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Clinical Research Article

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Decreased Monocyte Count Is Associated With Gestational Diabetes Mellitus Development, Macrosomia, and Inflammation

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Abbreviations: 1h BG, 1-hour blood glucose after oral glucose tolerance test; 2h BG, 2-h blood glucose after oral glucose tolerance test; ALT, alanine aminotransferase; AST, aspartate transaminase; AUC, area under the curve; BMI, body mass index; CHO, cholesterol; Cr, creatinine; DBP, diastolic blood pressure; FBG, fasting blood glucose; GDM, gestational diabetes mellitus; Glucose AUC, glucose area under the curve based on oral glucose tolerance test; HbA_{1c}, glycated hemoglobin A_{1c}; HOMA-β, homeostasis model assessment of pancreatic β-cell function index; HOMA-IR, homeostasis model assessment of insulin resistance index; IL-6, interleukin 6; IL-10, interleukin 10; IR, insulin resistance; MLR, monocyte-to-lymphocyte ratio; NLR: neutrophil-to-lymphocyte ratio; OGTT, oral glucose tolerance test; OR, odds ratio; T2DM, type 2 diabetes mellitus; TGF-β1, transforming growth factor-β1; TNF-α, tumor necrosis factor-α.

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Abstract

Context: The immune system plays a central role in the pathophysiology of gestational diabetes mellitus (GDM). Monocytes, the main innate immune cells, are especially important in the maintenance of a normal pregnancy.

Objective: Here, we investigated the potential effect of monocytes in GDM.

Methods: Monocyte count was monitored throughout pregnancy in 214 women with GDM and 926 women without in a case-control and cohort study. Circulating levels

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of inflammatory cytokines, placenta-derived macrophages, and their products were measured.

Results: Throughout pregnancy, monocyte count was significantly decreased in women with GDM, and was closely associated with glucose level, insulin resistance, and newborn weight. First-trimester monocyte count outperformed that of the second and third trimester as a risk factor and diagnostic predictor of GDM and macrosomia both in the case-control and cohort study. In addition, our cohort study showed that as first-trimester monocyte count decreased, GDM and macrosomia incidence, glucose level, and newborn weight increased in a stepwise manner. Risk of GDM started to decrease rapidly when first-trimester monocyte count exceeded 0.48×10^9/L. Notably, CD206 and interleukin 10 (IL-10) were significantly lower, whereas CD80, CD86, tumor necrosis factor α (TNF-α), and interleukin 6 (IL-6) were higher both in GDM placental tissue and peripheral blood. First-trimester monocyte count was positively related to IL-10 and CD206, but negatively related to CD80, CD86, TNF-α, and IL-6.

Conclusion: Decreased monocyte count throughout pregnancy was closely associated with the development of GDM, macrosomia, and the chronic inflammatory state of GDM. First-trimester monocyte count has great potential as an early diagnostic marker of GDM.

Key Words: gestational diabetes mellitus, monocytes, macrophages, inflammation, insulin resistance

Gestational diabetes mellitus (GDM) is one of the leading complications of pregnancy, with a high prevalence (5.4%-25.1% in various countries) (1-4). It is characterized by glucose intolerance with onset or first recognition during pregnancy and associated with maternal and fetal complications. Long-term consequences include type 2 diabetes mellitus (T2DM), obesity, and cardiovascular events (5-8). Like T2DM, insulin resistance (IR) may play a vital role in the pathogenesis of GDM (9, 10). Accumulating evidence (11-19) shows that the immune system plays a vital role in IR and the development of GDM. Nonetheless, the exact effect and mechanism of immune-induced GDM remains unclear.

Monocytes, the most important innate immune cells and inflammatory effectors, can produce inflammatory cytokines (20, 21) and have been demonstrated to be associated with GDM (15, 16, 22, 23). Nonetheless, results are inconsistent and it is unclear whether such an association can be used for early prediction of GDM, or whether monocyte-derived cytokines are involved in the chronic inflammatory state of GDM.

This study aimed to investigate the potential association of monocytes with GDM and its adverse pregnancy outcomes. First, we established that monocyte count was decreased throughout pregnancy in women with GDM, and that continuously decreased monocyte level was closely associated with glucose level, IR, and newborn weight. First-trimester monocyte count outperformed that of the second and third trimester as a risk factor and diagnostic predictor of GDM and macrosomia both in the case-control and cohort study. In addition, our cohort study showed that as first-trimester monocyte count decreased, the incidence of GDM and macrosomia, glucose level, and newborn weight increased in a stepwise manner. Spline regression revealed that the risk of the developing GDM decreased rapidly when monocyte count exceeded 0.48×10^9/L. Finally, we found that decreased monocyte count was closely associated with the chronic inflammatory state of GDM.

Materials and Methods

Study Population

This retrospective study included 1418 women who underwent prenatal examination and normal delivery from January 2016 to July 2019 at the GDM care center of the Fifth People's Hospital of Shanghai, Fudan University, and the Department of Obstetrics at Wujing Hospital in Minhang District, Shanghai. The retrospective analysis is described in Fig. 1. Individuals were excluded if they had any of the following: (1) any infectious disease during the 2 weeks before blood testing; (2) abnormal liver or renal function; (3) presence of viral infection or positive carrier status (hepatitis B virus, syphilis, or HIV); (4) preexisting DM; (5) chronic hypertension; (6) multiple pregnancy; (7) malignant tumor; or (8) preexisting pancreatic exocrine disease. Finally, 1140 women (926 without GDM and 214 with GDM) were identified for study.

The criteria for GDM were those of the 2020 American Diabetes Association guidelines (24): fasting blood glucose (FBG) greater than or equal to 5.1 mmol/L and 1-hour blood glucose (1h BG) after oral glucose tolerance test (OGTT) greater than or equal to 10.0 mmol/L or 2h BG

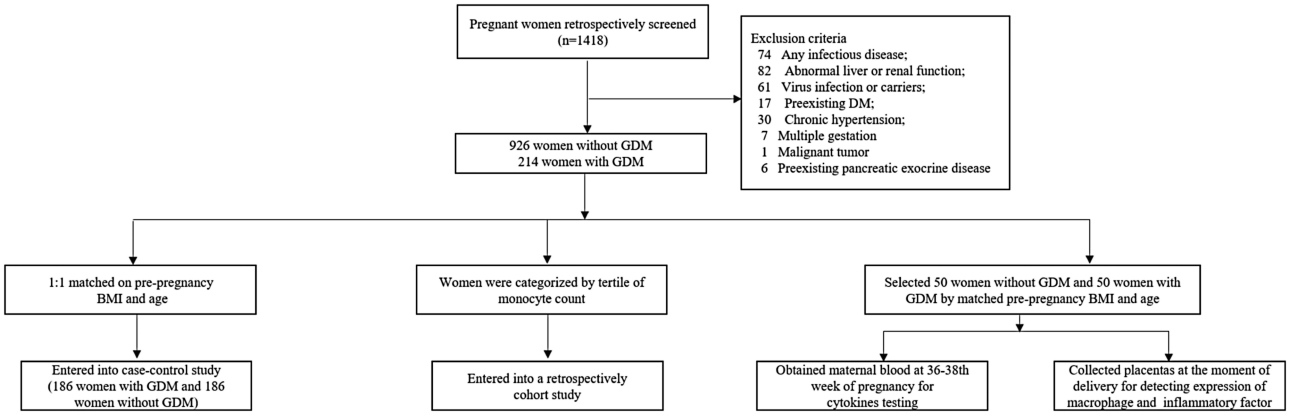

Figure 1. Flowchart of the study.

greater than or equal to 8.5mmol/L. The study protocol was approved by the ethics committee of the Shanghai Fifth People's Hospital, Fudan University (No. 2016081) and all participants gave written informed consent. The study was conducted in accordance with the Declaration of Helsinki.

Data Collection and Laboratory Determinations

At the first visit, gestational age was calculated based on the last menstrual period and confirmed by ultrasound. Blood pressure and anthropometric parameters were measured at the same time. A questionnaire was used to record clinical data of participants and included age, information about last menstruation, method of conception, parity, previous history of GDM, prepregnancy height, and prepregnancy weight. A 75-g OGTT was administered between the 24th and 28th week of gestation after an overnight fast of at least 8 hours to all participants who had no overt diabetes or GDM in early pregnancy.

After an overnight fast of 12 hours, blood samples were collected for measurement of blood cell count (Automatic Blood Cell Analyzer, Sysmex XN9000) and biochemical parameters (Automatic Biochemical Analyzer, Roche Cobas 8000).

Calculation of Body Mass Index, Homeostasis Model Assessment of Insulin Resistance Index, Homeostasis Model Assessment of Pancreatic β-Cell Function Index, and Glucose Area Under the Curve

Body mass index (BMI) was calculated as weight (in kilograms) divided by the square of height (in meters). Primary methods to evaluate insulin resistance and pancreatic β-cell function were as follows: (1) homeostasis model assessment of insulin resistance index (HOMA-IR) = FBG (mmol/L) × fasting insulin (mU/L)/22.5; (2) glucose area under the curve (glucose AUC) based on OGTT = FBG (mmol/L)/2 + 1h BG (mmol/L) + 2h BG (mmol/L)/2; and (3) homeostasis model assessment of pancreatic β-cell function index (HOMA-β) = 20 × fasting insulin (mU/L)/[FBG (mmol/L) – 3.5].

Intervention for Gestational Diabetes Mellitus

Lifestyle modification was initiated first in women diagnosed with GDM. If the goals of glycemic control were not achieved after 1 week (FBG < 5.1 mmol/L combined with 1h BG < 7.8 mmol/L and 2h BG < 6.7 mmol/L), insulin therapy was supplemented.

Immunohistochemistry and Cytokine Testing

Maternal procoagulant serum (4 mL) was collected in the morning during the 36th to 38th week of pregnancy and before the beginning of labor from 50 women with GDM and 50 women without GDM to evaluate changes in the level of inflammatory cytokines. Serum level of interleukin 10 (IL-10) and tumor necrosis factor-α (TNF-α) was measured by chemiluminescent immunoassay, while that of transforming growth factor-β1 (TGF-β1) and IL-6 were measured by immunoenzymatic assay and electrochemiluminescence immunoassay, respectively, according to the manufacturers' protocols. GDM and controls for cytokine testing were matched for prepregnancy BMI and age.

The placenta was obtained at delivery from 50 GDM patients and 50 non-GDM patients matched for prepregnancy BMI and age. Placentas were immediately washed with a saline solution and cut into 4-μm sections using a conventional formalin-fixed, paraffin-embedded method. Sections were then dewaxed, boiled for 20 minutes in citrate buffer (10 mM, pH 6.0) to retrieve antigens, and subjected to immunohistochemical staining for CD163 (catalog No. 16646-1-AP; antibody

dilution 1:1000; Proteintech), CD206 (catalog No. 60143-1-lg; antibody dilution 1:500; Proteintech), CD80 (catalog No. ab254579; antibody dilution 1:500; Abcam), CD86 (catalog No. ab269587; antibody dilution 1:100; Abcam), IL-10 (catalog No. 60269-1-lg; antibody dilution 1:200; Proteintech), TGF-β1 (catalog No. 21898-1-Ap; antibody dilution 1:100; Proteintech), IL-6 (catalog No. ab6672; antibody dilution 1:400; Abcam), and TNF-α (catalog No. 60291-1-lg; antibody dilution 1:500; Proteintech) according to the manufacturers' protocol. In brief, primary antibodies were incubated overnight at 4 °C followed by the addition of horseradish peroxidase–labeled antirabbit or antimouse antibodies for 50 minutes (Dako) and visualized using diaminobenzidine tetrachloride (Dako). The sections were then photographed using a computer-assisted video-imaging system (NIKON DP controller).

A total of 5 random fields of each stained specimen (200×) were used to calculate the product of immunoreactivity intensity and the proportion of macrophages staining positive, and the mean integrated optical density of inflammatory cytokines using Image-Pro Plus 6.0 software (Media Cybernetics Inc) calculated. Immunoreactivity intensity was stratified into 4 categories: 0, no immunoreactivity; 1, canary yellow immunoreactivity; 2, pale brown immunoreactivity; and 3, tan immunoreactivity. The proportion of positive cells was classified into 5 groups: 0, 0% to 5%; 1, 6% to 25%; 2, 26% to 50%; 3, 51% to 75%; and 4, greater than 75%. All slides were evaluated independently by 2 investigators blinded to patient identity and clinical outcomes.

Statistical Analysis

To eliminate potential bias due to uneven distribution of covariates between women with or without GDM, a case-control matching method was applied to match variables, including prepregnancy BMI and age. Matching tolerance was 0.5 and 2, respectively. To compare the predictability of GDM and macrosomia for monocyte count at different gestational stages, logistic regression analysis was performed in the case-control study.

To further validate the association of monocyte count with GDM and pregnancy outcomes, a cohort that included the same individuals as the case-control study was established in which patients were divided into 3 groups based on tertile of first-trimester monocyte count: the highest group ($> 0.55 \times 10^9$/L), middle group (0.42 to 0.55×10^9/L), and lowest group ($< 0.42 \times 10^9$/L).

Data are expressed as mean ± SD or as a number (percentage). Data with a nonnormal distribution were analyzed following logarithmic transformation. Continuous variables were compared using independent sample *t* test and one-way analysis of variance test followed by least significant difference. Categorical variables were compared using the chi-square test. Spearman rank correlation was used to evaluate the relationship between inflammatory blood cell parameters and metabolic indexes, monocyte count, and inflammatory cytokines. Monocyte association with HOMA-IR, glucose AUC, and newborn weight was assessed by simple and multivariable linear regression analysis. To demonstrate whether monocyte count was an independent risk factor of GDM, a binary logistic regression analysis, spline regression analysis, and receiver operating characteristic curves were performed in the cohort study. All data were analyzed using SPSS 23.0 (IBM SPSS Inc) and R software (version R 4.0.1). A 2-tailed *P* value of less than .05 was considered statistically significance.

Results

Characteristics of Women With and Without Gestational Diabetes Mellitus in all Participants and Matched Case-control Study

Among all 1140 participants, 214 women (18.77%) who had older age, previous GDM history and higher level of prepregnancy BMI developed GDM ($P < .001$, Table 1). Those with GDM had a much lower first-trimester monocyte count (0.46 ± 0.13 vs $0.52 \pm 0.20 \times 10^9$/L, $P < .001$, Fig. 2A) and monocyte-to-lymphocyte ratio (MLR) (0.26 ± 0.08 vs $0.29 \pm 0.11 \times 10^9$/L, $P = .003$), and much higher first-trimester white blood cell count ($P = .002$), neutrophil count ($P < .001$), and neutrophil-to-lymphocyte ratio (NLR) ($P < .001$) than women without GDM. Similarly, whether in the second trimester or third trimester, women with GDM consistently had a much lower monocyte count (0.60 ± 0.17 vs $0.66 \pm 0.17 \times 10^9$/L, $P = .002$; 0.64 ± 0.23 vs $0.69 \pm 0.21 \times 10^9$/L, $P = .035$, respectively; see Fig. 2A) than those without although there were no significant differences in white blood cells, neutrophils, MLR, or NLR. In addition, women with GDM had a markedly higher second trimester FBG, 1h BG, 2h BG, glycated hemoglobin A_{1c} (HbA_{1c}), Ln (fasting insulin), glucose AUC, Ln (HOMA-IR) (all $P < .001$), triglycerides ($P = .014$), high-density lipoprotein cholesterol ($P = .019$), and weight gain before GDM screening ($P = .002$), and lower Ln (HOMA-β) ($P < .001$). We identified only 22 women with GDM (10.28%) who required insulin treatment. Most (89.72%) could be managed with lifestyle intervention. Expectedly, women with GDM had a heavier newborn (3501.53 ± 433.05 vs 3363.24 ± 439.48 g, $P = .002$) and a higher rate of newborn Apgar score of less than 8 (2.14% vs 0.22%, $P = .002$) than women without GDM (see Table 1).

Table 1. Characteristics of women with and without gestational diabetes mellitus in all participants and matched case-control study

	All participants			Matched case-control		
	Women without GDM	Women with GDM	*P*	Women without GDM	Women with GDM	*P*
No.	926	214		186	186	
Anthropometric parameters						
Age, y	26.84 ± 4.81	29.63 ± 4.98	< .001	28.90 ± 4.57	29.26 ± 4.65	.459
Parity						
Nulliparous	257 (27.75)	70 (32.71)	.149	32 (17.20)	63 (33.87)	< .001
Parous	669 (72.25)	144 (67.29)		154 (82.80)	123 (66.13)	
Previous GDM						
No	666 (71.92)	121 (56.54)	< .001	153 (82.30)	100 (65.36)	< .001
Yes	3 (0.32)	23 (10.75)		1 (0.50)	23 (12.37)	
Nulliparous	257 (27.76)	70 (32.71)		32 (17.20)	63 (33.87)	
Pregnancy BMI	22.4 ± 3.2	24.0 ± 4.2	< .001	23.59 ± 3.26	23.28 ± 3.37	.358
First trimester						
SBP, mm Hg	115.74 ± 9.63	115.34 ± 14.42	.746	115.71 ± 9.79	114.98 ± 14.81	.739
DBP, mm Hg	68.51 ± 7.51	69.91 ± 7.60	.090	68.14 ± 7.11	69.57 ± 7.57	.248
WBC, × 10^9/L	8.66 ± 2.01	9.16 ± 2.12	.002	8.47 ±1.86	9.2 ± 2.15	.001
Neutrophils, × 10^9/L	6.12 ± 1.61	6.77 ± 1.74	< .001	6.03 ± 1.59	6.79 ± 1.89	< .001
Lymphocytes, × 10^9/L	1.88 ± 0.51	1.83 ± 0.46	.194	1.83 ± 0.5	1.83 ± 0.47	.913
MLR	0.29 ± 0.11	0.26 ± 0.08	.003	0.29 ± 0.11	0.26 ± 0.08	.007
NLR	3.47 ± 1.44	3.87 ± 1.28	< .001	3.51 ± 1.23	3.87 ± 1.27	.005
Platelets, × 10^9/L	216.44 ± 53.26	219.46 ± 50.27	.457	216.29 ± 55.76	220.44 ± 49.52	.450
Second trimester						
WBC, × 10^9/L	9.60 ± 2.34	9.46 ± 2.04	.697	9.93 ± 2.32	9.49 ± 2.03	.160
Neutrophils, × 10^9/L	6.93 ± 1.89	6.88 ± 1.67	.812	7.21 ± 2.01	6.89 ± 1.65	.226
Lymphocytes, × 10^9/L	1.93 ± 0.51	1.82 ± 0.49	.083	1.94 ± 0.5	1.84 ± 0.5	.180
MLR	0.36 ± 0.11	0.35 ± 0.11	.446	0.37 ± 0.1	0.35 ± 0.11	.110
NLR	3.79 ± 1.40	4.09 ± 1.93	.087	3.89 ± 1.38	4.08 ± 1.95	.417
Platelets, × 10^9/L	216.18 ± 56.46	211.26 ± 52.49	.455	215.2 ± 62.35	211.05 ± 52.57	.618
FBG, mmol/L	3.82 ± 0.45	4.74 ± 0.96	< .001	3.84 ± 0.47	4.69 ± 0.95	< .001
1h BG, mmol/L	6.53 ± 1.33	9.66 ± 1.99	< .001	6.9 ± 1.37	9.67 ± 2.04	< .001
2h BG, mmol/L	5.96 ± 1.06	8.40 ± 1.68	< .001	6.24 ± 1.06	8.41 ± 1.68	< .001
HbA_{1c}, %	4.93 ± 0.35	5.30 ± 0.50	< .001	4.93 ± 0.31	5.3 ± 0.51	< .001
HbA_{1c}, mmol/mmol/L	30	34		30	34	
Ln, fasting insulin	4.12 ± 0.46	4.42 ± 0.46	< .001	4.18 ± 0.48	4.42 ± 0.45	.004
Glucose AUC	11.46 ± 1.71	16.01 ± 2.61	< .001	12 ± 1.72	16.08 ± 2.7	< .001
Ln (HOMA-IR)	0.38 ± 0.50	0.88 ± 0.48	< .001	0.46 ± 0.51	0.87 ± 0.48	< .001
Ln, HOMA-β	6.34 ± 0.97	5.71 ± 1.25	< .001	6.28 ± 1.15	5.77 ± 1.25	.027
ALT, U/L	13.72 ± 7.51	14.97 ± 8.61	.066	14.23 ± 8.6	15.1 ± 8.66	.418
AST, U/L	17.08 ± 6.78	17.99 ± 6.19	.130	18.24 ± 6.86	18.2 ± 6.29	.955
Cr, mmol/L	42.31 ± 7.12	43.05 ± 8.60	.231	42.06 ± 6.72	42.56 ± 8.35	.574
TGs, mmol/L	2.63 ± 1.19	2.91 ± 0.97	.014	2.86 ± 1.18	2.96 ± 0.99	.477
CHO, mmol/L	5.41 ± 1.12	5.49 ± 1.27	.428	5.38 ± 1.09	5.48 ± 1.28	.495
HDL-C, mmol/L	1.61 ± 0.37	1.70 ± 0.43	.019	1.58 ± 0.33	1.67 ± 0.41	.050
LDL-C, mmol/L	3.09 ± 0.88	3.01 ± 1.00	.383	3.02 ± 0.83	3.01 ± 1.02	.948
Third trimester						
WBC (× 10^9/L)	10.14± 3.11	9.83 ± 2.79	.367	10.2 ± 3.47	9.86 ± 2.8	.426
Neutrophils, × 10^9/L	7.68 ± 2.90	7.44 ± 2.62	.448	7.78 ± 3.24	7.45 ± 2.63	.420
Lymphocyte, × 10^9/L	1.69 ± 0.53	1.62 ± 0.59	.226	1.65 ± 0.52	1.62 ± 0.59	.742
MLR	0.43 ± 0.16	0.42 ± 0.15	.435	0.44 ± 0.14	0.41 ± 0.14	.161
NLR	5.04 ± 3.22	5.30 ± 3.21	.470	5.12 ± 2.77	5.32 ± 3.24	.618
Platelets, × 10^9/L	211.54 ± 59.78	203.82 ± 62.67	.251	204.25 ± 60.92	204.03 ± 62.98	.979
Weight gain, kg						
Before GDM diagnosed	5.58 ± 3.18	7.05 ± 2.56	.002	6.93 ± 2.42	7.14 ± 2.57	.833

Table 1. Continued

	All participants			Matched case-control		
	Women without GDM	Women with GDM	*P*	Women without GDM	Women with GDM	*P*
Whole pregnancy	13.56 ± 4.96	13.18 ± 5.41	.412	12.97 ± 4.78	13.12 ± 5.39	.787
Treatment						
Lifestyle intervention	NA	192 (89.72)	NA	NA	166 (89.25)	NA
Insulin	NA	22 (10.28)	NA	NA	20 (10.75)	NA
Pregnancy outcome						
Delivery time, wk	39.18 ± 1.52	38.48 ± 2.04	.124	39 ± 2.12	38.49 ± 2.09	.602
Fetus sex						
Male	512 (55.29)	74 (64.35)	.065	124 (66.67)	66 (62.26)	.448
Female	414 (43.71)	41 (35.65)		62 (33.33)	40 (37.74)	
Birth length, cm	49.92 ± 0.69	49.82 ± 1.93	.430	49.98 ± 0.82	49.76 ± 2.02	.461
Newborn weight, g	3363.24 ± 439.48	3501.53 ± 433.05	< .001	3360.17 ± 460.07	3501.36 ± 423.75	.007
Macrosomia						
No	829 (93.04)	124 (86.11)	.004	164 (93.18)	109 (87.20)	.078
Yes	62 (6.96)	20 (13.88)		12 (6.82)	16 (12.80)	
Newborn Apgar score < 8						
No	924 (99.78)	137 (97.86)	.002	184 (98.92)	126 (97.67)	.383
Yes	2 (0.22)	3 (2.14)		2 (1.08)	3 (2.33)	

Data are mean ± SD or No. (%). Continuous variables were compared using independent *t* tests. Categorical variables were compared using the chi-square test.
Abbreviations: 1h BG, 1-hour blood glucose after oral glucose tolerance test; 2h BG, 2-hour blood glucose after oral glucose tolerance test; ALT, alanine aminotransferase; AST, aspartate transaminase; BMI, body mass index; CHO, cholesterol; Cr, creatinine; DBP, diastolic blood pressure; FBG, fasting blood glucose; GDM, gestational diabetes mellitus; Glucose AUC, glucose area under the curve based on oral glucose tolerance test; HbA_{1c}, glycated hemoglobin A_{1c}; HDL-C, high-density lipoprotein cholesterol; HOMA-β, homeostasis model assessment of pancreatic β-cell function index; HOMA-IR, homeostasis model assessment of insulin resistance index; LDL-C, low-density lipoprotein cholesterol; LSD, least significant difference; MLR, monocyte-to-lymphocyte ratio; NA, not available; NLR, neutrophil-to-lymphocyte ratio; SBP, systolic blood pressure; TG, triglycerides; WBC, white blood cell.

A 1:1 case-control matching procedure was performed to eliminate the potential bias of covariates that were unevenly distributed between women with and without GDM. After matching for age and pregnancy BMI, there remained a significantly higher glucose level ($P < .001$), glucose AUC ($P < .001$), Ln (HOMA-IR) ($P < .001$), newborn weight ($P = .007$), and lower HOMA-β ($P = .027$) among women with GDM compared with controls (see Table 1). Further, monocyte count throughout pregnancy, during the first (0.46 ± 0.12 vs $0.51 \pm 0.22 \times 10^9$/L, $P = .004$), second (0.60 ± 0.17 vs $0.69 \pm 0.16 \times 10^9$/L, $P < .001$), and third trimester (0.61 ± 0.19 vs $0.68 \pm 0.20 \times 10^9$/L, $P = .007$) remained significantly decreased in women with GDM compared with those without (Fig. 2B).

Decreased Monocyte Count Throughout Pregnancy Was Closely Associated With Glucose, and Lower First-Trimester Monocyte count Outperformed That of the Second and Third Trimester as an Independent Risk Factor and Diagnostic Predictor for Gestational Diabetes Mellitus Development and Incidence of Macrosomia

To further confirm the relationship between inflammatory blood cell parameters and metabolic indexes, Spearman rank correlation was performed in all participants (Supplementary Table 1) (25). Throughout pregnancy, during the first ($r = –0.118$, $P < .001$; $r = –0.152$, $P < .001$, respectively), second ($r = –0.155$, $P < .001$; $r = –0.095$, $P = .018$, respectively), and third trimester ($r = –0.099$, $P = .006$; $r = –0.102$, $P = .007$, respectively) monocyte count was negatively correlated with FBG and glucose AUC. Only first-trimester monocyte count ($r = –0.094$, $P = .006$; $r = –0.128$, $P = .013$, respectively) was negatively corelated with 1h BG and HOMA-IR, and first ($r = –0.105$, $P = .003$) and second trimester ($r = –0.077$, $P = .040$) monocyte counts both were negatively correlated with 2h BG. We also demonstrated a negative relationship between monocyte count throughout pregnancy ($r = –0.089$, $P = .010$ for first trimester; $r = –0.089$, $P = .024$ for second trimester; $r = –0.082$, $P = .021$ for third trimester) and newborn weight.

To compare the predictive capability of monocyte count during different gestational stages as a risk factor for GDM development, logistic regression analysis with enter selection was performed separately in a matched case-control study. First-trimester monocyte count had the highest odds ratio (OR) as an independent risk factor for the development of GDM (OR = 2.58; 95% CI, 1.45-4.60 in the lowest tertile vs the highest tertile, $P = .001$ in all mothers), regardless of GDM history (OR = 2.49; 95%

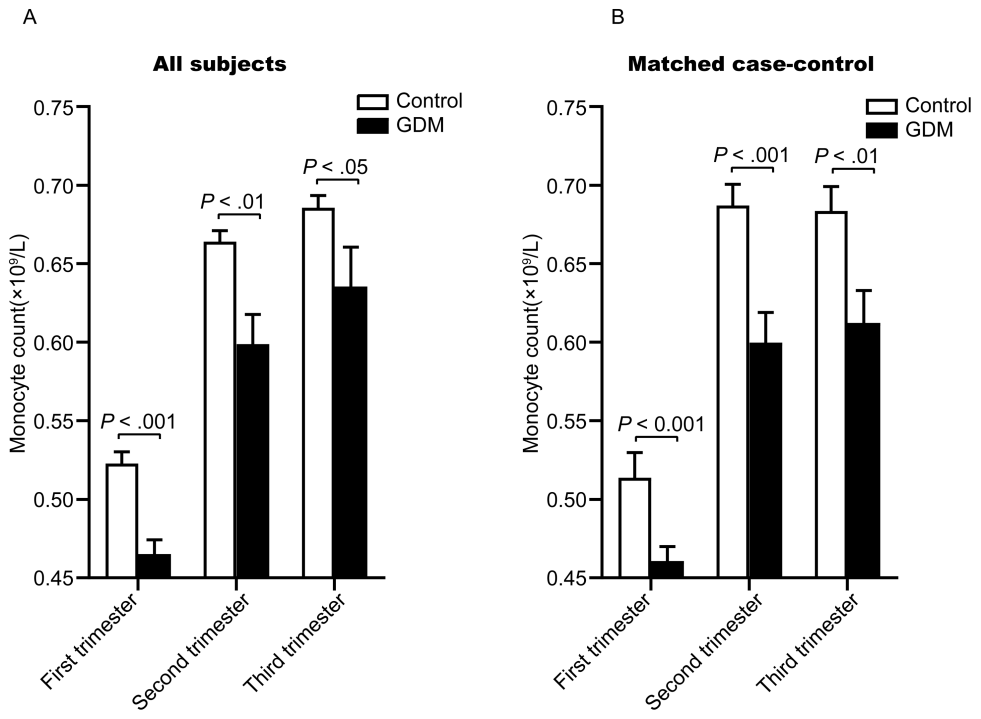

Figure 2. Difference of monocyte count of throughout pregnancy between women with and without gestational diabetes mellitus (GDM) in A, all participants, and B, a matched case-control study. Data are mean ± SE and were analyzed by independent-sample *t* test.

CI, 1.39-4.43; $P = .002$ in mothers without GDM history) compared with second-trimester monocyte count (OR = 2.21; 95% CI, 1.02-4.81, $P = .045$ in all mothers; OR = 2.22; 95% CI, 1.01-4.90, $P = .048$ in mothers without GDM history) (Supplementary Table 2) (25). First-trimester monocyte count (OR = 10.81; 95% CI, 1.18-99.06 in the lowest tertile vs the highest tertile, $P = .035$) also outperformed that of the second (OR = 9.47; 95% CI, 1.05-85.47, $P = .045$) and third trimester (OR = 5.82; 95% CI, 1.11-30.59, $P = .038$) as an independent risk factor for the incidence of macrosomia adjusted by age, prepregnancy BMI, weight gain during the whole pregnancy, and HbA1c (Supplementary Table 3) (25).

Comparison of Parameters in the First and Second Trimester, and Delivery Among 3 Groups Categorized by Tertile of Monocyte Count in the Cohort Study

Participants were divided into 3 groups based on tertile of monocyte count in the first trimester: the highest group ($> 0.55 \times 10^9$/L), middle group (0.42 to 0.55×10^9/L), and lowest group ($< 0.42 \times 10^9$/L). As shown in Table 2, there was a stepwise increase in the incidence of GDM (15.41%, 24.48%, 28.74%, $P < .001$), as well as age, level of FBG ($P < .001$),1h BG ($P = .007$), 2h BG ($P = .012$), glucose AUC ($P < .001$), and HOMA-IR ($P = .040$) across the highest, middle, and lowest groups, respectively (see Table 2). Likewise, newborn weight and incidence of macrosomia increased as monocyte count decreased. (Supplementary Table 4) (25).

First-Trimester Monocyte Count Was Closely Associated With Homeostasis Model Assessment of Insulin Resistance Index, Glucose Area Under the Curve, and Newborn Weight in the Cohort Study

Simple and multiple correlation analysis was performed to explore the relationship between monocyte count and insulin resistance and newborn weight. There was a negative linear correlation for first-trimester monocyte count with Ln (HOMA-IR) ($\beta = -0.127$; F (1, 376) = 6.083; adjusted $R^2 = 0.013$; $P = .014$), glucose AUC ($\beta = -0.145$; F (1, 794) = 17.037; adjusted $R^2 = 0.021$; $P < .001$), and newborn weight ($\beta = -0.121$; F (1, 846) = 12.490; adjusted $R^2 = 0.013$; $P < .001$) according to simple linear regression analysis. Multiple linear regression analysis adjusted for confounding factors showed that monocyte count maintained a negative linear correlation with Ln (HOMA-IR) ($\beta = -0.132$; F (4, 375) = 22.278; adjusted $R^2 = 0.185$; $P = .006$), glucose AUC ($\beta = -0.087$; F (4, 776) = 36.584; adjusted $R^2 = 0.155$; $P = .01$), and newborn weight ($\beta = -0.095$; F (5, 795) = 17.274; adjusted $R^2 = 0.093$; $P = .006$) (Supplementary Table 5) (25).

Table 2. Comparison of parameters in the first trimester and the second trimester among 3 groups categorized by tertile of monocyte count in the cohort study[c]

	Highest group	Middle group	Lowest group	*P*
Monocyte range, × 10^9/L	> 0.55	0.42 to 0.55	< 0.42	
No.	305	290	334	
Anthropometric and first-trimester parameters				
Women with GDM, No. (%)	47 (15.41)	71 (24.48)	96 (28.74)	< .001
Age, y	26.21 ± 4.87[a,b]	27.57 ± 4.66	28.10 ± 4.93	< .001
Prepregnancy BMI	22.42 ± 3.09	22.55 ± 3.53	22.61 ± 3.48	.787
First trimester				
SBP, mm Hg	116.6 ± 10.44	115.42 ± 10.14	114.74 ± 13.15	.449
DBP, mm Hg	68.60 ± 7.38	69.23 ± 6.97	68.64 ± 8.12	.779
Second trimester				
OGTT				
FBG, mmol/L	3.88 ± 0.60[a,b]	4.04 ± 0.75	4.12 ± 0.81	< .001
1h BG, mmol/L	7.04 ± 1.82[c]	7.18 ± 2.13[d]	7.54 ± 2.09	.007
2h BG, mmol/L	6.37 ± 1.42[c]	6.57 ± 1.71	6.78 ± 1.72	.012
HbA_{1c}, %	5.01 ± 0.37	4.98 ± 0.48	5.01 ± 0.41	.756
HbA_{1c}, mmol/mmol/L	31	31	31	
Ln, fasting insulin	4.14 ± 0.39	4.15 ± 0.58	4.25 ± 0.42	.186
Glucose AUC	12.00 ± 2.24[a,b]	12.49 ± 3.00[d]	13.11 ± 2.96	< .001
Ln, HOMA-IR	0.41 ± 0.43[c]	0.44 ± 0.62	0.58 ± 0.48	.040
Ln, HOMA-β	6.28 ± 0.98	6.17 ± 1.21	6.05 ± 0.92	.321
ALT, U/L	15.42 ± 9.64	13.88 ± 6.90	13.99 ± 7.41	.121
AST, U/L	18.55 ± 6.28	17.91 ± 6.29	18.99 ± 7.60	.253
Cr, mmol/L	42.39 ± 7.01	41.75 ± 7.03	43.00 ± 8.55	.197
TG, mmol/L	2.84 ± 1.19	2.78 ± 1.37	2.88 ± 1.51	.765
CHO, mmol/L	5.48 ± 1.21	5.43 ± 1.08	5.50 ± 1.17	.795
HDL-C, mmol/L	1.55 ± 0.37[b]	1.66 ± 0.39	1.62 ± 0.37	.013
LDL-C, mmol/L	3.16 ± 0.96	3.05 ± 0.79	3.10 ± 0.90	.475
Weight gain, kg				
Before GDM diagnosis	6.77 ± 2.81	6.62 ± 2.38	7.48 ± 2.68	.378
Whole pregnancy	12.89 ± 4.93	13.47 ± 5.19	13.63 ± 4.56	.159
Treatment for GDM				
Lifestyle intervention	42 (21.9)	60 (31.2)	90 (46.9)	.150
Insulin	5 (22.7)	11 (50.0)	6 (27.3)	

Data are mean ± SD or No. (%). Continuous variables were compared using one-way analysis of variance followed by LSD. Categorical variables were compared using chi-square tests.

Abbreviations: 1h BG, 1-hour blood glucose after oral glucose tolerance test; 2h BG, 2-hour blood glucose after oral glucose tolerance test; ALT, alanine aminotransferase; AST, aspartate transaminase; BMI, body mass index; CHO, cholesterol; Cr, creatinine; DBP, diastolic blood pressure; FBG, fasting blood glucose; GDM, gestational diabetes mellitus; Glucose AUC, glucose area under the curve based on oral glucose tolerance test; HbA_{1c}, glycated hemoglobin A_{1c}; HDL-C, high-density lipoprotein cholesterol; HOMA-β, homeostasis model assessment of pancreatic β-cell function index; HOMA-IR, homeostasis model assessment of insulin resistance index; LDL-C, low-density lipoprotein cholesterol; LSD, least significant difference; MLR, monocyte-to-lymphocyte ratio; NA, not available; NLR, neutrophil-to-lymphocyte ratio; OGTT, oral glucose tolerance test; SBP, systolic blood pressure; TG, triglycerides; WBC, white blood cell.

[a]Highest group vs lowest group, *P* less than .001.

[b]Highest group vs middle group, *P* less than .05.

[c]Highest group vs lowest group, *P* less than .05.

[d]Middle group vs lowest group, *P* less than .05.

Decreased First-Trimester Monocyte Count Was an Independent Risk Factor for Development of Gestational Diabetes Mellitus in the Cohort Study

To identify independent risk factors for the development of GDM, binary logistic regression analysis was performed. Model 1 was unadjusted for confounding factors and showed that the risk for development of GDM in women with first-trimester lowest or middle tertile monocyte count was 2.12 (OR = 2.12; 95% CI, 1.42-3.16, *P* < .001) or 1.64 times (OR = 1.64; 95% CI, 1.08-2.51, *P* = .021) that of women with the highest tertile monocyte count, respectively. After adjusting for potential confounding factors, lower first-trimester monocyte count remained an independent risk factor for development of

GDM (OR = 2.06; 95% CI, 1.31-3.22, in the lowest tertile vs highest tertile, $P = .002$) (Supplementary Table 6) (25). Analysis of independent risk factors in women without a history of GDM (including those with no previous GDM and those who were nulliparous) revealed that monocyte count (OR = 2.17; 95% CI, 1.42-3.31, in the lowest tertile vs the highest tertile for unadjusted confounding factors, $P < .001$; OR = 1.85; 95% CI, 1.20-2.88, in the lowest tertile vs highest tertile for adjusted prepregnancy BMI and age, $P = .006$) remained a risk factor for development of GDM, regardless of adjustment of confounding factors.

More notably, we used restricted cubic splines adjusting for GDM history, prepregnancy BMI, and age to flexibly model and visualize the association of first-trimester monocyte count with development of GDM. Risk of developing GDM was relatively flat until monocyte count was around 0.48×10^9/L but decreased rapidly thereafter ($P = .02$; Supplementary Fig. 1) (26). In addition, first-trimester monocyte count combined with basal factors (age, previous GDM history, prepregnancy BMI) had a high area under the receiver operating characteristic curve for prediction of GDM (AUC = 0.763; 95% CI, 0.724-0.802; $P < .001$; see Supplementary Fig. 1) (26).

Decreased Monocyte Count Was Closely Associated With the Chronic Inflammatory State of Gestational Diabetes Mellitus

To determine why a decreased monocyte count induced development of GDM, we first analyzed the expression difference of inflammatory cytokines in peripheral blood of women with and without GDM. As shown in Fig. 3, the level of IL-10 ($P < .01$, Fig. 3) was significantly lower in women with GDM than without, whereas that of TNF-α ($P < .001$; see Fig. 3) and IL-6 ($P < .001$; see Fig. 3) were higher. Furthermore, first-trimester monocyte count in peripheral blood was positively correlated with level of IL-10 ($r = 0.349$, $P = .013$) but negatively correlated with level of TNF-α ($r = –0.333$, $P = .018$) and IL-6 ($r = –0.445$, $P = .001$) in women with GDM (see Fig. 3).

It is well known that monocytes migrate into tissues to become macrophages. We detected the expression difference of macrophages and their products in the placenta of women with and without GDM by immunohistochemistry. Expression of CD206, as markers of M2 macrophages, and IL-10 was significantly lower in women with GDM than without ($P < .001$; Fig. 4 and Supplementary Fig. 2) (27). In contrast, women with GDM exhibited significantly higher expression of CD80 and CD86, which represented M1 macrophages in the placenta ($P < .001$; see Fig. 4 and Supplementary Fig. 2) (27), than women without GDM. Both TNF-α and IL-6, produced by M1 macrophages, were increased in the placenta of women with GDM compared with those without ($P < .001$; see Fig. 4 and Supplementary Fig. 2) (27). In addition, first-trimester monocyte count was positively correlated with expression of CD206 ($r = 0.315$, $P = .026$) and IL-10 ($r = 0.317$, $P = .025$) in the placental tissue of women with GDM but negatively correlated with that of CD80 ($r = –0.314$, $P = .026$), CD86 ($r = –0.302$, $P = .033$), TNF-α ($r = –0.326$, $P = .021$), and IL-6 ($r = –0.294$, $P = .038$) (see Fig. 4).

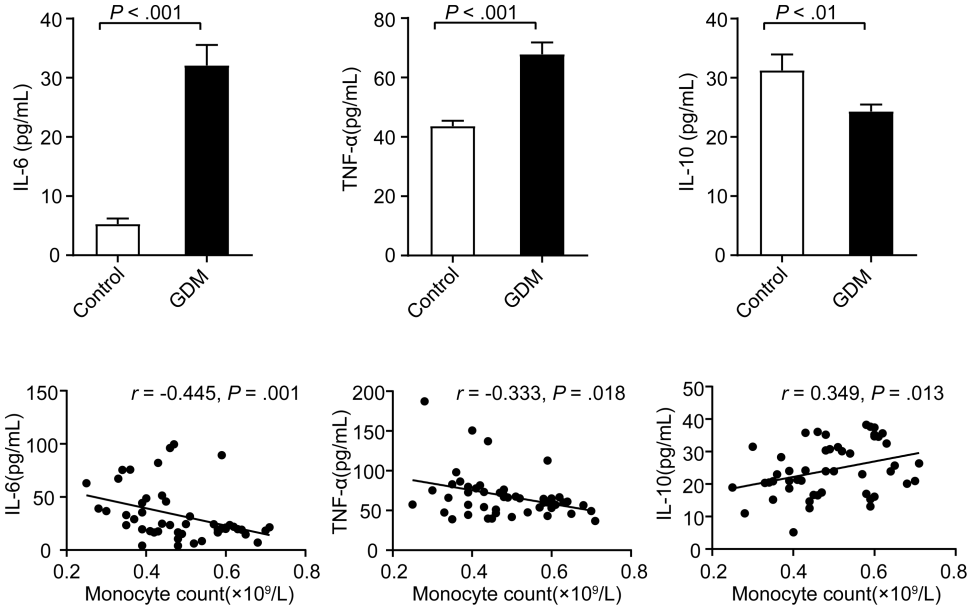

Figure 3. Circulating level of peripheral blood interleukin 10 (IL-10), tumor necrosis factor-α (TNF-α), and interleukin 6 (IL-6) in gestational diabetes mellitus (GDM; n = 50) and controls (n = 50), and relationship between first-trimester monocyte count and peripheral blood inflammatory cytokines in women with GDM (n = 50). White bars = control, black bars = GDM; black dots = first-trimester monocyte count and peripheral blood inflammatory cytokines in women with GDM. Data are mean ± SE and were analyzed by independent-sample *t* test and Spearman rank correlation analysis.

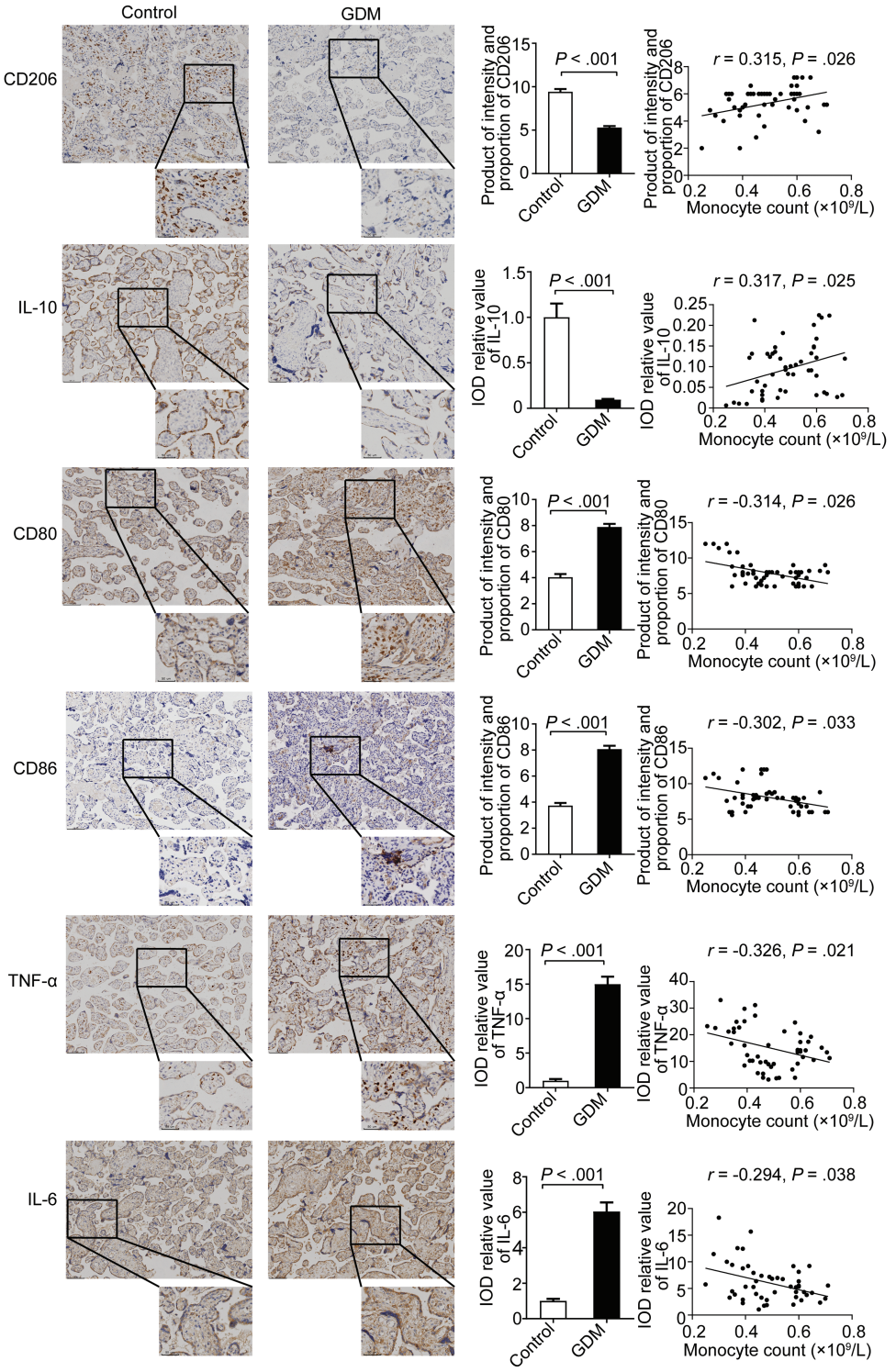

Figure 4. Immunohistochemistry of placenta-derived macrophages and their products in gestational diabetes mellitus (GDM; n = 50) and controls (n = 50), and relationship between the first-trimester monocyte count, placenta-derived macrophages, and their products in women with GDM (n = 50). White bars = control, black bars = GDM; black dots = first-trimester monocyte count, and placenta-derived macrophages and their products in women with GDM. Data are mean ± SE and were analyzed by independent-sample *t* test and Spearman rank correlation analysis. IL-10, interleukin 10; TNF-α, tumor necrosis factor-α; IL-6, interleukin 6.

Discussion

Our study is the first to confirm the close association of monocyte count throughout pregnancy with GDM in a large combined case-control and cohort study. Several studies have demonstrated that monocytes are involved in IR and metabolic disease (28-32). A study by Šiklová

et al (28) reported that nondiabetic first-degree relatives of T2DM patients, with existing IR, had lower intermediate monocytes than control individuals. Circulating CD163-expressing monocytes had a close inverse relationship with IR in patients with T2DM (29). On the contrary, an increased level of monocytes was reported by Shim et al (30) in patients with T2DM and features of metabolic syndrome. In other studies (31, 32), the association of monocytes with incident diabetes was not found.

Monocytes are the main cells of the innate immune system of peripheral blood and are especially important in the maintenance of a normal pregnancy (33). Circulation of peripheral blood through the placenta results in direct or indirect contact of maternal immune cells with the placenta. This may activate circulating immune cells, especially monocytes (34). A few small sample studies have demonstrated that human peripheral monocytes are associated with GDM (15, 16, 22, 23). Wang et al (15) found that second-trimester neutrophil and monocyte counts were higher in women with hyperglycemia first detected during pregnancy than in those in a control group. On the contrary, Daci et al (22) reported no difference in monocyte count between patients with GDM and healthy pregnant women in Kosovo. Furthermore, a study performed by Angelo and colleagues (16) demonstrated that the level of intermediate monocytes was lower in women with GDM compared with healthy controls. Although we did not analyze profile changes of monocytes in GDM, we confirmed that monocyte count was decreased throughout pregnancy in women with GDM, and that a continuously decreased monocyte level was closely associated with glucose level, IR, and newborn weight. In addition, first-trimester monocyte count was a strong independent risk factor for GDM both in the case-control and cohort study. The risk of developing GDM decreased rapidly when the monocyte count exceeded 0.48×10^9/L. Therefore, monocytes appear to have great potential as an early diagnostic marker for GDM and play a vital role in its development. Nonetheless, studies that focus on how monocytes affect GDM are scarce.

Previous studies (12, 35-42) have shown that low-grade chronic inflammation plays a vital role in the pathophysiology of GDM and that women with GDM have higher TNF-α and IL-6 levels, and lower IL-10 levels, in serum or placenta than non-GDM individuals. These findings were corroborated by our study. In addition, monocytes can be divided into anti-inflammatory and proinflammatory subsets by functional classification and produce cytokines such as TNF-α and IL-10 (20, 21, 29). In our study, we hypothesized that decreased monocyte count in the presence of GDM might be due to severely reduced anti-inflammatory monocyte subsets and slightly elevated proinflammatory subsets leading to downregulation of anti-inflammatory and upregulation of proinflammatory factors in peripheral blood. We investigated the association of monocyte count with several inflammatory cytokines in peripheral blood and determined that first-trimester monocyte count was positively correlated with level of IL-10, but negatively correlated with that of TNF-α and IL-6. Moreover, monocytes are macrophage precursors that can be recruited into tissues where they mature into macrophages. Similar to monocytes, macrophages can also be a proinflammatory M1 or anti-inflammatory M2 subtype (43). In a normal pregnancy, most macrophages at the maternal-fetal interface of the placenta are the M2 type and are vital to immunomodulation (44). Several studies (17-19) have shown that macrophages with altered polarization at the maternal-fetal interface, named placental Hofbauer cells, may be involved in the development of GDM. In our study, we also observed that M2 macrophage marker CD206 and its product IL-10 was significantly lower and M1 macrophage markers CD80, CD86, and its products TNF-α and IL-6 were significantly higher in women with GDM than in those without GDM. Interestingly, anti-inflammatory monocyte subsets are speculated to differentiate into M2 macrophages. Therefore, decreased monocyte count in GDM might also contribute to altered placenta-derived macrophage differentiation and products. Subsequently, the hypothesis was confirmed by our study: First-trimester monocyte count was positively correlated with the expression of M2 macrophage marker and IL-10 in the placental tissue of women with GDM but negatively correlated with the expression of M1 macrophage markers TNF-α and IL-6. All these results indicate that decreased monocyte count may contribute to GDM development by mediating insulin resistance via downregulation of anti-inflammatory factors, up-regulation of inflammatory factors and changing placenta-derived macrophage differentiation.

This study has some limitations. First, all participants were from The Fifth People's Hospital of Shanghai and Wujing Hospital, and may lack representativeness, leading to biased results. Additionally, although we have indicated that the pathophysiological role of monocytes in GDM development may be associated with an inflammatory state, it is unclear whether these changes are a cause or consequence of GDM development. Further studies using primary culture macrophage cells and a reliable rodent GDM model to elucidate the function of monocytes should be conducted.

Conclusions

We demonstrated that monocyte count throughout pregnancy was decreased in GDM. Monocyte count was closely associated with glucose level, IR, and newborn

weight. Decreased first-trimester monocyte count was an independent risk factor for the development of GDM and macrosomia. The risk of GDM started to decrease rapidly if monocyte count exceeded 0.48×10^9/L. Moreover, decreased monocyte count during pregnancy might be linked to the chronic inflammatory state of GDM.

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Author Contributions: X.H., B.Z., and M.Z. wrote the manuscript, completed the testing of macrophage makers and inflammatory cytokines, and prepared the study data for statistical analysis. Y.L., R.Z., L.S., Z.Y., C.G., Z.C., J.X., and L.M. provided access to the study data. R.Z. and H.D. performed the statistical analyses. Z.Y., L.S., Y.Z., C.G., and L.M. provided general study input. X.H. and J.L. obtained funding. S.Z., T.N.Z., and J.L. conceived of the research and approved the final manuscript. J.L. is the guarantor of this work and as such has 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.

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