

**USEFULNESS OF CRP IN DIFFERENTIATING INFECTED FROM UNINFECTED NEONATES AS AMONG THOSE AT RISK OF INFECTION**Somaiah<sup>1</sup>, Vamsidhar<sup>2</sup>, Sujitha<sup>3</sup>, A. Mohan Rao<sup>4</sup>, K. Srikanth<sup>5</sup>**HOW TO CITE THIS ARTICLE:**

Somaiah, Vamsidhar, Sujitha, A. Mohan Rao, K. Srikanth "Usefulness of CRP in Differentiating Infected from Uninfected Neonates as among those at Risk of Infection". Journal of Evolution of Medical and Dental Sciences 2014; Vol. 3, Issue 07, February 17; Page: 1724-1731, DOI: 10.14260/jemds/2014/2049

**ABSTRACT:** Neonatal sepsis remains an important diagnostic consideration in many neonates, either because of risk factors or clinical manifestations associated with bacterial infection, which are unspecific and vague. It is important to differentiate infected from uninfected neonates among those at risk of infection due to maternal risk factors. Several surrogate markers of inflammation like CRP, Cytokine levels have been evaluated as possible tools for early diagnosis of bacterial sepsis. Such test could be of special importance in a newborn that is asymptomatic or has only equivocal signs at birth but has risk factors for infection. The present study was conducted to evaluate the effect of the intrapartum risk factors for early onset sepsis on CRP levels and utility of CRP in the diagnosis of EOS. A study of 108 neonates at risk of developing infection over a period of one year was done at tertiary Hospital, Khammam. CRP levels in cord blood and neonatal blood at 24-36 hrs. were estimated using commercial kits. Babies were observed for signs of sepsis for at least 72 hrs. Ten (9.3%) neonates had elevated CRP levels in the cord blood. Elevated cord CRP level showed positive association with primiparity, rupture of membranes more than 24 hrs, prolonged labor, maternal fever, more than three vaginal examinations, foul smelling liquor, urinary tract infection and babies extracted by LSCS. The negative predictive value for elevated CRP levels at 24 hrs. was 97%. Several intrapartum risk factors for EOS can cause elevation in CRP levels however; this test may be useful in excluding infection. CRP level is a good indicator, in diagnosing the early sepsis and early intervention in management of neonatal sepsis, intern it prevents mortality in neonates.

**KEYWORDS:** C-Reactive protein; Intrapartum risk factors; Neonatal sepsis.

**INTRODUCTION:** Neonatal sepsis is a clinical syndrome of bacteremia characterized by systemic signs and symptoms of infection with or without accompanying bacteremia in the first month of life<sup>1</sup>. Neonatal sepsis was one of the common causes of neonatal mortality contributing to 16% of all intramural deaths.<sup>1</sup> Other direct causes of neonatal death are preterm birth, severe infection, and asphyxia.<sup>2</sup> The immune system of newborns is immature.<sup>3</sup> They lack the antibody-mediated protection against E.coli and enterobacteriaceae.<sup>4</sup> This account for newborn vulnerability to microbial attack Neonatal Sepsis has been a challenge for urgent need to know whether baby has sepsis, to institute treatment as quickly as possible. Confirmation of the diagnosis by definitive culture is not rapid or particularly sensitive. Of all the newborns only 3-5 per 1000 admitted in NICU have culture proven sepsis.<sup>5</sup> Hence early and efficient diagnosis and management remains a difficult task. It is costly, time consuming, demands a well-equipped laboratory, requires more blood and has success rate of about 40% only. The ideal investigation for neonatal sepsis should clearly differentiate between infected and non-infected neonates. It should be economical and with minimal technical errors and above all should have low rate of false positive and false negative values.

During the last few decades, there has been an increasing interest in the value of acute phase

## ORIGINAL ARTICLE

---

reactants for the diagnosis and follow up of neonatal sepsis. Among the acute phase reactants, it has been shown that concentration C - reactive protein (CRP) is a sensitive and responsive monitor of neonatal sepsis.

It has the advantage of being performed easily, quickly and is also subject to less technical errors and inter-observer variability with low false positive and false negative values. The study undertaken with following aim and objectives:

1. To document effects of intrapartum risk factors for early onset sepsis (EOS) on CRP levels in neonates.
2. To assess the suitability of this test in diagnosing EOS

**MATERIALS AND METHODS:** The present study was conducted in the department of Pediatrics, Mamata Medical College and General Hospital, Khammam.

### **INCLUSION CRITERIA:**

1. One of the following risk factors is considered for neonates.
  - Premature rupture of membranes
  - More than 3 vaginal examinations after rupture of membranes
  - History of maternal fever one week before delivery
  - Foul smelling liquor
  - Prolonged labor
  - Meconium stained liquor
  - Untreated or partially treated urinary tract infection.
2. Neonates born in tertiary care hospital, Khammam

### **EXCLUSION CRITERIA:**

- Newborn babies with gestation age less than 28 weeks.
- Neonates with birth weight less than 1 kg.
- Neonates with congenital anomalies.
- Out born neonates.

All neonates born in a tertiary Hospital between 1<sup>st</sup> January 2012 and 31<sup>st</sup>

The cord blood of all the neonates born to mothers with at least one of the risk factors for neonatal sepsis was collected and sent for CRP analysis. Neonates were followed upto 72 hrs. from the time of birth for the development of any symptoms and signs suggestive of sepsis. Between 24 and 36 hrs. the blood was collected from the neonate and was sent for:

CRP, Total leucocyte count, Absolute neutrophil count, Band cell count. Blood culture and sensitivity.

**SAMPLE COLLECTION:** Approximately 5ml of blood was collected from the umbilical cord after clamping and cutting of the cord. About 24 hrs. later an area of approximately 5cm over the venepuncture site was disinfected with 70% alcohol rubbing vigorously and allowed to dry. This was followed by of povidine iodine in concentric circles over the site and allowed to dry for at least 1 minute. About 3-4ml blood was drawn using a sterile syringe, out of which 1ml of the blood sample was inoculated aseptically in to a blood culture bottle, 1ml of blood, was allowed to clot in a sterile

## ORIGINAL ARTICLE

---

bottle to collect serum for the estimation of C-reactive protein and the remaining 2ml of blood was collected in a sterile bottle containing the anticoagulant EDTA (2-2.5 mg/ml) for estimation of Total WBC count, Absolute neutrophil count, Band cell count.

**C-Reactive protein assay:** The test was done by using the diagnostic kit for the in-vitro detection of CRP in human serum by the rapid slide latex agglutination quantitative method and semi quantitative method.

### TEST PROCEDURE:

**QUALITATIVE:** A drop of undiluted patient's serum is mixed with a drop of latex reagent on a slide provided in the kit with the help of disposable applicator stick spreading the fluid over the entire area of circle. The slide was gently rocked to and fro for 2 minutes and observed for macroscopic agglutination of the latex particles under a direct light source. Agglutination of latex particles within 2 minutes was taken as a positive reaction. No agglutination was taken as negative test and indicated the absence of detectable levels of CRP in the test specimen. Controls were run along with the test.

The sensitivity of the antigen in the kit for a visible agglutination is 0.6mg/dl. So in our study, a CRP value of >0.6mg/dl was taken as a positive test. The semiquantitative assay was done by repeating the test in higher serum dilution of 1:2, 1:4, 1:8, 1:16 etc. using isotonic saline. Agglutination in the highest serum dilution corresponds to the amount of CRP in mg/dl present in the test specimen.

Concentration of CRP is calculated as follows:  $CRP (mg/dl) = S \times D$

Where S= Sensitivity of the reagent i.e. 0.6mg/dl D= Highest dilution of serum showing agglutination

In present study if the CRP content was equal to (or) more than 0.6mg/dl was considered to be significant.

**RESULTS:** This is a prospective study conducted from 1<sup>st</sup> Jan 2012 to 31 Dec 2012. 108 babies were included in the study. Males were 64(59.3%) and females were 44(40.7%). 20 cases (18.5%) were <2.5kgs and 88 cases(81.5%) were >2.5kgs. 15 cases (13.9%) were  $\leq$ 37wks and 93 cases (86.1%) were >37wks. 72 cases (66.7%) were born to primi gravida mother and 36 cases (33.3%) were born to multi gravida mother. 58 cases (53.7%) had duration of ROM between 12 and 24hrs, whereas 30 cases had <12 hrs. and 20 cases had >24hrs.

In other intrapartum risk factors, 12 cases (11.1%) had maternal fever, 50 cases (46.3%) had >3 VE after ROM, 8 cases(7.40%) had FSL, 52 cases (48.1%) had MSL, 39 cases (36.1%) had prolonged labor and 8 cases(7.4%) had UTI. 63 cases (58.3%) had normal delivery and 45(41.7%) cases had LSCS.

Most common clinical features observed were respiratory distress (20.4%), lethargy (15.7%), poor feeding (13.0%) and chest retraction (6.5%). Among the 108 babies, 10(9.3%) neonates had CRP levels of  $\geq$  0.6mg/dl in cord blood. Among the 108 babies, 46 babies (42.6%) had elevated CRP levels at 24-36 hr. after birth, 8 babies (7.4%) had TLC<5000/cu.mm, 4 babies (3.7%) had ANC<1500/cu.mm, 5 babies (4.6%) had BCC<20%, 4 babies (3.7%) had blood culture positives. Klebsiella isolated from blood culture in 2 babies, E.coli in one baby and Staphylococcus aureus in 1 baby. Of the total 4 babies, 3 were male babies and one female baby.

## ORIGINAL ARTICLE

CRP level in cord blood  $\geq 0.6$ mg/dl showed positive association with primiparity, ROM >24 hrs, prolonged labor, maternal fever, > 3 VE after ROM and LSCS. At 24 hrs, elevation in CRP level  $\geq 0.6$  mg/dl showed positive association with primiparity, ROM >24 hrs, prolonged labor, maternal fever, FSL, MSL and LSCS. At 24hrs elevation in CRP >1.2mg/dl, positive association is seen with ROM <12 hrs. and 24 hrs, prolonged labor, maternal fever, > 3 VE after ROM, FSL, MSL and LSCS. CRP blood at 24 hrs. is positive in babies with no sepsis with 21.42% cases  $\geq 0.6$ mg/dl and 12.24% cases with >1.2mg/dl.

Among 108 babies studies with risk factors, there were 10 babies with cord blood CRP  $\geq 0.6$ mg/dl and 46 babies with neonatal blood CRP  $\geq 0.6$ mg/dl at 24 hrs. after birth. When the cut off of CRP level was increased to >1.2mg/dl in the neonatal blood 22 out of 46 babies had the values.

CRP Levels (mg/dl)	Sepsis	Probable Sepsis	No sepsis	P value
<b>Cord blood &lt; 0.6 (n=98) At 24-36 hrs.</b>				
< 0.6	0	1(1.02%)	58(59.18%)	0.0205*
$\geq 0.6$	0	0	21(21.42%)	0.7943
> 1.2	3(3.06%)	3(3.06%)	12(12.24%)	0.0001**
<b>Cord blood &gt;6 (n=10) At 24-36 hrs.</b>				
< 0.6	0	1(10%)	2(20%)	0.9999
$\geq 0.6$	0	1(10%)	2(20%)	0.999
>1.2	1(10%)	2(20%)	1(10%)	0.3333

**Table – 2 : Shows association between CRP levels and neonatal sepsis**

Among those 108 babies, there were 4 babies diagnosed to have sepsis, 8 babies were diagnosed to have probable sepsis, 96 babies were diagnosed to have no sepsis. The above table shows correlation of CRP levels with neonatal sepsis. When cord blood CRP was < 0.6 mg/dl 98 babies were observed out of 108 babies with risk factors to have negative CRP.

Of these at 24-36 hrs. 59 babies had CRP <0.6 mg/dl of which 1(1.02%)baby was diagnosed to have probable sepsis and remaining 58(59.18%) babies were diagnosed as no sepsis with P value 0.0205(suggestive significant).21(21.42%) babies had CRP  $\geq 0.6$  mg/dl and were diagnosed to have no sepsis. 18 babies had CRP > 1.2 mg/dl of which 3(3.06%) babies were diagnosed to have sepsis. 3(3.06%) babies were diagnosed to have probable sepsis and remaining 12 babies were to have no sepsis with P value 0.0001(strongly significant).

When cord blood CRP value was > 0.6mg/dl 10 babies were observed out of 108 of babies studied with risk to have positive CRP. Of these 24-36 hours 3 babies had CRP <0.6 mg/dl of which 1(10%) baby had probable sepsis and remaining 2(20%) babies had no sepsis. 3 babies had CRP  $\geq 0.6$ mg/dl of which one baby (10%) was diagnosed to have probable sepsis and remaining 2(20%) babies no sepsis. 4 babies had CRP > 1.2mg/dl of which 1(10%) baby was diagnosed to have sepsis and 2(20%) babies were diagnosed to have probable sepsis and 1(10%) baby no sepsis.

The sensitivity, specificity, positive and negative predictive value of CRP estimation at 24 hrs. for diagnosis of EOS using 0.6mg/dl as the cut off for 83%, 64%, 23% and 96.8% respectively.

## ORIGINAL ARTICLE

---

**DISCUSSION:** Neonatal sepsis with its high mortality rate, still remains a diagnostic and treatment challenge for neonatal health care providers, developing countries have the highest incidence and mortality rates. This may be due to problems in utero by anatomical defects, infections and infected birth passage etc. Early diagnosis of neonatal septicemia helps the clinician in instituting antibiotics therapy at the earliest thereby reducing mortality in neonates. Early identification of an infected neonate also helps in abandoning unnecessary treatment of a non-infected neonate.

In the present study an attempt has been made to document the effects of intrapartum risk factors for early onset sepsis on CRP levels in neonates and to assess the suitability of this test in the diagnosing EOS.

Male babies were more than the female babies in the present study, showing a ratio 1.5:1. These results are comparable with earlier observations.<sup>6</sup> Male preponderance in the neonatal septicemias may be linked to the X-linked immunoregulatory gene resulting in the host's susceptibility to the infection in males.

In the present study the higher proportion of cases were with birth weight >2.5 kg. The results of other studies showed higher proportion of cases with birth weight <2.5 kg. As during our study there were less babies with weight <2.5kg delivered in the hospital, less cases were observed.

In the present study the higher proportion of cases were found to with gestational age >37 weeks. The result of our study was comparable to the earlier studies <sup>6</sup>. The higher proportion of cases among the term neonates compared to the preterm neonates in our study probably reflect difference in the population characteristics and the occurrence of the predisposing factors(preterm incidence) among them. Preterm are more susceptible to the infections due to lack of inherent defensive mechanism. According to Barbara J Stoll et al,<sup>7</sup> 1991 the incidence of septicemia is inversely proportional to gestational age of the neonates. In the present study higher proportions of the mothers were primi gravida.

In the present study, duration of rupture of membranes <12hrs as a risk factors is 27.8% of cases which is comparable to study done by earlier studies, who showed 23.2%.<sup>6</sup> Duration of rupture of membranes was 12-24 hrs. seen in which was comparable to study done by Mathai et al<sup>7</sup> who showed 46.8%. Duration of rupture of membranes >24 hrs. was seen in 18.5% as a risk factor which was comparable to earlier studies.<sup>6,8</sup>

In the present study maternal fever as a risk factor was observed in 11.1% cases, which was in accordance with earlier studies.<sup>6,8</sup> >3 VE after ROM as a risk factors was observed in 46.3% cases, similar observation was also made by earlier authors.<sup>6,9</sup> FSL as risk factors was observed in 7.4% cases, and MSL as a risk factor was observed in 48.1% cases, which were in comparable with earlier studies.<sup>10</sup> Prolonged labor as a risk factor was observed in 6.1%% cases, and UTI as a risk factor was observed in 7.4%% cases, which was in accordance with earlier studies.<sup>6,8,9</sup> The variations in the occurrence of intrapartum risk factors probably reflect differences in the rates of occurrence of the predisposing risk factors in the various studies.

In the present study higher proportion of cases were delivered vaginally. The result of our study is comparable to the earlier studies.<sup>6,10</sup> In the present study higher proportion of babies had respiratory distress, lethargy, poor feeding as predominant clinical features. The results are in agreement with earlier studies.<sup>11,12</sup>

In the present study cord CRP was elevated ( $\geq 0.6$  mg/dl) in 10 babies (9.3%).

The result in our study is comparable to the study done by Chiesa et al. In the present study

## ORIGINAL ARTICLE

---

TLC < 5000/ cu.mm is considered to be leucopenia. In the present study it is observed that 7.4% babies with maternal risk factors and CRP  $\geq 0.6$ mg/dl done at 24 hrs. after birth in babies with neonatal risk factors was 42.6%, absolute neutrophil count <1500/cu mm was observed in only 3.7% babies these findings are in agreement with earlier studies.<sup>6, 13</sup>

In the present study band cell count >20% in proven sepsis was 50% and probable sepsis babies 37.5%, which was comparable to earlier studies.<sup>14</sup> Blood culture is +ve in 3.7% of the babies born with maternal risk factors which is comparable with the other studies.<sup>15</sup> The success of isolating bacterial pathogens from blood depends upon the quantum of blood cultured, frequency of culture and duration of incubation and volume of blood required for the isolation of the pathogen depends on the magnitude of septicemia, which is directly related to age of the patient. The above reasons say why blood culture positivity is low.

Datta and co-workers<sup>5</sup> in their study showed that confirmation of the diagnosis by definitive culture is not rapid or particularly sensitive. Of all the new born only 3-5 per 1000 admitted to a newborn intensive care unit have culture proven septicemia Referring to above table, The present study showed a positive association between elevated cord blood  $\geq 0.6$ mg/dl and maternal risk factors like primiparity, ROM >24 hrs., prolonged labor, maternal fever, >3 VE after ROM, FSL, UTI and babies extracted by LSCS. This is comparable to the study done by Mathai et al<sup>6</sup> which showed positive association between elevated CRP level with ROM >24hrs, prolonged labor.

In present study, at 24 hrs. elevation in CRP level  $\geq 0.6$ mg/dl showed positive association with maternal risk factors like primiparity, prolonged labor, maternal fever, FSL, MSL and in babies extracted by LSCS. This is comparable to the study done by Mathai et al<sup>6</sup> which showed elevated CRP level  $\geq 0.6$ mg/dl with primiparity, >3 VE after ROM. In the present study, at 24 hrs. when the cut off CRP level was increased to >1.2mg/dl, showed positive association with maternal risk factors like ROM <12 hrs. and > 24 hrs, prolonged labor, maternal fever, > 3 VE after ROM, FSL, MSL and LSCS. Mathai et al<sup>6</sup> showed in their study that when cut off of CRP was increased to >1.2mg/dl, positive association was noted only with maternal fever. In the present study several risk factors caused elevated CRP levels in the absence of infection.

In the present study, 21.42% cases with no evidence of sepsis had CRP levels >0.6mg/dl at 24 hrs. which is comparable with Mathai et al<sup>6</sup> study. 12.24% cases with no evidence of sepsis had CRP level  $\geq 1.2$ mg/dl at 24hrs which is comparable with Mathai et al<sup>6</sup> study. Only 3.06% cases with sepsis with probable sepsis had CRP level >1.2mg/dl at 24 hrs. which is comparable with Mathai et al<sup>6</sup> study. From the above inference it can be stated that CRP elevated has no significant association with presence or number of signs.

In the present study, single CRP value has sensitivity of 83%, specificity of 64%, positive predictive value 23% and negative predictive value of 96.7% which is comparable to observation made by Mathai et al<sup>6</sup> and other studies. The difference in various studies is due to the different cut off value used in the qualitative test.

We had higher sensitivity because our cut off was 0.6mg/dl.

Hence we conclude that intrapartum risk factors for early onset sepsis can cause elevation of cord and neonatal CRP levels in the absence of infection. A CRP level of <0.6mg/dl at 24 hrs. has good negative predictive value for neonatal sepsis. CRP level is a good indicator, in diagnosing the early sepsis and early intervention in management of neonatal sepsis.

**REFERENCES:**

1. Jeeva SM, Rajiv A, Nupur S, Ashok K. Deorari, Vinod KP. Sepsis in the New born. *Indian J Pediatr* 2008; 75 (3): 261-266.
2. Jeremy Shiffman. Issue attention in global health: the case of newborn survival. *The Lancet* 2010;375(9730):2045-2049
3. Rennie JM, Robertson NRC. *Textbook of Neonatology*. 5<sup>th</sup> ed. Churchill Livingstone 2012.
4. Richard EB, Robert MK, Hai BJ. *Nelson Textbook of Pediatrics*. 19<sup>th</sup> ed. Philadelphia: WB Saunders Company 2011.
5. Datta S, Oberoi JK, Chugh TD. Laboratory diagnosis of Neonatal sepsis. *Journal Neonatology* 2006; 20(1): 18-23.
6. Mathai E, Christopher U, Mathai M, Jana AK, Rose D, Bergstorm S. Is C-reactive protein level useful in differentiating infected from uninfected neonates among those at risk of infection? *Indian Pediatrics* 2004; 41: 895-900.
7. Stoll BJ, Gordon T, Sheldon BK, et al. Late onset sepsis in very low birth weight Neonates: A report from the NICHD Neonatal Research Network. *J Pediatrics* 1996; 129: 63-71.
8. Betty C, Inderpreet S. Early onset Neonatal sepsis: *Indian J Pediatr* 2005; 72: 23-26
9. Yancey MK, Patric D, Paul K et al. Risk factors for neonatal sepsis. *Obstetrics and Gynecology* 1996; 87: 188-94.
10. Raghavan M, Mondal GP, Bhatt V, Srinivasan S. Perinatal risk factors in neonatal infections. *Indian J Pediatr* 1992; 59: 335-440.
11. Remington JR, Klein JO. *Infectious diseases of the fetus and newborn infants*. 7<sup>th</sup> ed. Philadelphia: WB Saunders Company; 2010.
12. Dawodu A, Umran K, Twum- Danso K. A case study of Neonatal Sepsis: Experience from Saudi Arabia. *J Trop Pediatrics*.1997; 43: 84-88.
13. Ashok KY, Wilson CG, Prasad PL, Menon PK. Polymerase Chain reactions in Rapid diagnosis of neonatal sepsis. *Indian J Pediatrics* 2005; 42: 681-685.
14. Misra PK, Kumar R, Malik GK, Mehra P, Awasthi S. Simple hematological tests for diagnosis of neonatal sepsis. *Indian Pediatr*. 1989 Feb; 26(2):156-60.
15. Rodwell RL, Anton LL, David IT. Early diagnosis of Neonatal sepsis using a hematological scoring system. *J Pediatrics* 1987; 112: 761-67.

## ORIGINAL ARTICLE

---

### **AUTHORS:**

1. Somaiah
2. Vamsidhar
3. Sujitha
4. A. Mohan Rao
5. K. Srikanth

### **PARTICULARS OF CONTRIBUTORS:**

1. Professor, Department of Pediatrics, Mamata Medical College & General Hospital, Khammam.
2. Professor, Department of Pediatrics, Mamata Medical College & General Hospital, Khammam.
3. Assistant Professor, Department of Pediatrics, Mamata Medical College & General Hospital, Khammam.

4. Professor, Department of General Medicine, Mamata Medical College & General Hospital, Khammam.
5. Professor, Department of General Medicine, Mamata Medical College & General Hospital, Khammam.

### **NAME ADDRESS EMAIL ID OF THE CORRESPONDING AUTHOR:**

Dr. Somaiah,  
Department of Padiatrics,  
Mamata Medical College & General Hospital,  
Khammam, Andra Pradesh.  
E-mail: mamatakhmm@gmail.com

Date of Submission: 23/12/2013.

Date of Peer Review: 24/12/2013.

Date of Acceptance: 31/01/2014.

Date of Publishing: 13/02/2014.