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Original Research Article

Evaluation of the Role of CRP and Gastric Aspirate Polymorphs in Early Onset Neonatal Sepsis: An Observational Study

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Abstract

Aim: The aim of the present study was 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 Upgraded Department of Pediatrics, 100 babies who had clinical symptoms and signs of suspected neonatal sepsis/high risk factors for developing the sepsis, were included in the study.

Results: Only 25 patients had TLC more than 25000 /dl. Maximum TLC value in the study was 42500 /dl. 70 patients showed positive CRP values. 65 patients had polymorphs in the GA more than 5 per high power field. By combination of any CRP and TLC specificity increased to 82%. 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 0.015 respectively. When all the three parameters were combined together, both the sensitivity and specificity increased to 100% and 92.68% respectively with p values of 0.001.

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.

Introduction

Sepsis, an important cause of morbidity and mortality among newborn infants, is responsible for about 30–50% of the total neonatal deaths in developing countries. [1] It is estimated that 20% of all neonates develop sepsis and approximately 1% die of sepsis related causes. [2] However the incidence varies with the geographical area, the socio-economic status and various practices in the perinatal period. [3] Sepsis is defined as life-threatening organ dysfunction caused by a dysregulated host response to infection. [4] Neonatal sepsis is diagnosed when generalized systemic features of sepsis are associated with growth of bacteria from one or more sites. [5] Sepsis related morbidity, mortality is largely preventable with rational antimicrobial therapy and aggressive supportive care.

To diagnose septicaemia various investigations are performed such as complete blood count, C-reactive protein (CRP), gastric aspirate for polymorphs, blood culture ,immature to total neutrophil (I:T) ratio, urine culture, lumbar puncture, micro erythrocyte sedimentation rate (ESR) and high vaginal swab. Blood culture is the gold standard for diagnosis of septicaemia and should be performed in all cases of suspected sepsis prior to starting antibiotics. A positive blood culture is the best guide to antimicrobial therapy. [6] Examination of gastric aspirate has been used in the newborn for diagnosis of neonatal infection. The presence of more than five polymorphs per high power field is correlated with increased risk of neonatal infection. Gastric fluid sample represent amniotic fluid protected from vaginal contamination. Gastric aspirate polymorphs have

been assumed to represent intra amniotic fetal response to inflammation. [7]

Acute phase proteins are produced principally by the liver as part of an immediate inflammatory response to infection or tissue injury. The most extensively used and investigated acute phase reactant is CRP. CRP is more sensitive as well as specific than TNC and I/T ratio. Moreover serial measurements at 1st day and 2nd day significantly improve its sensitivity (82% and 84%, respectively). [8] It also depicts high predictive values for diagnosis of neonatal sepsis as compared with CBC. [9] During first 72 hours of life, CRP, leucopenia and neutropenia were comparably good tests; however after that CRP was single best test in early diagnosis of neonatal sepsis. [10]

The aim of the present study was to evaluate the role of C-reactive protein and gastric aspirate polymorphs in early onset neonatal sepsis.

Materials and Methods

This was a prospective observational study conducted in the Upgraded Department of Pediatrics, Patna Medical College and Hospital, Patna, Bihar, India from Jan 2016 to December 2016. 100 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 out born 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, obstetrics 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

Table 1: Comparing TLC with blood culture positive neonates

TLC	N	Blood culture		Sensitivity	Specificity	NPV	PPV	Accuracy	P value
		Positive	Negative						
<25000	75	40	35						
>25000	25	18	7	78.82	48.82	28.52	88.00	53.37	0.135
Total	100	58	42						

Only 25 patients had TLC more than 25000 /dl. Maximum TLC value in the study was 42500 /dl.

Table 2: Comparing CRP with blood culture positive neonates

CRP	N	Blood culture		Sensitivity	Specificity	NPV	PPV	Accuracy	P value
		Positive	Negative						
<6.0	30	5	25						
>6.0	70	50	20	89.11	48.00	92.28	72.38	74.66	0.001
Total	100	55	45						

70 patients showed positive CRP values.

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

GA	N	Blood culture		Sensitivity	Specificity	NPV	PPV	Accuracy	P value
		Positive	Negative						
<5.0	35	0	35						
>5.0	65	55	10	100.00	82.00	100.0	89.51	92.68	0.001
Total	100	55	45						

65 patients had polymorphs in the GA more than 5 per high power field.

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

TLC+CRP	N	Blood culture		Sensitivity	Specificity	NPV	PPV	Accuracy	P value
		Positive	Negative						
Positive	25	19	6						
Negative	25	4	21	91.92	82.00	93.34	75.95	83.67	0.001
Total	50	23	27						

By combination of CRP and TLC specificity increased to 82%.

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

TLC+GA	N	Blood culture		Sensitivity	Specificity	NPV	PPV	Accuracy	P value
		Positive	Negative						
Positive	15	13	2						
Negative	5	0	5	100.00	65.69	100.0	90.91	93.34	0.001
Total	20	13	7						

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

CRP+GA	N	Blood culture		Sensitivity	Specificity	NPV	PPV	Accuracy	P value
		Positive	Negative						
Positive	45	38	7						
Negative	15	0	15	100.00	74.36	100.00	88.42	92.85	0.015
Total	60	38	22						

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 0.015 respectively (Tables 5 and 6).

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

TLC+CRP +GA	N	Blood culture		Sensitivity	Specificity	NPV	PPV	Accuracy	P value
		Positive	Negative						
Positive	15	12	3						
Negative	15	0	15	100.00	92.68	100.0	90.91	96.44	0.001
Total	30	12	18						

When all the three parameters were combined together, both the sensitivity and specificity increased to 100% and 92.68% respectively with p-values of 0.001.

Discussion

Neonatal sepsis is an inflammatory response to bacteremia occurring during the first month of life and it remains a big problem in developing countries. [11] Septicaemia in newborns is a systemic inflammatory reaction to local infection that may lead to the development of more serious conditions. [12] Sepsis often presents a diagnostic challenge in the resource poor setting of most developing countries. Successful treatment depends on early initiation of antibiotics, but early diagnosis of neonatal infections is difficult because clinical signs are non-specific and may initially be subtle. Respiratory distress, apneic spells, episodes of bradycardia, feeding intolerance, lethargy, and the clinical signs of early onset sepsis are usually apparent in the first hours of life; 90% infants are symptomatic by 24 hours of age. Respiratory distress is the most common presenting symptom.

Temperature instability, as well as minor changes on physical examination or in clinical status is some of the conditions that suggest a possible neonatal infection and needs sepsis evaluation. [13]

Only 25 patients had TLC more than 25000 /dl. Maximum TLC value in the study was 42500 /dl. 70 patients showed positive CRP values. 65 patients had polymorphs in the GA more than 5 per high power field. By combination of any CRP and TLC specificity increased to 82%. 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 0.015 respectively. When all the three parameters were combined together, both the sensitivity and specificity increased to 100% and 92.68% respectively with p values of 0.001. Studies have shown presence of polymorphs in GA to represent a fetal intra-amniotic inflammatory response. [14,15] 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. [16] In the present study, we evaluated the utility of GA cytology as a screening tool for neonatal sepsis.

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. 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. [18] 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.

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. [19] 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%. [16,20] GA polymorphs also showed high sensitivity and specificity in the present study and also with the combination of GA polymorphs and CRP. 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). [21] Combination of various parameters in our study showed high sensitivity and specificity.

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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