ORIGINAL ARTICLE

EARLY DIAGNOSIS OF NEONATAL SEPSIS THROUGH HAMETOLOGICAL AND BIOCHEMICAL MARKERS

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ABSTRACT

Background: Neonatal sepsis is a common occurrence in our part of the world characterized by signs and symptoms of bacterial infection during first 28 days of life. This study was carried out to evaluate the hematological parameters and C-reactive protein estimation in combination for early diagnosis in patients with neonatal sepsis.

Material & Methods: This cross sectional study was conducted at the Pediatrics Department, District Headquarters Hospital, Dera Ismail Khan, from October 2008 to April 2009. Seventy-five neonates having clinical features of sepsis and 35 clinically normal (asymptomatic) neonates were evaluated with a set of investigations. Total leukocyte count (TLC) absolute neutrophil count (ANC) platelet count (PLT) and C-reactive protein (CRP) estimation were used for diagnosis of neonatal sepsis.

Results: TLC had sensitivity of 75% for group A (proven sepsis) and 76% for group B (probable sepsis); and had a negative predictive value (NPV) of 80% and 65% respectively. The sensitivity of ANC was 65% and 76% in group A and B respectively. For proven sepsis, the sensitivity of CRP was 75% and 76% for probable sepsis. The sensitivity and NPV for the combination of TLC, ANC and CRP were 100% each in group A and 79% and 80% in group B.

Conclusion:: The combination of TLC, ANC and CRP is more sensitive in detection of culture positive than culture negative cases of neonatal sepsis.

KEY WORDS: Neonatal sepsis; Culture; TLC; CRP.

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INTRODUCTION

Sepsis is defined as systemic inflammatory response of the body to an infection. When it occurs in first 28 days of life, it is known as "Neonatal Sepsis" (NS).[1] Evaluation of a neonate for possible infection is a challenging clinical problem in newborn nurseries.[2]

Annually five million neonates die in Asia and Africa, out of which 1.6 million (20%) are due to neonatal sepsis.[3] The incidence of neonatal sepsis in developed countries is 1-5 per 1,000 live births. In Pakistan, three times higher incidence has been reported. Neonatal sepsis has non-specific symptoms and signs and a delay in diagnosis and initiation of treatment results in high morbidity and mortality rate.[4]

The causes of NS include, intrauterine infection,[5] ascending bacterial infection, and postnatal infection.[5-7] The patient presents with respiratory distress or grunting, lethargy or irritability, fever or hypothermia, hypo or hyperglycemia, acidosis, hypotonia, vomiting, poor feeding, apnoea, cyanotic spells, seizures, persistent pulmonary hypertension, poor perfusion or shock, petechiae or purpura, unexplained jaundice, or a very sick-look.[8]

Many attempts have been made to develop a set of screening tests, which can rapidly diagnose infected neonates, thus, preventing delay.[9] The diagnosis based on culture of blood, cerebrospinal fluid or urine is established after delay of 24 hours. However, many patients with bacterial infection have negative blood cultures.[10] It has been suggested that a combination of hematological tests (total leucocyte count (TLC), absolute neutrophil count (ANC), immature to total neutrophil ratio(I/T ratio), platelet count and C-reactive protein (CRP) estimation provide early diagnosis of bacteremia.[11]

This study was carried out to evaluate the hematological parameters TLC, ANC and CRP estimation in combination for the early diagnosis in patients with neonatal sepsis.

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MATERIAL AND METHODS

This cross-sectional study was carried out in Nursery Section at Pediatrics Department, District Headquarters Teaching Hospital, Dera Ismail Khan, from October 2008 to April 2009.

Seventy-five patients suspected of having sepsis on the basis of history and clinical examination were included in the study after having informed consent from parents. Thirty-five clinically normal subjects from postnatal ward were included to define the normal ranges of hematological parameters in our population. Patients with history of antibiotics use within 48 hours of admission and those with history of exposure to viral and fungal infections in perinatal period were excluded from the study. Neonates with culture positive for coagulase negative Staphylococcus, Corynebacterium diphtheriae, and Baillus (contaminants) were also excluded. A written consent of the parents of all study groups was obtained for inclusion in the study.

In each case 5ml of blood sample was collected once only by venipuncture out of which 1.5ml was used for culture and 3.5ml for estimation of TLC, ANC, Platelet count, peripheral smear and CRP. As blood culture was not required for control group, 3.5ml of blood sample was collected only. Blood culture was observed for growth after 48 hours,then sub-cultured for next 72 hours and read after five to seven days.

For description of results the neonates were divided into three groups: Group A: Confirmed cases of neonatal sepsis with positive blood culture. Group B: Symptomatic neonates having clinical diagnosis of sepsis but negative blood culture. Group C: Normal asymptomatic neonates.

Automated hematology analyzer Sysmex KX-21 (Nihon Kohden, Tokyo) was used to study full blood count. CRP was measured by latex agglutination kit (SPAN Diagnostic Ltd, Gujrat).[12]

The data obtained was analyzed using SPSS version 13.0.Qualitative variables were presented in the form of frequencies and percentages. The sensitivity, specificity, positive and negative predictive values were determined for each quantitative variable individually and in combination.

RESULTS

In the present study, 75 (33 boys and 42 girls) neonateswere admitted with clinical features of neonatal sepsis and 35 (22 boys and 13 girls) normal neonates from postnatal ward were investigated. The common clinical features of sepsis were poor feeding in 60(80%), fever 54 (72%), inactivity 53 (70%), vomiting 50 (66%), diarrhea 34 (45%) and respiratory distress 25 (33%).

Blood culture was positive out of 75 diseased subjects in 11 (15%) cases. Gram-negative organisms contributed 72% of the total number of neonates with proven sepsis. Klebsiell apneumoniae was the commonest organism (58%) followed by E. coli (10%) and Pseudomonas aeruginosa (4%). Among gram-positive coagulase positive Staphlococcus aureus accounted for 28%. No other microorganism was isolated.

TLC showed sensitivity of 75% for blood culture positive cases of NS (group A) (Table 1) and 76% for

Table 1: Sensitivity, specificity and predictive values of tests in proven sepsis (Group A) at defined cut-off values.

Tests	Sensitivity	Specificity	Positive predictive values	Negative predictive values
TLC <5000 or >20,000/mm3	75%	57%	50%	80%
ANC <1750mm3	65%	78%	58%	80.5%
CRP >5 mg/dl	75%	85%	72%	85.8%
TLC+ANC+CRP	100%	84%	62.3%	100%

Table 2: Sensitivity, specificity and predictive values of tests in probable sepsis (Group B) at defined cut-off values.

Tests	Sensitivity	Specificity	Positive predictive values	Negative predictive values
TLC <5000 or >20,000/ mm3	76%	82%	62%	65%
ANC <1750mm3	76%	78%	70.4%	83%
CRP >5 mg/dl	76%	89%	87%	71%
TLC+ANC+CRP	79%	82%	78%	80%

Table 3: Haematological parameters and C- reactive protein in normal neonates (n=35).

Parameters	Normal Range
Total leucocyte count	5,000- 20,000 /mm3
Absolute neutrophil count	1750-6000 /mm3
C reactive protein	< 5mg /dl

blood culture negative cases (group B). (Table 2) Its specificity was 57% in group A and 82% in group B respectively. The positive predictive value was 50% and 62%, whereas its negative predictive value was 80% and 65% for group A and B respectively.

ANC had sensitivity of 65% for group A and 76% for group B. Its negative predictive value was 80.5% for culture proven sepsis and 83% for culture negative cases of NS.

CRP had sensitivity of 75% in proven sepsis and 76% in probable sepsis. The negative predictive value of CRP was 85.5% and 71% in group A and B respectively.

The sensitivity, specificity and predictive values of three tests in combination, TLC, ANC and CRP were also calculated for both groups. This combination had sensitivity of 100% for group A and 79% for group B. Its negative predictive value was 100% and 80% for group A and B respectively. (Table 1)

DISCUSSION

The evaluation of screening tests for neonatal sepsis is essential because the infection may present a very serious threat to the baby. Confirmation of diagnosis by blood culture may take time, and diagnostic tests are used to obtain a rapid indication of the infection status.[13]

A screening test would detect all the patients with the disease and exclude all the cases, which do not have that specific disease. That means, a screening test should have high sensitivity and high negative predictive value. However, a low specificity and positive predictive value are acceptable, because the risk of missing a patient with a certain infection is greater than the risk of over treatment with antibiotics.[14]

The present study has re-evaluated the markers of sepsis in neonates. No doubt, blood culture is gold standard for the diagnosis and treatment of neonatal sepsis, but there are some setbacks. It can be falsely negative, because of intermittent or low-density bacteremia, suppression of bacterial growth by intrapartum antibiotic administration,[15] improper inoculation and incubation of blood culture. Statistaical analysis would be simplified if culture negative neonates were excluded from the study but we cannot ignore these cases because fatal infection has been reported in presence of negative blood culture.[22] In present study, 15% cases had positive blood cultures. Similarlly, Manuchaet al[16] documented 14% blood culture positive cases of NS. Multiple studies have documented blood culture positivity in 20%, 42% and 17% of cases. None of the reports exceed 42%. Thus it can be understood that more than half the cases of neonatal sepsis are missed if only blood culture is made the basis of diagnosis.[17,18]

In the present study, when TLC, ANC and CRP were included as the screening tests, it showed the following data.

TLC was in the abnormal range (either too low / too high) in 88% diseased cases. The breakdown of values is shown in table 1. The cut off values of TLC for positive test were taken either $<5000/mm^3$ or $>20,000/mm^3$ in the present study as suggested by Ahmed et al.[4]

The current study showed high sensitivity and high negative predictive value for TLC in both group A (75% and 80%) and group B (76% and65%). It was similar to another study conducted by Berger et al[20] who reported that leuocopenia ($TLC \leq 5,000/mm^3$) and leucocytosis ($TLC \leq 20,000/mm^3$) had high sensitivity i.e. 67% and 74% respectively for the detection of NS.TLC was found to have optimal sensitivity 86% and NPV 96% in a study carried out at Delhi Hospital.[16] Contrary to this, Ahmed et al[4] reported low sensitivity of TLC 39.3% and 27.8% for blood culture proven sepsis group and culture negative but clinically diagnosed sepsis group.

The normal range of absolute neutrophil count had been suggested by Schelonkaet al[20] who studied larger number of healthy neonates and found ANC range to be $9500/mm^3$ to $21,500/mm^3$. In our study the normal range of ANC was found to be 1750 to $6000/mm^3$ and the cut off value for positive test was taken <1750/mm3 which were similar to that of Gerdes et al.[21] The present study revealed the sensitivity for ANC 65% in blood culture positive case and 76% in blood culture negative cases of NS. Ahmed et al[4] also reported moderately high sensitivity for the same parameters.

Manuchaet al[16] documented optimal sensitivity and NPV of ANC for the diagnosis of NS. Anwer et al[22] found ANC to be more sensitivity than TLC as indicator of bacterial sepsis in neonates. The sensitivity was 62% in proven sepsis and 48% in culture negative cases.

There are some factors, which contribute to discrepancy in sensitivity of WBC indices. TLC can be higher in capillary than in arterial or venous blood. To avoid this discrepancy all sampling was carried out from vein. Timing is also important as WBC indices in the septic infant may be normal at the time of initial evaluation, but abnormal 4 to 12 hours later.[22,23]

C-reactive protein (CRP) is a non-specific acute phase protein, released in response to sepsis. Good evidence exists to support the use of CRP measurement in conjunction with other diagnostic tests such as TLC, ANC, platelet count and blood culture to establish or exclude the diagnosis of sepsis in full term or near term infants.[26] Benitz and colleagues found that if CRP was >5.0 mg/dl, it was highly suggestive of NS.[27]

The sensitivity, specificity, positive and negative predictive values of CRP for blood culture positive sepsis, a cut off point >5mg/dl were 100%, 94%, 91.6% and 100% respectively.

In NS, the range of reported statistical outcomes is as follows, sensitivity 70-93%, specificity 41-98%, positive predictive value 6-83% and negative predictive value 97-99%.[28,29]

In this study, sensitivity and NPV of CRP were 75% and 85.8% in blood culture positive cases (group A). In blood culture negative cases of NS (group B), these were 76% and 71% respectively. Likewise, Ahmed et al[4]reported 85.7% sensitivity and 95.9% NPV for CRP in group A, whereas, 80.5%sensitivity and 87.1% NPV for CRP in group B. Similarly, Arshad et al[27] documented 86.7% and 80.6% sensitivity of CRP for blood culture positive and negative cases of NS respectively.

In the present study, we also analyzed the combination of TLC, ANC and CRP and found that the sensitivity and NPV were improved to 80-100% each. This was very significantly high sensitivity and NPV as compared to those of the individual parameters. Therefore, we report that none of the tests used alone were as reliable, as when, employed in combination to diagnose NS. Individual tests with low sensitivity and low NPV were not considered for combination tests because of their poor value as screening tests. Except for cytoplasmic vacuolization, I/T ratio and toxic granulation in neutrophils were found to be poor indicators of sepsis. The results obtained were almost similar to those of a study by Ahmad et al.[4]

The tests used, were readily available, reliable and in combination found to be highly sensitive for the detection of NS. It is recommended that when the screening tests show a high NPV, the neonate could be discharged immediately from the hospital, after stopping the antibiotics, therapy reducing the hospital stay, potential exposure of neonate to hospital acquired infections, unnecessary antibiotic exposure and anxiety of the family.

It is suggested that the combination of TLC, ANC and CRP with the new modality like Interleukein-6 (IL-6) be further analyzed to improve the sensitivity and NPV to even higher values for blood culture negative cases of NS.

CONCLUSION

The combination of TLC, ANC and CRP is more sensitive in detection of culture positive than culture negative cases of neonatal sepsis.

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CONFLICT OF INTEREST
Authors declare no conflict of interest.
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None declared.