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Original Research

Evaluating the Predictive Potential of Neutrophil-to-Lymphocyte and Platelet-to-Lymphocyte Ratios in the Second-Trimester Preterm Premature Rupture of Membranes (PPROM): A Retrospective Case-Control Study

Pınar Tuğçe Özer[1], Mehmet Özer[2], Süleyman Cemil Oğlak[3,*], Adnan Budak[4], Abdurrahman Hamdi İnan[4], Gökhan Tosun[4], Emrah Töz[4], Emine Zeynep Yılmaz[5], Varol Gülseren[6], Gökhan Bolluk[7], Mehmet Özeren[2]

[1]Department of Obstetrics and Gynecology, Ministry of Health Kemalpaşa State Hospital, 35170 Izmir, Turkey
[2]Department of Perinatology, Health Sciences University, Tepecik Training and Research Hospital, 35020 Izmir, Turkey
[3]Department of Obstetrics and Gynecology, Health Sciences University, Gazi Yaşargil Research and Training Hospital, 21070 Diyarbakır, Turkey
[4]Department of Obstetrics and Gynecology, Health Sciences University, Tepecik Training and Research Hospital, 35020 Izmir, Turkey
[5]Department of Obstetrics and Gynecology, Bahçelievler Memorial Hospital, 34180 Istanbul, Turkey
[6]Department of Gynecologic Oncology, Erciyes University Faculty of Medicine, 38039 Kayseri, Turkey
[7]Department of Perinatology, Health Sciences University, Başakşehir Çam and Sakura City Hospital, 34480 Istanbul, Turkey
*Correspondence: sampson_21@hotmail.com (Süleyman Cemil Oğlak)
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Abstract

Background: Proteolytic enzymes and specific cytokines have been associated with the underlying mechanism of preterm premature rupture of membranes (PPROM), contributing to weakened amniotic membranes. This study aims to elucidate the predictive role of neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR) during the early stages of PPROM, given the limited existing literature exploring this relationship in second-trimester cases. **Methods**: This retrospective case-control study was performed from June 2018 to June 2023. We included 159 pregnant women diagnosed with mid-trimester PPROM and 573 pregnant women who gave birth at term. We obtained complete blood cell counts in all patients. We used a receiver operating characteristic (ROC) curve to evaluate the cut-off, sensitivity, and specificity values. **Results**: Complete blood count evaluations revealed that in the mid-trimester PPROM group, neutrophil count, leukocyte count, hemoglobin (Hb) levels, NLR, and PLR were notably higher than those delivering after the 37th gestational week. These observations were identified not only in the first-trimester but also within 24 hours after membrane rupture. Contrary to prior research and to conventional patterns, our study identified a diminished mid-trimester NLR in cases of PPROM compared to the control group. **Conclusions**: In our study, we identified significant differences in lymphocyte counts, platelet levels, NLR, and PLR values between the PPROM group and the control group. Our study suggests that the NLR and PLR values from the first-trimester might be powerful indicators of PPROM risk.

Keywords: neutrophil-to-lymphocyte ratio; platelet-to-lymphocyte ratio; mid-trimester preterm premature rupture of membranes

1. Introduction

Preterm premature rupture of membranes (PPROM) is characterized by the premature rupture of the fetal membrane occurring before the 37th week of pregnancy. This condition is associated with significant complications that can give rise to serious health issues in both the mother and the baby [1]. Furthermore, mid-trimester PPROM refers to a condition in which the rupture of the membranes occurs before reaching the fetal viability threshold, prior to the 24th week of pregnancy and accounting for less than 1% of pregnancies. Fetuses born at the 22nd week of pregnancy have only a 1% chance of survival, irrespective of the presence of neurodevelopmental disorders [2,3].

It is believed that proteolytic enzymes, presumed to play a role in acute inflammation, along with certain cytokines in the amniotic fluid that initiate apoptosis and activate matrix metalloproteinase (MMP), are also associated with the PPROM mechanism. As a consequence of such mechanisms, it is hypothesized that the amniotic membranes become weakened and ultimately rupture [4].

The control of the immune response and the regulation of the immune system are believed to be dependent on the mobility of circulating lymphocytes. However, it has been reported that platelet aggregation is not only vital for hemostasis but also holds crucial significance for the immune system. The neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR) have been evaluated in numerous clinical studies as indicators of systemic inflammation when patients do not exhibit a distinct infection [5]. Moreover, findings that support the potential of increased levels of NLR and PLR have been reported to

be predictive markers in cases with PPROM [6,7]. Additionally, in chronic inflammatory processes, an increase in megakaryocytic series and a decrease in lymphocytes due to apoptosis can affect indicators such as the PLR [6].

In this study, we conducted a retrospective analysis to evaluate the relationship between serum NLR and PLR in patients diagnosed with PPROM and the predictive ability of these markers. The current literature provides limited data regarding the relationship between NLR and PLR in second-trimester PPROM cases. Therefore, we aimed to investigate the predictive role of NLR and PLR in the early stages of PPROM.

2. Materials and Methods

Our research study was conducted at the Health Sciences University Tepecik Training and Research Hospital. Ethical committee approval was obtained with the number 2023/07/18. Our study focused on pregnant women who experienced PPROM in the second-trimester of pregnancy and a healthy control group who gave birth after the 37th week of gestation (third-trimester of pregnancy) between June 2018 and June 2023. In our study, we assessed the demographic data of the patients with second-trimester PPROM. We compared complete blood count (CBC) parameters obtained during the first-trimester visits of pregnancies resulting in PPROM and those leading to term births (control group). Additionally, we evaluated blood parameters taken within the first 24 hours after membrane rupture in PPROM patients. Furthermore, we compared these parameters with CBC data obtained during delivery for patients who gave birth at term. Comparisons were made between the two groups.

Hemogram values of the PPROM-diagnosed women were meticulously examined during both the first-trimester and their presentation time, while the control group, composed of healthy pregnant women, had their hemogram values reviewed during the first-trimester and at childbirth.

Exclusions from our study encompassed patients with factors such as multiple pregnancies, cervical cerclage, fetal anomalies, and specific risks for preterm birth. Furthermore, individuals who had PPROM without a birth record, those with hematological disorders, and those showing active infection symptoms were excluded from the study. Moreover, neither group included cases with potential sources of inflammation, such as gestational diabetes, *in vitro* fertilization (IVF) pregnancies, and gestational hypertension.

Cases with PPROM before 24 weeks were hospitalized for the entire expectant management period. Upon admission, a single dose of 1 gram of oral Azithromycin was administered, in addition to 2 grams of intravenous Ampicillin every 6 hours for 7 days. Antenatal corticosteroids were recommended. Tocolysis aimed to delay delivery for 48 hours for corticosteroid administration. Daily non-stress test (NST) and CBC were performed, along with twice-weekly C-reactive protein (CRP) analysis. If NST results were concerning, a biophysical profile was conducted. Intrauterine growth restriction (IUGR) was defined as estimated fetal weight (EFW) $<$3rd centile based on sonographic measurements of fetal biometry along with end-diastolic flow loss on Doppler examination [8].

Statistical Analysis

Data analysis was conducted using the Statistical Package for the Social Sciences (SPSS) version 26 (IBM Corp, Chicago, IL, USA). The normality of continuous variables was assessed considering graphical investigation, normality tests, and sample size. Comparisons of independent groups were made using the Student's *t*-test for variables with a normal distribution and the Mann-Whitney U test, a non-parametric method, for those without a normal distribution. To calculate the cutoff value, a receiver operating characteristic (ROC) analysis was performed, and the most suitable cutoff values were determined based on Youden's index. For all statistical comparison tests, the type I error rate α was set at 0.05, and two-tailed tests were conducted. A p-value less than 0.05 was considered statistically significant for differences between groups.

3. Results

In our study, we evaluated the demographic data of patients with second-trimester PPROM and compared it with corresponding control groups that resulted in to term births. We compared CBC parameters obtained during the first-trimester visits of pregnancies resulting in PPROM and those from the control group. We also assessed the blood parameters taken within the first 24 hours after membrane rupture in PPROM patients, as well as the CBC parameters taken during delivery for patients from the control group. Comparisons were made between the two groups.

In the study, the PPROM group comprised 159 of the patients, whereas the control group consisted of 573 individuals. As shown in Table 1, the median maternal age for those with PPROM was 29 years (range 18–44 years). In this group, the average gravidity was found to be 2.30 $\pm$ 1.2 and the average parity was 1.13 $\pm$ 1.08. Additionally, the proportion of those with a history of one prior abortion was approximately 20% (n = 33), instead of the rate of those with a history of recurrent abortions was 9.4% (n = 15).

Furthermore, in the PPROM group, the average membrane rupture occurred at 18.7 $\pm$ 2.3 weeks, and the average gestational age at birth for these patients was determined to be 19.4 $\pm$ 2.7 weeks (data not shown). Additionally, in this group, 15 babies (9.4%) were born alive (data not shown). During the research, antepartum hemorrhage was detected in 3 individuals (1.8%) of the PPROM group, with 2 of these cases (1.2%) determined to be due to placental abruption (data not shown). Furthermore, chorioamnionitis was observed in 15 individuals (9.4%) of the PPROM group (data not shown).

Table 1. Comparison of the two groups based on the demographic parameters and complete blood evaluations taken during the first-trimester visits.

Variables	Mid-trimester PPROM group (n = 159)	Control group (n = 573)	*p*-value
Maternal age, years	29 (18–44)	28 (18–43)	0.191
Gravidity, n	2.30 ± 1.2	2.42 ± 1.3	0.282
Parity, n	1.13 ± 1.08	2.11 ± 1.05	**0.001**
Previous abortion, n (%)	33 (20%)	95 (16.6%)	0.080
Neutrophil count (mm^3)	9820.1 ± 3378.1	5442.4 ± 1644.1	**0.001**
Platelet ($10^3/\mu L$)	202.7 ± 57.7	254.82 ± 62.6	**0.001**
White blood cell (mm^3)	11,191.2 ± 2443.1	8000.9 ± 1933	**0.001**
Lymphocyte count (mm^3)	1375.5 ± 505.9	1881.8 ± 629.4	**0.001**
Hb (g/dL)	10.6 ± 0.9	12.3 ± 1.1	**0.001**
NLR	8.6 ± 6.3	3.1 ± 1.5	**0.001**
PLR	166.7 ± 80	146.1 ± 53.3	**0.021**

Hb, hemoglobin; NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio; PPROM, preterm premature rupture of membranes. Bold values denote statistical significance at the $p < 0.05$.

In the study, 573 patients were included in the control group. The median maternal age in this group was 28 years (range 18–43 years). As shown in Table 1, the average gravidity and parity were 2.42 ± 1.3 and 2.11 ± 1.05, respectively. Notably, 16.6% (n = 95) had a history of one prior abortion, while 7.1% (n = 41) had a history of recurrent abortions. The study and control groups were similar regarding maternal age, gravidity, and a history of previous abortions. However, the average parity was significantly higher in the mid-trimester PPROM group compared to the control group ($p = 0.001$).

Based on the CBC evaluations taken during the first-trimester visits for both groups, the mid-trimester PPROM group exhibited significantly higher neutrophil count, leukocyte count, NLR, and PLR, while the hemoglobin (Hb) level was significantly lower compared to the control group. In contrast, platelet and lymphocyte counts were significantly higher in the control group compared to the PPROM group (Table 1).

For the control group, the CBC parameters taken during delivery were compared with those taken within the first 24 hours after membrane rupture in the PPROM group. Accordingly, the platelet ($p = 0.001$), lymphocyte counts ($p = 0.001$), and Hb values ($p = 0.008$) were significantly higher in the mid-trimester PPROM group compared to the control group. However, the neutrophil and leukocyte counts, and NLR ratio were significantly higher in the term birth group ($p = 0.001$). Additionally, the PLR ratio did not produce a statistically significant result when comparing the blood values obtained in the first 24 hours after membrane rupture between the two groups (Table 2).

Using ROC analysis, the threshold values for NLR and PLR parameters were calculated based on the Youden index. The NLR was 3.92 with an area under the curve (AUC) of 0.86 (95% confidence interval (95% CI) = 0.82–0.90, $p < 0.001$), and the PLR was 141.83 with an AUC of 0.56 (95% CI = 0.50–0.61, $p = 0.21$) (Fig. 1).

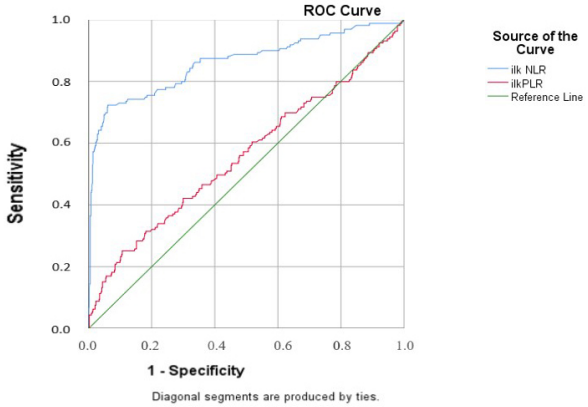

Fig. 1. ROC analysis curve of NLR and PLR values. ROC, receiver operating characteristic.

Comparisons based on the NLR cutoff value showed statistically significant differences between the first-trimester Hb levels and viability values ($p = 0.001$, and $p = 0.028$, respectively). However, comparisons of the NLR cutoff value with histories of IUGR (1.8%), PPROM (2.5%), and preterm birth (7.5%) were statistically insignificant ($p = 0.136$, $p = 0.273$, and $p = 0.218$, respectively) (data not shown).

Comparisons based on the PLR cutoff value indicated that there was no statistically significant relationship between the first-trimester Hb levels, viability values, and previous histories of IUGR, PPROM, and preterm birth ($p = 0.607$, $p = 0.593$, $p = 0.427$, $p = 0.0248$, and $p = 0.725$, respectively) (data not shown).

4. Discussion

In this study, patients with second-trimester membrane rupture (PPROM group) were compared to term-birth controls (control group). First-trimester CBC parameters were analyzed for both groups, with PPROM patients also

Table 2. Comparison of the two groups based on the complete blood count (CBC) parameters taken within 24 hours after membrane rupture.

Variables	Mid-trimester PPROM group (n = 159)	Control group (n = 573)	*p*-value
Neutrophil count (mm^3)	9205 ± 3303.1	10,592 ± 2858.8	**0.001**
Platelet ($10^3/\mu L$)	223.68 ± 53.816	195.7 ± 58.8	**0.001**
White blood cell (mm^3)	11,420.1 ± 2755.6	12,819.2 ± 3147	**0.001**
Lymphocyte count (mm^3)	16,245 ± 5175.1	13,888 ± 4892	**0.001**
Hb (g/dL)	10.8 ± 1	10.5 ± 1.3	**0.008**
NLR	6.4 ± 3.8	8.5 ± 3.8	**0.001**
PLR	145.1 ± 62.2	154.3 ± 63.3	0.071

Hb, Hemoglobin; NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio; PPROM, preterm premature rupture of membranes. Bold values denote statistical significance at the $p < 0.05$.

evaluated 24 hours post-rupture and controls at delivery. Significant differences were found in first-trimester neutrophil, leukocyte, Hb, NLR, and PLR values, while post-rupture values showed variations in platelet, lymphocyte, NLR, and Hb levels.

In both the United States of America (USA) and Europe, preterm births are a significant concern, constituting approximately 12% in the USA pregnancies and 5–9% in Europe, contributing to 40–50% of all premature deliveries. Globally, early births not only result in neonatal deaths but also increase the risk of neurological issues and educational challenges in children [9,10].

Current methods to predict premature delivery, especially among first-time mothers, are limited by their low sensitivity and reliability. Hence, there is an urgent need to improve the identification of individuals at heightened risk for premature births, aiming to decrease its occurrence [10].

In cases of PPROM, inflammation plays a pivotal role. Amniotic membranes, acting as a barrier between fetal and maternal tissues to safeguard the fetus, can exhibit an inflammatory response under specific circumstances [11–14]. The surge of inflammatory mediators may compromise membrane integrity, resulting in premature rupture. Moreover, cytokines and enzymes released during inflammation, particularly MMPs, can disrupt the structure of the amniotic membranes, causing them to weaken and ultimately rupture [11,12,15].

In cases of PPROM, acute-phase reactants typically rise as a sign of inflammation, serving as a potential indicator of intra-amniotic infection or inflammation. Interleukin-6 (IL-6), CRP, and prostaglandins are among the prominent acute-phase reactants associated with PPROM. Among the prostaglandins, especially prostaglandin E2, holds critical importance not only in inflammation but also in the initiation of labor. Its concentrations increase in the amniotic fluid in PPROM situations [16,17].

Neutrophils are myeloid-derived leukocytes that provide the initial response during acute inflammation and provide a primary defense mechanism against many pathogens [18]. In various studies, including those by Ozel *et al*. [19], Esercan *et al*. [7], and Toprak *et al*. [6], it was consistently observed that the neutrophil count in the PPROM group was higher than in the control group, especially in the early stages of pregnancy. However, according to the results of our study, while the neutrophil levels in the PPROM group were higher during the first-trimester, they were found to be lower than in the control group in the mid-trimester, a finding that deviates from earlier research. This latter observation is statistically significant and contrasts with previous findings in the literature.

Peripheral blood lymphocytosis is commonly seen in hematological tests, with lymphocyte levels being crucial for disease diagnosis and management [20]. Research by Ozel *et al*. [19], Esercan *et al*. [7], and Toprak *et al*. [6] consistently found that the lymphocyte count in the PPROM group was lower than in the control group, with specific variations across gestational periods. In line with these previous observations, our research also found a statistically significant lower lymphocyte count in the PPROM group during the first-trimester ($p = 0.001$).

Compared to the first-trimester, when examining the blood parameters obtained within 24 hours after membrane rupture and at birth, we found the lymphocyte counts in the PPROM group to be higher than in the control group. This difference is statistically significant ($p = 0.001$). However, this finding diverges from earlier literature.

When activated, platelets release a range of proteins and factors that bolster the onset of the inflammatory reaction. This release includes growth factors, enzymes, and protease inhibitors. Once activated, platelets stimulate the attraction of cells via chemotaxis and aid in the formation of new tissue [21,22].

Ozel *et al*. [19] and Esercan *et al*. [7] studies found slight platelet count differences in PPROM groups compared to control groups, but these differences were not statistically significant. In contrast, Toprak *et al*. [6] study reported a notably higher platelet count in the PPROM group. Our research found higher platelet levels in the PPROM group post-rupture, but a lower count during the first-trimester. This discrepancy was also statistically significant but deviates from the trends observed in other studies.

Inflammatory processes are becoming increasingly significant in understanding PPROM. Elevated levels of cytokines such as IL-6, IL-8, and tumor necrosis factor-α (TNF-α), which mediate inflammation, have been noted in PPROM, potentially contributing to complications like membrane rupture. Hematological markers, especially NLR, indicate inflammation, with higher NLR values observed in PPROM patients, signifying enhanced inflammatory reactions [19].

Similarly, values of PLR greater than 117.14 have been associated with the onset of PPROM. PLR is another inflammatory marker that reflects the balance between platelet activation and lymphocyte function [6]. No significant differences were found between the groups in terms of platelet counts and PLR values [19].

Furthermore, patients in the PPROM group have been found to have higher NLR values when compared to individuals at risk of preterm birth and healthy controls. Additionally, there is an increased rate of neonatal sepsis risk and admissions to the neonatal intensive care unit in this group. This suggests that assessing inflammatory biomarkers could be significant in predicting potential complications of PPROM.

In studies by Ozel *et al.* [19], Esercan *et al.* [7], and Toprak *et al.* [6], the NLR values in PPROM group were consistently higher than in control group, and these differences were statistically significant [6,7,19]. On the contrary, in our study, the NLR value in the mid-trimester PPROM group was lower than the control group, deviating from prior findings. However, the NLR value in the first-trimester was consistent with the other studies, being higher in the PPROM group compared to the control group.

Studies by Ozel *et al.* [19] and Esercan *et al.* [7] found PLR values in the PPROM group differing from the control group, but these differences were not statistically significant. Toprak *et al.* [6] research noted a statistically significant difference in PLR values between the PPROM and control groups. In our study, although the PLR values did not show a statistically significant difference between the PPROM and control groups, the values examined in the first-trimester were statistically significant between the patient and control groups.

The main limitation of this study study is its retrospective nature. NLR and PLR are increased or decreased in many underlying conditions. Since this was a retrospective analysis these factors could have developed bias during the analysis. Prospective observation studies are recommended to eliminate this bias.

5. Conclusions

This study has conducted an in-depth examination of the clinical profiles of PPROM patients and identified differences in various CBC parameters. Furthermore, no significant associations were found between the latent periods leading up to delivery in PPROM patients and elevated PLR and NLR. In particular, CBC values obtained in the first-trimester may be a potential indicator for predicting the risk of preterm birth. Considering the limitations of current prediction methods, these data can contribute to the development of new assessment methods. It has been observed that neutrophil values increase in PPROM. Further research is needed on NLR and PLR values, and how these findings can be used in clinical practice. This information can guide the development of strategies to reduce the risk of preterm birth and to protect maternal and infant health.

Availability of Data and Materials

Data is available on request from the authors.

Author Contributions

PTÖ, MÖzer, SCO, EZY, VG, and GB designed the research study. MÖzer, AB, AHİ, GT, ET, and MÖzeren performed the research and experimental protocols. PTÖ, MÖzer, SCO, AB, AHİ, GT, ET, EZY, VG, GB, and MÖzeren analyzed the data and statistics. PTÖ and MÖzer wrote the manuscript. PTÖ and SCO made the critical review of the manuscript. All authors contributed to editorial changes in the manuscript. All authors have participated sufficiently in the work to take public responsibility for appropriate portions of the content and agreed to be accountable for all aspects of the work in ensuring that questions related to its accuracy or integrity. All authors read and approved the final manuscript.

Ethics Approval and Consent to Participate

The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Ethics Committee of University Tepecik Training and Research Hospital (approval number: 2023/07-18). We did not obtain informed consent due to the retrospective nature of the study.

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Conflict of Interest

The authors declare no conflict of interest. Süleyman Cemil Oğlak is serving as one of the Guest editors of this journal. We declare that Süleyman Cemil Oğlak had no involvement in the peer review of this article and has no access to information regarding its peer review. Full responsibility for the editorial process for this article was delegated to Michael H. Dahan and Paolo Ivo Cavoretto.

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