Research Article (Open access)

Evaluation of CRP as a Preindicative Marker in Women with Preterm Labour and Preterm Prelabour Rupture of Membrane (PPROM)

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ABSTRACT- **Background:** Early detection of infection is most important during the conservative management of patients with PROM and preterm labour. Hence CRP is suitable markers for predicting risk of preterm delivery. To determine the diagnostic accuracy of C-reactive protein in the detection of chorioamnionitis in women with Preterm labor and PPROM and to test sensitivity/specificity/positive predictive valve of CRP in diagnosing chorioamnionitis against gold standard of histopathological examination of placenta.
Method: A prospective case-control study conducted on total 240 antenatal women, 120 cases of PROM and 120 cases of normal term pregnancy were used as a control. A detailed obstetrical and menstrual history was recorded along with systemic and local examination. Subjects were evaluated prospectively and managed expectantly with use of tocolytics, antibiotics and Steroids. Frequent vital signs monitoring and hematological investigation were done. CRP levels were determined by qualitatively. After delivery placenta was sent for histopathological examination for the presence of chorioamnionitis.
Result: CRP emerged as an early and sensitive predictor of chorioamnionitis in diagnosing histopathological chorioamnionitis.CRP had sensitivity and specificity of 100% and 50% respectively .the positive predictive value was 29.27% and negative predictive value 100% whereas TLC has sensitivity only 37.93%.
Conclusion: CRP is early and reliable indicator of histopathological and clinical chorioamnionitis in comparison of leucocyte counts and clinical parameter. Thus CRP can prove useful markers in identify early and subclinical infection which could lead to preterm labour and premature rupture of membrane.

Key-Words: Preterm birth, C-reactive protein, PPROM, Chorioamnionitis

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INTRODUCTION

The management of patients with preterm labour and premature rupture of membrane poses one of the most serious dilemmas in obstetrics since they significantly increase the likelihood of prematurity and serious perinatal infection. Potential pathogens largely arise from the ascending route and the endogenous vaginal flora, and may cause chorioamnionitis [(1)].

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Preterm birth rates have been reported to range from 5% to 7% of live births in some developed countries, but are estimated to be substantially higher in developing countries [(2)]. Etiology of preterm birth is thought to be multifactorial. It is, however, unclear whether preterm birth results from the interaction of several pathways or the independent effect of each pathway. Causal factors linked to preterm birth include medical conditions of the mother or fetus, genetic influences, environmental exposure, infertility treatments, behavioural and socioeconomic factors and iatrogenic prematurity [(3)].

The choice between immediate active or expectant management for women with PROM from 34 to 37 weeks of gestation remains controversial [(4)] and may require active management, mainly because of the risk of neonatal infection [(5)].

By gestational age, 5% of preterm births occur at less than 28 weeks (extreme prematurity), 15% at 28–31 weeks (severe prematurity), 20% at 32–33 weeks (moderate

prematurity), and 60–70% at 34–36 weeks (late preterm) [3]. The incidence of preterm delivery was 5.4% when neither chorioamnionitis nor premature rupture of membranes (PROM) was present, 11.9% when chorioamnionitis was present without PROM, and 56.7% when both chorioamnionitis and PROM were present and suggests that occult antepartum infection of the genital tract is an important cause of preterm delivery [6].

Intrauterine infection and inflammation are frequently associated with preterm labor and delivery, and at least 40% (positive, amniotic fluid & chorioamniotic space culture) of all preterm births have been estimated to occur with mothers who have an intrauterine infection, which is largely subclinical. The lower the gestational age at delivery, the greater the frequency of intrauterine infection [7].

Preterm labour can be prevented by early and prompt diagnosis of infection. Identification of high risk pregnancies can be done by various methods including clinical and biochemical markers of preterm delivery, clinical methods are: change in the cervix, uterine contraction and vaginal bleeding and identification of various epidemiological risk factors [8]. Measurement of inflammatory markers can be an alternative method to detect early infection of preterm labour. One of the markers in maternal serum, which indicates an increased risk of preterm delivery, is the C-reactive protein (CRP) [9].

CRP is annular (ring shaped) pentameric protein in blood plasma. It has five identical polypeptide chains and is synthesized in the liver. Their main roles is to identify potentially toxic autogenous substances released from damaged tissues, to bound them, and then detoxify them and remove from the blood. For its detection in the blood used are immunochemical methods such as laser nephelometry or sensitive homogenous enzyme assay with subclinical amnionitis and premature delivery [10-11]. The level of CRP in plasma greater than 1.5 mg/dl (15 mg/l) showed a highly significant correlation with levels of interleukin-6 (IL-6) in the amniotic fluid greater than 1500 Pg/ml [12]. Watts *et al.* (1992) also showed that the value of CRP in the serum of a mother which is greater than 1.5 mg/dl (15 mg/l) showed a highly significant correlation with positive amniotic fluid culture [13] C-reactive protein can be used in prediction and as a screening test to detect the risk of premature delivery [14].

MATERIALS AND METHODS

A prospective study was carried out in Department of Obstetrics & Gynaecology, in collaboration with Department of Pathology and Microbiology, King George Medical University, Lucknow, India for one year from June 2013- May 2014.

The present study comprises of a total 240 antenatal women, who were admitted in the hospital. 120 cases were of the study group, and 120 of the control group. An informed consent was obtained from the patients.

Study group comprised of 120 pregnant women between >20 week to <37 week of gestation with singleton pregnancy with preterm labour and or preterm premature rupture of membrane (PPROM) & control group comprised of 120 pregnant women at term $\geq$37 weeks to 40 weeks of gestation with singleton pregnancy without any other complication.

Exclusion criteria of study group were pregnancy complications (twin pregnancy, malpresentation, preeclampsia, antepartum, hemorrhage, polyhydramnios, uterine anomalies (cervical incompetence, malformation of uterus), chronic diseases (hypertension, hepatitis, diabetes) and any medical condition such as rheumatic fever rheumatoid arthritis, SLE, history of recent bacterial or viral infection. A detailed obstetrical and menstrual history was recorded along with local and systemic examination. Obstetrical examination done and signs of chorioamnionitis were specially looked for example maternal tachycardia, PR >100 BPM, fetal tachycardia FHR >160 BPM maternal fever >100. 4^{o}F, uterine tenderness, foul smelling discharged per vaginum. Subjects were evaluated & managed with use of tocolytics, antibiotics and steroid as per hospital protocol.

C-reactive protein estmation was done in each patient of both groups. Blood sample was collected by venupuncture for routine hematological investigation like Hb%, TLC, DLC and C-reactive protein determination. Urine and vaginal swab culture and sensitivity and routine antenatal investigation (if previously not done) were done. C-reactive protein determination was done by using latex agglutination method with the help of C-reactive protein reagent kit. 2 ml blood sample was drawn in a non-oxalated vial at the time of admission. The blood sample was stored in refrigerator at 4^{o}C and CRP was later assessed by lipid latex agglutination method by RapiTex-CRP kit.

After delivery placenta in 10% formalin was sent to pathology department for histopathological examination with membrane. Chorioamnionitis was identified by the presence of Polymorphonuclear leucocyte infiltration of placental membrane and villi. Baby was examined for signs of prematurity, apgar score at birth, any congenital anomaly and weight were noted.

Statistical Analysis: The statistical analysis was done using SPSS (Statistical Package for Social Sciences) Version 15.0 statistical Analysis Software. The values were represented in Number (%) and Mean±SD. To test the significance of two means the student't'- test was used.

RESULTS

Age-wise distribution of the patients was compared in the study group and control group. The highest number of cases of preterm labour and PPROM were primigravida (45.83%) and mean gestational age was 33.02±2.43 weeks. Leucocytosis (>12000/m^3) was present in 5% cases and vaginal swab culture was present in 5.83% cases of study group. CRP level was raised (>6 mg/dl) in 68.33% in study group and 47.5% in control group. Histopathological chorioamnionitis was present in 20% cases of study group and 5.83% cases in control group (Table 1).

Table 1: Characteristics of patients of study and control group

	Study (n=120)	Control (n=120)	'p' value
Age (years)	25.33±3.09	24.95±3.29	0.36 (NS)
Parity (Primiparae)	45.83%	55.83%	0.42 (NS)
Leucocytosis	5%	0	
Vaginal swab culture	5.83%	0	
CRP level (positive)	68.33%	47.5%	0.0006 (Sig)
Histopathological change chorioamnionitis (present)	20%	5.83%	

***CRP positive =≥6mg/dl**

Table 2 showed mean CRP level in both group. In study group mean CRP level in CRP positive cases (68.33%) were 10.39±3.33 mg/dl while in CRP negative cases (31.67%) it was 2.68±1.84 mg/dl. In control group mean CRP level in CRP positive cases (47%) was 6.6±2.75 while in CRP negative cases it was 2.24±1.68 mg/dl. Although CRP was raised in both study and control group but mean CRP level was higher in study group (10.39±3.33 mg/dl) than control group (6.6±2.75 mg/dl) and this difference was statistically significant (p<0.001).

Table 2: Mean CRP in study and control group

Group	CRP level	Range	Mean	't'	'p'
Study Group (n=120)	Positive (≥6 mg/dl) n=82 (68.33%)	6.2-18.4	10.39±3.33	14.986	<0.001 (S)
	Negative (<6 mg/dl) n=38 (31.67%)	0.8-5.8	2.68±1.84		
Control Group (n=120)	Positive ≥6 mg/dl n=57 (47%)	6.2-13	6.60±2.75	13.175	<0.00 (S)
	Negative (<6 mg/dl) n=63 (52.5%)	0.5-5.4	2.24±1.68		

Table 3 was shown mean CRP levels in study group in relation to clinical chorioamnionitis. Among 120 patients of study group. 14.17% patients had features of clinical chorioamnionitis (*i.e.* maternal fever, tachycardia, leucocytosis and fetal tachycardia uterine tenderness and foul smelling discharge) with elevated C-reactive protein levels (>6 mg/L). They had mean CRP level of 12.16±2.96 mg/litre. 65 patients in study group *i.e.* 54.17% had no evidence of clinical chorioamnionitis but they had elevated CRP level with mean level of CRP level is 10.64±3.33. 38 patients *i.e.* 31.67% had no evidence of clinical chorioamnionitis and their C-reactive protein levels were also in normal range.

Table 3: Mean CRP levels in study group in relation to clinical chorioamnionitis

Clinical chorioamnionitis and CRP level	Patients in study group (n=120)		Range and Mean CRP level in study group (n=120)	
	No.	%	Range	Mean
Clinical Chorioamnionitis with elevated CRP levels	17	14.17	7.4-16.2	12.16±2.96
No clinical chorioamnionitis with elevated CRP levels	65	54.17	6.0-18.4	10.64±3.33
Non-clinical chorioamnionitis with normal CRP level	38	31.67	0.8-5.8	2.68±1.84
Total	120	100		

Significance 'p' 1 vs. 2 = 0.091 (NS); 1 vs. 3<0.001 (S), 't'- test employed

Table 4 shown that 24 out of 120 patients with preterm delivery and PPROM showed histopathological chorioamnionitis *i.e.* neutrophilic infiltration of chorioamnion on their placental examination. They also had elevated CRP value with mean of 12.38±2.77 mg/dl. 58 patients *i.e.* 48.3% had no evidence of histopathological chorioamnionitis with elevated CRP level. 38 patients *i.e.* 31.67% had no evidence of histopathological chorioamnionitis with normal CRP levels.

Table 4: Relationship of clinical symptoms in study and group with histopathological chorioamnionitis

Diagnostic Group	Patients in study group (n=120)		Range and Mean CRP level in study group (n=120)	
	No.	%	Range	Mean
Histopathological chorioamnionitis with elevated CRP levels	24	20	8.6-16.2	12.38±2.77
No histopathological chorioamnionitis with elevated CRP levels	58	48.3	6.0-18.4	10.39±3.33
No histopathological chorioamnionitis with normal CRP level	38	31.67	0.8-5.8	2.68±1.84
Histopathological chorioamnionitis with normal CRP	–	–	–	–
Total	120	100		

Significance 'p' 1 vs. 2 = 0.054 (NS); 1 vs. 3= 0.009 (S) 't' test employed

Table 5 shown sensitivity and specificity of CRP level with different clinical features and laboratory test of chorioamnionitis in cases *i.e.* fever, maternal tachycardia, fetal tachycardia and total leucocyte counts. It showed that CRP level to be more sensitive (100%) but less specific (36.89%) in identifying clinical chorioamnionitis CRP also had the highest predictive value (100%) but least positive predictive value 20.73% among all parameters.

Table 5: Sensitivity and Specificity of CRP with different clinical features in study group

Clinical feature	CRP level				Sensitivity	Specificity	Positive predictive value	Negative predictive value
	With clinical features		Without clinical features					
	Positive	Negative	Positive	Negative				
Fever (100.4^{0}F)	17	0	65	38	100	36.89	20.73	100
Maternal tachycardia ≥100	9	0	73	38	100	34.23	10.98	100
Fetal tachycardia ≥160	13	0	69	38	100	35.51	15.85	100
TLC ≥12000	6	0	76	38	100	33.33	7.32	100

***CRP ≥6 mg/dl positive**

Table 6 shown the comparison of C-reactive protein determination and other tests in the identification of histopathologically diagnosed chorioamnionitis. It was found that histopathological chorioamnionitis present in 24 patients in study group and in 7 patients in control group. CRP was raised in all patients who had positive histopathological chorioamnionitis. Maternal fever and fetal tachycardia significantly higher in patient who had positive histopathological chorioamnionitis where as total leucocyte count (>12000/mm^3) was raised only in 5 patients with positive histopathological chorioamnionitis. CRP level was highly sensitive *i.e.* (100%) and less specific (50%) in identification of histopathological chorioamnionitis.

Table 6: Comparison of CRP determination and other test in the identification of histopathological chorioamnionitis

Test	Histopathological chorioamnionitis		Without histopathologies		Sensitivity	Specificity	Positive predictive value	Negative predictive value
	Normal	Abnormal	Normal	Abnormal				
CRP (≥6 mg/dl)	0	24	38	58	100	50	29.27	100
Maternal fever	0	12	103	5	100	95.37	70.59	100
WBCs count (>12000/mm^3)	19	5	95	1	66.7	94.4	57.14	96.23
FHR (≥160)	0	12	107	1	100	99.07	92.31	100

DISCUSSION

Expectant management for preterm labour and preterm premature rupture of membranes is now an accepted modality of treatment. Nevertheless, the main clinical concern is still the danger to the mother of acquiring chorioamnionitis.

Therefore, an approach to expectant management is based on monitoring for symptoms and signs of impending infection. The laboratory indicators most often used to predict infection are total leucocyte count, differential leucocyte count, urine culture, vaginal culture, the tests are by and large, unreliable. C-reactive protein appears to be the most sensitive acute phase protein, rising thousand folds in the initial stages of information. A short half-time of less than 24 hours makes it suitable to serve as a marker for diagnosing an infective process in early stage.

In the present study, total 240 patients were studied. The control group consist of 120 antenatal patients with full term pregnancy (>37 completed weeks) whereas study group consist of 120 antenatal patients with preterm labour and preterm premature rupture of membrane >20-37 weeks. Demographic, socioeconomic were comparable between control and study group.

In our study maximum number of patients in study groups belongs to 29-34 weeks of gestation. The results are in accordance with the previous studies [15-18] .

In our study total leucocytes C count (≥12000/mm^3) was present in 6 patients of study group (5%) whereas CRP was raised (≥6 mg/dl) in 82 patients (68.33%). Mean CRP level for CRP positive patients were 10.39±3.3 mg/dl. All patients who had TLC ≥12000/mm^3, CRP was also raised in those patients. Thus, all the patients with leucocytosis (≥12000/mm^3) had raised CRP level but not all patients with raised CRP level had leucocytosis. So that CRP is more reliable indicator in diagnosing chorioamnionitis, than TLC and DLC. Researchers found that very high levels of maternal plasma CRP level in early pregnancy was associated with preterm delivery [15-16,19]. A study found that pregnant women has higher level of CRP > 1 mg /and women were at high risk of preterm delivery is associated with premature uterine contractions. [14]

According to a study on C-reactive protein as a biochemical marker of idiopathic preterm delivery found that C-reactive protein (CRP) is a reliable marker of idiopathic preterm delivery in pregnant women who do not have any of the known risk for preterm delivery [20].

Another study **s**hown that Higher level of CRP in the 1st trimester is associated with preterm delivery. [21] Similar results found in a study that very high levels of maternal plasma CRP in early pregnancy was associated with increased risk of preterm delivery [22].

On comparing C-reactive protein levels with other laboratory tests and indicators of infection (e.g. total leucocyte count DLC, maternal fever, maternal tachycardia, foetal tachycardia) we found CRP level to be more sensitive (100%) but less specific (36.89%) in identifying clinical chorioamnionitis. The positive predictive value was 35.7% and negative predictive value was 100%. Our results were in accordance with other studies [14-15,23].

As we know that for diagnosing the chorioamnionitis gold standard test is histopathological examination of placenta. In our study histopathological chorioamnionitis present in control group. CRP raised in all patients with positive histopathological chorioamnionitis. Maternal fever and fetal tachycardia also present in all patients with positive histopathological chorioamnionnitis whereas TLC (>20200/mm^3) was raised only in 5 patients. The sensitivity of CRP is 100%, specificity is 50%, positive predictive value of CRP 29.27% and negative predictive value of CRP is 100% in detection of histopathological chorioamnionitis [7] recruited six reports for meta analysis excluding 466 cases and showed that CRP had sensitivity, specificity FPV, FNV of 72.8%, >6.4%, 23.6% and 27.2% respectively in diagnosis of chorioamnionitis. Our study is also supported by other studies [15-17,24].

CONCLUSIONS

Our study showed that many of the traditional laboratory test relied upon to predict the development of chorioamnionitis is unreliable. Pregnancy affects the WBC in a variable fashion. Physiological stress and

beta-methasone administration significantly elevate WBC. Only CRP determination accurately reflected chorioamnionitis with high sensitivity but less specific. However, elevated CRP levels correlated better with histopathological evidence of chorioamnionitis than with clinical features.

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