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Original Research Article

Role of C-Reactive Protein (CRP) as a screening tool in early diagnosis of neonatal septicemia

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Abstract

Background: Neonatal septicemia refers to generalized bacterial infection of neonate, which includes septicemia, pneumonia and meningitis. In developing countries one of leading factors for neonatal morbidity and mortality is bacterial sepsis.

Aim: Early diagnosis of sepsis in the neonate is often difficult because symptoms and signs are usually non-specific. This study was conducted to evaluate C-reactive protein (CRP) as a screening tool for neonatal sepsis.

Material and Methods: The study was conducted in the Department of Biochemistry at Guru Gobind Singh Medical College and Hospital, Faridkot retrospectively from November 2013 to August 2014. 50 neonates were included with the age group of first 28days (4week) of life (infant age) in study. All of which were suspected to have sepsis in clinical settings. Patient with suspected sepsis having two or more of the following clinical features were used to identify patients: Respiratory and cardiovascular compromise, metabolic and neurologic changes. Blood samples were drawn prior to administration of antibiotic therapy on day one of admission for blood culture and CRP by trained staff with all aseptic precautions. Sample for blood culture was taken in blood culture bottle and the growth of bacteria was observed for 5 days after that they were reported and for CRP the investigation was performed by immunometric assay. Absolute neutrophil count and total leucocyte count was done by fully automated cell counter.

Results: Among 50 septic screens, 39 (52%) patients had positive cultures, the sensitivity and specificity of ANC (Absolute Neutrophil Count) was 75% and 65.34%, TLC (Total Leucocyte Count) was 62% and 70.41%, CRP was 90 % and 83.21% respectively. This study also found that premature and low birth weight babies are more prone to neonatal sepsis.

Conclusion: CRP is one of the most widely available, most studied, and most used laboratory tests for neonatal bacterial infection and despite the continuing emergence of new infection markers and it still plays a central role in the diagnosis of early-onset sepsis of the neonate.

Keywords: Oxidative protein modification, Advanced Oxidative Protein Products, Ischemia Modified Albumin, metabolic syndrome

1. Introduction

Neonatal sepsis is defined as an invasive bacterial infection which occurs in the first 4 weeks of life. The incidence of neonatal sepsis varies from 11-24.5 /1000 live births in India [1]. In developing countries, sepsis is the commonest cause of mortality responsible for 30-50% of the 5 million total neonatal deaths each year. The reported incidence of neonatal sepsis varies from 7.1 to 38 per thousand live births in Asia [2]. National Neonatal Perinatal Database (NNPD, 2002-2003) from India has reported an

incidence varying from 0.1% to 4.5%. The database comprising of 18 tertiary care neonatal units across India found sepsis to be one of the commonest causes of neonatal mortality contributing to 19% of all neonatal deaths. Septicemia was the commonest category with an incidence of 23 per 1000 live births [3].

Neonatal septicemia refers to generalized bacterial infection of neonate, which includes septicemia, pneumonia and meningitis. In developing countries one of leading factors for neonatal morbidity and mortality is bacterial sepsis[4]. Bacterial infections are the commonest cause of morbidity and mortality during the neonatal period. Fulminant and fatal course of infection may result from complications such as shock, disseminated intravascular coagulation and multi-system organ failure, mandating early diagnosis of this life threatening condition for a timely treatment and favorable outcome [5].

The clinical manifestation of sepsis in newborn infants is usually non-specific. Because of the high morbidity and mortality which is associated with neonatal sepsis[6],[7] an antibiotic therapy is commenced soon after the onset of the symptoms before the diagnosis is confirmed by blood culture.

The diagnosis of neonatal sepsis on the basis of the clinical symptoms is not possible[9]. Although isolation of the causative microorganisms by using blood culture has been the golden standard method for its diagnosis[10], the result is ready only 24- 72 hrs after the sampling and during this period, it is necessary to treat the suspicious infants for sepsis with antibiotics on the basis of the clinical symptoms and the risk factors. It is also possible that a pseudo-negative result may be obtained in some cases [10]. The present trend which is being applied for infants who are suspected to have neonatal sepsis may lead to unnecessary and increased antibiotic consumption, a higher incidence of the side- effects due to their use, increased resistance to the antibiotics, a long hospitalization, the separation of the infants from their mothers and increased health costs[11]**.** Therefore, using fast diagnostic methods including laboratory markers could be beneficial for the diagnosis of neonatal sepsis [12].

In addition to the blood culture, other tests that are usually used for the diagnosis of neonatal sepsis include estimations of the white blood cell count (WBC), the absolute neutrophil count (ANC), micro ESR. Unfortunately, these tests do not have a high sensitivity and specificity in diagnosing neonatal sepsis. Subsequent studies have suggested that additional markers such as C-reactive protein (CRP) may be useful.

1.1 Plausible mechanism

The principal ligand to CRP is phosphocholine, which is found in lipopolysaccharide, bacterial cell walls, as well as in most biological membranes [13]**.** After binding, CRP is recognized by the complement system; CRP activates it, and promotes phagocytosis of the ligand by neutrophil granulocytes, macrophages, and other cells. CRP further activates monocytes and macrophages, and stimulates the production of proinflammatory cytokines [13][14].

CRP is part of the acute-phase response, a physiological and metabolic reaction to an acute tissue injury of different etiologies (trauma, surgery, infection, acute inflammation, etc.) which aims to neutralize the inflammatory agent and to promote the healing of the injured tissue [15]. After a trauma or the invasion of microorganisms, an acute inflammatory reaction is initiated by activation of local resident cells which promote the recruitment and activation of further inflammatory cells, including fibroblasts, leukocytes, and endothelial cells. Once activated, they release proinflammatory cytokines including IL-1, TNF-α, and IL-6. These cytokines induce the production of proteins of the acute-phase response in the liver. These include but are not limited to components of the complement system, coagulation factors, protease inhibitors, metal-binding proteins, and CRP [14][15]**.**

During the acute-phase-response, CRP's hepatic synthesis rate increases within hours and can reach 1,000- fold levels [13][16]. Levels remain high as long as the inflammation or tissue damage persists and then decrease rapidly. The half-life time has been reported by Vigushin *et al*[17] to be 19 h in any of the diseases studied, being the fractional catabolic rate independent of the plasma CRP concentration. From this information, the synthesis rate of CRP therefore appears as the only significant determinant of its plasma level, supporting the clinical use of CRP measurements to monitor disease activity in all disorders characterized by a major acute-phase response.

CRP is an acute-phase reactant which is synthesized by the liver, diagnose and follow the course of infection in neonates [18][22]. Its advantages include its very low serum levels in normal infants, a rapid rise within 12 to 24 hours of sepsis and a large incremental increase thereafter.

C-reactive protein (CRP) is the most extensively studied acute-phase reactant so far, and

despite the ongoing rise (and fall) of new infection markers, its wide availability and its simple, fast, and cost-effective determination make it one of the preferred indices in many neonatal intensive care units (NICUs)[23].

2. Material and methods

The study was conducted in the Department of Biochemistry, Guru Gobind Singh Medical College and Hospital, Faridkot retrospectively from November 2013 to August 2014. 50 neonates were included with the age group of first 28days (4week) of life (infant age) in study. All the neonates were suspected to have sepsis in clinical settings. Patient with suspected sepsis having two or more of the following clinical features were used to identify patients for suspected neonatal sepsis: respiratory and cardiovascular compromise, metabolic and neurologic changes.

2.1 Exclusion criteria include:

- ✓ age at the time of admission is greater than 28 days,
- ✓ neonates who received antibiotic dose prior to septic workup and
- ✓ Neonates diagnosed to have congenital malformations.

Blood samples were drawn prior to administration of antibiotic therapy for blood culture by trained staff with all aseptic precautions in blood culture bottle and the growth of bacteria was observed for 5 days after that they were reported. Investigation for CRP in newborn was sent after 12 hour if neonate was admitted on day one and it was performed by immunometric assay. Absolute neutrophil count and total leucocyte count was done by fully automated cell counter.

CRP was done by Nyco Card Reader which is a solid phase, sandwich- format, immunometric assay. In the test well of the device there is a membrane coated with immobilized CRP-specific monoclonal antibodies. A diluted sample is applied to the test device. When the sample flows through the membrane, the C-reactive proteins are captured by the antibodies. CRP trapped on the membrane will then bind the gold-antibody conjugate added, in a sandwich type reaction. Unbound conjugate is removed from the membrane by the washing solution. A paper layer underneath the membrane absorbs excess liquid. In the presence of a pathological level of CRP in the sample, the membrane appears red brown with colour intensity proportional to the CRP concentration of the sample. The colour intensity is measured quantitatively with the Nyco Card Reader II.

All the values were checked and analysed through SPSS software version.

3. Results

During study period, total 50 neonates admitted in neonatal intensive care unit were studied. They were divided in three groups based on clinical features and blood culture reports - proven sepsis, probable sepsis and clinically sepsis.

Proven Sepsis: These were the patients among suspected neonatal sepsis in which blood culture confirms sepsis or there is definite evidence of localized infection and CRP >0.6 mg/dl.

Probable Sepsis: These were the patients among suspected septic patient with CRP suggestive (≥0.6mg/dl) of septicemia but negative blood culture.

Clinically Sepsis: These were the suspected septic patients with CRP <0.6 mg/dl and sterile blood culture.

Among 50 septic screens, 39 (52%) patients had positive cultures. Our present study found that low birth weight babies are more prone to develop neonatal sepsis as represented in **Figure 1.** Majority of the low birth weight babies have proven sepsis as compared to normal birth weight babies which is a very high percentage and therefore a matter of concern to reduce neonatal mortality and morbidity. Also, there was a strong association of neonatal sepsis with prematurity. In proven sepsis group, rate of prematurity was very high which is far more than incidence of prematurity in general population which may indicate predilection of suspecting sepsis more in case of premature neonate as depicted in **Figure 2.** This study further showed that sensitivity and specificity of ANC and TLC are low as compared to CRP; therefore these indices render them less valuable than CRP for screening purposes. The scenario is shown in **Figure 3**.

Figure 1: Relation between birth weight and sepsis

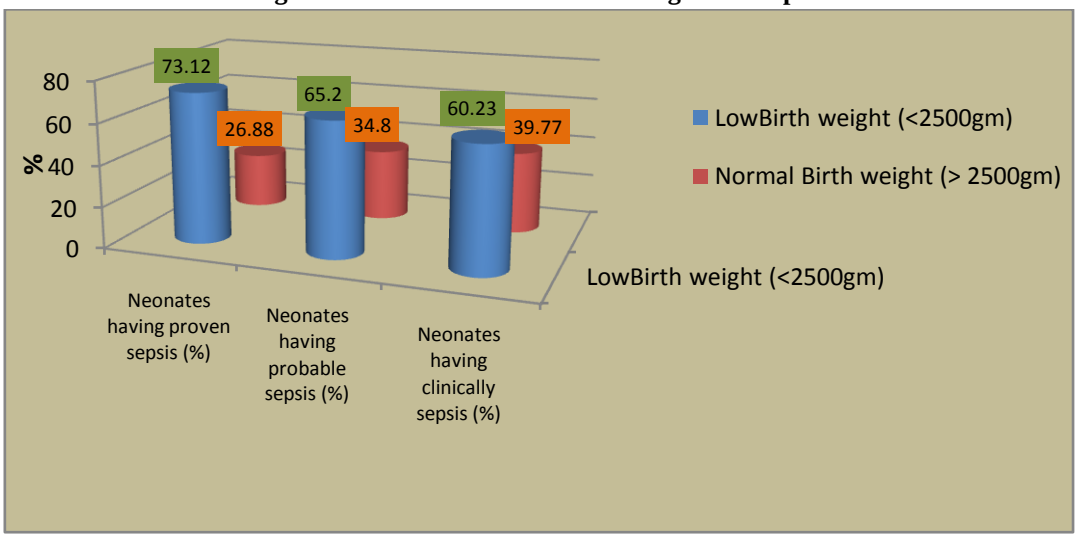


Figure 2: Relation between gestational age and sepsis

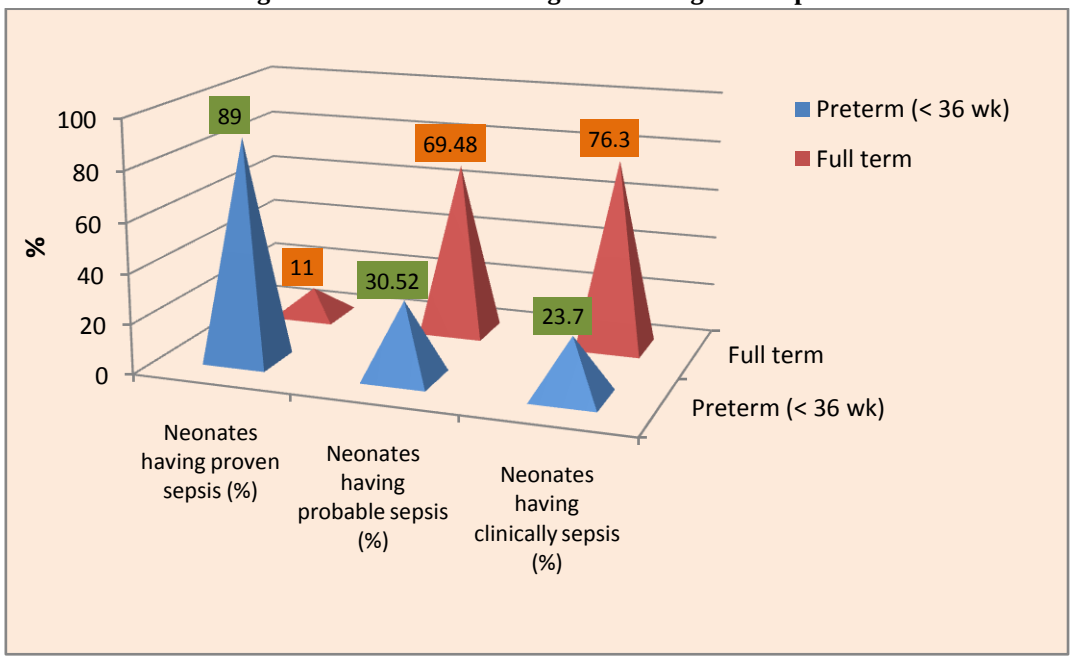
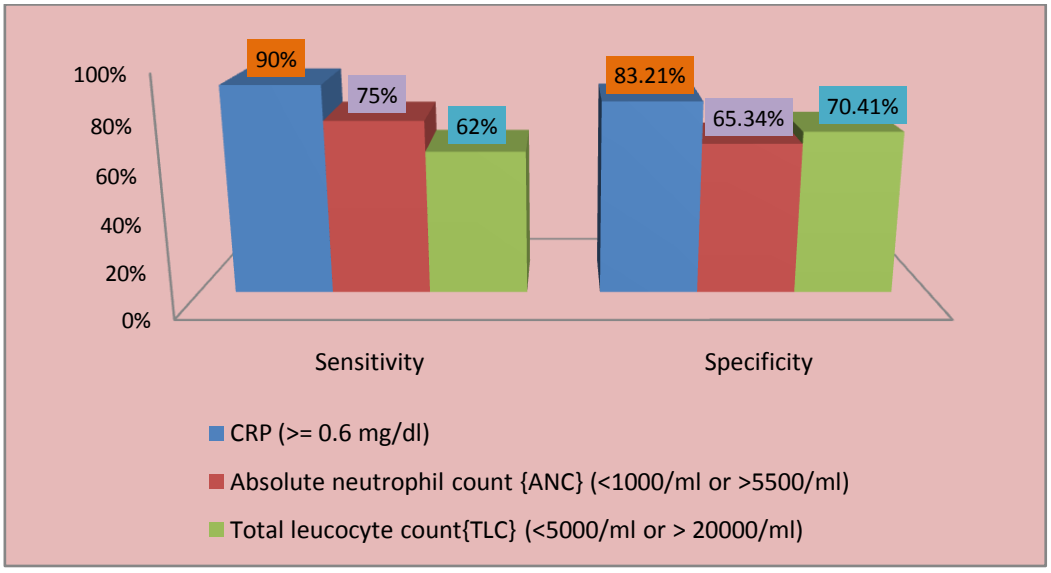


Figure 3: Bar diagram showing sensitivity & specificity of various parameters in detecting sepsis



4. Discussion

Neonatal sepsis with its high mortality rate still remains a diagnostic and treatment challenge for the neonatal health care providers. An early diagnosis of neonatal septicemia helps the clinician in instituting antibiotic therapy at the earliest, thereby reducing the mortality rates in the neonates. An early identification of an infected neonate also helps in avoiding the unnecessary treatment of a non-infected neonate.

The blood culture not only takes time, but it is also complicated, with a low yield. The readily achievable complete blood count and the leukocyte differential assays have a relatively poor specificity for diagnosing sepsis. The associated band count and a leftward shift of the myeloid immaturity measurements may improve the diagnostic yield, but their subjective measurement is problematic. Therefore, the need persists for improved diagnostic indicators of neonatal sepsis [24].

The calculation of both sensitivity and specificity depend on knowing which infants were already septic when CRP assay was performed. Parikh M and Singh N[25] reported sensitivity and specificity of CRP test as 81.4% and 75.5% respectively in culture proven cases. Anuradha DE and co-workers [26] in 1998, reported sensitivity of 100% and specificity of 87.3%. Our present study showed that the sensitivity and specificity of ANC was 75% and 65.34%, TLC was 62% and 70.41%, CRP was 90 % and 83.21% respectively as represented in **Figure 3.**

Therefore, as sensitivity and specificity of CRP are both high, so measuring C-reactive protein is a useful adjunctive tool in screening for neonatal sepsis in comparison to the earlier studies done. And moreover, CRP is a good POCT (point of care testing) as it is simple to perform at the bedside by medical staff. It is readily completed within 10 minutes, utilizing only 5 μL of the infants' blood. The optimum CRP value for screening of neonatal sepsis appeared to be 0.6 mg/dl[27]. Mention the basis of taking cut off value of CRP as 0.6mg/dl.

Furthermore, Manroe *et al*[28] had reported that abnormal hematology may be affected by non-septic processes like steroid treatment as part of therapy for chronic lung disease. CRP on the other hand, was unaffected by arterial catheterization, intraventricular bleeding or steroid therapy[29][30]. There is no single test for the diagnosis of early or late onset neonatal sepsis. Current sepsis markers like neutrophil indices, CRP are useful adjunct tests in identifying infants with sepsis and CRP is a good diagnostic marker for neonatal sepsis because of its high sensitivity and specificity, easy to perform and also not time consuming [27].

Therefore, sound clinical judgment combined with CRP assay should provide a rational basis for treatment decisions in the management of neonatal sepsis[31]. Such a strategy may significantly reduce unnecessary antimicrobial therapy which could otherwise permit the emergence of resistant strains of organisms as well as place these immature infants at risk for allergic and adverse side-effects with increased hospitalization costs. The half-life time has been reported by Vigushin *et al*[17] to be 19 hours in any of the diseases studied, being the fractional catabolic rate independent of the plasma CRP concentration. From this information, the synthesis rate of CRP therefore appears as the only significant determinant of its plasma level, supporting the clinical use of CRP measurements to monitor disease activity in all disorders characterized by a major acute-phase response.

5. Conclusion

CRP is one of the most widely available, most studied, and most used laboratory tests for neonatal bacterial infection and despite the continuing emergence of new infection markers, it still plays a central role in the diagnosis of early-onset sepsis of the neonate. CRP has the advantage of being well characterized in numerous studies, and the extensive knowledge of its properties and limitations makes it safer compared to other, newer markers.

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