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Association of leukocyte counts in the first trimester with glucose intolerance during pregnancy

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ABSTRACT

Aims/Introduction: We investigated the association between leukocyte counts and glucose challenge test (GCT) level during pregnancy.

Materials and Methods: We collected prenatal information of women who had their first clinic visit in early pregnancy. Women underwent GCT at 24–28 gestational weeks, and a result of ≥7.8 mmol/L was considered positive. Participants were divided into quartiles of leukocyte counts, and association with GCT results and positive rate were analyzed by logistic regression.

Results: Among 20,707 pregnant women, the median of leukocyte counts was higher in the positive group than the normal group (8.5×10^9/L vs 8.2×10^9/L, $P < 0.01$). There was a linear trend in GCT results and positive rate with increasing leukocyte quartiles. Compared with the lowest quartile, the highest leukocyte quartile (>9.70×10^9/L) was significantly associated with positive GCT results (adjusted odds ratio 1.378, 95% confidence interval 1.246–1.524), and the linear relationship between increased risk of positive result and increasing leukocyte quartiles persisted (P for linear trend <0.01). In multivariable analysis, the risk of a positive result increased by 2.2% with each 1-unit increase in leukocyte counts (adjusted odds ratio 1.022, 95% confidence interval 1.011–1.033).

Conclusions: Elevated leukocyte counts in early pregnancy were independently and linearly associated with the risk of positive GCT levels, indicating that inflammation might play an important role in the development of gestational diabetes mellitus.

INTRODUCTION

Gestational diabetes mellitus (GDM) is defined as glucose intolerance that occurs for the first time in the second and third trimester of pregnancy, resulting in hyperglycemia of variable severity[1]. Over the past two decades, the prevalence of GDM has increased by >30% worldwide, including developing countries[2]. However, it is difficult to estimate the global prevalence of GDM. It was reported that the prevalence of GDM varied from 1.8% to 25.1% depending on the study population and diagnostic criteria applied[2]. In Tianjin, a cosmopolitan city in north China, the prevalence of GDM has increased from 2.3% in 1999 to 8.1% in 2011–2012 by using the 1999 World Health Organization criteria, which increased to 9.3% if the International Association of Diabetes and Pregnancy Study Group criteria were applied[3]. As a major public health problem, GDM has significant negative effects on both the mother and offspring's health outcomes. It is well recognized that GDM is associated with pregnancy complications, such as pre-eclampsia, pre-term birth and macrosomia, and greater risk of type 2 diabetes in the future[4]. Furthermore, children of GDM mothers are at higher risk of overweight or obese, higher blood pressure, insulin resistance, dyslipidemia and cardiovascular disease in adulthood[5–7]. Therefore, discovering novel screening indicators for GDM is important to identify women at high risk and prevent potential pregnancy complications.

It is well documented that GDM is a consequence of insulin resistance, which is facilitated by increased placenta secretion of hormones and other mediators, making pregnancy a diabetogenic condition[8]. Evidence suggested that inflammation, which is involved in the pathogenesis of insulin resistance, played a potential role in the development of dysglycemia[9–12]. Several

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studies have shown that elevated levels of pro-inflammatory proteins have been related to the incidence and development of type 2 diabetes mellitus and GDM[10,11,13–15]. Inflammatory cytokines secreted by adipose tissue have been shown to induce insulin resistance[16], and other inflammation proteins, including interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), C-reactive protein and plasminogen activator inhibitor-1, could impair β-cell function gradually and augment insulin resistance, resulting in poor blood glucose control and dysglycemia[14]. However, because of the high costs and difficulties, it is difficult to test these inflammatory markers as GDM biomarkers in daily outpatient clinics, especially in developing countries. Hematological parameters, including leukocyte counts, neutrophil counts and lymphocyte counts, alter in response to inflammation. The neutrophil-to-lymphocyte ratio and the platelet-to-lymphocyte ratio, indicators of inflammation as well, have also been proved useful as markers of inflammation-associated diseases, prognostic markers in ischemic heath disease and screening tests for complications of diabetes[12,17,18]. Recent studies focused on the utility of these hematological parameters as predictors of insulin resistance disorders, including type 2 diabetes and GDM. Evidence has shown that increased leukocyte counts could be used in predicting the incidence of GDM[9,12,19]. To our knowledge, however, there were few studies on the association between inflammation and the development of GDM in China. Therefore, we carried out a prospective cohort study to discover whether inflammation during early pregnancy was independently associated with GDM, and the utility of hematological parameters in predicting hyperglycemia in the second trimester.

In the present prospective cohort study, we aimed to investigate the association between leukocyte counts and glucose intolerance during pregnancy by comparing leukocyte counts in pregnant women with normal GCT results and in pregnant women with positive GCT results, and whether increased leukocyte counts are linearly correlated with the risk of positive GCT levels.

MATERIALS AND METHODS

Tianjin is a metropolitan city in northern China. As one of the four province-level municipalities directly under the Central Government, Tianjin consists of 16 districts, covering >11,000 km^2, with a resident population of >15 million. Prenatal care is shared by a three-tier care system, consisting of the following: (i) 267 primary care hospitals (level 1); (ii) 16 district-level women and children's health centers, and other secondary obstetric hospitals (level 2); and (3) a city-level Tianjin Women and Children's Health Center, and other tertiary hospitals (level 3). All pregnant women are suggested to register at primary hospitals in the first trimester, and receive regular prenatal clinic visits and checkups until the 28th week of pregnancy. Then, they are referred to a secondary or tertiary delivery hospital of their choice to receive prenatal care until delivery.

Women's prenatal care and delivery records have been collected since 2009, and are stored in an electronic information system, Tianjin Women and Children Health Information System, which is managed by Tianjin Women and Children's Health Center. In the present study, we collected the prenatal checkup information of 48,802 pregnant women who registered at primary hospitals from January 2009 to December 2010. During this period, a total of 47,394 pregnant women registered at primary hospitals and had their first prenatal examination at the gestational age of 4–12 weeks. Among them, 31,834 women with singleton pregnancies had a glucose challenge test (GCT) at 24–28 weeks' gestation. After excluding 6,385 women without complete blood cell counts results and 826 women with suspicious results (i.e., obviously unreasonable data), just 24,623 pregnant women had reliable and accurate blood test results at their first prenatal clinic visits. The exclusion criteria also included: (i) women who had any condition that might affect leukocyte counts, including recent infection, recent anti-infection drug intake, hematological disease, autoimmune disease and so on; (ii) women who had pre-existing diabetes; and (iii) women with missing data, including maternal age, education, smoking, ethnicity, height, pre-pregnancy weight, parity, blood pressure, weight change between pre-pregnancy and GCT. Finally, 20,707 pregnant women were included in the analysis (Figure 1). The study was approved by Peking University The Third Hospital Medical Science Research Ethics Committee, and all participants provided verbal consent.

Laboratory tests and screening for GDM

Venous blood samples were collected at the first prenatal clinic visit at primary hospitals for a complete blood count test. Hematological parameters were measured by an auto-hematology analyzer at primary hospitals. All pregnant women underwent a 50-g GCT between 24 and 28 gestational weeks at a primary hospital. Venous blood were taken 1 h after intake of 200 mL glucose solution, and women whose plasma glucose level was ≥7.8 mmol/L were defined as having a positive result and were categorized as the group at high risk of GDM.

Data collection

Maternal age, pre-pregnancy weight, height, parity, ethnicity, education, smoking and blood pressure were collected at the first prenatal clinic visit at primary hospitals. Because weight change during the first trimester was minimal and negligible, weight at the first prenatal clinic visit was used as the pre-pregnancy weight. Weight was also measured at GCT to calculate the weight gain between pre-pregnancy and GCT. The height and weight were measured while wearing light clothing and no shoes. Weight was measured to the nearest 0.1 kg, and height was measured to the nearest 0.5 cm. Blood pressure was measured by a calibrated mercury sphygmomanometer in the resting state. Pre-pregnancy body mass index (BMI) was calculated as pre-pregnancy weight divided by height square (kg/m^2). Pre-pregnancy BMI groups were

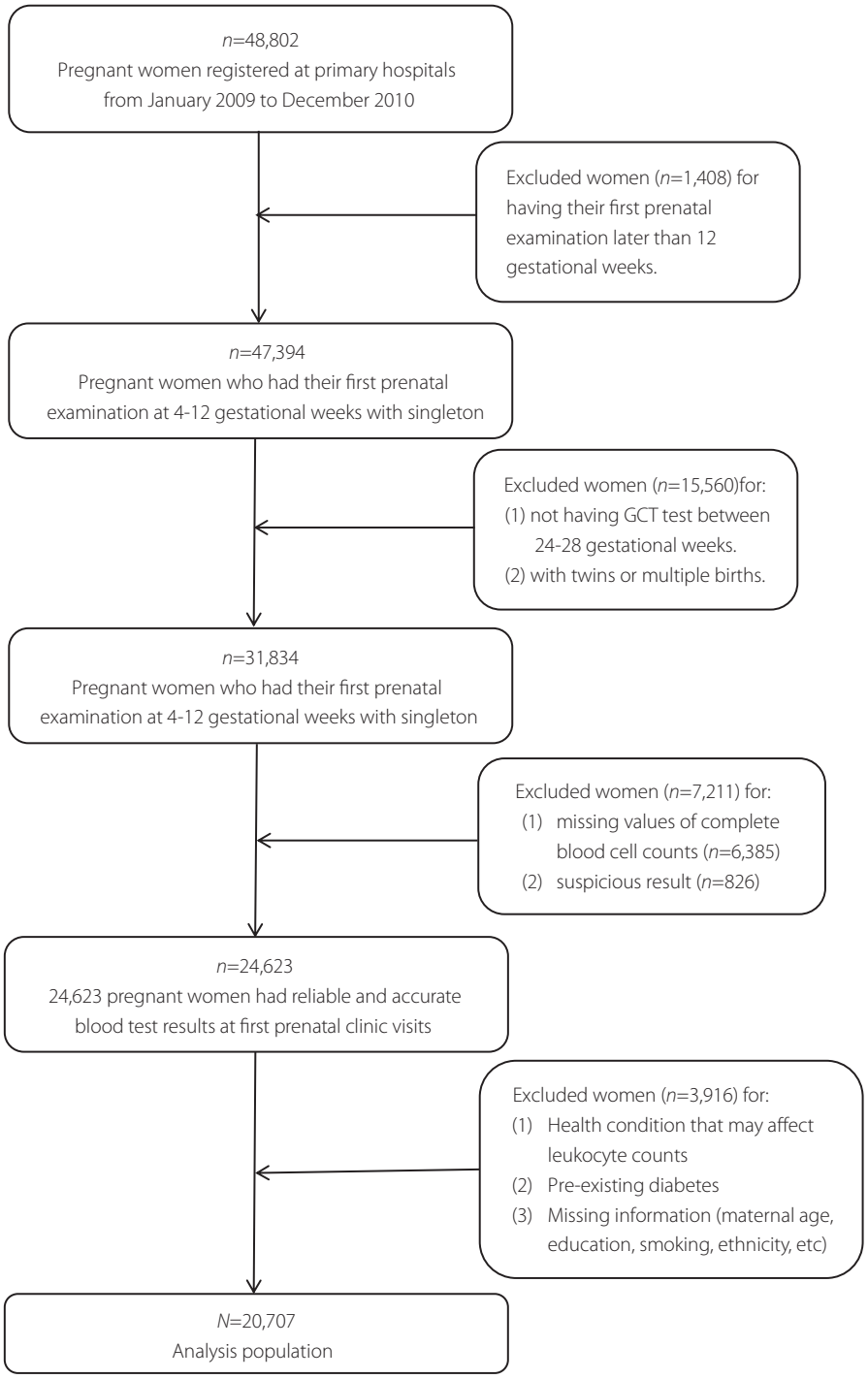

Figure 1 | Inclusion and exclusion of participants. In total, 48,802 pregnant women registered at primary hospitals. Women who did not register in the first trimester, or did not have their glucose challenge test (GCT) test between 24 and 28 gestational weeks were excluded. Other exclusion criteria included: having missing or suspicious results of complete blood cell test, having missing general information or having a pre-existing health condition that might influence the study. Finally, 20,707 women with singleton pregnancies were included in the study.

categorized according to the BMI classification standard of Chinese adult developed by the Working Group on Obesity in China[20]: underweight (<18.5 kg/m^2), normal weight (18.5–23.9 kg/m^2), overweight (24–27.9 kg/m^2) and obesity (≥28 kg/m^2). Other information was collected using a questionnaire at the primary hospital. Education level was classified as >12 years and ≤12 years of schooling, and ethnicity was classified as Han and others.

Statistical analysis

SPSS 21.0 (IBM Corp., Chicago, IL, USA) was used for statistical analyses. Quantitative data were analyzed by the Kolmogorov–Smirnov test for a normal distribution test. Descriptive statistics are presented as the mean ± standard deviation (SD), the median (interquartile ranges) for continuous variables and frequencies or ratios for categorical variables. Comparisons between groups of continuous variables were carried out by Student's *t*-test or Mann–Whitney *U*-test for normally or non-normally distributed variables, and comparisons of categorical variable were carried out using the χ^2-test or Fisher's exact test. The correlation between leukocyte counts and GCT results was examined by Spearman's correlation coefficient, and linear regression analysis was carried out to confirm the association. The linear trend in mean GCT results across different leukocyte quartiles was examined by analysis of variance (ANOVA). To determine the influence of risk factors, univariate and multivariate logistic regression analysis were applied to calculate the odds ratios (ORs) and 95% confidence intervals (CIs). The multivariate regression analyses were adjusted for potential confounders known to be associated with GDM, which were examined in multivariable analysis and confirmed by other studies[3,9,21], including maternal age, gestational age at first prenatal clinic visit, height, pre-pregnancy BMI, parity, ethnicity, education, blood pressure and weight change from pre-pregnancy to GCT. A two-sided *P*-value <0.05 was considered statistically significant.

RESULTS

General characteristics

In total, the present study involved 20,707 pregnant women who had their first prenatal clinic visit at primary hospitals in the first trimester, and had reliable and complete information. At the first clinic visit, the mean age of all women was 28.07 years (SD 3.01 years), the mean gestational age was 12.86 weeks (SD 1.85 weeks), the mean BMI was 22.14 kg/m^2 (SD 3.34 kg/m^2) and mean diastolic/systolic blood pressure was 104.94 (SD 10.51)/67.76 (SD 7.46) mmHg, the median leukocyte count was 8.29×10^9/L (P_{25}–P_{75}: 7.00×10^9/L–9.70×10^9/L). Among all women, 18.8% were overweight and 5.9% were obese. The mean gestational week of taking the GCT test was 26.55 weeks (SD 1.30 weeks), and the mean GCT result was 6.58 mmol/L (SD 1.53 mmol/L). A total of 3,853 women (18.6%) had positive GCT results and were considered at high risk of GDM.

Women with positive GCT results showed increased leukocyte counts at their first prenatal clinic visit compared with women with normal GCT results. Women with positive GCT results were also older, shorter and heavier, and had higher blood pressure and BMI. In addition, women with positive GCT results were more likely to be overweight or obese at pre-pregnancy. No statistically significant differences were found in education level, parity, smoking and ethnicity between the two groups. Detailed comparison information is shown in Table 1.

Association of leukocyte counts with GCT results

Correlation of leukocyte counts during early pregnancy was examined with GCT results. In Spearman's correlation analysis, leukocyte count was positively and significantly correlated with GCT result (Spearman's $r = 0.098$, $P < 0.001$). Linear regression analysis was carried out to confirm the association of leukocyte count with GCT results. Leukocyte count was linearly correlated with GCT glucose levels (coefficient = 0.036, $P < 0.001$). In multivariate regression analysis, the association still existed (coefficient = 0.025, $P < 0.001$; Table 2).

Quartiles of leukocyte counts for GCT results

To further examine the effect of leukocyte level in early pregnancy on GCT levels, women were divided into four groups according to quartiles of leukocyte counts based on distribution (quartile 1, $\leq 7.0 \times 10^9$/L; quartile 2, 7.01–8.29×10^9/L; quartile 3, 8.30–9.70×10^9/L; quartile 4, $>9.70 \times 10^9$/L). We found that there was a statistically significant increase in mean GCT results according to quartiles of leukocyte counts (*P* trend < 0.001; Figure 2). The rates of GCT-positive results were also elevated with the increase in quartiles of leukocyte count (Table 3).

Leukocyte counts for risks of positive GCT results

In univariate analysis, increase in leukocyte counts were associated with increased risk of positive GCT results. (OR 1.032, 95% CI 1.020–1.043; Table 4). After adjusting for maternal age, gestational age, BMI at the first prenatal clinic visit, height, systolic blood pressure at the first prenatal clinic visit, ethnicity, parity, education and weight gain from pre-pregnancy to GCT, the association still existed (adjusted OR 1.022 95% CI 1.011–1.033).

Quartiles of leukocyte counts for risk of positive GCT results

In univariate analysis, elevated leukocyte quartiles were associated with an increased rate of positive GCT results compared with quartile 1 as the reference group (*P*-value for trend < 0.001; Table 4). In multivariable analysis, after adjusting covariates, compared with the reference group, quartile 2 and quartile 3 were no longer statistically associated the positive GCT results (adjusted OR 1.104, 95% CI 0.994–1.225 and adjusted OR 1.063 95% CI 0.960–1.178), respectively; but the OR of quartile 4 was still statistically significant (adjusted OR 1.378, 95% CI 1.246–1.524; *P*-value for trend < 0.001; Table 4).

DISCUSSION

The present population-based study examined the association of leukocyte count at early pregnancy with positive GCT results between 24 and 28 gestational weeks. The findings showed that elevated leukocyte level, even within the normal range, was associated with increased risk of positive GCT results. With the increase in leukocyte counts, there was a linear increase in GCT level and risk of positive GCT results. Consistent with other previous studies, the present results showed that

Table 1 | Comparison of basic characteristics according to glucose challenge test results

	GCT ≥7.8 mmol/L	GCT <7.8 mmol/L	*P*-value
n	3,853	16,854	
Variables at first prenatal visit			
Gestational age (weeks)	10.235 ± 1.695	10.271 ± 1.642	0.232*
Age (years)	28.761 ± 3.208	28.008 ± 2.884	<0.001*
Height (cm)	162.674 ± 4.654	163.000 ± 4.697	<0.001*
Weight (kg)	61.047 ± 10.262	58.337 ± 9.414	<0.001*
BMI (kg/m^2)	23.046 ± 3.599	21.931 ± 3.239	<0.001*
BMI group (kg/m^2)	0.001**		
<18.5	267 (6.93%)	1,967 (11.67%)	
≥18.5-<24	2,243 (58.22%)	11,120 (65.98%)	
≥24-<28	988 (25.64%)	2,896 (17.18%)	
≥28	355 (9.21%)	871 (5.17%)	
Systolic blood pressure (mmHg)	106.320 ± 10.885	104.630 ± 10.398	<0.001*
Diastolic blood pressure (mmHg)	68.940 ± 7.861	67.480 ± 7.335	<0.001*
Leukocyte count (×10^9/L)	8.50 (7.50 ~ 10.01)	8.20 (6.90 ~ 9.20)	<0.001#
Parity			0.084**
0	3,710 (96.29%)	16,321 (96.84%)	
≥1	143 (3.71%)	533 (3.16%)	
Education			0.626**
≤12 years	900 (23.36%)	3,875 (22.99%)	
>12 years	2,953 (76.64%)	12,979 (77.01%)	
Smoking			0.943**
Yes	80 (2.08%)	353 (2.09%)	
No	3,773 (97.92%)	16,501 (97.91%)	
Ethnicity			0.223**
Han	3,700 (96.03%)	16,110 (95.58%)	
Others	153 (3.97%)	744 (4.42%)	
Variables at GCT			
Gestational age (weeks)	26.544 ± 1.319	26.549 ± 1.296	0.825*
Weight change (kg)	8.650 ± 3.463	8.964 ± 3.325	<0.001*
GCT result (mmol/L)	8.930 ± 1.177	6.041 ± 1.000	<0.001*

Data are reported as the mean ± standard deviation, median (P_{25}–P_{75}) or number (%). *Derived from Student's *t*-test; #derived from the Mann–Whitney *U*-test; **derived from the χ^2-test. BMI, body mass index; GCT, glucose challenge test.

Table 2 | Linear regression analysis of leukocyte counts and glucose challenge test glucose levels

Leukocyte counts	GCT glucose levels			
	B† (95% CI)	*t*	B‡	*P*-value
Univariate analysis	0.036 (0.028–0.043)	9.749	0.068	0.000
Multivariate analysis§	0.025 (0.018–0.032)	6.951	0.048	0.000

†Unstandardized coefficient. ‡Standardized coefficient. §Adjusted for maternal age, gestational age, body mass index at the first prenatal clinic visit, height, systolic blood pressure at first prenatal clinic visit, ethnicity, parity, education, weight gain from pre-pregnancy to glucose challenge test (GCT).

inflammation might play an important role in the development of hyperglycemia during pregnancy.

Although the pathophysiological mechanisms of altered glucose metabolism and insulin sensitivity in GDM are not completely understood, previous studies have proved that two main pathways – insulin resistance and chronic subclinical inflammation – are involved in GDM, as well as type 2 diabetes[14]. Hormonal changes during pregnancy result in a state of insulin resistance, and when the increased insulin secretion cannot compensate, GDM occurred as a result[22].

Evidence showed that prolonged acute and chronic subclinical inflammatory might play an important role in the pathogenesis of insulin resistance, type 2 diabetes and metabolic disorders[23]. Elevated leukocyte counts might be used as an indicator of clinical or subclinical inflammation[19]. Several studies examined the relationship between leukocyte counts and insulin resistance involved in type 2 diabetes and GDM. In a study of Pima people, high leukocyte counts predicted a worsening of insulin sensitivity and the development of type 2 diabetes after adjustment for established risk factors of diabetes[24].

In the Atherosclerosis Risk in Communities study consisting of 12,330 middle-aged adults, individuals in the highest quartile

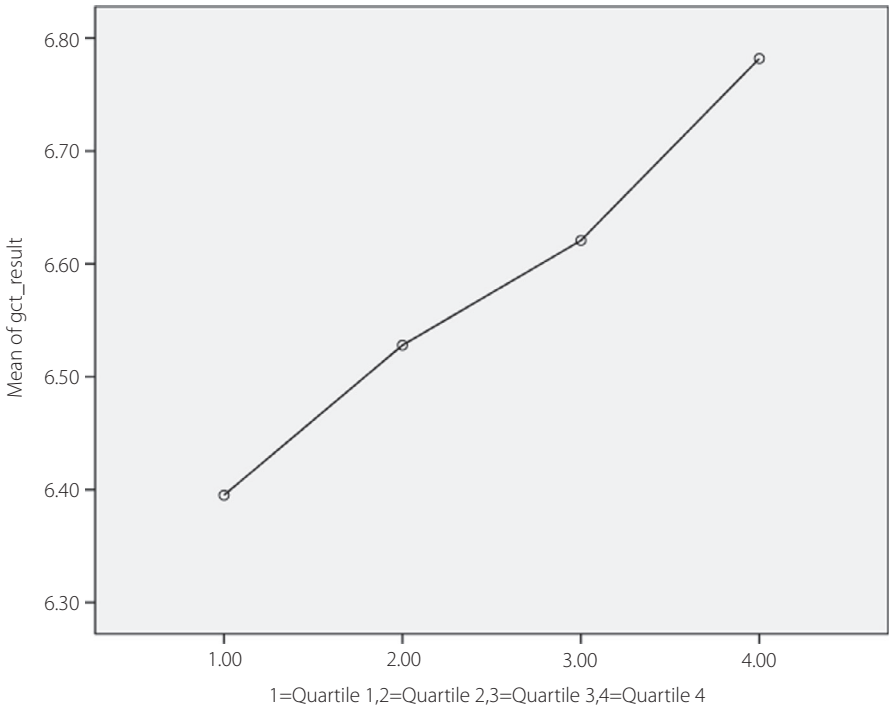

Figure 2 | Horizontal ordinate shows leukocyte count quartiles. Quartile 1 represents leukocyte counts ≤7.0 × 10^9/L; quartile 2 represented leukocyte counts 7.01–8.29 × 10^9/L; quartile 3 represented leukocyte counts 8.30–9.70 × 10^9/L and quartile 4 represented leukocyte counts >9.70 × 10^9/L. Longitudinal ordinate shows the mean glucose challenge test (GCT) result of each quartile group. The figure suggests that there was a statistically significant increase in mean GCT results according to quartiles of leukocyte counts (*P* trend < 0.01).

Table 3 | Glucose challenge test results and positive rate of glucose challenge test according to quartiles of leukocyte counts

	Quartile1 (≤7.0 × 10^9/L)	Quartile 2 (7.01–8.29 × 10^9/L)	Quartile 3 (8.30–9.70 × 10^9/L)	Quartile 4 (>9.70 × 10^9/L)	*P* for linear trend
n	5,417	4,938	5,328	5,024	
GCT results (mmol/L)†	6.40 ± 1.47	6.53 ± 1.48	6.62 ± 1.53	6.78 ± 1.61	<0.001
GCT positive rate (%)‡	15.97%	17.82%	17.98%	22.89%	<0.001

†Data are the mean ± standard deviation; ‡Glucose challenge test (GCT)-positive: ≥7.8 mmol/L.

of leukocyte counts had a higher risk of developing diabetes after follow up for 7 years[10]. After approximately 20 years' follow up of 8,352 participants from a prospective study of the National Health and Nutrition Examination Survey Epidemiologic Follow-up Study, data showed that a leukocyte count of ≥9.1 × 10^9/L was significantly associated with an increased incidence of diabetes compared with a leukocyte count of ≤5.7 × 10^9/L (OR 1.75, 95% CI 1.05–2.93), and the association was slightly stronger in women than in men, but was not statistically significant[25].

Results from the cross-sectional study of individuals without diabetes in the Prospective Metabolism and Islet Cell Evaluation (PROMISE) cohort also showed that all leukocyte subtypes were significantly associated with insulin resistance, supporting the role of inflammatory processes in the mechanisms of type 2 diabetes involving insulin resistance[26].

Data from current studies supported that leukocyte levels in early pregnancy, even within the normal range, play an important role in dysglycemia during pregnancy. In a case–control study from a Tertiary Care Hospital in India, white blood cell count was higher in the GDM group than the non-GDM control (11.95 ± 2.51 × 10^9/L vs 9.09 ± 1.90 × 10^9/L, *P* < 0.01), and was significantly associated with glycated hemoglobin after adjusting pre-pregnant BMI[27].

Sargin *et al.*[12] retrospectively examined the leukocyte, neutrophil and lymphocyte counts in 762 pregnant women. They found that compared with the control group, leukocyte counts were higher in all of the groups with abnormal blood glucose

Table 4 | Odds ratios of leukocyte counts for the risk of positive glucose challenge test results

	Reference group	β	*Sx*	Wald χ^2 value	*P*-value	OR	95% CI
Univariable analysis							
Leukocyte counts†		0.031	0.006	30.772	<0.001	1.032	1.020–1.043
Quartile 2	Quartile 1‡	0.132	0.053	6.323	0.012	1.141	1.030–1.265
Quartile 3		0.143	0.051	7.713	0.005	1.154	1.043–1.276
Quartile 4		0.446	0.050	79.481	0.000	1.562	1.416–1.723
Multivariable analysis*							
Leukocyte counts†		0.021	0.006	15.215	<0.001*	1.022	1.011–1.033
Quartile 2	Quartile 1‡	0.099	0.053	3.433	0.064	1.104	0.994–1.225
Quartile 3		0.061	0.052	1.376	0.241	1.063	0.960–1.178
Quartile 4		0.321	0.051	38.979	<0.001*	1.378	1.246–1.524

*Adjusted for maternal age, gestational age, body mass index at first prenatal clinic visit, height, systolic blood pressure at first prenatal clinic visit, ethnicity, parity, education, weight gain from pre-pregnancy to glucose challenge test. †Leukocyte counts were analyzed as continuous variable; ‡Leukocyte counts were analyzed in quartiles. CI, confidence interval; OR, odds ratio.

results, including the GDM group, impaired glucose tolerance group and only screen-positive group, and therefore asserted that leukocyte count was an important marker for GDM[12].

A retrospective cohort study[28] of 1,190 South Asian women found that increased leukocyte counts had a significant impact on GDM development, and the risk of GDM increased with the range of leucocyte level (≥9,300/μL) in early pregnancy.

Wolf *et al.*[9] prospectively examined the leukocyte counts at the first prenatal visit and the risk of GDM. Data showed that leukocyte count in early pregnancy was linearly associated with the results of GDM screening tests, and the risk of GDM was significantly increased with an elevation of leukocyte $>9.1 \times 10^3$/mL.

In the present study, we also found that an increased leukocyte count ($>9.7 \times 10^9$/L) had an impact on positive results of GCT tests after adjusting established risk factors. This evidence suggests that in clinic, elevated leukocyte counts, even within the normal range, should be given enough attention for glucose intolerance later in pregnancy.

The mechanism of elevated leukocyte counts as an independent determinant of insulin resistance, and GDM might be interpreted by the effects of pro-inflammatory cytokines on insulin sensitivity and secretion[10]. Those pro-inflammatory cytokines could enhance leukocyte differentiation and activation[28], and therefore cytokine-induced insulin resistance might play a central role in underling the relationship between increased leukocyte counts and GDM. Previous data showed that elevated pro-inflammatory markers, such as IL-6, C-reactive protein and TNF-α, mainly produced by adipose tissue, had an important impact on the progress of metabolic disorder[29]. Several observational studies provided evidence of the important role of inflammatory cytokines in insulin resistance, and they were highly related to elevated blood glucose level and decreased glucose tolerance.

In a prospective, nested case–control study[11], the results showed that the risk of future diabetes mellitus for women in the highest quartile versus lowest IL-6 and C-reactive protein were 2.3 and 4.2 respectively, asserting that elevated IL-6 and CPR predicted the development of diabetes mellitus. The elevation in IL-6 was also found to have a strong correlation with GDM, indicating a role of low-grade inflammation in the pathogenesis of GDM[30,31].

Data from women in inner Mongolia, China, showed that serum IL-6 was significantly associated with GDM (OR 4.66, 95% CI 1.53–14.59, $P = 0.008$), suggesting that abnormal levels of IL-6 might be diagnostic biomarkers[31].

Current evidence supported that TNF-α, secreted by adipose tissue as well as macrophage and other cells, played an important role in insulin resistance by several pathways[32]. Serum levels of TNF-α were more significantly increased in obese patients with non-insulin-dependent diabetes mellitus than healthy individuals ($P < 0.01$) or in non-obese patients with non-insulin-dependent diabetes mellitus ($P < 0.01$)[33]. Kirwan *et al.*[34] reported that circulating TNF-α was significantly correlated with insulin resistance during late pregnancy, indicating that the regulation of TNF-α could be used as an intervention target to reduce the risk of adverse pregnancy outcomes related to insulin resistance.

Various pathways were proposed to explain the inflammatory and insulin resistance. IL-6 causes insulin resistance by inducing the expression of suppressor of cytokine signaling 3, which inhibits insulin signaling, impairing phosphorylation of insulin receptor and insulin receptor substrate-1, and promoting polymorphism at position 174 (G > C), which is linked to insulin sensitivity[35]. TNF-α might impair insulin signaling by several separate molecular modifications: a serine phosphorylation of insulin receptor substrate-1; phosphorylation and activation of the protein tyrosine phosphatase, SH-PTPase; and phosphorylations of the protein phosphate, PP-1[32]. TNF-α and IL-6 might also alter β-cell function, impairing insulin secretion, through stimulation of free fatty acid production and low-grade tissue-specific inflammation in pancreatic islets[10,35].

The source of inflammation in pregnant women might be related with obesity, as cytokines are mainly expressed by adipose tissue[36]. However, previous research found that the association between inflammation and GDM persisted after adjusting for BMI or body fat[9,28,34]. In the present study, we also found that after adjustment for pre-pregnant BMI, the association still existed between the highest quartile of leukocyte counts and the risk of positive GCT results.

Another hypothesis is the placenta-derived cytokines. The placenta is recognized as a primary organ mediating inflammation change in pregnancy. Placenta macrophages are present in placental villous stroma across the gestational period, and are most numerous in the first and second trimesters[37]. Several reports confirmed that the changes of macrophages function, characterized by an increased expression of the pro-inflammatory cytokines, were associated with altered maternal metabolic status and pregnancy complications[38,39]. Results showed that pro-inflammatory cytokines in the placenta were significant different between GDM and healthy pregnant women[31,40–42]. An accumulation of resident macrophages, including CD14 and CD68, with increased expression of cytokines, were found in the placenta of women with GDM[43]. Therefore, it is suggested that inflammation contributes to the development of glucose intolerance independent of obesity.

A limitation of this study was that we only included leukocyte counts, but not all indicators of routine blood test in the study because of availability of complete data. Leukocyte count is not a specific marker of inflammation, which is influenced by many factors, including infection, gestational age and so on[9], and we cannot exclude all the confounding factors in the study.

However, we tried to minimize the effects of confounding by involving gestational age when taking the blood test in the multivariable analysis. As blood sampling for leukocyte counts was taken as a routine examination rather than required by a physician, pregnant women who had clinical symptoms of concurrent infection were few and excluded. Second, we used GCT instead of an oral glucose tolerance test to evaluate the blood glucose level during pregnancy, which is a screening test rather than a diagnostic method for GDM. Third, we did not have information regarding diet, family history and exercise, which have obvious effects on blood glucose level, and, therefore, might influence the research results. Fourth, a large number of individuals with incomplete information or who did not have routine prenatal examinations on time were excluded from the present study. We compared the general characteristics, and found that there was no difference in height, leukocyte results, diastolic blood pressure, ethnicity and smoking status between the study group and excluded group. However, differences were found in systolic blood pressure, BMI at first clinic visit, weight change between first clinic visit and GCT test, and GCT results between the two groups, which might be caused by delayed gestational ages when the excluded group had those examinations. There were significant differences in maternal age and educational level as well, which might confuse the relationship between leukocyte counts and risk of positive GCT results. Furthermore, considering the homogeneity of the participants, the present results need to be proven in other populations.

Despite the aforementioned limitations, the present study had several strengths. First, it was a large population-based study. Our study also reflected the time sequence between inflammation and glucose intolerance, as the blood sample for leukocyte count was taken in early pregnancy, and GCT was carried out in the second trimester. Third, information of GDM traditional risk factors was collected in detail at the first prenatal clinic visit, and adjusted in our analysis.

In conclusion, the present results showed that women with a leukocyte count of $>9.7 \times 10^9$/L in early pregnancy were at higher risk of positive GCT results after adjustment for obesity and other risk factors. This was consistent with other studies that showed chronic inflammation might play a role in the pathogenesis of insulin resistance. As leukocyte count, as a part of a complete blood count, is a routine and economical blood test in pregnancy clinics, it could be used as an early non-specific indicator for women at high risk of glucose intolerance that might develop several months later. The association between leukocyte counts and positive GCT results helped to point out that the direction of further studies would be exploring the mechanisms of inflammatory status on altered glucose tolerance, and investigating more specific biomarkers for early pregnancy diagnosis, and potential targets on inflammation mitigation for the prevention and therapy of GDM.

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DISCLOSURE

The authors declare no conflict of interest.

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