RESEARCH ARTICLE

C-reactive protein - as an early diagnostic marker of early onset sepsis and its correlation with blood culture

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ABSTRACT

Objective: To document effects of intrapartum risk factors for early onset sepsis (EOS) on C-reactive protein (CRP) levels in neonates, and to assess the suitability of this test in diagnosing EOS and its correlation with blood culture in a tertiary care centre. **Design:** Prospective cohort study. **Setting:** Labour room and post natal wards in a tertiary level teaching hospital in India. **Subjects:** 298 neonates at risk of developing infection. **Methods:** CRP levels in cord blood and neonatal blood at 24 hrs & 48 hrs were estimated. Babies were observed for signs of sepsis for at least 48 hours. If any newborn developed signs of sepsis or sepsis screen done at birth was positive, blood culture was sent and antibiotics was started. **Results:** 20(6.7%) babies had elevated CRP level in cord blood. At 24 hrs, this elevation was seen in 170(57%) babies while at 48 hrs 100(33.6%) babies showed positive results. Elevated cord CRP levels was significantly associated with rupture of membrane (ROM) >24 hrs, prolonged labour >12 hrs, and maternal fever (p=<0.05). At 24 hours, elevated CRP levels were associated with primiparity, ROM >18hrs, maternal fever, more than three vaginal examinations after membrane rupture, and meconium staining of amniotic fluid (p =<0.05). Fifteen (5.03%) babies developed EOS. The negative predictive value for elevated CRP levels at 24 hrs was 99.2%. Sepsis screen parameters that was significantly associated with proven sepsis were neutropenia <1500/cmm (p=0.01) and immature to total neutrophil ratio (I/T ratio) > 0.2 (p=0.04). **Conclusion:** Several intrapartum risk factors for early onset sepsis can cause elevation in CRP levels. However, this test may be useful in excluding infection.

Keywords: Foetal blood, newborn, C-reactive protein.

Of the 130 million babies born every year globally, about 4 million die in the first 4 weeks of life [1]. Neonatal sepsis accounts for 30-50% of these deaths [2]. It is difficult to differentiate neonatal sepsis from other conditions as the symptoms and clinical signs are non-specific. The gold standard for diagnosis of sepsis is a positive blood culture, which is costly and time consuming. As a result, early diagnosis of neonatal sepsis has been a frustrating experience in developing and developed countries. Serum proteins like CRP, haptoglobin and fibrinogen, can be used as non-specific indicators of bacterial sepsis. However, the utility of

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CRP for the diagnosis of neonatal infection has been the subject of controversy because of its low sensitivity [3]. Serial measurement of CRP is considered more useful than single titer in diagnosis of sepsis [4]. Such tests could be of special importance in a newborn that is asymptomatic or has only equivocal signs at birth and risk factors for infection [5].

Keeping in mind these facts, the present study was designed to evaluate the effect of intrapartum risk factors for early onset sepsis (EOS), the utility of CRP in the diagnosis of EOS and its correlation with blood culture.

Methods

This was prospective cohort study conducted in the department of Pediatrics and Obstetrics & Gynecology of Silchar Medical College and Hospital during the period from July 2013 to June 2014.

Inclusion and exclusion criteria

Hospital born neonates were included if they were born with 2 or more of the following risk factors [6] : low birth weight, preterm, febrile illness in mother within 2 weeks of delivery, 3 or more per vaginal examinations after rupture of membranes, foul smelling liquor, prolonged rupture of membranes, prolonged and difficult delivery and peri-natal asphyxia. Newborn babies born at less than 28 weeks, babies having lethal congenital anomalies and neonates born with history of antibiotics usage by the mother during labour were excluded from the study.

Primary outcome

The primary outcome was EOS, defined as sepsis occurring within 48 hours of birth [7]. The following were considered to be signs suggestive of sepsis: lethargy/ poor feeding, temperature: $<36^0$ C or $>38^0$ C for >30min, significant Jaundice (in the absence of blood group incompatibility), apnoea, respiratory distress, CRT: >3sec, heart rate (corrected for elevation of body temp) >160/min, vomiting, diarrhoea, abdominal distension, ileus, omphalitis, bulging fontanel, shrill cry, convulsion, tachypnea, sub costal retraction, grunt, petechiae/bleeding diathesis and pyoderma [8]. Laboratory markers considered abnormal were: C-Reactive protein (CRP): > 6mg/L, Total leucocyte count < 5000/cu.mm, Absolute neutrophil count < 1500/cu.mm and I/T ratio > 0.2

Newborn babies developing signs suggestive of sepsis were categorized as having sepsis or probable sepsis. Sepsis was diagnosed if the newborn baby had signs suggestive of sepsis and a positive blood culture. Probable sepsis was diagnosed in a newborn baby with negative blood culture, if it had two or more signs suggestive of sepsis and one or more abnormal laboratory markers, or two or more abnormal laboratory markers with one or more signs suggestive of sepsis [8]. Newborn babies with sepsis or probable sepsis received antibiotics for about 10-14 days. The remaining newborn babies were classified as at risk of infection and received antibiotics for an average of 5-7 days.

Sample size estimation

Taking the incidence of EOS to be 2-8% for India and taking confidence level of 95%, cases were selected. Altogether 5960 babies were born in the hospital during July 2013 to June 2014. Out of this 298(5%) babies meeting the inclusion, exclusion criteria were included in the study.

Laboratory techniques

Approximately 5 ml of blood was collected from the umbilical cord after clamping and cutting of the cord. At 24 and 48 hrs approximately 3-4 ml of blood was collected by venepuncture from the new born. out of which 1ml of the blood sample was inoculated aseptically in to a blood culture bottle, 1ml of blood collected for the estimation of C-reactive protein and the remaining 2ml for estimation of Total WBC count, Absolute neutrophil count, I/T ratio.CRP levels were determined by turbidimetric immunoassay supplied commercially by Transasia Bio-Medicals Ltd. Daman, Erba Diagnostics Mannheim GmbH, Germany. The investigator performing the CRP test was blinded to the clinical status of the newborn babies.

Data collection and analysis

Newborn babies were observed for signs of sepsis for at least 48 hrs. Data were collected in a pre-tested proforma. Proportions were compared by 2x3 Fisher Exact test. Relative risks were calculated for the risk

factors for sepsis. The predictive values of CRP for diagnosing neonatal sepsis were also calculated.

Results

There were 298 newborn babies enrolled for the study. Twenty (6.7%) neonates had CRP levels of ≥ 6 mg/L in cord blood while 170 babies (57%) had elevated levels at 24 hours. CRP levels in cord blood of ≥ 6 mg/L was significantly associated with rupture of membranes >24 hrs, prolonged labour >12 hrs, and maternal fever (Table-1). At 24 hours, elevated CRP levels were associated with primiparity, ROM >18hrs, maternal fever, more than three vaginal examinations after membrane rupture, and meconium staining of amniotic fluid. When the cut-off CRP level was increased to 12 mg/L, significant association was noted only with rupture of membranes >24 hrs. There was no association between Apgar score, birth weight and CRP levels.

Out of 298 babies, only seven babies were diagnosed to have proven sepsis. *Acinetobacter* spp was isolated in 3 cases followed by *E-coli*, *Pseudomonas*, *Klebsiella Pneumoniae* and *Staphylococcus Aureus* (MRSA) (each 1case). An additional eight babies were diagnosed to have probable sepsis. The sensitivity, specificity, positive and negative predictive values of CRP estimation at 24 hr for diagnosis of EOS using 6 mg/L as the cut off 93.3%, 44.9%, 8.2% and 99.2% respectively. The corresponding values for a cut off level of 12 mg/L were 40.0%, 80.6%, 9.8% and 96.2% respectively. Table-2 provides association between CRP levels and sepsis. CRP elevation was not significantly associated with the presence or number of signs. It was also noted

Table 1: Association of risk factors with CRP levels.

		CRP (mg/L)			Risk ratio (95%CI)		
Risk factors	**Number of cases observed**	**Cord Blood (≥6mg/L) (n=20)**	**Neonatal Blood (≥6 mg/L) (n=109)**	**Neonatal Blood (>12 mg/L) (n=61)**	**Cord Blood (≥6 mg/L)**	**Neonatal Blood (≥6 mg/L)**	**Neonatal Blood (>12mg/L)**
Primiparity	170	6 (30%)	81 (74.3%)	25 (40.9%)	0.32 (0.12 to 0.81)	2.17* (1.51 to 3.13)	0.52 (0.33 to 0.82)
ROM < 18 hrs	62	3 (15%)	18 (16.5%)	7 (10.4%)	0.67 (0.20 to 2.2)	0.75 (0.49 to 1.14)	0.47 (0.22 to 0.98)
ROM 18-24 hrs	193	7 (35%)	90 (82.6%)	41 (67.2%)	0.29 (0.12 to 0.71)	2.57* (1.66 to 3.97)	1.11 (0.69 to 1.80)
ROM >24 hrs	43	11 (55%)	25 (22.9%)	14 (22.9%)	7.53* (3.31 to 17.1)	1.76* (1.29 to 2.40)	1.76* (1.06 to 2.91)
Prolonged labour	91	16 (80%)	35 (32.1%)	21 (34.4%)	9.09* (3.12 to 26.4)	1.07 (0.78 to 1.47)	1.19 (0.74 to 1.90)
Maternal fever	30	10 (50%)	22 (20.1%)	9 (14.8%)	8.93* (4.04 to 19.7)	2.25* (1.71 to 2.97)	1.54 (0.84 to 2.81)
>3VE after ROM	143	6 (30%)	70 (64.2%)	31 (50.8%)	0.46 (0.18 to 1.17)	2.28* (1.70 to 3.06)	1.12 (0.71 to 1.75)
FSL	22	3 (15%)	8 (7.3%)	1 (1.6%)	2.21 (0.70 to 6.98)	0.97 (0.55 to 1.73)	0.20 (0.03 to 1.43)
MSL	104	4 (20%)	60 (55.0%)	15 (24.6%)	0.46 (0.16 to 1.35)	2.28* (1.70 to 3.06)	0.60 (0.35 to 1.03)
Gestation <37wks	50	2 (10%)	13 (11.9%)	6 (9.8%)	0.55 (0.13 to 2.30)	0.67 (0.41 to 1.10)	0.54 (0.25 to 1.20)
Male	188	7 (35%)	75 (68.8%)	40 (65.6%)	0.31 (0.12to 0.76)	1.29 (0.92 to 1.79)	1.11 (0.69 to 1.78)
SVD	169	3 (15%)	67 (61.5%)	28 (45.9%)	0.13 (0.04 to 0.45)	1.21 (0.89 to 1.66)	0.64 (0.41 to 1.01)

*Risk ratio - > 1 - Positive association, ≤ 1 - No association, *p value <0.05*

Table 2: CRP levels and neonatal sepsis.				
CRP Levels (mg/dl)	**Proven Sepsis**	**Probable Sepsis**	**No sepsis**	**p value**
Cord blood < 6 (n=278) At 24 hrs				
<6	0	1(0.36%)	119(42.8%)	0.14
≥6	4(1.43%)	2(0.72%)	95(34.2%)	0.21
>12	2(0.72%)	2(0.72%)	53(19.0%)	0.24
Cord blood > 6 (n=20) At 24 hrs				
<6	0	0	8(40%)	0.11
≥6	0	2(10%)	6(30%)	0.99
>12	1(5%)	1(5%)	2(10%)	0.16
Cord blood < 6 (n= 278) At 48 hrs				
<6	0	3(1.08%)	179(64.4%)	0.07
≥6	5(1.79%)	1(0.36%)	50(17.9%)	0.19
>12	1(0.36%)	1(0.36%)	38(13.7%)	0.66
Cord blood > 6 (n=20) At 48 hrs				
<6	0	2(10%)	14(70%)	0.16
≥6	0	1(5%)	2(10%)	0.50
>12	1(5%)	0	0	0.20

that 10 of the 12 babies with CRP levels of 48 mg/L or more did not have evidence of infection. Only three of the 48 babies with CRP levels above 12 mg/L were diagnosed to have EOS. Table 2 provides association between CRP levels and sepsis. CRP elevation was not significantly associated with the presence or number of signs. Only six of the 63 babies with CRP levels above 12 mg/L were diagnosed to have EOS. Eight babies among those with sepsis or probable sepsis had abnormal total leukocyte, seven babies had abnormal absolute neutrophil counts in the cord blood. Seven had abnormal immature to total leukocyte ratio. Table 3 provides correlation of sepsis screen parameters with the blood culture status. Sepsis screen parameters that was significantly associated with proven sepsis were Neutropenia <1500/cmm (p=0.01) and I/T ratio > 0.2 (p=0.04) If two or more positive tests combined as sepsis screen parameters then also there is significant association with proven sepsis with p =0.0001.

Table 3: Sepsis screen parameters and blood culture status					
Sl. No	**Screening parameters**	**Culture positives n=7(%)**	**Culture negative n =8 (%)**	**Total cases n = 15**	**p-value**
1	CRP positive	7(100%)	7(87.5%)	14(93.3%)	1.00
2	Leucopenia (<5000/cmm)	3(42.9%)	1(12.5%)	4(26.7%)	0.28
3	Neutropenia (<1500/cmm)	6(85.7%)	1(12.5%)	7(46.7%)	0.01
4	I/T ratio > 0.2	6(85.7%)	2(25%)	8(53.3%)	0.04
5	Two or more tests positive	7(100%)	1(12.5%)	8(53.3%)	0.0001

Discussion

The prevalence of blood culture proven sepsis in the present study was 46.6%. This is similar to the 41.7% reported by Chako and Sohi [9], 42% by Mustafa et al. [10] and 47.5% by Roy et al. [11]. The commonest organism isolated was Acinetobacter spp, which was resistant to commonly used antibiotics including carbapenems. This shows a changing pattern of bacterial isolates over the years. Among the risk factors for neonatal sepsis, CRP performance was highest in neonates born to mothers with ROM >24 hrs, Prolonged labour >12 hrs, and Maternal fever.

This study showed that several such risk factors can cause elevated CRP levels in the absence of infection. This is in agreement with previously published reports [3,4]. Since CRP does not cross placenta, the elevated levels are due to production of CRP in the neonate. Stimuli other than infection, like hypoxia, trauma and metabolic changes can also induce production of proinflammatory mediators [3]. There are few longitudinal studies examining CRP changes in healthy babies with intrapartum risk of infection. Cytokine elevation seen in the early neonatal period in such babies probably reflects physiological stress induced at birth [4]. Since CRP levels rise during the initial 24 hours in many babies irrespective of infection or administration of antibiotics, serial determinations in this period may not be of much use in

diagnosis but may help in identifying uninfected babies and restricting antibiotic use [12,13].

Various studies utilizing varying protocols have suggested different values as upper limit of normal [14]. In the present study, we found that CRP has a low PPV and high NPV (99.2%). This signifies that CRP is not of much use in diagnosing neonatal sepsis, but lower levels (<6 mg/l) are helpful in excluding sepsis.

Cord blood CRP levels estimated using a kit with 6 mg/L as detection limit, could not satisfactorily predict EOS. Recent studies show that cut off values may be different for cord and 24 h samples [3]. More sensitive techniques like nephelometry may help. With sensitivity of 93.3%, CRP levels at 24 h proved to be best indicator for diagnosing EOS. If utilised with caution, this test can help in reducing antimicrobial use in the new-born.

Conclusion

Presence of high level of C- reactive protein alone does not indicate that the child is infected as specificity and positive predictive value are very low. Since CRP levels rise during the 24 hours in many babies irrespective of infection or administration of antibiotics, serial determinations in this period may not be of much use in diagnosis. However, absence of raised C- reactive protein can definitely exclude the infection as negative predictive value is very high (>95%). C-reactive protein alone cannot be used for early detection of septicemia, as in present study CRP was not statistically significant with proven sepsis. Antibiotic resistance has emerged as a concern especially in developing countries. Controlling the use of broad spectrum antibiotics and implementation of infection control measures can results in decreased microbial resistance.

Conflict of interest: None. **Disclaimer:** Nil.

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