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Association of maternal platelet-to-lymphocyte ratio with preterm prelabour rupture of membranes

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Abstract
Background: Preterm prelabour rupture of membranes (PPROM) is one of the common obstetric complications linked to significant maternal and fetal morbidity and mortality. In the developing nations like Bangladesh, where the maternal and child mortality rate is still considerably high, early detection of PPROM and understand-ding its etiology is vital for better management and prevention of complications.
Objective: This study was conducted to determine the association of maternal platelet-to-. Lymphocyte ratio (PLR) with preterm prelabour rupture of membranes.
Methodology: This was a case-control study among purposively selected pregnant women matched for age and gestational age attending inpatient department of Obstetrics and Gynecology, ICMH, Matuail, Dhaka from October 2020 to September 2021. A total 60 pregnant women between 18-35 years of age were included in this study in their 28-36 weeks of gestation. Among them 30 diagnosed women with PPROM were considered as the cases and rest of the 30 matched healthy pregnant women without PPROM were selected as controls. After taking consent, and matching eligibility criteria, data were collected from patients on variables of interest using the predesigned semi-structured questionnaire by interview, observation, relevant clinical examination, and laboratory investigation of the participants. Complete blood count in order to investigate platelet- to-lymphocyte ratio, was measured in the laboratory of ICMH, Dhaka. Statistical analysis was done using Microsoft Excel 2010 and the analytic software SPSS v27.0, where required.
Result: The overall PLR level was found significantly higher among the PPROM cases 159.05±49.45 compared to the controls 108.68±18.30 (p<0.001). Considering PLR level of 117.24 as cut-off value, odd's ratio calculation showed PPROM was 5.68 times more likely in pregnant women with elevated PLR level of ≥117.24 than those with < 117.24 (OR=5.68; 95% CI=1.84-17.49, p-value=0.002). The sensitivity, specificity, positive predictive value, negative predictive value, and accuracy rate of PLR was 76.67%, 63.33%, 67.65%, 73.08% and 70.0% respectively.
Conclusions: Raised maternal platelet-to-lymphocyte ratio was found strongly associated with preterm prelabour rupture of membranes.

Keywords: Preterm Prelabour Rupture, Platelet-To-Lymphocyte Ratio, Maternal Morbidity, Fetal Morbidity, Obstetric Complications, Bangladesh

Introduction
Prelabor rupture of membranes (PROM), previously known as premature rupture of membranes, is breakage of the amniotic sac before labor onset [1]. Preterm prelabour rupture of membranes (PPROM) is defined as spontaneous rupture of fetal membranes between 24 and 37 weeks of gestation diagnosed. Women usually experience a painless gush or a steady leakage of fluid from the vagina. It is confirmed with a positive test of insulin growth factor-binding protein-1 in vaginal discharge and or the presence of gross pooling of amniotic fluid in the vagina by sterile speculum examination, which affects approximately 3% of all pregnancies [2]. About 8% of term pregnancies are complicated by PROM, while about 20% of these become prolonged PROM and 30% of preterm births are complex by PROM, and rupture of membranes before viability (before 24 weeks) occurs in less than 1% of all pregnancies. Since there are significantly fewer preterm deliveries than term deliveries, the number of PPROM cases makes up only about 5% of all cases of PROM [3]. PPROM isone of the most common causes of preterm delivery, closely related to significant maternal and fetal morbidity and mortality and associated with maternal and neonatal infections [4, 5]. Preterm labor is an obstetric condition that complicates 5-9% of pregnancies. Factors associated with preterm PROM include lower socioeconomic status, cigarette smoking, urinary tract and sexually transmitted infections,

low maternal body mass index, prior cervical conization, prior preterm delivery, prior preterm labor in the current pregnancy, uterine distention (eg, twins, hydramnios), cervical cerclage, amniocentesis, previous or current cervical surgical procedures and vaginal bleeding in pregnancy. Irrespective of causes, inflammation is the only pathologic process for a strong causal relation with preterm labour and PPROM has been defined [6]. The pathophysiologic mechanism of PPROM has not been clearly defined and is multifactorial. Inflammation plays a crucial role in the rupture of membranes [7]. During inflammation pro-inflammatory Cytokines such as interleukin (IL)-8, IL-6, IL-1 and tumor necrosis factor (TNF) were released in maternal blood. This Cytokines subsequently weakens the fetal membranes and put them at risk for rupture. Choriodecidual infection or inflammation appears to play an important role in etiology of preterm PROM, especially at early gestational ages. Several studies reported that platelets and lymphocytes share regulatory mechanisms in the pathophysiology of inflammation, immunity, thrombosis and atherosclerosis. The effect of platelets on lymphocyte function may be via direct contact or by soluble mediators such as P-selectin and L-selectin. Platelets enhance adhesion and cell migration of lymphocytes and affect other functional aspects of lymphocytes in a complex manner [8]. PLR is a widely available, effective, and simple marker. Unfortunately, despite our developing ability to identify women at increased risk for preterm PROM, testing like fetal fibronectin screen is expensive and inconvenient to the patient, and will identify only a small fraction of those ultimately delivering preterm. Because of this, our clinical efforts remain focused on treatment of preterm PROM. Early diagnosis and appropriate management is very important for preventing its poor outcomes. As there are many inflammatory markers have been evaluated for their ability to diagnose membrane rupture at early stages, PLR might be a cost effective, easy to use, and practical marker for the early diagnosis of PPROM, which can help to determine the appropriate waiting time for delivery and provide maternal and fetal well-being.

Materials and methods

Study design: Case control study.

Place of study: In the Department of Obstetrics and Gynecology of the Institute of Child and Mother Health (ICMH), Matuail, Dhaka, Bangladesh.

Period of study: From October 2020 to September 2021.

Study population: The study population included pregnant women between 28 to 37 weeks of gestation admitted in the inpatient department of Obstetrics and Gynecology of ICMH. Recruited pregnant women were divided into two groups (Case and Control groups).

Case: Pregnant women with gestational age between 28 to 37 weeks with PPROM (aged - 18 to 35 years).

Control: Matched normal healthy pregnant women with gestational age between 28 to 37 and aged between 18 to 35 years.

Sampling technique: Purposive sampling was done according to the availability of the study subjects who fulfilled the inclusion criteria.

Sample size determination

A calculation of sample size is done by the following formula: [9].

$$n = \frac{(Z_\alpha + Z_\beta)^2 X\ (\sigma_1^2 + \sigma_2^2)}{(\mu_1 - \mu_2)^2}$$

So the estimated total sample size was 60 (30 X 2); Case = 30 and Control = 30.

However, as my data collection period was one year, I aimed to recruit as many subjects as possible during this one year.

Selection criteria

Inclusion criteria

For case: Pregnant woman with PPROM with gestational age between 28 to 37 weeks

Age more than 18 years pregnant women who have given consent to participate in the study.

For Control: Age- matched pregnant women with spontaneous preterm labor (SPL)

Pregnant women who have given consent to participate in the study.

Exclusion criteria

Women with history of diabetes mellitus, heart disease, renal disease, liver disease, thyroid disease, hematologic disorders, malignancies acute or chronic infectious or inflammatory diseases and autoimmune disease.

Women with multiple gestations, gestational diabetes mellitus and preeclampsia, pregnancies with fetal chromosomal anomalies, intrauterine growth restriction, fetal infection

Women who underwent any invasive procedures such as amniocentesis.

Women who did not give consent

Study procedure: After obtaining approval from the Institutional Review Board, this case control study was conducted in the Institute of Child and Mother Health (ICMH), Matuail, Dhaka, from April 2019 to March 2021. The study population was pregnant women between 28 to 37 weeks of gestation admitted in the inpatient department of Obstetrics and Gynecology of ICMH, fulfilling the inclusion and exclusion criteria. Recruited pregnant women were divided into two groups (Case and Control groups). During the period (after approval of protocol), the purpose and procedure of the study were discussed with the pregnant women and informed written consent was taken from those who agreed to participate in the study. A total 60 pregnant women was included in this study, among which 30 pregnant woman with PPROM with gestational age between 28 to 37 weeks was enrolled in case and the rest of the 30 pregnant women with spontaneous preterm labor were included in control matched for their age between 18-35 years and gestational age within 28-37 weeks. For each subject, a separate data collection sheet was used. Socio-demographic data, obstetric history, and family history were recorded in a predesigned data sheet. All the participants underwent standardized anthropometric measurements. The maternal age, gravidity, parity and the gestational age at admission, birth weight, APGAR score, neonatal intensive care unit (NICU) admission rate and the presence of neonatal sepsis were recorded. All laboratory investigations were evaluated. With all aseptic precautions, 2 ml blood was collected from antecubital vein by 5cc disposable plastic syringe from each subject before administration of

betamethasone and antibiotic prophylaxis. Blood was taken in a tube with EDTA anticoagulant for estimation of complete blood count. Collected blood samples was analyzed by Automated Hematology Analyzer (Sysmex XT- 2000) in the laboratory of Institute of Child and Mother Health (ICMH).Collected data were analyzed and compared using Microsoft Excel and the latest SPSS software version (v23.0).

Measurement of weight and height

The body weight was measured on barefoot with bathroom weighing scale. The average weight (0.5kg) of the clothes was subtracted from the measured weight. The measurement of weight was done after the bladder had been emptied and before a meal. The heights of the subjects were measured on bare feet in the standing position with meter scales.

Statistical analysis

Statistical analyses were carried out by using Windows-based Microsoft Excel and Statistical Package for Social Sciences (SPSS-26) where required. For qualitative variables, distribution was expressed by frequency and their percentage. An unpaired t-test was done to find out the difference in mean serum platelet and lymphocyte level between the case and control. Chi-square tests were done to observe the association between maternal serum platelet and lymphocyte level. Maternal platelet and lymphocyte level were categorized based on the cut off value. Strength of association was determined by estimating odds ratio (OR) and their 95% confidence interval (CI).To understand the relationship between maternal platelet lymphocyte ratio Pearson's correlation coefficient (P) was done. The p-value <0.05 was considered as statistically significant.

Results

A total of 30 pregnant women with PPROM (Case) and 30 pregnant women without PPROM (Control) were enrolled in this study to find out the association between maternal PLR and PPROM.

Table 1: Comparison of socio-demographic variables between cases and controls (case=30 and control=30).

Socio-demographic variables	Group		Total N (%)	p-value
	Case (n=30) N (%)	Control (n=30) N (%)		
Age (years)				
Up to 20	5 (16.7)	4 (13.3)	9 (15.0)	0.915[a]
21 - 29	18 (60.0)	18 (60.0)	36 (60.0)	
30 - 35	7 (23.3)	8 (26.7)	15 (25.0)	
Mean±SD	25.03±4.99	26.02±4.75		0.786[b]
Place of Residence				
Urban	10 (33.3)	15 (50.0)	25 (41.7)	0.190[a]
Rural	20 (66.7)	15 (50.0)	35 (58.3)	
Education				
Illiterate	8 (26.7)	4 (13.3)	12 (20.0)	0.054[a]
Up to SSC/Equivalent	15 (50.0)	10 (33.3)	25 (41.7)	
Higher secondary & above	7 (23.3)	16 (53.3)	23 (38.3)	
Occupation				
Housewife	20 (66.7)	19 (63.3)	39 (65.0)	0.731[a]
Student	2 (6.7)	3 (10.0)	5 (6.3)	
Wage earner	4 (13.3)	6 (20.0)	10 (16.7)	
Service holder	4 (13.3)	2 (6.7)	6 (10.0)	
Monthly family income (in BDT)				
< 15,000 tk	9 (30.0)	11 (36.7)	20 (33.3)	0.850[a]
15,000 - 30,000 tk	17 (56.7)	15 (50.0)	32 (53.3)	
> 30,000 tk	4 (13.3)	4 (13.3)	8 (13.4)	
Mean±SD	18700±8436.99	18866±8278.49		0.939[b]

a Chi square test was done to measure the level of significance
b Unpaired t-test was done to measure the level of significance

Table 1 for this study purpose, respondent's age were matched according to selection criteria and there was no statistically significant difference between case and control groups regarding residence, education, occupation and economic status (p > 0.05).

Table 2: Comparison of Gestational age and Gravidity between cases and controls (case=30 and control=30).

Obstetric variables	Group			p-value
	Case (n=30) N (%)	Control (n=30) N (%)	Total (n=60) N (%)	
Gestational age (in weeks)				
Mean± SD	32.6±1.75	32.73±2.48		0.811[b]
Gravida				
Primigravida	19 (63.3)	12 (40.0)	31 (51.7)	0.071[a]
Multi gravida	11 (36.7)	18 (60.0)	29 (48.3)	

a Chi square test was done to measure the level of significance.
b Un-paired t-test was done to measure the level of significance

Table 2 illustrates the distribution of the patients according to the obstetric history of the groups. Gestational age of the controls were taken matched with the cases, therefore their mean (±SD) difference was found statistically not significant (p=0.811). In regards of gravidity, the difference in distribution of the respondents were also found statistically not significant (p=0.071).

Table 3: Comparison of BMI between cases and controls (case=30, control=30).

BMI (kg/m^2)	Group		p value
	Case (n=30) N (%)	Control (n=30) N (%)	
Normal (18.5-24.9)	7 (23.3)	13 (43.3)	0.112[c]
Over weight (25.0-29.9)	21 (70.0)	17 (56.7)	
Obese (≥30)	2 (6.7)	0 (0.0)	
Mean±SD	26.89±2.21	25.67±2.53	0.051[b]

b Unpaired t-test was done to measure the level of significance.
c Fisher's Exact test was done to measure the level of significance.

Table 3 shows that there was no statistically significant difference in the distribution of the respondents according to BMI (p=0.112) and in the mean±SD between case and control groups of patients' body mass index (p=0.051).

Table 4: Comparison of previous history of PROM and preterm labour between cases and controls (case = 30, control=30).

Parameters	Group		p value
	Case (n=30) N (%)	Control (n=30) N (%)	
P/H of PROM			
Yes	4 (13.3)	1 (3.3)	0.353[c]
No	26 (86.7)	29 (96.7)	
P/H of Preterm Labor			
Yes	3 (10.0)	1 (3.3)	0.612[c]
No	27 (90.0)	29 (96.7)	

cFisher's Exact test was done to measure the level of significance.

There was no statistically significant difference in the distribution of the respondents according to history of PROM and preterm labor in both the case and control groups (p>0.05).

Table 5: Comparison of mean (±SD) hemoglobin percentage between cases and controls (case = 30, control = 30).

Hb% (g/dl)	Group		p-value
	Case (n=30)	Control (n=30)	
Mean ±SD	11.31±1.09	11.85±1.10	0.058[b]
Range (min - max)	9.3 - 13.3	10.0 - 14.2	

bUnpaired t-test was done to measure the level of significance.

Though the mean (±SD) hemoglobin level was observed slightly lower in the PPROM group of women 11.31±1.09 g/dL (range: 9.3-13.3) than pregnant women without PPROM 11.85±1.10 g/dL (range: 10.0-14.2): but there was no statistically significant difference in the distribution of the study subjects according to mean (±SD) hemoglobin percentage (p>0.05).

Table 6: Comparison of mean (±SD) Platelet-to- lymphocyte ratio (PLR) between cases and controls (case = 30, control = 30)

PLR	Group		p-value*
	Case (n=30) Mean ±SD	Control (n=30) Mean ±SD	
PLR	159.05±49.45	108.68±18.30	<0.001
Min - Max	82.74 - 285.63	74.02 - 139.27	

*Un-paired t-test was done to measure the level of significance

Mean (±SD) PLR among the cases were much higher 159.05±49.45 (range: 82.74 - 285.63) than the control group 108.68±18.30 (range: 74.02-139.27). This result was statistically highly significant (p<0.001).

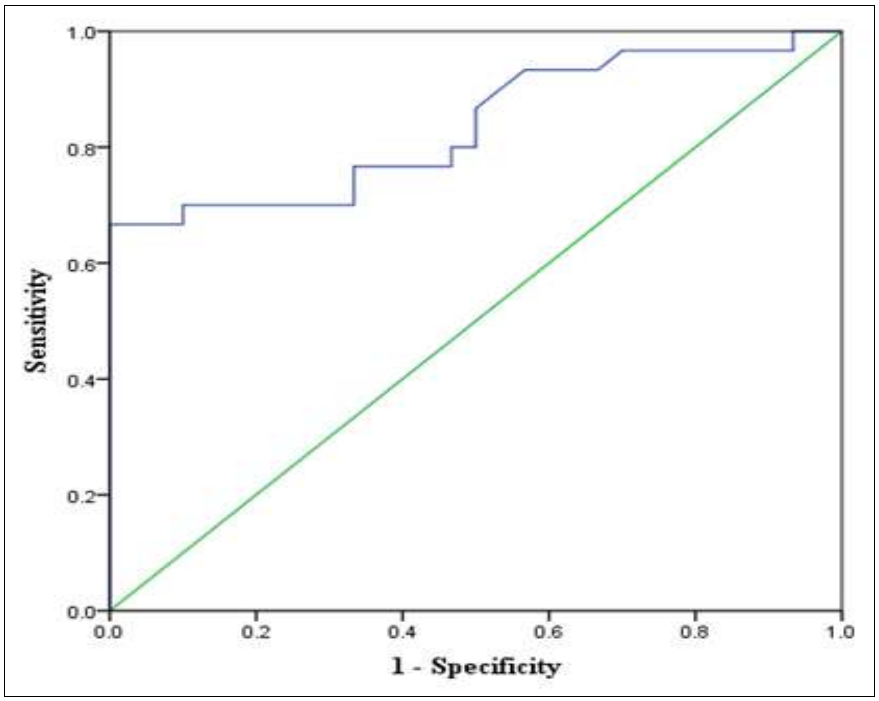

Fig 1: ROC curve for PLR in patients with PPROM (area = 0.836, SE = 0.053, asymptomatic significance = 0.000, lower bound = 0.731, upper bound = 0.941)

The ROC curve shows that area under the curve is. 84 means 84% of the total subjects have been diagnosed correctly. For this study purpose the ideal cut-off value for PLR in PPROM patients was subjected to 117.14 mg/dL which showed 76.6% sensitivity and 63.3% specificity. For this study purpose PLR level 117.14 was selected as cut off value.

Table 7: Odds ratios (OR) and 95% confidence intervals (CI) for PPROM according to Platelet-to- lymphocyte ratio in pregnancy (case = 30, control = 30)

PLR	Groups		p-value*	Odds Ratio (95% CI)
	Case (n=30)	Control (n=30)		
≥ 117.14	23 (76.7)	11 (36.7)	0.002	5.68 (1.84-17.49)
< 117.14	7 (23.3)	19 (63.3)		

*Chi-square test was done to measure the level of significance.

CI = Confidence Interval
Case: Pregnant women with PPROM

Control: Pregnant women without PPROM
There was significant difference in regards of platelet to lymphocyte ratio in between case and control groups (p=0.002) and the respondents with PLR ≥ 117.14 had 5.68 times more chance to develop PPROM compared to that of the respondent with PLR < 117.14 (OR=5.675; 95% CI=1.841- 17.494).

Table 8: Diagnostic performance and accuracy of PLR in detection of PPROM (case = 30, control = 30)

Statistics	Value	95% CI
Sensitivity	76.67%	57.72% to 90.07%
Specificity	63.33%	43.86% to 80.07%
Positive Predictive Value	6.07%	3.74% to 9.72%
Negative Predictive Value	98.87%	97.75% to 99.44%
Accuracy	63.73%	50.30% to 75.76%

The sensitivity, specificity, positive predictive value, negative predictive value and accuracy rate of PLR was 76.67%, 63.33%, 6.07%, 98.87% and 63.73% respectively.

Discussion

A raised platelet-to-lymphocyte ratio (PLR) might be a reliable marker for predicting PPROM. It has been evident that PLRraises during inflammatory conditions. Therefore, this case-control study was conducted with an aim to determine the association of PLR with PPROM by estimating the level of PLR in women with PPROM and women without PPROM and thereby comparing the PLR level between the two groups using a cut-off value. A total of 60 women aged 18-35 years and gestational age between 28-36 weeks, attending inpatient department of Obstetrics and Gynecology, ICMH were included in this study. Among them 30 diagnosed cases of PPROM were the cases and rest of the 30 matched women without PPROM were the controls. In this study, the mean age of the controls was found slightly higher than the cases (controls: 26.02±4.75 years vs. cases: 25.03±4.99 years) but this difference was statistically not significant. In another study by Toprak *et al.* [10], the mean (±SD) age in PPROM group was 28.7±5.1 years compared to control group 29.4±5.0 years (p=0.56). This finding was similar with the present study. Majorities (66.7%) of the cases belonged from rural residence compared to half (50.0%) of the controls. Half (50.0%) of the cases were educated up to secondary school certificate / equivalent level compared to 33.3% of the control group of respondents. Among the case majorities (66.7%) were house-wife compared to controls (63.3%) with monthly family income of majorities (53.3%) within Taka 15000 - 30000. None of this distribution according to socio-demographic characteristics were found significant (p>0.05). On determining association of socio-demographic parameters and PPROM by Hackenhaar *et al* [11], it was observed that 18.8% of the mothers were adolescents, 44.7% had eight years or less of schooling, 69.9% were white and 20.1% were smokers. The occurrence of PPROM was significantly higher in women of lower socioeconomic status, lower educational level and those older than 29 years. This difference in study finding was probably due to geographical and life-style variations. The mean (±SD) gestational age of the cases was 32.60±1.75 weeks which was taken matched in the controls with the mean (±SD) representing 32.73±2.48 weeks (p-value 0.811). Above three-fifths (63.3%) of the cases were primigravida compared to two-fifths (40.0%) of the control group of respondents. This difference in distribution of the respondents were found statistically not significant. On evaluation of the risk factors a study in India revealed primigravida women constituted 61% of the PPROM [12]. This was probably because primigravida is a risk factor for PPROM due to increased sexual activity & increased genital infection. In comparison of the Body Mass Index between cases and controls, the mean (±SD) difference BMI was found slightly higher among the cases 26.89±2.21 kg/m^2 than the controls 25.67±2.53 kg/m2 with the majorities (70.0%) of the cases distributed in overweight group compared to 56.7% controls. Obesity was observed only among the 6.7% of the cases. But these differences in distribution of the respondents were found statistically not significant (p>0.05). Previous history of PROM was observed in 13.3% of the cases compared to 3.3% of the controls; while history of preterm labor was also found in slightly higher proportion among the cases (10.0%) than the controls (3.3%). But none of these differences were found statistically significant (p>0.05). On evaluation of the past obstetric history in PPROM Akter *et al.* [13] demonstrated that fifty 56% had previous history of PROM, preterm delivery, abortion, MR and dilatation and curettage. Addisu *et al.* [14] also found abnormal vaginal discharge (AOR 5.30, 95% CI=2.07-13.52), vaginal bleeding in current pregnancy (AOR 2.58, 95% CI=1.14-5.82), previous history of PROM (AOR 3.31, 95% CI=1.32-8.27), MUAC of the mother (AOR 6.26, 95% CI=3.21-12.20), and UTI (AOR 2.62, 95% CI=1.32-5.19) remained significant (p<0.05) and independently associated with PPROM in the multivariable analysis. This difference in the findings with the present study was probably because of the geographical and study designs variation.

The average hemoglobin level among the cases (11.31±1.09 g/dl) were slightly lower than the control group of respondents (11.85±1.10 g/dl) but this was statistically not significant (p>0.058). In the present study the mean (±SD) PLR level was significantly higher among the cases (159.05±49.45) compared to the controls (108.68±18.30) which was found statistically highly significant (p<0.001). On Odd's ratio calculation, it revealed that respondents with PLR ≥117.14 had 5.68 times more chance to develop PPROM compared to that of the respondents< 117.14 (OR=5.675; 95% CI=1.84-17.49, p=0.002). This cut-off value of 117.14 had 76.67% sensitivity, 63.33% specificity and accuracy rate 63.73%. Ekın *et al.* [15]

demonstrated that compared with controls, women with PPROM had significantly increased levels of platelet count and significantly decreased levels of MPV in the first trimester ($P < 0.001$). The area under the receiver-operator curve was 0.642 for MPV and 0.579 for platelet count. The cut-off values of MPV ≤ 8.6 fL and platelet count $\geq 216 \times 103/\mu L$ predicted PPROM with a sensitivity of 58% and 65% and specificity of 62% and 44% respectively. Akgun *et al* [16] established that there was a statistically significant difference in the PLR ($p<0.001$). Toprak *et al*. [10] demonstrated a relation between PLR values and the occurrence of PPROM and a condition that leads to adverse maternal and neonatal events. They found the PLR and neutrophil-to-lymphocyte ratios (NLR) were both significantly higher in the PPROM group ($p<0.001$). Correlation analysis revealed that the PLR was positively correlated with the NLR (r=0.10, p=0.031). The ability of the PLR to diagnose preterm premature rupture of membranes was evaluated using an ROC curve. The sensitivity and specificity of the PLR was 57.8% and 73.7% respectively at a threshold >117.14 ($p<0.001$.[10] In a similar study Sharami *et al* [17] on performing Mann Whitney U test between the groups found PLR was significantly higher in PROM group (160.8±34.3) than the controls (226±52.2), p-value = 0.0001. Based on the ROC diagram which was used to detect PROM, the appropriate cut-off point for the PLR index was 142.2 with a sensitivity value of 62.7% and a specificity value of 63.3% which is an acceptable point and represents an association between PLR values and PROM Sharami *et al* [17]. In contrast, Ekın *et al* [6] for studying to identify risk factors and perinatal outcomes associated with the duration of latency period in women who experience PPROM, shown that there is no significant relation with PLR between the groups according to latency period (PLR in ≤72 h latency period: 123.6±44.7 vs. PLR in >72h latency period: 133.6±48.5, p=0.130) Ekin *et al* [6]. Daglar, *et al*. [18] also did not find any significant difference in on comparison of average PLR level among the Control (136±88), threatened preterm labor (139±56) and preterm delivery (137±40) groups ($p>0.05$). Were also not able to confirm that there is a relationship between PLR and PPROM which is an inflammatory condition. They found the PLR in PPROM group149.5±74.9 and in control group 131.3±56.2 (p=0.40) [2].

Conclusion

The study findings suggest that patients with PPROM have higher PLR in comparison to normal pregnant women. High level of maternal PLR in early to mid-pregnancy may be a predictor of the development of PPROM. So, raised level of maternal PLR is associated with PPROM.

Limitations of the study

- The study was conducted in a selected tertiary hospital.
- Sample size was small.
- Short study period.
- Sample was not randomly selected.
- There was no consensus on a cut-off value for PLR in the Pregnant population
- Only one inflammatory marker was evaluated.

Therefore, the study findings cannot be generalized to the entire population.

Recommendations

In the light of the findings of the present study and discussion; therefore, it is recommended:

1. Furthermore study can be undertaken nationwide with large number of population in multiple centres and for long duration.
2. Random sampling techniques can be used.
3. Follow up study can be done to observe whether high PLR that took place in PPROM affect the pregnancy outcome and sustain after pregnancy is over.

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