Elevated First-Trimester Neutrophil Count Is Closely Associated With the Development of Maternal Gestational Diabetes Mellitus and Adverse Pregnancy Outcomes

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Diabetes 2020;69:1401–1410 | https://doi.org/10.2337/db19-0976

Chronic low-grade inflammation plays a central role in the pathophysiology of gestational diabetes mellitus (GDM). To investigate the ability of different inflammatory blood cell parameters in predicting the development of GDM and pregnancy outcomes, 258 women with GDM and 1,154 women without were included in this retrospective study. First-trimester neutrophil count outperformed white blood cell count and the neutrophil-to-lymphocyte ratio in the predictability for GDM. Subjects were grouped based on tertiles of neutrophil count during their first-trimester pregnancy. The results showed that as the neutrophil count increased, there was a stepwise increase in GDM incidence as well as in glucose and glycosylated hemoglobin levels, HOMA for insulin resistance (HOMA-IR), macrosomia incidence, and newborn weight. Neutrophil count was positively associated with prepregnancy BMI, HOMA-IR, and newborn weight. Additionally, neutrophil count was an independent risk factor for the development of GDM, regardless of the history of GDM. Spline regression showed that there was a significant linear association between GDM incidence and the continuous neutrophil count when it was >5.0 × 10^9/L. This work suggested that the first-trimester neutrophil count is closely associated with the development of GDM and adverse pregnancy outcomes.

Gestational diabetes mellitus (GDM), one of the most common metabolic disorders in pregnant women, is defined as any degree of glucose intolerance with onset or first diagnosis during pregnancy (1). Over the past few decades, the prevalence of GDM has increased, coinciding with rising rates of obesity and type 2 diabetes (T2DM). In 2010, GDM prevalence in the U.S. was estimated to be 4.6–9.2% (2). In China, GDM prevalence has been reported to be 9.3–18.9% (3). The presence of GDM is associated with higher risk of adverse consequences for both the mother (preeclampsia, cesarean section, development of T2DM after delivery) and infant (macrosomia with consequent shoulder dystocia and birth injury, neonatal hypoglycemia, and childhood obesity) (4–8). Several traditional factors, including a family or personal history of diabetes, previous adverse pregnancy outcome, glycosuria, and obesity, are associated with GDM, but the exact pathophysiology of GDM remains elusive.

Previous studies have shown that low-grade chronic inflammation plays a crucial role in the pathophysiology of GDM and T2DM (9–14). The abnormal increase of the inflammatory blood cell parameters, such as white blood cell (WBC) and neutrophil count, neutrophil-to-lymphocyte ratio (NLR), and platelet count, usually serve as simple markers of inflammation, and all have been investigated for their ability to predict GDM in a previous study with a small sample size, but results were inconsistent (15–19).

This study investigated the potential correlation of inflammatory blood cell parameters with GDM and adverse pregnancy outcomes. First, we found that the first-trimester neutrophil count outperformed WBC count and NLR as a risk factor and showed better diagnostic

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Received 30 September 2019 and accepted 16 April 2020

This article contains supplementary material online at https://doi.org/10.2337/db20-4567/suppl.12145296.

T.S. and F.M. are co-first authors.

predictability for GDM. In addition, our cohort study showed that as the first-trimester neutrophil count increased, the incidence of GDM, blood glucose level, HOMA for insulin resistance (HOMA-IR), and adverse pregnancy outcomes increased in a stepwise manner. The first-trimester neutrophil count was closely associated with prepregnancy BMI, HOMA-IR, and newborn weight and was also an independent risk factor for development of GDM. Finally, a significant linear association between continuous neutrophil count and the incidence of GDM was analyzed by spline regression.

RESEARCH DESIGN AND METHODS

Study Population

From May 2015 to July 2018, 1,781 pregnant women were retrospectively screened at the GDM Care Center of Shanghai Fifth People's Hospital, Fudan University. The retrospective analysis process followed the procedure described in Fig. 1. Women were excluded from the study for any of the following: *1*) any infectious disease 2 weeks before the blood cell test; *2*) abnormal liver or renal function; *3*) presence of viral infection or positive carrier status (hepatitis B virus, syphilis, and HIV); *4*) preexisting diabetes; *5*) chronic hypertension; or *6*) multiple gestation. Finally, 1,412 women (1,154 without GDM and 258 with GDM) were collected for the analysis.

Data Collection and Laboratory Assessments During Pregnancy

At the first visit, gestational age was calculated based on the date of last menstruation or first-trimester ultrasonography. After an overnight fast for 12 h, blood samples were collected for counts of blood cells (XN9000 Automatic Blood Cell Analyzer; Sysmex, Kobe, Japan) and biochemical parameters tests (Cobas 8000 Automatic Biochemical Analyzer; Roche, Basel, Switzerland). Blood pressure and anthropometric parameters were measured, and a questionnaire was also completed. The patient questionnaire obtained information of last menstruation, method of conception, parity, obstetric history, family history of diabetes, previous history of GDM, and prepregnancy weight. Prepregnancy BMI was calculated as the prepregnancy weight in kilograms divided by the square of height in meters. After delivery, details including gestational age at delivery, mode of delivery, newborn weight, and sex of the neonate were recorded by medical staff.

Oral Glucose Tolerance Test

All subjects, with the exception of those diagnosed with overt diabetes or GDM in early pregnancy, underwent routine screening for GDM at 24–28 weeks' gestation according to a 75-g oral glucose tolerance test (OGTT) (1). OGTT was performed in the morning after an overnight fast of at least 8 h. Diagnosis of GDM was made when fasting blood glucose (FBG) was $\geq$5.1 mmol/L, the 1-h level was $\geq$10.0 mmol/L, or the 2-h level was $\geq$8.5 mmol/L, respectively.

Intervention for GDM

Therapeutic regimen started as soon as the individual was diagnosed with GDM. At first, lifestyle intervention was initiated, and insulin was then supplemented in addition to lifestyle intervention if the goals of glycemic control were not reached (fasting glucose $<$5.3 mmol/L, 1-h postprandial glucose $<$7.8 mmol/L, or 2-h postprandial glucose $<$6.7 mmol/L).

Calculation of HOMA-IR, HOMA of β-Cell Function, and QUICKI

The values for HOMA-IR, HOMA of β-cell function (HOMA-β), and QUICKI were determined from FBG and insulin concentration using the following formula (20): HOMA-IR = (I_0 [μIU/mL] × G_0 [mmol/L])/22.5; HOMA-β = 20 × (I_0 [μIU/mL]/[G_0 (mmol/L)] − 3.5; QUICKI = 1/(logI_0 [μIU/mL] + logG_0 [mg/dL]). I_0 is the level of fasting insulin, and G_0 is the level of FBG.

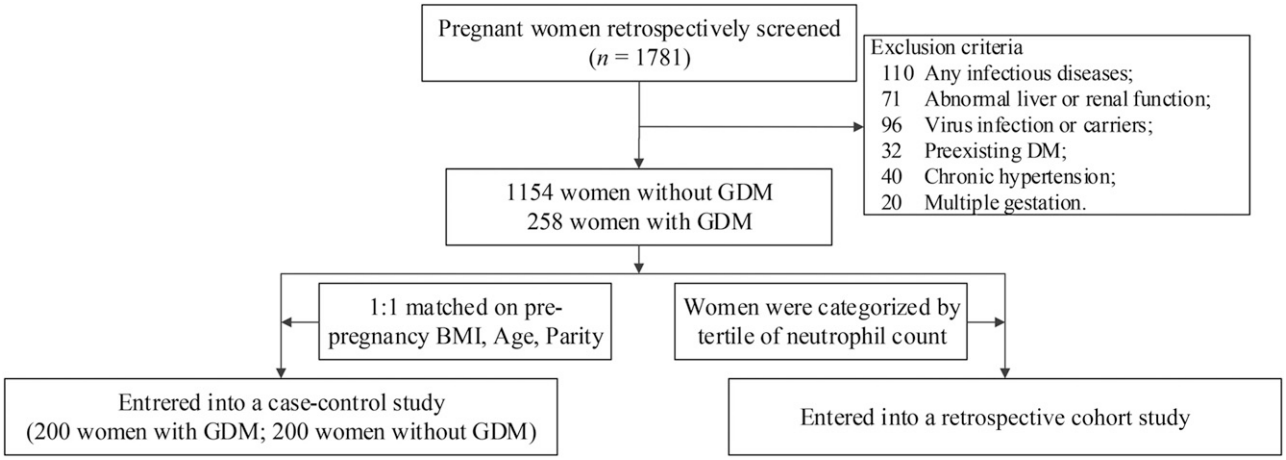

Figure 1—Flowchart of this study. DM, diabetes.

Table 1—Characteristics of women with and without GDM in all subjects and in the matched case-control study

	All subjects			Matched case-control study		
	Women without GDM (n = 1,154)	Women with GDM (n = 258)	P	Women without GDM (n = 200)	Women with GDM (n = 200)	P
Anthropometric parameters						
Age (years)	27 ± 5	30 ± 5	**<0.001**	29 ± 5	30 ± 5	0.607
Parity						
Nulliparous	276 (26.1)	86 (37.2)	**0.0001**	65 (32.2)	65 (32.2)	1.000
Parous	783 (73.9)	145 (62.8)		137 (67.8)	137 (67.8)	
Previous GDM						
No	871 (75.5)	152 (58.9)	**<0.001**	135 (66.8)	117 (57.9)	**<0.001**
Yes	7 (0.6)	20 (7.8)		2 (1.0)	20 (9.9)	
Nulliparous	276 (23.9)	86 (33.3)		65 (32.2)	65 (32.2)	
Family history of diabetes						
No	117 (99.5)	236 (93.7)	**<0.001**	193 (99.5)	188 (94.9)	**0.011**
Yes	6 (0.5)	16 (6.3)		1 (0.5)	10 (5.1)	
Pregnancy BMI (kg/m^2)	22.4 ± 3.2	24.0 ± 4.2	**<0.001**	23.4 ± 3.5	23.4 ± 3.5	0.921
First trimester						
SBP (mmHg)	116 ± 10	117 ± 11	0.446	117 ± 9	116 ± 11	0.359
DBP (mmHg)	68 ± 7	69 ± 8	0.228	69 ± 7	68 ± 8	0.980
WBCs ($\times10^9$/L)	8.57 ± 2.00	9.37 ± 2.07	**<0.001**	8.57 ± 1.92	9.27 ± 2.04	**0.001**
Neutrophils ($\times10^9$/L)	6.03 ± 1.70	7.06 ± 1.76	**<0.001**	6.05 ± 1.61	7.00 ± 1.74	**<0.001**
Lymphocytes ($\times10^9$/L)	1.88 ± 0.51	1.88 ± 0.52	0.906	1.86 ± 0.51	1.86 ± 0.52	0.969
NLR	3.36 ± 1.13	3.93 ± 1.32	**<0.001**	3.45 ± 1.21	3.93 ± 1.34	**0.001**
Platelets ($\times10^9$/L)	216 ± 53	221 ± 49	0.257	217 ± 53	220 ± 50	0.649
Second trimester						
OGTT time (weeks)	25.7 ± 2.1	25.6 ± 2.3	0.669	25.5 ± 1.9	25.6 ± 2.1	0.674
OGTT						
FBG (mmol/L)	3.80 (3.50–4.09)	4.80 (4.14–5.37)	**<0.001**	3.86 (3.57–4.25)	4.78 (4.15–5.33)	**<0.001**
1-h BG (mmol/L)	6.52 ± 1.37	9.84 ± 2.00	**<0.001**	6.68 ± 1.38	9.78 ± 1.91	**<0.001**
2-h BG (mmol/L)	5.99 ± 1.08	8.51 ± 1.98	**<0.001**	6.17 ± 1.04	8.44 ± 1.90	**<0.001**
HbA_{1c} (%)	4.9 ± 0.4	5.4 ± 0.9	**<0.001**	4.9 ± 0.3	5.4 ± 0.9	**<0.001**
HbA_{1c} (mmol/mol)	30	36		30	36	
HOMA-IR*	1.46 (1.00–2.09)	2.47 (1.54–3.45)	**<0.001**	1.64 (1.09–2.19)	2.44 (1.56–3.60)	**0.001**
QUICKI*	0.55 (0.54–0.56)	0.52 (0.50–0.54)	**<0.001**	0.54 (0.53–0.56)	0.52 (0.50–0.54)	**<0.001**
HOMA-β*	306.16 (258.91–802.39)	182.90 (83.93–426.71)	**<0.001**	258.91 (241.62–715.71)	165.05 (85.20–396.73)	**<0.001**
ALT (units/L)	14.0 (11.0–21.0)	17.0 (11.0–28.0)	0.073	17.0 (12.0–24.0)	17.5 (11.0–28.0)	0.86
AST (units/L)	18.0 (15.0–22.0)	19.0 (15.0–24.5)	0.685	19.0 (15.0–23.0)	19.0 (15.0–25.0)	0.76
Creatinine (mmol/L)	40 ± 7	40 ± 9	0.713	40 ± 8	40 ± 9	0.845
TG (mmol/L)	2.51 (1.98–3.20)	2.86 (2.09–3.65)	**0.010**	2.62 (2.10–3.21)	2.90 (2.09–3.86)	0.84
Cholesterol (mmol/L)	5.47 ± 1.09	5.30 ± 1.27	0.116	5.53 ± 1.22	5.28 ± 1.27	0.106
HDL (mmol/L)	1.59 ± 0.35	1.69 ± 0.48	**0.011**	1.59 ± 0.34	1.64 ± 0.47	0.287
LDL (mmol/L)	3.11 ± 0.88	2.81 ± 1.07	**0.001**	3.15 ± 0.99	2.73 ± 1.10	**0.003**
Weight gain (kg)						
Before GDM diagnosis	5.78 ± 2.61	6.63 ± 4.72	**0.022**	5.93 ± 2.75	6.74 ± 4.95	0.079
Whole pregnancy	13.48 ± 5.04	13.08 ± 5.94	0.386	13.24 ± 5.41	13.14 ± 6.14	0.876
Treatment						
Lifestyle intervention	NA	224 (86.8)	NA	NA	172 (86.0)	NA
Insulin	NA	34 (13.2)	NA	NA	28 (14.0)	NA
Pregnancy outcome						
Delivery time (weeks)	38.4 ± 1.3	38.2 ± 2.1	0.390	37.7 ± 1.5	37.9 ± 2.4	0.705
Fetus sex						
Male	645 (56.5)	83 (50.3)	0.135	117 (58.2)	69 (51.9)	0.254
Female	497 (43.5)	82 (49.7)		84 (41.8)	64 (48.1)	
Birth length (cm)	49.9 ± 0.9	49.9 ± 1.8	0.834	50.0 ± 0.6	49.7 ± 2.1	0.206
Newborn weight (g)	3,361.6 ± 476.5	3,527.2 ± 562.7	**<0.001**	3,409.5 ± 465.0	3,520.9 ± 581.6	0.061
Macrosomia						
No	1,054 (92.3)	136 (78.6)	**<0.001**	180 (89.6)	107 (77.0)	**0.002**
Yes	88 (7.7)	37 (21.4)		21 (10.4)	32 (23.0)	

Data are means ± SD, median (IQR), or n (%). Boldface P values are statistically significant ($P < 0.05$). ALT, alanine aminotransferase; DBP, diastolic blood pressure; NA, not applicable; SBP, systolic blood pressure. *Log-transformed for t test.

Table 2—Logistic regression analysis to determine the risk factors for development of GDM in matched case-control study

	In all mothers (n = 400)		In mothers without GDM history (n = 309)	
	OR (95% CI)	P	OR (95% CI)	P
Tertiles of neutrophils ($\times 10^9$/L)				
Lowest	Reference		Reference	
Middle	1.70 (0.93–3.09)	0.083	1.87 (1.02–3.44)	**0.044**
Highest	3.60 (2.02–6.41)	**<0.001**	3.70 (2.05–6.66)	**<0.001**
GDM history				
No	Reference			
Yes	12.55 (2.80–56.19)	**0.001**		
Nulliparous	1.01 (0.62–1.60)	0.982		
Tertiles of WBCs ($\times 10^9$/L)				
Lowest	Reference		Reference	
Middle	1.67 (0.96–2.90)	0.069	1.53 (0.86–2.70)	**0.148**
Highest	2.40 (1.38–4.17)	**0.002**	2.75 (1.56–4.86)	**<0.001**
GDM history				
No	Reference			
Yes	12.50 (2.81–55.57)	**0.001**		
Nulliparous	1.01 (0.62–1.62)	0.982		
Tertiles of NLR				
Lowest	Reference		Reference	
Middle	1.52 (0.87–2.68)	0.069	1.86 (1.06–3.25)	**0.003**
Highest	2.77 (1.58–4.88)	**<0.001**	2.45 (1.40–4.30)	**0.002**
GDM history				
No	Reference			
Yes	14.38 (3.24–63.92)	**<0.001**		
Nulliparous	1.02 (0.63–1.66)	0.945		

The boldface P values are statistically significant ($P < 0.05$).

Statistical Analysis

To avoid the potential bias due to uneven distribution of covariates between women with or without GDM, a case-control matching method was used to match variables that included prepregnancy BMI, age, and parity. Matching tolerance was 0.5, 2, and 0, respectively. To compare the predictability for GDM among the inflammatory blood cell parameters, logistic regression analysis and receiver operating characteristic curves were performed.

To further validate the association of neutrophil count with GDM and pregnancy outcomes, a cohort including the same subjects as the case-control study was established in which patients were divided into three groups by tertiles of neutrophil count: lowest group ($<5.30 \times 10^9$/L), middle group ($5.30–6.80 \times 10^9$/L), and highest group ($>6.80 \times 10^9$/L). Descriptive statistics for the studied variables are presented as means ± SD for normally distributed variables, median (interquartile range [IQR]) for nonnormally distributed variables, and frequency (percentage) for categorical variables. ANOVA and the Student t test were used to identify the difference in the mean between groups. Bonferroni correction was applied in multiple comparisons. Nonnormally distributed variables were analyzed by Kruskal-Wallis one-way ANOVA or Wilcoxon tests. HOMA-IR, HOMA-β, and QUICKI were log-transformed previously for t tests or ANOVA. Linear correlation between neutrophil count and HOMA-IR and prepregnancy BMI and newborn weight were assessed by simple and multivariate linear regression analysis. Continuous association of neutrophil count with GDM incidence was determined by spline regression analysis. To determine whether neutrophil count was an independent risk factor, logistic regression analysis was performed with GDM classified in a binary manner (presence/absence) as the dependent variable. Neutrophil count and traditional risk factors, including age, previous GDM history, prepregnancy BMI, triglyceride (TG) level, and weight gain before GDM was diagnosed as the possible risk factors, were entered into logistic regression analysis in all mothers, and the same analyses were repeated in the subgroup of mothers with no previous GDM (women without GDM history and nulliparous). All data were analyzed using SPSS 24.0 software (IBM, Armonk, NY). A two-tailed $P < 0.05$ was considered to indicate statistical significance.

Data and Resource Availability

The data sets generated during and/or analyzed during the current study are not publicly available but are available from the corresponding author on reasonable request.

RESULTS

Characteristics of Women With and Without GDM in All Subjects and Matched Case-Control Study

GDM developed in 258 women (18.27%) among the 1,412 subjects, and women with older age, previous GDM

Table 3—Comparison of parameters in the first trimester and the second trimester among three groups categorized by tertiles of neutrophil count in the cohort study

	Lowest group (n = 372)	Middle group (n = 385)	Highest group (n = 348)	P
Neutrophil range ($\times 10^9$/L)	<5.30	5.30–6.80	>6.80	
Anthropometric and first-trimester parameters				
Women with GDM	33 (9.1)	56 (14.7)	97 (28.2)	**<0.001**
Age (years)	27 ± 5	28 ± 5	27 ± 5	0.316
Prepregnancy BMI (kg/m^2)	21.7 ± 2.8	22.7 ± 3.5*	23.2 ± 3.6†	**<0.001**
First trimester				
SBP (mmHg)	116 ± 10	114 ± 10	117 ± 10	0.084
DBP (mmHg)	69 ± 7	68 ± 8	69 ± 8	0.411
Second trimester				
OGTT				
FBG (mmol/L)	3.96 ± 1.52	3.94 ± 0.64	4.12 ± 0.88	0.054
1-h BG (mmol/L)	6.68 ± 1.60	6.99 ± 1.77*	7.60 ± 2.29†‡	**<0.001**
2-h BG (mmol/L)	6.07 ± 1.29	6.39 ± 1.45*	6.87 ± 1.81†‡	**<0.001**
HbA_{1c} (%)	4.9 ± 0.3	5.0 ± 0.3*	5.1 ± 0.5†‡	**<0.001**
HbA_{1c} (mmol/mol)	30	31	32	
HOMA-IR§	1.37 (0.88–1.82)	1.56 (1.04–2.39)	1.71 (1.19–2.40)†	**<0.001**
QUICKI§	0.54 (0.53–0.56)	0.55 (0.53–0.55)	0.54 (0.53–0.55)	0.090
HOMA-β§	257.04 (230.08–578.52)	332.35 (256.00–830.36)	244.61 (184.80–783.45)	0.209
ALT (units/L)	14.0 (11.0–20.0)	15.0 (12.0–22.0)	16.0 (11.0–25.0)‡	**0.006**
AST (units/L)	18.0 (15.0–21.0)	18.0 (15.0–22.0)	18.0 (15.0–24.0)‡	**0.012**
Creatinine (mmol/L)	40 ± 8	39 ± 7	39 ± 6	0.516
TG (mmol/L)	2.48 (1.98–3.09)	2.50 (1.92–3.34)	2.66 (2.05–3.50)	0.052
Cholesterol (mmol/L)	5.44 ± 1.12	5.57 ± 1.12	5.29 ± 1.07‡	**0.014**
HDL (mmol/L)	1.56 ± 0.31	1.59 ± 0.35	1.58 ± 0.43	0.517
LDL (mmol/L)	3.10 ± 0.88	3.21 ± 0.88	2.88 ± 0.89†‡	**<0.001**
Weight gain (kg)				
Before GDM diagnosis	6.08 ± 2.48	5.71 ± 2.54	6.06 ± 3.69	0.158
Whole pregnancy	13.82 ± 4.81	13.24 ± 4.50	13.52 ± 5.86	0.295
Treatment for GDM				
Lifestyle intervention	32 (19.2)	51 (30.5)	84 (50.3)	0.166
Insulin	6 (17.6)	13 (38.2)	15 (44.2)	

Data are means ± SD, median (IQR), or n (%).The bold P values are statistically significant ($P < 0.05$). ALT, alanine aminotransferase; DBP, diastolic blood pressure; SBP, systolic blood pressure. §Log-transformed for t test. *Middle group vs. lowest group, $P < 0.001$. †Highest group vs. lowest group, $P < 0.05$. ‡Highest group vs. middle group, $P < 0.05$.

history, or GDM family history were more likely to develop GDM (Table 1). Compared with women without GDM, patients with GDM had a much higher level of prepregnancy BMI ($P < 0.001$), first-trimester WBC count (9.37 ± 2.07 vs. 8.57 ± 2.00 × 10^9/L, $P < 0.001$), neutrophil count (7.06 ± 1.76 vs. 6.03 ± 1.70 × 10^9/L, $P < 0.001$), and NLR (3.93 ± 1.32 vs. 3.36 ± 1.13, $P < 0.001$), whereas the difference in lymphocyte count or platelet count was not significant. In addition, patients with GDM had much higher second-trimester TG ($P = 0.010$), HDL ($P = 0.011$), FBG ($P < 0.001$), 1-h blood glucose (BG) ($P < 0.001$), 2-h BG ($P < 0.001$), HbA_{1c} ($P < 0.001$), HOMA-IR ($P < 0.001$), and weight gain before GDM screening ($P = 0.022$) and lower QUICKI ($P < 0.001$) and HOMA-β ($P < 0.001$) than women without GDM. Unexpectedly, patients with GDM had lower LDL than women without GDM (2.81 ± 1.07 vs. 3.11 ± 0.88 mmol/L, $P = 0.001$). There was no difference in weight gain during the whole pregnancy between women with and without GDM, and we found most patients with GDM (86.8%) simply needed lifestyle intervention, while only 34 women (13.2%) with GDM required insulin treatment. Obviously, mothers with GDM tended to deliver heavier newborns (3,527.2 ± 562.7 vs. 3,361.6 ± 476.5 g, $P < 0.001$) and had a higher rate of delivering macrosomic infants than mothers without GDM (21.4% vs. 7.7%, $P < 0.001$) (Table 1).

A 1:1 case-control matching procedure was performed to avoid the potential bias of covariates that were not evenly distributed between women with and without GDM. After matching for age, pregnancy BMI, and parity, there were no differences in TG and weight gain before GDM screening between women with and without GDM. There remained a significantly higher WBC count (9.27 ± 2.04 vs. 8.57 ± 1.92 × 10^9/L, $P < 0.001$), neutrophil count (7.00 ± 1.74 vs. 6.05 ± 1.61 × 10^9/L, $P < 0.001$), and NLR (3.93 ± 1.34 vs. 3.45 ± 1.21, $P = 0.001$) as well as higher glucose level ($P < 0.001$) and HOMA-IR ($P = 0.001$) and lower HOMA-β ($P < 0.001$) and QUICKI ($P < 0.001$) in women with GDM compared with control subjects (Table 1).

Table 4—Comparison of parameters at delivery among three groups categorized by tertile of neutrophil count in the cohort study

	Lowest group (n = 372)	Middle group (n = 385)	Highest group (n = 348)	P
Neutrophil count range ($\times 10^9$/L)	<5.30	5.30–6.80	>6.80	
Fetal characteristics				
Fetus sex				
Male	213 (58.0)	191 (52.2)	172 (54.6)	0.278
Female	154 (42.0)	175 (47.8)	143 (45.4)	
Birth length (cm)	49.9 ± 0.8	49.8 ± 1.6	49.9 ± 1.1	0.592
Newborn weight (g)	3,320.3 ± 478.0	3,369.0 ± 473.1	3,447.9 ± 523.2*	**0.003**
Macrosomia				
No	343 (93.5)	339 (92.4)	276 (86.0)	**0.001**
Yes	24 (6.5)	28 (7.6)	45 (14.0)	
Preterm birth				
No	346 (96.4)	340 (96.9)	279 (95.9)	0.798
Yes	13 (3.6)	11 (3.1)	12 (4.1)	
Placenta weight (g)	619.2 ± 118.9	628.6 ± 111.9	642.7 ± 123.8*	**0.035**
Umbilical cord length (cm)	56.0 ± 10.4	56.5 ± 10.5	56.1 ± 9.9	0.765
Maternal adverse outcome				
Hypertension in pregnancy				
No	99 (99.0)	128 (97.7)	111 (98.2)	0.759
Yes	1 (1.0)	3 (2.3)	2 (1.8)	
Cesarean delivery				
No	364 (98.9)	360 (97.3)	306 (95.0)	**0.009**
Yes	4 (1.1)	10 (2.7)	16 (5.0)	
Postpartum hemorrhage				
No	321 (90.2)	305 (88.4)	248 (86.4)	0.333
Yes	35 (9.8)	40 (11.6)	39 (13.6)	

Data are means ± SD or n (%). The boldface P values are statistically significant ($P < 0.05$). Macrosomia was defined as birth weight >4,000 g. Preterm birth was defined as delivery <37 weeks of gestation. Gestational hypertension was defined as a systolic blood pressure ≥140 mmHg and/or a diastolic blood pressure ≥90 mmHg on two occasions at least 4 h apart after 20 weeks of gestation in a woman with previously normal blood pressure. *Highest group vs. lowest group, $P < 0.05$.

Higher Neutrophil Count Outperformed WBC Count and NLR as an Independent Risk Factor and Diagnostic Predictive Factor for GDM Development and Incidence of Macrosomia

To compare the predictive capability of metric WBC count, neutrophil count, and NLR as risk factors for GDM development, logistic regression analysis with enter selection was performed separately in a matched case-control study. We found neutrophil count had the highest odds ratio (OR) value as an independent risk factor for the development of GDM (OR 3.60; 95% CI 2.02–6.41 in the highest tertile vs. the lowest tertile; $P < 0.001$), regardless of GDM history (OR 3.70; 95% CI 2.05–6.66; $P < 0.001$) compared with WBC count (OR 2.40; 95% CI 1.38–4.17; $P = 0.002$ in all mothers; OR 2.75; 95% CI 1.56–4.86; $P < 0.001$ in mothers without GDM history) and NLR (OR 2.77; 95% CI 1.58–4.88; $P < 0.001$ in all mothers; OR 2.45; 95% CI 1.40–4.30; $P = 0.002$ in mothers without GDM history) (Table 2). Furthermore, we also found neutrophil count and combined basal factors (age, previous

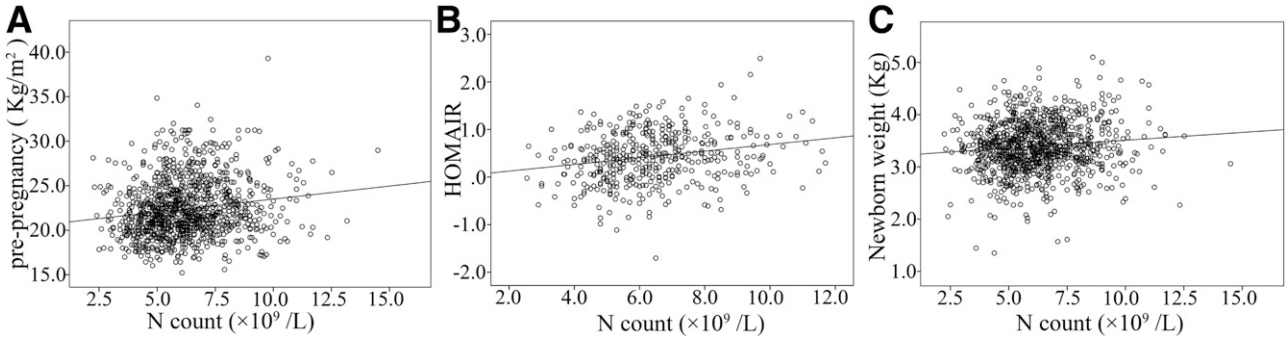

Figure 2—Simple linear regression analysis between the first-trimester neutrophil (N) count and prepregnancy BMI, HOMA-IR, and newborn weight. The neutrophil count showed a significant and moderate linear correlation with prepregnancy BMI [β = 0.29; F(1, 1,085) = 27.51; adjusted $R^2 = 0.02$; $P < 0.001$] (*A*), HOMA-IR [β = 0.07; F(1, 426) = 19.88; adjusted $R^2 = 0.04$; $P < 0.001$] (*B*), and newborn weight [β = 0.03; F(1, 1,039) = 10.27; adjusted $R^2 = 0.01$; $P = 0.001$] (*C*).

Table 5—Logistic regression analysis to determine the risk factors for development of GDM in the cohort study

	In all mothers (n = 756)		In mothers without GDM history (n = 734)	
	OR (95% CI)	P	OR (95% CI)	P
Tertile of neutrophils ($\times 10^9$/L)				
Lowest	Reference		Reference	
Middle	1.15 (0.52–2.53)	0.733	1.14 (0.52–2.53)	0.739
Highest	3.71 (1.80–7.63)	**<0.001**	3.66 (1.78–7.56)	**<0.001**
GDM history, n (%)				
No	Reference			
Yes	58.16 (18.60–181.86)	**<0.001**		
Nulliparous	4.77 (2.47–9.21)	**<0.001**		
Prepregnancy BMI (kg/m^2)	1.12 (1.04–1.20)	**0.004**	1.09 (1.01–1.17)	**0.027**
Age (years)	1.16 (1.09–1.23)	**<0.001**	1.11 (1.05–1.17)	**<0.001**
TG (mmol/L)	1.19 (1.03–1.37)	**0.020**	1.23 (1.07–1.41)	**0.003**
Weight gain before GDM diagnosis (kg)	1.05 (0.97–1.13)	0.236	1.08 (1.00–1.17)	0.065

The boldface P values are statistically significant ($P < 0.05$).

GDM history, prepregnancy BMI, and TG) had the highest area under receiver operating characteristic curve for predicting GDM compared with WBC count and NLR (0.787, 0.776, and 0.774, respectively) (Supplementary Fig. 1).

Besides, neutrophil count was also an independent risk factor for the incidence of macrosomia (OR 4.09; 95% CI 1.04–16.13 in the highest tertile vs. the lowest tertile; $P = 0.044$) corrected by prepregnancy BMI and weight gain during the whole pregnancy, rather than WBC count and NLR (Supplementary Table 1).

Comparison of Parameters in the First Trimester, the Second Trimester, and at Delivery Among Three Groups Categorized by Tertiles of Neutrophil Count in the Cohort Study

Subjects were divided into three groups according to tertiles of neutrophil count in the first trimester: lowest group ($<5.30 \times 10^9$/L), middle group (5.30–6.80 $\times 10^9$/L), and highest group ($>6.80 \times 10^9$/L). There was a stepwise increase in the incidence of GDM (9.1%, 14.7%, and 28.2%; $P < 0.001$), level of prepregnancy BMI, alanine aminotransferase, 1-h BG (6.68 ± 1.60 vs. 6.99 ± 1.77 vs. 7.60 ± 2.29 mmol/L; $P < 0.001$), 2-h BG (6.07 ± 1.29 vs. 6.39 ± 1.45 vs. 6.87 ± 1.81 mmol/L; $P < 0.001$), HbA_{1c} (4.9 ± 0.3 vs. 5.0 ± 0.3 vs. 5.1 ± 0.5%; $P < 0.001$), and HOMA-IR ($P < 0.001$) across the lowest, middle, and highest groups, respectively (Table 3). Likewise, macrosomia, neonatal weight, placental weight, and the incidence of cesarean delivery increased as neutrophil count increased (Table 4).

First-Trimester Neutrophil Count Was Closely Associated With Prepregnancy BMI, HOMA-IR, and Newborn Weight

To investigate the correlation between neutrophil count and insulin resistance or newborn weight, correlation analysis was performed. Simple linear regression analyses were performed to determine the association of neutrophil count during the first trimester with prepregnancy BMI, HOMA-IR, and newborn weight. There was a significant and linear correlation for neutrophil count with prepregnancy BMI [$\beta = 0.29$; $F(1, 1{,}085) = 27.51$; adjusted $R^2 = 0.02$; $P < 0.001$] (Fig. 2*A*), HOMA-IR [$\beta = 0.07$; $F(1, 426) = 19.88$; adjusted $R^2 = 0.04$; $P < 0.001$] (Fig. 2*B*), and newborn weight [$\beta = 0.03$; $F(1, 1{,}039) = 10.27$; adjusted $R^2 = 0.01$; $P = 0.001$] (Fig. 2*C*). Multiple linear regression analysis adjusting for confounding factors was performed to analyze the association between neutrophil count and prepregnancy BMI, HOMA-IR, and newborn weight. There was a significant linear association of neutrophil count with prepregnancy BMI ($P < 0.001$) and HOMA-IR ($P = 0.045$) (Supplementary Table 2).

Neutrophil Count Was an Independent Risk Factor for the Development of GDM

To determine independent risk factors for the development of GDM, tertiles of neutrophil count, GDM history (divided into no previous GDM, previous GDM, and nulliparous), prepregnancy BMI, age, TG, and weight gain before GDM was diagnosed were entered into logistic regression analysis with enter selection. The risk of developing GDM in the highest tertile neutrophil count increased 3.71-fold compared with the lowest tertile neutrophil count ($P < 0.001$). Risk of developing GDM in women with a previous history of GDM was significantly higher than in those without (OR 58.16; 95% CI 18.60–181.86; $P < 0.001$), and women with a higher prepregnancy BMI (OR 1.12; 95% CI 1.04–1.20; $P = 0.004$), age (OR 1.16; 95% CI 1.09–1.23; $P < 0.001$), and TG level (OR 1.19; 95% CI 1.03–1.37; $P = 0.020$) also had a tendency to develop GDM. Furthermore, the independent risk factors in women without a history of GDM (including those with no previous GDM and nulliparous) were also determined, and neutrophil count (OR 3.66; 95% CI 1.78–7.56 in highest tertile vs. in lowest tertile; $P < 0.001$) remained a risk factor for development of GDM independent of prepregnancy BMI, age, and TG level (Table 5).

Continuous Neutrophil Count in the First Trimester Was Closely Associated With the Incidence of GDM

After adjusting for GDM history, prepregnancy BMI, age, and TG, a spline model showed a significant relationship between continuous neutrophil count during the first trimester and GDM incidence. The risk of developing GDM increased when the neutrophil count was $>5.0 \times 10^9$/L (Fig. 3).

DISCUSSION

This retrospective case-control and cohort study is the first one to confirm the closest association of neutrophil count with development of GDM in a large sample size. Many studies have demonstrated increased inflammatory markers during pregnancy compared with a nonpregnant state characterized by elevated WBC count and neutrophil count (19,21). Nevertheless, pregnant women generally have a steady state of pro- and anti-inflammatory cytokines, although this balance is disturbed in some pathological states, including obesity and insulin resistance (22–24). A growing number of studies have described the central role of inflammation in GDM. In their 2004 cohort study, Wolf et al. (17) showed that women who developed GDM had a much higher leukocyte count than those who did not.

Neutrophils, which constitute the largest fraction of WBCs, have been found to be involved in chronic metainflammatory states such as diabetes, nonalcoholic fatty liver disease, and atherosclerosis (25–27). Although previous studies have produced inconsistent results, Yilmaz et al. (15) showed that NLR was significantly higher in patients with GDM compared with pregnant women with normal glycemic levels and was a powerful predictor of GDM, whereas Sargın et al. (16) showed no predictive ability of NLR. In our case-control study, after matching the possible confounder factors, we found that neutrophils, WBCs, and NLR were all associated with the development of GDM but that the neutrophil count had the highest OR and possessed the most predictive value. As we know, the WBC count is largely equal to the sum of the neutrophil count and the lymphocyte count, NLR is the ratio of neutrophil count to lymphocyte count. Because there is no difference of lymphocytes, which may dilute the impact of neutrophils on GDM, the WBC count and NLR were inferior to the neutrophil count in the role of GDM development and its outcomes. These results support the important pathological role of innate immune cells in the development of diabetes (28,29). Further analyses of the relationship between neutrophil count and GDM were performed in the cohort study. We found the incidence of GDM increased progressively with the increase of the neutrophil count, which was also an independent factor for GDM development. Moreover, fully adjusted spline regression showed a significant correlation of continuous neutrophil count with GDM incidence, and the risk abruptly increased when the neutrophil count was $>5.0 \times 10^9$/L. All of these demonstrated a close association of the first-trimester neutrophil count with GDM development.

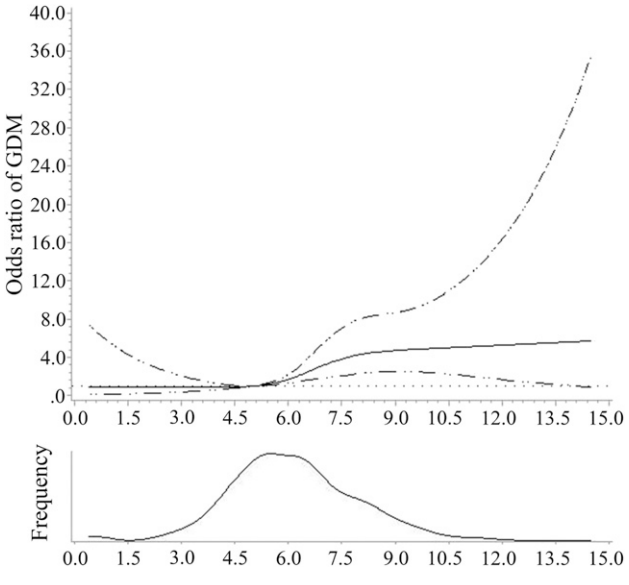

Figure 3— Continuous association of the neutrophil count in the first trimester with the incidence of GDM. Adjusted for GDM history, prepregnancy BMI, age, and TG.

From a functional perspective, Talukdar et al. (30) and Mansuy-Aubert et al. (31) both revealed that neutrophils contribute to the etiology of chronic inflammation and insulin resistance via secreted neutrophil elastase (NE) by the degradation of insulin receptor substrate 1 (IRS1). Recently, Stoikou et al. (32) reported that patients with GDM had increased neutrophil activity with elevated neutrophil extracellular traps (NETs) and NE levels in vitro. Lou et al. (33) found that high levels of neutrophil gelatinase-associated lipocalin (NGAL) in plasma and subcutaneous adipose tissue were associated with insulin resistance in GDM. Our study showed a significant positive association between neutrophil count and HOMA-IR, supporting the crucial role of neutrophils in insulin resistance. All of these results demonstrate that neutrophils may contribute to GDM development by mediating insulin resistance and that neutrophil-derived NE, NETs, and NGAL may serve as the potential targets.

Another important finding in our study was that an increased neutrophil count was also associated with adverse pregnancy outcomes. A higher neutrophil count was an independent risk factor for macrosomia corrected by prepregnancy BMI and weight gain during the whole pregnancy in the case-control study. Women with the highest tertile of neutrophil count had the highest risk for macrosomia and cesarean delivery in the cohort study. The developmental overnutrition hypothesis suggests that maternal hyperglycemia and obesity predispose offspring to obesity and metabolic dysfunction and may have been transferred from the mother through the placenta (34,35), although the underlying mechanism is elusive. An increased neutrophil count may lead to a rise in NE and NETs

in the placenta, as suggested in the Stoikou et al. (32) study; therefore, we hypothesize that neutrophil count may play a crucial role in this programming process via NE and NETs.

Moreover, our study found that patients with GDM had much higher levels of TG and lower levels of LDL, consistent with previous studies (36–40). The precise mechanism of lower LDL in women with GDM was unclear. This might be attributed to higher concentration of estrogen and insulin resistance in women with GDM.

There were some limitations of this study. First, all subjects were derived from one center, which may have led to biased results. We also acknowledge that a mechanistic insight into the potentially pathophysiological role of neutrophils in GDM development and offspring metabolic dysfunction is lacking in this clinical study. Further studies using reliable rodent GDM models to delineate the function of neutrophils, especially NE, NETs, and NGAL, are warranted.

Conclusions

This study demonstrated that the first-trimester neutrophil count was closely associated with GDM development and adverse pregnancy outcomes, especially macrosomia. The neutrophil count was an independent risk factor for GDM development when it was $>5.0 \times 10^9$/L.

Funding. This study received support from the Natural Science Foundation of China (81700510), the Medical Key Faculty Foundation of Shanghai (ZK2019B15), Health Profession Clinical Funds of the Shanghai Municipal Health Commission (201940295), the Science Foundation of the Fifth People's Hospital of Shanghai (2019WYZD02, 2018WYZD04), and the Science and Technology Planning Project of Hangzhou City (20170533B43).

Duality of Interest. No potential conflicts of interest relevant to this article were reported.

Author Contributions. T.S. wrote the manuscript and researched data. F.M. and H.D. contributed to discussion and reviewed and edited manuscript. H.Z. contributed to data collection. M.Y., R.Z., Z.Y., and X.H. contributed to data collection and database establishment. J.L. and S.Z. reviewed and edited the manuscript. S.Z. researched data. S.Z. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Prior Presentation. Parts of this study were presented in abstract form at the 79th Scientific Sessions of the American Diabetes Association, San Francisco, CA, 7–11 June 2019.

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