International Journal of Contemporary Pediatrics
Kaur S et al. Int J Contemp Pediatr. 2021 Jun;8(6):987-992
http://www.ijpediatrics.com
pISSN 2349-3283 | eISSN 2349-3291

DOI: https://dx.doi.org/10.18203/2349-3291.ijcp20211938

Original Research Article

Role of C-reactive protein and gastric aspirate polymorphs in early onset neonatal sepsis

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Received: 25 April 2021
Revised: 10 May 2021
Accepted: 11 May 2021

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ABSTRACT

Background: Neonatal septicemia is a major cause of morbidity and mortality in the neonates. It presented a diagnostic challenge in the resource poor setting of most of the developing countries of world.
Methods: This prospective observational study included all term and preterm babies inborn and outborn referred cases. We included neonates less than 7 days of age with clinical suspicion of sepsis. Significant values for screening tests were taken as total leucocyte count (TLC) of >25,000/<5000, C-reactive protein>0.6 mg/dl and gastric aspirate polymorphs>5 per HPF. Sepsis screen was considered positive for two or more positive tests. Blood culture was used as the gold standard. The statistical analysis was done using SPSS 22.0 version.
Results: A total number of 60 subjects were included in the study with 45 (75%) as outborn neonates. Most of them presented with tachypnea followed by difficulty in feeding and lethargy. Significant p values were observed using CRP and gastric aspirate polymorphs as independent sepsis screening markers and when combined together ($p<0.001$).
Conclusions: Sepsis screen in neonates is required for detection of infection as blood culture may be negative and even positive result takes time. CRP showed high sensitivity. Gastric aspirate cytology with its relatively high specificity and negative predictive values serves as a good screening tool to rule out neonates unaffected by sepsis. When all the three parameters were combined together, sensitivity and specificity increased to 100% and 91.67% respectively with p values of 0.001.

Keywords: Sepsis, Neonates, CRP, TLC, Blood culture

INTRODUCTION

Neonatal sepsis is a clinical syndrome resulting from the pathophysiological effects of severe bacterial infection in the first month of life. Worldwide many neonates with sepsis die due to lack of early diagnosis.[1,2]

Neonatal sepsis is classified depending on the hours of presentation into early onset: within first 72 hours of life; late onset: occurring after 72 hours of life. Early onset neonatal sepsis is often due to organisms present in the maternal vaginal flora.[2] Mortality in this condition is much higher than in late onset sepsis.[3] In contrast to bacteremia (bacteria in blood), septicemia usually consists of bacteriemia plus a constellation of signs and symptoms caused by microorganisms or their toxic products in circulation. There may be progression of bacteriemia to septicemia depending on clinical manifestations. However, septicemia may also occur without bacteriemia such as in culture-negative sepsis

associated with pyelonephritis or pneumonia due to endotoxemia. The clinical diagnosis of neonatal sepsis is difficult because the signs and symptoms are not always specific. There is no laboratory test with 100% sensitivity and specificity.[4,5] Moreover, in many cases blood culture fails to detect the offending organism/bacteria. So, the search for a reliable test continues, especially one that is useful in culture-negative cases.[1]

Isolation of the infecting organism from blood provides the definitive diagnosis and is considered as the gold standard. However, this culture procedure takes at least 48 hours to confirm the diagnosis. Therapy cannot wait this long in a critically sick neonate. On the other hand the indiscriminate overuse of antibiotics on the basis of clinical suspicion alone is hazardous for any neonatal unit because it will lead to emergence of resistance. Hence, to rationalize antimicrobial therapy in neonatal sepsis, certain indirect early markers of neonatal infections have been identified.[2]

There are various predisposing factors that lead to an increased neonatal susceptibility to infection. These include VLBW(<1500 gms) or preterm baby; febrile illness in the mother during or within two weeks of delivery; foul smelling liquor; PROM >12 hours; frequent vaginal examinations (>3); prolonged and difficult labor with instrumentation; birth asphyxia and difficult resuscitation.[6]

Markers for infection which are used for diagnosing are direct markers of neonatal infection which include cultures of blood, cerebrospinal fluid (CSF) and urine are taken before initiating antibiotic therapy; indirect markers of neonatal infection: a number of early indirect markers of infection can be used as a mean of suspecting diagnosis of neonatal septicemia which include leukocyte counts: ratios and morphology, micro erythrocyte sedimentation rate (m-ESR) and acute phase proteins.[6]

Recently, microscopic appearances of fluid from the ear canal and gastric aspirate (GA) have been used as evidence of bacterial sepsis acquired in utero. Blanc detected evidence of inflammation from a smear of the fetal surface of the placenta and presence of leucocytes in the GA of the fetus.[7] Bernirschke introduced the technique of microscopic examination on rapid frozen sections of the umbilical cord and correlated umbilical wall inflammation with infection.[8] Oliver has suggested a relationship between the presence of polymorph nuclear cells in the GA and the possibility of subsequent infection of the newborn.[9] This method of using criteria of cells in the gastric fluid of an infant appears to be the simplest and one of the most readily carried out investigation without the requirement of specialized personnel and equipment. It can be done in a side laboratory. Thus, demonstration of bacteria and inflammatory cells in the GA on the first day of life (within an hour of life) may reflect maternal amnionitis.

Gastric polymorphs have thus been assumed to represent a fetal intra-amniotic inflammatory response. This test is simple and can be done without specially trained staff and in a rural district hospital. This is of great importance in a developing country with limited resources and high infection rates.[4] Respiratory tract secretions are usually swallowed by the new born the study on gastric aspirates may be more helpful for the diagnosis of pulmonary infection in this age group. Examination of gastric contents is a rapid and reliable method of early diagnosis of neonatal sepsis, provided the aspiration is done within an hour of birth.[10] 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.[11]

Berger prospectively studied the diagnostic value of CRP and white blood cell counts for detection of neonatal septicaemia. Sensitivity and specifity in receiver operating characteristics and positive and negative predictive value of CRP and white blood cell count were calculated. He concluded that CRP has high sensitivity and specificity in detecting neonatal septicemia.[12] Jaswal et al studied the CRP levels to evaluate the duration of antibiotic in 50 consecutive neonates with suspected septicemia. The negative predictive value of serial CRP was 100% in deciding duration of antibiotics therapy in suspected neonatal septicemia upto 7 days.[13]

Present study was conducted to correlate the GA polymorphs, TLC and CRP with blood culture in early onset neonatal sepsis.

METHODS

The study was a prospective observational study done on 60 patients over a period of one year (January 2005 to December 2005). The study was conducted at the neonatal intensive care unit attached to the department of paediatrics and neonatology, Jaipur Golden Hospital, Rohini, New Delhi, a tertiary care teaching hospital. It included all term and preterm babies inborn and outborn referred cases. The babies who had the 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, chorioamnionitis, 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 sample 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 2 cc 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 22.0.

RESULTS

A total number of 60 subjects were included in the study with 45 (75%) 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 13 patients (21.67%) had TLC more than 25000 /dl. Maximum TLC value in the study was 41300 /dl. 47 (78.33%) patients showed positive CRP values, whereas 40 patients (66.67%) had polymorphs in the GA more than 5 per high power field. Only 35 (58.33%) patients had positive blood culture and sensitivity report.

Table 1: Comparing TLC with blood culture positive neonates.

TLC	Number	Blood culture		Sensitivity	Specificity	NPV	PPV	Accuracy	P value
		Positive	Negative						
<25000	47	25	22	76.92	46.81	28.57	88.00	53.33	0.124
>25000	13	10	3						
Total	60	35	25						

Table 2: Comparing CRP with blood culture positive neonates.

CRP	Number	Blood culture		Sensitivity	Specificity	NPV	PPV	Accuracy	P value
		Positive	Negative						
<6.0	13	1	12	87.14	48.00	92.31	72.34	76.67	0.001
>6.0	47	34	13						
Total	60	35	25						

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	20	0	20	100.00	80.00	100.00	87.50	91.67	0.001
>5.0	40	35	5						
Total	60	35	25						

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	13	10	3	90.91	80.00	92.31	76.92	84.62	0.001
Negative	13	1	12						
Total	26	11	15						

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	11	10	1	100.00	66.67	100.00	90.91	92.31	0.001
Negative	2	0	2						
Total	13	10	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	38	34	4	100.00	73.33	100.00	89.47	91.84	0.012
Negative	11	0	11						
Total	49	34	15						

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	11	10	1	100.00	91.67	100.00	90.91	95.45	0.001
Negative	11	0	11						
Total	22	10	12						

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 80% (Table 4). While sensitivity approached to 100% when TLC with GA polymorphs and CRP with GA polymorphs were combined with significant p values of 0.001 and 0.012 respectively (Tables 5 and 6). When all the three parameters were combined together, both the sensitivity and specificity increased to 100% and 91.67% 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*.

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.[14] 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.[7,15] 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.[12,16,17] In the present study the blood culture was positive in only 58.22% 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 87.14% and specificity of a (82.14%). 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.[12,17] 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.[17] 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.[2]

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).[18] 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.[19] 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.[20-23] Similarly in our study CRP was raised in 47 (78.33%) 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.[24] 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%.[11,25] 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.[26] 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.[27]

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 only percent in our stydy. Similar study done by Shah et al revealed 59-82% blood culture positivity in neonatal sepsis.[28,29]

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.[30]

The present study revealed that simple biochemical tests like CRP and GA polymorphs can help in diagnosing early onset neonatal sepsis.

Limitations

The limitation of the study was that they had a small study group. So there were few neonates with positive blood culture.

CONCLUSION

Sepsis screen in neonates is required for detection of infection as blood culture may be negative and even positive result takes few hours. TLC showed intermediate sensitivity and less specificity with insiginficant correlation with the blood culture sensitivity. 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. When all the three parameters were combined together, both the sensitivity and specificity increased to 100% and 91.67% respectively with p values of 0.001.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

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Cite this article as: Kaur S, Singh KP. Role of C-reactive protein and gastric aspirate polymorphs in early onset neonatal sepsis. Int J Contemp Pediatr 2021;8:987-92.