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PREDICTION OF SUB-CLINICAL CHORIOAMNIONITIS IN PRETERM PREMATURE RUPTURE OF MEMBRANE USING BIO-MARKER C-REACTIVE PROTEIN

Obstetrics & Gynaecology

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ABSTRACT

Background: Preterm premature rupture of the fetal membranes (PPROM) is associated with intra-uterine infection. Early and definitive diagnosis of subclinical chorioamnionitis, particularly in cases of PPROM, has crucial importance to prevent both maternal and neonatal morbidity and mortality. A biochemical biomarker, which has high diagnostic accuracy and can determine subclinical chorioamnionitis early, would be very useful in clinical practice. **Aim:** To evaluate the predictive value of maternal serum C- reactive protein in maternal sub-clinical chorioamnionitis in Preterm Premature Rupture of Membranes (PPROM), in deliveries after 28 weeks of gestation but before 37 weeks of gestation. **Methods:** 300 study subjects all satisfying our inclusion criteria with no uterine contractions, no tocolysis, no antibiotics, no steroids administration within 14 days of admission were recruited in this study. Maternal serum C- reactive protein and subclinical chorioamnionitis with the help of histopathology of placenta were assessed and observed. The association of the variables which were quantitative and not normally distributed in nature were analyzed using Mann-Whitney Test and variables which were quantitative and normally distributed in nature were analyzed using independent t test. Point-biserial correlation coefficient was used for correlation between Sub-clinical chorioamnionitis and C-reactive protein. **Results:** There was a significant difference in the levels of CRP between the subjects stratified by the presence or absence of subclinical chorioamnionitis at the time of admission with CRP levels being consistently higher in those women who had subclinical chorioamnionitis as compared to those who did not have histopathology diagnosed subclinical chorioamnionitis. The sensitivity and specificity were very high even at after 24- and 48-hours post-admission and both PPV and NPV were very promising, PPV being highest at admission (51.40%) and NPV being highest at 48 hours (99.50%). Among all the parameters, C-reactive protein (mg/dL) at 48 hours was the best predictor of Subclinical Chorioamnionitis. **Conclusion:** The CRP should be used in relevant clinical scenarios after ruling out other causes of inflammation. In women who have high CRP levels, the chances of having subclinical chorioamnionitis are higher. CRP levels should be repeated at 48 hours as seen in our study, the sensitivity 97.6%, specificity 84.6%, PPV 50%, NPV 99.5% respectively being higher at this duration.

KEYWORDS

C - reactive protein, Subclinical Chorioamnionitis, PROM, Sensitivity, Specificity.

INTRODUCTION:

Preterm Premature Rupture of Membranes is defined as spontaneous rupture of the fetal membranes before 37 completed weeks and before labor onset, [1] while extreme PPROM occurs before 26 weeks gestation.[2] Preterm birth occurs in approximately 10% of all births in the United States and is a major contributor to perinatal morbidity and mortality.

Pre-labor rupture of membranes (PROM) that occurs preterm complicates approximately 2-3% of all pregnancies in the United States, representing a significant proportion of preterm births, whereas term PROM occurs in approximately 8% of pregnancies. 40-50% of patients with peri-viable PROM will give birth within the first week and approximately 70-80% will give birth within 2-5 weeks after membrane rupture.[3] Disease burden of PPROM on India: 3% of all pregnancies. Babies born preterm per year 3,341,000.[4]

Chorioamnionitis is an infection that can occur before labour, during labour, or after delivery. CAM is the leading cause of preterm birth. It can be acute, sub-acute or chronic.

Acute inflammation of the placenta is responsible for adverse neonatal outcomes in PPROM.[5] Placental inflammation is often clinically silent and can signal the normal physiologic process of parturition. During labour an inflammatory process but it can also be a sign of sub-clinical infection. Sub-clinical/ chronic/ Histological chorioamnionitis (HCA),[6] which is defined as inflammation of the placenta and the inflammatory changes are neutrophil infiltration, mainly poly-morphonuclear leukocytes, in any of these parts. This is without any clinical signs like high fever, maternal or fetal tachycardia, elevated white blood cells (WBC) or C- reactive protein (CRP) level, uterine tenderness, and foul odour of amniotic fluid in cases of sub- clinical chorioamnionitis. This is seen in 40-70% of all PPROM cases.[7]

A biochemical biomarker, which has high diagnostic accuracy and can determine sub-clinical chorioamnionitis early, would be very useful in clinical practice. While some cases remain sub-clinical, if chorioamnionitis is diagnosed, prompt efforts to affect delivery, preferably vaginally, are initiated. Because maternal leukocytosis alone is not a consistent finding, fever is the only reliable indicator for the diagnosis of clinical chorioamnionitis.

METHODS:

This study was conducted in the Department of Obstetrics & Gynaecology at Lalla Ded Hospital GMC Srinagar, after obtaining Ethical clearance from the Institutional Ethical Committee keeping in view the accessibility, availability, affordability and finally the accuracy of chosen method.

After thorough explanation of objective of study, including the methods, tools, finances, and dos and don'ts of this prospective study; in common native language of participants, informed consent was taken from all the participants. This study consisted of pregnant women who were admitted to Lalla Ded Hospital through OPDs/emergency, between June 2020 and June 2023 with the diagnosis of preterm premature rupture of membranes (PPROM) at or after 28 weeks but less than 37 weeks of gestation, who met the above stated inclusion criteria. A sample size of 300 pregnant women was studied.

Estimation of C- reactive protein was done by Quantitative Immuno-turbidimetric method (supplied by Roche Integra fully automated method), was done. Estimation of C- reactive protein was done on admission, after 24 hrs, and 48hrs. Sample for CRP estimation was taken from ante-cubital vein at the time of admission. CRP determination was done using latex agglutination method with the help of CRP reagent kit. The detection limit of this assay was 0.1mg/L. CRP values were considered abnormal (positive), when the values exceeded 1.2mg/L.

Following delivery, the placental membranes were cut and sent for

histo-pathological examination in formalin 10% to the department of Pathology, GMC Srinagar and sub-clinical chorioamnionitis was identified as intra-cytoplasmic neutrophilic infiltration. Tissue samples were obtained from each placenta and roll of membranes from rupture point to placental margin.

Statistical Analysis

The presentation of the Categorical variables was done in the form of number and percentage (%). On the other hand, the quantitative data were presented as the means ± SD and as median with 25th and 75th percentiles (interquartile range). The data normality was checked by using Kolmogorov-Smirnov test. The cases in which the data was not normal, we used non parametric tests. The following statistical tests were applied for the results:

1. The association of the variables which were quantitative and not normally distributed in nature were analyzed using Mann-Whitney Test and variables which were quantitative and normally distributed in nature were analyzed using independent t test.
2. Receiver operating characteristic curve was used to find cut off point, sensitivity, specificity, positive predictive value and negative predictive value of C-reactive protein (mg/dL) at admission, 24 hours, 48 hours for predicting sub-clinical chorioamnionitis.
3. Point-biserial correlation coefficient was used for correlation between Sub-clinical chorioamnionitis and C-reactive protein.

The data entry was done in the Microsoft EXCEL spreadsheet and the final analysis was done with the use of Statistical Package for Social Sciences (SPSS) software, IBM manufacturer, Chicago, USA, ver 25.0. For statistical significance, p value of less than 0.05 was considered statistically significant.

Conflict of interest: Nil

Funding: Nil

RESULTS:

A profile of 300 patients was studied during the study period. Mean value of age (years) of study subjects was 29.64 ± 2.3 with median (25th-75th percentile) of 29 (28-31) [Table 1]. Majority [185(61.67%)] of patients were multigravida. 115 out of 300 patients (38.33%) were primigravida [Table 2].

In majority [223(74.33%)] of patients, gestational age at admission (weeks) was 34 weeks to 36 weeks + 6 days followed by 28 weeks to 31 weeks + 6 days [44(14.67%)]. Gestational age at admission(weeks) was 32 weeks to 33 weeks + 6 days in only 33 out of 300 patients (11.00%). Mean value of gestational age at admission (weeks) of study subjects was 34.73 ± 2.8 with median (25th-75th percentile) of 36(33.86-36.86) [Table 3].

Mean value of C-reactive protein (mg/dL) at admission, at 24 hours and at 48 hours of study subjects was 1.46 ± 1.47, 1.55 ± 1.64 and 1.61 ± 1.9 with median (25th-75th percentile) of 0.88(0.32-2.322), 0.69(0.315-3) and 0.65(0.25-2.8) respectively [Table 4].

In majority [120(40.00%)] of patients, gestational age at delivery was 34 weeks to 36 weeks + 6 days followed by >=37 weeks [106(35.33%)] and 28 weeks to 31 weeks + 6 days [47(15.67%)]. Gestational age at delivery was 32 weeks to 33 weeks + 6 days in only 27 out of 300 patients (9.00%). Mean value of gestational age at delivery (weeks) of study subjects was 35.19 ± 2.87 with median (25th-75th percentile) of 36.43(34.214-37.161) [Fig 1].

Interpretation of the area under the ROC curve showed that the performance of C-reactive protein (mg/dL) at admission (AUC 0.902; 95% CI: 0.863 to 0.934), C-reactive protein (mg/dL) at 24 hours (AUC 0.907; 95% CI: 0.868 to 0.937) and C-reactive protein (mg/dL) at 48 hours (AUC 0.929; 95% CI: 0.894 to 0.956) was outstanding. Among all the parameters, C-reactive protein (mg/dL) at 48 hours was the best predictor of Sub-clinical chorioamnionitis at cut off point of >2.23 with area under curve of 0.929 for correctly predicting Sub-clinical chorioamnionitis [Fig 2, 3,4].

Table I: - Distribution Of Age (years) Of Study Subjects.

Age(years)	Frequency	%
25-30	211	70.33
31-35	89	29.67
Mean ± SD= 29.64 ± 2.3 (25-35)		

Table 2: - Distribution Of Gravidity Of Study Subjects.

Gravida	Frequency	%
Primi	115	38.33
Multi	185	61.67

Table 3: -Distribution Of Gestational Age At Admission (weeks) Of Study Subjects.

Gestational age at admission(weeks)	Frequency	%
28 weeks to 31 weeks + 6 days	44	14.67
32 weeks to 33 weeks + 6 days	33	11.00
34 weeks to 36 weeks + 6 days	223	74.33
Mean ± SD = 34.73 ± 2.8 (28-36.86)		

Table 4: - Descriptive Statistics Of C-reactive Protein (mg/dL) Of Study Subjects.

C-reactive protein (mg/dL)	Mean ± SD	Median (25th-75th percentile)	Range
At admission	1.46 ± 1.47	0.88(0.32-2.322)	0.11-8.78
At 24 hours	1.55 ± 1.64	0.69(0.315-3)	0.12-7.42
At 48 hours	1.61 ± 1.9	0.65(0.25-2.8)	0.08-7.55

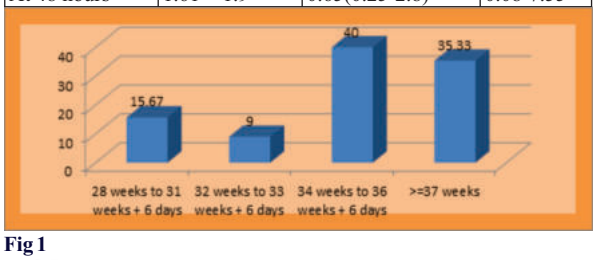
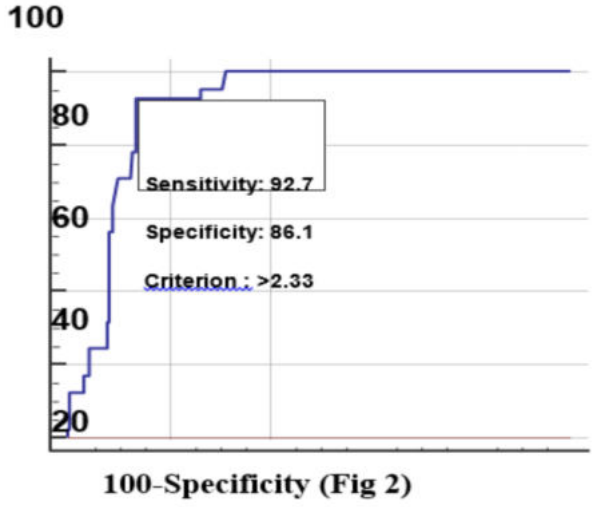
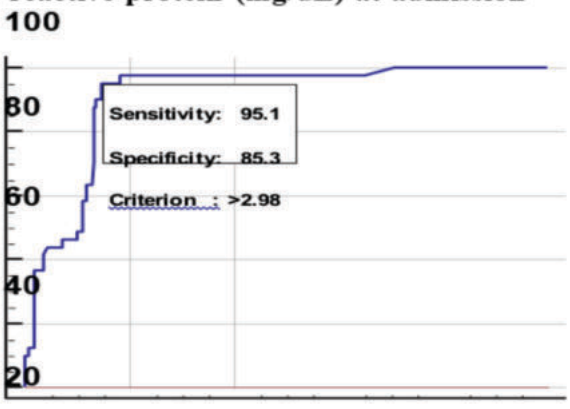


Fig 1



100-Specificity (Fig 2)

C-reactive protein (mg/dL) at admission



100-Specificity (Fig 3)

C-reactive protein (mg/dL) at 24 hours

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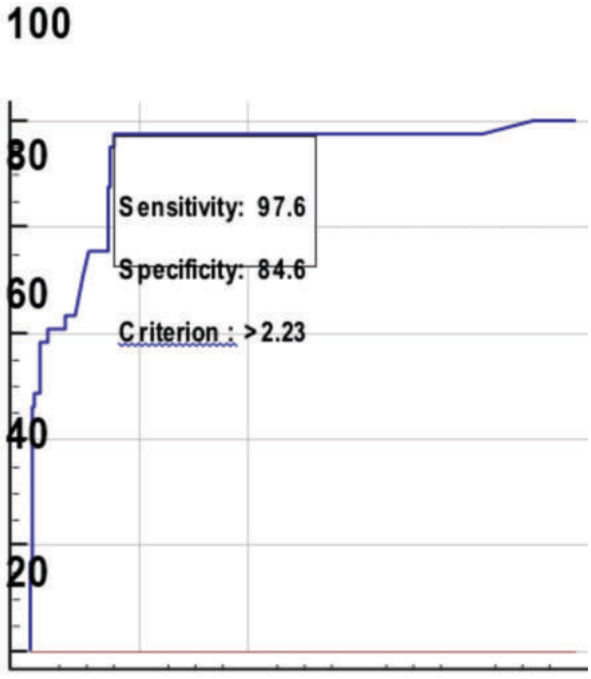

100-Specificity (Fig 4)

C-reactive protein (mg/dL) at 48 hours

DISCUSSION:

The pathogenesis of spontaneous preterm labour and PPROM in the setting of sub-clinical infection is not fully elucidated though host mediated response to bacterial products and tissue injury through activation of the macrophage-monocyte system, may cause preterm birth and PPROM.

In our study among all the parameters, C-reactive protein (mg/dL) at 48 hours was the best predictor of Subclinical chorioamnionitis at cut off point of >2.23 with area under curve of 0.929 for correctly predicting Subclinical chorioamnionitis. C-reactive protein (mg/dL) at 48 hours had sensitivity of 97.56% followed by C-reactive protein (mg/dL) at 24 hours (95.12%), C-reactive protein (mg/dL) at admission (92.68%). On the other hand, C-reactive protein (mg/dL) at admission had specificity of 86.10% followed by C- reactive protein (mg/dL) at 24 hours (85.33%), C-reactive protein (mg/dL) at 48 hours (84.56%). Highest positive predictive value was found in C-reactive protein (mg/dL) at admission (51.40%) and highest negative predictive value was found in c-reactive protein (mg/dL) at 48 hours (99.50%). Our results are in agreement with other studies conducted by **Aggarwal et al** (sensitivity 100%, specificity 69.56%, PPV 22.22%, NPV 100%)[8] and **Garg P**. (sensitivity 83.33%, specificity 80.76%, PPV 80%, NPV 84%)[9] wherein they concluded that CRP was the earliest and most reliable diagnostic marker of clinical as well histological chorioamnionitis in patients with PPROM. However, **Smith et al** found that CRP levels were not effective independent predictors of clinical or histological chorioamnionitis, nor was sequential CRP testing statistically significant for the identification of both clinical CAM and HCA in patients with PPROM. [10]

In our study majority [185(61.67%)] of patients were multigravida and only 115 out of 300 patients (38.33%) were primigravida. Similarly, **Garg et al** also reported a greater number of PPROM cases with multigravida (22 out of 31 and 9 out of 19 were multigravida in PPROM and PROM cases whereas 9 cases in PPROM and 10 cases in PROM were primigravida) in their study.[9] However, **Agarwal et al** reported primigravida both in study (40%) and control group (56%) as the prevalent group in their PPROM cases. [8]

In our study Majority [223(74.33%)] of our patients were admitted, at gestation age of (weeks) was 34 weeks to 36 weeks + 6 days followed by 28 weeks to 31 weeks + 6 days [44(14.67%)]. Gestational age at admission(weeks) was 32 weeks to 33 weeks + 6 days in only 33 out of 300 patients (11.00%). Mean value of gestational age at admission (weeks) of study subjects was 34.73 ± 2.8 with median (25th-75th percentile) of 36(33.86-36.86). This is comparable to **Kayem et al** (their study covered 689 women out of whom 184 women were admitted before 37 weeks gestation and 505 women after 37 weeks gestation)**, [11]Park et al** [out of 180 women 91(75.2%) were admitted at a gestation ≥29^{+0} weeks and 30(24.8%) at □29 weeks gestation] [12] but **Wang et al** reported a mean gestational age at admission to be 30.47±1.40 for 104(24.47%) out of 425 patients studied. [13]

In the current study, we observed that only 35.67% subjects had history of PPROM in previous pregnancy. Studies have persistently linked a previous history of PROM and PPROM with the predisposition to developing PPROM. **Samejima et al** found a ~15-fold risk of PPROM in the subjects who had a history of PPROM when compared to term-PROMs. [14]While, like many others, **Tolera at al** found that women who had a history of PROM were ~3 times more likely to develop PROM when compared to those who did not have it.[15] This might be due to untreated genitourinary infection and a short cervical length. Moreover, it has been observed that obstetric problems are recurrent by nature. Given the poorly understood etiopathology PPROM, it remains to be established how the history of PPROM modulated its risk in the future pregnancies. However, the genetic factors involved (if any) cannot be ruled out.

In our study mean value of C-reactive protein (mg/dL) at admission, at 24 hours and at 48 hours of study subjects was 1.46 ± 1.47, 1.55 ± 1.64 and 1.61 ± 1.9 with median (25th-75th percentile) of 0.88(0.32-2.322), 0.69(0.315-3) and 0.65(0.25-2.8) respectively. In the current study, we found that levels of CRP were inversely correlated with the time post-admission which may be attributed to the policy of prompt antibiotic administration, which is followed in our hospital. In the study done by **Lee AY et al**, mean value of CRP at admission, at 24 hours, at 48 hours, at 72 hours and at 96 hours of the study subjects were 9.85, 6.10, 6.45, 3.50 and 2.3 respectively.[16] While as per the study conducted by **Varsha Sinha et al**, the mean value of CRP at admission was 4, at 24 hours 6 and at 48 hours 3 respectively.[17]

However, **Lee et al.,** reported, abnormally high C-reactive protein levels (9.85) on admission [26(21.13%)], at 24 hours (6.10) [13(21.31%)] and at 48 hours (6.45) [16(26.22%)] respectively. This difference in the mean values of our study with **Lee et al** could be explained on the basis of subclinical chorioamnionitis patients selected in our study who had mean CRP values lower as compared to the patients who had clinically evident chorioamnionitis. [16]

In majority [120(40.00%)] of our study subjects, gestational age at delivery was 34 weeks to 36 weeks + 6 days followed by >=37 weeks [106(35.33%)] and 28 weeks to 31 weeks + 6 days [47(15.67%)]. Gestational age at delivery was 32 weeks to 33 weeks + 6 days in only 27 out of 300 study subjects (9.00%). Mean value of gestational age at delivery (weeks) of study subjects was 35.19 ± 2.87 with median (25th- 75th percentile) of 36.43(34.214-37.161). The above observations maybe attributed to inclusion of a greater number of study subjects at gestations 34 weeks to 36 +6 weeks presenting with PPROM. Many patients went into spontaneous labor and some were induced because of obstetric reasons like decreasing AFI/ NRCTG/ clinical suspicion of chorioamnionitis.

Significant association was seen in C-reactive protein (mg/dL) at admission, at 24 hours, at 48 hours with subclinical chorioamnionitis. (p value <.05)

Mean ± SD of C-reactive protein (mg/dL) at admission, at 24 hours, at 48 hours in patients with subclinical chorioamnionitis was 3.4 ± 1.29, 4.01 ± 1.04, 4.77 ± 1.38 respectively which was significantly higher as compared to patients without subclinical chorioamnionitis (1.16 ± 1.25(p value<.0001), 1.16 ± 1.36(p value<.0001), 1.11 ± 1.43(p value<0.0001) respectively. Significant positive correlation was seen between subclinical chorioamnionitis with c-reactive protein (mg/dL) at admission, c-reactive protein (mg/dL) at 24 hours, c-reactive protein (mg/dL) at 48 hours with correlation coefficient of 0.524, 0.596, 0.664 respectively.

CONCLUSION

In our study of use of CRP as a predictive marker with a relatively large sample size, we found it to be a useful marker to predict subclinical chorioamnionitis.

1. The sensitivity and specificity are higher at 48 hours.
2. The CRP should be used in relevant clinical scenarios after ruling out other causes of inflammation.
3. In women who have high CRP levels, the chances of having subclinical chorioamnionitis are higher.
4. CRP levels should be repeated at 48 hours as seen in our study, the sensitivity 97.6%, specificity 84.6%, PPV 50%, NPV 99.5% respectively being higher at this duration.

We propose that more studies should be performed to further evaluate the exact levels of CRP at which chorioamnionitis can be diagnosed. More studies are also warranted to shed some more light on the predictive value of CRP for chorioamnionitis so that prompt management of the patients can be planned , thereby improving the outcomes of patients presenting with PPROM.

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