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The value of complete blood count parameters in predicting preterm delivery

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Abstract. – OBJECTIVE: The association between inflammation and threatened preterm labor (TPL) is well known. The aim of this study was to investigate a possible relation between TPL and new defined systemic inflammatory markers, neutrophil to lymphocyte ratio (NLR), lymphocyte to monocyte ratio (LMR) and platelet to lymphocyte ratio (PLR).

PATIENTS AND METHODS: Fifty-three healthy pregnant women as the control group and 55 pregnant women diagnosed with TPL as the study group were included in this prospective controlled study. The study group was divided into two groups according to the gestational age at delivery: pregnant with TPL who delivered < 37 weeks and pregnant with TPL who delivered ≥ 37 weeks. The clinical and laboratory data were compared among the groups.

RESULTS: LMR was found to be significantly higher in patients complicated with TPL who delivered prematurely when compared to the women whose pregnancies continued to term ($p = 0.02$). Furthermore, a significant negative correlation was also observed between the gestational week at delivery and LMR in women who delivered < 37 weeks.

DISCUSSION: We demonstrated that LMR was significantly increased in pregnant women with preterm birth and threatened preterm labor than healthy subjects.

CONCLUSIONS: Although increased LMR levels were demonstrated to be associated with preterm birth and threatened preterm labor to be able to extrapolate these findings into clinical daily practice, further studies are needed.

Key Words:
Inflammation, Neutrophil, Lymphocyte, Monocyte, Preterm delivery, Pregnancy.

Introduction

Preterm delivery a multifactorial syndrome is defined as birth between 20 0/7 and 37 0/7 weeks of gestation regardless of the birth weight[1]. Preterm delivery is still one of the most important reason of neonatal mortality and morbidity. Nearly 75% of perinatal deaths occur in infants born prematurely[2]. While spontaneous preterm labor and preterm rupture of membranes (PROM) constitute 80% of preterm births, the rest is caused by the maternal and fetal disorders[3].

Labor is defined clinically by regular, painful uterine contractions with progressive cervical effacement and dilation. Inflammation has been associated with the mechanisms responsible for the onset and maintenance of both term preterm labor[2,3].

It has been reported that 75% of women presenting with threatened preterm labor (TPL) remain initially undelivered after an initial course of tocolytics of 48 hours, their risk of preterm delivery after this period is still increased; 65% of women deliver before 37 weeks[3,4]. However, it is not easy to identify women presented by TPL who will give birth preterm.

Clinical inflammation is defined by fever; pain, redness and swelling that reflect the effects of inflammatory mediators on local blood vessels and tissues. Unlike it, subclinical, inflammation is defined by the infiltration of tissue by neutrophils, macrophages and lymphocytes[4]. Both clinical and subclinical infection and inflammation play an important role in the etiology of preterm birth[4,5]. It has been reported that most cases of histopathological inflammation and chorioamnionitis, both in preterm and term delivery are sub-clinical[4].

Although the specific tests and monitoring modalities, such as measurement of cervical length with transvaginal ultrasound, bacterial vaginosis testing or fetal fibronectin screening have been proposed in the prediction of preterm

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labor, none has been demonstrated to improve the perinatal outcomes[1,5].

Systemic inflammation can be measured using a variety of biochemical and hematological markers. Recent evidence indicates that measurement of the ratio of sub types of blood cells like neutrophil to lymphocyte ratio (NLR), Lymphocyte to monocyte ratio (LMR) and platelet to lymphocyte ratio (PLR) might have prognostic significance for diseases related to chronic low-grade inflammation[6,7].

Inflammatory markers have been suggested previously to be potential predictors of preterm deliveries, therefore, in this study, we aimed to study the association between preterm birth and LMR, NLR and PLR levels in a group of women who were hospitalized with the diagnosis of threatened preterm labor.

Patients and Methods

This prospective case-control study was conducted at Gaziantep Maternity Hospital, Gaziantep, Turkey, between January 2011 and March 2012. The local Institutional Board of Ethics approved the study and signed informed consent was obtained from participants. The universal principles of the Helsinki Declaration were applied.

TPL was defined as the presence of at least 4 regular and painful uterine contractions in 20 minutes or 6 contractions in 60 minutes, which continued at least 30 seconds, as measured by an electronic cardiotocography device, with the absence or ≥ 20 mm of cervical dilatation and/or ≥ 80% of cervical effacement between 24 and 37 0/7 weeks of gestation[1]. Gestational age was determined based o0n the first day of the last menstrual period and first trimester ultrasonographic measurement of the crown-rump length. Patient with signs and symptoms of active infection (urinary infection, chorioamnionitis) or fever of unknown origin, or any pregnancy complication or severe anemia, who smoke and also with a history of having preterm labor, second trimester abortion, repeated pregnancy loss, cervical incompetence, uterine anomalies, patients with any chronic inflammatory diseases like ulcerative colitis, Crohn's diseases, rheumatoid arthritis, familial Mediterranean fever and pre-pregnancy obesity (body mass index [BMI] ≥30, kg/m^2); and pregnant with preterm rupture of membranes were not included the study.

During the study period, 120 consecutive singleton pregnant women with intact membranes 60 of whom diagnosed of having TPL and 60 of whom as the matched control cases without any complication of pregnancy, aged 17-35 years between 24 0/7 and 37 0/7 week of gestation, were included in this study. Routine blood tests were performed in patients at the admission. All of the pregnant were examined for infection, routine urine cultures were obtained and body temperature was measured by commercially available thermometer (Thermoscan IRT 4520; Braun, Kronberg, Germany). Patient with any signs and symptoms of active infection (pain, fever, or vaginal discharge), were excluded from the study. The clinical data including the age, body mass index (BMI), gravidity, parity, the clinical findings of antenatal follow-up and gestational age at delivery, also the laboratory data and vital findings (body temperature, blood pressure) were recorded for each participant. All pregnant were followed until delivery.

A total of 4 pregnant excluded from the study group due to noncompliance and missing data during the study period. Another one in the study group had to be discarded from the study due to developed preeclampsia. A total of 2 pregnant excluded from the control group due to noncompliance and missing data. 5 pregnant in the control group were excluded since delivered prematurely.

In the end, the data of 55 pregnant in the study groups and 53 healthy pregnant in the control group were evaluated for the final analysis. The study group was further divided into two groups according to the gestational age at delivery; women with TPL who delivered before 37 weeks and the ones who gave birth at term, respectively. Then the clinical and laboratory data were compared to the control group, which comprised women with normal healthy pregnancy.

High sensitive C-Reactive Protein (CRP) measurements were performed by Tina-quant CRP immunoturbidimetric assay (Roche COBAS INTEGRA, 6343 Rotkreuz, CH) at the biochemistry laboratory of the hospital. Complete blood count including white blood cells (WBC), hemoglobin, hematocrit, and platelets were measured by an automatic hematology analyzer at the central laboratory of the hospital. Measurements were completed within one hour of blood sampling. LMR value was calculated by dividing the absolute lymphocyte to absolute monocyte count. NLR and PLR values were calculated by dividing the absolute neutrophil and platelet counts, respectively, by the absolute lymphocyte counts.

Statistical Analysis

Statistical analyses were conducted with Statistical Package for the Social Sciences, version 11,5 (SPSS Inc., Chicago, IL, USA). The sample size was determined according to the results of the central limit theorem[8]. Values were expressed as the means± SD. Student's t test for continuous variables and chi-square statistics for categorical variables were used to investigate the differences between the groups. The one-way analysis of variance (ANOVA) was applied to determine the differences between the means of independent groups. Spearman linear correlation analysis was used to investigate the relationships between LMR, NLR and PLR levels and neonatal birth weight and delivery week. The best cut-off points of LMR to discriminate patients and control groups each other was evaluated by ROC analysis calculating AUC as giving the maximum sum of sensitivity and specificity for the significant test. Sensitivity, specificity, positive and negative predictive values were also calculated at the best cut-off point for LMR. *P*-values < 0.05 were considered statistically significant.

Results

In this study, a total of consecutive 108 singleton pregnancies, 53 (49.1%) were in the control group (Group 1) and 55 (50.9 %) were in the threatened PTL groups. In PTL group 25 pregnant reached term pregnancy (Group 2), and 30 pregnant delivered prematurely (Group 3).

The demographic data of the groups are depicted in Table I. Age, gravidity, parity, maternal weight and height; body temperature and educational status were comparable between the groups. The mean birth weights in the control (group 1), threatened PTL group (group 2) and preterm delivery group (Group 3) were 3458 ± 461 g, 3270 ± 510 g and 2550 ± 745 g, respectively (Group 1 *vs*. Group 2 and Control $p < 0.001$).

The biochemical data of the groups are showed in Table II. As seen in the table, admission levels of hemoglobin, hematocrit, WBC, platelet, and PLR values were comparable among the groups. CRP values significantly elevated study groups compared to control group. The NLR values were significantly higher in the group 2 compared to the group 1 ($p < 0.01$) and group 1 compared to the group 2 ($p < 0.01$). There was no significant difference between group 2 and 3. The mean LMR was significantly different in each group. LMR levels were higher in the group 2 group than in the control group and higher in the group 3 than in both of the other groups (Table II).

At a cutoff level of 4.25, LMR accurately predicted preterm delivery {AUC = 0.728 (95% confidence interval 0.721-0.912), $p = 0.003$} with sensitivity and specificity rates of 75.2% and 78.7% and positive and negative predictive values of 75% and 71%, respectively. Pearson's correlation analysis revealed a significant negative correlation between LMR and gestational age at delivery (r = -0.271, $p = 0.04$).

Table I. Demographic and obstetric characteristics of the Control (Group 1), TPL reached term pregnancy (Group 2) and TPL delivered prematurely (Group 3) groups.

Parameter	Group 1 (n = 53)	Group 2 (n = 25)	Group 3 (n = 30)	*p*-value
Maternal age, yrs	26.7± 8.4	24.4± 4.2	26.4 ± 8.2	NS
Gravidity, range	2 (0-2)	2 (0-2)	2 (1-2)	NS
Parity, range	1 (0-1)	1 (0-1)	1 (0-1)	NS
Weight, kg	76.9 ± 20.1	77.7 ± 3	74.7 ± 6	NS
Height, cm	161.8 ± 4.8	163 ± 4.8	161 ± 7	NS
Body temperature, °C	36.7± 0.8	36.8 ± 0.5	37 ± 0.6	NS
Educational status, n (%)				
Primary	15 (28.3%)	7 (28%)	8 (26.6%)	NS
Secondary	28 (52.8%)	13 (52%)	16 (53.4%)	NS
Post secondary	10 (18.8%)	5 (20%)	6 (20%)	NS
Gestational week at assessment	32.1 ± 2.4	32.4 ± 2.1	31.1 ± 1.9	NS
Gestational week at delivery	40.1 ± 1.2[a]	39.4 ± 1.4[a]	35.1 ± 1.4[b,c]	< 0.001
Birth weight, g	3458 ± 461[a]	3270 ± 510[a]	2550 ± 745[b,c]	< 0.001

Data expressed as number (%), mean ± SD. TPL, threatened preterm labor. *The mean difference is significant at the 0.05 level. NS: Non-significant. [a]Different from the Preterm Delivery Group. [b]Different from the Term Delivery Group. [c]Different from the control group.

Table II. Comparison of serum levels of inflammatory markers and the other blood parameters among the Control (Group 1), Threatened PTL (Group 2) and Preterm Delivery (Group 3) groups.

Parameters, ± SD	Group 1 (n = 53)	Group 2 (n = 25)	Group 3 (n = 30)	*p*-value
WBC count, $\times 10^3/mm^3$	8.7 ± 4.5	10.1 ± 3.4	10.8 ± 3.9	NS
Hemoglobin, g/dL	12.1 ± 0.2	11.7 ± 0.3	12.4 ± 0.6	NS
Hematocrit	37.1 ± 4.1	34.1 ± 2.7	36.8 ± 3.6	NS
Platelet count, $\times 10^3/mm^3$	220 ± 56	239 ± 76	229 ± 58	NS
NLR	4.77 ± 3.18[a,b]	5.21 ± 2.77[c]	5.29 ± 2.98[c]	< 0.01
PLR	136 ± 88	139 ± 56	137 ± 40	NS
LMR	2.62 ± 2.4[a,b]	3.18 ± 1.75[b,c]	3.67 ± 2.9[a,c]	0.02
CRP, mg/dl	0.59 ± 1.7[a,b]	1.4 ± 2.5[c]	1.6 ± 1.1 c	0.01

CRP: C-reactive protein, NLR, Neutrophil to lymphocyte ratio; PLR, Platelet to lymphocyte ratio; WBC, white blood cell. *The mean difference is significant at the 0.05 level. NS: Non-significant. a Different from the Group 2. b Different from the Group 3. c Different from the group 1.

Discussion

Preterm delivery continues to be the main participator to long-term morbidity and mortality. Although multiple different mechanisms and factors have been identified in the pathophysiology of preterm delivery – including inflammation and infection[3,4,10,11]; defective placentation; malfunction in genetic, immunological, hormonal, and angiogenic mechanisms[3,10,12;] and increased oxidative stress[13] an understanding of the exact pathophysiology of the disease remains elusive.

Normal human parturition and labor are multifactorial physiologic events involving an integrated set of changes within the maternal tissues and fetal membranes. There is mounting evidence that labor is an inflammatory process that accompanied by increased expression of cell adhesion molecules, chemotactic agents such as interleukin (IL)-8, and proinflammatory cytokines and WBC activation. Despite its significance as a marker of infection, WBC count is normally increased during pregnancy and is strongly affected by factors like stress or physical activity[14-16]. Yuan et al[16] conclude that WBC in peripheral blood is primed in preparation for activation during term and preterm labor. They suggested that this might contribute to the pathophysiological events of parturition. Furthermore, it has been shown that inflammatory cells mainly neutrophils and macrophages massively infiltrate myometrium and cervix during parturition[17,18]. Although WBC count was suggested to predict upcoming preterm birth[18,19], mean WBC count was comparable within the groups, in the present study.

In addition to normal variations in the WBC count, the distribution of cell types is altered significantly during pregnancy. Specifically, the ratio of granulocytes and T helper (Th)-1 lymphocytes are significantly elevated with a concomitant reduction in the ratio of Th-2 lymphocytes and monocytes, during the third trimester[15]. Macrophages and monocytes play a major role in placental development. They promote invasion of the extravillous trophoblast and spiral artery remodeling, and the parturition process. It has argued that deregulation of these cells may lead to many complications of pregnancy, including abortion, and preeclampsia, preterm labor. Probably stem from deviant immunological and inflammatory responses in either the mother or the fetus[20].

LMR has been proposed as a surrogate marker for inflammation and it also has prognostic and predictive value. A high monocyte count or a low lymphocyte count has separately been shown as an adverse effect of prognosis in various disorders[6,8]. In this study, LMR was found to be significantly higher in patients complicated with TPL who delivered prematurely when compared to the women whose pregnancies continued to term ($p = 0.02$). Furthermore, a significant nega-

Table III. Correlations of gestational week at delivery with hematological markers.

	WBC	NLR	LMR	CRP
r*	-0,01	-0,003	-0,273	-0,03
p	0,82	0,96	0,04	0,08

*Spearman's correlation coefficient. NLR, Neutrophil to lymphocyte ratio; LMR, lymphocyte to monocyte ratio; WBC: white blood cell, CRP; C-reactive protein.

tive correlation was also observed between the gestational week at delivery and LMR in women who delivered prematurely.

To the best of our knowledge, this is the first study to explore the possible relationships among TPL and NLR, PLR and LMR and other routine hematologic parameters.

Conclusions

Although increased LMR levels were demonstrated to be associated with preterm birth and threatened preterm labor, respectively, to be able to extrapolate these findings into clinical daily practice, further studies are needed.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

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