Original Article

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"C-Reactive Protein :An Important Diagnostic Tool in Neonatal Sepsis"

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ABSTRACT

Background: Global burden of pediatric mortality during the very first month of life is around four million per year. In developing country like India, neonatal mortality is as high as one fourth (25%) of the global burden means around one million per year. Among the various common causes, infection or sepsis alone contributes for almost 30-40% of total neonatal deaths. The study is aimed to determine the effectiveness of C-reactive protein (CRP) as a diagnostic tool in neonatal sepsis.

Materials and Methods: This is a cohort prospective study. Study included total 123 patients considering inclusion and exclusion criteria. Blood samples were taken from all the patients for blood culture and CRP measurements. Results of blood culture and CRP were recorded and statistical analysis was performed by GraphPad Instat Demo.

Results: Out of 123 patients included in the study blood culture was positive in 71 (57.72%) patients and CRP was raised in 72 (58.54%) patients. Sensitivity, specificity, positive predictive value and negative predictive value for CRP were found to be 98.59%, 96.15%, 97.23% and 98.04% respectively.

Conclusion: C-reactive protein has high sensitivity and specificity with good positive predictive value and negative predictive value for establishing diagnosis of neonatal sepsis and results with CRP are comparable to those with blood culture.

Keywords: *C-reactive Protein, Neonatal Sepsis, Blood Culture.*

Introduction

Neonatal mortality gives idea about country's primary health status.[1] Infection, birth asphyxia, prematurity, birth trauma are the leading causes of neonatal deaths. Among these only sepsis alone claims 32.8% of neonatal deaths in India.[2] Approximately 41% of all under 5 year child deaths happen in the first week of life and the risk of deaths during neonatal period is at least 68 times higher than the rest of childhood.[3] Neonatal sepsis is a clinical syndrome showing systemic signs of circulatory disturbances like pallor, poor peripheral perfusion, poor responsiveness and hypotonia. It is caused by invasion of the blood stream by bacteria often in the first month of life.[4] Invasive medical procedures, prematurity, low birth weight and prolonged hospitalisation are important risk factors for neonatal sepsis. Neonatal sepsis presents with variety of clinical presentation like fever, tachypnea, diarrhoea, bradycardia or tachycardia, poor feeding, abdominal distention, hypotension, oliguria, irritability, convulsions, bleeding or bulging fontanelle.[5] Confirmed diagnosis of neonatal sepsis is made by culture of blood and other body fluids. Blood cultures are positive in 25-54% neonatal cases.[6] Total leukocyte counts and erythrocyte sedimentation rate (ESR) can be used as supportive diagnostic tests. Other laboratory parameters like acute phase proteins, cytokines, cell surface antigens and bacterial genomes have also been used alone or in combination to improve the diagnostic accuracy of neonatal sepsis.[7]

Studies have been carried out to determine the effectiveness of various interleukins (IL-6, 8, 10), Tumor necrosis factor alpha (TNFα) and procalcitonin (PCT) in diagnosing sepsis. But however in clinical practice, CRP is the most accessible, easy to use, interpret and so widely used marker of infection.[8] CRP is an acute phase serum glycoprotein produced in liver. As it is a part of innate immune system, its level increases within 24 to 48 hours after bacterial infection.[9] In proven cases of sepsis, CRP has 97.2% sensitivity and 95% specificity.[10] In a case of neonatal sepsis, blood culture is the gold standard for diagnosis and also allows a targeted anti microbial therapy. But its results are delayed up to 48 hours and it comes negative in many cases of septic shock. Also chances of contamination are high because of technical difficulties in deriving sterile samples from small babies. Considering high morbidity and mortality related to sepsis, it is utmost important to make decision timely and start antibiotics without waiting for culture results. On the other side unjustified usage of the antibiotics leads to development of microbial resistance and also increases the risk of drug reactions.

Measurements of CRP has several advantages over these tedious and expensive parameters like; sterile sample is not required, simple, easy to interpret, rapid and allows early identification of patients with sepsis. Serial CRP measurements can guide about response to antibiotic therapy and thus has prognostic importance also.[11]

Materials and Methods

This study is aimed to determine the effectiveness of CRP as a diagnostic tool in neonatal sepsis. Specified terms used in the study can be explained by following definitions. "Neonate" refers to the new born from 1st day to 28th day of life. "Neonatal sepsis" is a clinical syndrome characterised by systemic signs of circulatory disturbances leading to pallor, poor peripheral perfusion, hypotonia, poor responsiveness and often caused by invasion of blood stream by bacteria in the first month of life.[4] Diagnosis of neonatal sepsis is made by the presence of two or more of following clinical features: poor peripheral perfusion (capillary refilling time > 03 seconds), temperature instability (<35° C or >38° C), tachypnea (respiratory rate >60/minute at rest), tachycardia (>150 beats/minute at rest) and oliguria (urine output <0.5ml /kg of body weight/ hour). This diagnosis was confirmed positive or negative as neonatal sepsis on the basis of blood culture results. CRP level >6 mg/L was defined as raised CRP level.

Total 123 patients admitted at a private hospital in north gujarat during December 2014 to February 2017 were included in this study. Inclusion criteria: all patients age between 0 to 28 days, all genders, with two or more of the following clinical features: poor peripheral perfusion (capillary refilling time > 03 seconds), temperature instability (<35° C or >38° C), tachypnea (respiratory rate >60/minute at rest), tachycardia (>150 beats/minute at rest) and oliguria (urine output <0.5ml /kg of body weight/ hour). Exclusion criteria: new borns with major systemic malformation, congenital abnormalities, underlying surgical condition, weight <1 kilogram, received antibiotics or undergone any invasive medical procedure. A detailed history and physical examination of the neonates was carried out. Weight, skin perfusion, temperature, respiratory rate, heart rate and urine output for each patient were noted. The blood sample for CRP as well as culture were taken and sent to the laboratory. For blood culture, 2 ml blood was taken with proper aseptic measures and inoculated in blood culture bottle. For CRP, 1 ml blood was collected in plain vacuette. CRP was carried out at in-house laboratory by latex agglutination method while for culture, samples were sent to a referral laboratory. Results of blood culture and CRP were noted down. CRP value >6mg/L was considered raised.

Considering blood culture as a gold standard for neonatal sepsis, diagnostic accuracy of CRP was measured in terms of sensitivity, specificity, positive predictive value and negative predictive value. Sensitivity and specificity were calculated by using True Positive (TP): if CRP raised and blood culture is positive, False Positive (FP): if CRP raised but blood culture is negative, True Negative (TN): if CRP not raised and blood culture is negative, False Negative (FN): if CRP not raised but blood culture is positive. Statistical analysis was carried out by applying Chi-square test using GraphPad Instat Demo software.

Expected sensitivity and specificity of CRP were 97.2% and 95% respectively.[9]

Results

Total 123 patients were evaluated in the study. Among all of them fever was the most commonly noted (63.41%) symptom in neonates suspected to have infection. Blood culture was found to be positive in 57.72% cases, whereas CRP was raised in 58.54% cases. Relationship of raised CRP with positive blood culture was further evaluated by cross tabulation. It was found that among 72 cases with raised CRP (>6mg/L), 70 cases had positive blood culture (97.23%) and thus they were true positive. Whereas 2 cases (2.78%) had negative blood culture and hence false positive. When cases with negative CRP results were compared with their blood culture results it was found that among 51 patients with normal CRP (< or = 6mg/L) 50 (98.04%) had negative blood culture and hence they were true negative. Only 1 patient (1.96%) with normal CRP level had positive blood culture and it was false negative.

Sensitivity and specificity of CRP were found to be 98.59% and 96.15% respectively. Positive predictive value for the test was 97.23% where as Negative predictive value was 98.04%. Relationship between CRP and blood culture was also found to be statistically significant at $p < 0.05$.

Discussion

Neonatal sepsis is one of the leading causes responsible for deterioration of vitals in neonates. Gold standard for identifying this bacterial infection in neonate is blood culture. But it has a low yield because its time consuming and high chances of sample contamination due to technical difficulties in sterile sample collection in small babies. Therefore, pediatricians use certain surrogate tests to identify neonatal sepsis. This study was conducted with an aim to assess the usefulness of CRP as an indicator of neonatal sepsis.

Neonatal sepsis related mortality and morbidity is very high. It is a medical emergency; failure to indentify it may lead to mortality and over diagnosis may lead to

Table 1: Quantitative Variables of Study Population

Variable	Mean	Standard Deviation (SD)
Age (in days)	16.31	8.42
Weight (in kilograms)	3.05	0.38

Table 2:

	Blood Culture Result	
CRP Result	Positive	Negative
Raised (>6 mg/L)	70	2
Normal (<or=6 mg/L)	1	50

Table 3: Effects of Weight on sample characteristics.

	3 kilograms	>3kilograms	p value
Fever	30	48	0.00007
Tachypnea	39	19	0.0014
Tachycardia	36	39	0.59
Delayed Capillary Filling	30	39	0.0318
Oliguria	30	35	0.167
Positive Blood Culture	31	40	0.0299
Raised CRP	31	41	0.178

Table 4: Effects of Age on sample characteristics.

	15 days	> 15 days	p value
Fever	34	44	0.2
Tachypnea	36	22	0.0031
Tachycardia	36	39	0.993
Delayed Capillary Filling	30	39	0.259
Oliguria	29	36	0.431
Positive Blood Culture	35	36	0.73
Raised CRP	35	37	0.865

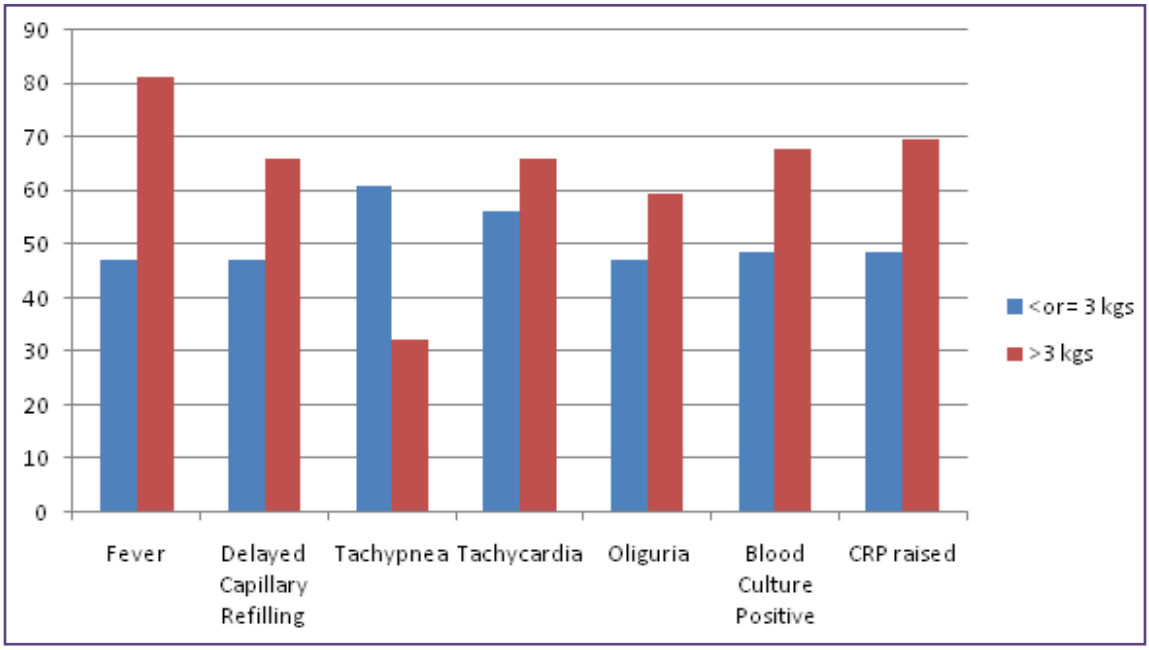

Fig. 1: Illustration of effects of Weight on sample characteristics.

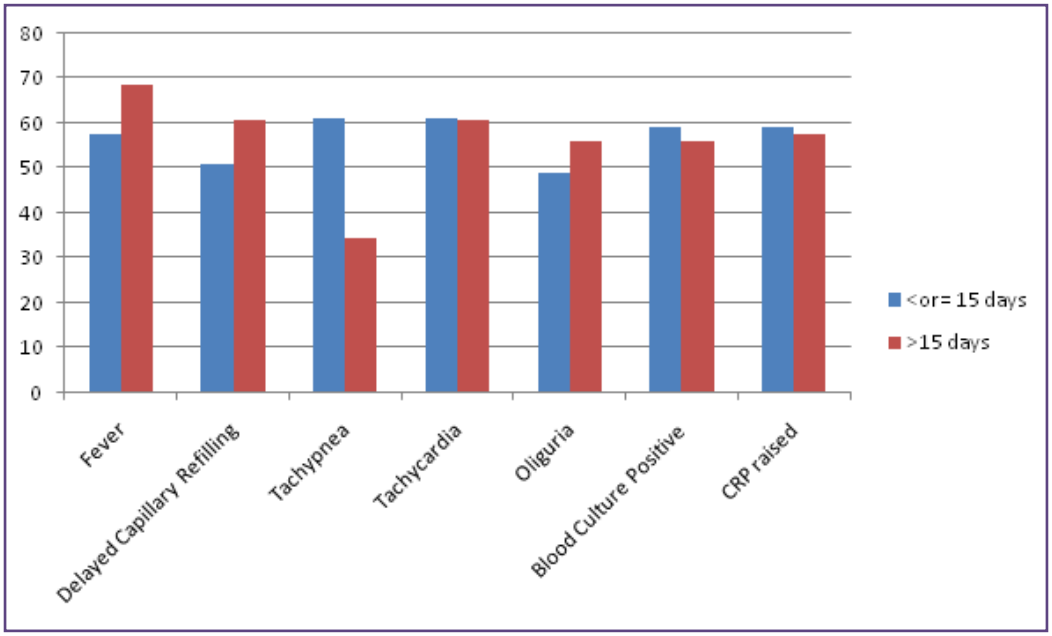

Fig. 2: Illustration of effects of Age on sample characteristics.

unnecessary use of antibiotics which ultimately lead to financial loss to the patient as well as development of drug resistance and to certain extent drug reaction also. Therefore it is utmost important to have a screening test for neonatal sepsis with high sensitivity and high negative predictive value so that it ideally recognizes all infected neonates and excludes all neonates with negative results. Various screening methods have been lately in use for early diagnosis of neonatal sepsis. They are microESR, ANC (absolute neutrophil count), band neutrophil ratio etc. In recent studies, parameters like NBT (nitro blue tetrazolium) and acridine orange have also been evaluated upto certain extent.

In our study, there were total 123 patients. Males were (52.03%), slightly on higher side than females (47.96%) which concludes the fact that male sex is predisposed to neonatal sepsis.[11] Mean age and weight for the study population were 16.31 ± 8.42 days and 3.05 ± 0.38 kilograms respectively (Table 1). These values are comparable to other studies. [12]

Effects of weight on various sample characteristics were noted (Table 3). For that sample were divided into two groups: >3kilograms and ≤ 3 kilograms. In group with >3kilograms there were more males (61%) than females (38.9%), where as in group with ≤ 3 kilograms there were more females (56.25%) than males (43.75%). This observation indicates that with lower birth weight females are more prone to develop sepsis where as with normal birth weight male gender is a risk factor for developing neonatal sepsis. Results similar to this were also observed in neonates with low birth weight.[13] Fever was found in 81.4% of patients in weight group >3 kilograms compare to only 46.9% patients in weight group ≤ 3 kilograms. This difference was statistically significant at $p < 0.05$. This would enlighten the fact that babies with low birth weight diagnosed with neonatal sepsis may not manifest fever because of not fully mature hypothalamic temperature control mechanism. This observation suggests that sepsis should be suspected even in the absence of fever, if other features suggestive of sepsis are present, particularly in low birth weight babies. Statistically important differences were also found for clinical features like tachypnea and delayed capillary refilling between two weight groups but they are unexplainable. Positive blood culture were also found to be more (67.8%) with weight group >3 kilograms than with group having ≤ 3 kilograms weight (48.4%). This difference is statistically significant but it could be by chance and not so much of clinical importance. Table 4 shows effects of age on various sample characteristics. Difference between two groups >15 days of age and ≤ 15 days of age for clinical feature tachypnea was found statistically significant but however it was by chance and not of clinical importance. Differences between other sample characteristics were not statistically significant. Effects of weight and age on various sample characteristics are illustrated also in figure 1 and 2 respectively.

Sensitivity of CRP is higher than specificity. These results are comparable with other studies which have shown a

high sensitivity, specificity, positive predictive value and negative predictive value in comparison to blood culture results. In one of the study, sensitivity and negative predictive value for CRP was found to be 100%.[12]

Proinflammatory cytokines (IL-2, IL-6, interferon gamma, TNF-α) and anti-inflammatory cytokines (IL-4 and IL-10) are elevated in cases with infection as compare to non-infectious cases. However, these parameters are not routinely used for detection of infection because of their high testing cost and no single parameter or their panel is sensitive enough to detect neonatal sepsis with reliability.[14]

Studies have also been conducted to evaluate procalcitonin as a useful marker for detecting bacterial infections. Data also suggest that elevated procalcitonin (>0.5ng/ml) is equivalent or somewhat better than CRP in detecting bacterial infection in preterm infants.[15] Though procalcitonin is emerging as a promising marker, its reliability as a sole diagnostic tool for neonatal sepsis is not clear and at this point of time it is not available routinely in all laboratories. There is still scope for a further research to develop sensitive and specific markers of inflammation after studying the neonatal inflammatory responses to infection in detail or to develop antigen/pathogen specific rapid diagnostic tests for early detection of neonatal sepsis.

Conclusion

C-reactive protein is a very good laboratory tool with high sensitivity and specificity for diagnosis of neonatal sepsis and its comparable to gold standard method blood culture. It has extra benefit of early availability of test result as compare to blood culture. It has also other advantages over blood culture like easy to perform, cheaper, does not require a sterile sample collection and does not require advanced technology. Serial measurements of CRP level are of prognostic significance as it gives idea about response to treatment. One disadvantage with CRP is that it is elevated sometimes in noninfectious inflammatory conditions which lead to reduction in its specificity and positive predictive value. But reduced specificity and positive predictive value is acceptable as the cost of treatment is much lesser and it would be much costlier to miss a case with potentially life threatening infection. Therefore, it is advisable to use C-reactive protein routinely for detection of sepsis in neonates with suggestive clinical presentation.

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