ISSN: 0975-1556

Available online on www.ijpcr.com

International Journal of Pharmaceutical and Clinical Research 2021; 13(3); 162-170

Original Research Article

The Role of C-Reactive Protein and Gastric Aspirate Polymorphs in Newborn Sepsis

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Received: 04-05-2021 / Revised: 02-06-2021 / Accepted: 22-06-2021
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Conflict of interest: Nil

Abstract

Aim: To evaluate the role of C-reactive protein and gastric aspirate polymorphs in early onset neonatal sepsis. **Methods:** This was a prospective observational study conducted in the Department of Pediatrics, Nalanda Medical College and Hospital, Patna, Bihar, India, from January 2020 to Feb 2021. 80 babies, who had the clinical symptoms and signs of suspected neonatal sepsis/high risk factors for developing sepsis, were included in the study. Each patient was subjected to detailed history and physical examination. Blood sample was taken at admission and subjected to TLC and CRP. The blood sample for blood culture and sensitivity was collected at the same time. Following this the decision to start antibiotic therapy was based on combination of clinical signs, obstetric risk factors and sepsis screen. **Results:** A total number of 80 subjects were included in the study with 61 (76.25%) as out born neonates. Most of the neonates had presented with tachypnea followed by difficulty in feeding and lethargy. Sepsis screening was done at admission for all neonates enrolled in the study. Only 20 patients (25%) had TLC more than 25000 /dl. Maximum TLC value in the study was 41300 /dl. 60(75%) patients showed positive CRP values, whereas 55 patients (68.75%) had polymorphs in the GA more than 5 per high power field. Only 48 (60%) patients had positive blood culture and sensitivity report. By combination of any CRP and TLC specificity increased to 81.25%. While sensitivity approached 100% when TLC with GA polymorphs and CRP with GA polymorphs were combined with significant p values of 0.001 and 0.014 respectively. When all the three parameters were combined together, both the sensitivity and specificity increased to 100% and 91.25% respectively with p values of 0.001. In this study the most common organism grown in blood culture was Klebsiella followed by Staphylococcus aureus and Pseudomonas. **Conclusion:** CRP showed high sensitivity while GA polymorphs showed high specificity. GA cytology as a screening tool for neonatal sepsis with intermediate sensitivity,

specificity, positive predictive value and negative predictive values serves as good tool, added to a detailed antenatal history and clinical examination of the neonate.

Keywords: CRP, Gastric Aspirate, Sepsis, etc.

Introduction

Neonatal sepsis (NS) may have subtle, diverse and non- specific symptoms and signs; Moreover, a delay in the diagnosis and commencement of treatment results in a high morbidity and mortality rates. Annually five million neonates die mostly in Asia and Africa, out of which 1.6 million (20%) are due to NS. The incidence of neonatal sepsis in developed countries is 1-10/ 1000 live births whereas it is three times more in Pakistan[1]. A definitive diagnosis based on culture of blood, cerebrospinal fluid (CSF), or urine is usually reached only after a delay of a day or two. Initiation of antibiotic therapy before diagnostic results are availableis recommended for neonates with clinical signs or epidemiologic factors associated with NS. However, some patients with bacterial infection may have negative blood cultures (clinical infection), and other approaches to identification of infection are required[2].

There have been many attempts to develop screening tests or scoring systems that can identify infected infants at the time of initial assessment, sparing others from invasive diagnostic procedures, intravenous antibiotic therapy, mother-infant separation and heightened parental anxiety[3]. Serum concentrations of many acute phase reactants rise in response to infection which can be used as non-specific indicators of bacterial sepsis including C-reactive protein (CRP), multiple leucocyte activation markers, interleukin 6, interleukin 8, tumour necrosis factor-alpha and procalcitonin in the diagnosis of NS[4-7]. As yet no international consensus regarding screening of neonatal sepsis has been made.

CRP is a non-specific, acute-phase protein that rises in response to inflammatory processes. Sufficient evidence exists to support the use of CRP measurements in conjunction with other established diagnostic tests, such as a total and differential leukocyte count (TLC and DLC) and blood culture to establish or exclude the diagnosis of sepsis in full-term or near-term infants[4]. Despite their limitations in sensitivity and specificity variations in TLC and DLC and immature neutrophils to total neutrophil count ratio (I/ T ratio) of 0.2 or greater suggests bacterial infection. Demonstration of bacteria and inflammatory cells in Gram-stained gastric aspirate cytology (GAC) on the 1st day of life may reflect maternal amnionitis, which is a risk factor for early-onset infection.2 Present study was conducted to correlate the GA polymorphs, TLC and CRP with blood culture in early onset neonatal sepsis.

Material and Methods

This was a prospective observational study conducted in the Department of Pediatrics, Nalanda Medical College and Hospital, Patna,Bihar, India, from January 2020 to February 2021. after taking the approval of the protocol review committee and institutional ethics committee.

80 babies who had clinical symptoms and signs of suspected neonatal sepsis/high risk factors for developing the sepsis, were included in the study. 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 7 days of life, inborn or outborn with suspected sepsis and with high risk factors (antenatal, natal, postnatal). The high-risk factors included preterm neonates, with history of fetal distress, maternal history of leaking P/V (more than 18 hours), maternal fever, history of any maternal infection like urinary tract infection, chorio-amnionitis, multiple obstetrical procedures or difficult labour.

Babies with age more than 7 days of life, having septic shock patients or rapidly deteriorating clinical condition, weighing <1500 gms, 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 samples were taken at admission and subjected to TLC and CRP. The blood sample for blood culture and sensitivity was collected at the same time. Following this the decision to start antibiotic therapy was based on combination of clinical signs, obstretic risk factors and sepsis screen. Furthermore, sepsis screen was repeated whenever new clinical signs of infection developed. The samples were collected in EDTA vial for TLC and in the plain vial for CRP. Under strict aseptic measures, samples for blood culture and sensitivity were collected. Gastric aspiration was sent for cytology in plain sterilized tubes. 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. RHELAX CRP reagent was used to detect CRP concentrations greater than 0.6 mg/dl. 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. Positive or negative results are determined by software contained in the BacT/ALERT microbial detection system. GA was obtained by infant feeding tube within 12 hours of life in a neonate and put in plain vial. One drop of GA was mixed with one drop of methylene blue on a slide and covered with a cover slip. Slide was seen under microscope for polymorphs/HPF.

Significant values for screening tests were taken as TLC of >25,000/<5000 and CRP positive (0.6 mg/dl) and GA polymorphs >5 /HPF. Sepsis screen positive was two or more positive tests. The babies were started on 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. The statistical analysis was done using SPSS 21.0.

Results

A total number of 80 subjects were included in the study with 61 (76.25%) as out born neonates. Most of the neonates had presented with tachypnea followed by difficulty in feeding and lethargy. Sepsis screening was done at admission for all neonates enrolled in the study. Only 20 patients (25%) had TLC more than 25000 /dl. Maximum TLC value in the study was 41300 /dl. 60(75%) patients showed positive CRP values, whereas 55 patients (68.75%) had polymorphs in the GA more than 5 per high power field. Only 48 (60%)

patients had positive blood culture and sensitivity report.

TLC was found to be least sensitive parameter in neonatal sepsis screening (Table 1). CRP and GA polymorphs were found to be highly sensitive parameters. Both these parameters showed positive correlation with blood culture (Table 2).

By combination of any CRP and TLC specificity increased to 81.25% (Table 4). While sensitivity approached 100% when TLC with GA polymorphs and CRP with GA polymorphs were combined with significant p values of 0.001 and 0.014 respectively (Tables 5 and 6). When all the three parameters were combined together, both the sensitivity and specificity increased to 100% and 91.25% respectively with p values of 0.001 (Table 7).

In the study the most common organism grown in blood culture was *Klebsiella* followed by *Staphylococcus aureus* and *Pseudomonas.*

Table 1: Comparing TLC with blood culture positive neonates.

TLC	Number	Blood culture		Sensitivity	Specificity	NPV	PPV	Accuracy	P value
		Positive	Negative						
<25000	60	33	27	77.50	46.25	30	88.75	50	0.15
>25000	20	15	5						
Total	80	48	32						

Table 2: Comparing CRP with blood culture positive neonates

CRP	Number	Blood culture		Sensitivity	Specificity	NPV	PPV	Accuracy	P value
		Positive	Negative						
<6.0	20	2	18	87.50	50	92.5	70	75	0.001
>6.0	60	46	14						
Total	80	48	32						

Table 3: Comparing gastric aspirate polymorphs with blood culture positive neonates.

GA	Number	Blood culture		Sensitivity	Specificity	NPV	PPV	Accuracy	P value
		Positive	Negative						
<5.0	25	0	25	100	81.25	98.75	88.75	91.25	0.001
>5.0	55	48	7						
Total	80	48	32						

Table 4: Comparing TLC and CRP with blood culture positive neonates.

TLC+CRP	Number	Blood culture		Sensitivity	Specificity	NPV	PPV	Accuracy	P-value
		Positive	Negative						
Positive	15	12	3	90	81.25	91.25	75	85	0.001
Negative	15	2	13						
Total	30	14	16						

Table 5: Comparing TLC and gastric aspirate polymorphs with blood culture positive neonates.

TLC+GA	Number	Blood culture		Sensitivity	Specificity	NPV	PPV	Accuracy	P-value
		Positive	Negative						
Positive	13	12	1	100	66.25	100.00	91.25	92.50	0.001
Negative	2	0	2						
Total	15	12	3						

Table 6: Comparing CRP and gastric aspirate polymorphs with blood culture positive neonates.

CRP+GA	Number	Blood culture		Sensitivity	Specificity	NPV	PPV	Accuracy	P-value
		Positive	Negative						
Positive	43	38	5	98.50	75	100	90	91.25	0.014
Negative	15	0	11						
Total	58	38	16						

Table 7: Comparing TLC, CRP and gastric aspirate polymorphs with blood culture positive neonates.

TLC+CRP +GA	Number	Blood culture		Sensitivity	Specificity	NPV	PPV	Accuracy	P-value
		Positive	Negative						
Positive	15	14	1	100	91.25	98.75	91.25	95	0.001
Negative	15	0	15						
Total	30	14	16						

Discussion

Neonatal sepsis is one of the important causes of mortality among neonates. An early diagnosis not only helps in early institution of antibiotic therapy to reduce mortality due to neonatal sepsis but also helps in avoiding the unnecessary treatment of non-infected neonates. Although the blood culture is gold standard in diagnosis, it takes time and often complicated and has low yield[8]. The readily achievable complete blood count and the differential leukocyte count have a relatively poor specificity for diagnosing sepsis[11].

Studies have shown presence of polymorphs in GA to represent a fetal intra-amniotic inflammatory response[9,10]. GA cytology is simple and can be done without specially trained staff even in rural hospital settings. This is of great importance in a developing country like ours with a high infection rate and limited resources[11]. In the present study, we evaluated the utility of GA cytology as a screening tool for neonatal sepsis.

In the present study, sensitivity, specificity of the CRP is similar to other studies[12,13,14]. In the present study the blood culture was positive in only 60% cases. The low positivity of blood culture underlines the need of other tests in diagnosing neonatal septicemia. Out of the various individual tests for rapid diagnosis of neonatal septicemia, in proved sepsis group GA polymorphs was the one with maximum sensitivity (100%) while CRP showed sensitivity of 87.50% and specificity of a (82.50%). Other workers have also observed similar high sensitivity and specificity with CRP, by Berger et al (75%, 86%) and Kite et al (61.80%, 81.20%), respectively[12,14] Of the rapid diagnostic tests, CRP was found to be most useful when taken singly. Its elevation and returning to normal levels once the infection is controlled occurs in a matter of a few hours. Kite et al have reported elevated CRP levels in 80% of cases of neonatal sepsis[14]. They further added that evaluation of sepsis screen markers is important in the diagnosis of neonatal septicemia, especially in areas where adequate micro-biological facilities are lacking.

CRP can be used for screening of early neonatal sepsis as its sensitivity, specificity and positive predictive value is high, 72.2%, 82.14% and 77.2% respectively. In conclusion, CRP and band forms are more useful than GA polymorphs and micro ESR in screening of early neonatal sepsis[15].

Kaur et al studied the role of CRP and immature to total neutrophil ratio in early onset neonatal sepsis and concluded that 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 ($p=0.016$)[16]. Combination of various parameters in our study showed high sensitivity and specificity.

Chatterjee et al studied the role of raised IL-6 and CRP in neonatal sepsis. The concluded that the IL-6 is the highly sensitive marker and CRP is the more specific marker for the identification of neonatal sepsis. The combination of IL-6 and CRP has the high sensitivity and negative predictive value when compared to other markers. Therefore, a combination of markers, IL-6 and CRP would be the better predictors of neonatal sepsis[17]. Similar results were obtained in our study where combination of various parameters showed high sensitivity and specificity.

The serum CRP level was significantly raised in the clinically suspected neonatal sepsis groups than the control groups which is consistent with other studies[18-21]. Similarly in our study CRP was raised in 60 (75%) patients.

Gyllensvärd et al studied the role of CRP and clinical symptoms guided strategy in term neonates with early- onset sepsis.

They concluded that CRP and clinical symptoms guided decision-making for early onset neonatal sepsis significantly decreased the duration of antibiotic therapy and hospital stay and hence reduced healthcare cost[22]. GA cellularity correlates directly with the occurrence of clinical infection with sensitivity of 75% and specificity of 70%. CRP with GA was found to be the best combination with sensitivity of 80% and specificity of 70%[11,13]. GA polymorphs also showed high sensitivity and specificity in the present study and also with the combination of GA polymorphs and CRP.

The combination of CRP (0.10 mg/l) with abnormal film and/or I/T ratio>0.2 and/or GA cytology has been reported to have a sensitivity of 97%, specificity of 61%, NPV of 98% and likelihood ratio of 49 for early onset neonatal sepsis[24]. The results of the study done by Leivobich et al GA cytology had a sensitivity of 75% and specificity of 68%, which is closely approaches with the results of present study[25].

GA cytology is a good screening tool for neonatal sepsis added to a detailed perinatal history and clinical examination but does not completely substitute the present-day available screening parameters. Blood culture was positive in 60 percent in our study. Similar study done by Shah et al revealed 59-82% blood culture positivity in neonatal sepsis[26,27].

Most of the patients in the present study had presented with tachypnea followed by difficulty in feeding and lethargy. Similar complaints were noted in the neonates in the study by Shah et al like refusal to feed, lethargy, respiratory distress and temperature changes[28].

Conclusion

CRP showed high sensitivity while GA polymorphs showed high specificity. GA cytology as a screening tool for neonatal sepsis with intermediate sensitivity, specificity, positive predictive value and negative predictive values serves as good tool, added to a detailed antenatal history and clinical examination of the neonate. GA cytology with its relatively high specificity and negative predictive values serves as a good screening tool to rule out neonates unaffected by sepsis and prevent unnecessary antimicrobial usage.

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