

A Study of Inflammatory Markers in Gestational Diabetes Mellitus

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ABSTRACT

OBJECTIVE: Gestational diabetes mellitus is a common disorder of carbohydrate metabolism, with onset or first recognition during pregnancy, resulting in hyperglycemia of variable severity. Insulin resistance and chronic subclinical inflammation are the underlying mechanisms of the disease. Soluble interleukin-2 receptor, neutrophil-to-lymphocyte ratio, and platelet-to-lymphocyte ratio are the markers of inflammatory disease processes such as type 1 and 2 diabetes mellitus, hepatitis, and neoplasms.

STUDY DESIGN: In our study, we measured complete blood count, serum soluble interleukin-2 receptor, serum glucose in blood samples from 52 women with gestational diabetes mellitus and 50 pregnant women with normal glucose tolerance. Pregnant women who were tested for oral glucose tolerance test (75 gr load) between 24 and 28 weeks of pregnancy were selected for the study. Gestational diabetes mellitus was defined according to the criteria provided by the National Institute for Health and Care Excellence.

RESULTS: In terms of age, gestational diabetes mellitus patients (mean±SD; 31±6 years) were older than controls (mean±SD; 25±5.3 years). Mean platelet volume values were lower in gestational diabetes mellitus patients (mean±SD; 10.3±1.4 fL) as compared to normal glucose tolerance group (mean±SD; 10.8±1fL). No statistically significant differences in serum glucose concentration, white blood cell count, neutrophil count, lymphocyte count, hemoglobin concentration, platelet count, neutrophil-to-lymphocyte ratio value, platelet-to-lymphocyte ratio value, and serum soluble interleukin-2 receptor concentration were found.

CONCLUSION: This study did not reveal an increase in the inflammatory markers, neutrophil-to-lymphocyte ratio, platelet-to-lymphocyte ratio, and serum soluble interleukin-2 receptor, in gestational diabetes mellitus. The mean platelet volume values were observed to be lower in gestational diabetes mellitus patients.

Keywords: Gestational diabetes mellitus, Mean platelet volume, Neutrophil-lymphocyte ratio, Platelet-lymphocyte ratio, Soluble interleukin 2 receptor

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Introduction

Gestational Diabetes Mellitus (GDM) is a common disorder of carbohydrate metabolism, with onset or first recognition during pregnancy, resulting in hyperglycemia of variable severity (1,2). The prevalence of GDM has been estimated to be 4-18% of all pregnancies, depending on the applied criteria (3). GDM is associated with a number of perinatal complications, such as neonatal clinical hypoglycemia, macrosomia, shoulder dystocia, and the need for neonatal intensive care. Maternal complications include an increased risk for cesarean delivery and preeclampsia (4). GDM patients are more likely to develop diabetes mellitus in the years following the pregnancy (5). Insulin resistance and chronic subclinical inflammation are the underlying mechanisms of the disease (6). Inflammation may configure the pathophysiological link between GDM and the risk for developing type 2 diabetes mellitus and cardiovascular disease (CVD) in the mother. The prevalence of GDM has been steadily increasing, which makes prevention of the disease and its complications the goal of patient management (6-8). Serum soluble interleukin-2 receptor (sIL2r) is a biomarker which is elevated in inflammatory disease processes, such as type I diabetes mellitus, type 2

diabetes mellitus, hepatitis, and neoplasms (9-12). Recently, tests of neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR) have emerged as new inflammatory biomarkers of GDM (13-15). Additionally, mean platelet volume (MPV) is a marker which is associated with platelet number and activity. The relationship between higher values of MPV and GDM has been previously established in several studies (16,17).

White blood cell (WBC) count, neutrophil count, lymphocyte count, hemoglobin concentration, platelet (PLT) count, NLR values, and PLR values are all analyzed in the complete blood count (CBC) test, which generates timely and accurate results at a low cost. Studies concerning the levels of serum glucose concentration, WBC count, neutrophil count, lymphocyte count, hemoglobin concentration, PLT count, NLR values, and PLR values in GDM patients vs. healthy pregnant women are scarce. We aimed to compare the levels of these biomarkers between the two groups.

Material and Method

Local Ethics Committee approved the study. Pregnant women who were tested for oral glucose tolerance test (OGTT 75 gr load) between 24 and 28 weeks of pregnancy were included. The study included 102 pregnant women (aged from 18 to 43 years). Venous blood samples were obtained. Based on the results of a 2-hour serum glucose concentration, the participants were divided into two groups: GDM and normal glucose tolerance (NGT). GDM was defined according to the criteria of the National Institute for Health and Care Excellence (NICE) 2015. NGT was diagnosed using the following criterion: serum glucose concentration a 2-h value of <140 mg/dL (7.80 mmol/L). A fasting serum glucose concentration of ≥ 100 mg/dL (5.6 mmol/L) or a 2-h value of ≥ 140 mg/dL was diagnostic for diabetes mellitus (18). Participants

with acute infections and chronic diseases such as pre-gestational diabetes mellitus, hypertension, and hyperlipidemia were excluded from the study.

The serum samples were kept at -80°C after centrifugation until the sIL2r test was carried out. Tests other than sIL2r were performed on the same day when samples were taken. The complete blood counts were measured using Sysmex XN-1000 automatic hematology analyzer (Sysmex Corporation, Kobe, Japan). NLR and PLR values were calculated for the two groups. Serum glucose concentrations were analyzed on Beckman Coulter Olympus AU2700 analyzer. Serum sIL2r concentrations were performed on the IMMULITE 2000 system (Siemens Healthcare, Germany). The two groups were compared for NLR, PLR, MPV, and IL2r concentrations.

Statistical analysis

Data were analyzed using Excel (Microsoft, USA) and MedCalc version 15.8 (Medcalc Software bvba Belgium). Descriptive statistics (mean, standard deviation, median, minimum, maximum, number) were generated for the two groups. The normality assumption was checked using the Kolmogorov-Smirnov test. Outlier analysis was performed using the Tukey test. Differences between three or more groups were compared by one-way analysis of variance (ANOVA) when the assumptions of this parametric test were met, and the Kruskal Wallis test was used when the assumptions of ANOVA were not met. Differences between the two groups were evaluated by the independent t-test when the assumptions of this parametric test were met and the Mann Whitney independent test was used when the assumptions of independent t-test were not met. The p-value of <0.05 was considered as statistically significant.

Results

The results of the analyses are shown in table 1. GDM pa-

Table 1: Age and laboratory parameters of the study population

Parameter	NGT (Mean \pm SD)	GDM patients (Mean \pm SD)	p value
Age (years)	25 \pm 5.3	31 \pm 6	<0.001**
Fasting glucose (mg/dL)	75 \pm 7.8	79 \pm 6.6	0.11
1 hour-glucose (mg/dL)	114 \pm 27.3	158 \pm 17.9	<0.001**
2 hour-glucose (mg/dL)	98 \pm 18.9	158 \pm 15.5	<0.001**
WBC (10^3 mm ³)	9.8 \pm 2.55	9.9 \pm 2.03	0.73
Hemoglobin (g/dL)	12.2 \pm 1.1	12.2 \pm 0.97	0.98
Platelet (10^3 mm ³)	238 \pm 56.5	239 \pm 48.7	0.94
Neutrophil ($10^3/\mu\text{L}$)	7.1 \pm 2.05	7.2 \pm 1.8	0.81
Lymphocyte ($10^3/\mu\text{L}$)	2.1 \pm 0.55	2.1 \pm 0.44	0.89
NLR	3.4 \pm 0.98	3.5 \pm 1.01	0.79
PLR	120 \pm 40.6	117 \pm 29.1	0.77
MPV (fL)	10.8 \pm 1	10.3 \pm 1.4	0.02*
sIL2r (U/mL)	352 \pm 100	382 \pm 189	0.56

* $p \leq 0.05$, ** $p \leq 0.001$. NGT: Normal glucose tolerance, GDM: Gestational diabetes mellitus, SD: Standard deviation, WBC: White blood cell, NLR: Neutrophil-to-lymphocyte ratio, PLR: Platelet-to-lymphocyte ratio, MPV: Mean platelet volume, sIL2r: Soluble interleukin-2 receptor

tients had higher serum 1-hour and 2-hour glucose concentrations as compared to NGT group. MPV values were lower in GDM patients (mean±SD; 10.3±1.4 fL) vs. healthy women (mean±SD; 10.8±1fL) (Figure 1). No statistically significant differences in fasting serum glucose concentration, WBC count, neutrophil count, lymphocyte count, hemoglobin concentration, PLT count, NLR value, PLR value, and serum sIL2r concentration were found between the two groups. The majority of GDM women were multiparas. Parity and gravidity rates correlated with age in both groups. Mean parity distribution was between minimum 0, maximum 5 (mean±SD; 2.67±1.37) and minimum 0, maximum 4 (mean±SD; 0.94±1.13) in GDM patients and controls, respectively. GDM patients (mean±SD; 31±6 years) were older than controls (mean±SD; 25±5.3 years) (Figure 2).

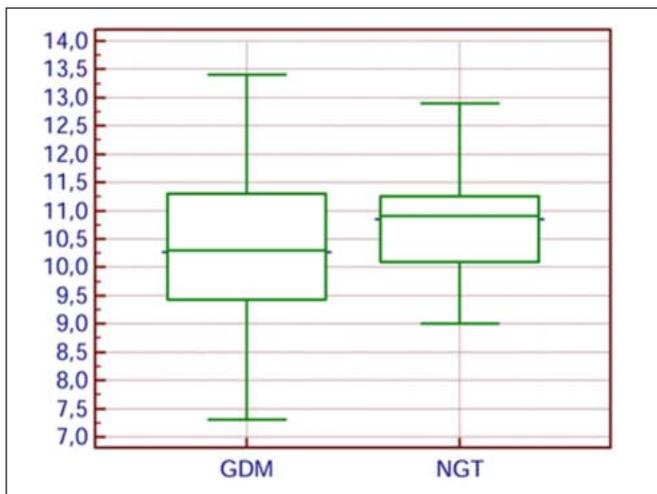


Figure 1: Box-plot graphic of two groups (with their 95% confidence intervals for mean) for MPV values

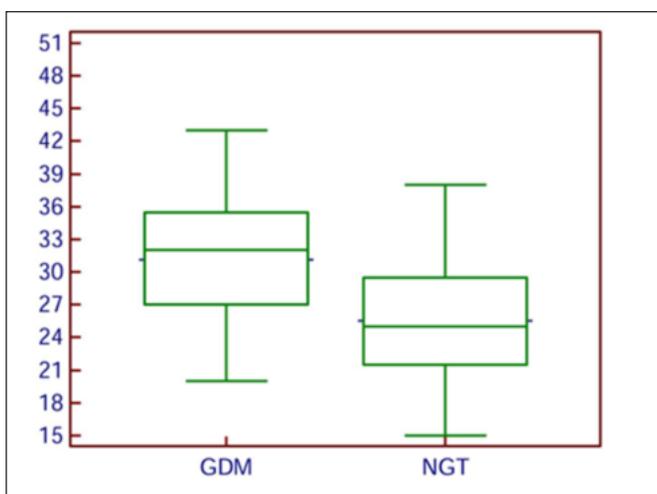


Figure 2: Box-plot graphic of two groups (with their 95 % confidence intervals for mean) for age in years

Body mass index (BMI) didn't differ between the groups (mean±SD; 28.6±5.3, 27.2±4.1 patients and controls, respectively). Gravidity, parity, abortion, dilatation, and curettage

rates were increasing with age. Both groups did not have a history of alcohol consumption during pregnancy. The rate of history of smoking during pregnancy was 2.19% and 2.47% in GDM patients and the control group, respectively. As far as comorbidities were concerned, no statistically significant differences were observed between the groups, with the exception of GDM.

Discussion

Gestational diabetes mellitus is a disorder of carbohydrate metabolism, with onset or first recognition during pregnancy. The diagnostic criteria may differ. According to the NICE 2015 criteria, GDM is diagnosed if the glucose level is ≥ 5.6 mmol/L (100 mg/dL) when fasting or ≥ 7.80 mmol/L (140 mg/dL) 2 hours after 75 g load. According to the WHO 2013 criteria, GDM is diagnosed again by 75 g glucose loading, but when one of the following plasma glucose value is met or exceeded: fasting, 5.10 mmol/L (92 mg/dL); 1 hour, 10.00 mmol/L (180 mg/dL); 2 hours, 8.50 mmol/L (153 mg/dL). Carpenter and Coustan criteria, on the other hand, are defined for the screening test with 50 g glucose load and the confirmatory test with 100 g glucose load. In the current study, NICE 2015 criteria were used for the diagnosis of GDM.

Lower MPV values in GDM patients as compared to healthy women were the most important finding of our study, especially in light of the fact that in most previous studies MPV values in GDM patients values were either higher (17,19,20), or the same as in healthy controls (16). Currently, the MPV test is not regarded as a marker of platelet function because of the test results may be affected by the following factors: time from obtaining the specimen until the analysis, the use of anticoagulated tubes, and the lack of a standardized method of measurement (21,22). Taking into consideration of the results of the above-mentioned studies, it seems safe to conclude that - regardless of the MPV values in GDM patients in these studies - the MPV values in GDM samples are not associated directly with the disease, as mentioned before, but are most likely attributable to pre-analytical factors and analytical limits. As far as the NLR, PLR, and sIL2r values are concerned, there were no statistically significant differences between the two groups.

Maternal age is one of the risk factors for GDM (18). In the present study, GDM patients (mean±SD; 31±6 years old) were also older than controls (mean±SD; 25±5.3 years) who were chosen randomly. The BMI of the two groups is similar. Adiposity may affect inflammatory status. So this may be a reason for the indifference of the levels of three inflammatory markers between the groups in this study.

Sargin et al. also found no statistical differences in NLR and PLR values between GDM patients and healthy pregnant women (15). Also, there were no statistical differences between the two groups in our study with regard to WBC, neu-

trophil, and lymphocyte count. Similarly, Ozyer et al., found no association between WBC count, serum c-reactive protein and IL-6 concentrations and GDM (23).

However maternal chemerin, a recently discovered adipokine, levels were found significantly increased in GDM (7). Another study found adiponectin, an anti-inflammatory protein, the level was significantly lower in pregnant women with GDM (8).

Yilmaz et al. found higher NLR values in GDM patients as compared to non-GDM pregnant women, with a sample of 42 GDM and 68 non-GDM pregnant women (14). They evaluated the results of the OGTT according to the criteria by Carpenter and Coustan. They considered higher NLR values to be the marker of subclinical inflammation and an independent risk factor for developing GDM and concluded that the NLR value can be used in early diagnosis of GDM (14). They excluded participants with WBC counts of >12000 cells/mL and set WBC of 12 000 cells/mL as the upper limit. In the GDM group, their WBC count was 7.715 ± 1.463 cells/mL (mean \pm SD). In our study, the upper limit of the WBC count was 15 000 cells/mL. In pregnancy, the WBC count of up to 15 000 cells/mL is regarded as a normal hematological finding (15). In our study, WBC counts in GDM and NGT women were 9.9 ± 2.03 cells/mL and 9.8 ± 2.55 cells/mL, respectively (mean \pm SD). In our study, mean and SD values were higher than in their study, which could be the reason why we found no statistically significant differences between the two groups. Also, our results are different from the results of the above mentioned studies and this may be because of the diagnostic criteria for GDM are different.

On the other hand, because of the fact that low-level inflammation occurs even in a physiological pregnancy (24), systemic inflammation occurring in GDM patients may not differ from a normal pregnancy.

Lapolla et al. found no statistically significant differences in blood IL2 and sIL2r concentrations between GDM group and control, which is consistent with our findings (25). Pereira showed that type 2 diabetes mellitus patients had higher blood tumor necrosis factor alpha and interleukin-2 soluble receptor concentrations as compared to the control group (10). Although most previous studies indicated that diabetes mellitus was related to inflammation, GDM is not exactly the same as diabetes mellitus because in some GDM pregnant women diabetes mellitus might not occur after pregnancy (5). In addition, according to the WHO criteria of GDM employed in our study, the GDM group involves not only patients diagnosed with GDM but also those with impaired glucose tolerance (1), which could be the reason why we found no differences between the two groups with regard to the inflammatory markers.

One of the limitations of this study is that maternal age is

higher in the patient group due to the unbalanced selection of the groups. So, this case should be taken into consideration when evaluating the results of this study.

In conclusion, according to the NICE 2015 criteria of GDM, serum sIL2r concentrations, NLR and PLR values are statistically not different in GDM patients as compared to healthy pregnant women. As a result, these parameters are not useful in the diagnosis of GDM. We can conclude that no special inflammatory condition occurs in GDM. MPV values decrease in GDM patients. As mentioned before, the MPV-GDM relationship results from pre-analytical factors and analytical limits. Therefore, we suggest that inflammatory markers should be evaluated using the diagnostic criteria for GDM other than the NICE 2015 criteria whose performance is not enough when compared to the Carpenter and Coustan criteria or the WHO 2013 criteria.

✉ : *Compliance with ethical standards*

Authorship declaration

All authors listed meet the authorship criteria according to the latest guidelines of the International Committee of Medical Journal Editors, and all authors are in agreement with the manuscript.

Conflict of interest The authors declare that they have no conflict of interest.

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Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

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