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Original Article

Use of hematological parameters as an early screening tool for neonatal sepsis

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ABSTRACT

Background: Neonatal sepsis continues to be a major public health problem in developing countries. Due to the nonspecific clinical manifestations of neonatal sepsis, the longer turnaround time for culture reports, an early diagnostic tool is essential for the initiation of appropriate and timely treatment.

Materials and Methods: This is a cross-sectional study of 470 clinically suspected cases of neonatal sepsis admitted in the pediatrics department of B.P. Koirala Institute of Health Sciences, Dharan, Nepal. Following hematological parameters: abnormal total leucocyte count, abnormal total polymorphonuclear count, elevated immature Neutrophils count, elevated immature to total neutrophil ratio, immature to mature neutrophils ratio ≥ 0.3, platelet count ≤ 150,000/mm3, and pronounced degenerative changes in Neutrophils were assessed and a score of 1 was assigned to each as per the hematological scoring system of Rodwell. A score ≥ 3 was considered positive and a score < 3 was considered negative for the diagnosis of sepsis. Blood culture was taken as a gold standard for diagnosis of neonatal sepsis.

Results: Hematological scoring system had fairly high sensitivity, specificity, NPV, and PPV; i.e. 85.96%, 84.26%, 84.52%, and 85.71% respectively. The diagnostic accuracy of HSS was high (85.1%) in comparison to the maximum diagnostic accuracy of individual hematological parameters, i.e. 74.46% for the total neutrophil count.

Conclusions: Hematological scoring system can be used as a convenient and early diagnostic tool against neonatal sepsis in developing countries with a high burden of neonatal sepsis because of its high sensitivity and easy availability.

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INTRODUCTION

Sepsis refers to the presence of physiological, pathological, and biochemical abnormalities occurring due to dysregulation of the mechanism of the host response to infection. It is a life-threatening situation and can lead to death if treatment is not initiated promptly.[1] An accepted definition for neonatal sepsis however is lacking, especially in Low and Middle-Income Countries (LMIC) due to resource-poor settings for laboratory evaluations. It is thus recommended that in cases with clinical signs of Possible Serious Bacterial Infection (PSBI), prompt initiation of antibiotics should be done so as to reduce the number of missed cases of sepsis.[2] On the other hand, neonatal sepsis

mostly presents with nonspecific clinical manifestations.[3] Neonatal sepsis is the most common diagnosis in the Neonatal Intensive Care Unit (NICU) and antibiotics are the most commonly used drugs in NICU. If antibiotic treatment is withheld until overt clinical manifestations occur, the outcomes are dismal. However, liberal use of antibiotics can lead to several adverse outcomes including antibiotic resistance. Three out of every 10 cases of deaths due to neonatal sepsis are now caused by resistant pathogens.[4] The development of an early and simple diagnostic tool would help establish a balance between deaths due to neonatal sepsis and emerging antimicrobial resistance.

Rodwell et al in 1988 utilized the change in a set of simple hematological parameters to establish a hematological scoring system (HSS).[5] Though several studies have been conducted to test the usefulness of HSS in neonatal sepsis, this scoring system is not yet used very commonly in routine practice. This study was undertaken to establish the usefulness of HSS in neonatal sepsis in the context of a tertiary care center in Nepal.

MATERIALS AND METHODS

This is a hospital-based cross-sectional study of clinically suspected cases of neonatal sepsis admitted in the pediatrics department of B.P. Koirala Institute of Health Sciences (BPKIHS) from December 2016 to November 2017. Ethical clearance was obtained from Institutional Ethical Review Board, BPKIHS. After taking all aseptic precautions, 2 ml of blood was withdrawn. One ml of sample was sent for blood culture and sensitivity and the other one ml of the sample was anticoagulated with EDTA for hematological study. Throughout the study period, a total of 235 cases showed positive blood culture reports, and an equal number of culture-negative cases were thus randomly taken as controls, thereby making the total sample size 470. For the hematological Study, values of total leucocyte count (TLC) and platelets count were noted using Lab Life D5 Supreme automated hematology analyzer and counter-checked manually. Peripheral blood smears were made and stained with Jenner- Giemsa stain. Differential leucocyte counts (DLC), absolute neutrophil count (ANC), immature neutrophil count (I) (including band form), mature neutrophil count (M) were then performed on stained smears. IT (immature to total neutrophil) ratio and IM (immature to mature neutrophil) ratio were calculated. IT ratio is calculated by dividing the total immature count by total neutrophil count (including both mature and immature neutrophil count). Degenerative changes including Dohle bodies, vacuolization, and toxic granulations were graded as shown in Table 1 and Table 2 respectively.[6] The hematological scoring system of Rodwell et al was used to calculate the score of each blood sample. (Table 3)

Table 1: Scoring for Dohle Bodies or Vacuolization

Score	Neutrophils involved (%)
0	None
1+	<25%
2+	25% to 50%
3+	51% to 75%
4+	>75%

Table 2: Scoring for Toxic Granulations

Score	Interpretation
0	Normal granulation
1+	Slight
2+	Dark granules in approximately 50% neutrophils
3+	Very heavy granulations in most cells
4+	'Gross' toxic granules causing obscuring of the nucleus

Table 3: Hematological Scoring System (HSS) [6]

Points	Abnormality	Score
I: T ratio (>2)	↑	1
Total PMN count *	↓ or ↑	1
I:M ratio	≥ 0.3	1
Immature PMN Count †	↑	1
Total WBC count*	↓ or ↑	1
Degenerative changes in PMN	≥3+	1
Platelet count	≤ 1,50,000/mm3	1

(≤ 5,000/mm3 or ≥ 25,000; 30,000 and 21,000/mm3 at birth, 12-24 hrs and day 2 onward respectively)*

RESULTS

Of the 470 neonates involved in this study, 253 were male and 217 were female with a male: female ratio of 1.16: 1. A history of preterm delivery was present in a total of 246 neonates. Out of these, 141 belonged to the culture-positive category and 105 belonged to the culture-negative category. Total 240 neonates had low birth weight (i.e.<2.5kg), of which 190 belonged to the culture positive and 50 belonged to the culture-negative group. Suspected cases of sepsis were mostly admitted with clinical manifestations of fever, poor feeding, lethargy, irritable cry, difficulty in breathing, diarrhea, convulsion, abdominal distension, hypothermia, or persistent vomiting. Fever and poor feeding were present in a majority of cases, i.e. 60%.

Hematological parameters were assessed in both culture-positive and culture-negative groups. The values that were beyond the normal range and thereby contributed to a score of 1 were taken as 'positive' while those lying within the normal range with zero contribution to the score were taken as 'negative'. Findings are summarized in Table 4. Hematological scores for each sample were then calculated using these hematological parameters. (Table 5)

Also, sensitivity, specificity, PPV and NPV, and accuracy of individual hematological parameters were calculated.

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Table 4: Individual hematological parameters in culture-positive and negative groups

Hematological parameter	Cases with positive hematological parameter in Culture positive group (n)	Cases with positive hematological parameter in Culture negative group (n)	Total cases with positive hematological parameters (n)
TLC	90	22	112
Total PMN	189	74	263
Immature PMN	200	116	316
I:T	50	3	53
I:M	58	3	61
Degenerative Changes	146	73	219
Platelet Counts	162	143	305

Table 5: Comparison of hematological scoring system with culture results

Scores	0	1	2	3	4	5	Total
Culture positive	0	10	37	23	105	60	235
Culture negative	3	69	133	18	10	2	235
Total	3	79	170	41	115	62	470

Table 6: Strength of individual hematological parameters

Hematologic parameters	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)	P value
Total WBC	38.3	90.6	80.36	59.50	64.46	<0.01
Total PMN	80.43	68.5	71.8	77.78	74.46	<0.01
Immature PMN	85.11	50.64	63.29	72.27	67.87	<0.01
I:T	21.28	98.72	94.34	55.64	60	<0.01
I:M	24.68	98.72	95.08	56.72	61.7	<0.01
Degenerative changes	62.13	91.73	66.67	90.1	65.5	<0.01
Platelet count	68.94	39.15	55.76	53.11	54.04	0.06

Table 7: Strength of HSS using Score ≥ 3 as cut off

Score	Culture negative	Culture Positive
<3	198	33
≥3	37	202
Total	235	235

Sensitivity= 85.96% *Specificity= 84.26%*
Positive predictive value= 84.52% *Negative predictive value= 85.71%*
Accuracy= 85.10% *P value < 0.01*

(Table 6) The Pearson Chi-square test was applied to calculate the p-value.

The results thus highlight the fact when all the hematological parameters are used in combination as in Rodwell's hematological scoring system, the diagnostic accuracy is quite high (85%) in comparison to the diagnostic accuracy of individual hematological parameters where the highest accuracy (74%) was of total PMN. HSS can also be used as a useful screening tool against sepsis because of its high sensitivity (86%) when HSS score ≥3 is used as a cut-off for the presence of sepsis. (Table 7)

DISCUSSION

Neonatal sepsis continues to be a major public health problem. Each year, around 3 million newborns suffer from sepsis worldwide[7] leading to around one million deaths per year, the majority of these occurring in developing countries.[8] Even in developed countries, four out of every ten infants with sepsis either die or experience a major disability such as permanent impairment of neurological development.[9]

In developing countries, infants are more likely to be exposed to several risk factors related to neonatal sepsis. Different studies have shown that factors like prolonged rupture of membrane (PROM), prematurity[3], low Apgar score at birth[10], and LBW[11] are linked with the incidence of sepsis. In our study, a history of preterm delivery was present in 246 (52%) of total neonates. Out of these, 141 belonged to the culture-positive category and 105 belonged to the culture-negative category. Total 240 neonates had a low birth weight of which 190 belonged to the culture positive and 50 belonged to the culture-negative group. Preterm and low birth weight babies have lower levels of immunoglobulin and less developed immune systems thereby being more susceptible to sepsis with a higher risk of mortality and lifelong neurodevelopmental handicap.[12] However, the accuracy of the pediatric consensus definitions has not yet been assessed in preterm infants, and using these criteria is untenable because of several limitations related to

the developmental maturity of preterm infants.[12] In a study by Adatara et al, no relation was seen between neonatal sepsis and gestational age, birth weight.[10]

The incidence of sepsis was higher (53.82%) in male neonates than in female neonates (46.1%) in our study. This finding is consistent with that of other studies.[13-16] In this study, neonates mostly presented with chief complaints of poor feeding (60%) and fever (60%). Studies have suggested that the clinical presentation of neonatal sepsis is quite nonspecific[3,16] and thus clinicians need to be more alert while handling these cases.

Blood culture is the gold standard test for the diagnosis of neonatal sepsis.[17] Factors like the small blood volumes obtained from neonates, maternal intrapartum antimicrobial exposure, and the presence of low or intermittent bacteremia can yield false-negative culture reports.[17] With the increased understanding of pathogenesis and immune mechanisms involved in neonatal sepsis, several newer inflammatory markers such as interleukin-6, interleukin-8, plasma elastase, neutrophil CD64 have been known to be useful in the diagnosis of sepsis. These markers are highly sensitive and specific, but they require expensive and sophisticated technologies, not easily affordable by developing countries.[18,19] On the other hand, in middle-income countries, the incidence rate of neonatal sepsis is nearly 40 times higher than in high-income countries and the mortality rate is two times.[7]

HSS utilizes parameters that can be assessed quickly and used as an early diagnostic tool for neonatal sepsis especially in developing countries where the burden of neonatal sepsis is higher and sophisticated labs are lacking at many places. Due to the insensitivity of physical examination and culture, even the modern diagnostic approaches for neonatal sepsis have chosen to use many of the parameters that are a part of HSS in combination with the acute phase reactants[17]. The different parameters used in the HSS have individual strengths and weaknesses [13,14,19-22]. The HSS uses them in combination to improve the accuracy of diagnosis of neonatal sepsis. Studies conducted on HSS to date have come to different conclusions regarding the strength of individual parameters used in the HSS. Most of these studies showed that these parameters are more reliable in the detection of sepsis when used in combination as HSS rather than individually.[13,14,23,24] A comparison between the strength of HSS as calculated by different studies has been shown in Table 8.

Apart from these, few other studies have also analyzed the strength of individual parameters used in HSS. Abnormal immature to total neutrophil ratio followed by an abnormal immature to mature neutrophil ratio was found to be the most sensitive indicators for the diagnosis of neonatal sepsis in the studies conducted by Ghosh et al, Narasimha et al and Majumdar et al.[28-30] In the study by Priyanka et al, absolute neutrophil count and the increased immature neutrophil count had the highest specificity of 91% and 92% respectively.[31]

The HSS may also be used with newer diagnostic modalities when possible to further increase the accuracy. In a study by Khair et al in 2012, HSS score ≥4 had a sensitivity of 100% and specificity of 60%. C-reactive protein (CRP) had a sensitivity of 75% and specificity of 74%. The combination of score ≥4 and CRP had a sensitivity of 75%, specificity 85%, positive predictive value (PPV) 41%, and negative predictive value (NPV) 96%. This combination was thus useful to differentiate the septicemic from nonsepticemic neonates and thereby help in the judicious use of antibiotics.[32]

In our study when the hematological score ≥ 3 was used as a positive cut-off for the diagnosis of neonatal sepsis, diagnostic accuracy was high. Saleem et al and Yusuf et al have also used this cut-off in their studies and thus found the sensitivity of HSS to be 71% and 90% respectively while the specificity was 72% and 74.5% respectively.[14,21] Whereas some other studies have used score ≥ 4 as their diagnostic cut off thereby leading to a maximum sensitivity of 100% and specificity of 86%.[23] Majumdar et al have concluded in their study that though the score ≥3 is highly sensitive, score ≥4 has a higher specificity and PPV and hence can be considered as the more reliable screening tool for the diagnosis of sepsis [30]. Studies have found that the certainty of sepsis being present increases with the increase in the score.[26,28]

An ideal screening test should have high sensitivity and negative predictive value that would enable it to detect all the patients with the disease and exclude all those cases without it.[26] HSS appears to have fulfilled these criteria in all the studies conducted so far and hence can be taken as an effective screening tool against neonatal sepsis.

CONCLUSIONS

When all the hematological parameters are used in combination as in Rodwell's HSS, the diagnostic accuracy is quite high in comparison to the diagnostic accuracy of individual hematological parameters. Because of its high sensitivity when HSS score ≥3 is used as a cut-off for the presence of sepsis, HSS can be used as a useful screening tool against neonatal sepsis. This test can be quickly assessed even in resource-poor setups and hence suitable for use in low-income countries with a greater burden of neonatal sepsis than their developed counterparts.

Conflict of Interest: None

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