Paediatrics Section

Role of C-Reactive Protein and Immature to Total Neutrophil Ratio in Early Onset Neonatal Sepsis

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ABSTRACT

Introduction: Neonatal septicaemia is a significant cause of morbidity and mortality in newborn infants. It often presents a diagnostic challenge in the resource poor setting of most developing countries.

Aim: To determine the efficacy of C-Reactive Protein (CRP) and Immature Neutrophil Count (INC) to Total Neutrophil Count (TNC) ratio (I/T ratio) in the early diagnosis of neonatal sepsis.

Materials and Methods: This prospective observational study included all term and preterm babies inborn and out born referred cases. The babies less than seven days of age with clinical symptoms and signs of suspected neonatal sepsis were included. Significant values for screening tests were taken as Total Leucocyte Count (TLC) of >25,000/<5000, I/T ratio >0.2 and CRP positive (>0.6 mg/dL). Sepsis screen was considered positive for two or more positive tests. Blood culture was used as gold standard. The statistical analysis was done by Chi-square, Fisher's exact and ANOVA tests using SPSS 20.0 version.

Results: A total number of 60 subjects were included in the study with 45 (75%) as outborn neonates. Most of the neonates presented with tachypnea 27 (45%), 11 (18.3%) with difficulty in feeding and 10 (16.7 %) with lethargy. Significant p-values were observed using CRP and ITR as independent sepsis screening markers. The combination of CRP with I/T ratio showed positive correlation with blood culture (p-value 0.016).

Conclusion: Sepsis screen in neonates is required for detection of infection as blood culture may be negative and even positive result takes few hours. C-reactive protein showed high sensitivity while I/T ratio was found to be highly specific.

INTRODUCTION

Neonatal sepsis is an inflammatory response to bacteremia occurring during the first month of life and it remains a big problem in developing countries [1]. Septicaemia in newborns is a systemic inflammatory reaction to local infection that may lead to the development of more serious conditions [2].

Neonatal septicaemia causes significant morbidity and mortality in newborns. Its incidence ranges from 11.0 to 24.5/1000 live births in India [3]. Sepsis often presents a diagnostic challenge in the resource poor setting of most developing countries. Successful treatment depends on early initiation of antibiotics, but early diagnosis of neonatal infections is difficult because clinical signs are non-specific and may initially be subtle. Respiratory distress, apneic spells, episodes of bradycardia, feeding intolerance, lethargy, and the clinical signs of early onset sepsis are usually apparent in the first hours of life; 90% infants are symptomatic by 24 hours of age. Respiratory distress is the most common presenting symptom. Temperature instability, as well as minor changes on physical examination or in clinical status is some of the conditions that suggest a possible neonatal infection and needs sepsis evaluation [4]. Respiratory symptoms can range in severity from mild tachypnea and grunting with or without supplemental oxygen requirement to respiratory failure. Other less specific signs of sepsis include irritability, lethargy, temperature instability, poor perfusion and hypotension. Gastrointestinal symptoms can include poor feeding, vomiting and ileus.

Though the commencement of illness is not noticeable, the clinical course may be terrifyingly fulminant, leading to septicaemic shock, disseminated intravascular coagulation and death within hours of the start of clinical manifestations. Detailed evaluation of sepsis is done and antibiotics were started even when there are remote chances of infection. A large number of screening markers have been investigated for sepsis evaluation. Haematological tests like TLC, TNC, INC, I/T ratio, morphological or degenerative changes in neutrophil and platelet count have been studied either individually or in combination. Study by Da Silva O et al., showed

wide range of WBC counts with sensitivity and specificity ranging from 17% to 90% and 31% to 100%, respectively [5].

Acute phase proteins are produced principally by the liver as part of an immediate inflammatory response to infection or tissue injury. The most extensively used and investigated acute phase reactant is CRP. CRP is more sensitive as well as specific than TNC and I/T ratio. Moreover serial measurements at 1st day and 2nd day significantly improve its sensitivity (82% and 84%, respectively) [6]. It also depicts high predictive values for diagnosis of neonatal sepsis as compared with CBC [7]. During first 72 hours of life, CRP, leucopenia and neutropenia were comparably good tests; however after that CRP was single best test in early diagnosis of neonatal sepsis [8].

Early and objective diagnosis of sepsis can be made using sepsis screen including parameters like elevated white blood cells with predominance of immature granulocytes, depressed total white cell count (<5000) and absolute neutropenia (PMN <1500), I/T ratio, CRP, micro ESR and gastric aspirate for polymorphs [4].

Blood cultures are definitive determinant of bacteremia. Most blood cultures are usually positive within 24 to 36 hours of incubation; therefore early identification of genuine sepsis is a major diagnostic problem. Due to non-specific signs of life threatening diseases in neonates, there is widespread use of antibiotics, which further aggravates antibiotic resistance. The careful evaluation of indications and treatment can reduce the duration of hospital stay and also side effects of various antibiotics. The aim of the present study was to determine the efficacy of CRP and I/T ratio in the early diagnosis of neonatal sepsis.

MATERIALS AND METHODS
This was a prospective observational study done on 60 patients from January 2005 to December 2005. The study was conducted in a Tertiary Care Teaching Hospital. It included all term and preterm babies inborn and out born referred cases.

Inclusion criteria: The babies, who had the clinical symptoms and signs of suspected neonatal sepsis/high risk factors for developing the sepsis, were included. Blood samples were taken for complete blood count, CRP (quantitative) and investigated as per the protocol. An informed written consent was taken from the parents/ attendants of the admitted neonates. The inclusion criteria were babies with age less than seven days of life, inborn or outborn with suspected sepsis and with high risk factors (antenatal, natal, postnatal). The high risk factors included preterm neonates, fetal distress, maternal history of leaking per vaginum (more than 18 hours), maternal pyrexia, history of any infection in mother like urinary tract infection, chorioamnionitis multiple obstetrical procedures, difficult delivery.

Exclusion criteria: Babies with age more than seven days of life, having septic shock patients or rapidly deteriorating clinical condition, weighing <1500 g, with history of severe perinatal asphyxia, any congenital malformations/chromosomal anomalies/ congenital metabolic defects or babies with family history of any immunodeficiency syndrome were excluded from the study.

Each patient was subjected to detailed history and physical examination. Blood sample were taken at admission and subjected to TLC, I/T ratio and CRP. The blood sample for blood culture and sensitivity was collected at the same time.

TLC was measured by manual method using Neubauer chamber as well as using an electronic cell counter. TLC report on coulter machine was verified by manual method. Immature neutrophil count divided by total neutrophil count was calculated using simple mathematical ratio. RHELAX CRP reagent was used to detect CRP concentrations greater than 0.6 mg/dL. Concentration of CRP was calculated as CRP (mg/dL)=S×D. Where S=Sensitivity of the reagent i.e., 0.6 mg/dL and D=Highest dilution of serum showing agglutination.

Blood culture sample was collected from venipuncture under aseptic measures, cleaning the skin with spirit- betadine- spirit and collected in a 2cc syringe and then transferred to BacT/ALERT PF bottle (20 mL) using another sterile needle. The BacT/ALERT Microbial detection system was used to determine microorganisms present in blood that provide both a microbial detection system and culture media. An inoculated bottle was placed into the instrument for incubation and monitoring to detect the growth of any microorganisms. BacT/ALERT PF (colour-coded YELLOW)- BacT/ALERT PF disposable culture bottles contain 12 mL of complex media and 8 mL of a 6.5% charcoal suspension. The media component consists of soybean-casein digest (2.0% w/v), brain heart infusion solids (0.1%W/V), sodium polyanetholesulfonate (0.05%w/v), pyridoxine HCl (0.001%w/v), menadione (0.0000725%w/v), hemin (0.000 725%w/v), L-cysteine (0.03%w/v) and other complex amino acid and carbohydrate substrates in purified water. Bottles contain an atmosphere of CO_2 in oxygen under vacuum. Positive or negative results are determined by software contained in the BacT/ALERT Microbial Detection System.

Significant values for screening tests were taken as: TLC of >25,000/<5000, Immature to TNC ratio >0.2 and CRP- positive (0.6 mg/dL) and above. Sepsis screen positive was two or more positive tests. The babies were started with intravenous (IV) antibiotics, while blood culture reports were awaited. Blood culture was used as gold standard and the decision to continue antibiotics was taken depending upon the blood culture report. Descriptive statistics were calculated.

STATISTICAL ANALYSIS
The statistical analysis was done by ANOVA tests using SPSS 20.0 version. Descriptive statistics were calculated. Chi-square test and Fisher's exact test were applied. Senstivity, specificity, NPV and PPV were calculated for each screening test.

RESULTS

A total number of 60 subjects were included in the study with 45 (75%) as outborn neonates. Most of the neonates had presented with tachypnea followed by difficulty in feeding and lethargy [Table/Fig-1].

S. No.	Signs and symptoms	No. of neonates (%)
1	Respiratory distress	27 (45)
2	Refusal to feeds	11 (18)
3	Lethargy	10 (16.7)
4	Jaundice	5 (8.0)
5	Abdominal distension	4 (7.0)
6	Convulsions	2 (3.0)
7	Cyanosis	2 (3.0)
8	Fever	2 (3.0)
9	Oliguria	1 (2.0)

[Table/Fig-1]: Signs and symptoms.
More than one sign or symptom were present together

The most specific parameter was found to be I/T ratio and most sensitive was CRP. Both these parameters showed significant association with blood culture [Table/Fig-2].

By combination of any two parameters, it was found that sensitivity decreased but specificity increased. Combination of CRP and I/T ratio was found to be significant with specificity of 100% and p-value of 0.016 [Table/Fig-3].

In the study, the most common organism grown in blood culture was Klebsiella seen in 10 (62.5%), followed by Staphylococcus aureus in four (25%) and Pseudomonas in two (12.5%) patients. In rest of the neoenates, the blood culture was sterile.

DISCUSSION

Neonatal septicaemia is accountable for 1.5 to 2.0 million deaths/year in the underdeveloped countries of the world [9]. Jaswal RS et al., studied the CRP levels to evaluate the duration of antibiotic in 50 consecutive neonates with suspected septicaemia. The negative predictive value of serial CRP levels was 100% in deciding the duration of antibiotic therapy in a patient with suspected septicaemia by up to one week [10]. Major challenge is prompt and accurate identification of infected infant. The task is frequently difficult because of non-specificity of clinical signs. A screening test should ideally detect all infected cases (high sensitivity) and high negative predictive value so that disease can easily be diagnosed. In the present study, 16 (26.6%) cases had positive blood cultures. Similarly, study by Jaswal RS et al., in 50 neonates revealed incidence of 42% blood culture positivity [10]. Mondal GP et al., studied 100 neonates and blood culture were positive among 32% of outborn and 34% of inborn babies [11]. Another similar study by Kumhar GD revealed blood culture positivity of 42% [12].

CRP estimation is now an established marker of neonatal septicaemia and many workers have concurred upon its utility in diagnosis and monitoring of treatment of neonatal septicaemia as it has very high sensitivity as well as specificity. Present study revealed high sensitivity and NPV of CRP. Garland SM and Bowman ED, also revealed similar results with CRP sensitivity of 67% and negative predictive value of 86% [13].

Haematologic findings including abnormal TLC, ANC, I/T ratio, thrombocytopenia and pronounced degenerative changes in neutrophils have been studied as screening tests for neonatal sepsis. The present study documented the specificity for TLC of 77.27% and of I /T ratio of 97.72%. Anwer SK and Mustafa S, also revealed high specificity of TLC up to 93% [14]. In the present study, the combinations of tests had much higher specificity but low sensitivity. Similar results were revealed by Manucha V et al., by combining CRP and haematological parameters in neonatal sepsis [15].

Sharma A et al., studied 50 clinically suspected cases of neonatal septicaemia for evaluating the role of sepsis screen. Serum markers for sepsis were studied individually and in combinations of two and three tests. The blood culture was positive in 20% only. So, there is need for a sepsis screening in neonatal septicaemia, especially in those areas where blood culture facilities are not available [16].

No. of positive parameters	Blood C/S								
Serial numbers	Positive	Negative	Total	Sensitivity	Specificity	PPV	NPV	Accuracy	p-value
1. CRP positive	14	25	39	87.5 %	43.18%	35.89%	90.47%	55.0%	0.034
CRP negative	2	19	21						
2. TLC positive	6	10	16	37.5%	77.27%	37.5%	77.27%	66.67%	0.325
TLC negative	10	34	44						
3. I/T positive	3	1	4	18.75%	97.72%	75.0%	76.78%	76.67%	0.054
I/T negative	13	43	56						

[Table/Fig-2]: Comparing sepsis screening with Blood Culture positive neonates.

Combination of parameters	Sensitivity	Specificity	PPV	NPV	Accuracy	p-value
CRP + IT ratio	18.75%	100%	100%	77.19%	78.33%	0.016

[Table/Fig-3]: Combination of two parameters.

Klebsiella pneumoniae was found to be the most common organism causing neonatal septicaemia in the present study. Similarly, Chugh K et al., found *Klebsiella* (68.87%) as the most common organism followed by *Staphylococcus aureus* (11.1%), *Pseudomonas* (8.8%) and *E. Coli* (4.4%) [17]. Kumhar GD et al., found gram negative organisms in 493 (60%) of 823 cases, with *Klebsiella* (33.8%), *Enterobacter* (7.5%), *Alcaligenes faecalis* (4.9%) and *E. Coli* (4.6%) being the common microbes. So, they concluded that *Klebsiella* and *Staphylococcous aureus* are the commonest microorganisms responsible for neonatal sepsis in a Tertiary Care Hospital [12]. Amongst gram negative organisms, *Klebsiella* was the commonest organism (23.8%) followed by *E. coli* (19.04%) and *Acinetobacter* (9.52%) in neonatal sepsis as revealed by Jaswal RS et al., [9].

Micro ESR, TLC and CRP are easy, quick and cost-effective screening tools for diagnosis of early onset neonatal sepsis. CRP is a good sensitive marker while Micro ESR and leucopenia being more specific markers in diagnosis of the same [18]. Although I/T ratio has good specificity in neonatal septicaemia, but it cannot be relied upon as a single marker. In a neonate with presumed sepsis, normal I/T Ratio and CRP values rule out infection [9]. Similar results were observed in the present study where combination of CRP and ITR showed high specificity. The present study revealed that simple biochemical tests like CRP and ITR can help in diagnosing early onset neonatal sepsis.

Limitation(s)

The limitation of the study was small study group and there were few neonates with positive blood culture.

CONCLUSION(S)

Sepsis screen in neonates is required for detection of infection as blood culture maybe negative and even positive result takes few hours. CRP showed high sensitivity while I/T ratio was found to be highly specific. The combination of CRP with I/T ratio showed significant association with blood culture (p-value 0.016).

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