Article

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Immature Granulocytes are Closely Associated with the Development of Maternal Gestational Diabetes Mellitus and Adverse Pregnancy Outcomes

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Background: Gestational diabetes mellitus (GDM) is defined as any degree of dysglycaemia that occurs for the first time or is first detected during pregnancy. GDM causes various complications for both the mother and fetus. Immature granulocytes (IGs) may enter the peripheral blood in response to infection, inflammation, or other stimuli. In this study, we delve into the role of IGs in the occurrence and development of GDM as well as their correlation with pregnancy outcomes.

Purpose: This study aimed to investigate the risk factors for gestational diabetes mellitus (GDM) as well as the correlation between immature granulocytes (IGs) and maternal pregnancy outcome.

Methods: This study was conducted between January 1, 2019 and December 31, 2019 at the Women's Hospital, School of Medicine, Zhejiang University. We collected maternal demographic data and clinical information on major adverse pregnancy outcomes from medical records. We implemented multiple logistic regression models to determine the association between maternal IGs and adverse pregnancy outcomes.

Results: A total of 9558 pregnant women, including 7613 controls (those without GDM) and 1945 pregnant women diagnosed with GDM. We found that compared to those without GDM (control group), GDM patients exhibited a significantly higher percentage of immature granulocytes (1.22% $\pm$ 1.03% vs. 1.34% $\pm$ 1.26%, $p < 0.01$), and absolute immature granulocyte count (0.12 $\pm$ 0.13 $\times$ 10^9/L vs. 0.14 $\pm$ 0.15 $\times$ 10^9/L, $p < 0.001$). Furthermore, GDM patients manifested substantially higher rates of premature birth (6.96% vs. 10.13%, $p < 0.001$), macrosomia (4.39% vs. 5.55%, $p < 0.05$), and cesarean section (34.6% vs. 41.8%, $p < 0.001$). We found that after adjusting for potential confounding variables, IGs were found to be associated with a high risk for GDM (absolute value of IGs, adjusted odds ratio [aOR] = 2.265; percentage of IGs [aOR = 1.100]), preterm birth (absolute value of IGs, aOR = 5.325; percentage of IGs, aOR = 1.209), and macrosomia (absolute IG count, aOR = 1.503).

Conclusions: Our study demonstrates an association between IGs and GDM. Furthermore, IGs can serve as a risk factor associated with preterm delivery and macrosomia.

Keywords: immature granulocytes; GDM; preterm birth; risk factors; macrosomia

Introduction

Gestational diabetes mellitus (GDM) is one of the most common metabolic disorders among pregnant women, characterized by a glucose-intolerance disorder emerging or being diagnosed during the second and third trimesters of pregnancy, without a pre-existing diabetic history [1]. GDM causes various complications for both the mother and fetus such as an increased risk for spontaneous preterm labor, neonatal hyperbilirubinemia and hypoglycemia, shoulder dystocia, stillbirth, prolonged hospitalization in the neonatal intensive care unit, and respiratory problems [2–6]. Due to the differences in ethnicity and socioeconomic conditions, the incidence rate of GDM varies across various countries and populations [7,8]. Epidemiologic evidence suggests a considerable increase in the prevalence of GDM over recent decades with predictions indicating a continued rise in the future [9]. Presently, there is a high prevalence of GDM in Asia, with a pooled prevalence of GDM of 11.5% [3]. Several studies conducted in China have reported a significant increase in the prevalence of GDM from 2.4% in 1999 to 8.2% in 2012 [10,11], presently resting at 14.8% [12]. GDM has become an epidemic in China, imposing substantial health challenges and economic burdens, particularly after the implementation of the "two-child policy" in 2015 [13,14]. While several traditional factors, including family or personal history of diabetes, previous adverse pregnancy outcome, glycosuria, age, low-grade inflamma-

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tion, immune function disorders, insulin resistance, and obesity, have been associated with GDM, its exact pathophysiology remains arcane. Some investigators currently suggest that low-grade chronic inflammation and immune-function disorders play a crucial role in the pathophysiology of both GDM and T2DM [15–21]. Inflammatory blood cell parameters such as white blood cell (WBC) count, neutrophil count, Neutrophil-to-lymphocyte ratio (NLR), and abnormally elevated platelet levels have usually been identified as markers of inflammation and have been investigated for their potential to predict GDM [22–24]. In the present study, we evaluated independent risk factors for GDM and their correlations with pregnancy outcomes. Our findings establish a link between immature granulocytes and both GDM and pregnancy outcomes.

Immature granulocytes (IGs) are neutrophils originating from the bone marrow progenitor cells, including promyelocytic cells, neutrophilic myelocytes, and neutrophilic metamyelocytes, which are typically not released or detected in the peripheral blood of healthy individuals. However, IGs may enter the peripheral blood in response to infection, inflammation, or other stimuli. IGs can serve as indicators of bone marrow activation, early innate immune response, and inflammation, offering a more accurate indication compared to pure neutrophil count measurement [25]. Previous studies have reported an association between IG count and percentage (IG%) with conditions like neonatal sepsis, sepsis, acute pancreatitis, and complex appendicitis [26–29]. Our previous study involving the peripheral blood IGs of 12,033 pregnant women revealed that the incidence of GDM exhibited a significant elevation with the increase in immature granulocytes (unpublished). Therefore, in this study, we delve into the role of IGs in the occurrence and development of GDM as well as their correlation with pregnancy outcomes.

Materials and Methods

Characteristics of the Subjects and Data Collection

This retrospective cohort study was conducted at the Women's Hospital, School of Medicine, Zhejiang University, Zhejiang, China. It was conducted after obtaining consent from Institute Ethical Committee, and the reference number is IRB-20200149-R. The clinical staff followed established diagnostic criteria for various obstetric diseases to ensure the effectiveness of our retrospective research. The women who gave birth between January 1 and December 31, 2019, were included in the analysis. Inclusion criteria comprised women with (1) singleton pregnancies, (2) maternal ages at delivery of $\geq$20 years, (3) live births, and (4) fetuses without known chromosomal or anatomical abnormalities. The exclusion criteria included manifestations of any of the following: (1) multiple pregnancies, (2) history of abortion or stillbirth, (3) diabetes mellitus or chronic hypertension before pregnancy; and (4) presence of concurrent diseases such as intrahepatic cholestasis, pregnancy-induced hypertension, eclampsia, and thyroid dysfunction.

A total of 9558 pregnant women, the GDM was diagnosed based on the oral glucose tolerance test (OGTT) conducted between the 24th and 28th weeks of gestation. According to the International Association of Diabetes and Pregnancy Study Groups (IADPSG) criteria [30], including 7613 controls (those without GDM) and 1945 diagnosed with GDM, were enrolled in this study. We collected whole blood samples from pregnant women in the second trimester (via EDTA-K2 anticoagulation), and performed a complete blood count and cellular identification (CBC + DIFF) for routine blood analysis using hematologic analyzers (XN-9000, Sysmex, Kobe, Japan). Moreover, the IG count and IG% were included in the routine analysis (Fig. 1).

The Diagnostic Criteria for GDM

All study participants underwent a 75-g oral glucose tolerance test (OGTT) to diagnose GDM, during which their IGs were assessed. The GDM was diagnosed based on the OGTT conducted between the 24th and 28th weeks of gestation. According to the International Association of Diabetes and Pregnancy Study Groups (IADPSG) criteria [30], at least one of the following cutoff values was met after a 75-g glucose load: 0-hour value of 5.1 mmol/L, 1-hour value of 10.0 mmol/L, or 2-hour value of 8.5 mmol/L.

Adverse Pregnancy Outcomes

Our clinical obstetricians identified adverse pregnancy outcomes in pregnant women according to current clinical practice guidelines, with data derived from electronic medical records. Subsequently, the study participants were classified into two groups based on their GDM status: the GDM group and the those without GDM (control group). Additionally, adverse pregnancy outcomes were recorded, including preterm birth (delivery before 37 weeks), macrosomia (newborn birth weight $\geq$4000 g), placental abruption, placental previa, placental implantation issues, premature rupture of membranes, fetal distress, abnormal amniotic fluid levels, cesarean section, dystocia, postpartum hemorrhage, and restricted fetal growth.

Statistical Analysis

Continuous variables were expressed as mean $\pm$ standard deviations. The differences in continuous variables were evaluated using the student's *t*-test, depending upon their distribution. However, categorical variables were expressed as frequencies and percentages. The differences among categorical variables were assessed using Pearson's chi-squared test. After adjusting for potential confounding variables, odds ratio (OR) with a 95% confidence interval (CI) were utilized to evaluate the impact of IGs on pregnancy outcomes. Statistical analyses were performed using SPSS version 19.0 (IBM, Chicago, IL, USA). A $p < 0.05$ was considered statistically significant.

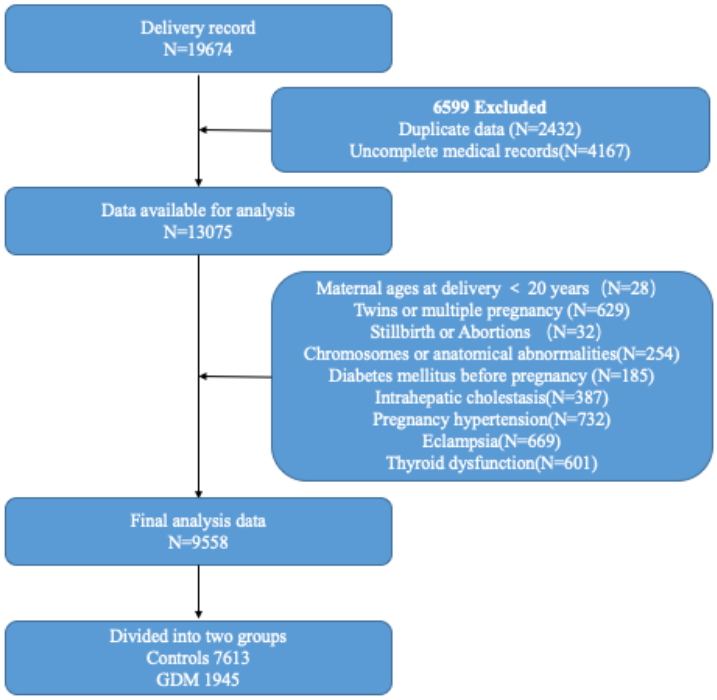

Fig. 1. A flow chart of study participants selection. N, number of included studies; GDM, gestational diabetes mellitus.

Results

Demographics and Clinical Characteristics of the Study Subjects

The present study aimed to explore the risk factors for GDM and the correlations between immature granulocytes and maternal pregnancy outcomes. A total of 9558 pregnant women, including 7613 controls and 1945 pregnant women diagnosed with GDM, were enrolled in our study. The clinical information and routine blood indices of the two groups are shown in Table 1. Compared to those without GDM (control group), GDM patients exhibited a significantly higher level of pre-parturient body mass index (BMI), age, abdominal girth, fasting blood glucose, 1-hour glucose, 2-hour glucose, glycosylated hemoglobin (HbA1c), percentage of immature granulocytes ($1.22 \pm 1.03\%$ vs. $1.34 \pm 1.26\%$, $p < 0.01$), absolute count of neutrophil granulocytes ($7.10 \pm 1.81 \times 10^9$/L vs. $7.27 \pm 1.86 \times 10^9$/L, $p < 0.001$), percentage of neutrophil granulocytes ($74.73 \pm 4.64\%$ vs. $74.94 \pm 4.54\%$, $p < 0.05$), leukocytes ($9.45 \pm 2.10 \times 10^9$/L vs. $9.65 \pm 2.18 \times 10^9$/L, $p < 0.001$), and absolute immature granulocyte count ($0.12 \pm 0.13 \times 10^9$/L vs. $0.14 \pm 0.15 \times 10^9$/L, $p < 0.001$). However, there were no significant differences in the percentage of basophilic cells, absolute eosinophil count, absolute lymphocyte count, percentage of lymphocytes, or percentage of monocytes (Table 1).

Adverse Pregnancy Outcomes

The study compared pregnancy outcomes between the control and GDM patient groups. The clinical outcomes included in this analysis were placental abruption, placental previa, placental implantation, premature rupture of membranes, fetal distress, abnormal amniotic fluid, cesarean section, dystocia, postpartum hemorrhage, premature delivery, fetal macrosomia, and restricted fetal growth. It was observed that compared to the control group, GDM patients manifested a substantially higher percentage of premature birth (6.96% vs. 10.13%, $p < 0.001$), macrosomia (4.39% vs. 5.55%, $p < 0.05$), and cesarean section (34.6% vs. 41.8%, $p < 0.001$). However, there was no significant difference in any other pregnancy outcome (Table 2).

Risk Factors for GDM, Preterm Birth, and Macrosomia

We used multivariable logistic regression to determine whether various factors, including the percentage of immature granulocytes, absolute neutrophil count, percentage of neutrophils, leukocytes, absolute immature granulocyte count, and the Neutrophil-to-lymphocyte ratio (NLR) served as independent risk factors for GDM, preterm birth, and macrosomia. This analysis revealed significant association between IGs and a high risk for GDM (absolute IG count, adjusted odds ratio [aOR] = 2.265; 95% confidence interval [CI], 1.595–3.215; percentage of IGs, aOR = 1.100; 95% CI, 1.053–1.141) (Table 3), preterm birth (absolute IG count, aOR = 5.325; 95% CI, 2.778–6.735; percentage of IGs, aOR = 1.209; 95% CI, 1.143–1.279) (Table 4), and macrosomia (absolute IG count, aOR = 1.503; 95% CI, 1.284–2.884) (Table 5).

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Table 1. Demographic and clinical characteristics of the study subjects.

Variables	Controls (N = 7613)	GDM (N = 1945)	*t*	*p*
Age, years	29.35 ± 3.18	30.51 ± 3.31	13.984	<0.001
Gravidity	1.86 ± 1.06	2.00 ± 1.15	5.025	<0.001
Parity	1.33 ± 0.51	1.39 ± 0.53	4.108	<0.001
Height (cm)	160.78 ± 5.07	160.10 ± 5.01	5.312	<0.001
Weight (kg)	67.54 ± 8.30	67.68 ± 9.37	0.603	1.00
Pre-parturient BMI, kg/m^2	26.12 ± 2.93	26.41 ± 3.29	3.431	<0.001
Abdominal girth (cm)	98.14 ± 5.83	98.63 ± 6.44	3.026	0.02
FBG (mmol/L)	4.36 ± 0.29	4.59 ± 0.45	19.048	<0.001
1-hour GLU (mmol/L)	7.56 ± 1.28	10.06 ± 1.37	61.913	<0.001
2-hour GLU (mmol/L)	6.56 ± 0.99	8.86 ± 1.31	61.555	<0.001
HbA1c (%)	4.98 ± 0.28	5.15 ± 0.32	18.847	<0.001
Delivery gestational age	38.79 ± 1.69	38.42 ± 1.77	8.388	<0.001
Neonatal weight (g)	3265.12 ± 472.76	3234.42 ± 517.94	2.214	0.01
Percentage of basophilic cells (%)	0.02 ± 0.038	0.02 ± 0.037	0.358	0.73
Absolute basophil count (10^9/L)	0.33 ± 0.15	0.32 ± 0.15	1.860	0.02
Absolute eosinophil count (10^9/L)	0.09 ± 0.085	0.09 ± 0.081	0.936	0.42
The percentage of eosinophils (%)	0.95 ± 0.79	0.92 ± 0.75	1.749	0.03
Hematocrit	0.34 ± 0.024	0.35 ± 0.025	9.024	<0.001
Hemoglobin (g/L)	114.72 ± 8.55	116.56 ± 8.82	8.253	<0.001
Percentage of immature granulocytes (%)	1.22 ± 1.03	1.34 ± 1.26	3.858	0.01
Absolute lymphocyte count (10^9/L)	1.67 ± 0.41	1.69 ± 0.42	2.024	0.08
Percentage of lymphocytes (%)	18.03 ± 4.08	17.87 ± 4.04	1.564	0.06
Mean corpuscular hemoglobin (pg)	31.24 ± 1.81	31.17 ± 1.82	1.551	0.05
Mean corpuscular hemoglobin concentration (g/L)	338.03 ± 8.81	337.70 ± 8.39	1.534	0.08
Mean corpuscular volume (fl)	92.39 ± 4.50	92.26 ± 4.52	1.087	0.18
Absolute monocyte count (10^9/L)	0.56 ± 0.16	0.57 ± 0.17	2.600	0.01
The percentage of monocytes (%)	5.95 ± 1.26	5.95 ± 1.25	0.098	0.87
Mean platelet volume (fl)	10.23 ± 1.15	10.21 ± 1.18	0.527	0.57
Absolute neutrophil granulocyte count (10^9/L)	7.10 ± 1.81	7.27 ± 1.86	3.520	<0.001
Percentage of neutrophil granulocytes (%)	74.73 ± 4.64	74.94 ± 4.54	1.814	0.04
Giant platelet ratio (%)	26.98 ± 7.82	26.91 ± 7.93	0.341	0.76
Thrombocytocrit (%)	0.21 ± 0.046	0.22 ± 0.047	1.657	0.10
Platelet distribution width	11.71 ± 2.29	11.73 ± 2.35	0.380	0.74
Blood platelet count (10^9/L)	211.51 ± 48.14	213.53 ± 49.53	1.616	0.23
Number of erythrocytes (10^{12}/L)	3.68 ± 0.31	3.75 ± 0.32	8.384	<0.001
Red blood cell distribution width	13.19 ± 0.88	13.21 ± 0.90	0.815	0.59
Red blood cell distribution width (SD)	44.27 ± 3.16	44.25 ± 3.15	0.203	0.40
Number of leukocytes (10^9/L)	9.45 ± 2.10	9.65 ± 2.18	3.553	<0.001
Absolute immature granulocyte count (10^9/L)	0.12 ± 0.13	0.14 ± 0.15	4.047	<0.001
Neutrophil-to-lymphocyte ratio	4.43 ± 1.33	4.47 ± 1.31	1.313	0.05
Immature granulocyte-to-lymphocyte ratio	0.07 ± 0.072	0.08 ± 0.088	3.840	<0.001
Immature granulocyte-to-neutrophil ratio	0.02 ± 0.0136	0.02 ± 0.0165	3.738	0.02

Continuous variables are expressed as mean ± one standard deviation. Differences in continuous variables were assessed using the student's *t*-test, depending upon the normality of variable distribution. The categorical variables are expressed as frequencies and percentages. Differences in categorical variables were evaluated using Pearson's chi-squared test. BMI, body mass index; FBG, fasting blood glucose; GLU, glucose; HbA1c, glycosylated hemoglobin.

Discussion

To the best of our knowledge, this retrospective investigation is the first to confirm the strong association between IGs and the development of GDM in a large sample set. We found that after adjusting for potential confounding variables-including age, pre-parturient BMI, gravidity, and parity-IGs were found to be associated with a high risk

Table 2. Adverse pregnancy outcomes among study participants.

	Controls		GDM		
Variables	Frequencies	Percentages (%)	Frequencies	Percentages (%)	*p*
Placental abruption	131	1.72	35	1.80	0.812
Placental previa	98	1.29	33	1.70	0.166
Placental implantation	14	0.18	5	0.26	0.518
Premature rupture of membranes	1833	24.08	471	24.22	0.898
Fetal distress	1459	19.16	342	17.58	0.112
Abnormal amniotic fluid	354	4.65	72	3.70	0.071
Cesarean section	2634	34.60	813	41.80	<0.001
Dystocia	497	6.53	135	6.94	0.513
Postpartum hemorrhage	413	5.42	95	4.88	0.343
Preterm birth	530	6.96	197	10.13	<0.001
Macrosomia	334	4.39	108	5.55	0.029
Fetal growth restriction	100	1.31	25	1.29	0.922

Categorical variables are expressed as frequencies and percentages. Differences in categorical variables were assessed using Pearson's chi-squared test.

Table 3. Risk factors for GDM.

Variables	*p*	OR	95% CI		*p*	aOR	95% CI	
Percentage of immature granulocytes	0.000	1.099	1.053	1.147	0.000	1.100	1.053	1.141
Absolute neutrophil count	0.000	1.050	1.022	1.079	0.000	1.055	1.027	1.085
Percentage of neutrophils	0.073	1.010	0.999	1.021	0.230	1.007	0.996	1.018
Leukocytes	0. 000	1.044	1.020	1.068	0.000	1.051	1.026	1.076
Absolute immature granulocyte count	0.000	2.197	1.559	3.096	0.000	2.265	1.595	3.215
Neutrophil-to-lymphocyte ratio	0.193	1.025	0.988	1.063	0.461	1.014	0.977	1.054

Multivariable logistic regression was adjusted for age, pre-parturient BMI, gravidity, and parity. The data are presented with an adjusted odds ratio, aOR (95% CI). aOR, adjusted odds ratio; OR, odds ratio; CI, confidence interval.

for GDM. Particularly, we found correlations between IGs and GDM, including absolute IG count and the percentage of IGs with preterm birth and the absolute IG count and the percentage of IGs with macrosomia.

The pathogenesis of GDM is yet to be fully understood. However, two potential factors, immune dysfunction [31–34] and persistent, chronic inflammation [15–21,35], have been found crucial in the onset and development of GDM.

Increasing evidence shows that the immune system, mediated by immune cells and their products, exhibits a critical role in regulating the occurrence and progression of GDM. Sheu *et al.* [31] reported GDM as an inflammatory condition involving $CD4^+$ Th subset cell-mediated immunity and resolution. $CD4^+$ T cells, as the principal component of the adaptive immune response, play a pivotal role regardless of the subgroup: helper T 2 cells (Th2) are essential for inducing humoral immunity, neutrophils, and natural killer cells by secreting IFN-γ or IL-17 [36]. Immune system disorders are characterized by phenotypic changes in peripheral blood mononuclear cells and regulatory T cells. An imbalance between helper T 1 cells (Th1) and helper T 2 cells (Th2) leads to a pro-inflammatory response. Schober *et al.* [32] reported decreased percentages of naive $CD45RA^+$ regulatory T cell (T_{regs}) but increased percentages of HLA-$DR^-CD45RA^-$ (DR^-), HLA-$DR^{low+}CD45RA^-$ (DR^{low+}), and HLA-$DR^{high+}CD45RA^-$ (DR^{high+}) memory T_{regs} in GDM patients compared to healthy pregnant women. This subgroup of $CD4^+$ T cells primarily regulates immunotolerance [37]. Moreover, a combined microarray analysis of circRNAs and their target mRNAs in GDM patients and healthy pregnant women showed that the upregulation of T cell receptor signaling pathway components could be the major pathological mechanism underlying GDM [38]. These studies indicate an impairment in both the function and quantity of immune cells in individuals with GDM.

An abnormal maternal immune system is a key factor in low-grade inflammation, and immune dysfunction and low-grade inflammation operate as independent but interrelated systems. For example, neutrophils serve as both immune and inflammatory cells. They act as the first line of defense against pathogen invasion through phagocytosis or the release of cytotoxic granular enzymes such as myeloperoxidase (MPO) or neutrophil elastase (NE) [39]. Recent evidence suggests that neutrophils exert long-term effects, including $CD4^+$ and $CD8^+$ T cell and B cell activation, through antigen presentation in lymph nodes or the

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Table 4. Risk factors for preterm birth.

Variables	*p*	OR	95% CI		*p*	aOR	95% CI	
Percentage of immature granulocytes	0.000	1.199	1.134	1.268	0.000	1.209	1.143	1.279
Absolute value neutrophil count	0.077	1.037	0.996	1.080	0.018	1.051	1.009	1.095
Percentage of neutrophils	0.458	0.994	0.978	1.010	0.404	0.993	0.977	1.009
Leukocytes	0.024	1.041	1.005	1.078	0.003	1.054	1.018	1.092
Absolute immature granulocyte count	0.000	3.993	2.565	6.216	0.000	5.325	2.778	6.735
Neutrophil-to-lymphocyte ratio	0.386	1.025	0.969	1.084	0.440	1.022	0.966	1.082

Multivariable logistic regression was adjusted for age, pre-parturient BMI, gravidity, and parity. The data are presented with an adjusted odds ratio, aOR (95% CI).

Table 5. Risk factors for macrosomia.

Variables	*p*	OR	95% CI		*p*	aOR	95% CI	
Percentage of immature granulocytes	0.005	1.115	1.034	1.202	0.104	1.069	0.986	1.158
Absolute neutrophil count	0.014	1.065	1.013	1.120	0.616	1.014	0.961	1.070
Percentage of neutrophils	0.320	1.011	0.990	1.032	0.474	1.008	0.986	1.030
Leukocytes	0.009	1.059	1.014	1.106	0.649	1.011	0.965	1.059
Absolute immature granulocyte count	0.007	2.248	1.242	4.068	0.002	1.503	1.284	2.884
Neutrophil-to-lymphocyte ratio	0.314	1.036	0.967	1.111	0.332	1.037	0.964	1.116

Multivariable logistic regression was adjusted for age, pre-parturient BMI, gravidity, and parity. The data are presented with an adjusted odds ratio, aOR (95% CI).

formation of neutrophil extracellular traps (NET), thereby contributing to the elimination of pathogens [40,41]. Additionally, it has also been found that neutrophils can promote insulin resistance through the release of neutrophil elastase (NE), which subsequently can affect the occurrence and progression of insulin resistance by altering the AMPK signal through IRS1 degradation [42]. Stoikou *et al.* [43] found a significant association between GDM and neutrophil activation, which then led to placental infiltration, enhanced NET formation, and NE release. Neutrophils not only play a role in the occurrence and progression of GDM, but also affect pregnancy outcomes regarding offspring. Sun *et al.* [35] observed an increased neutrophil count in the first trimester, along with a higher incidence of GDM, increased blood glucose, and glycosylated hemoglobin (HbA1c) levels; insulin resistance via the Homeostasis Model Assessment for Insulin Resistance (HOMA-IR); and higher incidence of macrosomia and neonatal weight. The neutrophil count also showed a positive correlation with pre-pregnancy body mass index (BMI), HOMA-IR, and newborn weight. Furthermore, regardless of the history of GDM, neutrophil count remained an independent risk factor for the occurrence of GDM. The increase in neutrophil count was also associated with adverse pregnancy outcomes. In case-control studies, higher neutrophil counts were identified as an independent risk factor for macrosomia, and women with the highest titer of neutrophil counts exhibited the highest risk for macrosomia and cesarean section [35]. Our results showed a substantial increase in absolute neutrophil granulocyte count in the GDM group compared to the control group ($7.10 \pm 1.81 \times 10^9$/L vs. $7.27 \pm 1.86 \times 10^9$/L, $p < 0.001$). However, we did not identify absolute neutrophil granulocyte count or NLR as an independent risk factor for GDM or macrosomia. This may be due to the fact that we used data from different trimesters. However, Sun's [35] study used absolute neutrophil counts in the first trimester, whereas we utilized data from the second trimester.

Previous studies have shown that neutrophils play a significant role in the onset and development of GDM. Immature granulocytes (IGs) are neutrophils originating from bone marrow progenitor cells in their mature stage, including promyelocytic cells, neutrophilic myelocytes, and neutrophilic metamyelocytes; and these are typically not released or detected in the peripheral blood of healthy individuals. IGs are currently recognized as immunologic and inflammatory markers in the occurrence and development of various diseases [26–29]. Moreover, our study found a significant association between IGs and increased risk of GDM, preterm birth, and macrosomia.

This study possesses certain limitations. Firstly, all our data were derived from a single hospital, potentially introducing bias into the dataset. Secondly, inflammatory parameters such as CRP and procalcitonin were not included. Thirdly, this study only observed the correlation between IGs and GDM, the underlying mechanism remains unclear and requires further research.

Conclusions

In conclusion, our study suggests that immature granulocytes constitute an independent risk factor for predicting GDM and an independent risk factor for preterm delivery

and macrosomia. However, further research is required to elucidate the potential role of IGs in the predisposition and onset of GDM.

Availability of Data and Materials

The data used to support the findings of this study are available from the corresponding authors upon request.

Author Contributions

Methodology, BZ, YFS, WW, and XJM; investigation, WW, XJM, LJD, BBY, LZ, MNM, and YNS; writing—original draft, WW, XJM, LZ; writing—review & editing, WW, XJM, YFS; supervision, BZ, YNS; funding acquisition, BZ. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

All the procedures performed herein involving human participants were in accordance with the ethical standards of the Women's Hospital, School of Medicine, Zhejiang University Committee (approval number, IRB-20200149-R), and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The informed consent of study participants was exemoted because this was a retrospective observational study without intervention.

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Conflict of Interest

The authors declare no conflict of interest.

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