

## Neutrophil-To-Lymphocyte and Platelet-To-Lymphocyte Ratios in Pregnant Diabetic Patients: A Hospital-Based Study

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### ABSTRACT:

#### BACKGROUND:

Diabetes in pregnancy has a major health risk to the mother and the fetus. Neutrophil-to-lymphocyte ratio (NLR) and the platelet-to-lymphocyte ratio (PLR) were shown to be as important markers of inflammation in neoplasms, cardiac diseases and diabetes-associated complications.

#### OBJECTIVE:

To estimate the mean Lymphocyte, Neutrophil, Platelet, PLR, NLR in diabetic and non-diabetic pregnant women and to identify the correlation between NLR and PLR with each type of diabetes in pregnancy.

#### PATIENTS AND METHODS:

A case control study was conducted to determine the relationship between (PLR) and (NLR) in 2 groups of pregnant women; in Diabetic Centre and outpatient clinics, Maternity Teaching Hospital, Kurdistan region, Iraq from 21<sup>st</sup> February 2019 to 15<sup>th</sup> September 2019. Study included 100 pregnant women 24-36 weeks gestation after exclusion of cardiovascular risk factors and underlying chronic inflammatory conditions. Group 1 included 50 diabetic women having positive 75g-OGTT (gestational DM) or known cases of type 1 or 2 diabetes mellitus. Group 2 were control group having negative 75g-OGTT.

#### RESULTS:

The neutrophil count was significantly higher in the diabetic group. No significant differences were detected between the two study groups regarding the platelets ( $p = 0.225$ ), lymphocytes ( $p = 0.251$ ), NLR ( $p = 0.121$ ), PLR ( $p = 0.784$ ) except for the difference between type II diabetes and the control group where the mean NLR among type II diabetics was 3.86 and that of the control was 3.16 ( $p = 0.029$ ).

#### CONCLUSION:

We could only find significant difference in terms of NLR in type 2 diabetes mellitus when compared to the comparison group and in terms of PLR in type 2 diabetes mellitus when compared to the gestational diabetes group.

**KEYWORDS:** Diabetes in pregnancy, NLR, PLR, Inflammation

### INTRODUCTION:

Diabetes in pregnancy has a major health risk to the mother and the fetus, and affects the future health of the child<sup>(1)</sup>, the worse the maternal hyperglycemia (before or during pregnancy); the worse perinatal and maternal outcomes will be<sup>(2-3)</sup>.

Many studies have explained the inflammatory role in the development of gestational diabetes mellitus (GDM) and type 2 diabetes mellitus (T2DM)<sup>(4,5)</sup> and It is believed that the obesity-induced low-grade chronic inflammation is the reason behind peripheral insulin resistance in T2DM.<sup>(6)</sup>

The neutrophil-to-lymphocyte ratio (NLR) is used to predict the impact of inflammation and many recent studies discussed that NLR is a valuable prognostic marker for patients affected by various diseases and has been correlated to bad outcomes in a numerous diseases like: myocardial infarction, atherosclerosis, renal cell carcinoma in obese individuals<sup>(7-10)</sup> and diabetes-associated complications.<sup>(11)</sup>

Platelets have abundant proinflammatory factors and have the ability to release potent micro-particles which are closely participating in the initiation and progression of different inflammatory disorders, platelet to lymphocyte ratio (PLR) was reported in many studies to have correlation with inflammatory diseases such as systemic lupus erythematosus, arthritis, cardiovascular, metabolic and infectious diseases<sup>(12-14)</sup>.

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In a study on DM and its complications such as retinopathy and nephropathy; they found that patients who had higher NLR and PLR showed increased albuminuria; and NLR and PLR were shown to be able to predict complications related to DM. This correlation may be the result of increased inflammation and endothelial malfunction in diabetic population.<sup>15</sup> for this reason NLR and PLR were regarded as being markers of inflammation that can be readily and inexpensively obtained and as screening tests for complications associated with DM.<sup>(16)</sup>

To our best knowledge this article is considered to be the first to be conducted in Maternity hospital to assess the ratio of PLR and NLR in diabetic pregnant women.

### OBJECTIVES:

This study was conducted to determine

1. The Mean Lymphocyte, Neutrophil, Platelet, Platelet/Lymphocyte ratio(PLR), Neutrophil/Lymphocyte ration(NLR) in diabetic and non-diabetic pregnant women
2. The correlation between NLR and PLR with each type of diabetes in pregnancy.

### PATIENTS AND METHODS:

A case control study was conducted on 2 groups of pregnant women diabetic and non-diabetic in outpatient and Diabetic Centre Clinic at Maternity Teaching Hospital, Erbil city, Iraq. The study was conducted between 21<sup>st</sup> February 2019 and 15<sup>th</sup> September 2019.

The study included 100 pregnant women. Group 1 (50 diabetic women) having positive 75g-OGTT, or being a known case of type 1 or 2 DM. Group 2 (50 pregnant women) were control group having negative 75g-OGTT and labeled as non-diabetic pregnant women.

All pregnant ladies who presented to the Diabetic Center Clinic at Maternity Teaching Hospital in their 24<sup>th</sup> -36<sup>th</sup> weeks gestation, having history of pre-gestational diabetes or referred on suspicion having diabetes and accept to participate in the research were included in the study.

Having cardiovascular risk factor such as hypertension, hyperlipidemia or coronary artery disease, chronic inflammatory condition such as collagen tissue disease and inflammatory bowel diseases, and the presence of other factors that may affect ratio of WBC subset and inflammation like: (using corticosteroids, Aspirin, smoking, impaired liver and kidney function, history of trauma, any evidence of active infection, peritonitis, pancreatitis, pelvic inflammatory disease, cancer, leukocytosis >17000/microL, leucopenia <3500/microL,

fever, and refused to participate were excluded from the study were regarded as exclusion criteria to participate in the research.

A questionnaire was constructed depending on the patients' bio-data and obstetrical history highlighting the risk factors mentioned by the NICE 2015 criteria<sup>17</sup>.

### Study procedure:

Pregnant woman in group 1 and 2 were interviewed in the diabetic center and outpatient clinic where a thorough history was taken including: age, obstetrical history, past medical history (diabetes, hypertension, cardiovascular disease, renal disease and collagen disease), past surgical history, drug history and family history (first degree relatives with diabetes). This was followed by general examination.

Then each lady was sent for complete blood count (CBC) and 75g-OGTT for those with negative history of diabetes. They were distributed according to the history and 75g-OGTT results into Group1 with diabetes whether type1, type2 or GDM and Group2 with no diabetes.

### NLR and PLR calculations:

The neutrophils count was calculated by multiplying the neutrophil percentage by the total WBC count, the same was done for the lymphocytes count as follows:

Neutrophils count = WBC x Neutrophil percentage.

Lymphocyte count = WBC x Lymphocyte percentage.

Then the NLR was calculated by dividing the Neutrophils count by the Lymphocytes count and the PLR was calculated by dividing the Platelets count by the Lymphocytes count as follows:

NLR = Neutrophils count ÷ Lymphocytes count.

PLR = Platelets count ÷ Lymphocytes count.

### Ethical considerations:

The study was approved by the scientific committee of Iraqi Board for Medical Specialties, approval taken from the Erbil directorate of health and from hospital's administration; a verbal consent was taken from each patient prior to participation in the study.

### Statistical analysis:

Data were analyzed using the Statistical Package for Social Sciences (SPSS, version 22). Chi square test of association was used to compare proportions. Fisher's exact test was used when the expected count of more than 20% of the cells of the table was less than 5. Student's t test of two independent samples was used to compare two means. One way analysis of variance (ANOVA) was used to compare three means. A post hoc test (LSD) was used to compare

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means of each two groups (after doing the ANOVA test). A p value of  $\leq 0.05$  was considered statistically significant.

### RESULTS:

The mean age and BMI of the diabetic women was significantly higher than the comparison group (Table 1). It is evident in Table 2 that the mean neutrophil was (7698.1 $\pm$ 2215.4) among the diabetics compared with (6251.9 $\pm$ 1612.6) among the comparison group ( $p < 0.001$ ). No significant differences were detected between the two groups regarding the means of the following parameters: platelets, lymphocytes, Neutrophil/lymphocyte ratio and Platelet/lymphocyte ratio.

Table 3 shows no significant differences in the mean NLR between the three types of the diabetes and the control group except for the difference between type II diabetes and the control group where the mean NLR among type II diabetics was (3.86 $\pm$ 1.22) and that of the control was (3.16 $\pm$ 1.22).

Regarding the PLR, no significant difference was detected between the three types of diabetes and the control group as presented in Table 4 except for the difference between the mean PLR of type 2DM and the GD.

The mean of the neutrophil/lymphocyte ratio among diabetic women with history of stillbirth was significantly higher than the mean among women with no history of stillbirth as presented in Table 5. The mean of the neutrophil/lymphocyte ratio was (4.93 $\pm$ 0.85) among women with fetal anomalies which was significantly higher than the mean among those with no anomalies ( $p = 0.011$ ). No significant association was detected between the neutrophil/lymphocyte ratio with miscarriage and macrosomia.

The same pattern was applied for the platelet/lymphocyte ratio as presented in Table 6, where it is evident that the means of this ratio is significantly high among those with history of stillbirth, and those with history of fetal anomalies but no significant associations were detected with miscarriage and macrosomia.

**Table 1. Basic characteristics of the study groups.**

	Diabetes		Comparison			Total	
	No.	(%)	No.	(%)	No.	(%)	p
Age (years)							
< 25	7	(14.0)	10	(20.0)	17	(17.0)	
25-34	18	(36.0)	29	(58.0)	47	(47.0)	
$\geq 35$	25	(50.0)	11	(22.0)	36	(36.0)	0.014*
Mean( $\pm$ SD)	33.1	( $\pm$ 6.3)	29.9	( $\pm$ 5.9)			0.010†
Gestational age (weeks)							
24-27	29	(58.0)	20	(40.0)	49	(49.0)	
28-31	9	(18.0)	19	(38.0)	28	(28.0)	
32-36	12	(24.0)	11	(22.0)	23	(23.0)	0.072*
Mean( $\pm$ SD)	28.1	( $\pm$ 3.8)	29.0	( $\pm$ 2.9)			0.169†
Parity							
Nulliparous	6	(12.0)	11	(22.0)	17	(17.0)	
Multi-parous	32	(64.0)	33	(66.0)	65	(65.0)	
Grand multi- parous	12	(24.0)	6	(12.0)	18	(18.0)	0.175*
Mean( $\pm$ SD)	3.0	( $\pm$ 2.1)	2.0	( $\pm$ 1.7)			0.010†
BMI (Kg/m <sup>2</sup> )							
< 25	5	(10.0)	14	(28.0)	19	(19.0)	
25-29	19	(38.0)	19	(38.0)	38	(38.0)	
$\geq 30$	26	(52.0)	17	(34.0)	43	(43.0)	0.046*
Mean( $\pm$ SD)	30.5	( $\pm$ 4.5)	27.9	( $\pm$ 4.7)			0.005†
Total	50	(100.0)	50	(100.0)	100	(100.0)	

BMI: Body Mass Index \*By Chi square test. †By t test of two independent samples.

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**Table 2. Blood parameters of the two study groups.**

Blood parameters	Diabetes		Comparison		P*
	Mean	(±SD)	Mean	(±SD)	
Neutrophil (cells/mL)	7698.1	(±2215.4)	6251.9	(±1612.6)	< 0.001
Platelets (cells/mL)	259720.0	(±59880.5)	243940.0	(±69018).0	0.225
Lymphocytes (cells/mL)	2307.3	(±640.7)	2161.0	(±625.5)	0.251
Neutrophil/lymphocyte ratio	3.5	(±1.2)	3.2	(±1.2)	0.121
Platelet/lymphocyte ratio	123.4	(±50.3)	120.8	(±44.0)	0.784

\*By student's t test.

**Table 3. Mean NLR by type of diabetes and control group.**

Group	N	Mean NLR	(±SD)	p by ANOVA	LSD groups	p (LSD)
Type 1 DM	6	3.15	(±0.95)		Type I X Type II	0.203
Type 2 DM	20	3.86	(±1.22)		Type I X GDM	0.692
GDM	24	3.37	(±1.14)	0.174	Type I X Control	0.979
Control	50	3.16	(±1.22)		Type II X GDM	0.174
Total	100	3.35	(±1.20)		Type II X Control	0.029
					GDM X Control	0.496

DM: Diabetes mellitus; GDM: Gestational Diabetes mellitus

**Table 4. Mean PLR by type of diabetes and control group.**

Group	N	Mean PLR	(±SD)	p by ANOVA	LSD groups	p (LSD)
Type I DM	6	108.03	(±40.25)		Type I X Type II	0.124
Type 2 DM	20	141.62	(±56.87)		Type I X GDM	0.848
GDM	24	112.12	(±43.32)	0.164	Type I X Control	0.525
Control	50	120.84	(±43.98)		Type II X GDM	0.039
Total	100	122.13	(±47.01)		Type II X Control	0.094
					GDM X Control	0.452

DM: Diabetes mellitus; GDM: Gestational Diabetes mellitus

**Table 5. Means of neutrophil/lymphocyte ratio by indicators of bad obstetric history among the diabetics.**

Neutrophil/lymphocyte ratio				
	N	Mean	(±SD)	P*
<b>Miscarriages</b>				
Yes	24	3.57	(±1.16)	0.850
No	26	3.51	(±1.19)	
<b>Stillbirth</b>				
Yes	11	4.34	(±1.13)	0.008
No	39	3.31	(±1.09)	
<b>Fetal anomalies</b>				
Yes	4	4.93	(±0.85)	0.011
No	46	3.42	(±1.12)	
<b>Macrosomia</b>				
Yes	20	3.56	(±1.27)	0.915
No	30	3.52	(±1.12)	

\*By t test of two independent samples.

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**Table 6. Means of platelet/lymphocyte ratio by indicators of bad obstetric history among the diabetics.**

Platelet/lymphocyte ratio				
	N	Mean	(±SD)	P*
<b>Miscarriages</b>				
Yes	24	123.08	(±48.43)	0.963
No	26	123.75	52.87)	
<b>Stillbirth</b>				
Yes	11	154.09	(±58.69)	0.020
No	39	114.78	(±44.76)	
<b>Fetal anomalies</b>				
Yes	4	174.64	(±50.32)	0.032
No	46	118.98	(±48.26)	
<b>Macrosomia</b>				
Yes	20	131.30	(±43.07)	0.372
No	30	118.18	(±54.62)	

\* By t test of two independent samples.

### DISCUSSION:

The findings from this study show that the NLR and PLR are not significantly different between diabetic ladies presenting at gestational ages of between 24 weeks and 36 weeks and non-diabetic pregnant women. According to a literature search with the related keywords in relevant topics, this is the first prospective study to compare both NLR and PLR in patients with Type1, Type2 and GDM.

A previous study results have showed that high WBC count is associated with GDM and T2DM. In a prospective study of 2,753 pregnant, Wolf et al<sup>18</sup> stated that in comparison with the non-diabetic pregnant ladies, the first trimester WBC count was significantly higher in the pregnant who have GDM at  $10.5 \pm 2.2 \times 10^3$  cells/mL; they also found linear correlation between the increment in the WBC count and the abnormal OGTT during the second and third trimesters. They finally stated that their results confirmed the presence of inflammation as an essential element of the etiology of GDM. However, the neutrophil and lymphocyte counts were not estimated individually in their study and, thus, the NLR and PLR were not calculated.

In our study, the neutrophil and the lymphocyte counts were higher in the Type1, Type2 and GDM groups compared to the comparison group, but the only statistically significant difference was found in the neutrophil count. The results of a study of 42 pregnant women with GDM and 68 non-diabetic pregnant ladies conducted by Yilmaz et al<sup>19</sup> showed that NLR was significantly higher in ladies with GDM.

The WBC count's upper limit in their study was 12,000cells/mL, and they excluded pregnant ladies whose WBC counts exceeded this limit. They found that the average WBC count was  $7,715 \pm 1,463$  cells/mL in the GDM group. Yilmaz et al distributed their subjects into the GDM group and non-GDM group but they did not further provide any subgroups. However, increments in WBC counts of up to 17,000 cells/mL during the second and third trimesters are considered normal hematological changes that are associated with pregnancy<sup>20</sup>. The WBC count upper limit in our study was 17,000 cells/mL, and Type1, Type2 and GDM groups comprised part of the study. While Yilmaz et al<sup>21</sup> retrospectively studied complete blood count parameters of 200 pregnant ladies in their first trimester, 100 of them with GDM and 100 healthy pregnant ladies; and found significant increment in platelet count ( $P=0.002$ ), neutrophil count ( $P=0.011$ ) and lymphocyte count ( $P=0.026$ ) while the NLR and PLR showed no significant difference between both groups. In our study the NLR and PLR showed also no statistically significant difference between both groups while in contrary to this study; our study showed no significant difference between groups regarding lymphocyte and platelets counts.

This difference may be attributed to the setting of the study where Vural Yilmaz undertook it at the first trimester while ours was undertaken at the second and third trimesters where platelets tend to decrease while the total WBC count to increase.

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A study by Sargin et al<sup>(22)</sup>, involved a retrospective analysis of 762 healthy and pregnant ladies with GDM. The subjects were distributed into four groups:

group 1: GDM, group 2: impaired glucose tolerance, group 3: only screen positive, and group 4: control. The results also showed no significant difference among groups in respect of NLR and PLR.

A study by Hussain et al<sup>(23)</sup> was conducted on 330 type 2 diabetic patients who were split into three groups depending on their control of diabetes: Patients in group A

with HbA1c  $\leq$  7% (excellent control), group B HbA1c 7.0-9.0 % (poor control) and group C HbA1c  $\geq$  9 % (worst control). They found that the value of NLR was significantly elevated in the worst-control (Group C) as compared to the poor-control (Group B) and excellent-control (Group A) diabetes; the worse control has more badly perinatal outcomes which are comparable with current study.

In our study we found comparable results where the NLR value in Type2 diabetic pregnant ladies was significantly higher than the NLR value in the non-diabetic control pregnant ladies.

### CONCLUSION:

There was no significant difference in terms of values of both NLR and PLR between the diabetic group and the comparison non-diabetic group. On the other hand, there was a significant difference in terms of NLR in T2DM when compared to the comparison group and in terms of PLR in T2DM when compared to the GDM group.

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