Original Article

Preterm PROM Inflammation Marker — Neutrophil-to-Lymphocyte Ratio (NLR) and Platelet-to-Lymphocyte Ratio (PLR)

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ABSTRACT

Introduction: *PPROM delivery is a significant event of obstetrical units of different centers. Usually, the feomaternal outcome related to this complication is not very satisfactory. Therefore, postulating any dependable investigation that can predict the forthcoming PPROM is essential.* ***Methods and materials:*** *The study place was the Department of Obstetrics and Gynaecology, Sir Salimullah Medical College (SSMC) & Mitford Hospital (MH), Dhaka, Bangladesh, from May 2019 to October 2019.* ***Results:*** *It was a case-control study. All mothers were selected by purposive sampling who were preterm PROM as cases. Age-matched non-preterm PROM pregnant women at term were included as control. Afterward, eligibility criteria were used to scrutinize and 200 mothers were included; 100 as cases, and the other 100 as control. A pre-tested, observation-based, peer-reviewed data collection regarding clinical, biochemical, and surgical profiles were done and recorded. The P-value was determined by the chi-square test (categorical variables) and the student's t-test (continuous variables). The p-value was significant at <0.05.* ***Conclusions:*** *The mean age of 100 patients from the case was 24.39±2.81 (age range: 18-36) years, and that of the control, like 100 normal pregnant women, was*

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24.31±2.34 (age range: 19-35). (P=0.49). Correlation analysis revealed that PLR levels were positively correlated with NLR (n=0.11, p=0.29). Therefore, NLR and PLR outcomes accurately and specifically predict spontaneous preterm birth. Preterm birth can be predicted, allowing for delicate and effective management of the pregnancy process.

Keywords: *Neutrophil, Platelet, Lymphocyte, PROM, NLR, PLR*

INTRODUCTION

The amnion and chorion are fetal tissues in origin and play significant roles in maintaining pregnancy by providing multi-level protection to the growing fetus. Fetal membranes accommodate constant challenges (immune, structural, mechanical, and endocrine) during pregnancy; they continue to grow and mechanistically and biochemically, maintaining elasticity to the stretch forces experienced during fetal growth. Although membranes overlaying the placenta and cervix face distinctly different environments and insults during pregnancy, the membranes maintain the homeostatic balance necessary to sustain fetal growth without interruption. This companionship between the fetus and the membranes continues until term, when the fetus reaches maturity, and the membranes reach longevity.

The development of amnion and chorion begins with embryogenesis, although they do not participate directly in forming the embryo or fetus. Like the fetus, early growth of the amnion and chorion layers is rapid and independent of each other. The formation of amnion-chorion as a unit structure is complete by the 12th week of gestation [1]. The membrane composition and its ability to produce a broad spectrum of biomarkers at different stages of gestation illustrate the possible role of the fetal membrane's influence on the growing fetus and in adverse pregnancy outcomes [2].

Specifically, the fetal membrane provides mechanical and immune protection and acts as a barrier to microbial access [3]. This protective role is supported by the biomarkers produced by fetal membranes during gestation and parturition. Compromise in the immune and mechanical properties of the fetal membranes allows for microbial invasion from the genital tract, activation of host inflammatory response leading to collagenolysis mediated mechanical disruption, and membrane weakening predisposing the membranes to Preterm PROM. Abruption-associated thrombin, matrix metalloproteinase (MMP) activation, and collagenolytic processes have also been reported in fetal membrane weakening and Preterm PROM [4]. Clearly, the dysfunctional status of fetal membranes is more evident in Preterm PROM than in spontaneous preterm birth with no rupture of membrane. Thus, Preterm PROM is considered a disease of the fetal membranes and likely a separate entity from spontaneous preterm birth with no ROM [5].

Approximately 70% of Preterm PROM cases are associated with intraamniotic infection (IAI), as documented by positive amniotic fluid (AF) cultures or clinical evidence of infection. However, whether the infection is a cause or consequence of Preterm PROM has been debated. Histological and microbiological findings indicate that focal infection and inflammation may play a primary or

secondary role in the pathogenesis of Preterm PROM [6]. Evidence of inflammatory changes is reported to be adjacent to the putative site of membrane rupture, suggesting that bacterial infection may be an initiator of Preterm PROM [7].

Recent reports have indicated that Preterm PROM may be associated with sterile inflammation in the fetal membranes (an inflammatory condition mimicking infection with no evidence of microbial presence either through culture or molecular methods). We hypothesize that certain risk factors of Preterm PROM (i.e., smoking, bleeding) can produce sterile inflammation in the fetal membranes leading to an immunocompromised state that allows for microbial invasion. Infection in Preterm PROM is likely a secondary phenomenon rather than a causal factor.

Over a decade ago, fetal membrane apoptosis was reported as a pathological mechanism associated with Preterm PROM [8]. Infection and other endotoxins are capable of inducing many of these proapoptotic factors during Preterm PROM that is generally absent in membranes collected after spontaneous preterm birth with no ROM [9,10]. Classic DNA fragmentation pattern (used in diagnosing apoptosis) have been reported in fetal membranes obtained from women with Preterm PROM. However, apoptosis is not often associated with the massive inflammatory response seen in Preterm PROM.

In summary, the roles of infection, inflammation, collagenolytic enzymes, and apoptosis in Preterm PROM have been independently characterized with the expectation that pathways responding to specific risk factors should indicate which initiators and effectors of Preterm PROM are operative in any given case. However, to date, findings from such studies have not produced useful biomarkers or improvements in the rates of Preterm PROM and spontaneous preterm birth.

METHODS AND MATERIALS

From May 2019 to October 2019, a case-control study titled "Preterm PROM Inflammation Marker — Neutrophil-to-Lymphocyte Ratio (NLR) and Platelet-to-Lymphocyte Ratio (PLR)" was conducted at Sir Salimullah Medical College & Mitford Hospital, Dhaka, Bangladesh. The study focused on women diagnosed with preterm premature rupture of membranes (PROM) who presented to the Department of Obstetrics & Gynecology. A total of 200 cases were enrolled, with 100 participants in both the case and control groups. Sample selection involved purposeful sampling of women attending the Gynecology & Obstetrics department of SSMC&MH during the study period to evaluate NLR and PLR as inflammatory markers for detecting preterm PROM. Inclusion criteria comprised preterm PROM patients (cases), age-matched term non-PROM pregnant women (controls), and mothers who consented to participate in the study. Exclusions were made for multiple gestations, hematologic disorders, malignancies, hepatic diseases, autoimmune diseases, inflammatory conditions of pregnancy (e.g., gestational diabetes mellitus, preeclampsia), infectious diseases, fetal chromosomal anomalies, intrauterine growth restriction, fetal infection, and women undergoing invasive procedures such as amniocentesis. Operational definitions included PROM (spontaneous rupture of membranes after the age of viability but before the onset of labor) and preterm-PROM (spontaneous

rupture of membranes after the age of viability but before 37 completed weeks of gestation). Main outcome variables encompassed sociodemographic, obstetric, and hematological profiles, with independent variables including age, parity, WBC count, occupation, gestational age, neutrophil count, lymphocyte count, platelet count, NLR, and PLR. The study procedure involved thorough history-taking, physical examinations, including speculum examinations, and recording of sociodemographic, obstetric, and hematological profiles. Laboratory investigations included complete blood counts using a Coulter LH 780 Hematology Analyzer, with NLR and PLR calculated accordingly. Data were collected through interviews and structured questionnaires, then processed using SPSS version 23, with statistical significance set at $p < 0.05$. Ethically, acutely ill convicts, children, and direct pupils were excluded from the study. Participants were informed about the study procedure and assured that their treatment would not be affected if they chose not to participate. Informed consent was obtained from each participant, and written consent was also secured from the relevant department overseeing the study. Participants had the right to withdraw from the study at any point without consequences.

RESULTS

We studied 200 patients where 100 women were in the case group and the rest 100 women were in the control group. Most were homemakers, and the maximum education level was up to primary. Besides, most candidates were from rural areas.

Table I: Demographic characteristics of respondents (N=200)

Variables	Case (n=100)	Control (n=100)	P-value
Age (in years) (Mean ± SD)	24.392.81	24.31±2.34	0.49^{NS}
Range (in years)	18 – 36	19 – 35	
Parity	2.1±0.9	1.98±0.2	0.35^{NS}
Range	0 – 5	0 – 3	
Occupation			
Housewife	67 (%)	72 (%)	-
Service holder	29 (%)	25 (%)	
Students	3 (%)	3 (%)	
Farmer	1 (%)	0 (%)	
Educational status			
Only can sign/nil	35 (%)	28 (%)	-
Primary	49 (%)	55 (%)	
SSC	10 (%)	13 (%)	
HSC	0 (%)	4 (%)	

Area of residence			
Urban	22 (%)	14 (%)	-
Rural	78 (%)	86 (%)	
P-value was calculated by student's t test (continuous variables) and chi square test (categorical variable) *NS: Not significant, P-value was significant at <0.05*			

Table I shows that in terms of the mean age and parity case and control match with each other and p-value is not significant.

Table II: Obstetric profile of the respondents (N=200)

Obstetric profile	**Case (n=100)**	**Control (n=100)**	**P-value**
Gravida (n)	3.1±1.2	3.4±1.4	0.53^{NS}
Para (n)	2±1.3	1.9±1.4	0.16^{NS}
Gestational age (week)	33.6±2.5	37.3±0.29	0.86^{NS}
P-value was calculated by student's t test, NS: Not significant, P-value was significant at <0.05			

Table II shows that there was no statistically significant difference between case and control regarding mean gravida (3.1±1.2 vs 3.4±1.4), mean para (2±1.3 vs 1.9±1.4) and mean gestational age (33.6±2.5 vs 34.7±1.2) (P=>0.05).

Table III: Hematological profile of the respondents (N=200)

Hematological profile	**Case (n=100)**	**Control (n=100)**	**P-value**
WBC Count (/mm^3)	9.1(6.31–10.7)	8.8 (6.1–9.9)	0.85^{NS}
Neutrophil count (/mm^3)	9936.5±3385.2	7311.1±1593.5	$<0.001^{S}$
Lymphocyte count (/mm^3)	1896.7±651.8	2144.7±673.2	0.5^{NS}
Platelet count (×1000/mm^3)	241.6±58.7	201.7±65.9	$<0.001^{S}$

P-value was calculated by student's t test, S: Significant, NS: Not significant,
P-value was significant at <0.05

Table III shows that the neutrophil count was significant higher in patients with preterm PROM as compared to controls (9936.5±3385.2 vs 7311.1±1593.5/mm^3 of blood, P<0.001). Likewise, the platelet count was found to be significantly higher in PROM or cases (241.6±58.7 vs 201.7±65.9×1000/mm^3, p <0.001).

Table IV: Ratio between platelet to lymphocyte and neutrophil to lymphocyte (N=200)

Hematological profile	Case (n=100)	Control (n=100)	P-value
NLR	5.27±3.2	3.86±2.1	<0.001^S
PLR	125.8±67.1	105.2±48.6	<0.001^S
P-value was calculated by student's t test , S: Significant, NS: Not significant *P-value was significant at <0.05*			

Table IV shows that NLR and PLR both are higher in cases (P=<0.001). Correlation analysis revealed that PLR levels were positively correlated with NLR (n=0.11, p=0.29)

DISCUSSION

Recent investigations showed that inflammation was the primary etiologic factor in preterm PROM [11]. An appropriate diagnostic for identifying preterm PROM that can show intra-amniotic or placental inflammation needs to be improved, despite several inflammatory markers being investigated for this purpose. This study's cases (n = 100) and controls (n = 100) were matched. Between the case and control, none of the sociodemographic factors displayed a statistically significant difference (P=>0.05).

In the current study, the mean age of cases was 24.39±2.81 years, while that of controls was 24.31±2.34 years. These results were consistent with the research conducted by Endale et al [12]. Case and control had respective mean parities of 2.10±9 (in the range of 0-5) and 1.98±0.2 (in the range of 0-3). There was no statistically significant difference between cases and controls in the mean of gravida, para, or gestational age (P>0.05). These results agreed with those of the Turkish study conducted by Jaffar DW et al [13].

A practical and widely recognized inflammatory marker is neutrophil. Neutrophil, which makes up 40% to 60% of WBC, is the body's first line of defense against any invasive invaders. TNF-, IL-8, interferon-gamma, other cytokines, and bacteria activate it. The activated neutrophil then travels to the inflammation area and eliminates the harmful substances. Activated neutrophils also release reactive oxygen species. These all contribute to the inflammatory process.

This investigation showed a slight variation in the WBC count between patients and controls. The P value was 0.85, which is not significant. However, the patient with preterm PROM had a considerably higher neutrophil count (9936.5±3385.2/mm^3) than the control (7311.1±1593.5/mm^3). The p-value is noteworthy because it is less than 0.001. These findings are consistent with the studies done by Toprak E et al [14] and Klement AH et al [15].

Megakaryocytes multiply more frequently in chronic inflammatory processes due to substances produced by both the agent and the host interactions with others. Additionally, inflammatory cytokines like IL 1 and IL 6 cause megakaryocytes to become active. Cytokines involved in inflammatory reactions have been linked to preterm PROM, which causes an increase in platelet count. This study discovered that the preterm PROM group (case) had a considerably higher platelet count than the control group (201.7±65.9 vs. 241.6±58.7 1000/mm^3). The p-value is <0.001, which is statistically significant. The research conducted by Satar et al. and Fldrová and Krejsek supports this [16,17]. Interleukin (IL)-8 levels in maternal serum and umbilical cord blood were shown to be elevated in preterm PROM, according to Satar et al. [16]. Similar findings were made with IL-6, which was only detected to be high in umbilical cord blood, particularly in preterm PROM with microbial invasion and histologic chorioamnionitis. Tumor necrosis factor (TNF), IL-8, IL-6, and IL-1 were reported to be upregulated in preterm delivery and preterm labor in the study by Fldrová and Krejsek [17].

The lymphocyte count was 1896.7±651.8/mm3 and 2144±673.2/mm3 in the case and control groups, respectively. It is not statistically significant because the P value is 0.5. The research conducted by Toprak E et al. and Klement AH et al. supported this conclusion [14,15].

A popular inflammatory marker is PLR (platelet to lymphocyte ratio). One of the most reliable indicators of systemic inflammation, particularly in chronic inflammatory illnesses. Platelet numbers increase during prolonged inflammatory conditions, while lymphocyte counts decline due to apoptosis. The inflammatory process consequently impacts PLR value. PLR has been studied in pregnant women with preterm PROM, acute pancreatitis, gestational diabetes, and other conditions (Ekin A et al., Yucel B et al., Dadhich S et al.) [18-20]. No matter the latency duration or amniotic fluid index, the preterm PROM group (cases) in this study had a significantly greater PLR than the controls. P value is <0.001 which is statistically significant. These findings were strongly supported by the study done by Toprah et al. and Ekin A et al [14, 21].

Neutrophil to lymphocyte ratio (NLR) is another measure that may be involved in inflammatory processes. Leukocyte subtypes proliferate and develop in diverse ways in settings of systemic inflammation in response to immunological response. Lymphocyte levels fall as neutrophil numbers rise. As a result, several systemic inflammatory disorders tend to change the NLR. In numerous pregnancy-related disorders, NLR was also discovered to be considerably altered. High NLR values were reported in preeclampsia by Kurtoglu et al [22].

In this study, the preterm PROM group (cases) had a greater NLR than the control group. The findings are in line with the work of Köseolu et al. and the P value was

statistically significant (0.001) [23]. In the study by Köseolu et al., the preterm PROM group's NLR was higher than that of the controls [23]. The NLR was found to be a reliable predictor for predicting preterm PROM.

CONCLUSION

According to this study, NLR and PLR are potential biomarkers that can be used to anticipate preterm birth. These results should be validated in prospective trials, despite the high sensitivity and specificity of these measures suggesting that they effectively predict premature labor.

LIMITATIONS AND RECOMMENDATIONS

The study period was short. If the observation period had been extended, we would have found more impressive outcomes. It was a single-centered study with a modest sample size. Multi-centered research may be carried out in Bangladeshi divisional/tertiary hospitals. A lengthy investigation is advised. The most accurate marker for preterm PROM will be made crystal evident by comparison research incorporating the many inflammatory biomarkers such as CRP (C Reactive Protein).

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