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ORIGINAL RESEARCH

Risk Factors Screening for Gestational Diabetes Mellitus Heterogeneity in Chinese Pregnant Women: A Case–Control Study

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Ning Wang[1,*]
Yanqi Peng[1,*]
Lu Wang[1]
Lin Song[2]
Bo Sun[2]
Junxiang Wei[3]
Ting Wang[4]
Yang Mi[3]
Wei Cui[1]

[1]Department of Endocrinology and Second Department of Geriatrics, The First Affiliated Hospital of Xi'an Jiaotong University, Xi'an, People's Republic of China; [2]Department of Physiology and Pathophysiology, School of Basic Medical Sciences, Xi'an Jiaotong University Health Science Center, Xi'an, People's Republic of China; [3]The Second Department of Obstetrics, Northwest Women and Children's Hospital, Xi'an, People's Republic of China; [4]Department of Respiratory Medicine, Xi'an No.4 Hospital, Xi'an, People's Republic of China

*These authors contributed equally to this work

Correspondence: Yang Mi
The Second Department of Obstetrics, Northwest Women and Children's Hospital, 1616 Yanxiang Road, Xi'an, Shaanxi, 710065, People's Republic of China
Tel +86-13700222172
Email miyangmm@163.com

Wei Cui
Department of Endocrinology and Second Department of Geriatrics, The First Affiliated Hospital of Xi'an Jiaotong University, 277 West Yanta Road, Xi'an, Shaanxi, 710061, People's Republic of China
Tel +86-13609281695
Email doctorweiweixjtu@126.com

Purpose: To study the risk factors of gestational diabetes mellitus (GDM) heterogeneity, and to evaluate the correlation between the risk factors and obesity.

Methods: We performed a case–control study of 452 women with GDM and 516 women with normal glucose tolerance (NGT) at the first and second trimester. We defined GDM women as GDM-resistance subtype, GDM-dysfunction subtype, and GDM-mixed subtype, according to their simultaneous insulin-release test with predominant insulin-sensitivity defect, insulin-secretion defect, or both defects.

Results: We found that higher maternal age, family history of diabetes, the elevated level of fasting blood glucose in the first trimester (≥5.1 mmol/L) were risk factors of all GDM subtypes. Pre-pregnancy overweight/obesity and the increased gestational weight gain (GWG) in the first-trimester are risk factors of the GDM-resistance subtype. Indicators including younger age at first menstruation, the elevated levels of alanine aminotransferase (ALT), total bile acid (TBA), triglyceride (TG), and the decreased level of high-density lipoprotein cholesterol (HDL-C) are risk factors of the GDM-resistance subtype. However, the associations between those risk factors and GDM-resistance subtype attenuated after adjusted by pre-pregnancy body mass index (pre-BMI) and gestational weight gain (GWG) in the first trimester. Nonalcoholic fatty liver disease (NAFLD) and the improved level of TG are independent risk factors for the GDM-resistance subtype and the GDM-mixed subtype, respectively.

Conclusion: Women with GDM exhibited heterogeneity based on glycemic physiology and their risk factors are not all the same. Some obesity-related risk factors are specific to the GDM-resistance subtype, which are mediated by pre-pregnancy overweight/obesity and the elevated GWG the first-trimester.

Keywords: pregnancy, pre-pregnancy BMI, gestational weight gain, obesity, insulin resistance, insulin secretion

Introduction

Gestational diabetes mellitus (GDM) is a common complication of pregnancy period in which spontaneous hyperglycemia usually happens during the second and third trimesters without known diabetes before pregnancy.[1] GDM increasing the risks for adverse perinatal outcomes such as metabolic disease for both mother and infants.[2] Insufficient insulin secretion and decreased insulin sensitivity have been verified as the two predominant drivers of hyperglycemia in non-pregnant diabetes individuals.[3] Powe[4] first observed and reported that heterogeneity existed in the physiologic and pathologic processes leading to hyperglycemia in women

with GDM. GDM heterogeneity refers to the variety in genetic factors, racial and demographic differences, beta-cell function, and some special clinical characteristics. In the current study, we specifically targeted on the heterogeneity of insulin sensitivity and beta-cell function and the pathogenesis of GDM, with our subtypes described as GDM with a predominant insulin-secretion defect, GDM with a predominant insulin-sensitivity defect, or GDM with both defects.

Many studies focus on risk factors of GDM, but these results lack consistency. A meta-analysis of European, North American and Australian cohorts found that maternal pre-pregnancy overweight/obesity is associated with the risk of GDM,[5] while another study uncovered that the effect of pre-pregnancy body mass index (pre-BMI) as a screening characteristic to predict GDM varied by race/ethnicity.[6] The effect of other reported risk factors on GDM such as maternal lipid profile and different diet/lifestyle changed according to BMI.[7–9] Variations in population and diagnostic criteria for GDM partly account for the differences in these findings. The heterogeneity of physiologic hyperglycemia among women with GDM may explain this inconsistency from a new perspective. Our previous research found that GDM women of the three subtypes had different clinical characteristics,[10] and we speculate that the discrepancy of risk factors also existed among the three GDM subtypes.

Obesity causes a systemic inflammatory response, with possible downstream metabolic sequelae, including insulin resistance and glucose dysregulation.[11] Some studies used stratified BMI, lean/obese phenotypes to determine risk factors of developing GDM,[9,12] however, none of these researches reflected the causes of hyperglycemia in pregnant women.

Recently, many studies uncovered some new risk factors of GDM, such as the increased level of total bile acid (TBA)[13] and ferritin,[14] nonalcoholic fatty liver disease (NAFLD)[15] and thyroid dysfunction[16] at the 1st trimester, earlier age at menarche,[17] and persistent vitamin D deficiency.[18] However, few studies analyzed the influence of these new risk factors on GDM according to the heterogeneity of physiologic hyperglycemia.

Since the heterogeneity could reflect the pathophysiologic state of GDM and the different clinical manifestations more objectively, we carried out a case–control study research to elucidate the variations in risk factors of the GDM subtypes, and to evaluate the correlation between GDM subtypes and obesity.

Patients and Methods

Study Population and Design

We performed a case–control study, and data were collected from Han Chinese women underwent routine gestational care visit at the Department of Obstetrics of the Northwest Women and Children's Hospital from November 2019 to March 2020. We collected clinical data at different follow-up times (1st-trimester study visit at 12 weeks of gestation, 2nd-trimester study visit at 24–28 weeks of gestation). Woman underwent the 75-g, 2-h oral glucose tolerance test (OGTT) at the 24th–28th gestational week, and GDM was diagnosed according to the International Association of Diabetes and Pregnancy Study Group (IADPSG) criteria[19] were included. The inclusion criteria is women of bearing age with full-term fetus and complete pregnancy data. From a total of 3829 women, the data of 1204 women who underwent OGTT were collected. The exclusion criteria included that: a history of diabetes (n = 12); OGTT results showed a fasting glucose ≥7.0 mmol/L, 2-hour post-meal blood glucose was ≥11.1 mmol/L, or glycated hemoglobin A1c (HbA1c) was ≥6.5% in the 1st trimester (n = 25); if they showed preeclampsia (n = 31), younger than 18 years of age (n = 35); had multiple gestations (n = 7), maternal disease (n = 23), or missing vital data (n = 23). Finally, 1048 subjects were eligible and included in further analysis.

Clinical Measurements and Definitions

The recorded weight and height at the first gestational care visit were used to calculate pre-BMI. Pre-BMI was categorized according to criteria specific for Chinese adults (BMI <18.5 kg/m^2, emaciated; BMI, 18.5–23.9 kg/m^2, normal weight; BMI, 24–27.9 kg/m^2, overweight; and BMI > 28 kg/m^2, obese).[20] Gestational weight gain (GWG) in the first-trimester was calculated as the maternal weight at the second-trimester visit minus pre-pregnancy body weight at the first gestational care. The presence or absence of NAFLD was evaluated utilizing liver ultrasound, and it was defined as the detection of bright echogenic patterns within the liver detected by ultrasonography. The Insulin Sensitivity Composite Index (ISI composite index) and the Stumvoll I index were used to measure insulin sensitivity and secretion according to the data from the OGTT and simultaneous insulin-release test.[21–23] We defined GDM subtypes using Powe's classification[4] according to the distributions of insulin sensitivity and secretion in women compared with the

normal glucose tolerance (NGT) group. We considered women with GDM to have an insulin secretion defect or sensitivity defect if insulin secretion or sensitivity was below the 25th percentile. According to the above standards, women with GDM were classified into the following subtypes: GDM with a predominant insulin-sensitivity defect (GDM-resistance, n = 202), GDM with a predominant insulin-secretion defect (GDM-dysfunction, n = 140), or GDM with both of the above-mentioned traits (GDM-mixed, n = 110). We excluded participants with GDM who had both insulin sensitivity and insulin secretion above the 25th percentile (n = 77), and women who were treated with insulin (n = 3).

Data Collection and Laboratory Measurements

The data included a questionnaire for pregnant women contained general information regarding their present and past medical histories, family history of diabetes, reproductive history, medication history, alcohol consumption, smoking status, and age at first menstruation. Fasting blood glucose (FBG) were tested using the glucose oxidase method (intra-assay and inter-assay coefficients of variation were 2.1% and 2.6%, respectively), high-performance liquid chromatography was used to measure hemoglobin A1c (HbA1c) percentage (Skylight Biotech, Akita, Japan). Reagents, which were provided by Chinese Shandong 3V Bioengineering Company, were used to test aspartate aminotransferase (AST), alanine aminotransferase (ALT) and TBA, and the detection was performed with the Hitachi 7600 automatic 49-biochemical analyzer. The enzymatic method (Parsazmun, Karaj, Iran) was used to detect lipid profiles [serum triglycerides, total cholesterol, low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C) concentrations]. Serum 25-hydroxyvitamin D_3 [25-(OH) D_3] was analyzed using the electro chemiluminescent immunoassay on Cobas e411 Elecsys 2010 (Roche Diagnostics GmbH, ranged 4–100 ng/mL). Serum insulin and ferritin levels were tested by electrochemiluminescent immunoassay kits of Chinese Shandong 3V Bioengineering Company (insulin, R-C-01-01, ranged 5–180μU/mL; ferritin, R-B-05-01, ranged 0–500ng/mL). Thyroid peroxidase antibodies (TPOAb) were measured by the radioimmunoassay of Chinese Shandong 3V Bioengineering Company (R-A-08-01, ranged 0.1–1000IU/mL). All the laboratory tests are performed by the standard laboratory methods in the certified laboratory of the Northwestern Women's and Children's Hospital in Xi'an, China. The ISI composite index = 10,000/√(fasting glucose × fasting insulin) × (mean glucose × mean insulin),[21] using glucose and insulin measurements expressed as mmol/L and uU/mL, respectively. The Stumvoll I index=2032+4.681×Ins0-135.0×Gluc120+0.995×Ins120+27.99×BMI-269.1×Gluc-0,[23] using glucose and insulin measurement expressed as mmol/L and uU/mL, respectively. The homeostasis model assessments (HOMA2-S and HOMA2-β) at http://www.dtu.ox.ac.uk (accessed on 11 January 2016) were used as indices to evaluate insulin sensitivity and secretion function, respectively.

Statistical Analysis

We used SPSS Statistics 22.0 (IBM SPSS, Chicago, IL, USA) to analyze our data. Data are expressed as means (standard deviation, SD) or median (interquartile range, IQR). Differences across the four groups (NGT and three GDM subtypes) were compared using one-way ANOVA for normally distributed continuous variables, the Kruskal–Wallis test for non-normally distributed continuous variables, and the Chi-squared test (or Fisher's exact-probability test) for categorical variables. When the *P*-value from the ANOVA, Kruskal–Wallis test, or Chi-squared test (or Fisher's exact test) was <0.05, pairwise comparisons between the NGT group and each GDM subgroup were made using the Tukey's test, Dunn's test, Chi-squared test, respectively. *P*-values for Chi-squared test pairwise comparisons were adjusted using the Bonferroni correction. Then, two adjusted polytomous logistic regression models were fit to identify potential risk factors of GDM-subtypes. The first one was adjusted for each other, alcohol consumption, and smoking status; the second included the first model plus pre-BMI and GWG in the first trimester. The reference value was the NGT group. The goodness of fit was tested using the Hosmer–Lemeshow test.

Results

Baseline Data

Baseline data are shown in Table 1. From a total of 3829 women, 1204 women underwent the oral 75g glucose tolerance test (OGTT), and 532 of them (13.9%) were diagnosed with GDM. Among women with GDM, 202 patients (38.0%) were classified into the GDM-resistance

Table 1 Characteristics of GDM Subtypes and NGT Group

	GDM-Resistance	*P*[a]	GDM-Dysfunction	*P*[a]	GDM-Mixed	*P*[a]	NGT
Number	202		140		110		516
Maternal age (years)	31 (28–34)	0.019	31 (29–34)	0.010	31 (28–33)	0.018	30 (28–33)
Smoking (n, %)	7 (3.5)	–	4 (2.9)	–	0 (0)	–	13 (2.5)
Alcohol use (n, %)	3 (1.5)	–	2 (1.4)	–	3 (2.7)	–	9 (1.7)
Family history of diabetes mellitus (n, %)	36 (17.8)	<0.001	19 (13.6)	0.001	14 (11.8)	0.004	27 (5.23)
Nulliparous (n, %)	111 (54.9)	–	82 (58.6)	–	64 (58.2)	–	303 (58.7)
History of macrosomia at delivery (n, %)	11 (5.4)	–	7 (5.0%)	–	3 (2.7)	–	16 (3.1)
Pre-BMI (kg/m^2)	23.73 (20.38–26.42)	<0.001	21.91 (18.35–24.83)	0.213	20.60 (18.34–23.14)	0.903	22.68 (20.53–24.50)
Height (cm)	162.36±4.74	–	161.43±5.30	–	161.53±4.74	–	161.83±4.70
Age at first menstruation (year)	13.0 (11.0–15.0)	<0.001	15.0 (13.75–15.0)	0.982	14.0 (14.0–16.0)	0.985	14.0 (13.75–15.0)

Notes: Data are presented as n (%), median (IQR, interquartile range), or mean (SD, standard deviation). [a]Differences across the four groups (NGT and three GDM subtypes) were compared using one-way ANOVA for normally distributed continuous variables, Kruskal–Wallis test for non-normally distributed continuous variables, or Chi-squared test (or Fisher's exact test) for categorical variables. When $P < 0.05$, pairwise comparisons between the NGT group and each GDM group were made using the Turkey's test, Dunn's test, Chi-squared test, or Fisher's exact test, respectively. P-values for pairwise comparisons were adjusted using the Bonferroni correction.
Abbreviations: GDM, gestational diabetes mellitus; NGT, normal glucose tolerance; pre-BMI, pre-pregnancy body mass index.

group, 140 patients (26.3%) were assigned to the GDM-dysfunction group, and 110 patients (20.7%) were classified into the GDM-mixed group. All women in the three GDM subtypes exhibited an elder maternal age ($P = 0.019$, $P = 0.010$, and $P = 0.018$, Table 1), and a higher percentage of family diabetes history ($P < 0.001$, $P = 0.001$, and $P = 0.004$) compared to NGT group. The GDM-resistance group showed an elevated pre-BMI [23.73 (20.38–26.42) vs 22.68 (20.53–24.50), P < 0.001] and a younger age of first menstruation ($P < 0.001$) relative to the NGT group, while women in the GDM-dysfunction and GDM-mixed group had pre-BMI comparable to the NGT group. Smoking status, alcohol consumption, percentage of macrosomia at delivery history, percentage of nulliparous women, and height showed no significant differences among the four groups.

Indicators at the First and Second Gestational Period

In their first trimester, women in the three GDM-subtype groups had higher plasma levels of FBG (all $P < 0.001$, Table 2), while only the GDM-dysfunction group exhibited higher HbA1c ($P = 0.028$) compared with the NGT group. Indicators regarding GWG in the first trimester ($P = 0.021$), the percentage of NAFLD ($P < 0.001$), ALT ($P = 0.029$), TG ($P = 0.012$), and TBA ($P = 0.009$) showed a significant increase, while HDL-C ($P = 0.024$) demonstrated a remarkable reduction in the GDM-resistance group when compared to the NGT group. The TG values and the percentage of NAFLD in the GDM-mixed group were higher than those in the NGT group ($P = 0.009$ and $P = 0.006$). Meanwhile, 25-(OH) D_3, AST, CHO, LDL-C, ferritin, and TPOAb showed no significant differences among the four groups.

In the second trimester, women in the three GDM-subtype groups manifested higher blood glucose at all the time points of the OGTT test, and larger glycemic area under the curve (AUC) compared with the NGT group ($P < 0.001$, Table 2). Plasma insulin levels at all time-points and AUC (insulin) presented to a significant increase (All $P < 0.001$) in the GDM-resistance group relative to the NGT group. However, insulin levels and AUC (insulin) were reduced in the GDM-dysfunction and GDM-mixed group (All $P < 0.01$) compared with the NGT group (except for fasting insulin level of GDM-dysfunction group). For insulin secretion indicators, the levels of HOMA2-β, Stumvoll I index were higher in the GDM-resistance group, but lower in GDM-dysfunction

Table 2 Clinical Data from GDM Subtypes and the NGT Group at the First and Second Trimester Study Visit

	GDM-Resistance	P^a	GDM-Dysfunction	P^a	GDM-Mixed	P^a	NGT
First-trimester study visit							
GWG in the first trimester (kg)	1.5 (1.0–2.0)	0.021	0.8 (0.5–1.4)	–	1.0 (0.8–1.6)	–	1.0 (0.7–1.5)
FBG (mmol/L)	5.03±0.43	<0.001	5.05±0.44	<0.001	5.04±0.38	<0.001	4.82±0.35
HbA1c (%)	5.01±0.33	–	5.12±0.35	0.028	5.01±0.45	–	4.99±0.30
HbA1c (mmol/mol)	31±3	–	32±4	0.028	31±5	–	30±4
25-(OH) D_3 (ng/mL)	13.48±7.24	–	14.10±8.38	–	13.83±8.28	–	12.66±7.79
ALT (U/L)	20.39±13.33	0.029	16.27±11.55	–	17.53±9.41	–	17.69±13.07
AST (U/L)	18.67±6.57	–	18.11±7.27	–	18.48±5.10	–	18.97±8.70
CHO (mmol/L)	4.44±0.69	–	4.37±0.75	–	4.49±0.68	–	4.34±0.74
TG (mmol/L)	1.76±0.77	0.012	1.64±0.66	–	1.85±0.83	0.009	1.50±0.61
HDL-C (mmol/L)	1.57±0.31	0.024	1.65±0.30	–	1.62±0.24	–	1.66±0.32
LDL-C (mmol/L)	2.35±0.55	–	2.19±0.50	–	2.39±0.54	–	2.22±0.56
TBA (umol/L)	2.80 (1.83–4.55)	0.009	1.70 (1.31–1.82)	–	1.68 (1.21–1.82)	–	1.67 (1.26–1.84)
NAFLD (%)	51 (26.2)	<0.001	10 (7.1)	–	17 (15.5)	0.006	38 (7.4)
Ferritin (ng/mL)	47.81 (19.41–77.38)	–	37.05 (22.84–56.05)	–	39.22 (28.31–50.40)	–	35.35 (19.76–65.52)
TPOAb (U/mL)	1.38 (0.53–5.39)	–	4.25 (1.45–44.23)	–	1.02 (0.00–2.45)	–	0.43 (0.10–16.99)
Second-trimester study visit							
OGTT							
Fasting glucose (mmol/L)	5.19 (4.89–5.33)	<0.001	5.22 (5.02–5.33)	<0.001	5.34 (4.76–5.54)	<0.001	4.77 (4.47–5.03)
1h glucose OGTT (mmol/L)	9.45 (7.32–10.43)	<0.001	10.71 (6.85–11.83)	<0.001	10.74 (9.74–10.81)	<0.001	8.28 (6.91–9.21)
2h glucose OGTT (mmol/L)	7.73 (6.23–8.60)	<0.001	7.87 (6.26–8.07)	<0.001	10.13 (8.35–10.24)	<0.001	6.42 (5.45–7.10)
AUC (glucose)	15.58 (13.50–16.84)	<0.001	16.81 (12.97–18.16)	<0.001	18.06 (16.47–18.72)	<0.001	13.68 (11.90–14.78)
Fasting insulin (uU/mL)	10.25 (7.90–12.80)	<0.001	7.15 (5.53–8.33)	–	7.30 (6.80–9.90)	0.002	9.60 (5.70–11.73)

(*Continued*)

Table 2 (Continued).

	GDM-Resistance	P[a]	GDM-Dysfunction	P[a]	GDM-Mixed	P[a]	NGT
1-h insulin OGTT (uU/mL)	67.9 (44.68–77.08)	<0.001	31.75 (26.78–48.23)	0.004	40.60 (27.7–53.18)	0.002	57.60 (36.30–71.80)
2-h insulin OGTT (uU/mL)	53.95 (37.58–88.60)	<0.001	28.85 (20.83–44.88)	<0.001	30.45 (19.80–41.70)	<0.001	37.0 (21.3–45.2)
AUC (insulin)	98.95 (71.06–118.04)	<0.001	50.67 (40.33–62.04)	<0.001	61.0 (40.25–79.93)	<0.001	75.35 (49.63–89.35)
HOMA2-β	114.60 (101.80–133.98)	<0.001	85.7 (77.80–101.40)	<0.001	88.60 (85.60–116.10)	<0.001	101.15 (102.78–127.55)
HOMA2-S	72.80 (52.30–86.53)	<0.001	92.10 (81.60–103.98)	–	74.70 (68.60–102.20)	<0.001	96.10 (76.73–124.30)
Insulin sensitivity (ISI composite index)	85.40 (60.91–94.82)	<0.001	122.04 (118.93–131.27)	–	89.41 (78.11–98.47)	<0.001	119.15 (85.10–145.48)
Insulin secretion (Stumvoll I index)	1030.80 (974.70–1314.22)	<0.001	753.65 (708.79–836.79)	<0.001	719.66 (424.60–780.78)	<0.001	919.42 (790.4–1094.63)

Notes: Data are presented as n (%) for categorical variables, median (IQR, interquartile range) or mean (SD, standard deviation) for continuous variables. [a]Differences across the four groups (NGT and three GDM subtypes) were compared using one-way ANOVA for normally distributed continuous variables, Kruskal–Wallis test for non-normally distributed continuous variables, or Chi-squared test for categorical variables. When $P < 0.05$, pairwise comparisons between the NGT group and each GDM group were made using the Tukey's test, Dunn's test, or Chi-squared test, respectively. *P* values for pairwise comparisons were adjusted using the Bonferroni correction.

Abbreviations: GDM, gestational diabetes mellitus; NGT, normal glucose tolerance; GWG, gestational weight gain; FBG, fasting blood glucose; HbA1c, glycated hemoglobin A1c; 25-(OH) D_3, 25-hydroxyvitamin D_3; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CHO, total cholesterol; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TBA, total bile acid; NAFLD, nonalcoholic fatty liver disease; TPOAb, thyroid peroxidase antibodies; OGTT, oral glucose tolerance test; AUC, area under the curve; HOMA, homeostasis model assessment.

and GDM-mixed groups when compared to the NGT group (all $P < 0.001$). Insulin sensitivity indicators (HOMA2-S and ISI composite index) were declined in the GDM-resistance and GDM-mixed groups relative to the NGT group (All $P < 0.001$).

Multivariate Polytomous Logistic Regression Analysis of Risk Factors for GDM

In the multivariate polytomous logistic regression, the risk factors in the first trimester concerning GDM are presented in Table 3. The elevation of maternal age and the family history of diabetes are both risk factors for all GDM-subtypes compared with the NGT group (All $P < 0.05$) in model 1 and model 2. Meanwhile, overweight/obese before pregnancy and GWG in the first trimester (All $P<0.05$) are verified as risk factors for the GDM-resistance subtype in model 2. For the indicators in plasma, increased FBG level (≥5.1 mmol/L) is a risk factor of all GDM subtypes regardless of whether values were adjusted or unadjusted for pre-BMI and GWG in the first-trimester (All $P<0.05$). However, the increased levels of ALT, TG, TBA, and the decreased level of age at first menstruation and HDL-C are risk factors of GDM-resistance subtype in model 1 (All $P<0.05$), but the connection between these risk factors and GDM-resistance subtype are attenuated in model 2 (model 1 plus pre-BMI and GWG in the first trimester as adjustments). The rising extent of TG increased the risk of GDM-mixed subtype in both model 1 and model 2 (all $P < 0.05$). While NAFLD in the first trimester is the risk factor of the GDM-resistance group (model 1 and mode 2) and GDM-mixed group (model 1) (All $P < 0.05$), the relationship between NAFLD and GDM-mixed subtype was diminished in model 2. The increased level of HbA1c was not a risk factor of all GDM subtypes in either model.

Discussion

Herein we implemented a case–control study in women who had GDM to investigate the risk factors of GDM subtypes that were defined by the validated indicators based on glycemic physiology.

We found that the GDM-resistance group showed higher pre-BMI and GWG in the first trimester relative to the NGT group, while women in GDM-dysfunction and GDM-mixed group had comparable characteristic above to the NGT group. Meanwhile, higher maternal age, family

Table 3 Multivariate Polytomous Logistic Regression Analysis of Risk Factors for GDM Subtypes

	GDM-Resistance vs NGT		GDM-Dysfunction vs NGT		GDM-Mixed vs NGT	
	Model 1[a]	**Model 2[b]**	**Model 1[a]**	**Model 2[b]**	**Model 1[a]**	**Model 2[b]**
Maternal age	1.10 (1.02–1.16)*	1.07 (1.01–1.10)*	1.13 (1.06–1.18)*	1.07 (1.03–1.14)*	1.17 (1.02–1.29)*	1.04 (1.01–1.09)*
Family history of diabetes mellitus (yes vs no)	4.01 (2.62–6.89)*	3.93 (2.31–6.67)*	3.14 (1.68–5.98)*	2.82 (1.51–5.23)*	2.61 (1.48–4.91)*	2.42 (1.20–4.85)*
Pre-BMI	–	1.47 (1.36–1.59)*	–	0.91 (0.82–1.07)	–	1.04 (0.92–1.16)
<18.5	–	0.15 (0.05–0.51)*	–	–	–	–
18.5–23.9	–	1 (Reference)	–	–	–	–
24–27.9	–	5.57 (3.71–8.33)*	–	–	–	–
≥28	–	12.93 (6.21–26.74)*	–	–	–	–
GWG in the first trimester		2.62 (2.09–3.36)*		1.34 (0.99–1.80)		0.92 (0.71–1.25)
HbA1c	1.40 (0.67–1.42)	1.02 (0.46–1.87)	1.53 (0.80–1.72)	1.13 (0.61–1.24)	1.17 (0.74–2.03)	1.04 (0.58–1.29)
FBG	3.86 (2.35–6.33)*	3.47 (1.21–9.95)*	4.25 (2.46–7.34)*	4.29 (1.53–12.08)*	4.03 (2.23–7.29)*	4.08 (1.15–14.45)*
≥5.1 vs.<5.1 (mmol/L)	–	1.98 (1.22–3.21)*	–	2.97 (1.81–4.88)*	–	2.52 (1.46–4.34)*
ALT	1.02 (1.01–1.03)*	1.02 (0.99–1.05)	0.99 (0.97–1.01)	0.99 (0.97–1.03)	0.99 (0.98–1.02)	1.01 (0.97–1.05)
TG	1.80 (1.25–2.60)*	1.43 (0.75–2.71)	1.44 (0.93–2.23)	1.55 (0.89–2.70)	2.06 (1.34–3.17)*	2.63 (1.28–5.38)*
The decrease of HDL-C	2.63 (1.12–5.88)*	1.52 (0.38–6.25)	1.08 (0.43–2.70)	1.21 (0.39–3.70)	1.56 (0.56–4.35)	1.64 (0.30–9.09)
The decrease of age at first menstruation	2.70 (2.33–3.13)*	0.85 (0.62–2.13)	0.95 (0.80–1.34)	1.41 (0.92–2.38)	0.93 (0.78–1.14)	1.85 (1.00–3.45)
TBA	1.681 (1.20–2.36)*	1.07 (0.60–1.91)	0.89 (0.61–1.31)	0.70 (0.42–1.18)	0.67 (0.37–1.22)	0.48 (0.21–1.09)
NAFLD	6.49 (4.74–8.89)*	3.67 (1.04–12.91)*	1.38 (0.87–2.19)	0.556 (0.11–2.92)	2.33 (1.51–3.60)*	1.36 (0.96–1.22)

Notes: Data are presented as odds ratios (OR) and 95% confidence interval (95% CI). *$P < 0.05$. [a]Model 1 was adjusted for each other, alcohol consumption and smoking status. [b]Model 2 was adjusted for each other, alcohol consumption, smoking status, pre-BMI, and GWG in the first trimester.

Abbreviations: GDM, gestational diabetes mellitus; NGT, normal glucose tolerance; pre-BMI, pre-gestational body mass index; GWG, gestational weight gain; HbA1c, glycated hemoglobin A1c; FBG, fasting blood glucose; ALT, alanine aminotransferase; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol; TBA, total bile acid; NAFLD, nonalcoholic fatty liver disease.

history of diabetes mellitus, and the elevated level of FBG (≥5.1mmol/L) in the first trimester were all risk factors of the three GDM subtypes compared with the NGT group. The increased level of TG is an independent risk factor of the GDM-mixed subtype. For the women in the GDM-resistance group, overweight/obesity before pregnancy and the increased GWG in the first trimester are risk factors. Younger age at first menstruation, ascended level of ALT and TBA, and lipid metabolism disorder are risk factors of the GDM-resistance group. However, the adjustment for pre-BMI and GWG in the first trimester attenuated the observed associations. NAFLD is the independent risk factor for the GDM-resistance group regardless of whether the values were unadjusted or adjusted for pre-BMI and GWG in the first trimester.

Many studies focus on the risk factors of GDM come up with inconsistent results.[5–9] There is limited research focuses on the risk factors of GDM subtypes according to the heterogeneity of physiologic hyperglycemia, and that might be one reason for this inconsistency. Many studies have verified that pre-pregnancy overweight/obesity and excess GWG during pregnancy contributes to the occurrence of GDM,[5,24,25] which is consistent with our finding on the GDM-resistance subtype. We speculate the reason might be that these studies had a majority of overweight/obese participants, whose characteristic was similar to the GDM-resistance subtype in our study. Excessive fat accumulation before and during pregnancy also explains the association between the following risk factors and the GDM-resistance subtype. Some studies found that the elevated ALT level and dyslipidemia in the first trimester increasing the risk of GDM,[26,27] and the higher first-trimester serum levels of TBA is also associated with GDM,[13,28] however, the association between these risk factors and GDM are attenuated by overweight/obesity.[26,29] ALT is a cytosolic enzyme that has a relatively higher concentration in the liver. When liver damage occurs, such as infection, toxins and ischemia, ALT is released into the blood from injured liver cells. On the other hand, a mild increased level of ALT within normal range usually reflect fat accumulation, which is a marker of NAFLD.[30] Bile acids (BAs) are consisted of primary BAs and secondary BAs. Primary BAs are produced from cholesterol in the liver. Most of them are conjugated with taurine and glycine, and stored in the gallbladder and secreted into the duodenum, digest lipids and fat-soluble vitamins after meal stimulation. A study found that BAs were nearly twofold elevated in T2DM patients relative to healthy subjects, and the disproportion in BAs was associated with insulin resistance,[31] which is consistent with our GDM-resistance subtype. Earlier age at menarche also found to be a risk factor for GDM,[17,32] however, another study uncovered that the associations between earlier age at menarche and the risk of GDM may be mediated by insulin resistance caused by overweight/obesity.[33] Lee et al[15] revealed that the risk of developing GDM was significantly increased in participants with NAFLD, and this relationship remained significant after adjusted by metabolic risk factors and insulin resistance. These results revealed that other mechanisms might be involved in the process besides insulin resistance and obesity. For instance, pro-inflammatory cytokines from other visceral tissues could transfer to the liver, then lead to NAFLD.[34]

Enquobahrie et al[35] found that the increased level of TG at the 1st trimester was a risk factor for GDM. We also observed this phenomenon in the GDM-resistance and GDM-mixed groups. However, this association weakened after adjusted by pre-BMI and GWG for GDM-resistance group. It suggests that the elevated TG level as a risk factor for GDM resistance group may mediated by obesity. Meanwhile, the combination of abnormal glucose metabolism and insulin resistance may cause more serious lipid metabolism disorder for the GDM-mixed group, which may explain the increased TG level in the 1st trimester is still a risk factor for this group before and after adjustment.

In our study, family history of diabetes is a risk factor for all GDM subtypes, and many researches have shown the same results in both obese and non-obese pregnant women.[36,37] The following reasons might explain the relationship between family history of diabetes and GDM. Maternal diabetes during pregnancy lead to a transgenerational transmission of diabetes risk.[38] Meanwhile, the cumulative incidence of type 2 diabetes was significantly increased in the first 5 years after the delivery of GDM women.[39] At the same time, diabetes is a kind of polygenetic disease with overlaps of many susceptible genes of GDM.[40,41]

Many studies considered the increasing level and cut-off points of FBS in early pregnancy as a risk factor for GDM, but their results are inconsistent.[42–44] The areas and ethnic variations among participants, and the different diagnostic criteria for GDM may explain the reasons for these inconsistencies. In this study, the association between the elevated level of FBG in the first trimester and GDM might be relevant to the high-carbohydrate diet of women in the northwest of China.

Limitations of our study included that this is a single-center study, which is restricted for further stratified analysis of different regions. Furthermore, the absence of data

on pre-pregnancy islet β-cell function prevented us from presenting the dynamic characteristics of glucose metabolism. Finally, we did not collect the blood sample to examine the genetic loci of GDM subtypes in this study, which we will focus in the future.

In conclusion, our study showed that women with GDM exhibited heterogeneity based on glycemic physiology. The risk factors of GDM were different for each subtype, and the effect of many obesity-related risk factors on GDM was mediated by pre-pregnancy overweight/obesity and the elevated GWG during the first-trimester. Our study partly explained the reason for the inconsistency of risk factors on GDM about current studies. With the deep knowledge of etiology and pathogenesis on GDM subtypes, we will have a deeper understanding of the discrepancy on risk factors of GDM.

Data Sharing Statement

We would like to share the clinical data of our participants. Please contact Wei Cui through doctorweiweixjtu@126.com.

Ethics Approval

This study was approved and supervised by the Ethics Committee of the Xi'an Jiaotong University First Affiliated Hospital, and it was reviewed and recorded by the Northwest Women and Children's Hospital. All participants signed informed consent form, and the study was performed according to the Declaration of Helsinki, with a clinical trial registration number of ChiCTR 1900026735.

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Author Contributions

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare that they have no known competing financial interests or personal relationships and conflicts of interest that could have appeared to influence the work reported in this paper.

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