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Evaluation of diagnostic value of nucleated red blood cell count in the early neonatal sepsis

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Abstract
Objective
1. To Evaluate diagnostic value of Nucleated Red blood cell count in the early diagnosis of neonatal sepsis.
2. To compare and correlate raised Nrbcs with blood culture in diagnosis of neonatal sepsis.

Material and Method: A prospective study was conducted from January2017 to January 2019, in a Tertiary care private Hospital of 120 neonatses neonates with predisposing perinatal risk factors or if there was clinical suspicion of sepsis. Peripheral Blood smears were examined for haematological parameters along with NRBCS count. The data collected was statistically analyzed, to find out the performance of the test individually and in comparison to gold standard that is blood culture under the following parameters. Sensitivity, specificity, positive predictive value and negative predictive value.

Results: Presence of Nrbcs in peripheral smears of culture positive sepsis noted in 82.14% cases. Sensitivity of Nrbcs is 78.57%. Specificity is 89.13%, Positive predictive value 68.75%, Negative predictive value 93.18%.

Conclusion: Nrbcs is a single, quick, cost-effective and readily available, feasible tool with good sensitivity and specificity in the early diagnosis of neonatal sepsis. It gives comparable results to blood culture and can be done even in small hospitals and in peripheral setups, allowing prompt treatment of infected neonates and thereby reducing morbidity and mortality.

Keywords: Nucleated red blood cells, neonatal sepsis, blood culture, Haematological tests

Introduction

Neonatal sepsis is a clinical syndrome of bacteremia characterized by systemic signs and symptoms of infection in the first month of life [1].

Globally of the 130 million babies born every year, about 4 million die in the first 4 weeks of life, i.e. neonatal period [2].

According to recent data from National Neonatal Perinatal Database (NNPD) 2002-03 collected from 18 centers from various parts of India, incidence of neonatal sepsis has been reported to be 29.9 per 1000 live births. The main direct causes of neonatal deaths are estimated to be preterm birth (26%), severe septicemia (16%), and birth asphyxia (28.8%). Early onset sepsis contributes 67% of all sepsis. Pneumonia contributes to 15.6% of all cases of neonatal deaths [3]. Clinical features of sepsis are nonspecific in neonates and a high index of suspicion is required for early diagnosis. Although blood culture is the "Gold Standard" for the diagnosis of sepsis, reports are available after 48-72 hrs and they may be affected by intrapartum antibiotic administration to the mother. The positive yield rate of blood culture is only 25 to 30% [4].

Hence in order to diagnose septicemia early, we need a more comprehensive test which is cost effective and simple. Nucleated Red Blood Cells (NRBC) which are the precursors of erythrocytes, are released from the bone marrow in response to stress. Studies have shown, association of an increase in the NRBC count with conditions such as asphyxia, gestational diabetes, and neonatal sepsis [5]. Nucleated RBCs are in the peripheral blood of normal infants up to the fifth day of life [6]. At birth, 3 to 10 NRBCs per 100 WBCs are present [7, 8]. Premature birth [9] and fetal hypoxia can cause this number to increase [10, 11]. Previous studies have also investigated the role of NRBCs in the prognosis of sepsis [12].

In the present study we have evaluated the utility of Nrbcs count based on simple peripheral smear and other Hematologic parameters in early diagnosis of neonatal sepsis in rural area.

Objective:-1. Evaluation of diagnostic value of Nucleated Red blood cell count in the early diagnosis of neonatal sepsis. 2. To compare and correlate raised Nrbcs with blood culture sin diagnosis of neonatal sepsis.

Materials and Methods

This is a cross-sectional prospective diagnostic study of 120 neonates with sepsis or clinical suspension of sepsis according to WHO.

Inclusion criteria

Neonates with predisposing perinatal risk factors or if there was clinical suspicion of sepsis were included in this study.

Exclusion criteria

Neonates having congenital anomalies diagnosed during antenatal period or at birth and neonates undergoing surgeries were excluded.

Methodology

After admission, a detailed history of each patient was recorded which includes clinical, demographic data (e.g: age, sex, maturity, weight, place and mode of delivery, maternal illnesses) informed and written consent from parents obtained.

Collection of samples

The blood samples were collected by peripheral venipuncture using aseptic precautions sent in EDTA bulbs. Routine hematological investigations included hemoglobin, hematocrit, red blood cell indices (MCV, MCH and MCHC), NRBCS, total WBC count, differential WBC count, band cell count, degenerative changes and platelet count. These investigations were performed on multichannel automated cell counter-Sysmex3100 with standard calibration.

For every sample a peripheral smear was prepared and the blood film was stained with Leishman's stain. Neonates were divided into 3 groups:

Group 1: (Proven sepsis): neonates with sepsis (with positive blood culture)

Group 2: (Probable sepsis): neonates with probable infection (with clinical signs and negative blood culture)

Group 3: (No sepsis): normal neonates (without signs of sepsis). Statistical tests:-The data collected was statistically analyzed, to find out the performance of the test individually and in comparison to gold standard that is blood culture for Sensitivity, specificity, Positive predictive value, Negative predictive value. SPSS software 16 version was used for analysis.

Results

Table 1: Show the demographic sex risk factors and birth weight

Demographic factors		Proven Sepsis	Probable Sepsis	Clinical Sepsis	Total
		28	42	50	120
sex	Male	16(57.14%)	31(25.83%)	32(26.6%)	79(65.83%)
	Female	12(10%)	11(9.16%)	18(15%)	41(34.16%)
Risk Factors	Maturity	28	56	36	120
	Preterm	15(12.5%)	30 (25%)	19(15.83%)	64(53.33%)
	Term	13(10.83%)	26 (21.66%)	17 (14.16%)	56(46.66%)
		35	38	47	120 (100.0%)
Birth weight	<2500gms	18(15%)	16(13.33%)	12(10%)	46 (38.33%)
	≥ 2500 gms	17(14.16%)	22(18.33%)	35(29.16%)	74(61.66%)

Table 2: Grouping the neonates according to sepsis status (n=120)

Interpretation	Finding	Frequency	Percent
Sepsis	Culture +ve	28	23.33
Probable Sepsis	Culture –ve but Clinical +ve	42	35
No sepsis	Culture & Clinical -ve	50	41.66
Total		120	100.0

Of 120 neonates in the study 79(65.83%) were male and 41 (34.16%) were female. Of 64 (53.33%) were premature neonates (<37 weeks) gestational age and 56% (46.66%) were fullterm babies. Of 56 term neonates 13(10.83) % had proven and 26(21.66%) had probable sepsis and 17(14.16%). 46(38.33%) had low birth weight. Out of 46/120 neonates with Low birth weight, 18(15%) had proven sepsis and 16(13.33%) had probable sepsis and 12(10%).

Of 120 neonates, in the study confirmed sepsis was noted in i.e 28(23.33%) neonates having suspected sepsis because they had negative culture reports. 50(41.66%) neonates were labeled no sepsis to be not having sepsis because they had culture negative.

Table 3: Haematological Parameters in different Categories

Sr. No.	Haematological parameters	No sepsis	Probable sepsis	Proven sepsis
		n=50	n=42	n=28
1	Increase NRbcs	29 (58%)	30 (71.42%)	23 (82.14%)
2	Total WBC count	41(82%)	33 (78.57%)	22 (78.57%)
3	Immature PMN	38(76%)	31 (73.8%)	22 (78.5%)
4	Toxic granules	28 (56%)	29 (69.04%)	21(75%)
5	Degenerative Changes	29(58%)	26 (61.90%)	18 (64.28%)
6	Platelet count	39(78%)	29 (69.04%)	21 (75%)

Out of the all haematological parameters studied, Nrbcs count had highest 82.14%, association in culture positive sepsis as compared to others like total Wbc count WBCs and Imature PMN 78.5%, toxic granules 75%,degenerative changes 64.28% and platelet count 75%.

Table 4: Relationship between NRBCS and SEPSIS group

Sepsis group Nrbcs/ 100WBCS	No Sepsis N=50	Probable sepsis N=42	Proven Sepsis N=28
	29	**30**	**23**
10-19	15	7	3
20-29	09	17	9
>30	05	6	11

Nrbcs counted on peripheral smears of neonates by Nrbcs per 100 white blood cells wbcs. NRBCs counted in neonates with proven sepsis were 23, probable sepsis were 30 and clinical were 29. The infant's NRBC count was directly correlated with infection status. Nucleated red cells (Nrbcs) are counted as white blood cells. For this reason the obtained wbc count is actually corrected by counting number of nrbcs /100 leucocytes while performing peripheral blood smear. NRBC count done by peripheral smear manually avoiding manual errors.

Corrected WBC = obtained nucleated cell count x 100 / (nrbcs+100)

Absolute nrbcs (x103/ul) = obtained nucleated cell count -- corrected WBC. N-RBC count at birth is reported as nRBCs per 100 white blood cells. Unfortunately, the variability of leucocyte count after birth results in a wide range of values for nRBCs when expressed in this way. The problem is magnified by the many pathological processes that significantly alter total leucocyte count and lead to a misleadingly low value of nRBCs per 100 white blood cells. After assessing the sensitivity, specificity and positive predictive value of NRBC in the prediction of neonatal infection, statistical tests was performed and compared with the gold standard test. The data analysis showed that NRBC count shows sensitivity, specificity, and positive and negative predictive values for the diagnosis of neonatal sepsis will be 78.57%, 89.13%, 68.75%, and 93.18% respectively.

Table 5: Correlation between NRBCS and positive blood culture

Parameters	Number of positive Out of n=120	Culture positive out of n=28
NRBCs	88(73.33%)	23(82.14%)
Total WBCs	96(80.0%)	22(78.57%)
Immature PMN	91(75.83%)	22(78.57%)
Toxic Granules	78(65.0%)	21(75%)
Degenerated changes	73(60.83%)	18 (64.28%)
Platelet count	89(74.16%)	21 (75%)

Comparision of NRBCs, Total WBC count Immature PMN, toxic granulation degenerative changes and platelet count in 120 cases and culture positive 28 cases Nrbcs were noted in 23 (82.14%), Total WBC and Immature PMN in 22 (78.57%), both toxic granules and platelet count 21 (75%).

In 120 total number of cases, Nrbcs were noted in 88 (73.33%), Total WBC 96(80.0%) neonates and Immature PMN 91(75.83%) toxic granules and degenerative changes 78(65.0%) and 73 (60.83%) respectively.

Discussion

Sepsis is the commonest cause of neonatal mortality. It is responsible for about 30-50% of the total neonatal deaths in developing countries. It is estimated that up to 20% of neonates develop sepsis and approximately 1% die of sepsis related causes [13].

The definite diagnosis of septicemia is made by a positive blood culture which requires a minimum period of 48-72hrs and yields positive result in 30-40% of cases [4].

An early and accurate etiological diagnosis is not always easy, especially since the disease may start with minimal or non-specific symptoms. Delayed treatment until clinical recognition of signs and symptoms of sepsis entails risk of preventable mortality, notwithstanding the fact that presumptive antibiotic therapy may result in overtreatment.

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 [4].

The current study was undertaken to evaluate the utility of elevated Nrbcs in utility in diagnosis of neonatal sepsis. NRBC are precursor of mature erythrocytes and are released from the bone marrow in response to various conditions like hypoxia asphyxia, gestational diabetes and sepsis [14].

In normal neonate, nRBC are rapidly cleared from the bloodstream after birth. By 12 hours of age, the counts fall by about 50%, and by 48 hours only 20-30 nRBCs/mm3 are found. In healthy term newborns, virtually no nRBCs are found after the 3rd or 4th day of life, although they may persist in small numbers up to 1 week in preterm newborns [14] cytokines mediated mechanisms are responsible for elevations of fetal nucleated RBCs [15]. Various studies have been conducted till date to assess the role of Nrbcs in early inflammatory marker in neonatal sepsis.

Dulay *et al.* 5 2008 conducted that inflammation alone plays an important role in increasing Nrbcs Boskabadi [12] *et al.* 2017 showed a strong correlation between elevated Nrbcs more than 7 per 100 100 WBCS and infant mortality rate.They also proved that Nrbcs count was more sensitive and specific than other haematologial parameters like I/T ratio and CRP.

Many other authors like Cremer M [16] and Baschat AA [17], Shah BA [18] and Desai S [19] and Abhishek MG [11] have evaluated usefulness of Nrbcs in early diagnosis of neonatal sepsis and predicting poor outcome in ill infants.

In our study shows presence of Nrbcs in peripheral smears noted in 82.14%cases. Sensitivity of Nrbcs is 78.57%. Specificity is 89.13%, Positive predictive value 68.75%, Negative predictive value 93.18%.Our findings were showing similarity with Dulay *et al.* 2008 [5] where Nrbcs showed increase number of Nrbcs in neonatal sepsis, Tripathi *et al.* 2010 [20] mentioning activation of cytokine released by macrophages stimulate Nrbc levels in septicemia. Abhishek MG *et al.* 2015 [11] and N. Muthukumaran *et al.* 2018 [21] also observed and confirmed Nrbcs as marker in diagnosing early onset neonatal sepsis.

Limitations of the study

Manual counting is presently the only way to quantify NRBCs, but it is time-consuming and inaccurate due to manual errors. Even though who tried to minimize counting errors it could have lead to variations. Variation limited by preparing and reading all the smears by trained technical person and double blind method.

We have studied limitated sample size done was small level, can be improved in the future study for more profound results.

Cord blood is better sample for Nrbc, collecting immediately after birth but all deliveries were not conducted at our institute and more were referral neonates.

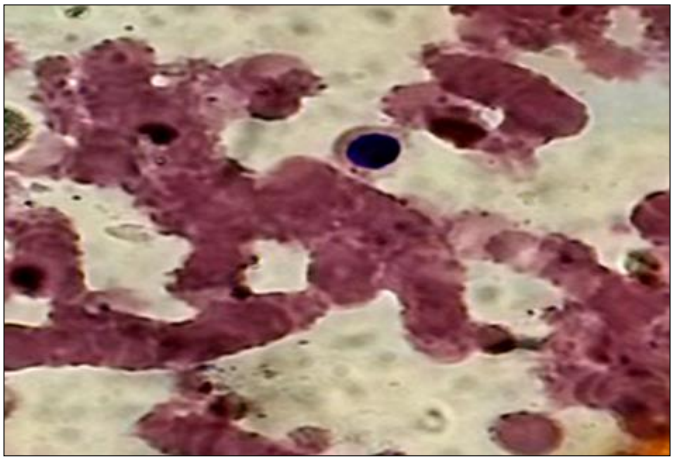

Fig 1: Peripheral Blood Smear Leishman's Stain (X1000) Showing Nucleated RBC (nRBC)

Conclusion

The valuable tests are those that are a simple, easily accessible, accurate, cost-effective, readily available and cause no harm to patients. As Nrbc count is seen in culture positive cases, Nrbcs can be with the early diagnosis of neonatal infections in combination with other laboratory tests, this test can be tool for timely diagnosis and prompt interventions especially in rural areas, even before blood culture positivity.

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