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ORIGINAL RESEARCH

Association of Serial C-Reactive Protein Levels with Development of Chorioamnionitis in Patients with Preterm Prelabour Rupture of Membranes in A Sub-Divisional Hospital in Eastern India: An Observational Study

Vipanchi Talukdar[1], Avishek Bhadra[2*], Rumela Biswas[3], Debasish Ganguli[4], Jitesh Satija[5]

[1]DNB Senior Resident, Department of Obstetrics and Gynaecology, Serampore Walsh Sub-Divisional Hospital, Hooghly, India.
[*2]Associate Professor, Department of Obstetrics and Gynaecology, R G Kar Medical College and Hospital, Kolkata, India
[3]Junior Resident, Department of Obstetrics and Gynaecology, Medical College and Hospital, Kolkata, India
[4]Senior Consultant, Dept. of Obstetrics and Gynaecology, Serampore Walsh Sub-Divisional Hospital, Hooghly, India
[5]Assistant Professor, Department of Obstetrics and Gynaecology, ESI Medical College and Hospital, Faridabad, India

Corresponding Author
Dr. Avishek Bhadra
Associate Professor, Department of Obstetrics and Gynaecology, R G Kar Medical College and Hospital, Kolkata, India
Email: drabhadra@gmail.com

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ABSTRACT

Background: Preterm premature rupture of membranes (PPROM) affects approximately 3% of pregnancies and significantly increases the risk of preterm birth and chorioamnionitis, a serious inflammatory condition. Early diagnosis of chorioamnionitis is crucial for improving maternal and neonatal outcomes. **Objectives:** This study aimed to investigate the association between serial C-reactive protein (CRP) levels and the development of chorioamnionitis in women with PPROM, to evaluate the diagnostic accuracy of CRP at admission, and whether serial CRP would improve diagnostic accuracy **Methods:** We conducted a prospective observational study involving 104 pregnant women with gestational ages between 28 to 34 weeks, diagnosed with PPROM. Participants underwent CRP testing at admission and again 48 hours later. Clinical signs of chorioamnionitis were monitored, and data were analysed using appropriate statistical methods. **Result:** The incidence of clinical chorioamnionitis was found to be 15.4%. Participants who developed chorioamnionitis had significantly higher mean CRP levels at admission (41.8 mg/L) compared to those without (12.1 mg/L, $p<0.001$). A CRP cut-off value of ≥17.9 mg/L at admission demonstrated high sensitivity (87.5%) and acceptable specificity (59.1%) for predicting chorioamnionitis. Serial CRP measurements improved diagnostic accuracy to 85.6%, compared to the previous diagnostic accuracy of 63.5%. **Conclusion:** This study reinforces the utility of CRP as a reliable biomarker for predicting chorioamnionitis in patients with PPROM. The findings support the use of serial CRP monitoring in clinical practice to enhance the management of at-risk pregnancies, ultimately improving maternal and neonatal outcomes. Further research with larger cohorts is needed to confirm these results and establish standardized protocols.

Key words: Serial, CRP, Chorioamnionitis, PPROM

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INTRODUCTION

Preterm premature rupture of membranes (PPROM) is defined as the spontaneous rupture of fetal membranes before 37 completed weeks of gestation[1]. PPROM complicates approximately 3% of pregnancies and is associated with 30%–40% of preterm births[1,2]. Several risk factors have been identified for PPROM, including a history of PPROM or preterm delivery (PTD), tobacco use, low body mass index (BMI), sexually transmitted infections, polyhydramnios, multiple gestation, and vaginal bleeding[3,4]. In many cases, the cause of PPROM remains unknown[4].

The most serious sequelae of PPROM is the development of chorioamnionitis, defined as inflammation of the chorioamniotic membranes of the placenta in response to microbial invasion or other pathological processes[5]. Clinical chorioamnionitis (CAM) is diagnosed before delivery based on clinical findings such as leukocytosis (white blood cell [WBC] count >15,000/μL), maternal tachycardia, fetal tachycardia, maternal fever (temperature >100.4°F), fundal or uterine tenderness, or foul-smelling amniotic fluid[6]. The incidence of chorioamnionitis also increases the risk of puerperal sepsis in mothers and early-onset neonatal sepsis in newborns[7]. Early diagnosis of chorioamnionitis can therefore directly reduce maternal mortality and morbidity, as well as improve neonatal outcomes and complications[8].

In cases of PPROM, deciding when to deliver involves carefully weighing the risks of preterm birth against the risks of infection from continuing the pregnancy[9,10]. If clinical features of infection or inflammation are present, delivery is usually initiated[11]. Early infection is not reliably predicted by commonly used laboratory tests such as erythrocyte sedimentation rate, white blood cell count, neutrophil count or vaginal bacterial culture, as clinical signs of CAM (fever and feto-maternal tachycardia) often appear late[12].

One maternal serum marker that indicates an increased risk of CAM is C-reactive protein (CRP)[13]. CRP is an acute phase protein synthesized in the liver. It serves as a sensitive marker of inflammation and remains stable in serum. Production of CRP is stimulated by the release of pro-inflammatory cytokines, including interleukin (IL)-1, IL-6, and interferon (IFN)-alpha[14].

Elevated CRP concentrations can be observed in peripheral circulation and amniotic fluid in cases of intrauterine infection [14]. CRP is utilized in various regions worldwide as an early predictor of chorioamnionitis[15]. Several studies have assessed maternal inflammatory markers for diagnosing chorioamnionitis in PPROM, yielding varying results and recommendations. Previous systematic reviews were based on a limited number of studies that demonstrated marked heterogeneity[12,16] and employed data analysis methods that are now considered inappropriate[12,17]. Several recent studies assessing CRP and other inflammatory markers have since been published[5,7,8,15].

Given the inconclusive and limited evidence, particularly from Eastern India, the aim of the present study was to investigate the association between serial C-reactive protein levels and the development of chorioamnionitis, to evaluate the diagnostic accuracy of CRP at admission in predicting CAM, and to determine if serial CRP estimation can increase the diagnostic accuracy, in pregnant women with preterm premature rupture of membranes presenting to the department of Obstetrics and Gynaecology at a sub-divisional hospital in Eastern India.

MATERIALS AND METHODS

This was an institutional-based prospective observational study conducted from 1st March 2023 to 29th February 2024, among 104 pregnant women selected conveniently, having gestational age between 28 to 34 weeks, and diagnosed with preterm PROM. The study setting was a sub-divisional hospital in Eastern India. The sample size for this study was calculated using the WHO defined standard formula for sample size determination of prevalence study.

As per the study by Suryavanshi et al (2019) [7], the proportion of patients with PPROM developing chorioamnionitis was reported to be 43.42%. In our study, P = 43.42%, Z = 1.96 (level of confidence is considered as 95%) and d = 10% absolute error. By applying the formula, the minimum sample size (n) was calculated to be 94. After considering 10% non-response rate, a total of 104 study participants were included in our study as per the inclusion criteria: all women with singleton pregnancy having gestational age between 28 to 34 weeks and diagnosed with PPROM. The exclusion criteria for this study were as follows: women with a latent period of less than 48 hours post-admission; women for whom two readings of C-reactive protein (CRP) taken 48 hours apart were unavailable; patients experiencing obstetric emergencies or those who were hemodynamically unstable; pregnant women with clinically proven infections; diabetic mothers or those with any medical complications; women with vaginal infections (such as candidiasis or trichomoniasis); those with significant congenital anomalies (including cardiac, renal, or pulmonary issues); women who received antibiotics before admission; any evidence of fetal compromise; women with a history of previous lower segment caesarean sections (LSCS); and women in established labour.

Upon inclusion, demographic details and antenatal history (including obstetric history) were noted. PPROM was confirmed clinically by per speculum examination. The time since rupture of membranes was noted. Maternal serum sample for CRP was taken at or soon after admission. A repeat serum CRP estimation was done after 48 hours. CRP estimation was conducted using RHELAX CRP reagent standardized to detect CRP concentration >0.6 mg/dl.

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This limit is traceable to the WHO International Reference Standard for Human CRP. Ultrasound evaluation of the amniotic fluid volume was performed at the time of admission, and before the administration of corticosteroids and antibiotics, to screen for incidence of oligohydramnios. The patients were initiated on prophylactic antibiotic therapy, and corticosteroids at admission as per standard guidelines for expectant management of PPROM. Expectant management was continued till 34 weeks, including clinical and laboratory monitoring for infection. Expectant management was also adopted at or beyond 34 weeks of gestation for 48 hours after PPROM to promote spontaneous onset of labour and vaginal delivery whenever possible. Development of any signs of maternal chorioamnionitis during the course of expectant management such as fever, maternal tachycardia (pulse >100/min), fetal tachycardia (>160/minute), raised WBC counts, uterine tenderness, and foul-smelling liquor, if present, were recorded. Pregnant women with any of the above two were considered as diagnostic for clinical chorioamnionitis, and the pregnancy was immediately terminated either through vaginal or caesarean delivery as indicated and maternal and fetal outcomes were noted. All details were recorded in a predesigned, pretested proforma.

The primary dependent variable in this study is the incidence of clinical CAM. The independent variables include maternal age, parity, gestational age, and the time since rupture of membranes at the time of admission. Other independent variables include the mode of delivery, latency period, and any perinatal complications. In addition to this, serum C-reactive protein (CRP) levels are assessed at admission and 48 hours after admission.

The collected data was tabulated in a Microsoft Excel 2016 spreadsheet (Microsoft Office 2016 package) and statistically analysed using Statistical Package for Social Sciences (SPSS) version 23 (IBM Corp., Illinois, Chicago). Independent sample T-test (for parametric data) and Mann Whitney U test (for non-parametric data) were used to compare continuous variables. Chi-square test was used to compare categorical variables. Fisher's Exact test was used for categorical data when at least one expected cell value is less than 5. Receiver operating curve (ROC) analysis was undertaken to identify cut-off value of serum CRP at admission in predicting incidence of chorioamnionitis. A "p-value" <0.05 was considered as statistically significant. Ethics approval was given by the institutional ethics committee.

RESULT

Analysis was conducted on a sample size of 104 patients. 33.6% were aged 18-24 years, 38.5% were aged 25-29 years, and 27.9% were aged 30 years or older. The mean (±SD) age of the study participants was 24.1 (±3.9) years, with a median age of 23 years; the ages ranged from 18 to 32 years.

97.1% of the study population were primigravidas, while 2.9% were multigravidas. The gestational age at presentation showed that 61.5% of participants were between 30 to 31+6 weeks, while 38.5% were between 32 to 33+6 weeks. (Table 1). Notably, there were no participants with gestational ages less than 30 weeks presenting with preterm premature rupture of membranes (PPROM).

The majority of participants (78.8%) presented within 24 hours of rupture of membranes, while 21.2% presented beyond 24 hours. The incidence of clinical CAM among the study participants was 15.4%. The mean age and gestational age at presentation did not significantly differ between patients with and without chorioamnionitis.

A significant association was observed between parity and chorioamnionitis; 93.8% of participants with chorioamnionitis were nulliparous compared to 63.6% of those without chorioamnionitis (p=0.017). Furthermore, delayed presentation to the hospital (>24 hours after rupture of membranes) was significantly associated with the incidence of chorioamnionitis (p<0.001).

Among participants who developed chorioamnionitis, the mean (±SD) serum CRP level at admission was 41.8 (±21.5) mg/L, compared to 12.1 (±7.9) mg/L for those without chorioamnionitis, with a statistically significant difference (p<0.001). (Table 2). Receiver operating characteristic (ROC) analysis indicated that the area under the curve for CRP levels at admission in predicting chorioamnionitis was 0.807. (Figure 1). A CRP cut-off value of ≥17.9 mg/L at admission demonstrated optimal sensitivity (87.5%) and specificity (59.1%) for predicting chorioamnionitis, along with a positive predictive value (PPV) of 28% and a negative predictive value (NPV) of 96.3%.

The criterion of CRP at baseline ≥17.9 mg/L with an increasing trend (positive ΔCRP) yielded 81.3% sensitivity, 86.4% specificity, a PPV of 52%, an NPV of 96.2%, and an overall diagnostic accuracy of 85.6% in predicting chorioamnionitis. (Table 3).

The majority of patients underwent vaginal delivery (82.7%), while the caesarean delivery rate was higher among those who developed clinical chorioamnionitis (18.7%) compared to those who did not (17.1%); however, this difference was not statistically significant (p=0.868).

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Table 1: Distribution of study participants according to incidence of chorioamnionitis and baseline characteristics (n=104)

Baseline characteristics	Chorioamnionitis		p-value[#]
	Yes	No	
Age	23.4 (±3.7)	24.6 (±5.2)	0.868
Parity (nulliparous)	15 (93.8)	56 (63.6)	0.017*
Gestational age at presentation	31.2 (1.1)	31.6 (1.3)	0.082
Time since rupture of membranes (>24 hours)	10 (62.5)	12 (13.6)	<0.001*
Total patients	16	88	

Values are presented as n (%) or mean (±SD)

Based on Chi-square test for categorical variable or Mann Whitney U test for continuous variable. P<0.05 is considered as statistically significant.

Table 2: Mean CRP levels at presentation among participants with and without clinical chorioamnionitis (N=104)

Category	Mean (± SD) CRP level (mg/L)	Median (IQR)	Minimum, maximum values
Chorioamnionitis present (n=16)	41.8 (± 21.5) *	26.5 (20.1-58.2)	13.0, 109.8
Chorioamnionitis absent (n=88)	12.1 (± 7.9)	12.5 (8.9-17.4)	1.0, 40.0

*** Significant at p<0.001**

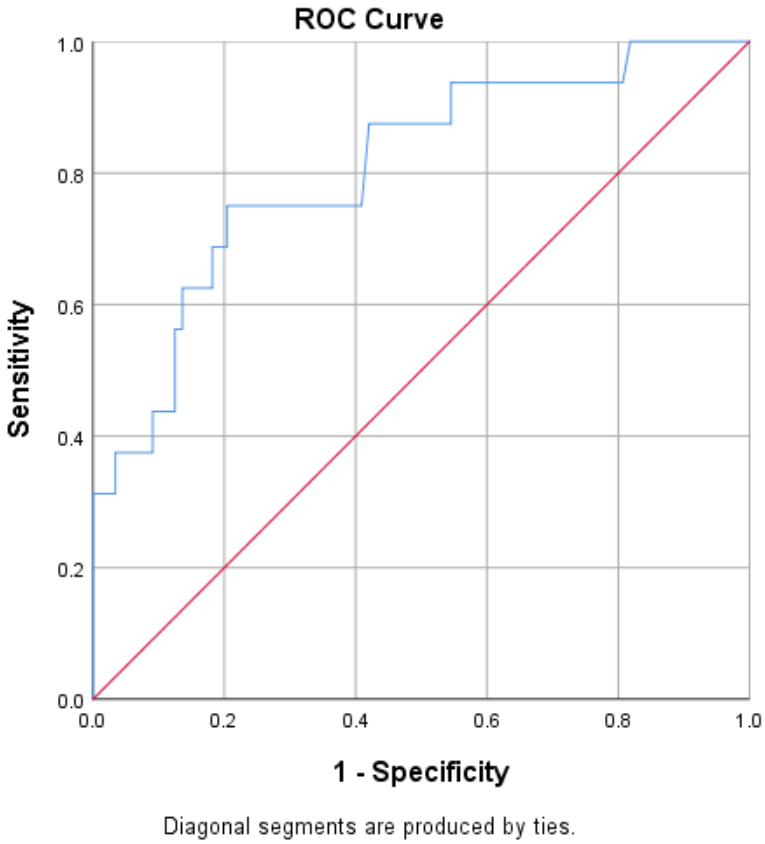

Figure 1: ROC curve of CRP level at admission predicting development of chorioamnionitis

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Table 3: Diagnostic test parameters of CRP level at admission in predicting development of chorioamnionitis

Cut-off value at maximum Youden's index	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)	Diagnostic accuracy (95% CI)
≥ 17.9	87.5% (61.7% - 98.5%)	59.1% (48.1% - 69.5%)	28.0% (22.2% - 34.7%)	96.3% (87.6% - 98.9%)	63.5% (53.5% - 72.7%)

Table 4: Change in CRP level at 48 hours as compared to baseline and chorioamnionitis (n=104)

Criterion	Total patients	Chorioamnionitis		p-value#
		Yes	No	
CRP at baseline ≥ 17.9 mg/L with positive ΔCRP (increasing trend)	25	13 (81.3)	12 (13.6)	<0.001
Otherwise	79	3 (18.7)	76 (86.4)	
Total patients	104	16	88	

Values are presented as n (%)

Based on Chi-square test for categorical data. P<0.05 is considered as statistically significant.

Table 5: Diagnostic test parameters of CRP criterion in predicting development of chorioamnionitis

Criterion	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)	Diagnostic accuracy (95% CI)
CRP at baseline ≥ 17.9 mg/L with positive ΔCRP (increasing trend)	81.3% (54.5% - 95.9%)	86.4% (77.4% - 92.7%)	52.0% (37.8% - 65.8%)	96.2% (90.1% - 98.6%)	85.6% (77.3% - 91.7%)

DISCUSSION

In our study, we examined the role of C-reactive protein (CRP) levels as a serum marker related to chorioamnionitis and whether serial CRP levels could predict chorioamnionitis before occurrence of clinical symptoms. We found that the mean CRP level at admission was significantly higher in those who developed chorioamnionitis (41.8 ± 21.5 mg/L) compared to those who did not (12.1 ± 7.9 mg/L).

Aggarwal et al. [15] reported that elevated CRP level of >6mg/L yielded 100% sensitivity, 69.56% specificity, 22.2% PPV and 100% NPV in predicting clinical chorioamnionitis. In their study, the incidence of clinical chorioamnionitis was 8% in which mean elevated CRP was 32 mg/L. Dutta et al [18] reported the mean CRP to be 43.5 mg/L among their cases with clinical chorioamnionitis. Elevated CRP (>6mg/L) was found to have 92% sensitivity and 58% specificity in predicting clinical chorioamnionitis. Our results from this study are also supported by Musilova et al. [19] which included a relatively large cohort of women with PPROM, reporting a positive association between presence of chorioamnionitis and with higher maternal serum CRP concentrations.

A repeat CRP in all the study participants at an interval of 48 hours after admission was done in our study, and for all those with second CRP value greater than 17.9 mg/L, an increasing trend was noted in a relatively lesser proportion of patients with no clinical chorioamnionitis, leading to an improved specificity of 86.4%, with a small decrease in sensitivity to 81.3%, improved PPV of 52%, no change in NPV and an overall improvement in diagnostic accuracy of 85.6% in predicting chorioamnionitis. There are limited studies conducted previously on the role of serial CRP levels in the identification of clinical CAM. Aggarwal et al. [15] reported that sequential CRP observations showed an increasing trend in 100% of the cases with chorioamnionitis which was consistent with our result.

A study by Smith et al. [14] stated that C-reactive protein levels were not effective independent predictors of clinical or histologic CAM, nor was sequential CRP testing statistically significant for the identification of clinical or histologic CAM in patients with PPROM. However, their observations were based on sequential CRP results available for only 17 out of 26 of their participants with chorioamnionitis; and also acknowledged that there was not enough power in the low sample size of 17 to reach statistical significance.

In all the aforementioned studies, CRP has been consistently found to have high sensitivity and low specificity, indicating that elevated CRP levels tend to include a larger proportion of false positive cases. This can be attributed due to the fact that pregnancy itself mimics a state of inflammation and median CRP values in normal pregnancies are found to be higher than standardized values for nonpregnant individuals [20].

Despite some limitations regarding sample size and the variability in CRP responses during pregnancy, our results align with existing literature on the utility of CRP as a biomarker in obstetric care. Future

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studies should explore larger cohorts and the integration of CRP monitoring in clinical practice to enhance the management of patients with PPROM and potential chorioamnionitis. Ultimately, measuring and interpreting serial CRP levels could play a crucial role in improving maternal and neonatal outcomes.

CONCLUSION

This study highlights the significant role of C-reactive protein (CRP) as a serum marker for predicting chorioamnionitis in patients with preterm premature rupture of membranes (PPROM). Our findings demonstrate that elevated CRP levels at admission correlate strongly with the development of clinical chorioamnionitis, with a threshold of ≥17.9 mg/L showing high sensitivity and acceptable specificity for diagnosis. Notably, serial measurements of CRP further improved predictive accuracy, underscoring the potential for early identification of at-risk patients. Despite some limitations regarding sample size and the variability in CRP responses during pregnancy, our results align with existing literature on the utility of CRP as a biomarker in obstetric care. Future studies should explore larger cohorts and the integration of CRP monitoring in clinical practice to enhance the management of patients with PPROM and potential chorioamnionitis. Ultimately, measuring and interpreting serial CRP levels could play a crucial role in improving maternal and neonatal outcomes.

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Competing Interests

None Declared.

Contribution to Authorship

VT and AB wrote the first draft of the paper. AB statistically analysed the data. RB, DG and JS edited and revised the article, and all the authors approved the final draft.

Details of Ethics Approval

The study received ethics approval from the institutional ethics committee (Reg. No. – ECR/287/Inst/WB/2013/RR-19) with Ref. No. MC/KOL/IEC/NON-SPON/1890/05/2023 dated 17/05/2023.

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Patient Consent

Informed written consent obtained from patient/guardians.

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