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To Enumerate the Nucleated Red Blood Cells in Neonatal Sepsis as an Early Response Element to Inflammation in Infection

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Abstract

Introduction: *Neonatal sepsis is a prevalent medical condition among the babies in all the regions of the world. According to WHO report, annually about 2.7 million neonatal deaths occur worldwide, with more than 35% of them being caused by infectious organisms.*

Aim: *To enumerate the nucleated RBC count in blood samples of neonates with sepsis. The objective was to relate the count of nucleated RBCs as an early response blood element in detecting the inflammation due to sepsis.*

Methodology: *A Descriptive study of a sample of 155 neonates divided into culture positive, clinical sepsis and no sepsis groups. The Nucleated Red Blood Cells (NRBC) count per 100 White blood cells (WBC) was counted carefully. A repeat peripheral smear was taken 3 days after admission and compared with the previous results.*

Results & Discussion: *Out of 115 study neonates, only 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.*

Conclusion: *NRBC count is a simple and valuable marker for detecting the earlier diagnosis of neonatal infection.*

Keywords: *Nucleated red blood cells, Neonatal sepsis.*

Introduction

Neonatal sepsis is a prevalent medical condition among the babies in all the regions of the world. According to WHO report, annually about 2.7 million neonatal deaths occur worldwide, with more than 35% of them being caused by infectious organisms[1]. Premature and very low-birth-weight (less than 1500 grams) neonates are at higher risk for infection[1, 2]. Various types of diagnostic tests have been developed for neonatal infection. Some of them are readily available, some are costlier and few are not available in all healthcare centers. The real most valuable tests are those that are easily accessible, accurate, cost-effective and minimal invasive to babies. The laboratory methods used for the diagnosis of sepsis include direct methods such as blood, urine, and CSF cultures and indirect way as Total leukocyte count, Immature cells / Total Neutrophil cells ratio (I/T ratio), Erythrocyte Sedimentation Rate (ESR), and C Reactive Protein(CRP) methods[3].

Nucleated Red Blood Cells (NRBC) which are the precursors of erythrocytes, are released from the bone marrow in response to stress. Many studies have been proved in the past, the role of NRBCs in the diagnosis of neonatal diseases. An increase in the NRBC count has been associated with conditions such as asphyxia, gestational diabetes, and adult sepsis[4]. Previous studies have also investigated the role of NRBCs in the prognosis of sepsis. Nucleated RBCs are in the peripheral blood of normal infants up to the fifth day of life [5]. At birth, 3 to 10 NRBCs per 100 WBCs are present[5,6]. Premature birth[7] and fetal hypoxia can cause this number to increase[8]. Beyond the neonatal period, the presence of NRBCs in the peripheral blood is usually associated with severe infections, malignant neoplasms, bone marrow diseases, and other serious disorders[5,9,10].

Mature red cell contains almost 95% of its proteins are hemoglobin, electrolytes and largely enzymes of its energy system approximately to live 4 months lifespan, enclosed by a cell membrane. In bone marrow disorders as infiltration / inflammation/ necrosis, nucleated red blood cells can be identified in the peripheral blood. Appearance of normoblasts in peripheral circulation is a serious concern[13]. 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.

Aim

The aim of the study was to enumerate the nucleated RBC count in blood samples of neonates with sepsis. The objective was to relate the count of nucleated RBCs as an early response blood element in detecting the inflammation due to sepsis.

Materials and Methods

This was a descriptive study of neonates admitted in the Newborn ward in a tertiary care hospital. The sample taken was 115. The babies were selected over a period of October 2017 to July 2018. The Institutional Ethics Committee approval was obtained. The inclusion criteria were term live neonates admitted to Newborn ward with clinical features and risk factors of sepsis. The clinical features of sepsis were fever, lethargy, poor cry, refusal to suck, poor perfusion, prolonged capillary filling time, respiratory distress, apnea, gasping respiration, absent neonatal reflexes, bradycardia /tachycardia, hypoglycemia/ hyperglycemia, hypothermia, metabolic acidosis and hypotonia. Risk factors received from mother's case file / history as febrile illness in the mother with evidence of bacterial infection within 2 weeks before delivery, foul smelling or meconium stained liquor, rupture of membranes more than 24 hours, single unclean or more than 3 sterile per vaginal examination during labour and prolonged labour, if stage 1 and

2 more than 24hrs. The exclusion criteria were maternal pre eclampsia or eclampsia, gestational diabetes mellitus, placenta previa, abruption or infarcts, maternal smoking and alcohol intake , intrauterine growth retardation, congenial malformation, ABO/ Rh incompatability, birth asphyxia, preterm or post term babies, hemolytic anemia, any suspected malignancy, bone diseases, arsenic therapy, metabolic disorders, granulomas.

The babies who fulfilled the inclusion criteria were enrolled in the study. Complete obstetric history of the mothers was obtained and clinical examination was done for all study neonates. Sepsis screening tests included Total Leucocytes Count, Absolute Neutrophil Count, Immature / Total Neutrophil ratio, micro- Erythrocyte Sedimentation Rate (ESR) and C-Reactive protein. Due to non availability of micro-ESR test, was not done in any study babies. The presence of two or more above laboratory parameters as positive were considered positive screen for neonatal sepsis. These babies were started on antibiotics immediately. If the screening was negative and the clinical suspicion persists, then it is repeated within 12 hours. If the screening was still negative, the diagnosis of clinical sepsis was ruled out.

Our aim was to prepare a good peripheral blood smear by placing a drop of blood through the needle, when blood was obtained on clean, dry, grease free glass slides. The following cells were identified: red blood cells, white blood cells and platelets. The Nucleated Red Blood Cells (NRBC) count per 100 White blood cells (WBC) was counted carefully. A repeat peripheral smear was taken 3 days after admission and compared with the previous results. These study neonates were followed up till their discharge and repeat peripheral smear examination done if the clinical condition deteriorated. The blood culture was done for all.

The study group was sub divided into the following groups based on the blood culture and clinical findings. Group IA were neonates with positive blood culture, Group IB were neonates with strong clinical features and positive sepsis screening, but negative blood culture and Group II neonates with negative both the sepsis screening and blood culture too.

Results

The study group of neonates who fit in the inclusion criteria was 115. Data were entered in to MS excel sheet and analyzed with SPSS v 20. The quantitative data were summarized as frequencies and percentages. Quantitative data were checked for normality which was summarized using mean and standard deviation. Pearson Chi square test and Fischer exact test has been used to find the significance of study parameters on categorical data between two groups. Significance is assessed at 5% level of significance. p value 0.05 taken as statistically significant. Out of them, 77 neonates (66.96%) were less than 72 hours old who were suspected to have early onset sepsis .The remaining 38 neonates (33.04%) were more than 72 hours old who were suspected to have late onset sepsis.

Table 1 Age Distribution among study population

Age of Neonates	Groups	
	Sepsis(IA&IB)	No Sepsis(II)
< 72 hours	40	37
> 72 hours	21	17
Total	61	54

p value 0.912 Not significant

Table 2: Gender Distribution among study population

Gender	Groups	
	Sepsis(IA&IB)	No Sepsis(II)
Male	34	32
Female	27	22
Total	61	54

p value 0.854 Not significant

The baseline characters as age and gender between the groups were not statistically significant as in table 1 and 2.

Table 3: Blood Parameters among study population

Variables	Sepsis(IA&IB) *Mean±SD*	No Sepsis(II) *Mean±SD*	p value
Hemoglobin	10.46± 2.12	13.21± 1.04	0.002
Total WBC Count	8425±5657	6756±1481	0.1184
Platelet	1,81,000±85,761	3,32,000±91,101	0.0001
NRBC	13.08±5.26	4.89±4.3	0.0002

Table 3 shows that the hemoglobin values are statistically significant between the sepsis babies and not proven sepsis group. There was much statistically significant difference as thrombocytopenia in sepsis babies. There was also statistically significant difference in increase of NRBCs in sepsis group of babies.

Table 4: Mean NRBC value in groups:

Groups	Mean
Culture positive sepsis(IA)	13.69
Sepsis screen positive sepsis(IB)	12.38
No sepsis(II)	04.87

Table 5: Mean values of NRBC

NRBC	Sepsis	No Sepsis
Positive	50	21
Negative	11	33

p value 0.0001

The sensitivity of NRBC in identifying sepsis was 81.96%, its specificity was 61.11%, positive predictive value was 70.42% and negative predictive value was 75%. Out of 115 study neonates, only 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.

Discussion

In total of 115 babies, 32 were culture positive, 29 were sepsis screen positive with blood culture negative while rest 54 had no sepsis. The mean value of NRBCs in culture positive sepsis was 13.69, in sepsis screen positive sepsis was 12.38 and 4.87 in no sepsis group of babies. In a previous study done, 47 babies out of 56 babies with proven sepsis had a NRBC score of > 10/ 100 WBCs accounting to about 83.9%. In our study sensitivity in identifying sepsis was 81.96%, its specificity was 61.11%, positive predictive value was 70.42% and negative predictive value was 75%. In a study done earlier, the sensitivity of the test in finding proven sepsis was 35%, specificity 53.4%, positive predictive value 23.07% and negative predictive value 67.64%. In another reference study, sensitivity of NRBCs was found to be 86.15%, specificity of 51.06%, positive predictive value 54.9% and negative predictive value of 84.21%. In our study also, the mortality was high in sepsis babies with increased NRBC count. In a previous study, it has been found that daily screening for NRBCs count was a really beneficial investigation in estimating the mortality risk.

In a study conducted on preterm infants with early sepsis caused by maternal chorioamnionitis, a significant difference was observed between healthy and infected groups. By excluding the effect of erythropoietin (EPO), cortisol, and acid-base disorders, the researchers finally came to the conclusion that inflammation alone plays an independent role in increasing NRBC count in preterm infants[(11)]. Sepsis is an inflammatory reaction that places the body under stress. In a number of studies, selective erythroid hyperplasia was observed in the bone marrow by administering interleukin-6 to adult animals after 12 hours (during this period, this event could not have been caused by the production of EPO). Similarly, an increased NRBC count associated with the elevated production of interleukins 3, 6 and 12 has been reported in critically-ill patients[(12)] There is therefore, a direct and positive correlation between the increased production of interleukin-6 (an inflammatory mediator) and a rise in NRBC count[(11)].

The red blood cell is the simplest cell of the human body. Formed as a nucleated cell in the bone marrow, it normally loses its nucleus before its release into the circulation. On entering the circulation, it still possesses residual ribosomes, mitochondria and Golgi apparatus. Cytoplasmic organelles are lost after a day or so, and then red blood cell assumes the shape of a flattened, biconcave disc[(13)]. Neonatal infections can be caused by various infectious agents transmitted from the mother to the fetus or new born infant by

diverse modes. The fetus and newborn infants are less capable of responding to infection because of immunologic immaturity particularly preterm infants are at particular risk always. Any co existing conditions often complicate the diagnosis and management of neonatal sepsis. The clinical manifestations of newborn infections vary and includes subclinical infections also and very rarely these days congenital syndromes resulting from in utero infection. The timing of exposure , inoculum size , immune status, and virulence of etiological agent influence the expression of disease. Maternal infection, the source of transplacental fetal infection is often undiagnosed during pregnancy because the mother was either asymptomatic or had non specific signs and symptoms at the time of acute infections[(14)]. Neonatal sepsis is a clinical syndrome characterized by signs and symptoms of infection with or without bacteremia in the first month of life. It encompenses septicemia, meningitis, pneumonia, arthritis, osteomyelitis and urinary tract infection[(15)]. Neonatal bacterial sepsis can definitely cause anemia on the basis of hemolysis, disseminated intravascular coagulation and / or hemorrhage. These babies have jaundice and hepatosplenomegaly. Some bacteria produce hemolytic endotoxin that result in red cell destruction. Congenital infection due to any type of viruses like cytomegalo virus, herpes simplex leads to hemolytic anemia[(16)]. Increased number of nucleated RBCs are seen in the neonates after hypoxia, hemorrhage, severe infection, Down's syndrome and congenital anomalies[(17)].

In our study, we have convinced about the hypothesis that there is significant increase in NRBCs in early newborn period if the neonates were physiologically stressed, hypoxic state which may be even by congestive heart failure, leads especially to the concept of bone marrow barrier necrosis due to infiltrative disease of marrow by septicemia[(18)]. Rarely nucleated RBCs enter the circulation. NRBCs in the blood symbolize a compensatory response to an excessive demand on the blood forming organs as either stress in the form of sepsis and severe anemia. In the inflammatory states, red blood cells may be destroyed at an accelerated rate. If production of new red blood cells can keep up with the accelerated rate of destruction, the red blood cell count will not fall. However the nucleated polychromotophilic macrocytes will be seen on the peripheral smear as an evidence of inflammatory mediators stimulated premature release of marrow nucleated normoblasts (younger RBCs) independent of hypoxia or stress[13]. Intravascular hemopoiesis or disruption of marrow structure which leads to inability of bone marrow screening mechanisms to prevent their passage in insulation leads to rise in NRBCs.

In an effort to avoid unnecessary antibiotic administration, attention has turned toward new diagnostic approaches such as the description of biomarkers and hematological indices designed to identify neonates at risk for sepsis and poor outcome. The importance of this concept is predicated by studies which demonstrate that general hematological indices and NRBCs are good predictors of short term neonatal outcome, independent of gestational age or birth weight. [19,20]

Limitations of the study

The sample size done was small and need to be improved in the future study. NRBC count done by peripheral smear manually in good steps, avoiding errors, but could have lead to variations. Inter observer variation limited by preparing and reading all the smears by same technical person. Micro ESR would have been done since a tool in screening list for neonatal sepsis.

Conclusion

Although the appearance of NRBCs in blood does not by itself provide a diagnosis of disease, it may give invaluable clues to the presence of a serious condition especially sepsis. Homeostatically, NRBCs in blood represent a compensatory response to an excessive demand on the blood-forming organs (marrow stress) such as in severe

anemia or hypoxia[19]. Any unexplained normoblastemia is important because it offers invaluable insight into disease processes or progressions that occur in conditions such as systemic infections (sepsis). According to the results of this study, NRBC count is a simple and valuable marker for detecting the earlier diagnosis of neonatal infection.

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