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Presepsin-Diagnostic marker for Neonatal sepsis in comparison with other sepsis markers

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A B S T R A C T

Background: To determine the Presepsin level in clinically suspected neonatal sepsis.To Evaluate the efficacy of Presepsin as a early diagnostic marker of sepsis with previously existing other neonatal sepsis markers like CRP, Total leucocyte count, Platlet count.To correlate Presepsin with conventional blood culture method for diagnosis of neonatal sepsis.

Methods: Blood samples were taken from 100 clinically suspected neonatal sepsis and were processed for blood culture, detection of serum level of CRP by latex agglutination test and detection of serum level of PRESEPSIN by ELISA

Results: In the present study, Serum C-Reactive protein was elevated in 16 out of 29 culture proven sepsis. The Abnormal WBC count was seen in 7 out of 29 cultures with proven sepsis and Platlet count was reduced in 12 out of 29 neonatal sepsis .Presepsin was elevated in 24 out of 29 cultures with proven sepsis. The sensitivity of Presepsin in detecting sepsis was 82.8%, its specificity was 77.5%, its positive predictive value was 60% and its negative predictive value was 41.4%.

Conclusion: The estimation of Presepsin also helps in avoiding unnecessary antibiotic usage where it is not required and thereby reducing the hospital cost and the occurrence of bacterial resistance. Estimation of Presepsin could be a milestone in the continuing research for a definitive diagnostic biomarker in neonatal sepsis.

Keywords: Presepsin, Neonatal sepsis, CRP

INTRODUCTION

Neonatal sepsis is a bacterial blood stream infection characterized by systemic signs and symptoms of infection in the first month of life. It includes systemic infections of the newborn including septicemia, meningitis, Osteomyelitis, Gatroenteritis and Pneumonia .It is one of the most common cause of mortality and morbidity particularly in developing countries. Neonatal sepsis is a significant problem of neonatal care unit as presents with nonspecific findings.Clinical signs and symptoms are basis of initial diagnosis, which are usually nonspecific, mimicking other non infectious conditions like aspiration and asphyxia. Consensus on the treatment of sepsis indicates that early anti-Microbial treatment should be given before comprehensive treatment. Though empirical antibiotic treatment is started early in many cases without specific diagnosis, it leads to antibiotic resistance which is of another important concern. Early diagnosis of neonatal sepsis remains a major challenge to the pediatricians and neonatologists. Though, due to the existence of non-infectious SIRS in many critical patients, how to differentiate sepsis from SIRS at the early stage has become a recent topic for many years. Prompt diagnosis and treatment of newborn infants with suspected sepsis are essential to prevent severe complications. A panel of tests(sepsis screen) consisting of total leukocyte count, absolute neutrophil count, immature to total neutrophil ratio, C-reactive protein,ESR constitutes a useful sepsis screen for clinically doubtful cases. But, it has low specificity and sensitivity.

Therefore biomarkers may play a vital role in the pathophysiology of sepsis &timely diagnosis and management of neonatal sepsis. They can indicate the presence, absence, or severity of sepsis and can differentiate bacterial from viral and fungal infection and systemic sepsis from local infection.Acute phase reactants, pro and anti-inflammatory mediators (cytokines, chemokines and cell-surface antigens) are nonspecific biomarkers that have been elaborately studied.Many

studies were conducted with already available C-reactive protein and Procalcitonin and Interleukins. CRP lacks specificity in differentiating non infectious inflammatory, bacterial and viralsituations. Procalcitonin is a promising marker for sepsis but the specificity and sensitivity vary with neonatal sepsis. Presepsin level are presumed to be elevated earlier than other sepsis markers. Presepsin consists of N-terminal 13kda fragment of CD-14, CD (cluster of differentiation) 14 is a cell-surface glycoprotein expressed in macrophages, monocytes, dendritic cells, and neutrophils [40]. This small polypeptide has been proposed as a novel, early and reliable biomarker for the diagnosis of sepsis. After the onset of infection the presepsin level rises within 2 hours and peaks by 3 hours, serum presepsin can be measured easily and rapidly. Therefore presepsin can be used as a biomarker for neonatal sepsis and thus could be helpful in early interventional strategies.In this study, we observed efficacy of presepsin in diagnosis of neonatal sepsis compared with other existing neonatal sepsis markers.

METHODOLOGY

This study was conducted at the Department of Microbiology, Tirunelveli Medical College, Tirunelveli. The study group included the neonates with clinically suspected neonatal sepsis from Neonatal intensive care unit, Tirunelveli Medical College, Tirunelveli. Informed consent was obtained from reliable informants of neonates who participated in the study.All neonates with history of poor cry, hypothermia, abdominal distension, vomiting, Apnoeic spells, Tachypnoea, chest retractions, grunting, fever, seizures, refusal to feed and lethargy were included. Neonates who had birth asphyxia and aspiration syndromes and who have congenital anomalies and inborn errors of metabolism were excluded

Ethical clearance was obtained from the college ethical committee before the commencement of the study.

Blood samples were taken from 100 clinically suspected neonatal sepsis and were processed for blood culture, detection of serum level of CRP by latex agglutination test and detection of serum level of PRESEPSIN by ELISA(Elk biotechnology ELISA kit, Wuhan, China). This test was based on sandwich enzyme-linked immune-sorbent assay. Capture antibody was pre-coated in 96-well plates. Biotin conjugated antibody issued as detection antibodies for this method. The standards, test samples and biotin conjugated detection antibody were added to the wells subsequently,washed with wash buffer. The HRP-Streptavidin was added and unbound conjugates were washed away with wash buffer. TMB substrates were used to visualize HRP enzymatic reaction. TMB was catalyzed by HRP to produce a blue color product that changed into yellow after adding acidic stop solution. The density of yellow is proportional to the target amount of sample captured in plate. Read the O.D. absorbance at 450nm in a microplate reader, and then the concentration of target can be calculated.

RESULTS

Among the 100 neonates with clinically suspected Neonatal sepsis, 51% had early onset (EONS) and 49% had late onset neonatal sepsis (LONS). Among the 100 neonates with clinically suspected neonatal sepsis, 57% were term (37 weeks completed) and 43% were preterm (< 37 weeks). Among the 100 neonates, 54% were males and 46% were females Out of 100 neonates included in the study, 39% had normal birth weight (Birth weight ≥ 2500 g) and 61% were of low birth weight (Birth weight < 2500 g). The mean birth weight of the study group was 2.3 kg with a standard deviation of 0.5 kg. The minimum weight was 0.8 kg and the maximum weight of the study group was 3.4 kg. However, this difference is statistically significant. Similar result had been reported in a comparative study by Rabindra N Misra[1] et al which showed that out of 75 culture positive cases, low birth weight babies are more prevalent. The immunocompromised state of low birth weight babies may be the cause of increased chance of sepsis in this group.

Out of 100 blood samples cultured, 29 samples were found to be positive for blood culture. Similar positivity % of blood culture had been reported in the study by Sucila Thangam et al[2] during April – September 2010 which revealed 28% positive blood culture. The most common organisms isolated were *Klebsiella pneumoniae* in 12 neonates (41.4%), *Escherichia coli* in 6 neonates (20.7%), Methicillin Resistant *Staphylococcus aureus* in 4 neonates (13.8%), *Pseudomonas aeruginosa* in 4 neonates (13.8%), Methicillin sensitive *Staphylococcus aureus* in 2 neonates (6.9%) and *Klebsiella oxytoca* in 1 neonates (3.4%).

Table 1: ASSOCIATION BETWEEN BLOOD CULTURE AND PRESEPSIN

Presepsin	**Blood culture**		**Total**	**p value**
	Positive	**Negative**		
Positive	24 (60%)	16 (40%)	40	<0.001, Chi square test
Negative	5 (8.3%)	55 (91.7%)	60	
Total	29 (29%)	71 (71%)	100	

Out of 100 clinically suspected sepsis cases 40 were positive for Presepsin with cutoff of 5ng/ml The sensitivity of Presepsin for proven sepsis (more than 5 ng/ mL)was 82.8%, its specificity was 77.5%, its positive predictive value was

60% and its negative predictive value was 91.7%.Similar observation was reported in study done by Mohammed yusef memr et.al[3], showed that sensitivity of Presepsin 81%, specificity was 80% with cutoff of 5ng/ml. In a similar study by Aahmed saied ozman et.al. showed that sensitivity and specificity respectively 95.7% and 87%, indicating that the level of Presepsin is a good marker for the diagnoses of neonatal sepsis. Blood culture is the gold standard method for detecting the presence of bacteria in the bloodstream, it has limited usefulness for early detection of sepsis because it usually requires several days for result.The sensitivity of blood cultures in neonatal sepsis is low and depends on the timing of cultures taken, blood volume, culture medium, technique, temperature, organism density as well as antibiotic administration. The current study confirms that sensitivity, Positive predictive value and negative predictive value for Presepsin value with cutoff of 5ng/ml was more higher than haematological indices and CRP.

TABLE 2: BIOMARKERS COMPARED WITH BLOOD CULTURE

Blood culture	Presepsin Positive	CRP Positive	Abnormal leucocyte count	Reduced platlet count
Positive (n=29)	24	16	7	12
Negative (n=71)	16	20	16	11

TABLE 3 : COMPARATIVE ANALYSIS OF BIOMARKERS

	Presepsin	CRP	Abnormal leucocyte count	Reduced platlet count
Sensitivity	82.8%	55.2%	24%	41.4%
Specificity	77.5%	71.8%	60.5%	84.5%
PPV	60%	44.4%	20%	52.2%
NPV	91.7%	79.6%	66.1%	77.9%

Out of 100 neonates 94% of neonates were improved and 6% neonates died.

CONCLUSION

Blood culture is a gold standard method to detect the organisms but there is a time delay of 3-4 days of for obtaining the results. The present study suggested that the serum levels of Presepsin could be a reliable marker of sepsis than the serum levels of CRP and the abnormal WBC counts and reduced platelet count in the early diagnosis of neonatal sepsis and in the evaluation of the response of the disease to the antibiotic therapy. It helps to improve the outcome of the neonates by starting Antimicrobial therapy at the earliest.

The estimation of Presepsin also helps in avoiding unnecessary antibiotic usage where it is not required and thereby reducing the hospital cost and the occurrence of bacterial resistance. Estimation of Presepsin could be a milestone in the continuing research for a definitive diagnostic biomarker in neonatal sepsis. Good Hospital infection control practices, strict antibiotic policy and earnest search for early diagnostic marker would widen the successful encounter with sepsis and lead the neonates to the road of health.

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