g)	n=13,958	3,296 ± 499	3,111 ± 507	3,344 ± 536	3,163 ± 581		0.0528	**<0.0001**	0.9483 **<0.0001**
Small-for-gestational-age infant	n=13,958	952 (9.1%)	135 (16.5%)	217 (8.4%)	11 (9.6%)		**0.0376**	**0.0165**	0.1115 **<0.0001**
Severe small-for-gestational-age infant		256 (2.4%)	43 (5.3%)	54 (2.1%)	5 (4.3%)		0.4837	**0.0023**	0.9348 **<0.0001**
Preterm delivery	n=13,958	507 (4.8%)	56 (6.8%)	180 (7.0%)	8 (7.0%)		0.3090	0.3740	0.3548 **<0.0001**
Neonatal intensive care unit	n=13,958	1,814 (17.4%)	146 (17.8%)	547 (21.3%)	28 (24.3%)		**0.0073**	0.3975	0.5611 **<0.0001**
Exploratory									
Shoulder dystocia	n=13,958	11 (0.1%)	2 (0.2%)	2 (0.1%)	0 (0.0%)				0.4379
Neonatal hypoglycemia	n=8,913	56 (0.9%)	5 (1.0%)	43 (2.0%)	1 (1.0%)		0.4660	0.5871	0.4929 **0.0017**
Neonatal death and stillbirth	n=13957	32 (0.3%)	3 (0.4%)	8 (0.3%)	1 (0.9%)				0.4983
Any malformation	n=13958	115 (1.1%)	7 (0.9%)	50 (1.9%)	4 (3.5%)		0.4691	0.3219	0.4861 **0.0010**
Maternal outcomes (secondary)									
Gestational weight gain (kg)	n=12,331	11.1 ± 5.4	13.1 ± 6.1	9.95 ± 5.55	12.0 ± 6.8		**<0.0001**	**<0.0001**	0.9292 **<0.0001**
Caesarean section	n=13,958	2,084 (19.9%)	157 (19.2%)	677 (26.3%)	27 (23.5%)		**0.0106**	0.4051	0.6660 **<0.0001**
Hypertensive disorders during pregnancy	n=13,958	442 (4.2%)	26 (3.2%)	163 (6.3%)	12 (10.4%)		**<0.0001**	0.5150	**0.0256** **<0.0001**
Pregnancy-induced hypertension	n=13,958	228 (2.2%)	17 (2.1%)	91 (3.5%)	7 (6.1%)		**<0.001**	0.2781	0.1948 **<0.001**
Preeclampsia	n=13,958	217 (2.1%)	9 (1.1%)	73 (2.8%)	5 (4.3%)		**<0.01**	0.7267	0.0621 **<0.001**

Data are *n* (percentage) or mean (standard deviation).
H1inP, hyperglycemia first diagnosed in pregnancy.

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TABLE 3 H1inP and smoking effects for large-for-gestational-age infant in multivariable analyses.

		Unadjusted	Model 1	Model 2	Model 3	Model 4
		OR (95%CI), *p*	OR (95%CI), *p*	OR (95%CI), *p*	OR (95%CI), *p*	OR (95%CI), *p*
Large-for-gestational-age infant	H1inP effect	**1.747 (1.539–1.983), *p* < 0.001**	**1.389 (1.21–1.595), *p* < 0.001**	**1.501 (1.298–1.736), *p* < 0.001**	**1.502 (1.299–1.737), *p* < 0.001**	**1.406 (1.211–1.632), *p* < 0.001**
	Smoking effect	**0.452 (0.331–0.617), *p* < 0.001**	**0.429 (0.309–0.596), *p* < 0.001**	**0.359 (0.252–0.511), *p* < 0.001**	**0.352 (0.247–0.503), *p* < 0.001**	**0.361 (0.252–0.518), *p* < 0.001**

Model 1: adjusted for age, body mass index, employment, ethnicity, parity, and hypertension before pregnancy; Model 2: model 1 + adjusted for gestational weight gain; Model 3: model 2 + adjusted for alcohol and drug consumption; Model 4: model 3 + adjusted for history of macrosomic infant. p < 0.05 are written in bold.
H1inP, hyperglycemia first-diagnosed in pregnancy; *OR*, odds ratio; *95%CI*, 95% confidence interval.

Smoking and H1inP both increase placental hypoplasia with fetal vascular perfusion lesions (33), "two pathways" that increase hypoxia and oxidative stress that may converge on preeclampsia, and a worse neonatal condition (likely expressed in a high rate of NICU admissions). Previous studies have shown the separate impacts of smoking (34) and of H1inP, particularly when the glucose values are high at diagnosis (12), on malformations. Our results, although exploratory, suggest that the combination of both is associated with the highest prevalence of malformations. This should be investigated further.

Strengths and limitations

A strength of this study is that it involved a large multi-ethnic cohort with prospective recruitment over a decade, allowing to explore the effects of smoking and H1inP and their combination on several adverse pregnancy outcomes, even if the event rates for neonatal hypoglycemia or stillbirth were low (35). We were also able to adjust for several cofounders, which also included the consumption of alcohol (36) and recreational substances (37).

The study also has several limitations. Firstly, smoking was self-reported. However, previous studies found a good validity of self-reported tobacco use when compared with measured plasma cotinine levels [31]. Secondly, we were unable to evaluate the impact of smoking at different gestational time points, and we had no quantitative data on cigarette smoking or a decrease in smoking quantity. In addition, despite the large cohort, the number of LGA babies in women with H1inP who smoked (10 out of 115) was relatively low. Moreover, we could not study placental lesions, whereas smoking-induced complications are likely driven by placental dysfunction (1–4). Finally, we had no data on paternal smoking and, thus, passive tobacco exposure (3).

Perspectives

Our adjusted data suggest that further studies should examine the role of earlier or stricter glucose management in women with H1inP. Smoking is associated with many adverse pregnancy outcomes, to which life span consequences for the future infant, such as metabolic diseases, attention disorders, respiratory dysfunction, and even sudden death, should be added (1, 3). Based on the data from this study and on previous data, women who smoke during pregnancy should be targeted as they have a higher GWG compared with non-smokers and nevertheless have a high rate of severely growth-restricted babies, which may even be underestimated (38).

Finally, the results of this study argue for a particular attentive screening for hypertensive disorders in smokers with H1inP. As fetal growth may be normal in these women, they should particularly be monitored for blood pressure and placental function (e.g., by Doppler ultrasound, biomarkers, or fetal tolerance to late-term contractions) on the one hand and the quality of dietary observance and glycemic level on the other hand.

Further research should investigate the pathophysiological mechanisms related to the impact of smoking on insulin resistance, inflammation, and placental function in the presence of normal and increased glucose levels throughout pregnancy.

Conclusion

Smoking and H1inP have opposing independent effects on fetal growth that therefore may appear normal in women with H1inP who smoke. Smoking among women with H1inP could mask the risk of maternal hyperglycemia for LGA babies. This might provide a false sense of security for women with H1inP who smoke, as it will hide a particular risk of hypertensive disorders during pregnancy and later severe SGA babies. These findings, together with the smoking- and H1inP-related life span consequences for both the child to be born and the mother, further argue for a timely smoking cessation in pregnant women.

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 Commission Nationale de l'Informatique et des Libertés, number 1704392v0. 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

EC: Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. LC: Data curation, Project administration, Supervision, Validation, Visualization, Writing – review & editing. ST: Validation, Visualization, Writing – review & editing. HB: Validation, Writing – review & editing. EV: Conceptualization, Resources, Supervision, Validation, Visualization, Writing – review & editing. IB: Writing – review & editing. SP: Validation, Writing – review & editing. IR: Writing – review & editing. MZ: Writing – review & editing. J-JP: Formal analysis, Writing – review & editing. MF: Writing – review & editing. JP: Conceptualization, Supervision, Validation, Visualization, Writing – review & editing. AB: Conceptualization, Supervision, Validation, Visualization, 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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The author(s) declare that no Generative AI was used in the creation of this manuscript.

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PDX1 in early pregnancy is associated with decreased risks of gestational diabetes mellitus and adverse pregnancy outcomes

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Aim: To investigate the association of pancreatic duodenal homeobox-1 (PDX1) in early pregnancy with the risks of gestational diabetes mellitus (GDM) and adverse pregnancy outcomes.

Methods: A total of 231 pregnant women were recruited at their initial antenatal care visit during 8-12 gestational weeks in this study. The 75g OGTT was performed during 24-28 gestational weeks. Blood samples were collected to measure PDX1 levels. Participants were followed throughout their pregnancy to monitor for the development of GDM and adverse pregnancy outcomes. The odds ratio (OR) was used to assess the risks of GDM and adverse pregnancy outcomes.

Results: Pregnant women in the GDM group had higher levels of HOMA-IR and TyG index, and lower PDX1 levels both in early and mid-pregnancy (P<0.05), but had lower HOMA-β levels only in mid-pregnancy (P<0.05). PDX1 in early pregnancy was negatively correlated with FPG, 2h PG, HOMA-IR, and TyG, while positively correlated with HOMA-β in mid-pregnancy (P<0.05). The adjusted analysis showed that elevated PDX1 levels in early pregnancy were associated with reduced risks of GDM (aOR 0.287, 95%CI 0.130-0.636, P=0.002), macrosomia (aOR 0.249, 95%CI 0.076-0.811, P=0.021) and composite adverse pregnancy outcomes (aOR 0.496, 95%CI 0.256-0.960, P=0.037).

Conclusion: Elevated PDX1 in early pregnancy was associated with decreased risks of GDM and adverse pregnancy outcomes.

KEYWORDS

pancreatic duodenal homeobox-1, gestational diabetes mellitus, adverse pregnancy outcomes, early pregnancy, mid-pregnancy

1 Introduction

Gestational diabetes mellitus (GDM), a form of diabetes, is identified during pregnancy in women who did not have diabetes before pregnancy. It is generally diagnosed at 24-28 weeks of pregnancy using an oral glucose tolerance test (OGTT) (1). Insulin resistance (IR) and pancreatic β-cell dysfunction are thought to be important mechanisms in the development of GDM (2). In fact, GDM is a common complication in pregnant women, with recent data indicating a prevalence of 20.8% in Southeast Asian women (3) and 21.1% in Chinese women (4). GDM can significantly and seriously impact both maternal and fetal health. It is linked to increased adverse pregnancy outcomes, including pre-eclampsia, preterm birth, macrosomia, and prenatal depressive symptoms (5). Women diagnosed with GDM face a risk of developing diabetes over 20 times higher than those without GDM (6). Additionally, the risks of cardiovascular diseases, hypertension, kidney disease, hyperlipidemia, and incident dementia all increase from one to six times, and these risks escalate with the duration of delivery (6, 7). Infants of mothers with GDM frequently experience hypoglycemia and jaundice, and they face a higher likelihood of becoming obese and developing type 2 diabetes later in life. Given the potential harms associated with GDM, it is imperative to identify GDM as early as possible.

Pancreatic duodenal homeobox-1 (PDX1) is a nuclear transcription factor expressed in both endocrine and exocrine cells before embryo maturation. However, as the pancreas matures, its expression becomes predominantly restricted to β-cells (8, 9). It plays a pivotal role in the development of the pancreas, the differentiation of β-cells, and the preservation of mature β-cell functions. PDX1 can bind to and activate the promoter of the insulin gene expression, thereby increasing the synthesis of insulin and maintaining glucose homeostasis (10). Existing studies indicate that the decreasing of PDX1 expression leads to abnormalities in blood glucose regulation, thereby impacting the onset and progression of diabetes (11, 12). Based on the role of PDX1 in glucose regulation, PDX1 may be implicated in glucose metabolic disorders during pregnancy.

In this prospective study, we explored the association of PDX1, GDM, and adverse pregnancy outcomes in Chinese women to identify early prediction and prevention strategies for GDM and adverse pregnancy outcomes.

2 Materials and methods

2.1 Study population and design

The study cohort and methods were described previously (13). Briefly, from October 2020 to March 2022, we established a preconception cohort of pregnant women based on a screening and management system in Taizhou People's Hospital. We initially recruited 315 singleton pregnant women aged 20 to 40 years old during their first prenatal examination in the hospital at 8-12 gestational weeks. Individuals with a history of abnormal glucose tolerance, diabetes, hypertension, polycystic ovary syndrome, hyperthyroidism, hypothyroidism, malignancies, autoimmune diseases, or severe cardiac, hepatic, or renal dysfunction were excluded. They were followed up from the initial prenatal examination until the completion of delivery. The OGTT was conducted during 24-28 weeks of pregnancy to diagnose GDM based on its results. Finally, 231 pregnant women were included in this study, 42 were diagnosed with GDM (GDM group), while 189 had normal glucose tolerance (non-GDM group). The flow chart is shown in Figure 1. This study complied with the Helsinki Declaration and was approved by the Institutional Ethics Committee of Taizhou People's Hospital.

2.2 Definition of GDM and adverse pregnancy outcomes

According to the 75g OGTT results, GDM was diagnosed based on World Health Organization 2013 criteria. Any one of the following criteria needs to be met: (1) fasting plasma glucose (FPG) ≥ 5.10 mmol/L; (2) 1-hour postprandial blood glucose (1h PG) ≥ 10.00 mmol/L; (3) 2-hour postprandial blood glucose (2h PG) ≥ 8.50 mmol/L (14).

Adverse pregnancy outcomes in this study were defined as pathological pregnancy and abnormal pregnancy, including pre-eclampsia, fetal growth restriction (FGR), preterm birth, macrosomia, and neonatal respiratory distress syndrome (NRDS). The composite adverse pregnancy outcomes included any one or a combination of the adverse events mentioned above. Pre-eclampsia was characterized by a systolic blood pressure (SBP) ≥140mmHg and/or a diastolic blood pressure (DBP) ≥90mmHg after 20 weeks of pregnancy. FGR was considered as ultrasonographic estimated fetal weight (EFW) or abdominal circumference (AC) below the 10th percentile of the normal gestational age (15). Preterm birth was defined as childbirth taking place between the 24th and 37th weeks of pregnancy. When a newborn's weight exceeded 4000g, it was classified as macrosomia. The diagnosis of NRDS is based on symptoms of respiratory distress, oxygen levels in the blood, and abnormal results from chest X-rays by professional pediatricians (16, 17).

2.3 Data collection and measurement of serum PDX1

A standardized procedure was performed since the initial antenatal care visit to the hospital. A questionnaire was administered to collect the information, including height, pre-pregnancy weight, smoking and alcohol habits, parity, family history of diabetes, and history of metabolic disorders. The pre-pregnancy BMI (Body Mass Index) was calculated by dividing pre-pregnancy weight(kg) by height squared(m2).

Blood samples were collected in the morning after at least 8 hours of fasting and analyzed in the hospital's central laboratory.

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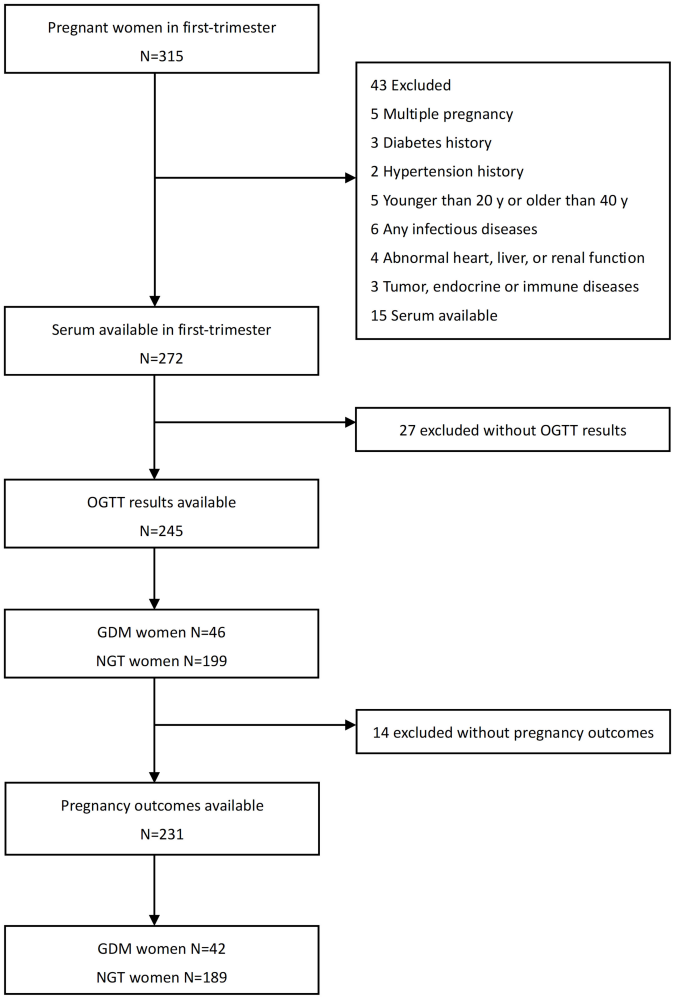

FIGURE 1
Flow chart of included subjects.

The results of the OGTT were recorded at weeks 24-28 of pregnancy from the electronic medical record system. The homeostatic model was used to assess insulin resistance (HOMA-IR=FIns×FPG/22.5), and insulin beta cell function (HOMA-β=20×Fins/(FPG-3.5)). Triglyceride and glucose (TyG) index was also calculated to assess insulin resistance (TyG index = Ln [fasting triglyceride (mg/dL)×fasting glucose (mg/dL)/2]) (18, 19). Serum aliquots were preserved for further analysis. For short-term storage, serum samples were maintained at 2–8°C for a maximum of 24 hours before being aliquoted and transferred to −80°C for long-term storage (up to 2 years). Hemolytic samples were excluded from the analysis to ensure data reliability. The enzyme-linked immunosorbent assay (ELISA) kit (Shanghai Zhenke Biology Co., Ltd., China) was used to quantify serum PDX1 levels. To minimize variability, each sample was measured in duplicate within the same analytical session, and the average value was used for further analysis. Intra- and inter-assay coefficients of variation (CVs) were controlled at 10% and 15%, respectively, by calibrating the equipment before each session and using standardized protocols across all measurements.

2.4 Statistical analysis

The analysis of statistical data and the creation of figures were realized by SPSS 26.0 (IBM SPSS Inc, Chicago, IL, USA) and GraphPad Prism 9.5. For variables following a normal distribution, the Student's t-test was used to calculate their mean and standard deviation (SD), while for variables not following a normal distribution, the Mann–Whitney test was used to measure their median and interquartile range. The Chi-square test was used for categorical variables between two groups, and percentages were calculated for categorical variables. We used Spearman correlation analysis to assess the relations of PDX1 with glucose metabolic indicators. Logistic regression analysis was performed to examine the associations of PDX1 with GDM and adverse pregnancy

outcomes. Receiver operating characteristic (ROC) curves measured by R language were used to assess the predictive ability of PDX1 for GDM. A p-value less than 0.05 was regarded as statistically significance.

3 Results

3.1 Characteristics of participants in GDM and non-GDM groups

In this study, 231 patients were recruited with an average age of 28 years and an average pre-pregnancy BMI of 22.11 kg/m^2. Participants were divided into two groups according to the results of OGTT. 42 pregnancies occurred GDM (GDM group), while the other 189 pregnancies exhibited glucose tolerance within the normal range (non-GDM group). There was a significant difference in age between the two groups, with the GDM group being older (30 vs 28 years, P=0.029). No statistical differences between the two groups in the number of male fetuses.

Patients in the GDM group had elevated FPG levels in the first trimester and second trimester (4.75 vs 4.63 mmol/L, 5.12 vs 4.36 mmol/L, P<0.05, respectively). Regardless of early or mid-pregnancy, HOMA-IR, TyG index and triglycerides (TG) were all higher in the GDM group (P<0.05). However, HOMA-β levels only showed lower levels in the GDM group in mid-pregnancy (148.97 vs 198.59, P=0.002). Additionally, PDX1 was lower in the GDM group in both two stages (123.21 vs. 132.15 pg/mL, P=0.013; 81.65 vs. 96.77 pg/mL, P<0.001, respectively, Table 1). Furthermore, from early pregnancy to mid-pregnancy, HOMA-IR, TyG index, and TG were all significantly increased in both GDM and non-GDM groups, while PDX1 was decreased (P<0.05) (Supplementary Table 1).

3.2 Characteristics, incidence of GDM and adverse pregnancy outcomes in two groups categorized by PDX1 in early pregnancy

We divided the participants into two groups based on the median PDX1 level in early pregnancy (131 pg/mL): a low PDX1 group (n=115) and a high PDX1 group (n=116). The prevalence of GDM was significantly higher in the low PDX1 group compared to the high PDX1 group (26.09% vs 10.34%, P=0.002). The low PDX1 group exhibited higher levels of FPG (4.57 vs 4.29 mmol/L, P<0.001) and HOMA-IR (1.83 vs 1.57, P=0.005), while HOMA-β levels were lower (175.27 vs. 207.16, P=0.022; Table 2). Pregnant women in the high PDX1 group had a lower incidence of preterm birth (11.30% vs. 4.31%, P=0.047), macrosomia (11.30% vs. 3.45%, P=0.022), and composite adverse pregnancy outcomes (28.70% vs. 16.38%, P=0.025). When grouped according to fetal sex, it was found that PDX1, FPG, HOMA-IR, HOMA-β, and other metabolic indicators and adverse pregnancy outcomes were not statistically different between the two groups (Supplementary Table 2).

3.3 Correlations of PDX1 in early pregnancy with glucose metabolic factors in mid-pregnancy

PDX1 in early pregnancy was negatively correlated with FPG (r=-0.320, P<0.001), 2h PG (r=–0.133, P=0.044), HOMA-IR (r=-0.179, P=0.007), and TyG index (r=-0.173, P=0.008) in mid-pregnancy, while positively correlated with HOMA-β (r=0.159, P=0.016). However, no correlation was found between PDX1 and 1h PG (Table 3). The scatter plot was further drawn in Supplementary Figure 1.

3.4 Association of PDX1 in early pregnancy and the risk of GDM

After adjusting for traditional risk factors (including age, preconception BMI, family history of diabetes, smoking exposure, and alcohol consumption), the logistic regression analysis revealed that PDX1 in early pregnancy was linked to a decreased risk of GDM (adjusted odds ratio [aOR] 0.287, 95%CI 0.130-0.636, P=0.002) (Table 4).

3.5 ROC curve analysis of diagnostic value of PDX1 and traditional factors in GDM

The area under the ROC curves (AUC) of PDX1 in early pregnancy for predicting the occurrence of GDM was 0.616 (P<0.05). The combination of PDX1 and traditional factors could improve the predictive value of GDM (AUC: 0.718, P<0.001, Figure 2, Supplementary Table 3).

3.6 Associations of PDX1 in early pregnancy with adverse pregnancy outcomes

After adjusting for traditional risk factors (age, preconception BMI, family history of diabetes, smoking exposure, alcohol consumption, and GDM), the logistic regression analysis showed that pregnant women with higher PDX1 levels in early pregnancy had a lower incidence of macrosomia (aOR 0.249, 95% CI 0.076-0.811, P=0.021) and composite adverse pregnancy outcomes (aOR 0.496, 95% CI 0.256-0.960, P=0.037, Table 5).

4 Discussion

GDM has been shown to have serious negative impacts on the health of both mothers and infants. A recent study with 53,649 participants revealed that GDM is a significant predictor of adverse pregnancy outcomes, leading to various complications for both mothers and newborns (20). Early detection and treatment of GDM

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TABLE 1 Characteristics of participants in GDM and non-GDM groups.

Index	All	GDM group	non-GDM group	Z/χ2	*P* value
	N=231	N=42	N=189		
Age (year)	28 (26,30)	30 (26,32)	28 (26,30)	-2.187	0.029
Preconception BMI (kg/m²)	22.11 (20.75,24.22)	23.23 (21.14,26.03)	22.04 (20.63,24.02)	-1.858	0.063
Family history of diabetes, n (%)	10 (4.33)	3 (7.14)	7 (3.70)	0.327	0.568
Smoking exposure,n (%)	9 (3.90)	3 (7.14)	6 (3.17)	2.699	0.100
Alcohol consumption,n (%)	18 (7.79)	5 (11.90)	13 (6.88)	0.021	0.885
Male fetuses,n (%)	121 (52.38)	23 (54.76)	98 (51.85)	0.117	0.733
Early pregnancy					
FPG (mmol/L)	4.65 (4.42,4.92)	4.75 (4.47,5.23)	4.63 (4.42,4.88)	-2.153	0.031
HOMA-β	113.87 (84.84,157.93)	110.26 (83.52,188.32)	114.42 (83.82,156.82)	-0.373	0.709
HOMA-IR	1.44 (1.02,1.85)	1.72 (1.07,2.34)	1.39 (1.02,1.79)	-2.341	0.019
TyG index	8.52 (8.36,8.79)	8.76 (8.52,9.08)	8.49 (8.31,8.75)	-4.623	<0.001
TC (mmol/L)	4.38 (4.03,4.94)	4.41 (4.02,5.06)	4.37 (4.04,4.89)	-0.867	0.386
TG (mmol/L)	1.36 (1.14,1.74)	1.62 (1.35,2.30)	1.33 (1.09,1.64)	-4.082	<0.001
HDL-C (mmol/L)	1.61 (1.42,1.82)	1.61 (1.43,1.85)	1.61 (1.42,1.81)	-0.416	0.645
LDL-C (mmol/L)	2.45 (2.18,2.85)	2.47 (2.15,2.86)	2.44 (2.18,2.85)	-0.870	0.385
PDX1 (pg/ml)	131.15 (118.29,142.96)	123.21 (108.38,139.78)	132.15 (119.35,143.10)	-2.48	0.013
SBP (mmHg)	114 (107,120)	117 (110,125)	113 (107,120)	-1.891	0.059
DBP (mmHg)	71 (65,77)	72 (65,79)	70 (65,76)	-0.82	0.412
Mid-pregnancy					
FPG (mmol/L)	4.42 (4.18,4.68)	5.12 (4.66,5.41)	4.36 (4.15,4.59)	-7.241	<0.001
1h PG (mmol/L)	7.54 (6.68,8.65)	9.88 (8.46,10.89)	7.33 (6.40,8.24)	-7.245	<0.001
2h PG (mmol/L)	6.75 (6.05,7.45)	8.53 (7.19,9.77)	6.60 (5.92,7.17)	-6.929	<0.001
HOMA-β	191.53 (132.65,285.65)	148.97 (112.21,209.16)	198.59 (140.82,298.03)	-3.058	0.002
HOMA-IR	1.66 (1.29,2.25)	2.27 (1.65,3.62)	1.57 (1.22,2.12)	-4.628	<0.001
TyG index	9.00 (8.80,9.25)	9.33 (9.03,9.68)	8.95 (8.77,9.19)	-5.417	<0.001
TC (mmol/L)	5.63 (5.14,6.29)	5.90 (5.06,6.37)	5.56 (5.15,6.27)	-1.011	0.312
TG (mmol/L)	2.32 (1.87,2.90)	2.70 (2.23,4.07)	2.21 (1.85,2.75)	-3.698	<0.001
HDL-C (mmol/L)	1.90 (1.72,2.13)	1.91 (1.72,2.10)	1.90 (1.73,2.14)	-0.107	0.915
LDL-C (mmol/L)	3.23 (2.87,3.61)	3.27 (2.85,3.66)	3.23 (2.88,3.58)	-0.236	0.813
PDX1 (pg/ml)	92.64 (74.93,109.58)	81.65 (71.34,92.75)	96.77 (77.43,111.56)	-3.523	<0.001
SBP (mmHg)	113 (108,120)	115 (109,122)	112 (108,119)	-1.371	0.170
DBP (mmHg)	70 (65,76)	71 (66,76)	70 (64,76)	-0.402	0.687

GDM, gestational diabetes mellitus; BMI, body mass index; FPG, fasting plasma glucose; PDX1, pancreatic duodenal homeobox-1; TyG index, triglyceride and glucose index; 1h PG, 1-hour postprandial blood glucose; 2h PG, 2-hour postprandial blood glucose; SBP, systolic blood pressure; DBP, diastolic blood pressure; TC, total cholesterol; TG, triglycerides; HDL-c, high-density lipoprotein cholesterol; LDL-c, low-density lipoprotein cholesterol.

have been proven to be more effective and cost-efficient (21). Currently, GDM is diagnosed based on the results of OGTT conducted during the mid-pregnancy. Detecting and predicting GDM early, followed by prompt intervention, is crucial for reducing adverse pregnancy outcomes and improving the health of both mothers and infants. In this prospective study, we investigated the association of PDX1 in early pregnancy with GDM and adverse pregnancy outcomes. The results showed that elevated PDX1 levels

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TABLE 2 Groups Categorized by PDX1 in early pregnancy.

Index	All	PDX1<131pg/mL	PDX1≥131pg/mL	Z/χ2	*P* value
	N=231	N=115	N=116		
Age (year)	28 (26,30)	28 (26,30)	28 (26,30)	-0.512	0.608
Preconception BMI (kg/m^2)	22.11 (20.75,24.22)	22.41 (21.03,22.41)	21.97 (20.42,24.01)	-1.394	0.163
Family history of diabetes,n (%)	10 (4.33)	6 (5.22)	4 (3.45)	0.114	0.736
Smoking exposure,n (%)	9 (3.90)	4 (3.48)	5 (4.31)	0.107	0.744
Alcohol consumption,n (%)	18 (7.80)	11 (9.57)	7 (6.03)	1.002	0.317
GDM	42 (18.18)	30 (26.09)	12 (10.34)	9.620	0.002
Male fetuses,n (%)	121 (52.38)	63 (54.80)	58 (50.00)	0.530	0.467
Mid-pregnancy					
FPG (mmol/L)	4.42 (4.18,4.68)	4.57 (4.32,4.82)	4.29 (4.08,4.51)	-5.098	<0.001
1h PG (mmol/L)	7.54 (6.68,8.65)	7.74 (7.00,9.00)	7.49 (6.33,8.35)	-2.630	0.009
2h PG (mmol/L)	6.75 (6.05,7.45)	6.86 (6.12,7.64)	6.63 (5.78,7.22)	-2.392	0.017
HOMA-β	191.53 (132.65,285.65)	175.27 (122.33,257.70)	207.16 (145.97,312.84)	-2.285	0.022
HOMA-IR	1.66 (1.29,2.25)	1.83 (1.31,2.61)	1.57 (1.22,2.02)	-2.828	0.005
TyG index	9.00 (8.80,9.25)	9.00 (8.82,9.29)	8.97 (8.74,9.23)	-1.671	0.095
TC (mmol/L)	5.63 (5.14,6.29)	5.55 (5.12,6.15)	5.72 (5.22,6.37)	-1.080	0.280
TG (mmol/L)	2.32 (1.87,2.90)	2.29 (1.88,2.90)	2.34 (1.84,2.89)	-0.160	0.873
HDL-C (mmol/L)	1.90 (1.72,2.13)	1.88 (1.71,2.13)	1.93 (1.74,2.14)	-0.529	0.597
LDL-C (mmol/L)	3.23 (2.87,3.61)	3.21 (2.83,3.50)	3.30 (3.00,3.66)	-2.049	0.040
SBP (mmHg)	113 (108,120)	113 (109,121)	112 (108,118)	-1.444	0.149
DBP (mmHg)	70 (65,76)	69 (62,76)	71 (66,76)	-1.985	0.047
Adverse pregnancy outcomes					
Pre-eclampsia,n (%)	8 (3.46)	6 (5.22)	2 (1.72)	1.192	0.275
Fetal growth restriction,n (%)	15 (6.49)	7 (6.09)	8 (6.90)	0.062	0.803
Preterm birth,n (%)	18 (7.80)	13 (11.30)	5 (4.31)	3.932	0.047
Macrosomia,n (%)	17 (7.36)	13 (11.30)	4 (3.45)	5.228	0.022
Neonatal respiratory distress syndrome, n (%)	7 (3.03)	4 (3.48)	3 (2.59)	0.156	0.693
Composite adverse pregnancy outcomes, n (%)	56 (24.24)	33 (28.70)	19 (16.38)	5.022	0.025

PDX1, pancreatic duodenal homeobox-1; GDM, gestational diabetes mellitus; BMI, body mass index; GDM, gestational diabetes mellitus; FPG, fasting plasma glucose; 1h PG, 1-hour postprandial blood glucose; 2h PG, 2-hour postprandial blood glucose; TyG index, triglyceride and glucose index; SBP, systolic blood pressure; DBP, diastolic blood pressure; TC, total cholesterol; TG, triglycerides; HDL-c, high-density lipoprotein cholesterol; LDL-c, low-density lipoprotein cholesterol.

in early pregnancy were associated with reduced risks of GDM (aOR 0.287, P=0.002) and composite adverse pregnancy outcomes (aOR 0.496, P=0.037). Besides, PDX1 in early pregnancy was negatively correlated with FPG, 2h PG, HOMA-IR, and TyG in mid-pregnancy, while positively correlated with HOMA-β (P<0.05).

GDM is a prevalent metabolic disorder initially diagnosed during pregnancy. This transient form of diabetes results from insulin resistance and pancreatic β-cell dysfunction. Normally, hormones produced by the placenta during pregnancy induce insulin resistance in the mother, ensuring adequate nutrient supply to the fetus. To maintain normal blood glucose levels, the maternal pancreatic β-cells must compensate by secreting more insulin (22). GDM develops when pancreatic β-cell function declines and cannot meet this increased demand. Late-stage pregnancy typically features maternal hyperinsulinemia and insulin resistance, which are especially pronounced in women with GDM (23). HOMA-IR and HOMA-β are considered to

TABLE 3 Correlations of PDX1 in early pregnancy with glucose metabolic factors in mid-pregnancy.

Items	r	p
FPG	-0.320	<0.001
1hPG	-0.120	0.068
2hPG	-0.133	0.044
HOMA-IR	-0.179	0.007
HOMA-β	0.159	0.016
TyG	-0.173	0.008

FPG, fasting plasma glucose; TyG index, triglyceride and glucose index; 1hPG, 1-hour postprandial blood glucose; 2hPG, 2-hour postprandial blood glucose.

assess insulin resistance and pancreatic β-cell function with high accuracy (24). In this study, we also use HOMA-IR and HOMA-β to evaluate insulin resistance and pancreatic β-cell function of pregnant women, and found that patients with GDM had higher HOMA-IR in both early and mid-pregnancy, but had lower HOMA-β only in mid-pregnancy. The TyG index is also an indicator calculated using TG and FPG, that can be used to assess insulin resistance (2). Guo Y et al. found that the TyG index was proportional to the risk of GDM (aOR=2.10, P<0.001), and concluded that the TyG index in early pregnancy could predict GDM (25). Another study showed that except for TyG, high levels of FPG and TG in the first trimester were associated with an increased risk of GDM. In this study, patients with GDM had higher FPG, TG, and TyG index both in the first trimester and second trimester.

PDX1, also known as insulin promoter factor-1 (IPF1), somatostatin transcription factor-1 (STF1), or glucose-sensitive factor-1 (GSF1), is located on human chromosome 13q12.1 and consists of 6284 base pairs (26). PDX1 is a transcription factor primarily expressed in the pancreas, particularly in β-cells, where it plays a critical role in pancreatic development, β-cell differentiation, and the regulation of insulin gene expression. Before the maturation of the embryo, PDX1 is extensively expressed in both endocrine and exocrine cells. As the pancreas develops, PDX1 expression becomes

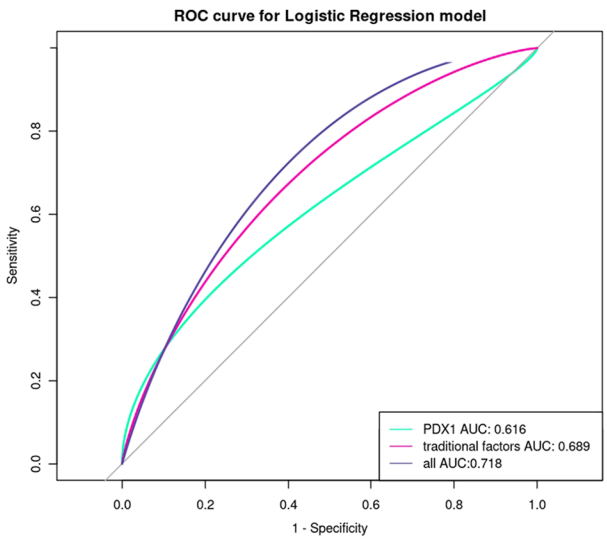

FIGURE 2
ROC curve analysis of the diagnostic value of PDX1 and traditional factors in GDM.

predominantly localized to the β-cells of the mature pancreas (27). Therefore, it can be said that PDX1 is a symbol of β-cells identity. A study showed that in PDX1 knockout mice, their β-cells mature poorly after birth and the expression of several β-cells related genes was impaired (28). The protein encoded by PDX1 activates the transcription of several genes essential for regulating glucose metabolism, such as insulin, glucokinase, somatostatin, and pancreatic amylin (29). PDX1 also increases insulin secretion indirectly by activating the transcription and expression of glucokinase and glucose-transporter 2 (9). Several important nuclear proteins, including MafA, HMGA1, and NeuroD1, play pivotal roles in maintaining pancreatic β-cell function. Notably, PDX1 exhibits synergistic effects with both NeuroD1 and MafA in regulating insulin biosynthesis. A study has shown that coordinated expression of these three transcription factors significantly upregulates insulin gene expression, promotes insulin synthesis

TABLE 4 Logistic regression analysis of PDX1 and GDM.

Items	β	SE	Wald χ2 value	OR	95% CI	P
Model 1						
Per 1 pg/mL	-0.021	0.009	4.954	0.979	0.092-0.998	0.026
PDX1<131pg/mL	Reference					
PDX1≥131pg/mL	-1.118	0.372	9.055	0.327	0.158-0.677	0.003
Model 2						
Per 1 pg/mL	-0.024	0.010	5.492	0.976	0.957-0.996	0.019
PDX1<131pg/mL	Reference					
PDX1≥131pg/mL	-1.248	0.406	9.466	0.287	0.130-0.636	0.002

Model 1: not adjusted; Model 2: adjusted for age, preconception BMI, family history of diabetes, smoking exposure, and alcohol consumption.

and secretion (30). Furthermore, HMGA1 can interact with PDX1 and MafA to enhance their transcriptional activation of the insulin gene promoter, thereby augmenting insulin production (31). Research reported that in adult pancreatic β-cells, short-term hyperglycemia enhanced the binding of PDX1 to the insulin gene, thereby increasing insulin mRNA levels. However, under the cytotoxic effects of prolonged hyperglycemia, both PDX1 and insulin levels decreased (32). In type 2 diabetes (T2DM), the expression levels of PDX1 are significantly compromised (33).

Considering the role of PDX1 in β-cell functionality, it is probable that PDX1 significantly contributes to the pathological process of GDM. Nasir I et al. discovered that prolactin could elevate the levels of PDX1 mRNA and protein in pancreatic islet cells of mice (34). A study showed that the high-fat diet during pregnancy in rats led to a significant reduction in PDX1 expression, damage to β-cells, and decreased insulin release (35). Furthermore, evidence from human studies further supports the close relationship between PDX1 and GDM. A study analyzing placental tissues from the fetal side demonstrated that the GDM group exhibited significantly reduced PDX1 mRNA expression levels compared to controls, and a negative correlation was observed between PDX1 mRNA levels and placental blood glucose levels (36). Additionally, another study investigated PDX1 mRNA expression in the peripheral blood of GDM patients and normal pregnant women. The results revealed that PDX1 mRNA expression was significantly lower in the GDM group (1.06 ± 0.18 vs. 1.35 ± 0.16, $P < 0.05$) and negatively correlated with neonatal blood glucose levels ($r = -0.390$, $P = 0.013$) (37). Our findings, which indicate lower serum PDX1 levels in GDM patients compared to those with normal glucose tolerance, are consistent

TABLE 5 Associations of PDX1 with adverse pregnancy outcomes.

Items	Model 1			Model 2		
	OR	95%CI	P	OR	95%CI	P
Pre-eclampsia						
Per 1 pg/mL	0.967	0.928-1.008	0.110	0.971	0.932-1.012	0.160
PDX1<131pg/mL	Reference			Reference		
PDX1≥131pg/mL	0.319	0.063-1.613	0.167	0.320	0.055-1.845	0.202
Fetal growth restriction						
Per 1 pg/mL	0.994	0.968-1.021	0.646	0.990	0.962-1.019	0.496
PDX1<131pg/mL	Reference			Reference		
PDX1≥131pg/mL	1.143	0.400-3.262	0.803	1.020	0.348-2.988	0.971
Preterm birth						
Per 1 pg/mL	0.974	0.948-1.001	0.059	0.971	0.942-1.000	0.049
PDX1<131pg/mL	Reference			Reference		
PDX1≥131pg/mL	0.353	0.122-1.026	0.056	0.326	0.104-1.021	0.054
Macrosomia						
Per 1 pg/mL	0.951	0.921-0.982	0.002	0.942	0.909-0.977	0.001
PDX1<131pg/mL	Reference			Reference		
PDX1≥131pg/mL	0.280	0.089-0.887	0.030	0.249	0.076-0.811	0.021
Neonatal respiratory distress syndrome						
Per 1 pg/mL	1.002	0.965-1.039	0.931	1.007	0.968-1.048	0.712
PDX1<131pg/mL	Reference			Reference		
PDX1≥131pg/mL	0.737	0.161-3.367	0.694	0.811	0.165-3.997	0.797
Composite adverse pregnancy outcomes						
Per 1 pg/mL	0.974	0.958-0.991	0.004	0.974	0.965-0.991	0.004
PDX1<131pg/mL	Reference			Reference		
PDX1≥131pg/mL	0.487	0.258-0.920	0.027	0.496	0.256-0.960	0.037

Model 1: not adjusted; Model 2: adjusted for age, preconception BMI, family history of diabetes, smoking exposure, alcohol consumption, and GDM.

with these studies. Although research on PDX1 expression in GDM remains limited, its role in other diseases has been explored. For instance, a study on pancreatic cancer utilized qRT-PCR to detect PDX1 transcripts in patient serum and reported significantly elevated PDX1 levels in pancreatic cancer patients compared to healthy controls (38). These findings suggest that PDX1 may serve as a potential biomarker across different pathological conditions, warranting further investigation into its role in GDM.

Under normal physiological conditions, PDX1 is a nuclear protein and is not secreted into the bloodstream (33). However, we found that PDX1 was present in serum in pregnant conditions. Firstly, hormones during pregnancy (such as prolactin) could elevate the levels of PDX1 mRNA and protein in pancreatic islet cells (34). Secondly, PDX1 might be secreted in extracellular vesicles (e.g., exosomes) under certain pathological stress or conditions (such as pregnancy). Thirdly, in cases of cellular stress, nuclear proteins like PDX1 could leak into the extracellular space and subsequently enter the bloodstream. Pregnancy is a special condition with stress and inflammation, during which PDX1 may present in serum. In this study, we found that the serum PDX1 levels in GDM patients were lower than those in women with normal glucose tolerance. This may be due to the damage to β cells caused by oxidative stress, inflammation, or autoimmune reactions, resulting in reduced release of PDX1. At the same time, there may be dysregulation of PDX1 gene expression, leading to decreased transcription or translation levels of PDX1, thereby reducing the release of PDX1 into the bloodstream. In addition, GDM patients may have impaired cellular secretion functions, resulting in decreased PDX1 secretion into the bloodstream via extracellular vesicles (such as exosomes). In this study, we also found that PDX1 levels were positively correlated with HOMA-β in pregnancy. HOMA-β serves as a crucial indicator for evaluating pancreatic β-cell function. Therefore, the levels of PDX1 might reflect the β-cell function in pregnancy.

This prospective cohort study first investigated the maternal serum PDX1 levels during pregnancy. The results showed that PDX1 in early pregnancy was negatively correlated with FPG, 2h PG, HOMA-IR, and TyG, while positively correlated with HOMA-β in mid-pregnancy. Moreover, the elevated PDX1 levels in early pregnancy were associated with reduced risks of GDM and adverse pregnancy outcomes. PDX1 had a modest predictive value for GDM. When PDX1 was incorporated into the predictive model for GDM, it slightly enhanced the predictive ability of traditional factors for GDM, but no significant statistical difference was observed ($P > 0.05$). Although the addition of PDX1 did not significantly augment the predictive value of conventional GDM risk factors, it offers a novel perspective for refining GDM prediction strategies.

Nevertheless, this study has several limitations. Firstly, the size of the sample was comparatively limited and exclusively drawn from an East Chinese population. We need a larger and more diverse sample to increase the persuasiveness of the findings. Secondly, we did not collect blood samples from pregnant women during childbirth, resulting in a lack of analysis of the complete trend of PDX1 throughout the pregnancy. Thirdly, we did not conduct follow-up monitoring after production, which prevented us from analyzing the long-term influence of PDX1 on the prognosis of GDM.

In summary, our results suggested that higher PDX1 levels in early pregnancy were associated with decreased risks of GDM and adverse pregnancy outcomes. It is suggested that PDX1 is significant for the early prediction of GDM and adverse pregnancy outcomes.

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 the Institutional Ethics Committee of Taizhou People's Hospital. 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

QiaZ: Investigation, Software, Writing – original draft, Writing – review & editing. QinZ: Data curation, Software, Writing – review & editing. SD: Data curation, Validation, Writing – review & editing. XL: Resources, Validation, Writing – review & editing. JW: Resources, Validation, Writing – review & editing. KL: Methodology, Supervision, Writing – review & editing. YL: Funding acquisition, Methodology, 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.2025.1486197/full#supplementary-material

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Bile acids and gestational diabetes mellitus: exploring the link and implications - a review

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Gestational diabetes mellitus (GDM) represents a prevalent metabolic disorder related to pregnancy, posing significant risks to both the expecting mother and the developing fetus. Recent research indicates a potential connection between bile acids (BAs) and GDM, such as lithocholic acid (LCA), β-muricholic acid (β-MCA), and 6,7-diketolithocholic acid (6,7-diketoLCA), have been found to be significantly increased in GDM individuals, thereby with the potential to reveal their involvement in glucose metabolism and the underlying mechanisms of GDM development. Additionally, BAs have emerged as vital signaling molecules that regulate glucose and lipid metabolism by interacting with Farnesoid X receptor (FXR) and Takeda G protein-coupled receptor 5 (TGR5), highlighting their potential as novel therapeutic targets for GDM management. The aim of this manuscript is to comprehensively review the current understanding of the relationship between BAs and GDM, delving into their potential mechanistic roles, diagnostic significance, and possible therapeutic applications.

KEYWORDS

gestational diabetes mellitus, bile acids, glucose homeostasis, offspring, therapeutic applications

1 Introduction

Gestational diabetes mellitus (GDM) classically denotes abnormal glucose tolerance that manifests or is first identified during pregnancy, featuring glycemia and insulin disorders (1). The worldwide prevalence of GDM is escalating at a rapid pace (2). This condition is not only linked to adverse perinatal outcomes (3) but also increases women's long-term risk of developing type 2 diabetes mellitus (T2DM) (4, 5) and metabolic syndrome (5, 6). Moreover, children born to mothers with GDM face a heightened risk of obesity, metabolic syndrome, future type diabetes (7), and brain development issues (8–10).

In recent years, numerous studies have delved deeper into the etiology and pathophysiology of GDM (11, 12). Emerging research has revealed a potential connection between bile acids (BAs) and GDM (13, 14) recently. BAs, amphipathic molecules synthesized in the liver from cholesterol and forming a crucial bile component (15), have traditionally been recognized for their role in the digestion and

absorption of dietary fats (16). However, modern perspectives view BAs as more versatile molecules with diverse functions (17), including promoting intestinal epithelial regeneration (18, 19), regulating gene expression (20, 21), influencing insulin secretion (22, 23), epigenetic mechanisms (24, 25), fibrogenesis (26), lipid metabolism (27)and glucose metabolism (28). Consequently, alterations in BAs are strongly linked to metabolic disorders.

2 Bile acid metabolism

BAs encompass both primary and secondary types, as outlined in Table 1. The biosynthesis of BAs commences with the formation of primary BAs, predominantly in the liver. This process involves a sequence of 17 enzymes, including cytochrome p450, which alter the steroid ring of cholesterol. These enzymes eliminate the short aliphatic side chain and conjugate it primarily with glycine (75%) and taurine (25%). The end result is the conjugated primary BAs, specifically cholic acid (CA) and chenodeoxycholic acid (CDCA) (29, 30). Secondary BAs come into being through enzymatic modification of primary BAs by colon-dwelling bacteria, which utilize them as substrates for microbial metabolism (31). The BA pool, encompassing all BAs circulating within the enterohepatic circulation, comprises BAs present in the intestine (~85%–90%), gallbladder (~10%–15%), and liver (<1%) (32). The ratio of glycine (G)- to taurine (T)-conjugated BAs stands at approximately 3 to 1, establishing a hydrophobic pool (32).

The synthesis of BAs can commence via several routes (Figure 1). The classic pathway involves the metabolism of cholesterol 7α-hydroxylase (CYP7A1) to form 7α-hydroxycholesterol, which is subsequently hydroxylated by sterol 12α-hydroxylase (CYP8B1) or sterol 27-hydroxylase (CYP27A1). Alternatively, the second (or alternate) pathway sees the formation of 27-hydroxycholesterol from cholesterol via CYP27A1, followed by hydroxylation via oxysterol 7α-hydroxylase (CYP7B1) (30). A third pathway involves the oxidation of cholesterol to 24- and 25-hydroxycholesterol by cholesterol 24-hydroxylase (CYP46A1), an enzyme predominantly expressed in the brain (33).

TABLE 1 The classification of bile acids.

	Unconjugated BAs	Conjugated BAs	
		+Taurine	+Glycine
primary BAs	CA CDCA	TCA TCDCA	GCA GCDCA
secondary BAs	DCA LCA UDCA HDCA	TDCA TLCA TUDCA THDCA	GDCA GLCA GUDCA GHDCA

BAs, bile acids; CA, cholic acid; CDCA, chenodeoxycholic acid; TCA, taurocholic acid; TCDCA, taurocholic acid; GCA, glycocholic acid; GCDCA, glycochenodeoxycholic acid; DCA, deoxycholic acid; LCA, lithocholic acid; UDCA, ursodeoxycholic acid; HDCA, hyodeoxycholic acid; TDCA, taurodeoxycholic acid; TLCA, taurolithocholic acid; TUDCA, tauroursodeoxycholic acid; THDCA, taurohyodeoxycholic acid; GDCA, glycodeoxycholic acid; GLCA, glycolithocholic acid; GUDCA, glycoursodeoxycholic acid; GHDCA, glycohyodeoxycholic acid.

3 Bile acids and glucose homeostasis

Recently, BAs have garnered attention due to their involvement in glucose metabolism and the secretion of glucoregulatory hormones (34, 35). Studies have shown that BAs regulate glucose homeostasis by directly interacting with the FXR (36) and the TGR5 (37, 38), or indirectly by promoting the synthesis of fibroblast growth factor 15 (FGF15) in the intestine, which is induced by FXR (39, 40). Specifically, certain BAs activate FXR in the intestine, triggering the production of FGF15/19 and enhancing the expression of pancreatic β cells (41). This mechanism exerts diverse effects on hepatic BA metabolism, lipid metabolism, protein metabolism, and glucose metabolism (42). Furthermore, BA-mediated TGR5 signaling boosts the release of intestinal glucagon-like peptide 1 (GLP-1), thereby increasing glucose-stimulated insulin secretion from pancreatic β cells (43). The receptors specific to BAs and the precise molecular mechanisms underlying their effects on glucose metabolism will be further explored (Table 2).

Given that different BAs exhibit unique affinities for FXR and TGR5, and they play varying roles in glucose metabolism, it becomes imperative to investigate whether the BA profile undergoes changes in patients with GDM. Determining the clinical significance of any such alterations is also crucial.

4 Bile acids in pregnant women with GDM and their offspring

The quantity of BAs differed significantly between mothers with GDM and those without. A study revealed that pregnant women with GDM had higher serum total bile acid (TBA) levels than their non-GDM counterparts during the first trimester (52). Notably, elevated serum TBA concentrations during pregnancy have been positively correlated with an augmented risk of GDM (13, 14).Although a causal relationship between GDM and serum TBA levels has not been conclusively established, it is apparent that GDM is often associated with higher serum TBA levels. Additionally, when maternal serum TBA levels surpass 40 mmol/L, the likelihood of fetal complications increases by 1%-2% for every additional mmol/L (53). Consequently, we postulate that altered serum TBA could be a potential influencing factor in the relationship between GDM and complications in offspring. However, some studies found no significant differences in TBA levels between GDM and non-GDM groups when measured in the second or third trimester (54). The substantial heterogeneity observed across studies, primarily attributable to variations in the timing of TBA measurement, suggests that the relationship between TBA levels and GDM is not straightforward. This indicates that the role of TBA as a biomarker for GDM may be highly sensitive to the gestational period during which it is measured (55).

Pregnant women with GDM not only encounter elevated serum TBA levels, but also demonstrate alterations in their BA profiles when compared to those without GDM. Research indicates that, in GDM pregnancies, serum concentrations of glycodeoxycholic acid

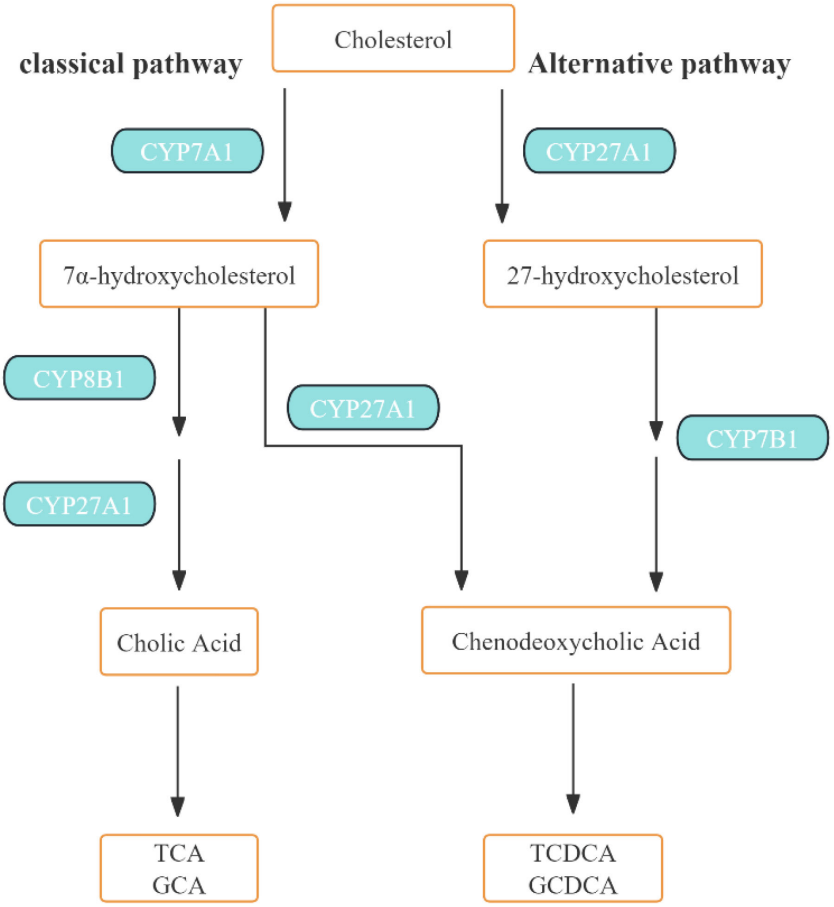

FIGURE 1
Bile Acid metabolism in liver. In the liver, cholesterol 7α-hydroxylase (CYP7A1) initiates the classical bile acid synthesis pathway by hydroxylation of the steroid rings at 7α-C for further modifications of the steroid rings, followed by steroid side chain oxidation and cleavage, whereas sterol 27-hydroxylase (CYP27A1) initiates the alternative bile acid synthesis pathway by oxidation of the steroid side chain followed by modifications of the steroid rings and cleavage of the side chain in the classic pathway. Cholic acid (CA) and chenodeoxycholic acid (CDCA) are the two major primary bile acids synthesized in the human liver.

(GDCA), taurodeoxycholic acid (TDCA), CA, dehydro-lithocholic acid (dehydro-LCA), and iso-deoxycholic acid (iso-DCA) are notably diminished (56). Conversely, certain BAs, such as glycohyodeoxycholic acid (GHDCA), taurohyodeoxycholic acid (THDCA), hyodeoxycholic acid (HDCA), LCA, β-MCA, and 6,7-diketoLCA, have been found to be significantly increased in GDM individuals (56). In summary, the modifications in BAs associated with GDM are intricate, underscoring the importance of understanding these changes to gain further insight into GDM.

Although numerous studies have established that the serum BA profiles of mothers with GDM undergo changes, the impact on fetal/neonatal serum BA profiles remains unclear. Recent studies have indicated that a higher prevalence of GDM among women with intrahepatic cholestasis of pregnancy (ICP), offering potential insights into this issue (57, 58). Previous studies have revealed that umbilical cord from ICP pregnancies exhibits elevated levels of CDCA, CA and LCA compared to controls (59). Based on these findings, we hypothesize that variations in BAs among GDM mothers may also lead to alterations in BA metabolism in their offspring. Furthermore, a study has documented significant changes in BA metabolism within the amniotic fluid (AF) during the second trimester of GDM-diagnosed pregnancies (60). Given that the AF primarily consists of fetal urine, this study lends credence to our hypothesis. However, direct evidence remains lacking and further investigation is warranted to elucidate the specific changes occurring.

5 Predictive value of BAs in GDM

Currently, the oral glucose tolerance test (OGTT) is widely regarded as the gold standard for diagnosing GDM (1). However, it is important to note that OGTT typically diagnoses GDM between 24–28 gestational weeks. By this time, irreversible fetal changes, such as epigenetic modifications (61), may have already occurred. Therefore, the identification of early predictors would be beneficial in improving the management of GDM and minimizing adverse outcomes for both the mother and the fetus.

ICP, characterized by elevated TBA levels, is strongly associated with an increased vulnerability to GDM (57, 58). This suggests a potential link between BA changes and the development of GDM.

TABLE 2 Effects of BAs and receptors on glucose metabolism and mechanisms.

BA Receptors	Function	Mechanism	References
FXR	Regulating hepatic glucose production and reducing serum glucose levels	Suppression of gluconeogenic genes, due to FXR activation of the transcriptional repressor SHP	Ma et al. (36)
		Protection from skeletal muscle lipotoxicity and improvement of peripheral insulin sensitivity, via FXR-dependent liver lipid metabolism	Ma et al. (36)
		Reduced weight gain due to adipose tissue browning, downstream of FXR-dependent alterations in BA composition	Fang et al. (44)
		Increased GLP-1 and insulin secretion, due to shifts in gut bacteria composition, which increase the TGR5 agonist TLCA	Pathak et al. (45)
		Increased secretion of FGF15 and/or FGF19, thereby repressing gluconeogenesis, and increasing glycogen synthesis and energy expenditure	Kir et al. (42); Potthoff et al. (46); Renga et al. (47)
		Expressed in human pancreatic β-cells and stimulates insulin gene transcription producing a positive control on glucose dependent insulin secretion	Renga et al. (47)
TGR5	TGR5 has a protective role in glucose homeostasis	TGR5 activation in enteroendocrine cells increases the release of GLP-1 which maintains homeostasis of blood glucose by promoting glucose-induced insulin secretion, suppressing glucagon release, delaying gastric emptying, promoting satiety, and increasing glucose disposal in the peripheral tissues	Cao et al. (48); Kuhre et al. (49); Lasalle et al. (50)
FGF15 and/or FGF19	Maintaining normoglycemia	Reduced hepatic gluconeogenesis, downstream of FGF15- and/or FGF19-dependent dephosphorylation of the gluconeogenic transcription factor CREB	Potthoff et al. (46)
		Increased hepatic glycogen synthesis, due to FGF15-/FGF19-dependent activation of an ERK-GSK3α/β phosphorylation cascade	Kir et al. (42)
		Reduced body weight and adiposity	Lan et al. (51)

BAs, bile acids; FXR, farnesoid X receptor; SHP, small heterodimer partner; GLP-1, glucagon like peptide 1; TGR5, takeda G-protein receptor 5; TLCA, taurolithocholic acid; FGF15, fibroblast growth factor 15; FGF19, fibroblast growth factor 19; CREB, Cyclic AMP-regulatory element-binding protein; ERK, extracellular signal–regulated protein kinase; GSK3α/β, glycogen synthase kinase 3α and 3β.

Based on this, we hypothesize that BAs could serve as valuable biomarkers for GDM diagnosis and risk stratification. Indeed, studies have shown that pregnant women with higher serum TBA levels during the first to second trimester face an increased risk of developing GDM. This indicates that TBA may represent a new risk factor for GDM (13), likely due to its correlation with insulin sensitivity (62). However, it's worth noting that Zhu et al. have found TBA levels to remain stable in the GDM group when compared to those with normal glucose tolerance (63). This discrepancy could be partially attributed to methodological differences, specifically the distinction between TBA measured by enzymatic cycling assay and individual BAs detected via mass spectrometry (MS). This finding underscores the importance of focusing on individual BA components related to glucose metabolism.

Individual BAs have emerged as promising biomarkers for the diagnosis and risk stratification of GDM (Table 3). Gao et al. have specifically highlighted β-MCA as a potential biomarker that can distinguish between GDM patients and healthy controls (54). Notably, β-MCA levels are elevated in GDM patients, possibly due to enhanced α-muricholic acid (α-MCA) C7-isomerase activity. This activity subsequently leads to increases in terminal GHDCA and THDCA levels through specific metabolic channels (54). GDCA, on the other hand, shows a significant decline in GDM patients. Its level is inversely correlated with insulin sensitivity and positively correlated with β-cell compensation, making it a valuable biomarker candidate for assessing these factors (63). Van Nierop et al. have indeed linked GDCA to insulin secretion and resistance, with increased GDCA triggering insulin secretion in a GLP-1-dependent manner (66). This explains why, despite an elevation in GDCA levels after glucose intake in GDM patients, the lower baseline GDCA levels are insufficient to promote insulin secretion via GLP-1, ultimately leading to glycemic dysregulation. Importantly, these markers have been identified post-diagnosis, and further studies are warranted to determine if they are altered in early pregnancy serum samples of women with GDM.

TABLE 3 The predictive value of BAs in GDM.

Predictive markers	The association with GDM risk	References
β-MCA	positive	Gao et al. (54)
GDCA	negative	Zhu et al. (63)
TCA	positive	Wu et al. (64)
LCA	negative	Wu et al. (64)
GUDCA	Negative (≤ 0.07 nmol/mL)	Li et al. (65)
DCA	Negative (≤ 0.28 nmol/mL)	Li et al. (65)

BAs, bile acids; GDM, gestational diabetes mellitus; β-MCA, β-muricholic acid; GDCA, glycodeoxycholic acid; TCA, taurocholic acid; LCA, lithocholic acid; GUDCA, glycoursodeoxycholic acid; DCA, deoxycholic acid.

Recent evidence also suggests that BAs could serve as early diagnostic marker for GDM. Circulating BAs levels during early pregnancy are associated with GDM risk. Specifically, taurocholic acid (TCA) is positively, while LCA negatively associated with GDM risk (64). Additionally, low serum levels of glycoursodeoxycholic acid (GUDCA) and deoxycholic acid (DCA) during early pregnancy are independently linked to an increased risk of GDM development (65). Secondary BAs are converted from primary BAs by gut microbiota (22), and an abnormal gut microbiome may reduce this conversion, particularly of GUDCA and DCA, which may contribute to the etiology of GDM. Furthermore, in a rodent model, an elevated serum CA concentration, coupled with reduced BA receptors, such as FXR and TGR5, is associated with GDM (67). Therefore, further validating the diagnostic value of these BA metabolites in the early stages of GDM through animal experiments holds significant promise for early and timely intervention in GDM, potentially reducing poor outcomes.

6 Potential values for BA intervention in the GDM

The treatment of GDM primarily aims to normalize hyperglycemia and mitigate the risk of unfavorable pregnancy outcomes. A crucial aspect of GDM management involves lifestyle interventions, such as dietary adjustments, physical activity, and weight control. If glycemic targets are not achieved through these interventions, it is necessary to introduce glucose-lowering pharmacologic therapy (68, 69). Although these treatments offer short-term benefits, their long-term effects on children exposed to antidiabetic medication during pregnancy remain uncertain. Hence, there is an urgent need for therapies that can improve both maternal and fetal glucose metabolism. BAs have emerged as vital signaling molecules that regulate glucose and lipid metabolism by interacting with FXR and TGR5 receptors (70–73). This suggests that therapeutic approaches targeting BAs could potentially be a powerful new strategy for GDM management.

The FXR agonist obeticholic acid (OCA) has been found to improve dyslipidemia and reduces the impact of pregnancy on insulin resistance in a mouse model of GDM, although it does not affect glucose tolerance (74). However, the limited effects of OCA in pregnant mice indicate that its agonistic action alone may not fully counteract the metabolic consequences of reduced FXR activity during pregnancy. Therefore, when considering FXR agonists for treating metabolic disorders during pregnancy, it is essential to consider the potential inhibition of FXR activity during gestation to ensure the safety of the pharmaceutical agent.

Studies have indicated that lower levels of GDCA are associated with increased risk of adverse pregnancy outcomes in GDM patients (63). Based on this, we hypothesize that GDCA supplementation may reduce these adverse outcomes, but further research is required to validate this hypothesis. Notably, UDCA has been shown to significantly lower fasting plasma glucose, hemoglobin A1c (HbA1c), and insulin concentrations, indicating a beneficial effect on glucose homeostasis (75). Preliminary data from studies involving UDCA treatment in women with ICP also suggest a reduction in insulin resistance (76). The study emphasizes that UDCA's potential as an effective therapy for improving maternal glycemia in GDM. Although direct evidence supporting UDCA's use in GDM treatment is lacking, some trial protocols have been designed (77), paving the way for future studies. Furthermore, animal studies have provided additional insights. For instance, mice fed a high-fat diet (HFD) exhibit elevated fasting glucose and a reduced BA pool size, but supplementation with CA improves insulin resistance (78). Another study found that secondary BAs exert a protective effect on pancreatic islet β-cells in diabetic rats (79).

BA sequestrants, which effectively disrupt the enterohepatic circulation of BAs and significantly reduce plasma cholesterol levels, provide evidence for a connection between BA and glucose metabolism (80). Numerous lipid-lowering studies have demonstrated that BA sequestrants, exemplified by colesevelam hydrochloride (81), cholestyramine (82) and colestilan (83), can also decrease plasma glucose and glycosylated hemoglobin levels. This suggests a potential role for these agents in the treatment of T2DM. Given the application of BAs in managing T2DM, it is reasonable to postulate that BAs may also hold promise in treating GDM. However, direct evidence supporting this hypothesis is currently lacking. Thus, further exploration into the therapeutic benefits of BA metabolites for GDM is crucial. While BA sequestrants demonstrate proven efficacy in T2DM management through TGR5/GLP-1 pathway (84, 85), their application in pregnancy warrants meticulous investigation. The placental transfer potential of BA sequestrants derivatives and their effects on fetal BA circulation remain undefined. The present study indicates that the use of BA sequestrants can impede the absorption of fat-soluble vitamins, such as vitamin K, potentially increasing the risk of neonatal cerebral bleeding (86), emphasizing the need for trimester-specific therapeutic development.

In summary, a novel approach to the treatment of GDM with BA has demonstrated significant potential. Evidently, future research should be directed towards three primary areas: first, conducting research on longitudinal BA profiling; second, performing randomized controlled trials (RCTs) of BA modulators; and third, investigating microbiome - BA interactions.

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CXL: Conceptualization, Investigation, Writing – original draft. CYL: Validation, Writing – review & editing. XL: Writing – review & editing, Conceptualization.

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Association between hypothyroidism and metabolic profile in gestational diabetes mellitus

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Introduction: Thyroid hormones exert many effects on glucose metabolism. Gestational diabetes mellitus (GDM) and hypothyroidism during gestation (HG) are the most common gestational endocrinopathies and seem to be associated. We therefore explored in women with GDM whether the presence of HG is associated with a different metabolic profile.

Materials and methods: We included 1,290 pregnant women with GDM [International Association of the Diabetes and Pregnancy Study Group (IADPSG)/ World Health Organization (WHO) criteria] and no history of hypothyroidism prior to pregnancy who had a measure of thyroid-stimulating hormone (TSH) and anti-thyroperoxidase antibodies during their hospital stay after GDM diagnosis. Patients with thyrotoxicosis and previous bariatric surgery were excluded. We evaluated concomitant blood pressure, fasting glycemia, insulinemia [with calculation of homeostatic model assessment for insulin resistance (HOMA-IR) index], glycated hemoglobin (HbA1c), and lipid profile according to the presence of HG (American Thyroid Association 2017 definition: TSH $\geq$ 4 mUI/L).

Results: The mean ($\pm$ standard deviation) age was 33 $\pm$ 5 years, the mean body mass index was 27 $\pm$ 5 kg/m^2, and 117 women (9%) displayed HG. HG was associated with higher HbA1c (5.35 $\pm$ 0.56% *vs.* 5.22 $\pm$ 0.52%, p = 0.009), even after adjustment for gestational age, age, and body mass index. TSH was also positively associated with HbA1c (p = 0.006) and HOMA-IR (p = 0.002). Patients with HG displayed less often

an early GDM, with their fasting glycemia before 24 weeks of amenorrhea being lower than that of patients with a TSH < 4 mU/L.
Conclusion: In our cohort of patients with GDM, women with HG showed higher HbA1c than those without and HOMA-IR was positively associated with the level of TSH.

KEYWORDS
gestational diabetes, hypothyroidism, TSH, thyroid, pregnancy

Introduction

Thyroid hormones are known to exert important effects on glucose homeostasis. These effects may be opposite according to the target organ, as they act as agonists of insulin in the muscle and as antagonists of insulin in the liver (1). Hypothyroidism has been shown to be associated with peripheral insulin resistance, which is characterized by reduced peripheral glucose utilization and, in addition, by a decrease in hepatic gluconeogenesis and glycogen synthesis (2).

In non-pregnant subjects, two studies reported an increased risk of type 2 diabetes in patients with hypothyroidism (3, 4). Furthermore, some studies have suggested that increasing thyroid-stimulating hormone (TSH) levels are associated with hyperglycemia and insulin resistance even in euthyroid patients (5, 6).

Hypothyroidism during gestation (HG) and gestational diabetes mellitus (GDM) are the most common endocrinopathies during pregnancy. Both conditions seem to be associated (7, 8). Moreover, having a TSH ≥4 mUI/L during pregnancy increases the risk of GDM independently from anti-thyroperoxidase antibodies (aTPO) status (9). The heightened risk may be attributed to the impact of hypothyroidism in exacerbating the physiologic gestational insulin resistance. It has been demonstrated that during the second half of pregnancy, the hormonal environment promotes a catabolic status in which there is a progressive increase in insulin resistance (10). In the presence of some pregestational conditions (i.e., obesity and advanced age), this insulin resistance may overcome the beta-cell capacity to increase insulin secretion and elicit a dysglycemic status, namely, GDM (10).

GDM was historically defined as any degree of glucose intolerance with an onset or first recognition during pregnancy. This definition has many limitations mainly because GDM is a heterogeneous condition.

According to the 2017 American Thyroid Association (ATA) guidelines on thyroid disease in pregnancy (11), an upper limit of normality (≈4.0 mUI/L for most TSH assays) should be used to diagnose HG in a pregnant patient. The presence or absence of positive tests for aTPO was suggested to be taken into account for treatment decision-making.

To the best of our knowledge, no studies have investigated the role of HG on glucose metabolism in women with GDM. The aim of our study was to correlate the presence of HG to metabolic parameters in a cohort of patients with GDM.

Materials and methods

Participant selection

The present retrospective, observational study was conducted at Jean Verdier University Hospital in a suburban area of Paris (Bondy), France. It was based on the electronic medical records of every woman who delivered between 1 January 2012 and 31 December 2018. Women were informed that their medical records could be used for research purposes unless they were opposed to such use; data were analyzed anonymously. Our database is registered in the French Committee for computerized data (Commission Nationale de l'Informatique et des Libertes, no. 1704392v0).

Exclusion criteria were no personal history of either pre-gestational diabetes or bariatric surgery and hypothyroidism. Inclusion criteria were the presence of GDM, age 18–50 years, singleton pregnancy, and measurement of TSH and aTPO during their hospital stay after GDM diagnosis. We then excluded the women with TSH level < 0.27 mUI/L.

Our policy was a universal screening of GDM at both the beginning of pregnancy and after 24 weeks of amenorrhea (WA) if previous screening either had been normal or had not been done. Early screening was based on fasting plasma glycemia (FPG) measurement, whereas late screening was based on a 75-g oral glucose tolerance test (OGTT) with measurement of fasting, 1-h, and 2-h plasma glucose levels. GDM was defined according to International Association of the Diabetes and Pregnancy Study Group (IADPSG)/World Health Organization (WHO) recommendations (12, 13), as these guidelines have been endorsed in France (14). We included both women with early fasting hyperglycemia (early-diagnosed GDM: FPG of 5.1–6.9 mmol/L before 24 WA) and patients with a pathological OGTT after 24 WA (FPG at 5.1–6.9 mmol/L and/or 1-h plasma glucose 10.0 mmol/L and/or 2-h plasma glucose at 8.4–11.0 mmol/L during

an OGTT) (14). Note that overt diabetes was defined as FPG ≥ 7 mmol/L or HbA1c ≥ 6.5%. In our department, after the diagnosis of GDM, the patient is invited to spend 1 day at hospital (DH), where she meets a diabetologist, a dietician, and a nurse, and a blood sample is taken. Women with HG received their DH workup later as compared to women without HG (30.7 ± 5.0 *vs.* 28.4 ± 5.6 weeks, $p \leq 0.001$), maybe because their screening after 24 WA was performed later too (27.8 ± 3.2 *vs.* 27.1 ± 3.1 WA, $p = 0.025$).

Blood pressures were measured after 10 min of resting.

Our local policy was a selective screening for HG according to ATA recommendations (15) at the first trimester, but first-trimester TSH values were not available in the dataset.

Laboratory assays

The serum levels of TSH and serum titers of aTPO were measured using electrochemiluminescence immunometric assay dedicated for cobas® e 601 analyzer (Elecsys TSH and aTPO assays, cobas®, Roche Diagnostics™, France). The sensitivity of the TSH and aTPO assays was 0.005 mIU/L and 5 IU/mL, respectively. According to TSH or aTPO levels, intra- and inter-assay coefficients of variation (CVs) reported by the manufacturer ranged from 1.3% to 11.1% and from 2.0% to 11.9% for the TSH assay, respectively. Intra- and inter-CV ranged from 2.8% to 4.8% and from 3.5% to 6.1% for the aTPO assay, respectively. Expected TSH serum levels range from 0.27 to 4.2 mUI/L. A borderline value of 34 IU/mL was defined for the aTPO assay.

Glucose values were measured on venous plasma using the enzymatic reference method with hexokinase (Cobas c 501 analyzer, Roche Diagnostics, France). Glycated hemoglobin (HbA1c) measurement was performed on hemolyzed whole blood using a turbidimetric inhibition immunoassay (c501 cobas®, Roche Diagnostics™, France).

The insulin level was measured in serum samples of some unselected women using the Roche Cobas electrochemiluminescence immunometric assay (Cobas e 601 analyzer, Roche Diagnostics, France). The intra-assay CV (repeatability) was 3.7% and the inter-assay CV (reproducibility) was 4.6%. The homeostatic model assessment for insulin resistance (HOMA-IR) index was calculated (16).

Total and high-density lipoprotein (HDL) cholesterol measurement was based on a colorimetric assay on the homogeneous phase and cholesterol dosage by cholesterol oxidase, measurement of triglycerides was based on a colorimetric assay, and low-density lipoprotein (LDL) cholesterol was calculated using the Friedewald formula. All these measurements were performed on plasma from fasting individuals using a Cobas 6000 analyzer (Roche Diagnostics, Meylan, France).

Adverse pregnancy outcomes

Levothyroxine therapy was prescribed in accordance with the 2011 ATA guidelines (15) if a TSH >2.5 or >3 mIU/L was found during or after the first gestational trimester, respectively. Because women with HG were eventually treated, the analysis of pregnancy outcomes by HG status was only exploratory.

Insulin treatment was prescribed only if, after 2 weeks of diet and physical activity, pre-prandial and/or 2-h post-prandial glucose levels were >5.0 mmol/L and/or 6.7 mmol/L, respectively, ≥3 times/week, as recommended by French guidelines (14).

Definitions of pregnancy outcomes are provided in previous publications (17–21). Gestational weight gain was defined as the weight measured before delivery minus self-reported pre-pregnancy weight.

Statistical analysis

Baseline continuous variables were expressed as mean ± standard deviation (SD). Categorical variables were expressed as frequencies (percentages). No data replacement procedure was used for missing data.

We analyzed the characteristics of the population according to the presence of HG defined as a TSH level >4 mU/L.

To compare the characteristics in the two groups (TSH ≤4 *vs.* >4 mUI/L), we used Student's *t*-test or the Mann–Whitney test for Gaussian and non-Gaussian continuous variables, respectively, and chi-squared (χ^2) or Fisher's exact test for categorical variables. We also evaluated TSH as a continuous variable and evaluated its association with metabolic parameters (FPG, HOMA-IR, and, in a subgroup of women, lipid profile and blood pressure) with linear regression. A multivariate linear model was designed including HbA1c and HOMA-IR as dependent variables and TSH (mUI/L), WA (weeks), BMI (kg/m^2), and age (years) as covariates.

All tests were two-sided. Analyses were conducted using the R 3.6.3 software (R foundation, Vienna, Austria, https://cran.r-project.org).

Results

Women characteristics

A total of 1,290 women (flowchart in Figure 1), 33 ± 5 years old, with a body mass index of 27 ± 6 kg/m², from multiple ethnicities were ultimately included in our observational study; their characteristics are shown in Table 1. Included patients had been admitted 1 day at hospital for education and care at 28.5 ± 5.6 WA, with a delay of 3.4 ± 3.3 weeks between GDM diagnosis and thyroid workup.

Percentage of HG and parameters associated with HG

A total of 117 women (9%) displayed HG. Table 1 shows that they were younger and with lower parity as compared to women without HG. Ethnicity also differed by HG status because of the

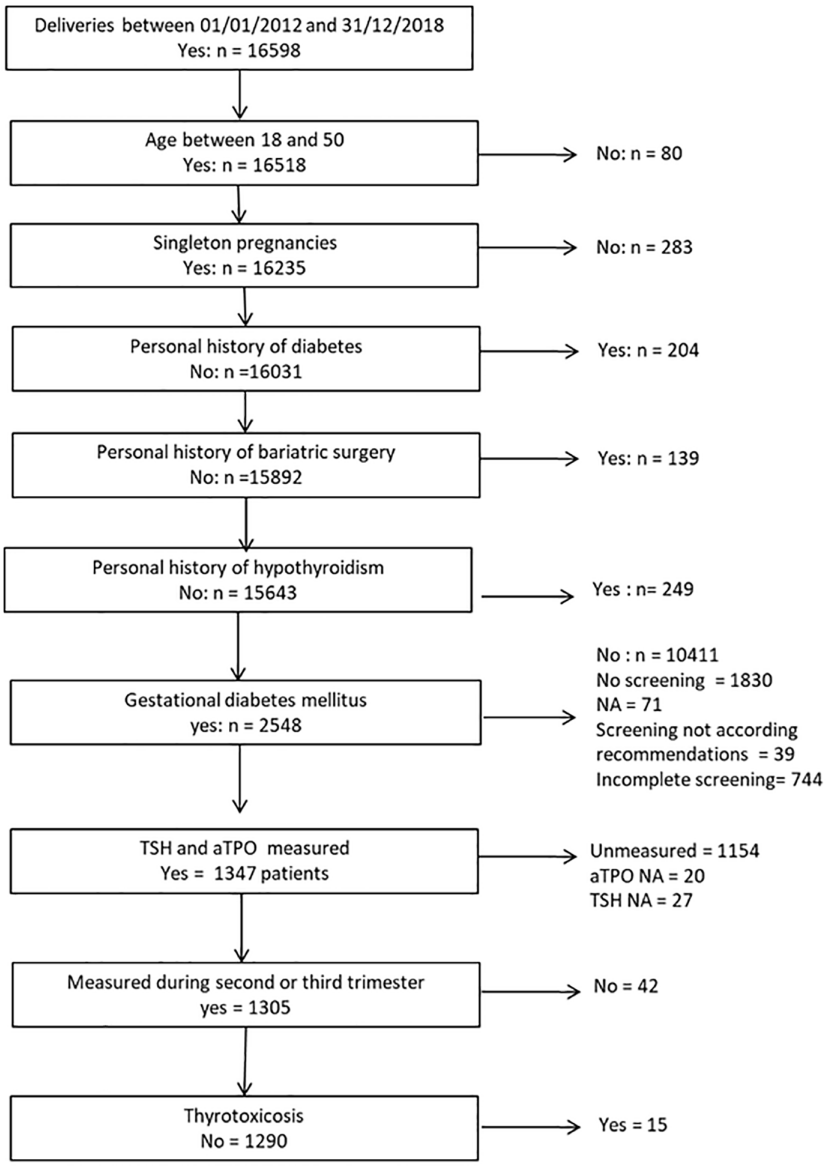

FIGURE 1
Flowchart of the study.

higher prevalence of women from India, Pakistan, Sri Lanka, and Haiti or DOM/TOM.

Table 1 also shows that women without HG more likely had an early-diagnosed GDM (29.6 *vs.* 17.1%, $p = 0.017$), and their FPG level before 22 WA was higher (5.2 ± 0.8 *vs.* 5.0 ± 0.5 mmol/L, $p = 0.0017$). Glucose profile at screening OGTT was similar in both groups.

Correlation between TSH and metabolic parameters at DH

As shown in Table 2, women with HG had a positive aTPO more frequently (16.2% *vs.* 5.3%, $p < 0.001$) and displayed slightly higher HbA1c (5.35 ± 0.6% *vs.* 5.2 ± 0.5%, $p = 0.0009$), even after adjustment for WA at DH, age, ethnicity, and BMI, as they were younger ($p = 0.0240$). No differences were found in terms of HOMA-IR. In a subgroup of women for whom these variables were available, lipids and blood pressure levels were similar by HG status.

When considering TSH as a continuous variable, we found a positive correlation between TSH and HbA1c ($p = 0.0058$) and HOMA-IR ($p = 0.002$), even after adjustment for WA at DH, age, and BMI ($p = 0.0240$, and $p = 0.002$, respectively, Table 3).

Discussion

The present study evaluates the association between metabolic parameters and TSH considered as both categorical (cutoff, 4 mUI/L) and continuous variables in a cohort of women with GDM.

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TABLE 1 Characteristics of population.

	Total (*N* = 1,290)	TSH [0.27–4.0] (*N* = 1,173)	TSH >4.0 (*N* = 117)	*P*
Age (years)	32.91 (5.40)	33.08 (5.31)	31.26 (5.98)	<0.001
Age (years), *n* (%)				<0.001
<30	354 (27.4%)	306 (26.1%)	48 (41.0%)	
≥30	936 (72.6%)	867 (73.9%)	69 (59.0%)	
BMI (kg/m^2)	27.29 (5.65)	27.35 (5.66)	26.75 (5.55)	0.2873
Ethnicity				<0.001
Sub-Saharian Africa	217 (16.8%)	199 (17.0%)	18 (15.5%)	
North Africa	446 (34.6%)	413 (35.2%)	33 (28.4%)	
Other	98 (7.6%)	89 (7.6%)	9 (7.8%)	
Europe	239 (18.6%)	230 (19.6%)	9 (7.8%)	
Haiti, DOM/TOM	60 (4.7%)	50 (4.3%)	10 (8.6%)	
Pakistan, India, Sri Lanka	228 (17.7%)	191 (16.3%)	37 (31.9%)	
Missing	2	1	1	
Family history of diabetes, *n* (%)				0.5665
No	836 (64.8%)	763 (65.0%)	73 (62.4%)	
Yes	454 (35.2%)	410 (35.0%)	44 (37.6%)	
Personal history of GD, *n* (%)				0.6148
1st pregnancy	378 (29.3%)	325 (27.7%)	53 (45.3%)	
No	693 (53.7%)	651 (55.5%)	42 (35.9%)	
Yes	219 (17.0%)	197 (16.8%)	22 (18.8%)	
Personal history of macrosomia, *n* (%)				0.3642
1st pregnancy	378 (29.3%)	325 (27.7%)	53 (45.3%)	
No	846 (65.6%)	786 (67.0%)	60 (51.3%)	
Yes	66 (5.1%)	62 (5.3%)	4 (3.4%)	
Personal history of fetal loss, *n* (%)				0.0706
1st pregnancy	219 (17.0%)	196 (16.7%)	23 (19.7%)	
No	1030 (79.8%)	936 (79.8%)	94 (80.3%)	
Yes	41 (3.2%)	41 (3.5%)	0 (0.0%)	
Smoking before pregnancy, *n* (%)				0.8528
No	1185 (91.9%)	1077 (91.8%)	108 (92.3%)	
Yes	105 (8.1%)	96 (8.2%)	9 (7.7%)	
Smoking during pregnancy, *n* (%)				0.5306
No	1231 (95.4%)	1118 (95.3%)	113 (96.6%)	
Yes	59 (4.6%)	55 (4.7%)	4 (3.4%)	
Parity	2.37 (1.27)	2.40 (1.27)	2.06 (1.24)	0.006
Fetal sex, *n* (%)				0.2470
Female	628 (48.7%)	577 (49.2%)	51 (43.6%)	

(Continued)

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TABLE 1 Continued

	Total (*N* = 1,290)	TSH [0.27–4.0] (*N* = 1,173)	TSH >4.0 (*N* = 117)	*P*
Fetal sex, *n* (%)				0.2470
Male	662 (51.3%)	596 (50.8%)	66 (56.4%)	
Diagnosis				0.017
Early GD	367 (28.4%)	347 (29.6%)	20 (17.1%)	
GD	835 (64.7%)	747 (63.7%)	88 (75.2%)	
Overt diabetes	88 (6.8%)	79 (6.7%)	9 (7.7%)	
WA of early screening *N* = 833	12.53 (5.77)	12.52 (5.90)	12.57 (4.27)	0.9312
Fasting glycemia (mmol/L) at early screening *N* = 807	5.17 (0.79)	5.19 (0.81)	4.97 (0.49)	0.0017
WA at OGTT *N* = 980	27.21 (3.10)	27.13 (3.08)	27.87 (3.20)	0.0247
Fasting glycemia (mmol/L) at OGTT *N* = 932	5.12 (0.73)	5.12 (0.73)	5.09 (0.78)	0.7055
1-hour glycemia (mmol/L) at OGTT *N* = 866	9.54 (2.01)	9.53 (1.99)	9.57 (2.17)	0.8665
2-hour glycemia (mmol/L) at OGTT *N* = 875	8.27 (1.99)	8.27 (2.00)	8.29 (1.93)	0.9255

BMI, body mass index; WA, week of amenorrhea; GD, gestational diabetes; OGTT, oral glucose tolerance test.

We found that women with HG displayed slightly higher HbA1c than those without and TSH levels were positively associated with HbA1c. These findings could be explained by a synergistic effect of HG and pre-gestational insulin resistance. Even if not associated with HG, HOMA-IR showed a correlation with increasing TSH without a cutoff. Only another study (22) explored HbA1c level in women with GDM according to the presence of euthyroidism or HG. It did not find any difference, maybe because the diagnosis of HG was made when TSH was ≥3 mUI/L and fT4 level was <0.76 ng/dL.

Together with the role of hypothyroidism in increasing peripheral insulin resistance, GH could promote the onset of GDM through an impairment of the placentation process (8). Indeed, the placenta is the main barrier between fetal and maternal environments and regulates fetal nutrition. Moreover, it has a central role in determining insulin resistance during pregnancy through its hormonal and cytokine secretion. Thyroid dysfunction and autoimmunity can cause alterations in the development of the feto-placental unit (23), as assessed by abnormalities in uterine artery pulsatility and in placental histology (23–25). Early-pregnancy hCG concentrations, which are reduced in abnormal placentation (26), are inversely related with GDM risk (27–29). These data suggest that placental abnormalities could be a possible physio-pathologic link between GH and GDM. In a small subgroup of women from our population where these parameters were available, no difference was found in terms of lipid and blood pressure levels. Indeed, a retrospective cohort study (30) evaluated the relationship between first-trimester thyroid function and lipid levels: as compared with the euthyroidism group, the hypothyroidism group (TSH > 3.52 mUI/L) had higher total cholesterol and LDL cholesterol levels; total cholesterol levels were positively correlated with TSH. The observed discrepancies between the former study and ours may be attributed to the varying gestational age when TSH measurement was performed.

In our study, women with HG were less likely to have an early-diagnosed GDM, because their FPG before 24 WA was lower as compared with women without HG. Actually, hypothyroidism is associated with reduced hepatic gluconeogenesis and glycogen synthesis. FPG did not differ between two groups after 24 WA neithr at OGTT during their hospital stay.

It was hypothesized that, since HG women displayed higher HbA1c levels than those without, they could require an increased insulin dosage, or even one that was initiated at an earlier stage in the pregnancy. This was not the case. Additional Supplementary Table 1 shows that the proportion of women needing insulin treatment was similar in the two groups. Insulin treatment was started later for women with HG probably because of late screening and subsequent DH. Only one study (31) evaluated the impact of HG on metabolic control in a GDM group of patients. The authors found that TSH was significantly associated with blood glucose levels and poor glycemic control but they did not provide treatment details.

We did not find any difference in terms of pregnancy outcomes, so the present exploratory results suggest that HG, when treated in some women, is not associated with adverse pregnancy outcomes. Nevertheless, we have to consider our results about pregnancy outcomes with caution as a number of women diagnosed with HG were treated with levothyroxine (our policy was to give

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TABLE 2 Hospital stay parameters according to the presence of hypothyroidism during gestation.

	Total (*N* = 1,290)	TSH [0.27–4.0] (*N* = 1,173)	TSH > 4.0 (*N* = 117)	*P*
WA at hospital stay	28.58 (5.60)	28.37 (5.62)	30.67 (5.03)	<0.001
Delay between OGTT and DH (weeks)	3.38 (3.31)	3.32 (3.33)	3.86 (3.11)	0.1261
Hospital stay trimester				<0.001
T2	461 (35.7%)	437 (37.3%)	24 (20.5%)	
T3	829 (64.3%)	736 (62.7%)	93 (79.5%)	
TSH (mUI/L)	2.27 (1.26)	1.99 (0.86)	5.05 (1.31)	<0.001
aTPO				<0.001
Negative	1209 (93.7%)	1111 (94.7%)	98 (83.8%)	
Positive	81 (6.3%)	62 (5.3%)	19 (16.2%)	
LT4 (>2.5 mUI/L at T1, >3 mU/L at T2 or T3)				<0.001
No	994 (77.1%)	994 (84.7%)	0 (0.0%)	
Yes	296 (22.9%)	179 (15.3%)	117 (100.0%)	
Fasting glycemia (mmol/L) at hospital stay *N* = 1,288	4.63 (0.78)	4.64 (0.78)	4.57 (0.81)	0.3534
HbA1c at hospital stay *N* = 1,287	5.23 (0.53)	5.22 (0.52)	5.35 (0.56)	0.0090
Insulin (mUI/L) *N* = 1,268	14.66 (10.26)	14.53 (10.26)	15.90 (10.17)	0.1730
HOMA-IR *N* = 1,266	3.13 (2.71)	3.11 (2.70)	3.37 (2.74)	0.3244
HDL-c (mmol/L) *N* = 243	1.74 (0.40)	1.74 (0.40)	1.74 (0.42)	0.9236
Non-HDL-c (mmol/L) *N* = 242	4.08 (1.13)	4.04 (1.14)	4.33 (1.05)	0.1951
Triglycerides, mmol/L *N* = 243	2.19 (0.87)	2.18 (0.88)	2.21 (0.79)	0.8591
DBP (mmHg) *N* = 990	68.10 (9.84)	68.07 (9.92)	68.46 (9.07)	0.7123
SBP (mmHg) *N* = 994	111.74 (11.30)	111.67 (11.39)	112.39 (10.45)	0.5630

WA, week of amenorrhea; LT4, levothyroxine treatment; DBP, diastolic blood pressure; SBP, systolic blood pressure; OGTT, oral glucose tolerance test.

levothyroxine in case of TSH ≥3 mUI/L after the first trimester, according to 2011 ATA recommendations). Indeed, treatment could have reset the metabolic differences between euthyroid and hypothyroid patients with GDM and have ameliorated pregnancy outcomes, masking HG adverse consequences. This is not consistent with the negative impact of HG in the first trimester, which has been shown to persist even after LT4 replacement (24, 32). The present study revealed that 9% of women with GDM exhibited HG. Assessing the prevalence of HG in women with GDM is also particularly challenging because the definitions and the indications for screening of both conditions have evolved throughout the years and vary worldwide. While several studies suggested that the prevalence of GDM could be increased in GH women (33–36), only few studies specifically assessed the prevalence of GH in GDM. A Pakistani group (31) found a prevalence of HG in GDM of 61.5% *vs.* 6%, $p < 0.001$, with 8.1% *vs.* 0% if only overt hypothyroidism is considered. This is unexpected, but it is a distinct population.

Vitacolonna et al. (37) did not find any difference in terms of TSH concentration or prevalence of HG in women with GDM. As in our study, the lack of data pertaining to the prevalence of HG in the non-GDM population constitutes a significant limitation in the interpretation of these findings.

Our study has several limitations. Firstly, this is a retrospective study. Secondly, as already mentioned, women with a TSH level ≥3 mUI/L after the first trimester were treated by levothyroxine replacement; thus, we could not draw conclusions about the role of HG on pregnancy outcomes in our GDM cohort. Thirdly, we did

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TABLE 3 Linear regression analysis.

Linear regression analysis				
	TSH			
	Regression coefficient	95% CI		P
Dependent variables		Lower	Upper	
HbA1c	0.183	0.053	0.313	0.006
HOMA-IR	0.040	0.015	0.066	0.002
WA at hospital stay	0.037	0.025	0.049	<0.001
Age	−0.028	−0.036	−0.011	<0.001
BMI	0.0006	−0.012	0.013	0.928
Multiple regression analysis using HbA1c and HOMA-IR as dependent variables and TSH (mUI/L), WA (weeks), BMI (kg/m^2), and age (years) as covariates.				
	HbA1c			
	Regression coefficient	95%CI		p
		Lower	Upper	
TSH	0.0262	0.0034	0.0489	0.0240
WA at hospital stay	0.0106	0.0055	0.0158	0.0001
Age	0.0070	0.0017	0.0123	0.0095
BMI	0.0192	0.0142	0.0242	<0.0001
	HOMA-IR			
	Regression coefficient	95% CI		p
		Lower	Upper	
TSH	0.1879	0.0690	0.3067	0.0020
WA at hospital stay	−0.0005	−0.0274	0.0264	0.9711
Age	0.0010	−0.0266	0.0287	0.9407
BMI	0.0987	0.0726	0.1249	<0.0001

WA, week of amenorrhea.

not have TSH levels in the first trimester; neither did we have fT4 levels at DH, but the increase in TSH in our population was mild (min–max: 4.01–13.83 mUI/L; median: 4.63 mUI/L; Q1, Q3: 4.25, 5.38 mUI/L) and overt hypothyroidism is not likely.

The strength of this study is that it shows that HG, known to be associated with an increased risk of GDM, may have a negative metabolic impact in the case of GDM, with TSH being associated with higher HbA1c and increased insulin resistance. Further studies are needed to prove the therapeutical implications of this metabolic profile.

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 Commission Nationale de l'Informatique et des Libertes. 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

SP: Writing – original draft. CN: Writing – original draft. LaC: Writing – review & editing. LiC: Writing – review & editing. AB: Writing – review & editing. EF: Writing – review & editing. MR: Writing – review & editing. EC: Writing – review & editing.

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Analysis of glucose metabolism outcomes 4–7 years postpartum in women with gestational diabetes mellitus using continuous glucose monitoring maternal risk factors: a Chinese cohort study

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Background: This study investigates glucose metabolism outcomes and glycemic variability in women with gestational diabetes mellitus (GDM) 4–7 years postpartum. It also identifies maternal risk factors for glucose metabolism abnormalities (GMA) to support early prevention strategies.
Methods: A bidirectional cohort study was conducted with 60 women with GDM and 60 without GDM, recruited from Peking University International Hospital between 2017 and 2019. Participants underwent oral glucose tolerance tests at 4–7 years postpartum and were categorized into GMA and normal glucose tolerance groups. Continuous glucose monitoring assessed glycemic variability, and logistic regression identified early pregnancy risk factors for postpartum GMA.
Results: (1) Women with a history of GDM have a higher incidence of GMA 4–7 years postpartum ($p< 0.001$). (2) They also showed increased cardiovascular risk factors 4–7 years postpartum, including diastolic blood pressure, body fat ratio, and interleukin-6 ($p<0.05$). (3) Blood glucose variability is significantly higher in all participants with a history of GDM, even in the normal glucose tolerance group. (4) Independent early pregnancy predictors of postpartum GMA included pre-pregnancy body mass index (BMI), the triglyceride-glucose index, and a history of GDM (AUC = 0.870, 95% CI: 0.808–0.931).
Conclusions: Women with a history of GDM are at a higher risk of GMA and glycemic variability 4–7 years postpartum. Pre-pregnancy BMI, the triglyceride-glucose index, and GDM history are strong predictors of postpartum GMA, highlighting the need for early intervention.Clinical trial registration: China Clinical Trials Registry, identifier ChiCTR2300067592.

KEYWORDS

gestational diabetes mellitus, postpartum period, continuous glucose monitoring, glycemic variability, risk factors

1 Introduction

Gestational diabetes mellitus (GDM) refers to hyperglycemia first detected during pregnancy that does not meet the diagnostic threshold for diabetes (1, 2). In recent years, the incidence of GDM has been steadily increasing, significantly impacting the long-term metabolic health of both mothers and their offspring. It has become a major global public health concern (3, 4). The Hyperglycemia and Adverse Pregnancy Outcome (HAPO) follow-up study reported that 52.2% of women with untreated GDM developed postpartum glucose metabolism abnormalities (GMA) (5). Studies have shown that women with a history of GDM have a 7-10-fold higher risk of developing postpartum GMA compared to those with normal blood glucose levels during pregnancy (6).

Evidence suggests that chronic low-grade inflammation persists after GDM, during which multiple physiological pathways are activated, exacerbating insulin resistance (IR). This further contributes to endothelial dysfunction, thereby progressively increasing the risk of GMA, hypertension, dyslipidemia, and atherosclerosis (7). This process may persist for several years or even decades, with insidious symptoms that make early detection challenging. In recent years, continuous glucose monitoring (CGM) has been recognized as a sensitive tool for the early detection of glucose metabolism dysregulation, potentially identifying metabolic changes before overt hyperglycemia becomes apparent (8). However, studies combining CGM with the traditional oral glucose tolerance test (OGTT) to assess the long-term prognosis of women with a history of GDM remain limited.

This study aims to evaluate the impact of GDM on postpartum 4–7 years glucose and lipid metabolism, glycemic variability (GV), and cardiovascular risk. Additionally, it seeks to identify maternal risk factors for postpartum GMA in women with GDM, develop a risk assessment model, and formulate early prevention and intervention strategies to provide a scientific basis for postpartum management of women with GDM.

2 Materials and methods

2.1 Participants

This study is a retrospective and prospective two-way cohort study. Based on a prospective cohort of pregnant women established at Peking University International Hospital from 2017-2019, from which GDM and non-GDM women meeting the inclusion and exclusion criteria were screened and matched 1:1 by age, gestational week, and parity, 120 consecutive participants were included to complete 4–7 years of postpartum follow-up (Figure 1). Participants underwent OGTT and were categorized into four groups: GDM-GMA group, Non-GDM-GMA group, GDM-normal glucose tolerance (NGT) group, and Non-GDM-NGT group. Additional tests assessed Hemoglobin (HbA1c), blood lipids, inflammatory factors, and cortisol levels, with CGM provided. Inclusion criteria: (1) age ≥18 years; (2) complete perinatal case data; (3) willingness to participate and consent to blood sample collection. Exclusion criteria: (1) pre-pregnancy diabetes and overt diabetes in pregnancy; (2) multiple pregnancies; (3) autoimmune diseases; (4) severe liver/kidney dysfunction; (5) long-term antidepressant/corticosteroid use; (6) use of hypoglycemic medications/insulin during follow-up. The research followed the guidelines of the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement.

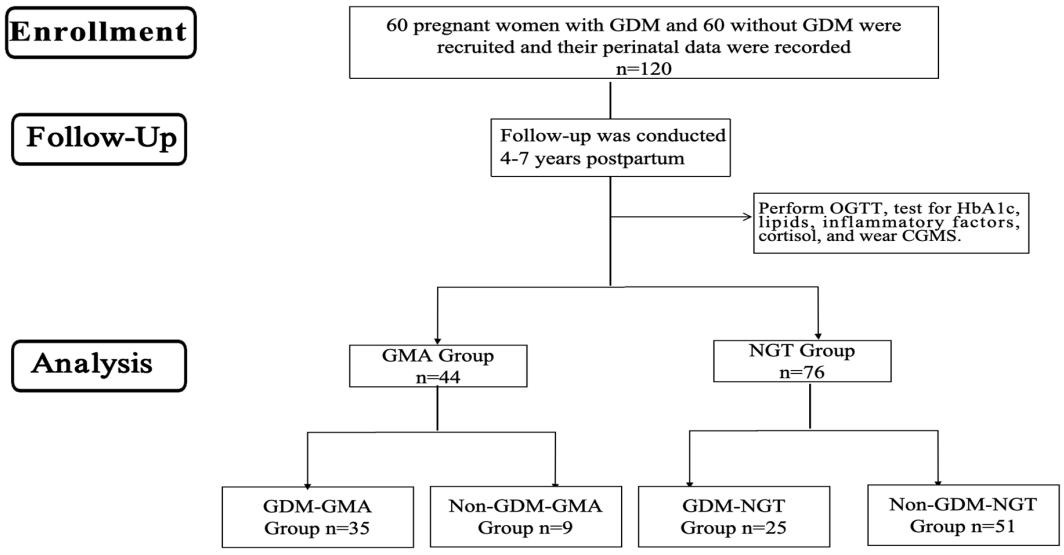

FIGURE 1
Flow diagram. GDM, Gestational diabetes mellitus; OGTT, oral glucose tolerance tests; HbA1c, glycosylated hemoglobin; CGMS, Continuous glucose monitoring systems; GMA, Glucose Metabolism Anomaly; NGT, Normal Glycemic Tolerance.

2.2 Sample size determination

The sample size calculation was based on previous literature parameters: the mean fasting blood glucose levels were 6.2 ± 1.9 mmol/L in the GDM group and 5.0 ± 1.6 mmol/L in the non-GDM group. Setting $\alpha = 0.05$ (two-sided) and $\beta = 0.10$, the required sample size for each group was calculated using PASS 11 software (independent samples t-test) to be 46 cases. Considering the 10% loss-to-follow-up rate, a minimum of 52 cases per group was required after correction. To ensure statistical efficacy, 60 cases per group were finally included in this study.

2.3 Perinatal information

Patient perinatal data was based on our previously established cohort (9), which was collected at the time of cohort creation and was available in the electronic medical record system.

2.4 Postpartum follow-up information

Basic information was collected from all participants, who were followed up 4–7 years postpartum. Anthropometric measurements, including systolic blood pressure (SBP), diastolic blood pressure (DBP), height, weight, body fat percentage (BFR), waist circumference, and hip circumference, were taken by the same researchers. Blood pressure was measured using an Omron electronic sphygmomanometer (model HEM-7201). Height and weight were measured using a Seca electronic height and weight scale (model 704). BFR was measured using bioelectrical impedance measurement (InBody 750). We gave each participant a retrospective ambulatory glucose monitoring system (Ipro2; Medtronic, Minneapolis, MN, USA) for data collection. Participants underwent fingertip glucose correction twice daily at fasting and bedtime, and the sensor was worn by 15:00 on the day of enrolment and for 7 consecutive 24-hour periods.

Venous serum samples were collected after fasting for 8 hours. The following measurements were made: total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), Lipoprotein (a)[Lp(a)], and sensitivity C-reactive protein (Hs-CRP). Cortisol (Cor) was collected at 9 am. HbA1c is determined by high-performance liquid chromatography. An OGTT was also performed: 75 g of glucose powder was dissolved in 250 mL of water and administered orally rapidly over 5 minutes. Fasting blood glucose (FBG), fasting insulin (FINS), 2-hour blood glucose, and 2-hour insulin levels after glucose administration were then tested. The above tests were performed in the laboratory of the Department of Laboratory Medicine of Peking University International Hospital, which strictly adheres to the health industry standards of the People's Republic of China for in-house quality control and has been certified by the National Center for Clinical Laboratories of China for external quality assessment.

Inflammatory factor detection: ELISA was used to detect the inflammatory factors in the serum of the study subjects, including interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), and tumor necrosis factor-β (TNF-β). The instrument used in this study was the MK3 ELISA kit (Thermo, America), which is Thermo's high-sensitivity human serum factor kit.

2.5 Definitions and calculations

The diagnostic criteria for GDM in this study were based on the criteria of the International Association of Diabetes and Pregnancy Study Groups (10). The history of GDM in the following text refers specifically to GDM diagnosed by OGTT performed at 24–28 weeks of this pregnancy.

Overt diabetes in pregnancy was defined as fasting blood glucose ≥126 mg/dl or 2-hour postprandial blood glucose ≥200 mg/dl (11).

The GMA encompasses Impaired Fasting Glucose (IFG), Impaired Glucose Tolerance (IGT), and Type 2 Diabetes Mellitus (T2DM). The diagnostic criteria for IFG, IGT, and T2DM adhere to the Chinese Guideline for Diabetes Prevention and Treatment and align with the 1999 World Health Organization (WHO) diagnostic standards (12).

Body mass index (BMI) was calculated as: weight (kg)/height^2 (m^2). Waist-to-hip ratio (WHR) was calculated as: waist/hip circumference.

TyG Index was calculated $\ln[\frac{TG(mg/dl)\times FBG}{2}]$; Homeostasis Model Assessment for IR (HOMA-IR) was calculated as: $\frac{FBG\times FINS}{405}$; Homeostasis Model Assessment for β-cell function (HOMA-β) was calculated as: $\frac{20\times FINS}{FBG-3.5}$; Matsuda index was calculated as: $\frac{10{,}000}{[(FBG\times FINS)\times(mean\ \ glucose)\times(mean\ \ insulin)]^{1/2}}$. In the above formula, blood glucose units are mg/dL, and insulin units are μU/mL.

2.6 Statistical analysis

Data were analyzed using SPSS 29.0 software. The Kolmogorov–Smirnov test assessed normality. Normally distributed data were expressed using the *mean ± standard* ($\bar{x} \pm s$), non-normally distributed data were expressed using the *median (interquartile range)*, and categorical variables were expressed using *absolute numbers and percentages*. Differences between the two groups were compared using the t-test, Mann-Whitney U test, and $\chi 2$ test. For multiple groups, one-way ANOVA, covariance ANCOVA, Kruskal-Wallis test, and $\chi 2$ test were used, with *post-hoc* comparisons via the Bonferroni method. A p-value<0.05 was considered significant. Binary logistic regression identified early pregnancy risk factors for postpartum glucose metabolism outcomes, and diagnostic performance was evaluated using the receiver operating characteristic (ROC) curve.

3 Results

3.1 Glucose metabolism outcomes in pregnant women 4–7 years postpartum and baseline

A follow-up study was conducted on women with GDM for 4–7 years postpartum, revealing that 58.3% (n=35) developed GMA.

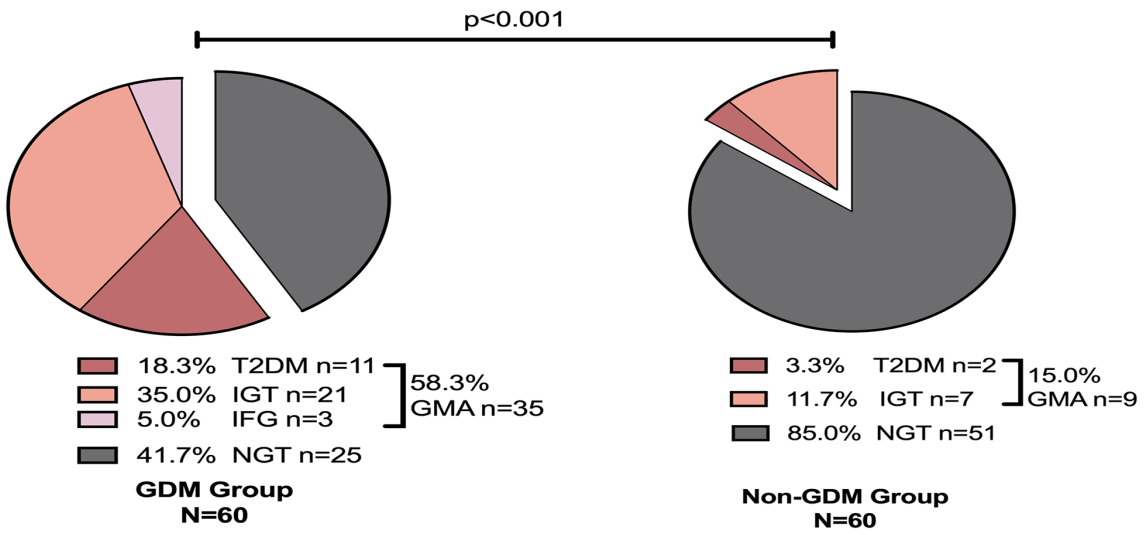

FIGURE 2
Glucose metabolism outcomes 4–7 years postpartum. GDM, Gestational diabetes mellitus; NGT, Normal Glycemic Tolerance; GMA, Glucose Metabolism Anomaly; T2DM, Type 2 Diabetes Mellitus; IGT, Impaired Glucose Tolerance; IFG, Impaired Fasting Glucose.

Among them, 18.3% (n=11) progressed to T2DM, 35.0% (n=21) developed IGT, and 5.0% (n=3) exhibited IFG, while 41.7% (n=25) maintained NGT. Follow-up in the non-GDM group showed that 15% (n=9) developed GMA. Among them, 3.3% (n=2) progressed to T2DM, 11.7% (n=7) developed IGT, and 85.0% (n=51) maintained NGT. No cases of IFG were reported in this group. Comparatively, the risk of developing GMA in the 4–7 years postpartum period was significantly higher in the GDM group (p<0.001) (Figure 2).

Table 1 summarizes the baseline characteristics of the four study groups (GDM-GMA, Non-GDM-GMA, GDM-NGT, and Non-GDM-NGT). Women with prior GDM, regardless of subsequent glucose metabolism status (GMA or NGT), exhibited significantly higher DBP compared to non-GDM groups [(75.25 ± 12.26,69.88 ± 7.25)vs. (66.55 ± 10.13,66.50 ± 6.99), p = 0.001]. BFR was elevated in the GDM group at 4–7 years postpartum[(34.12 ± 6.21,32.41 ± 5.37)vs. (30.15 ± 7.79,30.03 ± 6.01), p=0.049].IL-6 levels were significantly higher in the GDM group [(3.84 ± 2.27,3.67 ± 1.72)vs. (2.58 ± 1.58,2.52 ± 1.83), p=0.013]. The GDM-GMA subgroup showed elevated TC [(5.16 ± 0.86)vs. (4.79 ± 0.76,4.61 ± 0.62,4.48 ± 0.84),p=0.014], LDL-C[(3.12 ± 0.82)vs.(2.87 ± 0.47,2.73 ± 0.54,2.70 ± 0.72), p=0.040], and Lp(a)[(171.62 ± 99.81)vs.(124.45 ± 79.16,112.86 ± 72.43,111.55 ± 69.76),p=0.018]levels compared to other groups. Subjects with GMA (regardless of GDM history) demonstrated higher FBG compared to the NGT groups [(6.39 ± 2.21, 6.18 ± 2.60) vs. (5.26 ± 0.45,5.01 ± 0.47),p< 0.001]. HbA1c levels differed significantly only between GDM-GMA and Non-GDM-NGT groups (5.95 ± 1.12vs.5.42 ± 0.28,p = 0.001). GMA groups exhibited elevated cortisol levels [(10.50 ± 2.95,9.71 ± 3.42) vs.(8.21 ± 2.59,7.85 ± 3.80),p< 0.05] and increased IR indices: HOMA-IR [(4.84 ± 2.85,3.50 ± 2.76)vs.(2.38 ± 1.66,2.51 ± 1.50), p=0.004], TyG index [(2.08 ± 1.10,1.87 ± 1.22)vs.(1.41 ± 0.93,1.43 ± 0.91),p=0.019], and Matsuda index [(6.95 ± 4.35,9.59 ± 5.47) vs.(13.75 ± 7.46,15.01 ± 7.46),p<0.001].

3.2 CGM in pregnant women 4–7 years postpartum

Table 2 summarizes the CGM results for women 4–7 years postpartum. Regardless of GDM status, the GMA group had a higher mean blood glucose (MBG) level than the NGT groups [(6.31 ± 1.97,5.75 ± 0.59)vs. (5.35 ± 0.89,5.41 ± 0.51),p=0.004]. The GDM-GMA subgroup exhibited the highest maximum blood glucose (Max BG) levels among all groups[(11.28 ± 3.24)vs. (8.96 ± 1.56,9.66 ± 2.84,8.23 ± 1.23),p<0.001], even within the NGT group, women with prior GDM displayed elevated Max BG levels compared to their non-GDM counterparts(9.66 ± 2.84vs.8.23 ± 1.23, p< 0.001].

GMA subgroups with GDM history displayed significantly increased variability indices: Standard deviation (SD) (1.22 ± 0.52vs.0.76 ± 0.27,p<0.001), Coefficient of variation (CV) (19.71 ± 6.86vs.13.14 ± 3.79,p<0.001), Mean amplitude of glycemic excursions (MAGE) (2.88 ± 1.36vs.1.89 ± 0.87,p<0.001), Largest amplitude of glycemic excursions (LAGE) (7.54 ± 2.77vs.4.72 ± 1.70,p<0.001), Mean of daily differences (MODD) (1.08 ± 0.41vs.0.66 ± 0.26,p<0.001)and Average daily risk range (ADRR) (0.92 ± 0.29vs.0.39 ± 0.24,p<0.001). Even women with NGT but a history of GDM showed greater GV [SD(1.01 ± 0.49vs.0.71 ± 0.22, p<0.001), CV(18.93 ± 6.98vs.13.19 ± 4.31,p<0.001), MAGE (2.15 ± 0.75vs.1.61 ± 0.53,p<0.001), LAGE(6.56 ± 3.08vs.4.56 ± 1.71,p<0.001), MODD(0.87 ± 0.28vs.0.64 ± 0.19,p<0.001), ADRR (0.76 ± 0.28vs.0.39 ± 0.13,p<0.001)] over the 4–7 years postpartum. The GDM-GMA subgroup demonstrated the most pronounced SD, followed by GDM-NGT, then the non-GDM group[(1.22 ± 0.52)vs.

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TABLE 1 Baseline characteristics in pregnant women 4–7 years postpartum.

Characteristic	GDM-GMA	Non-GDM-GMA	GDM-NGT	Non-GDM-NGT	*F*	*P*
Age-offspring(years)	5.57 ± 0.96	5.67 ± 1.00	6.00 ± 1.04	5.98 ± 0.98	1.359	0.208
SBP (mmHg)	116.08 ± 17.61	109.11 ± 9.07	110.36 ± 10.26	109.02 ± 10.86	2.168	0.096
DBP (mmHg)	75.25 ± 12.26	66.55 ± 10.13*	69.88 ± 7.25*#	66.50 ± 6.99*&	5.557	0.001
BMI (kg/m^2)	24.42 ± 4.40	22.93 ± 5.89	22.39 ± 2.76	23.01 ± 2.98	1.738	0.163
Waist(cm)	84.00 ± 12.99	82.25 ± 11.89	79.52 ± 8.32	76.64 ± 7.42	2.173	0.095
WHR	0.87 ± 0.07	0.85 ± 0.07	0.84 ± 0.06	0.81 ± 0.04	2.861	0.054
BFR (%)	34.12 ± 6.21	30.15 ± 7.79*	32.41 ± 5.37#	30.03 ± 6.01*&	2.697	0.049
IL-6(pg/ml)	3.84 ± 2.27	2.58 ± 1.58*	3.67 ± 1.72#	2.52 ± 1.83*&	3.728	0.013
Hs-CPR (mg/L)	0.63 (0.40,1.60)	0.10 (0.10,2.22)	0.49 (0.29,0.94)	0.10 (0.10,0.40)	2.222	0.139
TNF-a(pg/ml)	10.51 ± 5.45	9.01 ± 5.39	10.17 ± 5.56	8.79 ± 4.78	0.556	0.645
TNF-β(pg/ml)	19.63 ± 6.60	13.64 ± 9.53	18.46 ± 7.21	13.21 ± 8.30	1.687	0.174
TC (mmol/L)	5.16 ± 0.86	4.79 ± 0.76*	4.61 ± 0.62*	4.48 ± 0.84*	3.680	0.014
TG (mmol/L)	1.67 ± 0.60	1.23 ± 0.93	1.00 ± 0.54	1.12 ± 0.61	2.045	0.111
HDL-C(mmol/L)	1.37 ± 0.30	1.29 ± 0.38	1.36 ± 0.30	1.37 ± 0.28	0.160	0.923
LDL-C(mmol/L)	3.12 ± 0.82	2.87 ± 0.47*	2.73 ± 0.54*	2.70 ± 0.72*	2.557	0.049
Lp(a) (mg/dl)	171.62 ± 99.81	124.45 ± 79.16*	112.86 ± 72.43*	111.55 ± 69.76*	1.639	0.018
FBG (mmol/L)	6.39 ± 2.21	6.18 ± 2.60	5.26 ± 0.45*#	5.01 ± 0.47*#	7.277	<0.001
HbA1c (%)	5.95 ± 1.12	5.53 ± 0.25	5.51 ± 0.30	5.42 ± 0.28*	3.999	0.001
Cor(ug/dl)	10.50 ± 2.95	9.71 ± 3.42	8.21 ± 2.59*#	7.85 ± 3.80*#	3.487	0.018
HOMA-β	122.31 ± 63.52	126.63 ± 76.18	145.03 ± 76.13	150.09 ± 109.87	0.734	0.534
HOMA-IR	4.84 ± 2.85	3.50 ± 2.76	2.38 ± 1.66*#	2.51 ± 1.50*#	4.765	0.004
TyG Index	2.08 ± 1.10	1.87 ± 1.22	1.41 ± 0.93*#	1.43 ± 0.91*#	3.464	0.019
Matsuda Index	6.95 ± 4.35	9.59 ± 5.47	13.75 ± 7.46*#	15.01 ± 7.46*#	11.011	<0.001

*p<0. 05*vs.*GDM-GMA Group; #p<0. 05*vs.*Non-GDM-GMA Group; &p<0. 05*vs.*GDM-NGT Group.
GDM, gestational diabetes mellitus; GMA, glucose metabolism anomaly; NGT, normal glycemic tolerance; SBP, systolic blood pressure; DBP, diastolic blood pressure; BMI, body mass index; WHR, waist-hip ratio; BFR, body fat rate; Cor, cortisol; FBG, fasting blood glucose; HbA1c, hemoglobin; TC, total cholesterol; TG, triglycerides; HDL-C, high-density lipoprotein cholesterol; LDL-L, low-density lipoprotein cholesterol; Lp(a), Lipoprotein (a); Hs-CRP, high sensitivity C-reactive protein; IL-6,interleukin-6; TNF-a, tumor necrosis factor-a; TNF-β, tumor necrosis factor-β; HOMA-β, homeostasis model assessment for β-cell function; HOMA-IR, homeostasis model assessment for insulin resistance; TyG, triglyceride glucose.

(1.01 ± 0.49)vs. (0.76 ± 0.27,0.71 ± 0.22),p<0.001]. Women with prior GDM (regardless of current GMA status) exhibited higher LAGE [(7.54 ± 2.77,6.56 ± 3.08)vs. (4.72 ± 1.70,4.56 ± 1.71), p<0.001]and ADRR[(0.92 ± 0.29,0.76 ± 0.28)vs.(0.39 ± 0.24,0.39 ± 0.13),p<0.001]compared to non-GDM groups. There was no statistically significant difference in GV between the non-GDM subgroups, regardless of whether they developed GMA (p< 0.001). After adjusting for postpartum BMI, the intergroup differences in glycemic variability parameters remained significant (all p< 0.05).

3.3 Baseline characteristics of pregnant women in the perinatal period

Table 3 compares the baseline characteristics of women in the GMA and NGT groups during the perinatal period. The pre-pregnancy BMI of the GMA group was significantly higher than that of the NGT group (25.52 ± 3.23 vs. 22.12 ± 3.12, p< 0.001). The uric acid (UA) level in the GMA group was higher than in the NGT group (233.59 ± 53.94 vs. 211.17 ± 58.47, p = 0.040). The incidence of GDM in the GMA group was significantly higher than that in the NGT group (79.5% vs. 32.9%, p< 0.001), and FBG was also elevated (5.21 ± 0.62 vs. 4.86 ± 0.40, p< 0.001). Similarly, the IR marker TyG index was significantly higher in the GMA group (1.27 ± 0.30 vs. 0.83 ± 0.49, p< 0.001) compared to the NGT group.

3.4 Analysis of maternal risk factors for the development of GMA in pregnant women 4–7 years postpartum

Figure 3 presents a logistic regression model with GMA as the dependent variable and the statistically significant indicators from

TABLE 2 Continuous blood glucose monitoring in pregnant women 4–7 years postpartum.

Metric Category	Characteristic	GDM-GMA	Non-GDM-GMA	GDM-NGT	Non-GDM-NGT	*F*	*P*
Glucose Levels	TIR (%)	94.49 ± 17.67	95.03 ± 11.01	97.78 ± 5.96	98.32 ± 0.94	1.269	0.288
	MBG (mmol/L)	6.31 ± 1.97	5.75 ± 0.59	5.35 ± 0.89*#	5.41 ± 0.51*#	4.738	0.004
	Max BG (mmol/L)	11.28 ± 3.24	8.96 ± 1.56*	9.66 ± 2.84*	8.23 ± 1.23*&	11.986	<0.001
	Min BG (mmol/L)	3.63 ± 1.64	3.84 ± 0.72	3.59 ± 0.82	3.66 ± 0.83	1.594	0.195
Glycemic Variability	SD (mmol/L)	1.22 ± 0.52	0.76 ± 0.27*	1.01 ± 0.49*#	0.71 ± 0.22*&	12.931	<0.001
	CV (%)	19.71 ± 6.86	13.14 ± 3.79*	18.93 ± 6.98#	13.19 ± 4.31*&	11.788	<0.001
	MAGE (mmol/L)	2.88 ± 1.36	1.89 ± 0.87*	2.15 ± 0.75*	1.61 ± 0.53*&	13.607	<0.001
	LAGE (mmol/L)	7.54 ± 2.77	4.72 ± 1.70*	6.56 ± 3.08#	4.56 ± 1.71*&	12.243	<0.001
	MODD (mmol/L)	1.08 ± 0.41	0.66 ± 0.26*	0.87 ± 0.28*	0.64 ± 0.19*&	16.664	<0.001
	ADRR (mmol/L)	0.92 ± 0.29	0.39 ± 0.24*	0.76 ± 0.28#	0.39 ± 0.13*&	26.232	<0.001

*p<0. 05*vs.*GDM-GMA Group; #p<0. 05*vs.*Non-GDM-GMA Group; &p<0. 05*vs.*GDM-NGT Group.
MBG, mean blood glucose; TIR, time in target range; SD, standard deviation; CV, coefficient of variation; MAGE, mean amplitude of glycemic excursion; LAGE, largest amplitude of glycemic excursions; Max BG, maximal blood glucose; Min BG, minimum blood glucose; MODD, mean of daily differences; ADRR, average daily risk range.

univariate analysis as independent variables. After adjusting for age, weight-add, parity and gestational weeks, the results showed that pre-pregnancy BMI (OR = 1.27; 95% CI: 1.04-1.50), a history of GDM (OR = 8.67; 95% CI: 2.91-25.77), and TyG index (OR = 8.17; 95% CI: 2.50-26.69) are independent risk factors for the development of GMA in women 4–7 years postpartum (p< 0.05).

Figure 4 evaluates the predictive performance of each indicator using ROC curves. The AUC for pre-pregnancy BMI was 0.723 (95% CI, 0.631-0.814), with a sensitivity of 71.1% and specificity of 63.6% at the optimal cutoff value of 23.015. The AUC for GDM was 0.733 (95% CI, 0.653-0.814), with a sensitivity of 67.1% and specificity of 79.5%. The AUC for the TyG index was 0.787 (95% CI, 0.705-0.869), with a sensitivity of 67.1% and specificity of 84.1% at the optimal cutoff value of 0.915. A predictive model named "Prediction" was established based on the three aforementioned risk factors. The AUC of this model was 0.870 (95% CI, 0.808-0.931), with a sensitivity of 73.7% and specificity of 88.6%. Decision curve analysis (DCA) further confirmed that this model offers the optimal clinical net benefit.

4 Discussion

This study found that women with a history of GDM had a significantly increased risk of developing GMA within 4–7 years postpartum (58.3% vs. 15.0%, p< 0.001). Among them, 18.3% developed T2DM, 35.0% had IGT, and 5.0% had IFG. These findings are consistent with the HAPO follow-up study, which also indicated that women with GDM remain at a higher risk of developing T2DM years after pregnancy (5). Furthermore, this study confirmed that women with a history of GDM had a 5- to 6-fold increased risk of developing postpartum T2DM, a finding consistent with the meta-analysis by Vounzoulaki et al (6). Several studies (13–15) have reported that the prevalence of GMA in women with GDM can range from 29% to 67% in the early (4–12 weeks) to mid-term (approximately 33 months) postpartum follow-up. In this study, the prevalence of GMA was 58.3% in women with GDM in the Chinese population up to 4–7 years postpartum, which is consistent with the trend of previous studies, and further revealed the cumulative effect of the risk at more distant follow-up. The results suggest that even with normal results on early postnatal glucose screening, women with GDM remain at significantly elevated metabolic risk over time, and the prevalence of GMA continues to increase over time. This finding further highlights the need to expand the metabolic management of the GDM population from short-term postnatal review to a systematic long-term follow-up mechanism for early warning and effective intervention of T2DM.

The GDM-GMA group exhibited a higher cardiometabolic risk, characterized by elevated DBP, BFR, IL-6, TC, LDL-C, and Lp(a) levels. Even in the NGT state, women with a history of GDM still exhibited higher cardiometabolic risk, primarily reflected in elevated DBP, BFR, and IL-6 levels. Studies have found that women with GDM maintain a heightened inflammatory state years after delivery, regardless of whether they develop postpartum GMA (16). The findings of this study, particularly the elevated IL-6 levels, further support this perspective. Participants in the GMA group exhibited higher FBG levels, accompanied by elevated cortisol levels. Additionally, women who developed GMA 4–7 years postpartum primarily exhibited greater IR, as indicated by higher HOMA-IR, TyG index, and Matsuda index, while β-cell function showed no significant difference between groups. Research suggests that the progression from GDM to T2DM and cardiovascular disease (CVD) in postpartum women is a dynamic process driven by shared pathogenic mechanisms, with chronic inflammation often being an early feature (17–20). The development of GDM may originate from an abnormal maternal immune adaptation to pregnancy and an upregulation of circulating inflammatory factors (21, 22), leading to immune pathway dysregulation. This, in turn, activates multiple metabolic

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TABLE 3 Baseline characteristics of pregnant women in the perinatal period.

Characteristic	GMA-Group		NGT-Group		*F/H/χ2*	*P*
Age(years)	31.40 ± 3.84		31.56 ± 3.49		1.580	0.820
SBP (mmHg)	112.18 ± 11.36		108.57 ± 11.88		0.408	0.107
DBP (mmHg)	68.68 ± 10.77		67.45 ± 14.05		0.518	0.616
BMI-pre (kg/m^2)	25.52 ± 3.23		22.12 ± 3.12		0.509	<0.001
GWG (kg)	11.80 ± 4.75		12.62 ± 4.20		0.124	0.327
Parity≥1	21	47.7%	33	43.4%	0.209	0.648
Gestational weeks	38.43 ± 1.48		38.28 ± 2.01		0.253	0.683
GDM	35	79.5%	25	32.9%	24.258	<0.001
WBC (10^9/L)	8.34 ± 3.47		7.24 ± 2.26		8.551	0.138
PLT (10^9/L)	250.05 ± 79.66		240.86 ± 45.03		2.229	0.421
HB(g/L)	130.13 ± 9.69		128.82 ± 11.46		0.880	0.526
NEU (10^9/L)	6.24 ± 2.17		8.39 ± 4.16		1.210	0.576
LYM (10^9/L)	1.85 ± 0.52		1.89 ± 0.55		0.045	0.733
MON (10^9/L)	0.41 ± 0.17		0.41 ± 0.15		1.885	0.893
Hs-CRP (mg/L)	0.37 (0.10,0.79)		0.27 (0.10,2.11)		-0.176	0.860
Ferritin(ng/ml)	61.80 (50.95,61.80)		61.80 (47.40,61.8)		-1.084	0.278
ALB(g/L)	43.27 ± 3.01		43.34 ± 2.42		1.256	0.887
ALT(U/L)	13.02 ± 5.47		12.68 ± 4.45		0.659	0.847
AST(U/L)	20.07 ± 10.01		19.19 ± 10.61		0.024	0.793
sCr(umol/L)	50.68 ± 9.04		50.40 ± 9.01		0.004	0.870
UA (umol/L)	233.59 ± 53.94		211.17 ± 58.47		0.051	0.040
Hcy(umol/L)	6.14 ± 1.89		6.16 ± 1.75		0.380	0.970
TSH (uIU/ml)	1.71 ± 1.01		1.69 ± 1.15		0.273	0.932
FT3(pmol/L)	4.69 ± 0.61		6.60 ± 1.54		2.184	0.413
FT4(pmol/L)	17.26 ± 2.08		17.63 ± 5.04		2.041	0.644
TPOAb(positive)	7	15.9%	10	13.2%	0.173	0.677
FBG (mmol/L)	5.21 ± 0.62		4.86 ± 0.40		3.857	<0.001
TC (mmol/L)	4.12 ± 0.66		3.85 ± 0.95		2.275	0.109
TG (mmol/L)	1.15 ± 0.92		1.07 ± 0.45		3.172	0.604
HDL-C(mmol/L)	1.39 (1.21,1.55)		1.39 (1.20,1.82)		-0.202	0.840
LDL-C(mmol/L)	2.20 (1.88,2.60)		2.09 (1.66,2.51)		-1.372	0.170
TyG Index	1.27 ± 0.30		0.83 ± 0.49		0.284	<0.001

GMA, glucose metabolism anomaly; NGT, normal glycemic tolerance; SBP, systolic blood pressure; DBP, diastolic blood pressure; BMI-pre, Pre-pregnancy body mass index; GWG, gestational weight gain; GDM, gestational diabetes mellitus; WBC, white blood cell count; PLT, platelet; HB, hemoglobin; NEU, neutrophil; LYM, lymphocyte; MON, monocyte; Hs-CRP, high sensitivity C-reactive protein; ALB, albumin;ALT, alanine aminotransferase; AST, aspartate transaminase; sCr, serum creatinine; UA, uric acid; Hcy, homocysteine; TSH, thyroid stimulating hormone; FT3, free triiodothyronine; FT4, free tetraiodothyronine; TPOAb, thyroid peroxidase antibody; FBG, fasting blood glucose; TC, total cholesterol; TG, triglycerides; HDL-C, high-density lipoprotein cholesterol; LDL-L, low-density lipoprotein cholesterol; TyG, triglyceride glucose.

pathways, promoting hyperinsulinemia and peripheral IR, accompanied by endothelial dysfunction and vascular lesions. Ultimately, this process progresses from glucose intolerance, hypertension, and dyslipidemia to atherosclerosis, and eventually to T2DM and CVD (7, 23–26). The findings of this study further confirm previous research while also identifying elevated Lp(a) levels, which may provide new insights into the atherosclerotic risk associated with GDM. In conclusion, the results of this study suggest that women with GDM may face an increased risk of CVD, further emphasizing the necessity of early intervention. It is

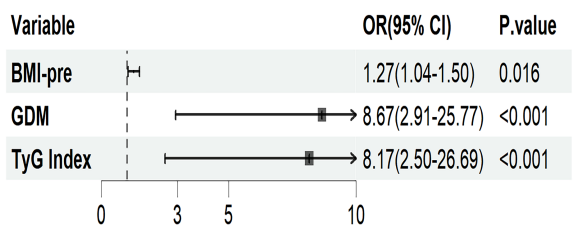

FIGURE 3
Logistic regression analysis of maternal risk factors for the onset of GMA in pregnant women 4–7 years postpartum. Adjusted for age, weight-add, parity, and gestational weeks. BMI-pre, Pre-pregnancy body mass index; GDM, gestational diabetes mellitus; TyG, triglyceride glucose; OR, odds ratio; CI, confidence interval.

recommended to enhance postpartum cardiovascular risk assessment and management to ensure continuous monitoring.

This study further revealed through CGM that GV was significantly elevated in the GDM subgroup. Even women in the GDM-NGT group exhibited greater GV (e.g., SD, CV, MAGE, LAGE, MODD, ADRR), suggesting that traditional HbA1c and OGTT may underestimate the early stages of metabolic dysregulation. This study found no statistically significant differences in TIR between groups, with glucose abnormalities primarily manifesting as increased GV. This may be because, in the 4–7 years postpartum period, IR is the predominant feature in women with GDM, while potential β-cell dysfunction has not yet become clinically evident. This characteristic aligns with the progression of T2DM (27). Studies have confirmed that GV is a core indicator of diabetes management, independent of HbA1c, and is closely associated with acute and chronic complications, cardiovascular risk, and patient quality of life (28–30). The potential mechanisms include GV accelerating β-cell apoptosis, exacerbating insulin secretion defects, and further promoting IR. Additionally, by increasing oxidative stress and inflammatory responses, GV may cause more severe endothelial cell damage than persistent hyperglycemia, accelerating atherosclerosis and leading to both microvascular and macrovascular complications. Moreover, it may also induce mitochondrial dysfunction, aggravating peripheral neuropathy. Previous studies have rarely focused on GV in postpartum women with GDM. This study provides new evidence through CGM, suggesting that CGM may serve as a more sensitive diagnostic tool than conventional OGTT for the early detection of metabolic abnormalities. The findings of this study support the perspectives of some researchers regarding the potential value of CGM in the early management of T2DM (8, 31). Furthermore, they suggest that CGM can serve as an early screening tool for identifying potential GMA, thereby reducing the long-term risk of T2DM and CVD.

This study found that pre-pregnancy BMI, the TyG index, and a history of GDM are independent predictors of GMA (AUC = 0.870). The predictive value of pre-pregnancy BMI (AUC = 0.723) aligns with global obesity trends (32, 33), further emphasizing the importance of pre-pregnancy weight management. The TyG index (AUC = 0.787), as a surrogate marker of IR (34), has particularly strong predictive value in Asian populations due to their heightened susceptibility to visceral fat accumulation. This finding also aligns with the previous research conducted by our group (9). This study developed a predictive model based on pre-pregnancy BMI, the TyG index, and a history of GDM, achieving an AUC of 0.870. The model demonstrated high predictive performance, providing a scientific basis for early intervention. For high-risk individuals with a pre-pregnancy BMI ≥23 kg/m², TyG ≥0.915, and a history of GDM, lifestyle interventions should be initiated as early as possible.

This study has certain limitations. Due to the single-center design and relatively small sample size, the generalizability of the study findings may be limited. Therefore, future multi-center, large-scale cohort studies are needed to further validate the stability and

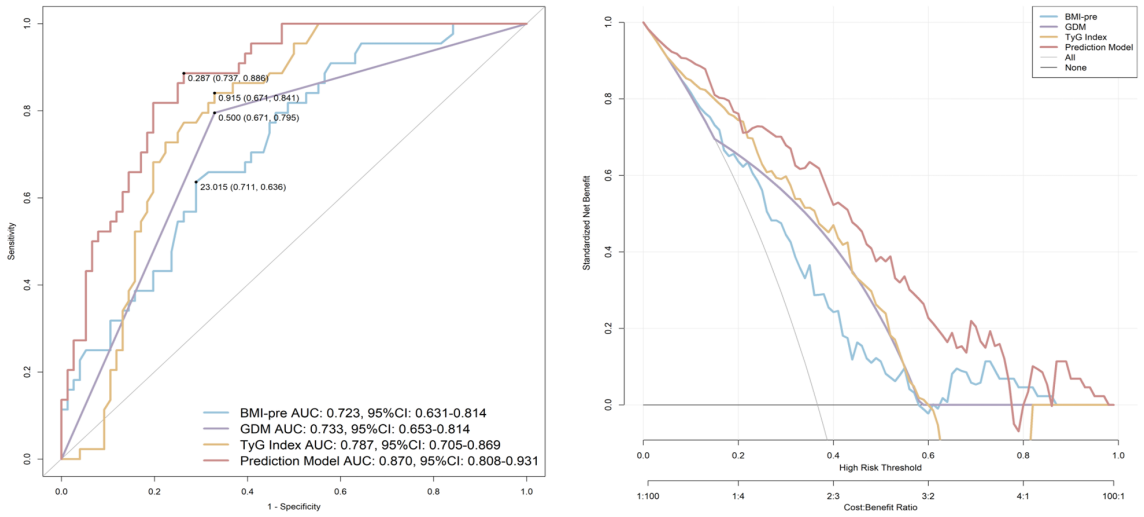

FIGURE 4
ROC curves to assess the predictive value of indicators for GMA 4–7 years postpartum. BMI-pre, Pre-pregnancy body mass index; GDM, gestational diabetes mellitus; TyG, triglyceride glucose; AUC, area under the curve; CI, confidence interval.

generalizability of these findings. The exclusion of potential influencing factors such as postpartum weight changes and breastfeeding duration may weaken the reliability of causal inferences to some extent. The current follow-up period of 4–7 years is still considered mid-to-short term. Thus, extending the follow-up period to over 10 years is necessary to comprehensively observe the natural progression of T2DM.

5 Conclusion

Women with a history of GDM exhibit greater GV within 4–7 years postpartum, accompanied by more pronounced cardiovascular risk factors. Pre-pregnancy BMI, TyG index, and a history of GDM are key independent predictors of GMA within 4–7 years postpartum. These findings underscore the critical role of continuous monitoring and early intervention in reducing the risk of long-term metabolic abnormalities and CVD.

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 Ethics Committee of Peking University International Hospital. 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

DZ: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Resources, Software, Validation, Visualization, Writing – original draft, Writing – review & editing. NY: Conceptualization, Data curation, Formal Analysis, Methodology, Software, Validation, Visualization, Writing – review & editing. XinZ: Formal Analysis, Software, Methodology, Validation, Visualization, Writing – review & editing. JS: Data curation, Investigation, Writing – review & editing. XX: Data curation, Investigation, Resources, Writing – review & editing. XiaZ: Conceptualization, 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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Comparison between 75-g and 100-g oral glucose tolerance tests using international association of diabetes and pregnancy study group one-step diagnostic threshold to detect gestational diabetes mellitus

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Background: The oral glucose tolerance test (OGTT) is the primary screening method for gestational diabetes mellitus (GDM), but global implementation criteria remain inconsistent.

Methods: This retrospective study analyzed data from 3,907 pregnant women at Tongchuan People's Hospital, including 1,925 in the 75g OGTT group (430 with GDM) and 1,982 in the 100g OGTT group (460 with GDM). A systematic comparison was conducted between the two groups regarding: blood glucose levels at each time point (0h, 1h, 2h);diagnostic rates, positive composition ratios of gestational diabetes mellitus, and risks of adverse maternal and neonatal outcomes based on the International Association of Diabetes and Pregnancy Study Groups (IADPSG) diagnostic criteria; Correlation analysis of blood glucose levels across time points; A glucose-level-adjusted continuous analysis to evaluate the dose-response relationship between dynamic glucose changes and adverse maternal and neonatal outcomes in the overall population.

Results: The 100g group had significantly higher 1h and 2h blood glucose levels than the 75g group ($p < 0.01$);Under the IADPSG criteria, there were no significant differences in GDM detection rates, positive case characteristics, or maternal-neonatal outcomes between the two groups ($p > 0.05$);Blood glucose levels at different time points were correlated within each group, no glucose rise difference occurred between groups at 0-1h [Difference in slope (95% CI): 0.127 (-0.092 to 0.346), p>0.05]. However, from fasting to 2h, the 100g group showed a steeper rise than the 75g group [Difference in slope (95% CI):0.412 (0.244 to 0.580), p<0.05], and a slower decline between 1-2h [Difference in slope (95% CI):0.047 (0.010 to 0.084), p<0.05].Glucose-adjusted continuous analysis showed that blood glucose levels were mostly associated with adverse outcomes, with the strength of association gradually decreasing from fasting to 1h and 2h. Both groups exhibited similar trends, no significant differences in

the risks of adverse outcomes (expressed as ORs) were observed between the 75g and 100g OGTT groups (all $p > 0.05$).

Conclusion: Under the IADPSG criteria, no significant differences in diagnostic efficacy were observed between the 75g and 100g OGTT glucose loads for GDM. Standardizing screening strategies to improve clinical consistency is warranted.

KEYWORDS

diagnostic accuracy, gestational diabetes mellitus, oral glucose tolerance test, adverse outcome, screening strategy, glucose dose

1 Introduction

Gestational diabetes mellitus (GDM) is a metabolic disease characterized by impaired glucose metabolism and is first detected or diagnosed during pregnancy. Its incidence increases with lifestyle and dietary changes. The prevalence of GDM is estimated at 9.3–25.5% worldwide (1, 2) and 9.3–18.9% in China (3, 4). Studies (5–7) have shown that GDM is associated with an increased risk of multiple adverse outcomes for both mother and baby, including cesarean section, neonatal hypoglycemia, and neonatal hyperbilirubinemia.

GDM is mainly diagnosed using the oral glucose tolerance test (OGTT), for which there is still a lack of consensus (8–10). There are two main strategies recommended internationally: the one-step strategy (2-h 75-g OGTT), which is recommended by the International Association of Diabetes and Pregnancy Study Group (IADPSG) (11), and the two-step strategy (50-g glucose loading test and 3-h 100-g OGTT), which is recommended by the American College of Obstetricians and Gynecologists(ACOG) (12). In addition to the two methods mentioned, other screening strategies are being used in some countries and regions (13–17). In mainland China, the IADPSG one-step 2-h 75-g OGTT was recommended to diagnose GDM by the Obstetrics Association of the Chinese Medical Association in 2014 (18). However, the latest version of the "National Guide to Clinical Laboratory Procedures, 4th edition (2014)" (19) was recommended by the National Health Commission of the People's Republic of China later in 2014. The procedure suggested a 100-g glucose dose to perform OGTT for pregnant women, but the blood collection time point and diagnostic threshold were not clear. As a result, some laboratories in mainland China, including Tongchuan People's Hospital, used the IADPSG one-step approach and the corresponding diagnostic threshold value to screen GDM for pregnant women, and the glucose load was 100 g. Although international recommendations for OGTT methods are inconsistent and lack the support or recognition of authoritative guidelines, the application of OGTT still exists objectively today. Evaluating these methods may play a positive role in the improvement of GDM screening strategies.

Abbreviations: BMI, body mass index; GDM, gestational diabetes mellitus; IADPSG, International Association of Diabetes and Pregnancy Study Group; OGTT, oral glucose tolerance test; OR, odds ratio; aOR, adjusted odds ratio.

This study employed a multidimensional analytical approach to systematically evaluate the following key metrics of 75g versus 100g OGTT: 1) Blood glucose levels at fasting (0h), 1h, and 2h post-load timepoints; 2) GDM screening performance based on IADPSG criteria, including diagnostic positivity rate, clinical characteristics of GDM population, and differential risks of adverse maternal-neonatal outcomes; 3) Correlation patterns of glucose values across different timepoints (0h, 1h, 2h); 4) Dose-effect relationship between dynamic glucose variations and adverse maternal-neonatal outcomes in the overall study population.

2 Materials and methods

2.1 Participant sources

OGTT data for GDM screening were available for 3,907 of 10,228 primiparas who gave birth in two districts of Tongchuan People's Hospital. This retrospective study covers the period from January 1, 2017, to September 30, 2022. The timeframe was selected based on comprehensive considerations including data availability, quality, consistency in clinical practice, and group sample size balance, with the aim of enhancing the scientific rigor and result reliability of the study. All primiparas who gave birth at the hospital during this period were enrolled, and their data were retrospectively analyzed using electronic medical records. Data extraction took place from April 16 to April 23, 2023.According to the actual screening strategy adopted, participants were divided into the 75-g and 100-g OGTT groups. Among these, the 75-g glucose dose recommended by the IADPSG was used in OGTT between October 1, 2019, and September 30, 2022, in the central southern campus, and between September 18, 2018, and September 30, 2022, in the northern campus. The 100-g glucose dose recommended in the guidelines was used in the OGTT experiments on the southern campus area from January 1, 2017, to September 30, 2019, and on the northern campus from January 1, 2017, to September 17, 2018. Women with maternal diabetes mellitus before pregnancy, multiple births, chronic kidney disease, and related endocrine diseases, such as hyperpituitarism, hyperthyroidism, and adrenal hyperfunction, were excluded from the study. The electronic medical records in this

study have clearly identified individuals who experienced vomiting, and we have verified and excluded all data from subjects who experienced vomiting through electronic medical record review.

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards. The study design was approved by the Ethics Committee of Tongchuan People's Hospital (approval number: TCSRMYY2022-01-03-005). The requirement for written informed consent was waived owing to the retrospective nature of the study. This retrospective study was conducted according to the STrengthening the Reporting of OBservational studies in Epidemiology guidelines. When we obtained the data, we obtained the patients' identifying information, including name, address, identification number, telephone number, clinical diagnosis and treatment information, various examination results, etc.; however, only age, sex, outcome, and treatment interventions are disclosed in the manuscript.

2.2 Main observation index

We obtained patient data from electronic medical records such as age, pregnancy duration at GDM screening and delivery, BMI at GDM screening and delivery and status of serum glucose management and treatment of GDM. Serum glucose levels at 0, 1 h, and 2 h time points during the 75-g and 100-g OGTT were analyzed. The correlations and regression lines for glucose levels (fasting vs. 1 h, fasting vs. 2 h, and 1 h vs. 2 h) were compared between the two groups. The GDM diagnosis rate and positive composition characteristics of the two groups were assessed using the IADPSG one-step diagnostic threshold. Further, 15 adverse maternal and 16 neonatal outcomes were evaluated. The 15 adverse maternal outcomes included abnormal fetal membranes, abnormal stage of labor, abnormal umbilical cord, abnormal amniotic fluid volume, placental abnormalities, cesarean section, cholestatic syndrome, dystocia, hypoproteinemia, perineal laceration, pregnancy-induced hypertension, poor uterine rejuvenation after childbirth, postpartum hemorrhage, and postpartum infection, as well as amniotic/chorionic abnormalities, induction of labor, postpartum fever, and postpartum anemia. The 16 adverse neonatal outcomes included abnormal fetal position, fetal distress, fetal growth restriction, low birth weight, large for gestational age, low Apgar score, macrosomia, neonatal cranial hematoma, neonatal asphyxia, neonatal hyperbilirubinemia, neonatal hypoglycemia, neonatal infection, neonatal respiratory distress syndrome, preterm delivery, small for gestational age, and stillbirth. These adverse outcomes are defined in Supplementary Methods 1.

2.3 GDM screening approach

GDM screening approaches were similar in the northern and southern regions of the hospital. Pregnant women maintained normal physical activity, a normal diet, and daily carbohydrate consumption of at least 150 g for 3 days before the test. Pregnant women fasted for 10–12 h on the day before OGTT (which was conducted no later than 9 am). During examination, the participants did not drink tea, drink coffee, smoke, or engage in strenuous exercise. OGTT was performed 2 h after ingesting a standard 75-g or 100-g glucose load.

2.4 Determination of serum glucose levels

Venous blood was collected in a procoagulant negative pressure tube, allowed to stand for 20 min, and centrifuged (3,000 rpm) for 5 min to separate the serum. The serum glucose level was detected using a Hitachi 008AS automatic biochemical analyzer (Toranomon, Minato-ku, Tokyo, Japan) in the south campus and a Hitachi 7,600 automatic analyzer (Toranomon) in the north campus. All procedures were completed within 2 h of blood collection. Hexokinase glucose detection reagents were produced by Ningbo Meikang Co., Zhejiang, China. The internal quality control data were controlled during the testing period. The external quality assessment data from the Shaanxi Provincial Clinical Laboratory Center and the Clinical Laboratory Center of the National Health Commission of China were qualified.

2.5 Diagnosis, management, and treatment of GDM

The diagnostic criteria for GDM in both groups were based on the 2010 IADPSG one-step screening method (11). Pregnant women were diagnosed with GDM if any of the following glucose thresholds were met: 0 h ≥5.1 mmol/L; 1 h ≥10.0 mmol/L; and 2 h ≥8.5 mmol/L. Pregnant women with GDM should undergo diet, exercise, and drug treatment according to the "Diagnosis and therapy guideline of pregnancy with diabetes mellitus (2014)" (20) (see Supplementary Methods 2 for details).

2.6 Statistical analyses

Data analysis was performed using SPSS Statistics 20.0 (IBM Corp., Armonk, NY, USA) for statistical computations and GraphPad Prism 8.0 (GraphPad Software Inc., La Jolla, CA, USA) for scatter plot generation. Continuous variables were assessed for normality via the Shapiro-Wilk test, with normally distributed data presented as mean ± standard deviation (mean ± SD) and compared using independent samples t-tests. Categorical variables were expressed as frequency (percentage), analyzed by chi-square tests. For OGTT glucose levels across timepoints (0h, 1h, 2h), intergroup comparisons were supplemented with Pearson correlation analyses and scatter plots.Employing a stratified analytical approach, we systematically evaluated 15 maternal and 16 neonatal adverse outcomes. In the GDM-positive cohort: 1) Potential determinants were screened through univariate analysis; 2) Multivariable unconditional logistic regression adjusted for baseline characteristics (age, pre-pregnancy BMI, gestational weight gain) to quantify outcome risk differences; 3)

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Log-linear modeling examined outcome interactions, with variance inflation factors (VIF <5) confirming absence of multicollinearity. For the full cohort, binary logistic regression modeled OGTT glucose levels (continuous) against adverse outcomes (dichotomous) to characterize dose-response relationships, adjusting for identical covariates. All analyses rigorously accounted for GDM diagnostic criteria and confounders—particularly excessive gestational weight gain per National Academy of Medicine standards (21, 22). Effects are reported as odds ratios (ORs) and adjusted odds ratios (aORs) with 95% confidence intervals. Statistical significance for primary outcomes was defined as $p < 0.05$ (two-tailed α=0.05). (Detailed protocols: Supplementary Methods 3).

3 Results

3.1 Baseline characteristics of the study population

After applying the exclusion criteria, this study included 1,925 pregnant women (430 with GDM) in the 75-g OGTT group and 1,982 pregnant women (460 with GDM) in the 100-g OGTT group. Maternal age, pregnancy duration at GDM, body mass index (BMI), and incidences of other abnormalities were calculated (Table 1). No significant difference was noted in these characteristics between the groups ($p > 0.05$). Similarly, pregnancy duration and BMI at the time of delivery showed no significant differences ($p > 0.05$; Table 1). There was no significant difference in serum glucose control among GDM-positive people between the groups ($p > 0.05$; Supplementary Table 1).

3.2 Comparison of serum glucose levels between the groups

There was no significant difference in fasting glucose levels between the two groups ($p > 0.05$). The serum glucose levels at 1 h and 2 h after oral glucose were significantly lower in the 75-g group than in the 100-g group ($p < 0.05$), as shown in Table 2.

3.3 Comparison of GDM diagnostic rates, positive composition ratio, and adverse outcomes between groups

Using IADPSG one-step criteria, no significant differences were observed in GDM diagnostic rates or positive case characteristics between groups ($p > 0.05$; Table 3). Similarly, maternal and neonatal adverse outcomes showed no significant differences ($p > 0.05$; Tables 4, 5). Given potential confounding by age, gestational age, BMI trajectory, and post-diagnosis interventions, we performed full covariate adjustment (Supplementary Table 2). Logistic regression analysis using the 75g group as reference demonstrated that the 100g group's risk profile for adverse outcomes (expressed as aORs) remained stable before versus after adjustment ($p > 0.05$; Tables 4, 5). In the GDM-negative population, there were no

TABLE 1 Intergroup differences in the baseline characteristics of the study population.

Characteristics	75-g OGTT, mean ± SD (n = 1,925)	100-g OGTT, mean ± SD (n = 1,982)	T/(χ^2)	*P*
Maternal age (years)	29.69 ± 4.02	29.55 ± 3.98	−1.141	0.254
Pregnancy duration at GDM screening (weeks)	26.02 ± 1.30	26.03 ± 1.34	0.246	0.806
BMI at GDM screening (kg/m^2)	23.37 ± 2.79	23.38 ± 2.81	0.124	0.902
Pregnancy duration of delivery (weeks)	39.07 ± 1.76	39.10 ± 2.02	0.525	0.599
BMI at the time of delivery (kg/m^2)	29.20 ± 2.63	29.32 ± 2.56	1.496	0.135
Incidence of other abnormalities* [% (n/n)]	1.87 (36/1,925)	2.12 (42/1,982)	0.309	0.648

t/χ^2: Student's *t*-test was performed for continuous variables and chi-square test for count data. *Other abnormalities included traumatic fractures, pregnancy with cholecystitis, pregnancy with pancreatitis, pregnancy with chronic nephritis, pregnancy with tuberculosis, and pregnancy with heart disease. The rate is expressed as a proportion (%; number of positives/total). The chi-square test was performed for the comparison of rates. BMI, body mass index; GDM, gestational diabetes mellitus; OGTT, oral glucose tolerance test.

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TABLE 2 Intergroup comparison of serum glucose levels at each time point.

Time point	75-g OGTT, serum glucose mean ± SD, mmol/L (n = 1,925)	100-g OGTT, serum glucose mean ± SD, mmol/L (n = 1,982)	*T*	*P*
Fasting	4.68 ± 0.49	4.69 ± 0.48	0.811	0.417
1 h	7.52 ± 1.90	7.72 ± 1.84	3.214	0.001
2 h	6.58 ± 1.50	6.75 ± 1.38	3.661	0.000

OGTT, oral glucose tolerance test.

significant differences in the risks of adverse outcomes between the 75g and 100g oral glucose tolerance tests, except for the "other" outcomes category ($p > 0.05$). Among those screened and diagnosed with GDM who received corresponding management, the risks of adverse pregnancy outcomes showed no significant difference compared to the GDM-negative group, except for cesarean delivery ($p > 0.05$). In contrast, the screened group demonstrated a statistically significant reduction in the risk of major adverse pregnancy outcomes compared to the unscreened group ($p < 0.05$); for detailed results, please refer to Supplementary Table 3.

3.4 Intergroup analysis of glycemic correlations

Significant positive correlations were observed between fasting vs. 1h, fasting vs. 2 h, and 1 h vs. 2 h blood glucose levels in two groups (see Supplementary Table 4). The effects of different glucose loads (75-g vs. 100-g) on glycemic kinetics demonstrated distinct phase-specific variations: During the fasting-to-1h phase, the rate of glucose elevation (slope) showed no statistically significant difference between the two groups [Difference in slope (95% CI): 0.127 (-0.092 to 0.346), p=0.254]; in the fasting-to-2 h phase, the 100-g group exhibited a significantly higher glucose elevation rate than the 75-g group [Difference in slope (95% CI): 0.412 (0.244 to 0.580), p<0.0001]; during the 1h-to-2 h phase, glucose decline occurred significantly more slowly in the 100-g group [Difference in slope (95% CI): 0.047 (0.010 to 0.084), p=0.013], see Figure 1.

3.5 Analysis of the continuous dose-response relationship between blood glucose levels and adverse outcomes in two groups

After adjusting for potential confounders, no significant differences were observed in the incidence of any adverse outcomes between the two groups (all $p > 0.05$; Tables 6, 7). The effects of glucose levels varied by timepoints.For example, for cesarean delivery risk, each 1 mmol/L increase in fasting glucose was associated with a 27.5% significantly higher risk (aOR=1.275, 95%CI:1.084-1.501, $p=0.003$), while 1-h postprandial glucose showed a 5.1% increased risk per 1 mmol/L (aOR=1.051, 95%CI:1.004-1.100, $p=0.032$), with no significant effect of 2-h glucose ($p = 0.649$); for macrosomia risk, although neither fasting (aOR=1.33, 95%CI:0.98-1.81, $p=0.072$), 1-h (aOR=0.99) nor 2-h glucose (aOR=0.97) reached statistical significance, the effect size and upper 95%CI limit of fasting glucose suggested potential clinical relevance. Detailed results for other adverse outcomes are shown in Tables 6 and Table 7.

4 Discussion

The international controversy regarding the standardization of GDM screening persists, primarily manifested in three aspects: First,

TABLE 3 Intergroup comparisons of the GDM diagnostic rate and positive composition ratio [%, (n/n)].

Positive modes (mmol/L)	75-g OGTT (%) (n = 430)	100-g OGTT (%) (n = 460)	χ^2	*P*
Only fasting ≥5.1	42.33 (182/430)	37.17 (171/460)	2.465	0.131
Only 1 h ≥10.0	11.63 (50/430)	12.39 (57/460)	0.122	0.758
Only 2 h ≥ 8.5	11.16 (48/430)	12.83 (59/460)	0.581	0.471
Fasting ≥ 5.1 and 1 h ≥ 10.0	7.21 (31/430)	8.70 (40/460)	0.669	0.458
Fasting ≥ 5.1 and 2 h ≥ 8.5	5.35 (23/430)	3.70 (17/460)	1.415	0.259
1 h ≥ 10.0 and 2 h ≥ 8.5	8.14 (35/430)	11.52 (53/460)	2.853	0.093
Fasting ≥ 5.1, 1 h ≥ 10.0, and 2 h ≥ 8.5	14.19 (61/430)	13.70 (63/460)	0.045	0.847
Total positive rate of GDM	22.34 (430/1,925)	23.21 (460/1,982)	0.421	0.517

GDM, gestational diabetes mellitus; OGTT, oral glucose tolerance test.

fundamental discrepancies exist in international guidelines—the IADPSG recommends the one-step 75g approach, while the ACOG advocates the two-step 50g+100g method, with significant differences in key parameters including glucose load, blood sampling timepoints, and diagnostic thresholds (13, 23, 24). Second, global implementation standards demonstrate regional variations: some countries rely solely on 2h glucose values while others incorporate both 1h and 2h measurements (14); within the United States alone, cutoff values for the 50g screening test vary between 7.2, 7.5, and 7.8 mmol/L across different states (23); and mainland China, while adopting the NDDG standard framework, employs IADPSG diagnostic cutoffs (13). Third, screening strategy selection is further influenced by multiple factors including regional epidemiological characteristics, healthcare resource allocation, and cultural acceptance (13, 14, 23). This global inconsistency in standards not only fuels diagnostic controversies regarding over- or under-diagnosis of GDM, but also severely compromises the comparability of epidemiological data, underscoring the urgent need for establishing internationally unified screening criteria. Against this backdrop, this study focuses specifically on evaluating differences between 75g and 100g glucose loads in OGTT-based GDM screening, aiming to provide evidence-based support for developing standardized protocols.

This study systematically evaluated the diagnostic performance of the 100g 2h OGTT for GDM screening and pregnancy outcome

TABLE 4 Intergroup comparison of maternal outcomes.

Maternal outcomes	Unadjusted		Adjusted※	
	OR (95% CI)	*P*	aOR (95% CI)	*P*
Abnormal fetal membranes	0.97 (0.68–1.40)	0.885	0.96 (0.67–1.40)	0.884
Abnormal stage of labor	0.93 (0.30–2.92)	0.906	0.97 (0.24–3.12)	0.901
Abnormal umbilical cord	0.97 (0.73–1.29)	0.828	0.98 (0.69–1.30)	0.830
Amniotic fluid volume abnormality	1.11 (0.69–1.79)	0.670	1.10 (0.41–1.79)	0.528
Cesarean section	0.86 (0.66–1.12)	0.260	0.89 (0.75–1.19)	0.301
Cholestatic syndrome	1.25 (0.52–3.00)	0.613	1.28 (0.48–3.01)	0.608
Dystocia	1.24 (0.64–2.41)	0.529	1.27 (0.79–2.45)	0.595
Hypoproteinemia	1.25 (0.52–3.00)	0.613	1.29 (0.68–3.02)	0.686
Perineal laceration	1.04 (0.76–1.42)	0.803	1.09 (0.69–1.48)	0.801
Pregnancy-induced hypertension	0.96 (0.57–1.62)	0.889	1.01 (0.84–1.19)	0.885
Placental abnormalities	1.28 (0.58–2.83)	0.535	1.34 (0.85–1.89)	0.517
Poor postpartum uterine rejuvenation	1.08 (0.58–1.99)	0.808	1.05 (0.62–1.94)	0.843
Postpartum hemorrhage	1.69 (0.74–3.86)	0.216	1.79 (0.91–2.95)	0.249
Postpartum infection	1.25 (0.52–3.00)	0.613	1.27 (0.48–3.01)	0.608
Other#	1.11 (0.49–2.50)	0.805	1.19 (0.71–2.57)	0.884

#Other conditions included amniotic/chorionic abnormalities, induction of labor, postpartum fever, and postpartum anemia. ※Adjusted for GDM and covariates associated with non-adherence: maternal age, BMI, pregnancy history, insulin treatment, and chronic hypertension. OR, odds ratio; CI, confidence interval; OGTT, oral glucose tolerance test.

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TABLE 5 Intergroup comparison of neonatal outcomes in progeny.

Neonatal outcomes	Unadjusted		Adjusted※	
	OR (95% CI)	*P*	aOR (95% CI)	*P*
Abnormal fetal position	1.10 (0.80–1.51)	0.560	1.11 (0.82–1.71)	0.561
Fetal distress	1.50 (0.49–4.64)	0.477	1.59 (0.21–4.75)	0.479
Fetal growth restriction	1.28 (0.58–2.83)	0.535	1.27 (0.55–2.20)	0.553
Low birth weight	1.07 (0.39–2.98)	0.898	1.05 (0.32–2.67)	0.891
Large for gestational age	0.93 (0.39–2.27)	0.879	1.00 (0.31–2.29)	0.892
Low Apgar score	1.70 (0.56–5.10)	0.347	1.81 (0.67–5.55)	0.374
Macrosomia	1.17 (0.71–1.92)	0.547	1.21 (0.74–1.93)	0.585
Neonatal cranial hematoma	1.15 (0.68–1.92)	0.607	1.14 (0.63–1.29)	0.603
Neonatal asphyxia	1.31 (0.41–4.17)	0.644	1.32 (0.84–4.21)	0.669
Neonatal hyperbilirubinemia	0.99 (0.72–1.36)	0.957	0.91 (0.65–1.01)	0.929
Neonatal hypoglycemia	1.22 (0.53–2.82)	0.639	1.29 (0.17–2.90)	0.801
Neonatal infection	1.02 (0.73–1.43)	0.906	1.05 (0.76–1.55)	0.959
Neonatal respiratory distress syndrome	1.25 (0.43–3.64)	0.681	1.16 (0.06–3.24)	0.620
Preterm delivery	1.38 (0.81–2.33)	0.237	1.41 (0.45–2.39)	0.298
Small for gestational age	1.41 (0.39–5.02)	0.598	1.61 (0.36–5.25)	0.601
Stillbirth	1.17 (0.31–4.39)	0.816	1.19 (0.35–4.41)	0.857

※Adjusted for GDM and covariates associated with non-adherence: maternal age, BMI, pregnancy history, insulin treatment, and chronic hypertension. OR, odds ratio; aOR, adjusted odds ratio, CI, confidence interval; OGTT, oral glucose tolerance test.

prediction, using the one-step 75g 2h OGTT recommended by the IADPSG as the reference standard. The results demonstrated that although the 100g group showed significantly higher postprandial glucose levels at 1h and 2h timepoints compared to the 75g group ($p < 0.05$, Table 2), no statistically significant differences were observed between the two groups in fasting glucose levels, GDM diagnosis rates, or clinical characteristics of GDM-positive individuals ($p>0.05$, Table 3). These findings likely reflect the physiological mechanisms of glucose homeostasis maintained through multi-organ coordination, including hepatic glucose metabolism regulation, compensatory insulin secretion, and peripheral tissue glucose uptake (24, 25). This suggests that the difference in glucose loads between 75-100g may not exceed the threshold required to disrupt the body's compensatory balance, thereby failing to induce significant metabolic disturbances. These results provide important physiological evidence for selecting appropriate OGTT glucose loads in clinical practice.

Current evidence demonstrates that clinical management of GDM exerts greater influence on pregnancy outcomes than screening method selection (26, 27). Our study revealed consistent clinical interventions between the two GDM groups, with potential confounders controlled through restriction to

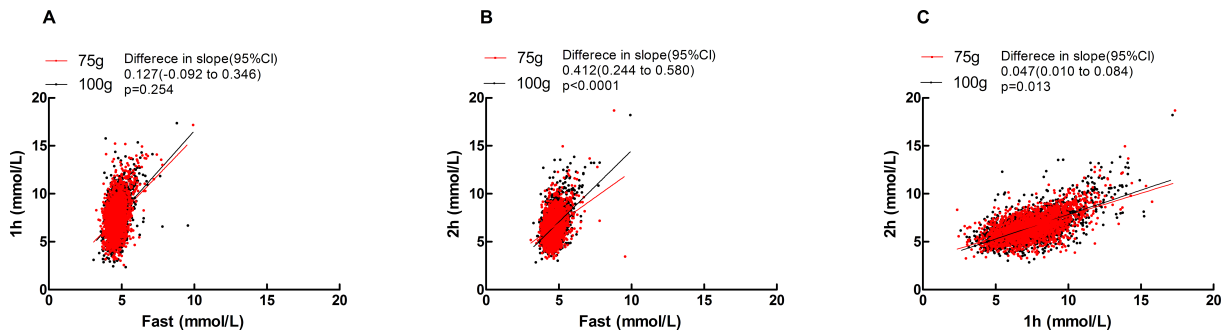

FIGURE 1
Scatters of fast Vs 1h, fast Vs 2h, 1h Vs 2h in two groups. **(A)**fast Vs 1h; **(B)** fast Vs 2h; **(C)** 1h Vs 2h. Solid lines represent regression fits for each group (red: 75 g group; black: 100 g group). Difference in slope was defined as the slope of the 100 g group minus that of the 75 g group.

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TABLE 6 Dose adjusted continuous analysis of the maternal outcomes (75g, n=1,925; 100g, n=1,982).

Outcomes	Variable	aOR (95% CI)	*P*
Cesarean section	groups	0.92 (0.81-1.05)	0.210
	fasting	1.28 (1.08-1.50)	0.003
	1hr	1.05 (1.00-1.10)	0.032
	2hr	1.01 (0.96-1.08)	0.649
Abnormal fetal membranes	groups	1.03 (0.87-1.23)	0.729
	fasting	1.01 (0.82-1.26)	0.904
	1hr	0.96 (0.90-1.02)	0.206
	2hr	1.08 (1.00-1.17)	0.054
Placental abnormalities	groups	1.03 (0.73-1.45)	0.883
	fasting	0.71 (0.45-1.12)	0.137
	1hr	1.07 (0.95-1.21)	0.259
	2hr	0.95 (0.81-1.11)	0.500
Abnormal umbilical cord	groups	0.93 (0.81-1.06)	0.272
	fasting	1.04 (0.88-1.24)	0.656
	1hr	0.99 (0.95-1.04)	0.779
	2hr	0.98 (0.92-1.04)	0.453
Amniotic fluid volume abnormality	groups	0.73 (0.55-0.97)	0.029
	fasting	1.21 (0.87-1.68)	0.256
	1hr	1.02 (0.93-1.13)	0.638
	2hr	1.01 (0.89-1.14)	0.931
Abnormal stage of labor	groups	0.33 (0.06-1.82)	0.204
	fasting	2.44 (0.71-8.38)	0.156
	1hr	0.70 (0.41-1.20)	0.192
	2hr	0.90 (0.45-1.82)	0.774
Dystocia	groups	0.99 (0.74-1.32)	0.938
	fasting	0.90 (0.62-1.30)	0.565
	1hr	0.91 (0.83-1.01)	0.084
	2hr	1.10 (0.96-1.25)	0.162
Pregnancy-induced hypertension	groups	1.65 (1.12-2.42)	0.011
	fasting	0.96 (0.58-1.59)	0.885
	1hr	1.00 (0.87-1.14)	0.969
	2hr	0.94 (0.79-1.12)	0.486
Cholestatic syndrome	groups	1.08 (0.57-2.06)	0.814
	fasting	0.55 (0.24-1.25)	0.154
	1hr	1.26 (1.02-1.57)	0.036
	2hr	0.99 (0.75-1.31)	0.939
Perineal laceration	groups	0.91 (0.78-1.06)	0.211
	fasting	0.92 (0.76-1.12)	0.391

(Continued)

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TABLE 6 Continued

Outcomes	Variable	aOR (95% CI)	*P*
	1hr	0.94 (0.89-0.99)	0.017
	2hr	1.04 (0.97-1.12)	0.262
Postpartum hemorrhage	groups	1.06 (0.45-2.51)	0.887
	fasting	0.44 (0.15-1.29)	0.135
	1hr	1.28 (0.97-1.70)	0.086
	2hr	1.02 (0.71-1.46)	0.934
Postpartum infection	groups	0.86 (0.37-2.01)	0.727
	fasting	1.43 (0.62-3.28)	0.403
	1hr	0.86 (0.64-1.15)	0.313
	2hr	1.25 (0.88-1.77)	0.221
Poor postpartum uterine rejuvenation	groups	1.14 (0.87-1.50)	0.348
	fasting	0.83 (0.58-1.19)	0.304
	1hr	1.07 (0.97-1.18)	0.173
	2hr	0.91 (0.80-1.04)	0.151
Hypoproteinemia	groups	0.34 (0.19-0.61)	0.000
	fasting	0.87 (0.46-1.66)	0.673
	1hr	0.89 (0.74-1.06)	0.192
	2hr	1.08 (0.85-1.37)	0.521

Adjusted for GDM and covariates associated with non-adherence: maternal age, BMI, pregnancy history, insulin treatment, and chronic hypertension. aOR, adjusted odds ratio; CI, confidence interval; OGTT, oral glucose tolerance test.

primiparous women and adjustment for covariates including BMI trajectory. Notably, GDM and excessive gestational weight gain exhibited significant interaction effects on both cesarean delivery rate and gestational hypertension incidence ($p < 0.05$; Supplementary Table 2). After comprehensive adjustment, both groups showed comparable risks of adverse outcomes ($p>0.05$, Tables 4, 5). In the GDM-negative population, no statistically significant differences were observed in the risks of adverse outcomes between the 75g and 100g oral glucose tolerance tests, except for the "other" outcomes category (Supplementary Table 3). This indicates that under the IADPSG criteria, the two OGTT loads have comparable predictive value. The observed difference within the "other" category may be due to the limited sample size, and further validation in larger studies is warranted.

Under a unified diagnostic criterion—that is, using identical glucose thresholds and cut-off values—the volume of the OGTT glucose load (75g versus 100g) does not significantly impact the diagnostic efficacy for GDM or alter the risks associated with adverse pregnancy outcomes. This result aligns with existing literature emphasizing the central importance of diagnostic thresholds (reference 14). Moreover, among those diagnosed with GDM through screening and subsequently managed, the risks for most adverse outcomes did not differ significantly from those in the GDM-negative population (Supplementary Table 3), highlighting the effectiveness of systematic GDM management. However, the higher rate of cesarean delivery observed in the GDM-positive group suggests that GDM may itself be an independent risk factor for cesarean section. The elevated risk of adverse outcomes in the unscreened group (Supplementary Table 3) further underscores the clinical importance of implementing OGTT screening and appropriate GDM management.

Dynamic glycemic correlation analysis revealed significant yet modest time-dependent correlations (fasting→1h→2h) within both 75g and 100g glucose load groups (all R^2=0.138-0.413, p<0.0001; Supplementary Table 4). These findings indicate that: (1) Fasting glucose levels, serving as metabolic baselines, partially predict subsequent glycemic responses but explain limited variation (≤24.0%); (2) The fasting vs. 2h glucose association was stronger under 100g loading (75g R^2=0.138 vs. 100g R^2=0.240), suggesting high-dose amplification of inter-individual baseline variations with potential implications for diabetes risk stratification; (3) Collinear effects between fasting and dynamic glucose levels (e.g., each 1 mmol/L fasting increase caused 0.412 mmol/L additional 2h glucose elevation specifically in 100g group) underscore the necessity of baseline adjustment in clinical trials, which could otherwise mask true intervention effects.

Figure 1 demonstrated comparable fasting-to-1h glucose elevation rates between 75g and 100g glucose loads (no dose-dependent difference in early-phase response). The 100g group exhibited significantly accelerated glucose rise during fasting-to-2h phase (indicating dose-amplified late-phase hyperglycemia) and attenuated glucose decline at 1h-to-2h phase. Collectively, 100g loading altered

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TABLE 7 Dose adjusted continuous analysis of the neonatal outcomes in progeny (75g, n=1,925; 100g, n=1,982).

Outcomes	Variable	aOR (95% CI)	*P*
Fetal distress	groups	0.59 (0.24-1.44)	0.242
	fasting	1.19 (0.43-3.31)	0.735
	1hr	1.15 (0.86-1.55)	0.335
	2hr	0.79 (0.53-1.17)	0.231
Abnormal fetal position	groups	1.84 (1.56-2.17)	0.000
	fasting	1.23 (1.01-1.51)	0.041
	1hr	0.92 (0.87-0.97)	0.002
	2hr	1.03 (0.96-1.11)	0.372
Stillbirth	groups	1.01 (0.52-1.99)	0.967
	fasting	0.53 (0.23-1.26)	0.152
	1hr	1.05 (0.83-1.33)	0.671
	2hr	1.20 (0.90-1.60)	0.221
Preterm infant	groups	0.86 (0.61-1.21)	0.392
	fasting	0.98 (0.65-1.48)	0.929
	1hr	1.11 (0.99-1.25)	0.073
	2hr	0.96 (0.82-1.12)	0.598
Small for gestational age (SGA)	groups	1.25 (0.27-5.72)	0.773
	fasting	1.18 (0.22-6.28)	0.847
	1hr	1.11 (0.66-1.88)	0.688
	2hr	1.04 (0.55-1.97)	0.904
Large for gestational age (LGA)	groups	1.32 (0.88-1.96)	0.177
	fasting	1.05 (0.64-1.70)	0.857
	1hr	1.11 (0.97-1.28)	0.119
	2hr	0.96 (0.81-1.15)	0.661
Low birth weight infant	groups	0.80 (0.50-1.30)	0.375
	fasting	0.81 (0.44-1.48)	0.491
	1hr	1.09 (0.92-1.28)	0.334
	2hr	0.98 (0.79-1.22)	0.886
Macrosomia	groups	0.83 (0.64-1.09)	0.183
	fasting	1.33 (0.98-1.81)	0.072
	1hr	0.99 (0.90-1.09)	0.862
	2hr	0.97 (0.86-1.10)	0.641
Neonatal hypoglycemia	groups	0.77 (0.50-1.18)	0.230
	fasting	1.05 (0.61-1.82)	0.864
	1hr	1.05 (0.90-1.22)	0.551
	2hr	0.88 (0.71-1.07)	0.199
Neonatal hyperbilirubinemia	groups	1.00 (0.86-1.17)	0.976
	fasting	1.08 (0.89-1.31)	0.446

(Continued)

TABLE 7 Continued

Outcomes	Variable	aOR (95% CI)	*P*
	1hr	1.00 (0.95-1.06)	0.968
	2hr	0.99 (0.92-1.06)	0.769
Neonatal asphyxia	groups	1.23 (0.45-3.35)	0.683
	fasting	1.70 (0.68-4.26)	0.260
	1hr	1.21 (0.86-1.69)	0.269
	2hr	0.78 (0.51-1.20)	0.254
Neonatal infection	groups	1.09 (0.92-1.29)	0.314
	fasting	1.31 (1.07-1.61)	0.009
	1hr	1.00 (0.94-1.06)	0.988
	2hr	0.97 (0.90-1.05)	0.401
Low Apgar score	groups	0.93 (0.41-2.14)	0.872
	fasting	0.98 (0.38-2.52)	0.969
	1hr	1.19 (0.90-1.57)	0.232
	2hr	1.00 (0.70-1.42)	0.989
Neonatal cephalohematoma	groups	1.09 (0.84-1.43)	0.519
	fasting	0.73 (0.52-1.04)	0.080
	1hr	0.99 (0.91-1.09)	0.904
	2hr	1.12 (0.99-1.26)	0.073
Fetal growth restriction (FGR)	groups	1.64 (0.59-4.54)	0.340
	fasting	0.98 (0.27-3.56)	0.969
	1hr	0.88 (0.62-1.25)	0.466
	2hr	1.14 (0.73-1.79)	0.571
Neonatal respiratory distress syndrome (NRDS)	groups	0.59 (0.24-1.44)	0.242
	fasting	1.19 (0.43-3.31)	0.735
	1hr	1.15 (0.86-1.55)	0.335
	2hr	0.79 (0.53-1.17)	0.231

Adjusted for GDM and covariates associated with non-adherence: maternal age, BMI, pregnancy history, insulin treatment, and chronic hypertension. aOR, adjusted odds ratio; CI, confidence interval; OGTT, oral glucose tolerance test.

glucose metabolism through enhanced late-phase glycemic surge and prolonged hyperglycemia, whereas 75g loading better maintained glucose homeostasis. These differential responses reflected more stable/efficient physiological regulation of 75g glucose.

Given the absence of statistically significant differences in outcome risks among women diagnosed with GDM based on diagnostic cutoff values, we conducted an in-depth analysis using binary logistic regression models. In these models, the occurrence of adverse outcomes served as the dichotomous dependent variable, while glucose levels at each time point were included as continuous independent variables. The analysis incorporated adjustments for potential confounding factors, including interactions between glucose levels at different time points, to systematically evaluate the risk of adverse outcomes in the entire study population across both groups. The results demonstrated that although glucose levels at various time points showed correlations with most adverse outcomes, with varying degrees of association for different outcomes, none of the adverse outcome rates exhibited statistically significant differences between the two groups (all $p > 0.05$; Tables 6, 7). These findings provide robust evidence that the glucose load is not a primary determinant influencing the occurrence of adverse outcomes.

The incidence of adverse outcomes in this study differed from those in other studies; for example, the incidences of hypoproteinemia in the 75-g and 100-g OGTT groups in our study were 2.09% (9/430) and 2.61% (12/460), respectively. Yuen et al. (28) reported that the incidence of hypoproteinemia was 4.6%. However, the incidence of macrosomia between the two groups in our study was 6.98% (30/430) and 8.04% (37/460), respectively. Moreover, Niroomand et al. (29) reported the incidence of macrosomia as 4.5%. These differences may be due to the

occurrence of GDM influenced by region, socioeconomic status, and nutritional status (1–4), not related to the OGTT glucose dose.

All data in this study were collected from two campuses in Tongchuan People's Hospital. The total number of primiparas in this region from 2017 to 2022 was 20,042 (http://www.tongchuan.gov.cn/), of whom 6,427 were at Tongchuan People's Hospital. Ultimately, a total of 3,907 primiparas (19.49%) were included in this study. Therefore, this research provides a good representation of this region. Moreover, the total numbers of adverse outcomes of pregnant women and newborns in this study were 15 and 16, respectively, more than those included in many other similar studies (27, 29).

This study has several limitations. Ideally, both the IADPSG and C&C criteria should have been applied for cross-analysis of the two groups. However, due to the retrospective design, the historical 100g OGTT tests did not include the 3-hour glucose measurement. Moreover, the 100g OGTT was intended to be performed only after a positive 50g GCT preliminary screening—a test not routinely conducted at our institution—making related data unavailable. Similarly, applying the C&C criteria was not feasible for the 75g OGTT group due to the lack of 3-hour glucose values. Given considerations of data accessibility and reliability, the IADPSG criteria (i.e., the 75g OGTT and its diagnostic thresholds) were uniformly used in this analysis. Additionally, information on the management and treatment of gestational diabetes mellitus (GDM) could only be obtained through retrospective medical record review, and statistical methods were employed to minimize inaccuracies. Nonetheless, lifestyle factors such as alcohol consumption, dietary quality, physical activity level, as well as socioeconomic indicators beyond education, were generally not systematically documented in medical records. This may have resulted in residual confounding and might have influenced the outcomes. Furthermore, since December 2019, the COVID-19 pandemic has affected both GDM screening and post-diagnosis management (30). This factor was not assessed in the present study and may also represent a potential source of interference.

In summary, under the IADPSG criteria, our study found no significant differences in GDM detection rates or adverse pregnancy outcomes between the 75-g and 100-g OGTT protocols. These results suggest that the two loads have comparable diagnostic and prognostic performance; however, a formal equivalence or non-inferiority trial is ultimately required to confirm true equivalence. To enhance clinical consistency and comparability across practices, we recommend that countries or regions move toward adopting a unified OGTT glucose load. The development of such a standardized screening strategy should be informed by multidisciplinary expertise, encompassing clinical, laboratory, health economic, and sociological perspectives.

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 Ethics Committee of Tongchuan People's Hospital. 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 The requirement for written informed consent was waived owing to the retrospective nature of the study. This retrospective study was conducted according to the STrengthening the Reporting of OBservational studies in Epidemiology guidelines. When we obtained the data, we obtained the patients' identifying information, including name, address, identification number, telephone number, clinical diagnosis and treatment information, various examination results, etc.; however, only age, sex, outcome, and treatment interventions are disclosed in the manuscript.

Author contributions

LZ: Data curation, Formal Analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. DL: Conceptualization, Data curation, Formal Analysis, Methodology, Project administration, Validation, Writing – original draft, Writing – review & editing. HS: Formal Analysis, Investigation, Writing – original draft. YW: Investigation, Writing – original draft. JFW: Resources, Writing – review & editing. JSW: Resources, Supervision, Writing – review & editing. CQ: Investigation, Writing – original draft. WH: Investigation, Writing – original draft. SL: Formal Analysis, Validation, Writing – review & editing.

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The author(s) declare financial support was received for the research and/or publication of this article. This study has certain limitations. Because it is a retrospective study, the management and treatment of the GDM population can only be managed through medical record tracking at a later stage and combined with statistical methods to reduce errors. However, lifestyle factors, such as alcohol consumption, diet quality, and physical activity level, as well as socioeconomic status other than education level, are not routinely recorded in medical records, and there may be some residual confounding factors, which may have affected our results. Moreover, beginning in December 2019, the coronavirus disease 2019 pandemic affected the screening and post-diagnostic management of GDM (30), which was not evaluated in this study.

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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 Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2025.1512499/full#supplementary-material

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Monochorionic-specific association between first-trimester serum ferritin and gestational diabetes in twin pregnancies: a retrospective cohort study

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Background: Previous studies have demonstrated that elevated serum ferritin (SF) levels in early pregnancy are significantly associated with the risk of developing gestational diabetes mellitus (GDM). However, these findings have primarily focused on singleton pregnancies, and evidence in twin pregnancies remains underexplored. This study aimed to explore the association between early-pregnancy SF levels and the risk of GDM in twin pregnancies, with a particular focus on different chorionicity types.

Methods: We conducted a retrospective cohort study involving 882 twin pregnancies delivered at our hospital between January 2019 and December 2021. The cohort included 700 dichorionic diamniotic (DCDA) and 182 monochorionic diamniotic (MCDA) pregnancies. Cases with gestational age at delivery less than 28 weeks, pre-existing diabetes, unknown GDM status, or mid-trimester fetal reduction in monochorionic-triamniotic (MCTA) pregnancies were excluded. GDM was diagnosed using a 75 g oral glucose tolerance test (OGTT) based on the IADPSG criteria. Serum ferritin (SF) levels were measured during the first prenatal visit in the first trimester. Logistic regression, linear correlation analyses and Receiver Operating Characteristic (ROC) curve were performed to assess associations between SF and GDM.

Results: In MCDA pregnancies, women with GDM had significantly higher mean SF levels compared to those without GDM (101.68 ± 59.72 vs. 79.87 ± 53.11 μg/L, $p<0.05$). However, no significant difference was observed in DCDA pregnancies. In MCDA cases, SF levels >71.4 μg/L were independently associated with an increased risk of GDM (adjusted OR = 2.775, 95% CI: 1.191–6.466; $p=0.018$), with a significant trend across SF levels (p for trend = 0.012). Additionally, SF was positively correlated with fasting blood glucose in early pregnancy ($r=0.17$, $p=0.025$) and 1-hour OGTT glucose at 24–28 weeks ($r=0.15$, $p=0.041$) among MCDA pregnancies.

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Conclusions: Elevated SF levels in early pregnancy are independently associated with a higher risk of GDM in MCDA twin pregnancies and may serve as a potential early biomarker for GDM prediction. In contrast, no significant association was found in DCDA pregnancies, indicating that the predictive value of SF may differ by chorionicity. Further studies are warranted to confirm these findings and investigate the underlying mechanisms.

KEYWORDS

serum ferritin (SF), gestational diabetes mellitus (GDM), twin pregnancy, chorionicity, monochorionic diamniotic (MCDA)

Introduction

Gestational diabetes mellitus (GDM) is a common pregnancy complication, defined as glucose intolerance with onset or first recognition during pregnancy (1). It is known to significantly elevate the risk of maternal and fetal complications, particularly in twin pregnancies, which are inherently associated with increased metabolic demand and placental complexity (2).

Accumulating evidence from experimental and clinical studies suggests that GDM is essentially a state of chronic insulin resistance, largely mediated by proinflammatory cytokines that impair insulin signaling and reduce insulin secretion from pancreatic β-cells (3, 4). In this inflammatory milieu, iron metabolism plays a pivotal role. Iron, a redox-active transition metal, can catalyze the formation of reactive oxygen species (ROS) when present in excess (5). These ROS promote oxidative stress, which in turn exacerbates insulin resistance and impairs β-cell function, ultimately contributing to the pathogenesis of GDM (6, 7).

High body iron stores have been consistently associated with increased diabetes risk in multiple epidemiological studies (8–12). Serum ferritin (SF), the primary intracellular iron-storage protein, is also an acute-phase reactant. Its circulating levels rise not only in response to iron overload but also under inflammatory conditions (13, 14). Elevated SF levels may further propagate the inflammatory response, leading to pancreatic β-cell dysfunction, heightened insulin resistance, and β-cell exhaustion, and may even contribute to hepatic insulin resistance and glucose dysregulation (15, 16). These pathophysiological changes eventually impair glucose uptake by skeletal muscle and promote hepatic gluconeogenesis, facilitating the development of diabetes (17).

As a result, numerous studies have investigated SF as a potential biomarker for GDM, and a consistent positive association has been observed between elevated SF levels in early pregnancy and subsequent GDM development in singleton pregnancies (12, 13, 18–25). Based on these findings, early-pregnancy SF levels are now recognized as a potential predictive marker for GDM in singleton gestations (26).

Twin pregnancies are associated with a higher incidence of GDM (3-9% morbidity statistically) (27–32), early prediction can help us identify and reduce its morbidity. But there is a noticeable lack of biochemical markers predicting the risk in this specific population. Studies have found that a certain proportion of GDM may likely result from the same pathogenesis as the singleton pregnancy: greater transient increase in insulin resistance (33, 34), therefore we could definitively establish the early predictive utility of SF in twin gestations. However, different types of twins have distinct hemodynamic changes, inflammatory responses and placental number due to the different chorionicity, which might lead to different mechanisms for GDM. We should evaluate the utility of SF particularly with respect to chorionicity-related differences and various risk factors of GDM.

In our research, we conducted a retrospective cohort study to evaluate the association between early-pregnancy SF levels and the risk of GDM diagnosed according to the criteria of the International Association of Diabetes and Pregnancy Study Groups (IADPSG) in twin pregnancies with different chorionicity. By exploring this relationship, we aim to facilitate earlier identification of high-risk individuals, thereby enabling timely interventions—such as dietary counseling and lifestyle modifications—to reduce GDM-related maternal and perinatal morbidity in the growing population of twin pregnancies.

Materials and methods

Study population and sample collections

This retrospective cohort study encompassed all twin pregnancies delivered at our institution in Eastern China from January 1, 2019 to December 31, 2021. A total of 882 eligible cases were identified. The exclusion criteria were: singleton pregnancies; deliveries before 28 weeks of gestation; absence of first-trimester ultrasound data to determine chorionicity or gestational age; twin pregnancies that became monochorionic diamniotic (MCDA) after mid-trimester fetal reduction in monochorionic triamniotic (MCTA) pregnancies; and pre-existing diabetes mellitus.

Upon enrollment, written informed consent was obtained from all participants, the institutional review board approved the study

(approval reference number: GKLW-A-2024-023-01), and maternal medical histories were documented. Blood samples were collected during the first prenatal visit in early pregnancy(<12 pregnant weeks, empty stomach, ECLIA, Roche Cobas analyzer, regular calibration using the standards provided by manufacturer) to measure SF levels. Screening for GDM was performed at 24–28 weeks of gestation using a 75 g oral glucose tolerance test (OGTT), and diagnoses were based on the IADPSG criteria: fasting plasma glucose ≥ 5.1 mmol/L, 1-hour glucose ≥ 10.0 mmol/L, or 2-hour glucose ≥ 8.5 mmol/L.

Data collection

Because of the unique physiologic characteristics of different chorionicity, we divided the included pregnancies into dichorionic diamniotic (DCDA,700 cases) and monochorionic diamniotic (MCDA,182 cases) twins. Chorionicity was initially assessed via prenatal ultrasonography and subsequently confirmed by intraoperative and pathological findings after delivery. Clinical and laboratory data were extracted from the hospital's electronic medical record system, including maternal demographic characteristics, obstetric and medical histories, and laboratory indices. Gestational age was confirmed based on first-trimester ultrasound.

Statistical analysis

Continuous variables were summarized as means ± standard deviations (SDs), and categorical variables were reported as frequencies and percentages. Comparisons between the GDM and non-GDM groups were performed using independent samples t-tests for continuous variables and chi-square tests for categorical variables.

Linear regression analyses were conducted to evaluate the associations between serum ferritin levels (as the dependent variable) and potential influencing factors, including maternal age, pre-pregnancy body mass index (P-BMI), geographical residence, educational level, mode of conception, family history of type 2 diabetes, hemoglobin level, HbA1c, fasting plasma glucose in early pregnancy, and OGTT results. These analyses were performed using the R programming language.

To determine the predictive value of SF for GDM, the optimal serum ferritin threshold was identified using the Youden index derived from receiver operating characteristic (ROC) curve analysis. Based on this cutoff, logistic regression models were applied to assess the association between elevated SF and the risk of GDM. Crude and adjusted odds ratios (ORs and aORs), along with their 95% confidence intervals (CIs), were calculated. The significance of trends across SF levels was also evaluated. All statistical analyses were conducted using SPSS software, version 29.0 (IBM Corp., Armonk, NY), and a two-sided p value < 0.05 was considered statistically significant.

Missing data were handled by complete-case analysis at the variable level. When a specific measurement was unavailable for a patient, that patient was excluded only from analyses involving that variable, without excluding the entire patient record. As the overall proportion of missing data was small (<3%), no imputation was performed.

Results

Baseline characteristics and early pregnancy SF levels in MCDA and DCDA twin pregnancies

A total of 182 MCDA and 700 DCDA twin pregnancies were included in the analysis. Tables 1, 2 show the baseline maternal characteristics stratified by GDM status in MCDA and DCDA groups. Tables 3, 4 summarize the early pregnancy laboratory results stratified by GDM status in MCDA and DCDA groups, respectively.

In MCDA pregnancies, women who developed GDM had significantly higher pre-pregnancy BMI (p = 0.01), a higher proportion of ART-conceived pregnancies (p = 0.03), and a greater frequency of family history of type II diabetes (p = 0.04) compared with non-GDM women. Notably, the mean serum ferritin (SF) level in early pregnancy was significantly higher in the GDM group than in the non-GDM group (101.68 ± 59.72 vs. 79.87 ± 53.11 μg/L, p = 0.04). Early pregnancy HbA1c was also elevated in the GDM group (p = 0.002).

In contrast, in DCDA pregnancies, although GDM was associated with older maternal age (p = 0.002), higher pre-pregnancy BMI (p = 0.002), and increased HbA1c levels (p = 0.003), no significant difference in SF levels was observed between GDM and non-GDM groups (87.79 ± 72.01 vs. 92.34 ± 70.14 μg/L, p = 0.49).

Association between early pregnancy SF and GDM risk in MCDA pregnancies

To assess the predictive value of SF for GDM, we conducted logistic regression analysis in MCDA pregnancies using the SF threshold of 71.4 μg/L, identified via ROC curve and Youden index.

As shown in Table 5, after adjustment for potential confounders (maternal age, parity, history of GDM, family history of diabetes, pre-pregnancy BMI, ART pregnancy, chronic hypertension, smoking, early Hb and HbA1c), women with SF > 71.4 μg/L had a significantly increased risk of developing GDM compared to those with SF ≤ 71.4 μg/L (adjusted OR = 2.775; 95% CI: 1.191–6.466; p = 0.018). A dose-response trend was also observed across SF categories (p for trend = 0.012), supporting a potential threshold effect.

The ROC curve of the prediction model of GDM in MCDA pregnancy was shown in Figure 1 (area under curve:0.77).The value of SF>71.4μg/L was found to be 72.5% sensitive and 50.7% specific. At the cutoff value, calculated positive predictive value and negative predictive values are 29.3% and 86.7% respectively.

TABLE 1 Demographic differences of women with MCDA pregnancies.

Characteristics	Non-GDM group (n=142)	GDM group (n=40)	p-value
Maternal age (year)	31.57 ± 4.52	32.45 ± 4.33	0.270
Maternal age≥35 years	38 (26.8%)	12 (30%)	0.840
Pre-pregnancy BMI (kg/m2)	21.33 ± 2.72	22.87 ± 3.41	**0.010**
Multiparity	42 (29.6%)	6 (15%)	0.100
Assisted Reproductive Technology (ART) pregnancy	28 (19.7%)	15 (37.5%)	**0.030**
chronic hypertension	1 (0.7%)	3 (7.5%)	0.050
History of GDM	1 (0.7%)	0 (0)	1.000
History of polycystic ovary syndrome(PCOS)	0(0%)	0(0%)	
Family history of type II diabetes	8 (5.6%)	7 (17.5%)	**0.040**
Geography			0.270
Shanghai	55 (38.7%)	20 (50%)	
Foreign/expatriate	87 (61.3%)	20 (50%)	
Educational level			0.720
Bachelor's degree or above	104 (73.8%)	27 (69.2%)	
specialist degree or below	37 (26.2%)	12 (30.8%)	
Smoking	1 (0.7%)	0 (0%)	1.000
Twin-to-twin transfusion syndrome (TTTS)	8(5.6%)	0(0%)	0.272
Twin anemia–polycythemia sequence (TAPS)	3(2.1%)	0(0%)	1.000
Selective intrauterine growth restriction(sIUGR)	12(8.5%)	2(5%)	0.698

*Bachelor's degree or above: further study at university after graduating from high school.
*specialist degree or below: further study at college after graduating from high school or below.
Bold values means p-value is < 0.05 with a statistically significant difference.

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TABLE 2 Demographic differences of women with DCDA pregnancies.

Characteristics	Non-GDM group (n=548)	GDM group (n=152)	p-value
Maternal age (year)	32.28 ± 3.7	33.3 ± 3.54	**0.002**
Maternal age≥35 years	140 (25.5%)	61 (40.1%)	**<0.001**
Pre-pregnancy BMI (kg/m2)	21.37 ± 2.89	22.22 ± 2.94	**0.002**
Multiparity	57 (10.4%)	19 (12,5%)	0.560
Assisted Reproductive Technology (ART) pregnancy	417 (76.1%)	109 (71.7%)	0.320
chronic hypertension	6 (1.1%)	3 (2%)	0.660
History of GDM	1 (0.2%)	3 (2%)	0.050
History of polycystic ovary syndrome(PCOS)	17 (3.1%)	9 (5.9%)	0.170
Family history of type II diabetes	32 (5.8%)	16 (10.5%)	0.070
Geography			**0.040**
Shanghai	248 (45.3%)	84 (55.3%)	
Foreign/expatriate	300 (54.7%)	68 (44.7%)	
Educational level			
Bachelor's degree or above	361 (67.1%)	101 (67.8%)	0.950
specialist degree or below	177 (32.9%)	48 (32.2%)	0.950
Smoking	4 (0.7%)	1 (0.7%)	1.000

Bold values means p-value is < 0.05 with a statistically significant difference.

Correlations between SF and glycemic parameters in MCDA pregnancies

To further explore the metabolic significance of serum ferritin, correlation analysis was performed between early pregnancy SF and glucose-related indices. As shown in Table 6 and Figure 2, SF levels were positively correlated with fasting blood glucose in early pregnancy (r = 0.17, p = 0.025) and 1-hour OGTT glucose at 24–28 weeks (r = 0.15, p = 0.041).

No significant correlations were observed with maternal age, pre-pregnancy BMI, or early pregnancy hemoglobin levels. These results indicate that elevated SF may be associated with early alterations in glucose metabolism in MCDA pregnancies.

TABLE 3 Blood sampling tests of women with MCDA pregnancies.

Characteristics	Non-GDM group (n=142)	GDM group (n=40)	p-value
Early pregnancy			
Ferritin	79.87 ± 53.11	101.68 ± 59.72	**0.040**
Fasting blood glucose	4.56 ± 0.45	4.66 ± 0.44	**0.240**
Glycated hemoglobin	5.24 ± 0.28	5.42 ± 0.31	0.002
hemoglobin	124.32 ± 10.71	126.62 ± 12.07	0.280
Folic.Acid	34.37 ± 9.33	37.08 ± 11.79	0.190
Vitamin.B12	351.88 ± 127.34	373.97 ± 114.14	0.300
Middle pregnancy (OGTT)			
Fasting blood glucose	4.12 ± 0.43	4.63 ± 0.59	<0.001
1h after	7.6 ± 1.24	10.21 ± 1.27	<0.001
2h after	6.29 ± 1.06	8.93 ± 1.57	<0.001
Glycated hemoglobin	12.71 ± 1.41	12.67 ± 1.15	0.860

*early pregnancy: <12 gestational weeks.
*middle pregnancy(OGTT): OGTT test performed at 24–28 weeks of gestation.
Bold values means p-value is < 0.05 with a statistically significant difference.

TABLE 4 Blood sampling tests of women with DCDA pregnancies.

Characteristics	Non-GDM group (n=548)	GDM group (n=152)	p-value
Early pregnancy			
Ferritin	92.34 ± 70.14	87.79 ± 72.01	0.215
Fasting blood glucose	4.54 ± 0.4	4.66 ± 0.43	0.002
glycated hemoglobin	5.23 ± 0.29	5.33 ± 0.39	0.003
hemoglobin	125.81 ± 9.62	127.51 ± 9.09	0.050
Folic.Acid	34.04 ± 8.38	35.16 ± 9.1	0.180
Vitamin.B12	363.56 ± 133.95	341.27 ± 118.4	0.050
Middle pregnancy (OGTT)			
Fasting blood glucose	4.17 ± 0.37	4.52 ± 0.51	**<0.001**
1h after	7.65 ± 1.19	10.15 ± 1.16	**<0.001**
2h after	6.45 ± 1.04	8.89 ± 1.46	**<0.001**
Glycated hemoglobin	12.73 ± 1.52	13.03 ± 1.49	**0.030**

*early pregnancy: <12 gestational weeks.
*middle pregnancy(OGTT): OGTT test performed at 24–28 weeks of gestation.
Bold values means p-value is < 0.05 with a statistically significant difference.

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TABLE 5 Association of early pregnancy SF level with GDM risk in MCDA pregnancies.

Variables in the Equation		OR		
	Sig.	Exp(B)	95% C.I.for EXP(B) (Lower)	95% C.I.for EXP(B) (Upper)
Step 1a(maternal age ≥35)	0.572	1.242	0.586	2.633
a. Variable(s) entered on step 1: maternal age≥35				
Variables in the Equation		OR		
	Sig.	Exp(B)	95% C.I.for EXP(B)	95% C.I.for EXP(B)
Step 1a(BMI divided into four groups)			Lower	Upper
Group 1	1.000	1	0.268	3.737
Group 2	0.028	4.978	1.189	20.845
Group 3	0.161	4	0.575	27.819
a. Variable(s) entered on step 1: BMI divided into four groups (BMI<18.5, 18.5≤BMI<24, 24≤BMI<28, BMI≥28).				
Variables in the Equation		OR		
	Sig.	Exp(B)	95% C.I.for EXP(B) (Lower)	95% C.I.for EXP(B) (Upper)
Step 1a	0.037	11.526	1.166	113.952
a. Variable(s) entered on step 1: chronic hypertension(without marked for 0, with marked for 1)				
Variables in the Equation		OR		
	Sig.	Exp(B)	95% C.I.for EXP(B) (Lower)	95% C.I.for EXP(B) (Upper)
Step 1a	1.000	1	0	0
a. Variable(s) entered on step 1: history of GDM(without marked for 0, with marked for 1)				
Variables in the Equation		OR		
	Sig.	Exp(B)	95% C.I.for EXP(B)	95% C.I.for EXP(B)
Step 1a			Lower	Upper
group1	0.067	0.416	0.163	1.063
group2	0.999	0	0	0
a. Variable(s) entered on step 1: parity(unipara marked for 1, delivery once marked for 2, delivery marked twice for 3).				
Variables in the Equation		OR		
	Sig.	Exp(B)	95% C.I.for EXP(B) (Lower)	95% C.I.for EXP(B) (Upper)
Step 1a	0.133	1.706	0.85	3.426
a. Variable(s) entered on step 1: geography (Shanghai marked for 0, foreign/expatriate marked for 1).				
Variables in the Equation		OR		
	Sig.	Exp(B)	95% C.I.for EXP(B) (Lower)	95% C.I.for EXP(B) (Upper)
Step 1a	1.000	1	453661135.8	0
a. Variable(s) entered on step 1: smoking (without marked for 0, with marked for 1).				
Variables in the Equation		OR		
	Sig.	Exp(B)	95% C.I.for EXP(B) (Lower)	95% C.I.for EXP(B) (Upper)
Step 1a	0.636	1.337	0.402	4.441

(Continued)

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TABLE 5 Continued

Variables in the Equation		OR		
	Sig.	Exp(B)	95% C.I.for EXP(B) (Lower)	95% C.I.for EXP(B) (Upper)
a. Variable(s) entered on step 1: hemoglobin and glycated hemoglobin level in early trimester (anemia marked for 1, without marked for 0).				
Variables in the Equation		OR		
	Sig.	Exp(B)	95% C.I.for EXP(B) (Lower)	95% C.I.for EXP(B) (Upper)
Step 1a	0.007	2.841	1.325	6.092
a. Variable(s) entered on step 1: SF: 71.4.				
Variables in the Equation		OR		
	Sig.	Exp(B)	95% C.I.for EXP(B) (Lower)	95% C.I.for EXP(B) (Upper)
Step 1a	<.001	9.286	3.401	25.354
a. Variable(s) entered on step 1: glycated hemoglobin level in early trimester:5.65				
Variables in the Equation		OR		
	Sig.	Exp(B)	95% C.I.for EXP(B) (Lower)	95% C.I.for EXP(B) (Upper)
Step 1a	0.009	2.72	1.284	5.761
a. Variable(s) entered on step 1: conception method (natural conception marked for 0, assisted reproductive technology pregnancy marked for 1).				
Variables in the Equation		aOR		
	Sig.	Exp(B)	95% C.I.for EXP(B)	95% C.I.for EXP(B)
Step 1a			Lower	Upper
Group 1	0.945	1.052	0.25	4.421
Group 2	0.081	4.108	0.841	20.067
Group 3	0.512	2.138	0.221	20.687
conception method	0.068	2.271	0.941	5.482
glycated hemoglobin level in early trimester:5.65	0.001	6.324	2.073	19.292
SF:71.4	0.018	2.775	1.191	6.466
a. Variable(s) entered on step 1: BMI divided into four groups, conception method, glycated hemoglobin level in early trimester:5.65, SF:71.4).				
Variables in the Equation		p for trend		
	Sig.	Exp(B)	95% C.I.for EXP(B)	95% C.I.for EXP(B)
Step 1a			Lower	Upper
conception method	0.008	3.08	1.338	7.091
BMI group median	0.026	1.205	1.022	1.42
Glycated hemoglobin group median	0.026	5.933	1.241	28.37
ferritin group median	0.012	1.01	1.002	1.018
a. Variable(s) entered on step 1: conception method, BMI group median, glycated hemoglobin group median, ferritin group median.				

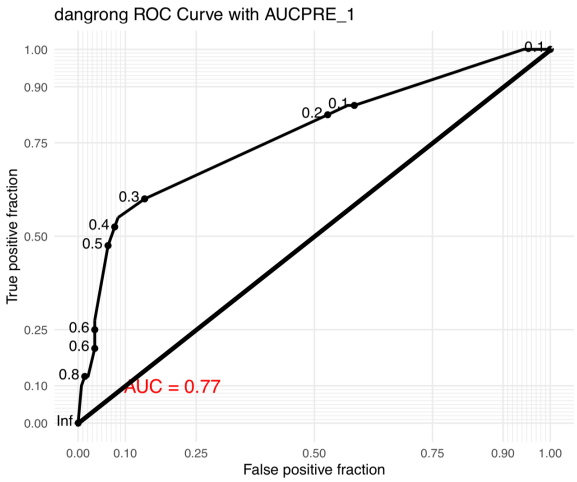

FIGURE 1
The ROC curve of GDM prediction model in MCDA pregnancies.

Taken together, these results demonstrate that elevated serum ferritin levels in early pregnancy are significantly associated with increased risk of GDM in MCDA twin pregnancies, but not in DCDA pregnancies. This association remains significant after adjustment for key clinical risk factors and correlates with both early and mid-gestation glycemic indices.

Discussion

In this retrospective observational study, we found that elevated SF in early pregnancy was significantly associated with an increased risk of GDM in MCDA pregnancies but not in DCDA pregnancies. After adjusting for key confounding factors, including maternal age, parity, history of GDM, family history of diabetes, pre-pregnancy BMI, assisted reproductive technology pregnancy, chronic hypertension, smoking, hemoglobin and glycated hemoglobin level in early trimester, high SF remained an independent predictor of GDM in the MCDA group. Moreover, early-pregnancy SF levels were positively correlated with fasting glucose and 1-hour OGTT glucose levels, indicating a potential link between iron metabolism and glucose dysregulation in MCDA pregnancies. So we can conclude that with the measurement of SF we can predict the risk of development of GDM even before its development.

TABLE 6 Correlations between SF and the characteristics of the MCDA pregnancy women in a simple correlation model.

Characteristics	Serum ferritin	
	r	p-value
Maternal age (years)	-0.12	0.110
Pre-pregnancy BMI (kg/m2)	0.0038	0.960
Geography(%)	-0.14	0.062
Educational level(%)	0.091	0.230
Conception method(%)	-0.11	0.140
Family history of type II diabetes(%)	-0.009	0.900
Hemoglobin in early pregnancy(g/l)	0.044	0.550
Glycated hemoglobin in early pregnancy(%)	0.084	0.260
Fasting blood glucose in early pregnancy (mmol/L)	0.17	**0.025**
Fasting blood glucose of OGTT(mmol/L)	0.14	0.052
1h after(mmol/L)	0.15	**0.041**
2h after(mmol/L)	0.073	0.330
Glycated hemoglobin (%)	-0.08	0.280

Bold values means p-value is < 0.05 with a statistically significant difference.

Several potential mechanisms may explain the association between elevated SF and GDM risk, particularly in MCDA twins. Unlike DCDA pregnancies, where two fetuses develop independently from separate ova and have distinct placentas, MCDA twins originate from a single fertilized ovum and share a common placenta, with a similar maternal inflammatory response like the single pregnancy, the greater increase in insulin resistance was observed (due to the greater placental mass) (33–37). Our findings consistent with previous studies conducted in singleton pregnancies further confirm the point. For instance, Cheng et al. (22) and Liu et al. (38) demonstrated that elevated SF in early pregnancy was significantly associated with impaired glucose tolerance and subsequent GDM especially linearly correlated with 1-hour OGTT. Notably, we observed a significant linear relationship between SF and 1-hour OGTT levels, which aligns with evidence suggesting that the 1-hour glucose value is more closely linked to insulin resistance and β-cell dysfunction than fasting or 2-hour values (39, 40). Conversely DCDA twins have two separate placentas, the interaction between two placental factors might result in the hemodynamic changes and inflammatory responses completely different.

In MCDA twins, unique complications such as twin-to-twin transfusion syndrome (TTTS), selective intrauterine growth restriction (sIUGR), and twin anemia–polycythemia sequence

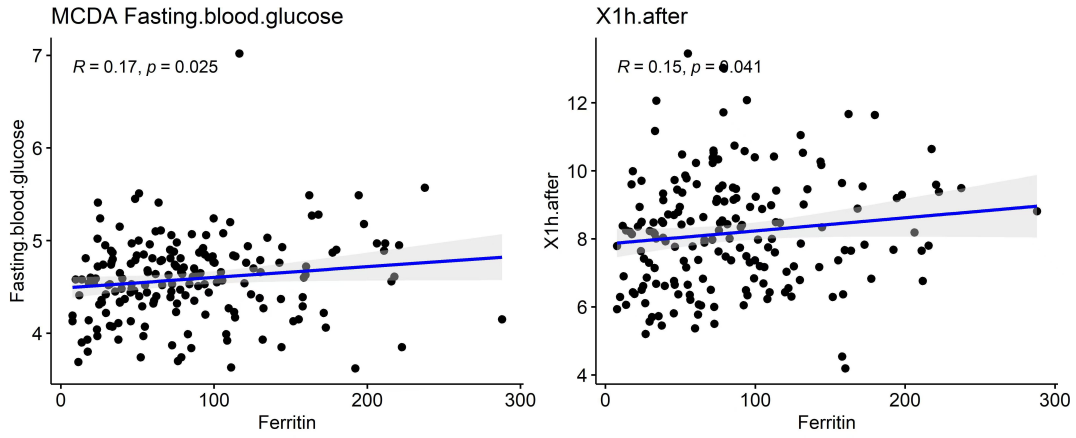

FIGURE 2
Correlations of SF between fasting blood glucose in early pregnancy and 1-h OGTT level.

(TAPS) are more frequent. These conditions may result in dynamic fluctuations in fetal and maternal hemoglobin levels, stimulating hepatic ferritin synthesis as a compensatory response. This increase in ferritin may reflect a state of subclinical inflammation or metabolic stress, both of which are known contributors to impaired insulin sensitivity and increased GDM risk (13–16).

Taken together, our results support the hypothesis that SF is not only a passive marker of iron status but also an active participant in the pathogenesis of GDM, particularly in MCDA pregnancies where placental structure and oxidative stress levels may amplify its impact. Early identification of high SF levels may allow clinicians to stratify GDM risk in twin pregnancies more precisely and implement timely interventions to reduce adverse outcomes.

Moreover, we identified a positive correlation between SF and fasting plasma glucose in the first trimester. Physiologically, insulin sensitivity is typically enhanced in early pregnancy to support maternal–fetal nutrient delivery, resulting in lower fasting glucose levels. However, elevated SF may contribute to early-onset insulin resistance, thereby blunting this adaptive mechanism and raising fasting glucose levels. This suggests that we should pay more attention to fasting glucose with increasing SF level in the early trimester, early screening and intervention when necessary.

Despite the strengths of our study, including a large sample size and stratified analysis by chorionicity, several limitations should be acknowledged. First, due to its retrospective nature, we could not obtain accurate data on dietary iron intake or iron supplementation, which may influence SF levels and confound associations. Second, SF concentrations were measured only in the first trimester, and dynamic changes in iron status during pregnancy were not captured. Third, we acknowledge that the MCDA GDM sample size is limited (n=40), leading to wide CIs. The proportion of MCDA in twin pregnancies is relatively low, especially in cases of GDM in MCDA, we only collected 40 cases during the two-year period. Given the limited sample size, the findings should be regarded as preliminary and exploratory, need to be further validated with more cases. Besides, we relied on a single biomarker (SF) rather than a panel of iron metabolism or inflammatory indicators, which limits the mechanistic interpretation of our findings. Future prospective studies incorporating broader iron indices and inflammatory markers are warranted to further elucidate these associations.

Conclusion

In conclusion, our study demonstrates that elevated serum ferritin in early pregnancy is independently associated with increased risk of GDM in MCDA twin pregnancies. SF may serve as a cost-effective and accessible early biomarker to predict GDM in this high-risk population, potentially guiding individualized screening and preventive strategies. In contrast, no such association was observed in DCDA pregnancies, highlighting the importance of considering chorionicity in the metabolic evaluation of twin gestations.

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 studies involving humans were approved by The International Peace Maternity and Child Health Hospital, School of Medicine, Shanghai Jiao Tong University. The studies were

conducted in accordance with the local legislation and institutional requirements (approval reference number: GKLW-A-2024-023-01). The participants provided their written informed consent to participate in this study.

Author contributions

YYN: Writing – original draft. YB: Writing – review & editing. XX: Investigation, Writing – review & editing. YH: Writing – review & editing, Data curation. JM: Conceptualization, Writing – review & editing, Data curation. YLW: Writing – review & editing, Funding acquisition.

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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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Study and validation on mitochondrial and immune-related hub genes in gestational diabetes mellitus based on bioinformatics

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Background: Mitochondria and immune function play pivotal roles in the pathogenesis of gestational diabetes mellitus (GDM). However, the intricate molecular mechanisms underlying their involvement remain elusive. Therefore, this study aimed to elucidate the interaction between mitochondria-related genes (MRGs) and immune-related genes (IRGs) in GDM.

Methods: In this study, GDM-related datasets (GSE103552, GSE154414, and GSE173193) were integrated along with MRGs and IRGs. Differential expression analysis was conducted on GSE103552 to identify differentially expressed genes (DEGs), which were then intersected with MRGs and IRGs. Correlations among the intersection genes were evaluated, and those with statistical significance and strong correlation were selected as candidate genes. Three machine learning algorithms were subsequently applied to further refine the selection of signature genes. The optimal model was determined, and genes within this model were designated as signature genes. Expression levels of these genes were then examined, and those showing significant differences and consistent trends between GDM and control groups in both GSE103552 and GSE154414 datasets were identified as hub genes. Further analyses included chromosomal and subcellular localization, enrichment, regulatory mechanism, and drug prediction analyses of hub genes. Key cell types were analyzed in GSE173193. Finally, the expression of hub genes was validated by reverse transcription quantitative polymerase chain reaction (RT-qPCR).

Results: Comprehensive analysis identified MRPL15, MRPL22, and MRPS18C emerged as pivotal hub genes, each showing significantly lower expression levels in the GDM group. Chromosomal localization revealed MRPS18C on chromosome 4, MRPL22 on chromosome 5, and MRPL15 on chromosome 8. Subcellular distribution analysis indicated that MRPL15 and MRPL22 were predominantly localized in the nucleus, whereas MRPS18C was mainly cytoplasmic. Enrichment analysis showed that spliceosome, proteasome, Parkinson disease, and ribosome pathways were enriched by the hub genes. Regulatory analysis revealed that YY1 regulated MRPS18C and MRPL22, ARID3A regulated MRPS18C and MRPL15, and FOXC1 regulated MRPL22 and MRPL15. Finally, results of RT-qPCR results confirmed that MRPL15, MRPL22, and MRPS18C were significantly downregulated in the GDM group.

Conclusion: Our findings highlight the significance of MRPL15, MRPL22, MRPS18C, monocytes, and villous cytotrophoblast cells in GDM. These insights provide valuable implications for the diagnosis and potential therapeutic interventions targeting of GDM.

KEYWORDS

gestational diabetes mellitus, mitochondria, immune, hub gene, bioinformatics

1 Introduction

Gestational diabetes mellitus (GDM) refers to abnormal glucose metabolism disorders of varying severity during pregnancy, and is one of the most common pregnancy complications. With changes in lifestyle and dietary patterns, the incidence of gestational obesity and GDM—closely related conditions—has been increasing yearly (1, 2), placing a heavy burden on affected patients. GDM is associated with an elevated risk of adverse pregnancy outcomes, including preeclampsia, preterm birth, postpartum depression, instrumental or surgical delivery, and birth trauma (3). Fetuses born to women with GDM are prone to fetal developmental abnormalities, such as macrosomia and have higher rates of congenital malformations, often accompanied by hypoglycemia and jaundice. Moreover, in the long term, children born to women with GDM have an increased risk of obesity and type 2 diabetes later in life (4). Therefore, the early detection and prevention of GDM are particularly important for maternal and infant health, and it is necessary to continuously explore new biomarkers to provide a theoretical basis for its treatment of GDM.

Mitochondria are the primary site of aerobic respiration in cells, providing energy for essential biological functions. They generate adenosine triphosphate (ATP) through oxidative phosphorylation (OXPHOS) and participate in key physiological processes, such as maintaining energy metabolism homeostasis, regulating cell survival and apoptosis, producing reactive oxygen species (ROS), and modulating calcium synthesis and homeostasis (1). Studies have shown that mitochondrial dysfunction reduces cellular energy utilization rate, and then the decrease of metabolic capacity, eventually leading to the excessive production of ROS production, oxidative stress, and metabolic diseases (such as diabetes) (5). The functions of mitochondria vary depending on the cell type in the unit (6). Screening for mitochondrial mutations and deletion polymorphisms in Asian Indian women with GDM revealed a relationship between mitochondrial mutations and GDM, suggesting that abnormal mitochondrial function plays a crucial role in the development of the disease (7).

The maternal immune system must balance key maternal immune mediators such as macrophages, natural killer (NK) cells, and regulatory T cells (Tregs) to prevent pathological conditions or pregnancy interruption (8). Both interleukin-6 (IL-6) and interleukin-8 (IL-8) are immune factors, and studies have shown that they influence the pathological processes of pregnancy-related diseases, including preeclampsia, GDM, and inflammation (9). Furthermore, studies have shown that in patients with type 1 diabetes and type 2 diabetes have shown that immune cells—including neutrophils, eosinophils, monocytes, NK cells, and lymphocytes—are altered, whether they are related to pregnancy is involved or not, indicating that these cells play an important role in disease pathogenesis of this disease (10). Although extensive research has focused on immune cells in tumors, but there are few studies have explored their roles in gestational metabolic diseases. Importantly, immune status is closely related to mitochondrial function. A key feature of mitochondria is their ability to regulate the activation, differentiation, and survival of immune cells. In addition, mitochondria can release mitochondrial DNA and mitochondrial ROS, among others, to modulate immune cell transcription of immune cells (10).

At present, the pathogenesis of GDM remains incompletely understood. The main contributing factors include insulin resistance, adipocytokine imbalance, inflammatory factor release, and genetic predisposition (11), but the involvement of mitochondrial and immune mechanisms is rarely investigated. To further elucidate the roles of mitochondria and immunity in GDM, this study screened the relevant hub genes associated with GDM, and conducted enrichment, regulatory mechanism, and drug prediction analyses to explore the pathways through which these hub genes act. Additionally, we examined cell populations in GDM at the single-cell level to identify cell types with crucial roles in the disease progression. Through this research design, we aim to better understand the relationships among mitochondria-related genes (MRGs), immune-related genes (IRGs), and GDM, thereby providing a scientific basis and guidance for future clinical practice.

2 Materials and methods

2.1 Data collection

GDM-related datasets—GSE103552 (sequencing platform: GPL6244) and GSE154414 (sequencing platform: GPL20301)—were obtained from the GEO database. The GSE103552 dataset, which contained 11 GDM and 8 control primary feto-placental arterial cell samples, served as the training set. The GSE154414

dataset included 4 GDM and 4 control placental tissue samples and served as the validation set.

The sample size was mainly limited by the sample collection period and strict sample inclusion criteria. However, for an exploratory study, this sample size meets the basic analytical requirements. Additionally, cross-validation between the two datasets provides a certain degree of reliability. Although the sample types of samples differ, both focus on the placenta—the key target organ in GDM—as the core research object. Thus, these datasets cross-validate gene expression changes from two perspectives—specific functional cells" and "whole tissue—thereby enhancing the comprehensiveness of the results.

GSE211617 was sequenced using the GPL24676 platform and contained two GDM placental tissue samples and two control placental tissue samples, serving as the single-cell RNA sequencing (scRNA-seq) dataset.

A total of 1,136 mitochondria-related genes (MRGs) were obtained from the MitoCarta 3.0 database (https://www.broadinstitute.org/), and 2,660 immune-related genes (IRGs) were collected from published literature (12).

2.2 Differential expression analysis

Differential expression analysis was performed to identify differentially expressed genes (DEGs) between GDM and control groups using the limma package (version 3.56.2) (13), with thresholds of *adjusted* $p < 0.05$ and $|\log^2$ fold change (FC)$| > 0.5$. Volcano map and heat maps of DEGs were generated using the ggplot2 (version 3.4.4) (14) and circlize package (version 0.4.15) (15) packages, respectively, to visualize DEG distribution.

It should be noted that, in exploratory studies, excessively strict FC thresholds (e.g., $|\log^2 FC| > 1$) may exclude genes with small fold changes but meaningful biological significance. Therefore, DEG screening of differentially expressed genes (DEGs) in this study adopted a dual-criterion approach combining both FC and statistical significance thresholds, which enhanced the stringency and biological relevance of the analysis.

2.3 Identification and analysis of candidate genes

Differentially expressed MRGs (DE-MRGs) and differentially expressed IRGs (DE-IRGs) were obtained by intersecting DEGs with MRGs and IRGs, respectively. The correlation between DE-MRGs and DE-IRGs was assessed using Spearman correlation analysis, and candidate genes were selected using thresholds of $p < 0.001$ and |correlation coefficient| > 0.6.

Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses were then conducted to explore the biological functions and pathways of the candidate genes using the clusterProfiler package (version 4.8.2) (16) with the org.Hs.eg.db background gene set in org.Hs.eg.db package (version 3.17.0) (17) (*adjusted* $p < 0.05$).

To further investigate the protein-level interactions of candidate genes, the STRING database was used to construct a protein–protein interaction (PPI) network (species: Homo sapiens, interaction score ≥ 0.4). The PPI network was visualized using Cytoscape software (version 3.7.1) (18). Four algorithms in CytoHubba were applied to select potential signature genes, and the intersection of the top 30 genes from all four algorithms was identified as the set of candidate signature genes.

2.4 Identification of hub genes

To obtain hub genes, three machine learning models—random forest (RF), support vector machine (SVM), and generalized linear model (GLM)—were constructed using the caret package (version 6.0.49) (19). These models were analyzed with the explain function in the DALEX package (version 2.4.3) (20), and the best-performing model was selected. Genes within the optimal model were identified as signature genes.

The expression of signature genes was compared between GDM and control groups was compared using the Wilcoxon test ($p < 0.05$), and differences were visualized with the ggpubr package (version 0.6.0) (21). Genes showing statistically significant difference and consistent expression trends were identified as hub genes ($p < 0.05$).

To assess the diagnostic ability of hub genes to distinguish between GDM and control samples, receiver operating characteristic (ROC) curves were plotted for the hub genes was drafted in the GSE103552 and GSE154414 datasets using the pROC package (version 1.18.4) (22).

2.5 Localization and function analysis of hub genes

Chromosomal localization of the hub genes was visualized using the RCircos package (version 1.2.2) (23). The FASTA DNA sequences of the hub genes were obtained from the NCBI database. Subsequently, subcellular localization of the hub genes was analyzed using the mRNALocater database.

To explore the potential relationships between hub genes and other genes, a co-expression network of hub genes was constructed using GeneMANIA (http://www.genemania.org/). Functional similarity among hub genes was evaluated by calculating the average semantic similarity between their Gene Ontology (GO) terms with the GOSemSim package (version 2.26.1) (24).

Gene set enrichment analysis (GSEA) was performed to investigate the biological pathways associated with hub genes involved in GDM. In the GSE103552 dataset, correlation coefficients between the expression levels of hub genes and all genes were calculated and ranked. Based on the background gene set, the top five pathways with the smallest adjusted p values were visualized using the clusterProfiler package (adjusted $p < 0.05$).

PhosphoSitePlus is a comprehensive protein phosphorylation database that contains extensive experimentally validated data,

including information on multiple post-translational modifications (PTMs), including phosphorylation, acetylation, and ubiquitination. The hub genes were imported the hub genes into this database to predict potential types of protein post-translational modifications.

2.6 Regulatory mechanism analysis and drug prediction

To explore the molecular regulatory mechanisms of hub genes in GDM, transcription factors (TFs) targeting the hub genes were predicted using JASPAR in NetworkAnalyst (https://www.networkanalyst.ca/). In addition, microRNAs (miRNAs) targeting the hub genes were predicted using the ENCORI database (https://rnasysu.com/encori/). Long noncoding RNAs (lncRNAs) targeting the hub genes were obtained from both miRNet (https://www.mirnet.ca/miRNet/home.xhtml) and the ENCORI database. The intersecting lncRNAs from the two databases were identified as key lncRNAs.

Based on the identified hub genes, miRNAs, and key lncRNAs, an lncRNA–miRNA–hub gene regulatory network was constructed and visualized using Cytoscape software.

Furthermore, potential therapeutic drugs for GDM were predicted using the Comparative Toxicogenomics Database (CTD) (https://ctdbase.org/) based on the hub genes. The results were also visualized using Cytoscape software.

2.7 scRNA-seq data analysis

The Seurat package (version 5.1.0) (25) was used for scRNA-seq data analysis in the GSE173193 dataset. Cells with fewer than 200 or more than 6,000 genes, genes expressed in fewer than three cells or with counts greater than 50,000, and cells with more than 15% proportion of genes expressed in mitochondria were removed from subsequent analyses. After quality control, the data were normalized using the *NormalizeData* function in the "Seurat package (version 5.1.0). Subsequently, the top 2,000 genes with the highest variability were identified using the FindVariableFeatures function. Next, the dimensionality reduction was performed through principal component analysis (PCA). The ElbowPlot function in the "Seurat" package (version 5.1.0) was used to draw the elbow plot, and the principal components (PCs) before the inflection point were selected for subsequent analysis. Subsequently, Based on the selected PCs, unsupervised clustering (resolution = 0.2) was conducted via uniform manifold approximation and projection (UMAP) for all cells using the FindNeighbors and FindClusters functions of the Seurat" package (version 5.1.0). Annotated analysis of cell clusters was performed to identify specific cell types based on marker genes (26) obtained from the literature. At the same time, the percentage of various cell types was also shown ($p < 0.05$). Cell types with a significant differences in proportion between GDM placental tissue samples and normal placental tissue samples were identified. Subsequently, key cells were determined based on the differential expression of hub genes in these distinct cell types. Cell–cell communication analysis among cell types was performed using the CellChat" package (version 1.6.1) (27) to study intercellular correlations. Functional enrichment analysis of cell types was carried out using the "ReactomeGSA" package (version 1.16.1) (28). Differentially enriched pathways among different cell types were identified, and the top 10 pathways with the greatest differences were visualized. The Monocle package (version 2.28.0; PMID: 28114287) was used to perform pseudotime analysis of key cells to investigate their differentiation trajectories and the expression changes of hub genes during this transition process of key cells.

2.8 Expression analysis of hub genes

A total of five pairs of samples (five control (1–5) and five GDM (6–10) placental samples) from mice were obtained from Peking University International Hospital. The study was approved by the Peking University Health Science Center Animal Ethics Committee (Ethics approval number: PUIRB-LA2023181).

Total RNA from the 10 samples (50 mg each) was extracted using 1 mL TRIzol reagent (Ambion, USA) according to the manufacturer's protocol. Then the RNA concentration was measured using a NanoPhotometer N50. Complementary DNA (cDNA) was synthesized by reverse transcription using the SureScript First-Strand cDNA Synthesis Kit, and the reverse transcription was performed with an S1000TM Thermal Cycler (Bio-Rad, USA).

Reverse transcription quantitative polymerase chain reaction (RT-qPCR) assay was performed using the CFX Connect Real-Time Quantitative Fluorescence PCR Instrument (Bio-Rad, USA) under the following conditions: pre-denaturation at 95 °C for 1 min; denaturation at 95 °C for 20 s, annealing at 55 °C for 20 s, and extension at 72 °C for 30 s, for a total of 40 cycles. The relative quantification of mRNA levels was calculated using the $2^{-\Delta\Delta CT}$ method.

2.9 Statistical analysis

R software (version 4.2.2) was used for data processing and analysis. Statistical significance between two groups was determined using the Wilcoxon rank-sum test. A p-value < 0.05 was considered statistically significant.

3 Results

3.1 A total of 148 GDM-related candidate genes were screened out

A total of 1,039 DEGs were identified between the GDM and control groups in the GSE103552 dataset. Among these, 391 genes were

upregulated and 648 genes were downregulated (Figures 1A, B). By overlapping the 1,039 DEGs with 1,136 MRGs and 2,660 IRGs, 93 DE-MRGs and 65 DE-IRGs were obtained, respectively (Figures 1C, D). After calculating the correlations between the 93 DE-MRGs and 65 DE-IRGs, 148 candidate genes were finally screened out ($p < 0.001$ and $|cor| > 0.6$) (Figure 1E).

3.2 Screening of candidate signature genes in GDM

To identify the biological functions and pathways associated with the candidate genes, GO and KEGG enrichment analyses were performed. The results showed that 132 GO terms were significantly enriched, including mitochondrial gene expression, mitochondrial inner membrane, and structural constituent of the ribosome, etc. were enriched (Figure 2A). Furthermore, the candidate genes were enriched in 12 KEGG pathways, involving in chemical carcinogenesis–reactive oxygen species, thermogenesis, and related processes (Figures 2B, C).

A PPI network was constructed containing 119 nodes and 535 edges. NDUFAB1 exhibited the highest degree of connectivity with other genes (Figure 2D). By intersecting the top 30 genes from four algorithms, 19 candidate signature genes—including MRPS18C, MRPL22, and MRPL15—were obtained (Figures 1A–D, Figure 2E).

3.3 MRPL15, MRPL22 and MRPS18C were identified as hub genes

After analyzing the RF, SVM, and GLM models, the GLM model was determined to be the best-performing model (Figures 3A, B). The top 10 genes in this model (MRPL9, MRPL47, MRPL15, MRPL21, MRPL22, MRPS18C, MRPL1, MRPS2, MRPL40, and MALSU1) were identified as signature genes (Figure 3C).

Expression analysis revealed that MRPL15, MRPL22, and MRPS18C had higher expression levels in the control group than in the GDM group in both the GSE103552 and GSE154414 datasets. Therefore, MRPL15, MRPL22, and MRPS18C were identified as hub genes (Figure 3D).

3.4 Corresponding localization and pathways of hub genes in GDM

Chromosomal localization analysis showed that MRPS18C was located on chromosome 4, MRPL22 was on chromosome 5, and MRPL15 was on chromosome 8 (Figure 4A). Subcellular localization analysis indicated that MRPL15 and MRPL22 were mainly expressed in the nucleus (proportion > 40%), whereas MRPS18C was mainly expressed in the cytoplasm (proportion > 50%) (Figure 4B).

The hub genes were found to share similar functions with MRPS18A, MRPS18A, RPL17-C18orf32, and other ribosomal proteins. Their main functions included the ribosomal subunit, ribosome, and translational termination processes (Figure 4C). Similarity analysis showed that MRPS18C and MRPL22 had higher functional similarity than MRPL15 (Figure 4D).

Additionally, GSEA was performed to explore biological pathways involving the hub genes in GDM. The top five pathways were enriched in spliceosome, proteasome, and ribosome-related processes (Figure 4E). Based on the PhosphoSitePlus database, we predicted the post-translational modification (PTM) types of the hub genes were predicted: MRPL15 was mainly modified by phosphorylation and ubiquitination, MRPL22 was primarily subject to phosphorylation and ubiquitination, and MRPS18C was mainly modified by phosphorylation and acetylation (Figure 4F).

3.5 Gene regulatory networks and potential drugs of hub genes in GDM

To clarify the regulatory mechanisms of hub genes in GDM, 11 transcription factors (TFs) were predicted. Among these TFs, YY1 regulated MRPS18C and MRPL22; ARID3A regulated MRPS18C and MRPL15; and FOXC1 regulated MRPL22 and MRPL15 (Figure 5A). According to the hub genes, 13 miRNAs and 43 lncRNAs were obtained, and an lncRNA–miRNA–hub gene network was constructed with 59 nodes and 128 edges. OIP5-AS1, NEAT1, and KCNQ1OT1 regulated MRPL22 through hsa-miR-1277-5p, hsa-miR-129-5p, hsa-miR-183-5p, hsa-miR-224-3p, and hsa-miR-522-3p. NEAT1, MALAT1, KCNQ1OT1, and XIST regulated MRPS18C through hsa-miR-140-5p, hsa-miR-154-3p, and hsa-miR-487a-3p. NEAT1 and KCNQ1OT1 regulated MRPL15 through hsa-miR-136-5p, hsa-miR-194-5p, hsa-miR-4712-5p, hsa-miR-770-5p, and hsa-miR-802 (Figure 5B).

Furthermore, potential drugs for GDM were predicted based on the hub genes. Acetaminophen, dicrotophos, lactic acid, and ribonucleotides were simultaneously predicted to target MRPL15, MRPL22, and MRPS18C (Figure 5C).

3.6 Cells were clustered into nine types

To explore the cell populations associated with GDM, scRNA-seq analysis was performed. After quality control, a total of 25,487 cells and 23,068 genes were retained (Figure 6A), and the top 2,000 highly variable genes were identified (Figure 6B). In PCA, 30 principal components (PCs) were selected for subsequent analyses according to the elbow plot (Figures 6C–E). The cells were then clustered into 14 clusters (Figure 6F).

Based on marker gene expression of marker genes, the clustered cells were classified into nine cell types: villous cytotrophoblast cells, syncytiotrophoblast cells, extravillous trophoblast cells, myelocytes, T/NK cells, B cells, monocytes, macrophages, and granulocytes

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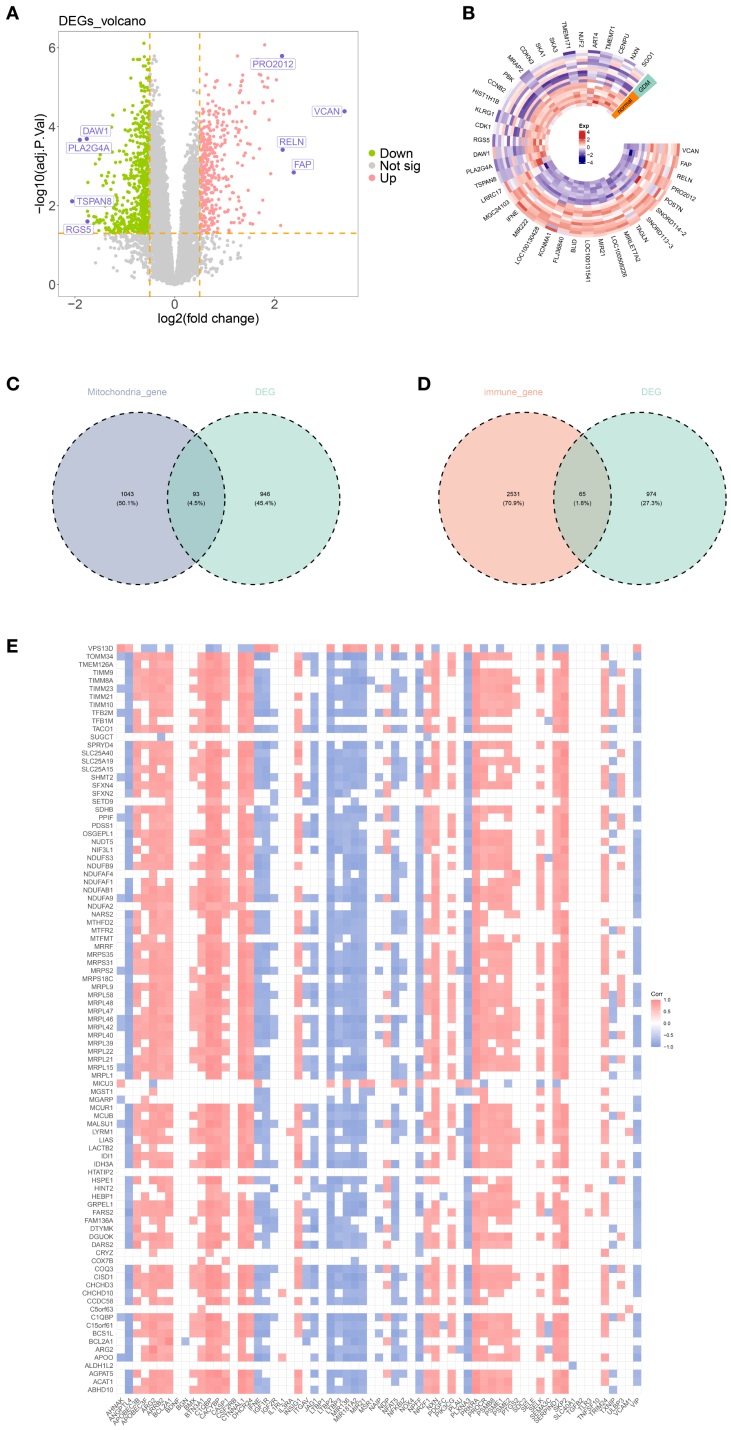

FIGURE 1
(A, B) Differential gene volcano plot and heatmap. **(C, D)** Venn diagram of differentially expressed genes, mitochondrial genes, and immune genes. **(E)** Correlation heatmap between differential mitochondrial genes and differential immune genes. Thresholds for differential analysis were adjusted p-value < 0.05 and $|\log_2 FC| > 0.5$; thresholds for correlation analysis were p-value < 0.05 and |cor| > 0.3.

(Figures 6G, H). Functional enrichment analysis of the nine cell types of cells was conducted to identify the pathways in which they were involved. The top 10 pathways showing the largest differences were visualized, including the TWIK-related acid-sensitive K^+ channel, hydrolysis of LPE, and ALKBH2-mediated reversal of alkylation damage, etc (Figure 6I).

3.7 Monocytes and villous cytotrophoblast cells were further defined as key cells

We first identified seven differential cell types between the GDM group and the control groups (Figures 7A, B). MRPL15 showed a significant expression difference in monocytes; MRPL22

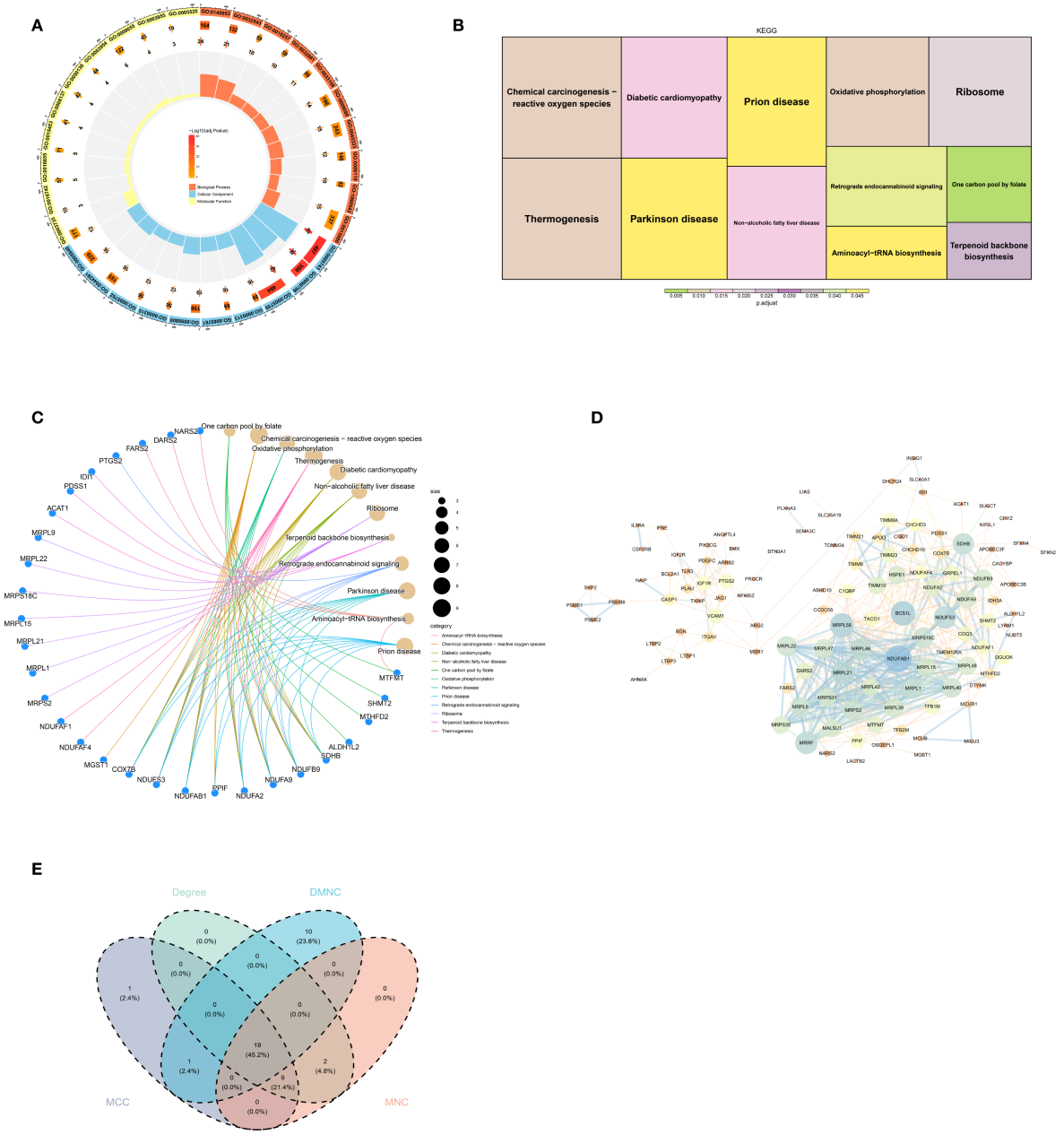

FIGURE 2
(A) GO enrichment circle diagram. **(B, C)** KEGG enrichment diagrams. **(D)** PPI network diagram. **(E)** CytoHubba screening gene Venn diagram.

exhibited a significantly higher expression difference in villous cytotrophoblast cells; and *MRPS18C* displayed significant expression differences in villous cytotrophoblast cells, monocytes, and granulocytes (Figure 7C). Therefore, monocytes and villous cytotrophoblast cells were selected and defined as key cells.

Next, the intercellular interaction network among all cells in the GDM and the control groups was analyzed. The results showed that, compared with the control group, the number of interactions between monocytes, T/NK cells, and other cells decreased in the GDM group decreased (Figure 7D). In addition, the receptor–ligand pairs MIF–(CD74+CXCR4) and MIF–(CD74+CD44) were more active in the GDM group than in the control group (Figure 7E). A heatmap of the intercellular interaction network further indicated that the total number of intercellular interactions was reduced in the disease group was reduced compared with the control group (Figure 7F).

Pseudotime analysis was then conducted for the key cells. During the differentiation and development of monocytes, one developmental node and three differentiation states were identified (Figure 7G). For villous cytotrophoblast cells, two developmental nodes and five differentiation states were observed during their differentiation and development (Figure 7H). The expression levels of MRPL15, MRPL22, and *MRPS18C* all showed a decreasing trend during the differentiation of both monocytes and villous cytotrophoblast cells (Figure 7I).

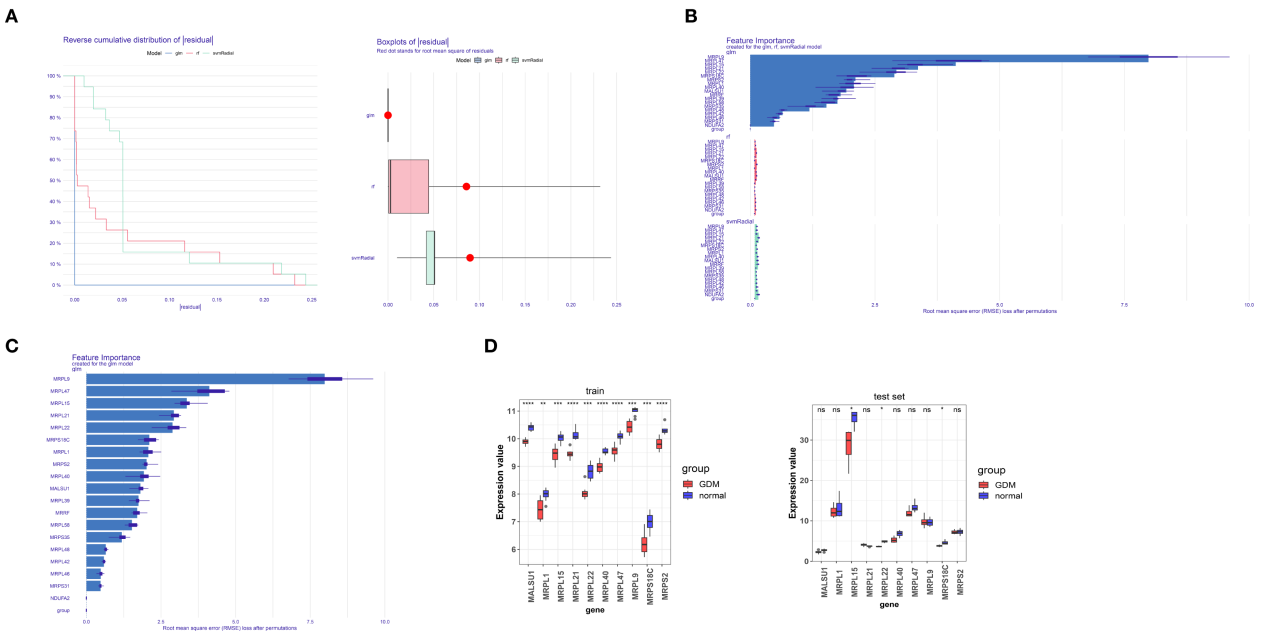

FIGURE 3
(A) Evaluation of machine learning models by sample cumulative residual distribution plots and sample residual box plots. **(B, C)** Importance of explanatory variables in the three models **(B)** and in the best model **(C)**. **(D)** Box plots of candidate gene expression levels. * represent $p < 0.05$, ** represent $p < 0.01$, *** represent $p < 0.001$, **** represent $p < 0.0001$.

3.8 Expression analysis results

RT-qPCR results showed that MRPL15, MRPL22, and MRPS18C had significantly lower expression levels in the GDM group (Figures 8A–C).

4 Discussion

With changes in social and economic life and dietary structure, the incidence of gestational obesity and its closely related GDM is increasing year by year (29). Studies have shown that the expression levels of mitochondrial electron transfer complexes I, II, III, and IV in GDM women with GDM treated with insulin or oral hypoglycemic drugs are lower than those in GDM women treated with normal pregnancy or those treated with diet control (30). Other studies have shown that some immune cells, including neutrophils, eosinophils, monocytes, NK cells, and lymphocytes, are regulated in patients with type 1 diabetes mellitus and type 2 diabetes mellitus, whether related to pregnancy-related or not, indicating that these cells play an important role in the pathogenesis of this disease (10). In this study, the hub genes related to mitochondria and immunity in the process of GDM, as well as the biological processes and mechanisms involved, were analyzed by bioinformatics to provide a theoretical basis for the treatment of GDM.

In this study, 1,093 differentially expressed genes between the GDM group and the normal group were screened, including 391 upregulated genes, 698 downregulated genes, and 148 candidate genes. Subsequently, GO and KEGG functional enrichment analyses were performed to obtain pathways such as mitochondrial gene expression, mitochondrial translation, NADH dehydrogenase complex assembly, and carbon pool by folate, chemical carcinogenesis–reactive oxygen species, and oxidative phosphorylation and so on. Previous studies have shown that the correlation between GDM and PM2.5 may be attributed to the possibility that high PM2.5 levels inducing mitochondrial gene dysfunction. Mitochondrial OXPHOS dysfunction affects the active growth of related genes and leads to mitochondrial damage in healthy premature infants (including newborns) through the changes in electron transport chain complex proteins (31). Another study found that endothelial dysfunction may be one of the mechanisms of GDM by comparing the difference of superoxide differences between GDM and healthy umbilical vein endothelial cells (32).

In this study, three hub genes in GDM were identified by machine learning and expression validation: MRPL15, MRPL22, and MRPS18C. MRPL15 belongs to the mitochondrial biomarker set of genes, which may encode mammalian mitochondrial ribosomal proteins and thus assist in protein synthesis within the mitochondrion. Previous research has shown that MRPL15 can be used as a companion diagnostic marker to determine which breast cancer patients might benefit most from clinical therapy (33). As a risk gene and potential biological target of Alzheimer's disease (AD), MRPL15 also plays an important role in regulating immune cells in AD (34). In addition, a recent study has confirmed that MRPL15 is significantly correlated with diabetic retinopathy (35). However, the abnormal expression of MRPL15 in

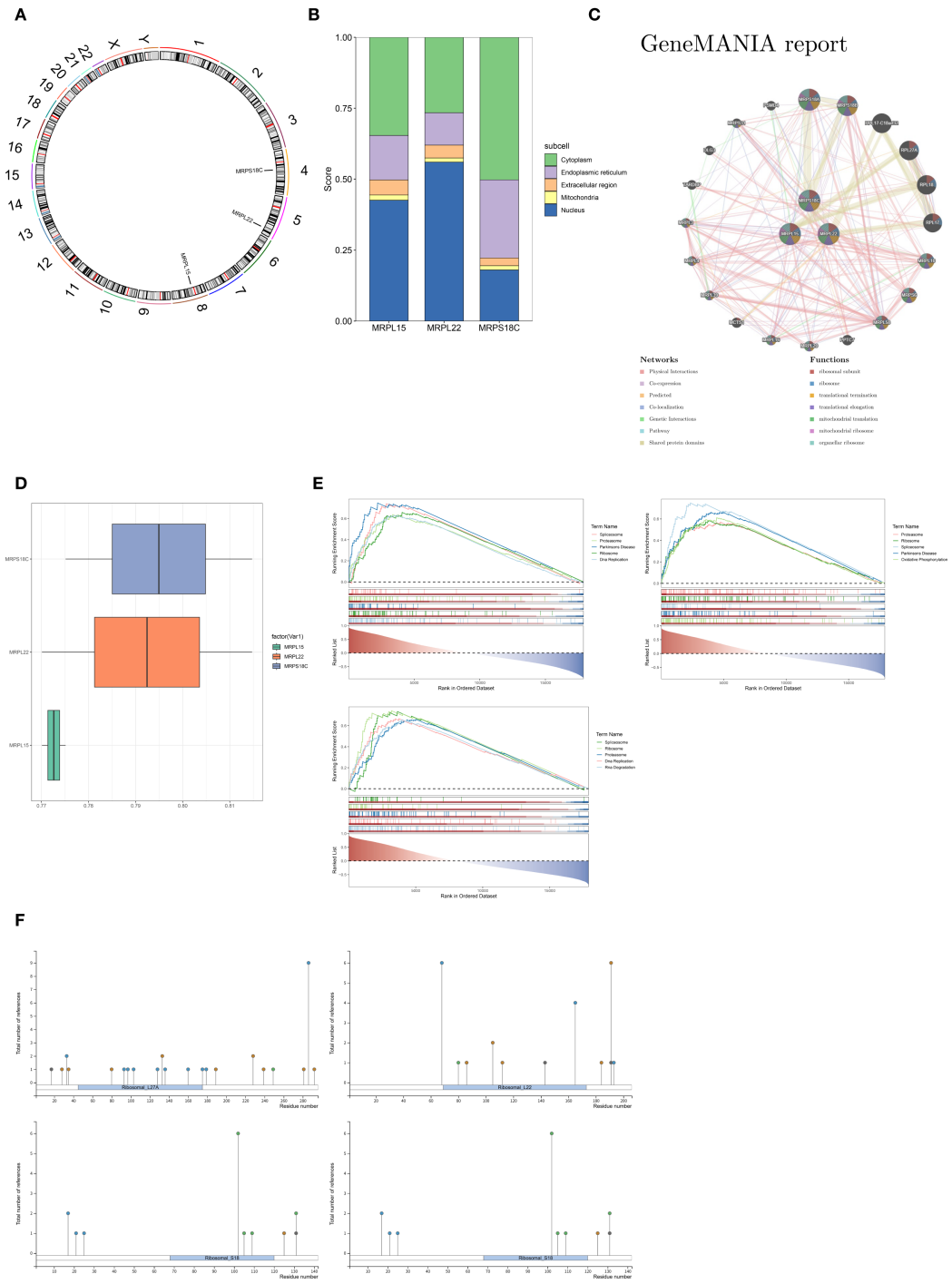

FIGURE 4
(A) Chromosomal localization of hub genes. **(B)** Subcellular localization of hub genes. **(C)** GeneMANIA network. **(D)** Functional similarity analysis of hub genes. **(E)** GSEA results. **(F)** Post-translational modification (PTM) analysis of hub genes.

GDM has not been confirmed. Previous studies have shown that MRPL22, as an immune-related gene, participates in the T cell receptor signaling pathway and was identified as a hub gene for the diagnosis of ischemic stroke (36). Recent studies have also shown that MRPL22 was identified as a shared gene signature for endometrial cancer and polycystic ovary syndrome (37). The MRPS18C gene belongs to the mitochondrial ribosomal protein (MRP) family, which is involved in mitochondrial translational termination, elongation, translation, and poly (A) RNA binding. Studies have shown that MRPS18C is negatively correlated with overall survival in breast cancer and may act as a biomarker for risk prediction and may serve as a potential genetic target in breast cancer patients (38). However, there is no known correlation between these three genes and the occurrence of GDM either

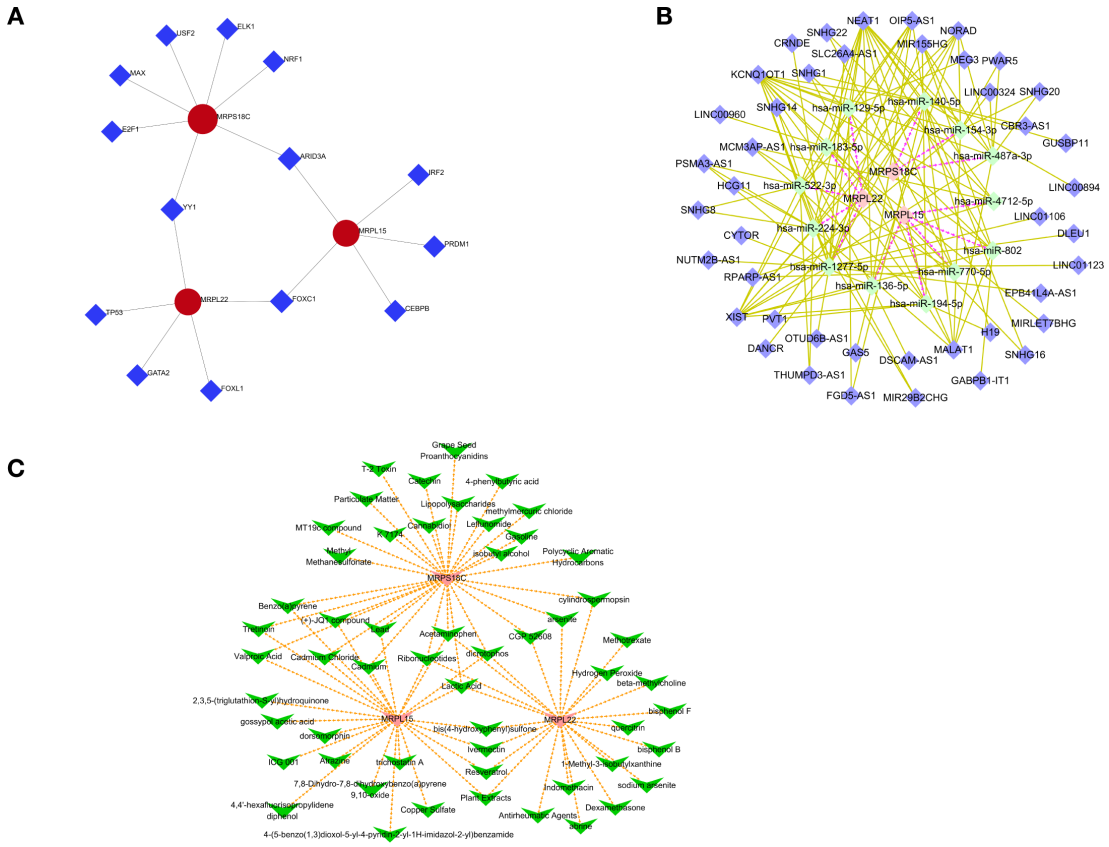

FIGURE 5
(A) Transcription factor (TF) network diagram. **(B)** mRNA–miRNA–lncRNA relationship network diagram. **(C)** Network diagram showing the relationship between genes and drugs.

domestically or internationally. This study is the first time to find that differences in the difference of expression of these three genes may contribute to the occurrence of GDM.

In this study, GSEA enrichment was used to explore the pathway functions of the hub genes. The results showed that the hub genes were mainly concentrated in the proteasome and Parkinson's disease (PD) pathways. Misfolded proteins are usually degraded by the ubiquitin–proteasome system. However, if this system is damaged, misfolded proteins will escape degradation and are released into the cytoplasm. Maternal hyperglycemia can lead to abnormal gene expression in the proteasome, resulting in the accumulation of misfolded cytotoxic proteins in cells and impaired organelle function. This may induce mitochondria to produce a large amounts of ROS, leading to oxidative stress and intracellular signaling disturbances that alter cell activity (39). Furthermore, studies have shown that gestational factors play an important role in shaping brain development. GDM may cause interindividual variation in neuronal and glial cell load at birth, potentially influencing acquired neurodegenerative diseases, including PD and Alzheimer's disease (AD) (40).

One of the core pathological features of Parkinson's disease (PD) is mitochondrial dysfunction in substantia nigra dopaminergic neurons, which is specifically manifested by decreased activity of mitochondrial respiratory chain complex I (NADH dehydrogenase), mitochondrial DNA (mtDNA) damage, excessive accumulation of reactive oxygen species (ROS), and ultimately neuronal apoptosis (41, 42). Similar mitochondrial pathological phenotypes have been reported in GDM placentas (43, 44). Hub genes such as *MRPL15* and *MRPL22* are enriched in the PD pathway, linking PD and GDM. We speculate that they share a core pathological mechanism of "mitochondrial functional defect–oxidative stress imbalance." MRPL15 and MRPL22 are both mitochondrial function–related genes. As core subunits of the mitochondrial ribosome, they are essential for mitochondrial oxidative phosphorylation and play crucial roles in regulating cell death–inducing factors (45–47). Abnormal expression of mitochondrial ribosomal proteins (MRPs) can lead to various disorders, such as mitochondrial metabolic defects and cellular dysfunction. Changes in the expression of these genes directly trigger a chain reaction of "decreased mitochondrial translation efficiency → OXPHOS complex assembly defect → decreased mitochondrial respiratory function → ROS accumulation," which represents not only the core pathogenesis of PD but also the key molecular basis of placental dysfunction in GDM.

In this study, monocytes and villous cytotrophoblast cells were identified as key cells in GDM. Monocytes are important innate immune cells in the maternal circulation. They can contribute to the pathological process of GDM by differentiating into macrophages

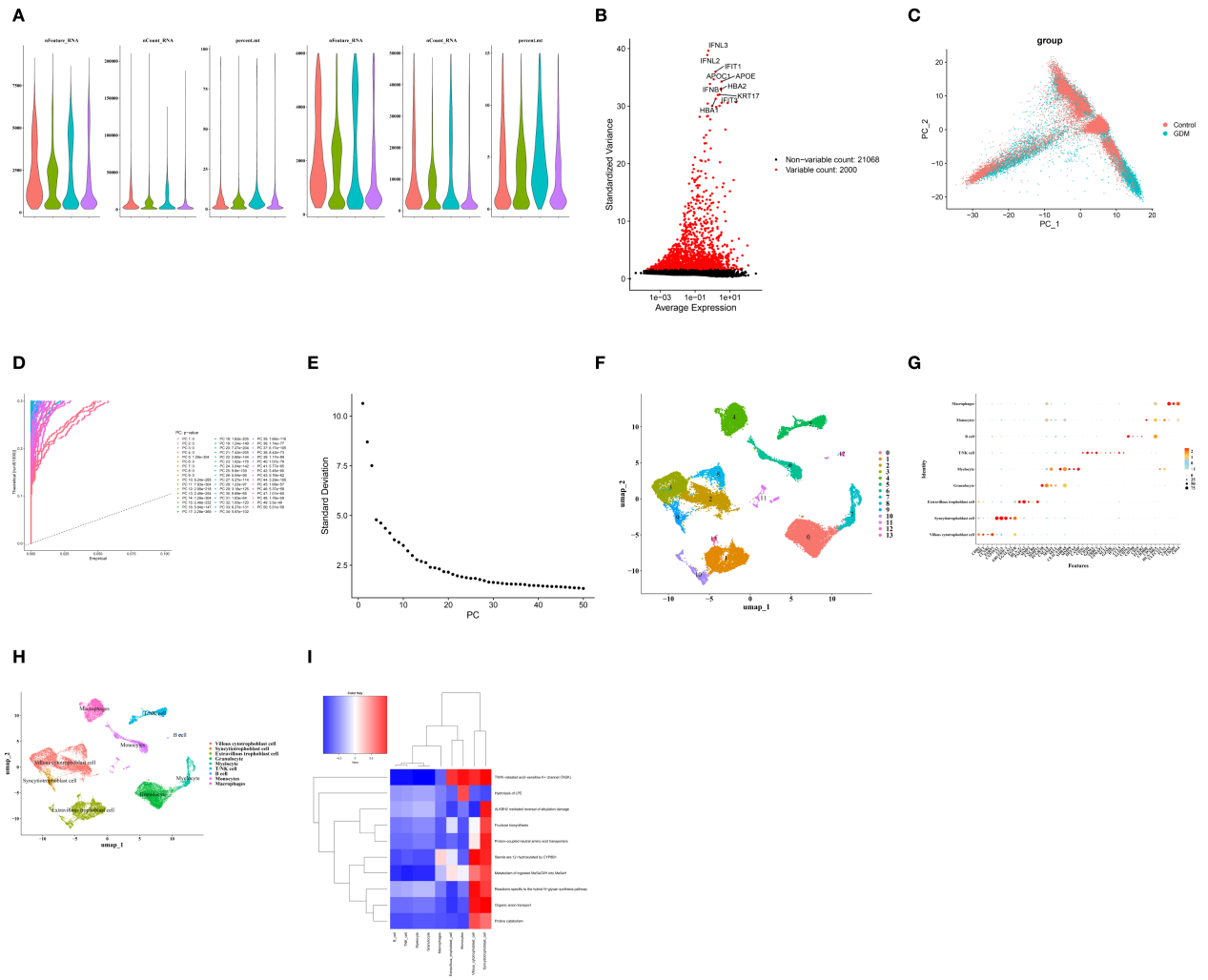

FIGURE 6
(A) Violin plots of the single-cell dataset before and after quality control. **(B)** Screening of highly variable genes. **(C–E)** Principal component analysis (PCA). **(F)** Cell clustering results. **(G, H)** Dot plot of marker gene expression in each cell type **(G)** and cell annotation results **(H)**. **(I)** Enrichment analysis of each cell type.

(such as extravillous macrophages in placental tissue), secreting inflammatory factors, and regulating metabolism-related pathways. Studies have shown that the monocyte-to-lymphocyte ratio in early pregnancy is a predictor of GDM (48). These activated monocytes oversecrete proinflammatory cytokines (such as IL-6, TNF-α, and IL-1β) (49), and there is a close link between the production of inflammatory biomarkers and the occurrence of GDM (50).

Villous cytotrophoblasts (VCTs) are the core cell type of placental villous lobules. Their main functions include differentiation into syncytiotrophoblasts (STBs), transport of materials (glucose, amino acids, and fatty acids), secretion of placental hormones (such as hCG and placental lactogen), and participation in placental vascularization (51, 52). Studies have shown that lipopolysaccharide (LPS) with 2.5–25 mM glucose can induce increased expression of autophagy proteins, inflammatory markers, and m6A levels in human villous trophoblasts (53). GDM alters the balance of paracrine factors regulating trophoblast-derived angiogenesis, which may lead to GDM-related pathological changes in placental angiogenesis and vascular structure (54). Under normal circumstances, placental development requires proper coordination of trophoblast proliferation, differentiation, and invasion, whereas in the context of diabetes, trophoblast proliferation, cell death, and cell-cycle control are altered (55). Both previous studies and our findings indicate the important role of these two cell types in the pathogenesis of GDM.

The regulatory network is a key component of the gene expression regulation process. It has important research value, and can reveal the complexity and diversity of gene expression regulation, thereby enabling a deeper understanding of the regulatory mechanisms involved. To study the potential regulatory mechanisms of the final hub genes in GDM, this study further constructed a regulatory network of these hub genes. Eleven TFs were predicted. The TF shared by MRPS18C and MRPL22 was YY1; the TF shared by MRPS18C and MRPL15 was ARID3A; and MRPL22 and *MRPL15* shared FOXC1. The GL-3/FOXC1 pathway

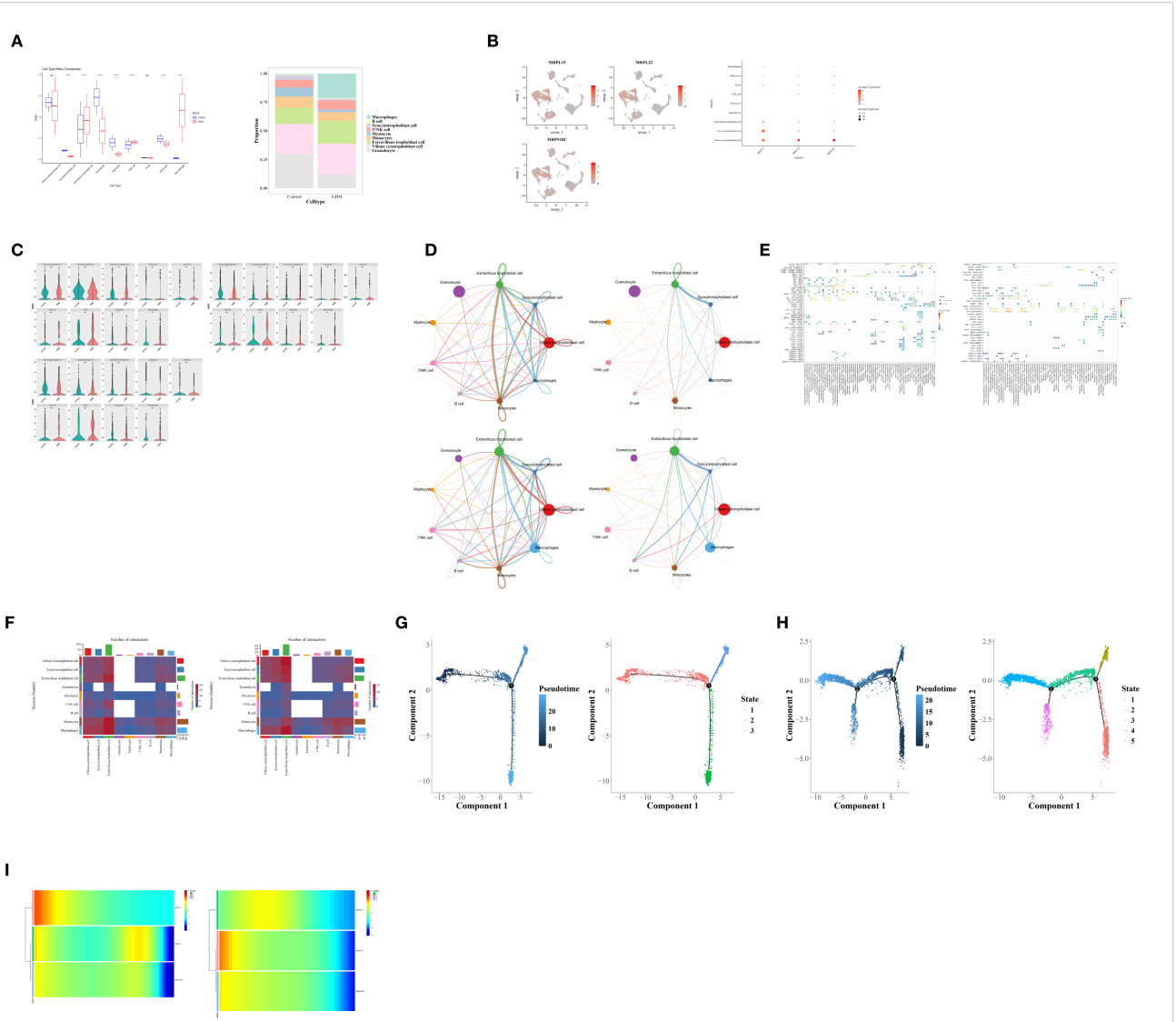

FIGURE 7
(A) Identification of differential cells between the GDM and control groups. **(B)** Expression of hub genes in each cell type. **(C)** Differential expression analysis of hub genes in each cell type (for identification of key cells). **(D)** Number and intensity of cell–cell communications between the control and GDM groups (1–2 represent communication number and intensity of the control group, respectively; 3–4 represent communication number and intensity of the GDM group, respectively). **(E)** Dot plot of cell–cell communication receptor–ligand pairs between the control and GDM groups. **(F)** Heatmaps of intercellular interaction networks in the control and GDM groups. **(G, H)** Pseudotime differentiation trajectories of key cells: monocytes **(G)** and villous cytotrophoblasts **(H)**. **(I)** Expression trends of hub genes during differentiation of key cells: monocytes (1) and villous cytotrophoblasts (2).

has been shown to protect HTR-8/SVneo cells from high glucose–induced apoptosis (56), suggesting that GL-3 and FOXC1 may play important protective roles in hyperglycemia during pregnancy. Studies have also shown that inactivation of YY1 impairs mitochondrial OXPHOS activity in mouse models and induces mitochondrial dysfunction and diabetes (57).

According to the hub genes, 13 miRNAs and 43 lncRNAs were identified. A recent study reported that the level of OIP5-AS1 levels decreased in GDM women with GDM. The OIP5-AS1/miR-137-3p/EZH2 axis may function in HTR-8/SVneo cells under high-glucose conditions (58), suggesting that OIP5-AS1 could be a potential target for the prevention and treatment of GDM. CEBPB is an important transcription factor involved in regulating immune inflammation and metabolic responses, playing significant roles in lipogenesis, glucose and lipid metabolism, liver regeneration, and hematopoiesis. Results have shown that the AKT phosphorylation level of insulin and glucose uptake in hepatocytes were significantly increase when CEBPB expression is eliminated by LIN (59). In addition, recent studies have confirmed that inhibiting the expression of CEBPB in trophoblasts can significantly enhance the insulin signaling by increasing AKT phosphorylation levels in the insulin pathway (60). These findings suggest that CEBPB affects glucose uptake by inhibiting AKT phosphorylation, which may further contribute to the development of GDM.

The miR-194-5p is a multifunctional miRNA involved in regulating cell differentiation and development, as well as

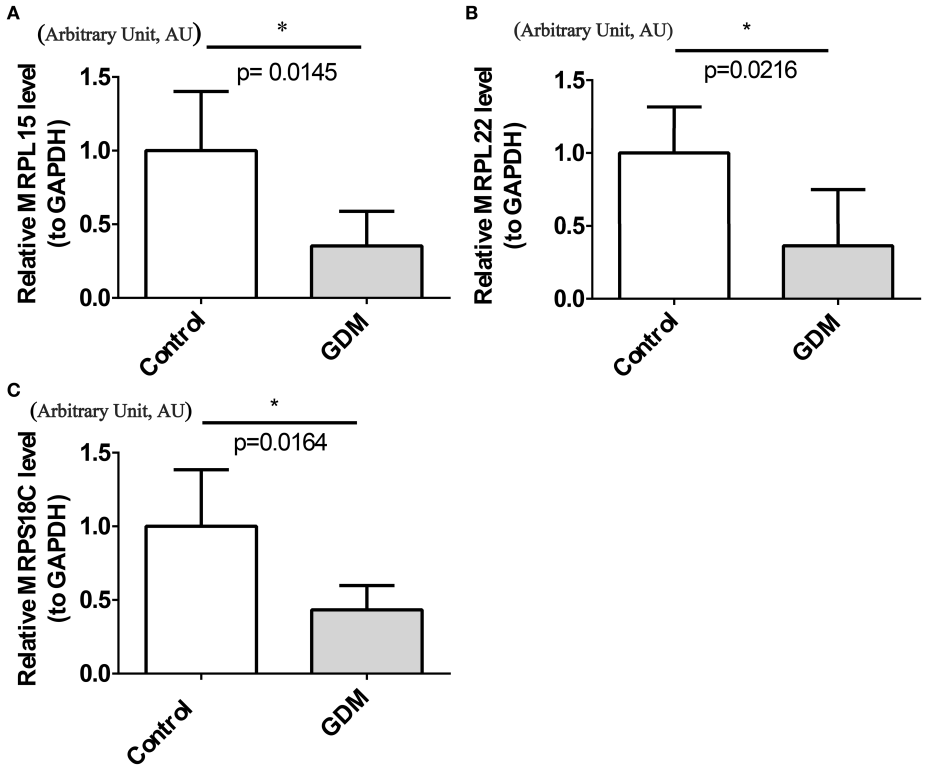

FIGURE 8
Confirmation of hub DEG expression in GDM mice. **(A)** MRPL15 expression in control (CON) and GDM mice. **(B)** MRPL22 expression in CON and GDM mice. **(C)** MRPS18C expression in CON and GDM mice. $p < 0.05$.

immune modulation of glucose and lipid metabolism and other biological processes, and is closely associated with diseases such as tumors, diabetes, and chronic inflammatory organ fibrosis (61). Previous studies have shown that miR-194-5p is closely related to residual β-cell function in children with type 1 diabetes mellitus (62). Recent studies have also found that miR-194-5p may participate in the progression of diabetic nephropathy by targeting ITGA9 to regulate macrophage migration and adhesion, thereby blocking the high glucose–induced upregulation of ITGA9 protein levels (63). Other studies have shown that the expression of *TGFB1*, *COL1A1*, and miR-139-5p changes in GDM patients, suggesting that miR-129-5p and miR-139-5p may play an important roles in GDM by regulating TGFB1 and COL1A1 gene networks (64). KCNQ1OT1 also plays an important role in regulating β-cell proliferation, scorching and insulin secretion, and cell death, as shown by Chen YL et al. (65). Studies have found that KCNQ1OT1 influences β-cell function by promoting its proliferation and insulin secretion, suggesting that it may serve as a new biomarker of islet function. However, in studies of type 2 diabetes caused by hepatitis C virus infection, it is shown that KCNQ1OT1 was found to promote the scorch death of β-cells infected by hepatitis C virus through the miR-223-3p/NLRP3 axis, thereby affecting insulin production and accelerating the onset of diabetes and (66). To date, there has been no study on the effect of KCNQ1OT1 on GDM, and its regulatory role of KCNQ1OT1 in GDM requires further investigation in the future.

In this study, three hub genes were used to predict related drugs. Four compounds—acetaminophen, nucleotide, dicrotophos, and lactic acid—were predicted by all three genes. Among these, dicrotophos is a highly toxic organophosphorus pesticide with teratogenic, embryotoxic, and neurotoxic properties. It is strictly prohibited for human or pregnancy-related research. Therefore, only the other three drugs will be discussed in the following section.

Previous studies have confirmed that prenatal use of acetaminophen is associated with adverse birth outcomes (67), but the correlation between acetaminophen and GDM still requires confirmation through animal experiments and clinical studies. Studies have shown that moderate administration of acetaminophen can activate the Nrf2 antioxidant pathway and reduce mitochondrial ROS generation (68). However, overdose induces hepatotoxicity. Targeted scavenging of mitochondrial ROS can significantly reduce drug-induced hepatotoxicity (69). These processes may correlate with the pathogenesis of GDM.

A recent study on the relationship between intestinal metabolic microflora and GDM in pregnant women showed that the changes in plasma lactate levels and hyperglycemia-related fecal microflora are associated with altered blood glucose levels in GDM patients, suggesting that modulation of intestinal microflora in pregnant women may help alleviate GDM (70). Lactic acid is an endogenous metabolite of glucose metabolism. When mitochondrial function declines, glycolysis is enhanced, leading to lactic acid accumulation. Lactic acid can activate the AMPK signaling pathway, thereby

promoting mitochondrial biosynthesis (71–73). Therefore, lactic acid may participate in metabolic compensation by regulating hub genes, providing new insights into the mechanism of "glycolytic compensation for mitochondrial function" in the placenta of GDM. Studies have also shown that there are significant differences in the taxonomic composition of the oral microflora between GDM and non-GDM women. Metabolic pathway analysis revealed that 5-aminoimidazole ribonucleotide biosynthesis and inosine-5′-phosphate biosynthesis were enriched in the GDM women with GDM (74), suggesting that the oral nucleotide level in pregnant women may be closely related to the occurrence of GDM and could serve as a target for prevention and treatment of GDM. Nucleotides are the precursors for RNA synthesis. Mitochondria are prone to oxidative stress–related DNA damage, and nucleotide imbalance can lead to mitochondrial depletion due to reduced replication fidelity. Supplementation with nucleotides can promote the synthesis of mitochondrial ribosomal proteins by increasing the supply of mitochondrial transcription materials (75). Therefore, theoretically, ribonucleotide supplementation may improve the expression of mitochondrial ribosomal proteins through "material support," potentially influencing the molecular mechanisms underlying GDM.

In this study, the hub genes related to mitochondria and immunity in GDM were identified using bioinformatics. By analyzing the relationship between the biological pathways of hub genes in bioinformatics and immune cells, we constructed the molecular regulatory network of these genes is constructed. However, there are still some limitations.

First, we used gene expression and co-expression network construction, but did not incorporate advanced data such as proteomics, which may limit a comprehensive understanding of the biological processes underlying GDM. To address this research gap, we plan to conduct detailed protein-level experiments in the future. Specifically, we will apply targeted proteomics techniques based on parallel reaction monitoring (PRM; high-sensitivity LC-MS/MS) and immunohistochemistry (IHC) or immunofluorescence (IF) to detect the protein abundance of MRPL15, MRPL22, and *MRPS18C*. Immunoprecipitation (Co-IP) will be used to verify key protein interactions and determine whether GDM disrupts mitochondrial ribosome assembly or its association with oxidative phosphorylation complexes.

At the same time, to correlate protein-level changes with actual mitochondrial function, we will use the Seahorse XF analyzer to assess mitochondrial respiratory parameters (such as basal respiration and maximal respiration), JC-1 staining to detect mitochondrial membrane potential, and the LC3B-II/LC3B-I ratio to evaluate mitochondrial autophagy levels.

Second, although the related hub genes were identified using machine learning algorithms and functional enrichment analyses, the dataset used in this study had a relatively small sample size, and the sample types between the training and validation sets were not consistent. Furthermore, no clinical validation was conducted on a large population or sample size. We recognize the necessity of more accurate and comprehensive clinical validation.

Therefore, further population-based experiments and clinical studies are essential. We plan to collaborate with three obstetrics and gynecology centers to collect samples from 150 women with GDM and 150 healthy pregnant women. Special attention will be given to paired sampling: collecting both primary placental artery cells and matched placental tissue samples from the same GDM/healthy participants. We will test whether the expression of MRPL15, MRPL22, and MRPS18C is consistent across sample types and extend the analysis to noncoding RNA and clinical levels by detecting the expression of related lncRNAs and miRNAs. Their association with clinical indicators—such as blood glucose and neonatal birth weight—will be analyzed to verify their potential as diagnostic biomarkers for GDM.

Finally, we currently lack experimental evidence directly linking hub gene expression changes to alterations in mitochondrial metabolic phenotypes. Therefore, we will conduct additional cellular-level experiments by constructing cell lines with gene knockdown or overexpression to verify the direct roles of these genes in regulating mitochondrial function and immune response, thereby further elucidating their mechanisms in GDM.

5 Conclusion

In this study, three hub genes related to mitochondrial and immune functions in GDM were identified using differential gene correlation and machine learning. The pathogenesis of GDM was explored through analyses of functional immune molecule regulatory networks and drug prediction, and further verified by animal models. These findings provide a foundation for the early diagnosis and treatment of GDM.

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 animal studies were approved by Peking University Health Science Center Animal Ethics Committee. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent was obtained from the owners for the participation of their animals in this study.

Author contributions

XZ: Conceptualization, Writing – original draft. YM: Data curation, Writing – original draft. JS: Methodology, Writing – original draft. XMZ: Formal analysis, 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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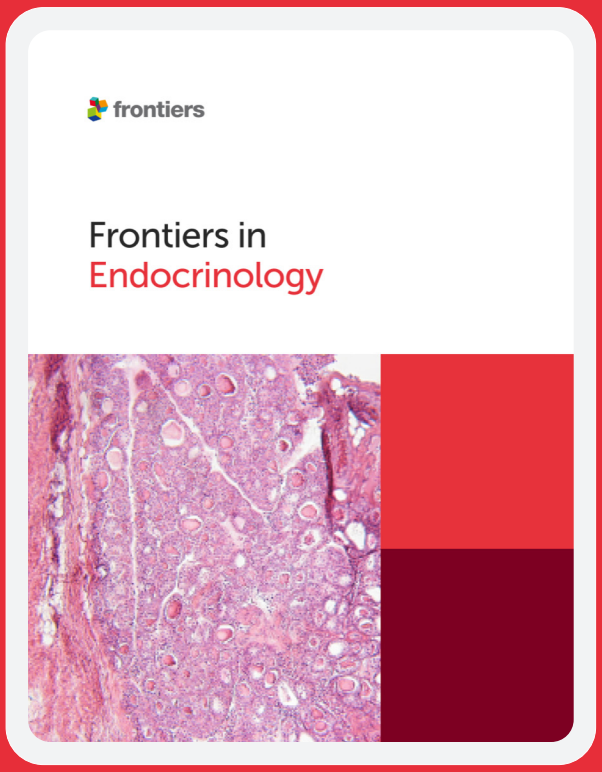

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