Inflammatory Markers and Gestational Diabetes Mellitus Risk: Investigating Neutrophil/Lymphocyte Ratio, Platelet/Lymphocyte Ratio, and Systemic Immune Inflammation Index

Original Article

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ABSTRACT

Aims of the work: In the present study, we aimed to investigate the correlation of neutrophil to lymphocyte ratio(NLR), platelet to lymphocyte ratio (PLR), systemic immune inflammation index (SII) with the risk of gestational diabetes mellitus.

Material and Method: In this controlled cross-sectional study, 90 pregnant women with GDM and 110 uncomplicated, healthy, age- and body mass index-matched control pregnant women were evaluated. Oral glucose tolerance test (OGTT) for GDM diagnosis was performed at 24-28 weeks of gestation. fasting blood glucose (FBSG), fasting insulin and hemostasis model assessment of insulin resistance (HOMA-IR) values were compared in both groups. Levels of NLR, PLR, and SII were assessed.

Results: This study compares the blood parameters of women with diabetes mellitus (GDM) with those of a group of healthy subjects. In the GDM group, there was an increase in blood cell (WBC) and platelet (PLT) levels (p=0.009, p<0.001, respectively), as well as an increase in neutrophil and monocyte levels. Moreover, the NLR, PLR, and SII were markedly increased in the GDM group (p<0.001 for all). Conversely, lymphocyte levels were found to be decreased (p=0.005).

Conclusion: In the study, NLR, PLR, SII were significantly higher in patients with GDM. These parameters, which can be calculated with a simple haemogram test, can be used to predict GDM in the first trimester of pregnancy.

Key Words: GDM, NLR, PLR, SII.

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INTRODUCTION

Gestational diabetes mellitus (GDM) is a significant and increasing health issue in numerous regions globally, characterized by impaired carbohydrate metabolism leading to varying degrees of high blood sugar that is first detected during pregnancy.[1] The state arises when blood sugar levels are elevated but do not reach the threshold for diabetes[2] Gestational diabetes can lead to problems in the short and long term for both the mother and the child. Furthermore, women with gestational diabetes (GDM) are at increased risk of developing type [2] diabetes mellitus (T2DM) after giving birth[3].

The number of white blood cells (WBC) rises during pregnancy, with the reference range for pregnant women having high lower and upper limits, typically ranging from (6-16 × 10^9/L).[4] To support the fetus's survival, pregnancy involves typical physiological changes. In a typical pregnancy, the innate immune system becomes active while the adaptive immune system is repressed. During pregnancy, the peripheral circulation changes include a higher amount of granulocytes and a decrease in platelet count, especially in the third trimester.[5,6] Inflammation has recently been found to be involved in the development of GDM.[7] Inflammatory markers like CRP, IL-6, and TNF-α are linked to both type 2 and gestational diabetes. While conflicting, current data on inflammation in women with GDM suggest that adipose tissue may trigger immune and inflammatory responses in white adipose tissue and the placenta, which could lead to systemic inflammation[8,9] Recently, there have been markers linked to white blood cells that are seen as warning signs for inflammation, including the NLR, PLR, and SII index. These markers are seen as signs of an response and are referred to as WBC based inflammatory markers. They are typically derived

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from a blood count (CBC) test. Studies have shown that they play a role, in identifying, monitoring and assessing systemic inflammatory conditions by indicating increased immune activity in cases of chronic inflammation[10].

The purpose of this research is to evaluate women with gestational diabetes and compare them with normal pregnant women, as well as investigate the correlation between NLR, PLR, SII, and the likelihood of developing gestational diabetes.

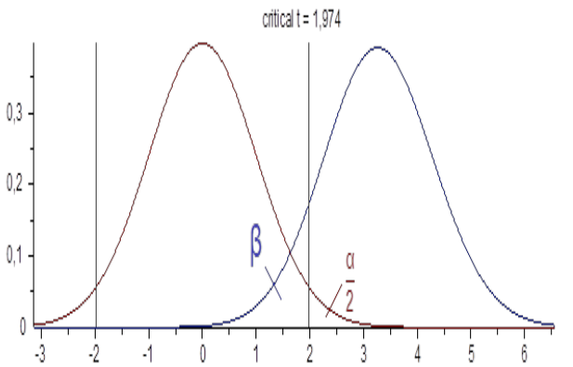
THE AIM OF THE STUDY

Was to investigate the relationship between neutrophil/lymphocyte ratio (NLR), platelet/lymphocyte ratio (PLR), systemic immune inflammation index (SII) and gestational diabetes mellitus (GDM) risk and to determine whether these inflammatory markers have any predictive significance.

MATERIAL AND METHOD

The Ethics Committee, at Samsun University Research and Training Hospital approved the research protocol (Protocol No; SÜGOKA 2024/2/14). A retrospective study conducted at the Womens Clinic of Samsun University Research and Training Hospital from January 2022 to December 2023 included 90 women with diabetes (GDM) and 110 healthy pregnant women. Gestational diabetes was diagnosed based on glucose level criteria. Participants diagnosed with GDM between the 28th weeks of pregnancy were selected to ensure consistency and comparability with the control group. All participants were assessed for active infections and those with any signs or symptoms of infection were excluded. Women with pre-existing conditions such as chronic hypertension, diabetes, cardiovascular disease or other medical conditions were also excluded. Also excluded were women taking certain medications that may affect blood glucose levels or women with pre-pregnancy obesity. Results for CBC parameters such as white blood cell count, neutrophil count, lymphocyte count, monocyte count, platelet count, and mean platelet volume (MPV) were acquired from the Laboratory Information System (LIS). The CBC parameters were assessed in blood samples taken while fasting between the 24th and 28th weeks of pregnancy, as part of the OGTT test. Glucose levels were determined by utilizing the hexokinase-based enzymatic technique with the BECKMAN COULTER AU5800 analyzer along with commercial reagents, and the plasma concentration was computed. Sysmex XN-1000 was used for carrying out CBC parameters. The NLR was determined by dividing neutrophil count by lymphocyte count, while the PLR was calculated by dividing platelet count by lymphocyte count. In addition, the systemic immune-inflammation index (SII) was determined through a specialized calculation that considers the levels of platelets, neutrophils, and lymphocytes[10]. The homeostatic model assessment of insulin resistance (HOMA-IR) index was calcu lated using the following formula: FG (mg/dl) × Fasting insulin level (μU/ml) / 405[11].

Power Analysis



Within the scope of the study, the sample number was calculated as a result of the power analysis performed with G*Power (Version 3.1.9.6). For the study conducted in 2-group research design, reliability was 95%, power was 90% and effect level was 0.50 in Power analysis and the minimum sample size was calculated as 172. Accordingly, 86 observations from each group is sufficient.

Statistical Analysis

All data was stored in a computer database and used for analysis The statistical analyses were performed using IBM SPSS Sta tistics version 22 (IBM Corp., Armonk, NY). Checks were made to ensure accuracy. Basic statistics [mean spread (SD), median, minimum and maximum values, count] were calculated for both data sets. A statistical test was then used to check the distribution of the data. Mean spread was used for data that followed the distribution, while median was used for those that did not. When comparing the two sets, Mann Whitney U test was used. Independent t-test was conducted to understand the relationship between variables that followed the distribution. Spearman correlation analysis was used to analyze the relationship between variables that did not follow the distribution. Finally, an evaluation was made to determine the ability of NLR, PLR, and SI index to predict the outcomes associated with pregnancy and GDM.

RESULTS

This study compared the baseline variables of GDM and healthy pregnancy groups in (Table 1). There was no notable distinction among the groups (p>0.05) regarding age, number of births, gestational age, and body mass index (BMI) (p=0.91, p=0.32, p=0.30, p=0.34, respectively).

Table 1: Demographic and clinical characteristics of study population.

Variables mean+SD	GDM (*n*=90)	Healthy (*n*=110)	*P-value*
Age (year)	29.3±5.9	27.9±6.2	0.91 (NS)
Parity	0.25±0.60	0.13±0.35	0.32 (NS)
Gestational age (week)	24.80±0.89	24.70±0.83	0.30 (NS)
BMI (kg/m^2)	31.08±3.62	30.78±3.27	0.34 (NS)

NS= Non significant *p value* > 0.05

Table 2: Comparison of full blood parameters between patient and control group.

Parameters	GDM (*n*=90)	Healthy (*n*=110)	*P-value*
50 gr OGTT (mg/DL)	168 27±	117±17	<0.001
Fasting blood glucose (mg/dl)	82.8±9.7	78.7±6.3	NS
Fasting insulin (μU/ml)	10.9± 2.9	7.2± 2.1	<0.001
HOMA-IR	3.66± 0.21	2.53± 0.33	= 0.02
WBC (×10^9/L)	6.95 ± 1.57	6.36 ± 1.20	0.009
Neutrophils (×10^9/L)	4.05 (3.34, 5.00)	3.42 (3.02, 4.20)	<0.001
Lymphocytes (×10^9/L)	1.97 (1.67, 2.38)	2.27 (2.00, 2.61)	<0.001
Monocytes (×10^9/L)	0.44 (0.35, 0.54)	0.37 (0.31, 0.43)	<0.001
PLT (×10^9/L)	245.40 ± 56.98	193.17 ± 47.65	<0.001
MPV (fL)	8.54 (4.93-15.8)	8.61 (5.29-15.6)	=0.645 (NS)
NLR	2.02 (1.57, 2.66)	1.54 (1.31, 1.90)	<0.001
PLR	116.18 (92.72, 156.08)	86.72 (70.97,106.74)	<0.001
SII (×10^9/L)	482.86(329.60, 661.93)	308.00 (217.32, 365.45)	<0.001

NS= Non significant *p value*> 0.05

This table compares blood parameters in women with diabetes mellitus (GDM) with blood parameters in a group of healthy individuals. In the GDM group, there was an increase in blood cell (WBC) and platelet (PLT) levels (p=0.009, p<0.001, respectively), as well as an increase in neutrophil and monocyte levels.

DISCUSSION

Subtle inflammation, during pregnancy is associated with diabetes mellitus (GDM) where insulin resistance plays a role[8]. When expecting the rise in hormones such as cortisol, progesterone and human chorionic growth hormone, coupled with increased storage contributes to insulin resistance. Adipose tissue has an impact on regulating insulin sensitivity through the release of cytokines like leptin, resistin, adiponectin, TNF alpha and IL 6[12]. Moreover previous studies have shown a connection, between inflammation and insulin resistance[13].

Elevated levels of blood cells indicate an inflammatory reaction and insulin resistance triggered by cytokines is the mechanism of the inflammatory response associated with gestational diabetes mellitus (GDM) and diabetes[13] Diabetes is primarily caused by inflammation and insulin resistance. Some writers have mentioned that a higher count of blood cells, in pregnancy could be linked to the outcomes of screening tests for GDM and an increased likelihood of developing GDM. They suggested that women who develop GDM may show signs of heightened inflammation during pregnancy 20 weeks before being diagnosed with GDM. The natural rise, in insulin resistance that comes with pregnancy combined with inflammation might contribute to the development of GDM[14]. White blood cell count serves as an employed indicator of inflammation, in settings. Pattanathaiyanon and his team found that an increase in blood cell numbers affected the onset of gestational diabetes mellitus[15]. In the same manner, our research discovered that white blood cell counts were elevated in groups with gestational diabetes mellitus compared to the group without the condition. It can be said that white blood cell count is a standalone factor for gestational diabetes mellitus.

The neutrophil, to lymphocyte ratio and PLR are seen as markers of inflammation. Are known to have strong connections with conditions, like coronary heart disease, inflammatory bowel disease, diabetes and various types of tumors[16–18]. Both of these elements are easily quantifiable in terms of cost and serve as convenient biological indicators of systemic inflammation. Subclinical inflammation and insulin resistance are fundamental in diabetes mellitus, so fluctuations in NLR and PLR levels in GDM are not surprising. A recent research conducted by Liu and colleagues found that NLR and PLR levels were elevated in GDM patients, indicating that these factors have the potential to individually forecast the development of GDM during pregnancy[19]. Likewise, in a study conducted by ***Yılmaz et al.,*** involving 42 patients diagnosed with GDM and 68 pregnant women with normal glucose levels, it was demonstrated that the NLR was notably elevated in

individuals with GDM[20]. This study confirmed that NLR is associated with GDM and was found to be consistent with the literature. The results support the hypothesis that NLR reflects the chronic inflammatory state of GDM.

Platelet count and platelet volume indicators, PLR and MPV respectively provide insights, into the size and activity of platelets. Larger platelets are known to exhibit increased metabolic and enzymatic functions as a higher prothrombotic capacity. The research conducted by Shah and colleagues showed a connection, between MPV levels and glucose levels well as glycated hemoglobin (HbA1c) measurements in people, with diabetes[21] Studies conducted in laboratory settings and within living organisms have demonstrated that insulin has the ability to reduce platelet aggregation and activation, in individuals who're responsive, to insulin[22] There have been limited studies examining the relationship, between MPV and GDM. Some earlier research has indicated that there is no difference in MPV levels, between the GDM group and the control group[23] One potential explanation could be that the research has been conducted with study groups and sample sizes. PLR serves as a marker indicating inflammation and several investigations have demonstrated its utility in assessing conditions such, as tumors, diabetes,

neurological disorders and more[24,25]. Sargın and his team found that an increase, in the platelet lymphocyte ratio (PLR) was linked to diabetes mellitus (GDM). They proposed that monitoring PLR after childbirth could help prevent the development of type 2 diabetes and its lasting harmful impacts[26]. In the present study, platelet-to-lymphocyte ratio (PLR) was significantly increased in the GDM group.

According to another study conducted by Huang and colleagues SII serves as an indicator of both immune response and overall systemic inflammation[27]. In a research conducted by Turgut and colleagues they investigated the potential of SII to forecast miscarriages. Elevated SII levels, during the stages of pregnancy have been emphasized as indicators for predicting miscarriages. Abortions take place due to reasons indicating a process within the uterus. The study also underscores the relevance of SII, in showcasing the reaction during this stage[28]. There is one research paper that delved into the connection, between SII and diabetes. This study showcased the association between depression and SII. In this study by ***Wang et al***. it was found that diabetic patients experiencing depression exhibited SII levels compared to those, without depression[29]. While there hasn't been a study linking GDM to SII previous research has examined the relationship, between NLR and PLR with GDM both being markers in the bloodstream. Given that SII reflects a response it was theorized that there could be a connection to GDM, which triggers inflammation. Past studies have shown associations between platelet count and size with conditions, like diabetes impaired fasting glucose levels and insulin resistance[30]. In our study, individuals with GDM had higher SII levels, based on these data, we can state that the measurement of SII value can be used as a tool to identify GDM.

It's important to note that our research has limitations. Firstly it was conducted at a location, with a group of participants. This means that the results may not apply broadly to the population. One of the strengths of our study is that the women in the groups had characteristics and it was designed prospectively. Additionally in this study since BMI and maternal age were similar across groups any potential factors causing confusion were removed. The laboratory procedures are straightforward and cost effective, with results available making it feasible to measure NLR, PLR and SII for prognostic insights.

CONCLUSION

To summarise, in this study, NLR, PLR and SII were found to be significantly higher in GDM patients. These parameters, which can be calculated with a simple haemogram test, can be used to predict GDM in the first trimester of pregnancy.

ETHICAL DE CLARATIONS

Ethics Committee Approval: Samsun University Non-Interventional Clinical Research Ethics Committee, Protocol No: SÜGOKA 2024/2/14.

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

CONFLICT OF INTERESTS

The authors have no conflicts of interest to declare.

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Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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