Section: Microbiology

Original Research Article

CRP AS A PROGNOSTIC MARKER IN NEONATAL SEPTICAEMIA

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Received *: 02/01/2024*
Received in revised form *: 09/02/2024*
Accepted *: 27/02/2024*

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DOI: 10.5530/ijmedph.2024.1.73

Source of Support: Nil,
Conflict of Interest: None declared

Int J Med Pub Health
2024; 14 (1); 401-405

ABSTRACT

Background: It has been documented that CRP is probably the best available diagnostic test. CRP elevation in neonates has been documented in non-infectious conditions including meconium aspiration, respiratory distress syndrome, foetal hypoxia and intraventricular haemorrhage.

Aim: CRP as a prognostic marker in neonatal septicaemia.

Materials and Methods: A study was conducted on 200 neonates clinically suspected cases of septicaemia recruited from the neonatal intensive care unit of Medical College from 1st January 2019 to 31th Dec 2019. Any neonate with signs and symptoms of suspected sepsis, in the form of respiratory distress, apnoea, oxygen dependence, feeding intolerance, poor feeding, hypotension shock, poor peripheral perfusion, tachycardia, lethargy, temperature instability, seizures, altered mental status, skin mottling and unexplained acidosis. Neonates clinically suspected of septicaemia, were tested for serum CRP and also blood culture was done. Positive cultures were the "gold standard" against which the performance of CRP, complete blood count, abnormal white blood cell counts (WBC) and platelets were compared. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of CRP were calculated.

Results: Out of 135 patients with blood culture growth, most patients belong to early neonates- 0 to 7 days- age group (60%) and then late neonates- 8 to 28 days- age group (40%). Out of 200 neonates with suspected septicaemia, blood from 135 patients yielded growth from bacterial culture. Blood culture growth has been considered as gold standard for septicaemia. In our study most common organism responsible for neonatal septicaemia was Escherichia coli (31.85%) followed by CoNS (20.74%), Klebsiella pneumoniae (16.29%) and Staphylococcus aureus (9.62%) and rest others. Out of 135 positive blood cultures, serum CRP test was positive in 125 patients, 19 CRP positive but culture negative. In my study Early neonates are 58.33% positive CRP test and 41.66% positive for Late neonates. Out of 200 neonates 94 Term neonates were showing positive Blood culture and 41 Preterm neonates with positive Blood culture. In term and preterm Sensitivity and Specificity of 89.36%, 78.26% and 82%, 100% respectively.

Conclusion: We concluded that the sensitivity of CRP was more for diagnosis of septicaemia than other inflammatory markers. Septicaemia is the major and common cause of morbidity and mortality in neonates. The incidence is much higher in the developing world. Early diagnosis and effective treatment are the best way to reduce morbidity and mortality.

Keywords: CRP, Blood culture, CoNS, Septicaemia.

INTRODUCTION

Sepsis is one of the major problems in neonates. Sepsis is the commonest cause of neonatal mortality and is probably responsible for 30-50% of the total neonatal deaths each year in developing countries.[1,2] It is estimated that 20% of all neonates develop sepsis and approximately 1% die of sepsis related causes.[2] Sepsis related mortality is largely preventable with rational antimicrobial therapy with aggressive supportive care. According to recent data from National Neonatal Perinatal Database (NNPD) 2000, the incidence of neonatal sepsis has been reported to be 38 per 1000 intramural live births in tertiary care institutions.[3] The neonatal sepsis at time is very difficult to diagnose on clinical criteria alone because of its non-specific and variable signs and symptoms. In view of the high mortality associated with this condition septic screening is carried out and empirical treatment with antibiotics started in the presence of two or more of these risk factors, resulting in a large number of babies receiving unnecessary antibiotics.[4] The use of safe and effective antimicrobial therapy has markedly reduced the neonatal mortality.[4] Though empirical therapy with antibiotics seems reasonable given the dire outcome of missed diagnosis but improvement in diagnostic accuracy should diminish the exposure of healthy neonates to the diagnostic accuracy should diminish the exposure of healthy neonates to the risk of un-wanted antimicrobial therapy, psychological stress of prolong stay and financial overburden. The C-reactive protein was first introduced in 1930 by Tillett and Francis.[5] C-reactive protein is an inflammatory marker that is synthesized in the liver in response to inflammatory cytokines and plays a major role in innate immunity. The level of C-reactive protein rises rapidly with a peak level in 6 hours, even up to thousands folds during an acute response. It has a short half-life of 19 hours, so the level falls rapidly once the source is removed.[6]

CRP is a useful infective marker as compared to leukocyte counts in the neonates, which varies significantly in the early neonatal period and hence cannot be set as a diagnostic test for confirmation of sepsis. In a recent comprehensive systemic review of the literature evaluating leukocyte ratios and C-reactive protein, there is wide range of sensitivity and specificity for leukocyte count and ratios, similarly C-reactive protein measurement showed variable accuracy though better than leukocyte count. Though blood culture is a gold standard test but due to some pit fall we need a test that is readily available, cost effective and less time consuming. We consider C-reactive protein as an indicator of neonatal sepsis, at the same time we cannot consider C-reactive protein as a sole indicator but may be regarded as a step-in approach to work up and in combination of other tests like blood culture, also with C-reactive protein we can determine the severity of neonatal sepsis while waiting for blood culture results that may even be negative in some fatal infections.[6]

C-reactive protein (CRP) is an annular (ring-shaped), pentameric protein found in the blood plasma, the levels of which rise in response to inflammation (i.e., C-reactive protein is an acute-phase protein). Its physiological role is to bind to phosphocholine expressed on the surface of dead or dying cells (and some types of bacteria) in order to activate the complement system via the C1Q complex.[7] CRP is synthesized by the liver [7] in response to factors released by macrophages and fat cells (adipocytes).It is a member of the pentraxin family of proteins. It is not related to C- peptide (insulin) or protein C (blood coagulation). C-reactive protein was the first pattern recognition receptor (PRR) to be identified.[7]

CRP is used mainly as a marker of inflammation. Apart from liver failure, there are few known factors that interfere with CRP production. Measuring and charting CRP values can prove useful in determining disease progress or the effectiveness of treatments. ELISA, immunoturbidimetry, rapid immunodiffusion, and visual agglutination are all methods used to measure CRP. A high sensitivity CRP (hs-CRP) test measures low levels of CRP using laser nephelometry.

CRP is a more sensitive and accurate reflection of the acute phase response than the ESR (Erythrocyte Sedimentation Rate). ESR may be normal and CRP elevated. CRP returns to normal more quickly than ESR in response to therapy. Several studies investigated differential diagnostic values of CRP in a series of inflammatory disease (including inflammatory bowel disease, Intestinal Lymphoma, Intestinal Tuberculosis and Behcet's Syndrome), and compared CRP to other inflammatory biomarkers, such as ESR and WBC.

MATERIAL AND METHODS

A study was conducted on 200 neonates clinically suspected cases of septicaemia recruited from the neonatal intensive care unit (NICU) of Medical College from 1st January 2019 to 31th Dec 2019. Any neonate with signs and symptoms of suspected sepsis, in the form of respiratory distress, apnoea, oxygen dependence, feeding intolerance, poor feeding, hypotension shock, poor peripheral perfusion, tachycardia, lethargy, temperature instability, seizures, altered mental status, skin mottling and unexplained acidosis. Neonates clinically suspected of septicaemia, were tested for serum CRP and also blood culture was done. Positive cultures were the “gold standard” against which the performance of CRP, complete blood count, abnormal white blood cell counts (WBC) and platelets were compared. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of CRP were calculated.

Methodology

The blood samples were received in red (Plain) vacutainer with laboratory request form after taking detailed clinical history of patients and checked for laboratory acceptance criteria. Samples not fulfilling criteria were rejected. Unique laboratory ID generated in Laboratory Information System for each sample. In laboratory, serum was separated after centrifugation at 1500 rpm (revolution per minute) for 10 minutes and aliquoted in two vials. One vial used for testing using immunopak CREACTIVE PROTEIN RECKON DIAGNOSTICS P.LTD and the other stored at -20◦C for tracing of the previous result.

QUALITATIVE TEST: Reagent and sample are brought to room temperature before use. Latex reagent is mix well before use. 25 µl of each sample are placed into center of the black circle of test card. Equal amount of the latex suspension is added to each sample or controls. It is then spread over the circle using separate mixing sticks for each sample. The card is placed on the shaker for two minutes per 50-250rpm.

SEMI QUANTITATIVE TEST: Sera showing positive results in quantitative test are retested in semi quantitative test for obtaining the titre. The specimens are serially diluted 1:2, 1:4, 1:8, 1:16, 1:32, 1:64 so on…using normal saline. One drop of each diluted sample is placed using plastic dropper in each circle of slide and proceeded further as in Qualitative Test.

Agglutination within 2 minutes is positive test. The highest dilution shows positive reaction within 2 minutes indicates CRP titre. No agglutination up to 2 minutes is negative test.

RESULTS

This study was conducted from 1st January 2019 to 31th Dec 2019. During this period samples from 200 clinically suspected cases of septicaemia in neonates were received and processed for serology and bacterial culture at respective section. [Table 1]

Septicaemia was more common in male (57.77%) than female (42.22%) neonates. [Table 2]

Out of 135 patients with blood culture growth, most patients belong to early neonates- 0 to 7 days- age group (60%) and then late neonates- 8 to 28 days- age group (40%). Out of 200 neonates with suspected septicaemia, blood from 135 patients yielded growth from bacterial culture. Blood culture growth has been considered as gold standard for septicaemia. In our study most common organism responsible for neonatal septicaemia was Escherichia coli (31.85%) followed by CoNS (20.74%), Klebsiella pneumoniae (16.29%) and Staphylococcus aureus (9.62%) and rest others (Enterococcus feacium, Candida spp, Acinetobacter baumanii). [Table 3]

Above table shows that, out of 135 blood culture positive patients, abnormal WBC count was found in 75 patients and platelet count was decreased in 47 patients. [Table 4]

Out of 200 neonates with suspected septicaemia, blood from 135 patients yielded growth from bacterial culture. Blood culture growth has been considered gold standard for septicaemia. Out of 135 positive blood cultures, serum CRP test was positive in 125 patients, 19 CRP positive but culture negative. [Table 5]

In my study Early neonates are 58.33% positive CRP test and 41.66% positive for Late neonates. [Table 6]

Out of 200 neonates 94 Term neonates were showing positive Blood culture and 41 Preterm neonates with positive Blood culture. In term and preterm Sensitivity and Specificity of 89.36%, 78.26% and 82%, 100% respectively. [Table 7]

In age group (0-7) days and (8-28) days 58.33%, 41.66% CRP positive, 80%, 20% abnormal WBC count,66.67%, 33.33% abnormal platelet counts and 56.8%, 43.20% positive blood culture respectively. [Table 9]

Table 1: Demographic data of neonates

Age	Male	Female	Total
0 to 7 days (Early neonate)	80	46	126
8 to 28 days (Late neonate)	39	35	74
Total	119	81	200

Table 2: Sex distribution of patients suspected of septicaemia

Sex	No of patients	Blood culture growth
Male	119	78(57.77%)
Female	81	57(42.22%)
Total	200	135

Table 3: Age distribution of patients with Blood culture growth

Age	Male	Female	Total
0 to 7 Days	52	29	81(60%)
8 to 28 Days	26	28	54(40%)
Total	78	57	135

Table 4: Comparison of Blood culture and other parameters

Parameter		Blood culture positive n=135	Blood culture negative n=65
Total WBC (10^3/cmm)	Normal	60	52
	<4	6	5
	>11	69	8
Platelet count (10^3/cmm)	Normal	79	44
	<150	47	12
	>450	9	9

Table 5: Comparison of blood culture and CRP

Bacterial culture → / CRP ↓	Growth	No growth	Total
Positive	125	19	144
Negative	10	46	56
Total	135	65	200

Table 6: Age Distribution with Positive CRP Test Result

Age	Male	Female	Total
0-7 (Days)	44	40	84 (58.33%)
8-28 (Days)	26	34	60 (41.66%)
Total	70	74	144

Table 7: Comparison of Gestational age with CRP and Blood culture

Bacterial Growth → / CRP ↓	Growth	No growth	Total
Term (37wk-42wk) Positive	84	10	94
Negative	10	36	46
Preterm (32wk-36wk) Positive	41	9	50
Negative	0	10	10
Total	135	65	200

Table 8: Age distribution in CRP positive patient with abnormal blood parameter and positive blood culture

Age Group	CRP Positive test result	Abnormal WBC Counts	Abnormal Platelet Counts	Positive Blood culture
0-7(Days)	84(58.33%)	40(80%)	42(66.67%)	71(56.8%)
8-28(Days)	60(41.66%)	10(20%)	21(33.33%)	54(43.2%)
Total	144	50	63	125

Table 9: Validity and predictive outcome of CRP in Neonates (Considering blood culture as a gold standard)

Sensitivity (Neonates)	92.59%
Specificity	70.76%
Positive predictive values	86.80%
Negative predictive values	82.21%
Diagnostic accuracy	85.50%

DISCUSSION

In this study, validity of serum CRP in the diagnosis of sepsis was studied on 200 neonates. 135 cases of neonatal sepsis confirmed on blood culture were evaluated. Most of the patients evaluated had the known risk factors and clinical features associated with sepsis.

In our study, sensitivity and specificity of serum CRP for diagnosis of septicaemia are 92.59% and 70.76% respectively, which can be comparable with study done by Kawamura et al,[15] Berger et al,[17] and also Anwar Zeb and collegues.[9]

Above table shows that for clinical evaluation of neonates with septicaemia, serum CRP test has highest sensitivity (92.59%) and specificity (70.76%) compare to abnormal WBC count and platelet count. In our study, sensitivity and specificity of abnormal WBC count for diagnosis of neonatal septicaemia was 55.55% and 80%, respectively which can be comparable with study done by Chauhan Setal B,[8] at NICU of V.S. Hospital, Ahmedabad, in which sensitivity and specificity of abnormal WBC count was 30.77% and 63.15%, respectively. In our study, sensitivity of decreased platelets count was 35.96%, which was comparable to study done by Chauhan Setal B.[8]

Table 10: Comparative study of Serum CRP for diagnosis of Neonates septicaemia

Author(s)	Testing Method	Sensitivity	Specificity
Our study	**Latex slide agglutination**	**92.59%**	**70.76%**
Chauhan Setal B [8]	Latex slide agglutination	92.30%	85.71%

Anwar Zeb Jan, et al [9]	Latex slide agglutination	88.35%	89.20%
Chan DK and colleagues [10]	-	56%	72%
M Himayua [11]	Semi-quantitative technique	70%	72.3%
Wagle S and Colleagues [12]	-	70.2%	87.7%
Effat Hisamuddin et al,[13]	Latex slide agglutination	76.92%	53.49%
Russel et al. [14]	Quantitative Laser nephelometry	71%	72%
Kawamura et al.[15] n = 108 term and n = 240 preterm infants with suspected sepsis	Quantitative laser nephelometry	Preterm: 61.5% Term: 75%	Preterm: 95% Term: 97.8%
Hafadh Jaleel Hussein,[16]	Latex slide agglutination	82.40%	93.00%
Berger et al. [17] n= 195 term and preterm infants	Qualitative immunoassay	75%	86%
Parviz Ayazi [18]	Qualitative latex agglutination	67%	80%

Table 11: Comparative analysis of tests used in study of septicaemia in neonates

Test parameter	Sensitivity	Specificity	PPV	NPV	Accuracy
Serum CRP	92.59%	70.76%	86.80%	82.21%	85.50%
Abnormal WBC count	55.55%	80%	85.22%	46.42%	63.50%
Abnormal Platelet counts	41.48%	67.69%	72.72%	35.77%	50%

CONCLUSION

We concluded that the sensitivity of CRP was more for diagnosis of septicaemia than other inflammatory markers. Septicaemia is the major and common cause of morbidity and mortality in neonates. The incidence is much higher in the developing world. Early diagnosis and effective treatment are the best way to reduce morbidity and mortality. The delay in diagnosis and initiating therapy are the main reasons for high mortality. The use of safe and effective antimicrobial therapy has markedly reduced the neonatal mortality. Though empirical therapy with antibiotics seems reasonable given the dire outcome of missed diagnosis but improvement in diagnostic accuracy should diminish the exposure of healthy neonates to the risk of un-wanted antimicrobial therapy, psychological stress of prolong stay and financial overburden.

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