Original Research Article

Clinical study of diagnostic efficacy of nucleated red blood cell count (NRBCc) in the early diagnosis of neonatal sepsis

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Abstract

Background: Early diagnosis of neonatal sepsis is a clinical challenge as clinical symptoms and signs of the disease are subtle, late, and nonspecific making it hard to be discriminated from that of noninfectious causes. Present study was aimed to evaluate diagnostic efficacy of nucleated red blood cell count (NRBCc) and platelet count in the early diagnosis of neonatal sepsis at a tertiary hospital. **Material and Methods:** Present study was a hospital based, single-center, prospective, observational study, conducted in term, live neonates admitted NICU with risk factors of sepsis or clinical features of sepsis. Peripheral Smear was prepared using Leishman stain and is then examined under microscope for the presence of NRBC as well as estimation of platelet count. **Results:** In present study, 92 neonates admitted in NICU in view of neonatal sepsis were considered. The study group was divided into the three groups based on the clinical findings and investigations. Both Groups I (20.65%) and II (36.96%) were included in the sepsis group (57.61 %). In present study majority of neonates were male (54.35%), had term maturity (55.43 %) and birthweight more than 2500 gms (53.26 %). Among various groups we compared nucleated red blood cell count (NRBCc), NRBCc > 30/100 WBCs was statistically significant in proven sepsis group (42.11%) as compared to probable sepsis and no sepsis groups($p<0.05$). We compared relation of thrombocytopenia (platelet < 50,000/mm^3) among various sepsis groups, NRBCc > 30/100 WBCs was statistically significant in proven sepsis group (73.68 %) as compared to probable sepsis and no sepsis groups($p<0.05$). **Conclusion:** Nucleated red blood cell count (NRBCc) and platelet count in the peripheral blood smear helps in the early diagnosis of neonatal sepsis.

Keywords: Nucleated red blood cell count, platelet count, peripheral blood smear, neonatal sepsis, early diagnosis.

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INTRODUCTION

Neonatal sepsis is a clinical manifestation of a systemic infection during the first 28 days of life, usually classified as early-onset (<48–72 h) and late-onset sepsis (>48–72 h), depending on the age at onset of the sepsis episode.[1] The estimates suggests that infections including sepsis, pneumonia, diarrhea, meningitis and tetanus are the most common causes of neonatal death in developing countries. The most common cause of neonatal mortality in the developing countries is neonatal sepsis, the diagnosis of which depends on blood culture, which has low sensitivity and takes time.[3] Early diagnosis of sepsis is a clinical challenge as clinical symptoms and signs of the disease are subtle, late, and nonspecific making it hard to be discriminated from that of noninfectious causes.[4] Nucleated Red Blood Cells (NRBC) which are the precursors of erythrocytes, are released from the bone marrow in response to stress. NRBCs count reflects high production of erythropoietin as a result of decreased arterial oxygen partial tension and/or increased

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concentrations of inflammatory cytokines. Studies have shown, association of an increase in the NRBC count with conditions such as asphyxia, gestational diabetes, and neonatal sepsis.[5] Also, association between platelets and sepsis is well established. The mean platelet volume (MPV), which refers to the average volume of individual platelets, has been considered a marker of platelet size, function, and reactivity.[5] Present study was aimed to evaluate diagnostic efficacy of nucleated red blood cell count (NRBCc) and platelet count in the early diagnosis of neonatal sepsis at a tertiary hospital.

MATERIAL AND METHODS

Present study was a hospital based, single-center, prospective, observational study, conducted in department of pathology, at a medical college and hospital. Present study period was from September 2020 to October 2021 (1 year). Study was approved by institutional ethical committee.

Inclusion criteria: Term, live neonates admitted NICU with risk factors of sepsis or clinical features of sepsis

Exclusion criteria: Maternal pre-eclampsia or eclampsia. Gestational diabetes mellitus. Intrauterine growth retardation. Birth asphyxia. Pre-term and post-term babies. Hemolytic anemia (ABO and Rh incompatibility). Maternal smoking.

Study was explained to parents of neonates considered for study and a written informed consent was taken for participation. Detailed history including maternal details and risk factors for sepsis, clinical examination, and relevant investigations (hematological/urine investigations, microbiological culture study, lumbar puncture, chest X-ray, abdominal X-ray, and other radiological studies whenever indicated) was noted in proforma. Peripheral Smear was prepared using Leishman stain and is then examined under microscope for the presence of NRBC. NRBC count was expressed relative to 100 WBCs. A value of 10 NRBCs/100 WBCs or more is considered elevated. Thrombocytopenia was labelled when platelet count was <1,50,00. Data was collected and compiled using Microsoft Excel, analysed using SPSS 23.0 version. Frequency, percentage, means and standard deviations (SD) was calculated for the continuous variables, while ratios and proportions were calculated for the categorical variables. Difference of proportions between qualitative variables were tested using chi- square test or Fisher exact test as applicable. P value less than 0.5 was considered as statistically significant.

RESULTS

In present study, 92 neonates admitted in NICU in view of neonatal sepsis were considered. The study group was divided into the three groups based on the clinical findings and investigations. Both Groups I (20.65 %) and II (36.96 %) were included in the sepsis group (57.61 %).

Table 1: Group allocation

Group	No. of cases (n=92)	Percentages
Proven sepsis (Group I) – Neonates with positive blood culture.	19	20.65
Probable/clinical sepsis (Group II) – Neonates with strong clinical features, a positive sepsis screen but a negative blood culture.	34	36.96
No sepsis (Group III) – Neonates with negative blood culture and a sepsis screen.	39	42.39

In present study majority of neonates were male (54.35 %), had term maturity (55.43 %) and birthweight more than 2500 gms (53.26 %).

Table 2: General Characteristics

Characteristics	Proven sepsis (Group I) (n=19)	Probable/clinical sepsis (Group II) (n=34)	No sepsis (Group III) (n=39)	Total (n=92)
Gender				
Male	11 (57.89 %)	18 (52.94 %)	21 (53.85 %)	50 (54.35 %)
Female	8 (42.11 %)	16 (47.06 %)	18 (46.15 %)	42 (45.65 %)
Maturity				0
Pre-term	12 (63.16 %)	13 (38.24 %)	16 (41.03 %)	41 (44.57 %)
Term	7 (36.84 %)	21 (61.76 %)	23 (58.97 %)	51 (55.43 %)
Birth weight				0
< 2500 gm	13 (68.42 %)	12 (35.27 %)	18 (46.15 %)	43 (46.74 %)
≥ 2500 gm	6 (31.58 %)	22 (64.71 %)	21 (53.85 %)	49 (53.26 %)

Among various groups we compared nucleated red blood cell count (NRBCc), NRBCc > 30/100 WBCs was statistically significant in proven sepsis group (42.11 %) as compared to probable sepsis and no sepsis groups($p<0.05$).

Table 3: Relation between NRBCc and sepsis group

Sepsis group (NRbc/100wbc)	Proven sepsis (Group I) (n=19)	Probable/clinical sepsis (Group II) (n=34)	No sepsis (Group III) (n=39)	Total (n=92)
<10	2 (10.53 %)	5 (14.71 %)	11 (28.21 %)	18 (19.57 %)
10-19	3 (15.79 %)	11 (32.35 %)	19 (48.72 %)	33 (35.87 %)
20-29	6 (31.58 %)	15 (44.12 %)	6 (15.38 %)	27 (29.35 %)
>30	8 (42.11 %)	3 (8.82 %)	3 (7.69 %)	14 (15.22 %)

We compared relation of thrombocytopenia (platelet < 50,000/mm^3) among various sepsis groups, NRBCc > 30/100 WBCs was statistically significant in proven sepsis group (73.68 %) as compared to probable sepsis and no sepsis groups($p<0.05$).

Table 4: Relation between platelet count and sepsis group

Sepsis group (platelet/mm^3)	Proven sepsis (Group I) (n=19)	Probable/clinical sepsis (Group II) (n=34)	No sepsis (Group III) (n=39)	Total (n=92)
< 50,000	14 (73.68 %)	6 (17.65 %)	3 (7.69 %)	23 (25 %)
50,000 – 1,50,000	3 (15.79 %)	17 (50 %)	12 (30.77 %)	32 (34.78 %)
> 1,50,000	2 (10.53 %)	11 (32.35 %)	24 (61.54 %)	37 (40.22 %)

DISCUSSION

Clinical features of sepsis are nonspecific in neonates and a high index of suspicion is required for early diagnosis. In order to diagnose septicemia early, several rapid diagnostic tests have been described, which are easily performed and have the benefit of quick availability of reports. Mahesh Ahirrao *et al.*,[6] noted that presence of Nucleated Red blood cell count (NRBCs) in peripheral smears of culture positive sepsis noted in 82.14% cases. Sensitivity of NRBCs was 78.57%. Specificity was 89.13%, Positive predictive value was 68.75%, Negative predictive value was 93.18%. NRBCs is a single, quick, cost-effective and readily available, feasible tool with good sensitivity and specificity in the early diagnosis of neonatal sepsis. Abhishek M G[7] studied 60 neonates with clinical suspicious of sepsis at birth and within 72 hours, NRBCs count was higher in all sepsis cases. Sensitivity of NRBc for detecting proven sepsis was 35%, its specificity 53.48%, its positive predictive value was 23.07% and its negative predictive value was 67.64%. Rozeta Sokou *et al.*,[8] studied 467 critically ill neonates, 45 (9.6%) had in-hospital mortality. No statistically significant difference was noted with regards to NRBCs count between survivors and non-survivors, although the median value for NRBCs was sometimes higher for non-survivors. ROC curve analysis showed that NRBCs is a good discriminator marker for the diagnosis of perinatal hypoxia in neonates with area under the curve (AUC) [AUC 0.710; 95% confidence interval (CI), 0.660–0.759] and predominantly in preterm neonates (AUC 0.921 (95% CI, 0.0849–0.0993)) by using a cut-off value of ≥ 11.2%, with 80% sensitivity and 88.7% specificity. NRBCs also revealed significant prognostic power for mortality in septic neonates (AUC 0.760 (95% CI, 0.631–0.888)) and especially in preterms with sepsis (AUC 0.816 (95% CI, 0.681–0.951)), with cut-off value ≥ 1%, resulting in 81.6% sensitivity and 78.1% specificity. The role of NRBCs count in critically ill neonates is confirmed by Morton *et al.*,[9] who reported that among critically ill neonates, NRBCs are associated with significantly elevated mortality risk. Boskabadi H *et al.*,[10] conducted a case-control study on 154 infants (78 infants with infection as the case group and 76 infants without infection as the control group). The mean NRBC counts in the infants with and without infection were 30 and 3 per 100 WBCs, respectively ($P<0.001$). In cases with an NRBC count of more than 10, sensitivity and specificity were reported as 45% and 83%, respectively, and the positive and negative predictive values were 29% and 91%, respectively. The infants' NRBC count was directly correlated with their mortality. Hemalatha AL *et al.*,[11] conducted a case-control study on asphyxiated and non-asphyxiated term neonates admitted in NICU. Nucleated RBCs (NRBCs) on 100 WBCs showed a mean value of 15.74 and standard deviation (SD) of 7.89 in the study group. The control group showed a mean value of 1.55 and SD of 0.78. The P = 0.001 was statistically significant and, therefore, a good predictor for birth asphyxia. Kulandaivel M *et al.*,[12] noted that sensitivity of NRBC in identifying sepsis was 81.5%, its specificity was 61.76%, positive predictive value was 70.4% and negative predictive value was 75%. In the neonates who expired, serial NRBC counts (mean – 22.4) were significantly increased from baseline value (mean 17.3). NRBC is significantly elevated in the neonatal sepsis and is a predictor of adverse neonatal outcome. N. Muthukumaran[13] studied 115 study neonates, 101 survived and 14 expired with a mortality of 12.17%. Of the expired 14 babies, 10 were blood culture positive. Mean NRBC in

the mortality group was17.1 on day 1, while a repeat count on day 3 showed an increase in the number of circulating NRBCs with mean value 23.1. In the group of babies who survived, NRBCs decreased on day 3 and were even undetected in most of them with a mean value of 3.51. Any unexplained normoblastemia is important because it offers invaluable insight into disease processes or progressions that occur in conditions such as systemic infections. Dulay AT *et al.*,[5] demonstrated that cytokines released in sepsis have an important role in stimulating Nucleated RBC (NRBC) production independent of hypoxia. In this study significantly elevated NRBC demonstrated in EOS (no EONS (n=49)) 1330 cells/ cmm (665-2630), EONS (n=19)3020 cells/cmm (1388-4558), p=0.011) along with significantly elevated IL-6 in EONS, but no increase in level of umbilical cortisol or erythropoietin. Normoblasts (younger nucleated RBCs) and immature granulocytes are less deformable and rarely enter the circulation. Their presence in the peripheral blood indicates that the bone marrow barrier has been disrupted or that extramedullary hematopoiesis has been triggered. Any condition that reduces the quantity of oxygen transported to the tissues causes an increase in the rate of RBC production. Increase NRBC count immediately after birth could be an interesting marker of EONS in absence of hypoxia and awaits further evaluation. In study of 100 suspected cases of neonatal sepsis, Majumder Ankur *et al.*,[14] noted that Platelet Distribution Width (PDW) showed sensitivity and specificity of 82% and 73 % respectively. Mean Platelet Volume (MPV) showed sensitivity and specificity of 76% and 40 % respectively. Thrombocytopenia showed sensitivity and specificity of 64% and 82 % respectively. Platelet Distribution Width was the most sensitive marker (82%). Thrombocytopenia was the most specific marker (82%). In a metanalysis, Wang J *et al.*,[15] included 11 studies on 932 neonates with sepsis, and noted that MPV was significantly higher in patients with neonatal sepsis compared with healthy controls. Therefore, in clinical practice, MPV could be used as an indicator for the early diagnosis of neonatal sepsis. Neonatal sepsis is a serious but treatable condition whose treatment should not be missed or delayed, a test or a combination of tests with a high sensitivity is desirable. A better understanding of the neonatal inflammatory response to sepsis and the identification of sensitive and specific markers of inflammation or rapid microbe-specific diagnostic tests are needed to assist the clinician in the early detection of neonatal sepsis.[16]

CONCLUSION

Nucleated red blood cell count (NRBCc) and platelet count in the peripheral blood smear helps in the early diagnosis of neonatal sepsis and can be used in conjunction with other laboratory tests as a simple and convenient method for early diagnosis of neonatal sepsis.

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