Role of C-Reactive Protein (CRP) in Establishing the Diagnosis of Sepsis in Neonates

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Abstract

Objective: To determine the accuracy of CRP in diagnosing neonatal sepsis when compared with blood culture as a reference.

Method: This cross-sectional study was conducted at the Neonatal Unit of Fatima Memorial Hospital, Lahore for 6 months. 300 Neonates presenting with complaints and clinical examination favouring sepsis were taken in this study through non-probability, purposive sampling. Informed consent and demographic information were obtained and a 2cc venous blood sample for blood culture and CRP were taken. CRP was measured quantitatively using the ELIZA method. All the information was recorded in a predesigned proforma and collected information was entered and analysed using SPSS version 17.0.

Results: The mean age of neonates was 10.68±7.45 days. There were 144(48%), neonates who were 1 week old, 75(25%) were 2 weeks old, 36(12%) were 3 weeks old and 45(15%) were 4 weeks old. There were 204(68%) male neonates and 96(32%) female neonates. The mean gestational age of neonates at the time of their birth was 35.88±3.64 weeks. There were 218(73%) neonates who were born at full term while 82(27%) were born preterm. The mean weight of all neonates was calculated as 2.42±0.62 kg. The calculated sensitivity, specificity, positive and negative predictive values were calculated as 93.18%, 71.43%, 71.93% and 93.02% respectively. The overall diagnostic accuracy of CRP was calculated as 81.0%.

Conclusion: This study depicts that we can rely on the results of CRP for confirmation of neonatal sepsis. The accuracy of CRP was sensitive enough based on haematological marker for early detection or confirmation of neonatal sepsis.

Keywords: Neonatal Sepsis, Prolonged rupture of membranes, Lethargy, Temperature instability, C-reactive protein

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Introduction

Sepsis in the neonatal age group is defined as the activation of the systemic inflammatory response syndrome to any infection in the first 28 days of life. Sepsis in this age group can lead to severe neonatal complications including death. Though great advances have been made in recent times but still the incidence of sepsis has been recorded to be from 1-4/1000 births in developed countries and the number for developing countries is around 5.5/1000 births (blood culture-confirmed) but gets as high as 170/1000 live births (clinically detected).[1,2,3]

Sepsis is categorized into early onset, occurring within 3 days of life, and late-onset that takes place after 3 days.[4] Early-onset sepsis results because of acquiring the offending organism vertically from the womb or mother's birth canal while the late-onset infection is the one that happens because of horizontal transmission from the

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environment or the Hospital settings.

It is difficult to describe sepsis in the early days of life as neonates don't manifest typical signs and symptoms that are usually associated with sepsis. Thus, the clinicians tend to keep a low threshold for starting antibiotics when it comes to managing babies suspected to have sepsis.[5]

Neonatal sepsis can have a wide spectrum of presentations as well as clinical pictures. Neonates harbouring sepsis may either have subtle signs and symptoms or obvious signs of infection including refusal to feed, temperature instability, lethargy, mottled skin, apnoea, grunting, cyanosis, jaundice, poor perfusion, pallor, tachycardia or bradycardia, shock, seizures, petechiae, purpura and bleeding.[2]

Isolation of microorganisms from blood culture growth is considered the best available standard for diagnosing sepsis but with a sensitivity of only 32% and it usually takes 72 hours at least or more to give a result.[6] White cell count and differential leukocyte count, neutrophilia, band cells, platelet count, C-reactive protein, Chest x-ray and inflammatory markers (IL-6, 1L-8, TNF-a and procalcitonin) are taken as reliable septic screening tools.[7]

C-reactive protein is an inflammatory marker, produced by the liver, that takes around four to six hours to increase after an infectious/inflammatory trigger and reaches a peak level at around 48 hours. In new-borns, where an infectious etiology is suspected, serial C-reactive protein measurements are taken one to two days apart and values of <10mg/dl are useful in excluding sepsis.[8] Kumar et al, showed that serum C-reactive protein is a reliable marker for sepsis. Previous studies enlighten us that sensitivity of 95%, specificity of 85%, PPV of 80% and NPV of 96 % are offered by this useful septic marker. A single level of C-reactive protein is not that accurate in establishing sepsis even a few studies have concluded C-reactive protein to be not a good investigation for neonatal sepsis.[9] The current study aims to establish the role of CRP in the diagnosis of sepsis in neonates.

Material and Methods

It was a cross-sectional study that took place at the Neonatal Unit of Fatima Memorial Hospital, Lahore, Pakistan. The duration of the study was about 6 months after the approval of the synopsis. The calculated sample size was 300 cases, with a 13% margin of error, 95% confidence level and taking 17% as an expected percentage of neonatal sepsis with sensitivity as 67.8% and specificity as 97% of C-reactive protein by taking blood culture as the gold standard in the diagnosis of neonatal sepsis. The sampling technique used was non-probability, purposive sampling.

Neonates of either genders. Gestational age 28-42 weeks. Birth weight of >1000g. Clinical signs and symptoms of neonatal sepsis i.e., babies of age <28 days presenting with one or more of the following symptoms: refusal to feed, temperature instability, lethargy, mottled skin, apnoea, grunting, jaundice, poor perfusion, pallor, cyanosis, tachycardia or bradycardia, hock and bleeding diathesis. Patients already on antibiotics. Babies having congenital alformations as following. Specific laboratory evidence of inborn error of metabolism. The asphyxia insult at the time of birth. Meconium aspiration syndrome (on clinical evaluation).

Three hundred cases with neonatal sepsis admitted to the neonatology unit of Fatima Memorial Hospital, Lahore (both inborn and outborn) were included for study purposes. Demographic information including name, age, sex and weight were recorded and informed consent was taken from their parents. There was no risk involved to the babies. Detailed history and physical examination were done. 2cc venous blood samples for blood culture were taken in a blood culture bottle under aseptic measures before starting antibiotics. 2cc clotted, venous blood samples for C-reactive protein were taken. C-reactive protein was collected under aseptic measures in a vacutainer at 24 hours of the onset of symptoms. C-reactive protein was measured quantitatively using ELIZA Method (Selective-E Autochemistry Analyzer). All the information was recorded in predesigned proforma to determine C-reactive protein level as raised / normal against blood culture report (positive/ negative)

The data gathered was entered and analysed by using SPSS version 17.0. The quantitative variables like age, gestational age, weight and height were presented by calculating mean and standard deviation. The qualitative variables like gender and positive cases of raised C-reactive protein were demonstrated as frequency and percentage. A 2×2 contingency table was generated to calculate specificity, sensitivity, PPV, NPV and accuracy of CRP in the diagnosis of neonatal sepsis by taking blood culture as the gold standard.

Results

A total of 300 neonates of both genders with clinical signs of sepsis were enrolled in the study with a mean age of 10.68±7.45 days. The minimum age was 2 days while the maximum age was 28 days (Table 1). There were 144 (48%), neonates who were 1 week old, 75 (25%) were 2 weeks old, 36 (12%) were 3 weeks old and 45 (15%) were 4 weeks old (Table 2).

Table 1: *Descriptive statistics of gestational age, age and weight of neonates*

Variable	Min.	Max.	Frequency [n]	Mean	Standard Deviation (SD)
Gestational Age	28 weeks	41 weeks	300	35.88	3.64
Age	2 days	28 days	300	10.68	7.45
Weight	1.2 kg	4.0 kg	300	2.42	0.62

Table 2: *Distribution of neonates by age*

No.	Age Groups	Frequency (n)	Percentage (%)
1.	1st Week (1-7 days)	144	48.0 %
2.	2nd Week (8-14 days)	75	25.0 %
3.	3rd Week (15-21 days)	36	12.0 %
4.	4th Week (22-28 days)	45	15.0 %
Total		**300**	**100.0 %**

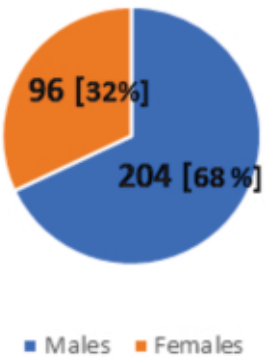

Figure 1: *Distribution of Gender of Neonates*

In this study, there were 204 (68%) male neonates and 96 (32%) female neonates. The male-to-female ratio was 2.1:1 (Figure 1). The mean gestational age of neonates at the time of their birth was 35.88±3.64 weeks. The minimum gestational age was recorded to be 28 weeks while the maximum duration of gestation was 41 weeks (Table 1). There were 218 (73%) neonates who were born at full term while 82 (27%) were born preterm (Figure 2). The mean weight of all neonates was calculated as 2.42+0.62 Kg. the minimum and maximum weights of neonates were 1.20 and 4.00 Kg respectively (Table 1). The calculated Sensitivity, Specificity, PPV and NPV of CRP were calculated as 93.18%, 71.43%, 71.93% and 93.02% respectively. The overall diagnostic accuracy of CRP was calculated as 81.0% (Table 3).

Table 3: *Diagnostic accuracy of C-reactive protein taking blood culture as the gold standard*

CRP	Blood Culture		Total
	Positive	Negative	
< 4.5 gm/dl	123	48	171
> 4.5 gm/dl	9	120	129
Total	132	168	300

Sensitivity = 93.18%; Specificity = 71.43%; PPV = 71.93%; NPV = 93.02%; Diagnostic accuracy = 81.0%

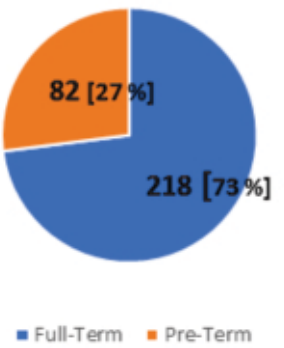

Figure 2: *Distribution of Neonates by Gestational Age*

Discussion

Sepsis in the neonatal period has serious implications in the life to come. World over, nearly 1.6 million neonatal deaths are caused by neonatal infections.[10] In developing Asian countries like India, the cases of neonatal sepsis reach as high as 30 per 1000 live births.[11] As neonatal sepsis has a wide spectrum of presentations ranging from subtle signs to robust critical presentation, it is quite difficult and challenging to diagnose it in the early stages.[12] Early start of antibiotic therapy is life-saving for new-borns in septic conditions. Though blood culture is the investigation of choice to establish the diagnosis of sepsis, it is time-consuming and at times can give false-positive results.[13] A few studies have been done that showed individual parameters to be unreliable and the sensitivity of diagnosing sepsis increases when different parameters are combined.[14,15]

Hence, the researcher used simple laboratory tests i.e., C-reactive protein (CRP) for early detection of neonatal septicaemia. Thus, it was included a total of 300 neonates with clinical signs of sepsis with a mean age of 10.68± 7.45 Almost 48% of neonates were 1 week old, 25% were 2 weeks old, 12% were 3 weeks old and only 15% were 4 weeks old. Thus 48% of neonates presented with early neonatal sepsis while 52% presented with late neonatal sepsis. The ratio of early-to-late neonatal sepsis 1:1.1 is slightly higher than early sepsis in our study. One study also reported that the frequency of early neonatal sepsis (54.53%) was higher than late neonatal sepsis (45.57%) but this deference has no statistical significance.[16] One study reported that 67% of cases develop septicaemia in less than seven days.[17]

There were 68% male and 32% female. The male-to-female ratio was almost 2.13:1. This showed that males are at two times more risk of developing sepsis as compared to females. These results match with another study which reported the incidence of neonatal sepsis was higher in males (60%) than female neonates.[17] But one study reported that the risk of developing neonatal sepsis is equal in both genders and reported the frequency of sepsis in 57% male and 43% female neonates. In this study; no statistical difference was found in the frequency of proven bacterial sepsis between males and females.[16] Gender predisposition to sepsis, remains controversial, while a study carried out in Winthrop hospital showed no significant gender difference in terms of increased susceptibility to bacterial sepsis; multiple other studies show male gender to be a risk factor for severe sepsis.[18]

The researcher also obtained the history of gestational age of neo-nates at the time of birth either from the mothers or the medical records available and the mean gestational age was observed as 35.88±3.64 weeks. Premature infants have an increased incidence of sepsis.[19] In preterm babies, inflammatory markers produced due to sepsis may contribute to poor neurodevelopmental outcomes. In the present study, it was noticed that 27% of neonates were pre-term i.e., born before 36 weeks of gestation while 73% were term babies.[20] But one study reported prematurity to be a great risk factor for neonatal sepsis with a frequency of almost 88.3%.[17] In the present study, the mean weight of all neonates was 2.42±0.62 kg. The Sensitivity, Specificity, PPV and NPV of CRP at cut-off >4.5gm/dl were 93.18%, 71.43%, 71.93% and 93.02% respectively. The overall diagnostic accuracy of CRP was calculated as 81.0%. One study reported the Sensitivity and Specificity of CRP were 84% and 65% respectively.[21]

Another study reported that the sensitivity, specificity and PPV and NPV of CRP for proven sepsis were 100%, 94%, 91.6% and 100% respectively. The researchers concluded that the Sensitivity of CRP could be improved by serial rather than a single reading. Serial CRP values showed better predictive values for confirming neonatal sepsis than leukocyte indices in CBC.[22]

At different cut-off values, different pattern in sensitivity, specificity was observed from the literature. A study reported at cut-off >8mg/L, the corresponding sensitivity, specificity, PPV and NPV values at 24 hours of the onset of sepsis were 70%, 72.3%, 28% and 94% respectively.[7] The sensitivity of CRP is lowest during the initial stages of infection with the sensitivity and specificity ranging from 22% to 69% and from 90% to 96%, respectively. Benitz et al. found that the Sensitivity of CRP done after two days improved when compared with that done on the first day.[23] In a large series of neonates, Pourcyrous et al. reported that CRP being done twelve hours after the initial septic profile was having a higher sensitivity (54% vs. 74%).[24] Generally speaking, the Sensitivity improved dramatically when serial values are determined 24-48 hours after the onset of symptoms.[23,25]

Conclusion

Through this study, it was proved that one can rely on the results of CRP for confirmation of neonatal sepsis. The accuracy of CRP was sensitive enough that one can rely on this haematological marker for early detection or confirmation of neonatal sepsis. So, in the future, neonates who will present with suspicion of neonatal sepsis will be diagnosed on basis of CRP values instead of waiting for blood culture, so that early medical interventions can be started and neonates can be prevented from hazardous events.

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Authors Contribution

SS: Conceptualization of Project
MAAB: Data Collection
BH: Literature Search
MMA: Statistical Analysis
SM: Drafting, Revision
MI: Writing of Manuscript