Section: Pathology

Original Research Article

ORIGINAL RESEARCH ARTICLE: A DESCRIPTIVE STUDY OF DIAGNOSTIC EFFECTIVENESS OF NUCLEATED RED BLOOD CELL COUNT IN THE EARLY DIAGNOSIS OF NEWBORN SEPSIS

Vaidehi Mehta[1], Ashish Jawarkar[2], Ashish P Jain[3]

[1]Associate Professor, Department of Micobiology, Parul Institute of Medical Science and Research, Parul University, Vadodara, Gujarat, India
[2]Associate Professor, Department of Pathology, Parul Institute of Medical Science and Research, Parul University, Vadodara, Gujarat, India
[3]Associate Professor, Department of Anaesthesiology, Parul Institute of Medical Science and Research, Parul University, Vadodara, Gujarat, India

Received : 01/02/2023
Received in revised form : 16/03/2023
Accepted : 31/03/2023

Keywords:
nRBC, Sepsis, Neonate, Marker.

Corresponding Author:
Dr. Ashish Jawarkar,
Email: pathology22222@gmail.com

DOI: 10.47009/jamp.2023.5.2.255

Source of Support: Nil,
Conflict of Interest: None declared

Int J Acad Med Pharm
2023; 5 (2); 1202-1206

Abstract

Background: In all parts of the world, neonatal sepsis is a common medical disease among infants. Over 2.7 million newborn fatalities globally occur each year, with infectious organisms accounting for more than 35% of these deaths, according to a WHO research. The objective is to determine the efficacy of Nucleated Red blood cell count in the Early diagnosis of neonatal sepsis. **Materials and Methods:** This Cross-sectional Descriptive Study includes 100 newborns with clinical suspicious of sepsis at birth and within 72 hours or had maternal history of infection. The cord blood was collected immediately after delivery for NRBCs count under peripheral smear. Statistical analysis was performed and Sensitivity, specificity, positive predictive value and negative predictive value was calculated. **Result:** Among total 100 cases, 41 neonates were female with 18 cases of proven sepsis,19 cases of suspected sepsis and 4 cases of clinical sepsis. The ratio of proven, suspected and clinical sepsis in Male and Female is 30:21:8 = 18:19:4. Among total 100 cases, 47 neonates were preterm and 53 were Term born. 43 neonates having birth weight less than 2500 gm(Low birth weight) and 57 having normal birth weight. Out of all 100 cases, an increase in NRBC count was detected in 30 cases whereas elevated ESR, increased total count was noticed only in 20 and 28 cases respectively. Out of 100 neonatal sepsis cases, 30(30%) were found to be culture positive cases. Among 30 positive culture cases, 12 neonates (40%) showed increase in NRBC count level, whereas increased total wbc count was seen in 9 neonates (30%). Another parameter which is Toxic granulation seen in neutrophils was revealed in only 5(30%) cases. **Conclusion:** For neonatal intensive care unit patients, NRBCs count may be included among the early diagnostic and prognostic markers. To evaluate trends in NRBC values for critically sick newborns, more research is required.

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] Neonatal sepsis incidence has been found to be 29.9 per 1000 live births, according to latest data from National Neonatal Perinatal Database (NNPD) 2002-03 collected from 18 facilities in various parts of India. According to estimates, birth asphyxia (28.8%), severe septicemia (16%), and preterm delivery (26%) are the three biggest direct causes of neonatal fatalities. 67% of all sepsis is caused by early onset sepsis. 15.6% of all newborn fatalities are caused by pneumonia.[3] Neonatal sepsis has non-specific clinical signs, making early identification difficult without a high index of suspicion. 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]

Normal foetal circulation contains nucleated red blood cells (nRBCs), however in healthy newborns, they vanish within the first month after birth. By gestational and chronological age, neonatal nRBC counts differ. nRBCs in neonates begin to form in

ISSN (O): 2687-5365; ISSN (P): 2753-6556

the bone marrow about 28 hours following a stressor like hypoxia. Increased nRBCs have been linked to events like foetal acidemia, meconium passage during birth, and perinatal problems. They have been described as an indicator of intrauterine and early postnatal stress in neonates. Hence, nRBCs have been examined as a predictive indicator for outcomes after prenatal hypoxia.[5]

Nucleated red blood cells (nRBCs), which are part of normal foetal circulation, disappear in healthy babies within the first month after birth. Neonatal nRBC counts differ according to gestational and chronological age. Neonatals' bone marrow starts to produce nRBCs about 28 hours after a stressor like hypoxia. Increased nRBCs have been connected to conditions such perinatal issues, meconium passage during delivery, and foetal acidemia. As a sign of intrauterine and early postnatal stress in newborns, they have been labelled. Hence, nRBCs have been investigated as a potential indication of future consequences following foetal hypoxia.[6]

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.

MATERIALS AND METHODS

This Cross sectional Descriptive Study was conducted From May 2021 to June 2022, at Department of Laboratory medicine, Parul Sevashram Hospital, Parul Institute of Medical Science and Research, Vadodara, Gujarat.

Cases: 100 Cases of clinically suspected neonatal sepsis.

Study Population

Neonates with clinical suspension of sepsis at birth and within 72 hours or had maternal history of infection.

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.

Exclusion Criteria

Neonates diagnosed with inborn errors of metabolism. Neonates with congenital anomalies

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.

Blood samples were collected in EDTA anti coagulated vials from umbilical cord blood or arterial or venous blood. Peripheral smear was prepared, stained using leishman's stain. NRbc count was done on peripheral smear.

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.

Data was expressed as mean values, Sensitivity, specificity, positive predictive value and negative predictive value were calculated.

RESULTS

Based on clinical findings and laboratory parameters, 60 Neonates were grouped into 3 categories. Namely 1) proven sepsis (14 cases) 2) suspected sepsis (20 cases) and 3) clinical sepsis (26 cases). This classification is based on Chandna A et al.[7]

Table 1: Comparison of Number of newborn of 3 sepsis groups

Type of sepsis	Number	Percentage(%)
Proven sepsis	48	48
Suspected sepsis	40	40
Clinical sepsis	12	12
Total	100 (100%)	

Table 2: No of Culture positive sepsis

Type of sepsis	Number (%)
Culture positive sepsis	30(30%)

Out of 100 neonatal sepsis cases, 30(30%) were found to be culture positive cases.

Table 3: Gender wise distribution of Sepsis Group

Type of sepsis	Number	Male	Female
Proven sepsis	48	30	18
Suspected sepsis	40	21	19

ISSN (O): 2687-5365; ISSN (P): 2753-6556

Clinical sepsis	12	8	4
Total	100		

[Table 3] depicts comparison of variable like Gender. Among total 100 cases, 41 neonates were female with 18 cases of proven sepsis,19 cases of suspected sepsis and 4 cases of clinical sepsis.
The ratio of proven, suspected and clinical sepsis in Male and Female is 30:21:8 = 18:19:4 (Male: Female) respectively.

Table 4: Maturity wise distribution of Sepsis Group

Type of sepsis	Number	Maturity	
		Preterm	Term
Proven sepsis	48	35	13
Suspected sepsis	40	8	32
Clinical sepsis	12	4	8

[Table 4] depicts comparison of variable like Maturity. Among total 100 cases, 47 neonates were preterm and 53 were Term born.

Table 5: Distribution of Sepsis Group based on birth weight

Type of sepsis	Number	Birth weight	
		Low birth weight (<2500 gm)	Normal Birth Weight
Proven sepsis	48	19	29
Suspected sepsis	40	19	21
Clinical sepsis	12	5	7

Comparison regarding birth weight of cases, 43 neonates having birth weight less than 2500gm (Low birth weight) and 57 having normal birth weight.

Table 6: shows comparison of hematological parameters with NRBC count in sepsis group.

Sepsis group	Proven sepsis (48)	Suspected sepsis(40)	Clinical sepsis (12)
Increase Nrbcs	13	12	5
Toxic granules in Neutrophils	4	5	6
Raised Total WBC count	13	11	4
Elevated ESR	5	12	3

The total WBC count was found to be increased in 13 neonates out of 48 proven sepsis cases, 11 neonates out of 40 suspected sepsis cases and 4 neonates out of 12 clinical sepsis cases
Raised ESR value and Toxic granulation in neutrophils were seen in 5 neonates and 4 neonates among proven sepsis cases (N=48). [Table 6]
Significant rise of Nrbc count was seen in 13 neonates among total 48 Proven sepsis neonates and 12 neonates among total 40 Suspected sepsis neonates. [Table 6]

Table 7: Relation between NRbc count and sepsis group

Sepsis group NRbc/100wbc	Proven sepsis (48)	Suspected sepsis(40)	Clinical sepsis (12)
10-19	2	1	3
20-29	4	2	6
>30	8	5	3

In this [Table 7] Depicts neonates shows more than 30NRBCs/100WBC out of 48 proven sepsis cases. 4 Cases showed 20-29 Nrbcs/100WBC among 48 cases of proven sepsis.

Table 8: Comparison of ESR, Total WBC count, Toxic granules in neutrophils, Nrbcs and positive blood culture

Sepsis group	No of Positive cases (out of N=100)	Culture positive (out of 30 cases)
Increase Nrbcs	30	12(40%)
Toxic granules in Neutrophils	15	5(30%)
Raised Total WBC count	28	9(30%)
Elevated ESR	20	3(15%)

Out of all 60 cases, an increase in NRBC count was detected in 30 cases whereas elevated ESR, increased total count was noticed only in 20 and 28 cases respectively. [Table 8]

Out of 100 neonatal sepsis cases, 30(30%) were found to be culture positive cases.
Among 30 positive culture cases, 12 neonates(40%) showed increase in NRBC count level, whereas

ISSN (O): 2687-5365; ISSN (P): 2753-6556

increased total wbc count was seen in 9 neonates (30%). Another parameter which is Toxic granulation seen in neutrophils was revealed in only 5(30%) cases [Table 8].

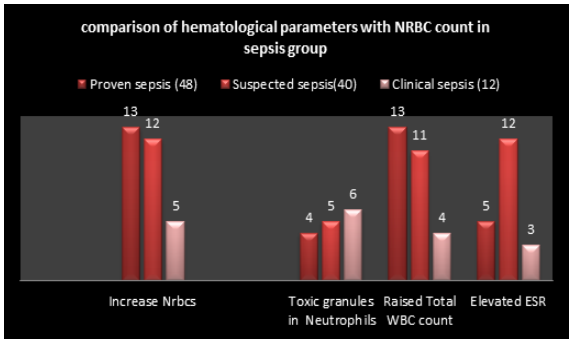

Figure 1: Graphical presentation of comparison of hematological parameters with NRBC count in sepsis group

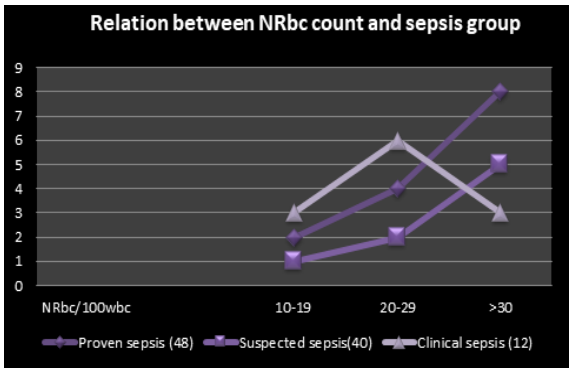

Figure 2: Graphical presentation of Relation between NRbc count and sepsis group

DISCUSSION

The most frequent reason for newborn death is sepsis. In underdeveloped nations, it is to blame for 30–50% of all newborn fatalities. According to estimates, up to 20% of newborns might develop sepsis, while only 1% of sepsis-related deaths occur.[8] A positive blood culture, which must be performed for at least 48 to 72 hours and produces a positive result in 30 to 40% of patients, is required for the definitive diagnosis of septicaemia.[9] It is not always simple to make an early and precise etiological diagnosis, especially given that the disease may initially present with few or vague symptoms. We conducted this investigation to identify early septicaemia and prevent negative outcomes, taking into account that delaying treatment until clinical diagnosis of sepsis symptoms and signs increases the risk of unnecessary fatality.

In a study on preterm children with maternal chorioamnionitis-induced early sepsis, a significant difference between the healthy and infected groups was found. The researchers finally got to the conclusion that inflammation alone has an independent role in boosting NRBC count in preterm infants by ruling out the impact of erythropoietin (EPO),[10] cortisol, and acid-base problems. Sepsis is an inflammatory response that stresses the body. Interleukin-6 administration to adult animals after 12 hours resulted in the selective erythroid hyperplasia in the bone marrow being shown in a lot of studies (during this period, this event could not have been caused by the production of EPO). Similar to this, in critically ill patients, an enhanced NRBC count has been linked to an increased production of interleukins 3, 6, and 12 has been reported.[11] There is therefore, a direct and positive correlation between the increased production of interleukin-6 (an inflammatory mediator) and a rise in NRBC count.

Neonatals with perinatal hypoxia have unfavourable short- and long-term outcomes when their NRBCs count is elevated at birth or persists.[12] According to Boskabadi et al,[13] neonates with birth asphyxia had NRBC counts that were considerably greater than those of healthy controls and were linked to worse short-term outcomes. These results led to the current study's conclusion that NRBC count might be used as a discriminator marker for early detection of prenatal hypoxia with exceptional performance in preterm newborns. In a more recent study, Boskabadi et al,[13] showed that the NRBC count can be utilised as a predictive marker for neonatal asphyxia. This marker, along with the HIE grade, can be used to predict high infant mortality and asphyxia-related problems. Moreover, it was discovered that NRBCs were particularly helpful in discriminating neonates with hypoxia ischemic encephalopathy, resulting in better care and outcomes for these infants.[14] Morton et al,[15] report that among critically unwell newborns, NRBCs are associated with noticeably enhanced mortality risk supports the significance of NRBCs count in critically ill neonates. Although the median NRBCs count for non-survivors in our investigation was found to be occasionally times higher, this parameter did not show good discriminative power for predicting mortality in all the study population, probably because of the great variability in NRBCs and the small number of cases.

In our investigation, Nrbc had a sensitivity of 38%, a specificity of 55.28%, a positive predictive value of 25.18%, and a negative predictive value of 68.470% for detecting sepsis. Our results agreed with those of the Tripathi et al. (2010) study.[16] They claimed that in the absence of hypoxia, active macrophages release cytokines that are crucial for activating Nrbc. She also disclosed that early and late neonatal sepsis were both associated with markedly elevated Nrbc. Another study, by Dulay et al (2008),[17] linked an early onset of newborn sepsis with a large increase in Nrbc. Total leukocyte count and differential leukocyte count were not shown to be important in the diagnosis of newborn sepsis by Mannon et al.[18] Sawankar et al,[19] observed that hematological parameters were poor predictors of neonatal sepsis.

ISSN (O): 2687-5365; ISSN (P): 2753-6556

CONCLUSION

For neonatal intensive care unit patients, NRBCs count may be included among the early diagnostic and prognostic markers. To evaluate trends in NRBC values for critically sick newborns, more research is required.

REFERENCES

1. Umlauf VN, Dreschers S, Orlikowsky TW. Flow cytometry in the detection of neonatal sepsis. Int J Pediatr. 2013:7631-91.
2. Haque KN. Neonatal Sepsis in the Very Low BirthWeight Preterm Infants:Part 1: eview of Pathophysiology. Journal of Medical Sciences2010;3(1):1-10.
3. Delanghe JR, Speeckaert MM. Translational research and biomarkers in neonatal sepsis. Clin Chim Acta. 2015; 451(Pt A):46-64.
4. Dulay AT, Buhimschi IA, Zhao G, Luo G, Abdel-Razeq S, Cackovic M, et al. Nucleated red blood cells are a direct response to mediators of inflammation in newborns with early-onset neonatal sepsis. Am J Obstet Gynecol. 2008; 198(4): 426.e1-9
5. Silva AM, Smith RN, Lehmann CU, Johnson EA, Holcroft CJ, Graham EM. Neonatal nucleated red blood cells and the prediction of cerebral white matter injury in preterm infants. Obstet Gynecol 2006;107:550e6.
6. Mandel D, Lubetzky R, Mimouni FB, Cohen S, Littner Y, Deutsch V, et al. Nucleated red blood cells in preterm infants who have necrotizing enterocolitis. J Pediatr 2004;144: 653e5.
7. Chanda a , Mimouni FB, Fink D, Sela H, Hammerman C.Elevated nucleated red blood cells at birth predict hemodynamically significant patent ductus arteriosus. J Pediatr 2016;177:313e5.
8. Kovalak EE, Dede FS, Gelisen O, Dede H, Haberal A. Nonreassuring fetal heart rate patterns and nucleated red blood cells in term neonates. Arch Gynecol Obstet 2011;283:1005e9
9. Cremer M, Roll S, Gra¨f C, Weimann A, Bu¨hrer C, Dame C. Nucleated red blood cells as marker for an increased risk of unfavorable outcome and mortality in very low birth weight infants. Early Hum Dev 2015;91:559e63.
10. Purtle SW, Horkan CM, Moromizato T, Gibbons FK, Christopher KB. Nucleated red blood cells, critical illness survivors and postdischarge outcomes: a cohort study. Crit Care 2017;21:154.
11. Christensen RD, Henry E, Andres RL, Bennett ST. Reference ranges for blood concentrations of nucleated red blood cells in neonates. Neonatology 2011;99:289e94.
12. Christensen RD, Lambert DK, Richards DS. Estimating the nucleated red blood cell "emergence time" in neonates. J Perinatol 2014;34:116e9.
13. Boskabadi H, Maamouri G, Tavakol Afshari J, Mafinejad S, Hosseini G, Mostafavi-Toroghi H, et al. Evaluation of serum interleukins-6, 8 and 10 levels as diagnostic markers of neonatal infection and possibility of mortality. Iran J Basic Med Sci. 2013; 16(12):1232-7.
14. Hanlon-Lundberg KM, Kirby RS. Nucleated red blood cells as a marker of acidemia in term neonates. Am J Obstet Gynecol 1999;181:196e201.
15. Mishra UK, Jacobs SE, Doyle LW, Garland SM. Newer approaches to the diagnosis of early onset neonatal sepsis. Arch Dis Child Fetal Neonatal Ed.2006;91:F208–12.
16. Tripathi M, Goel N, Chaudhary U, Aggarwal R, Arora DR. Bacteraemia in children. Indian J Pediatr.2002;69(12):1029-32.
17. Dulay AT, Buhimschi IA, Zhao G, Luo G, Abdel-Razeq S,Cackovic M, et al. Nucleated red blood cells are a direct response to mediators of inflammation in newborns with earlyonset neonatal sepsis. Am J Obstet Gynecol 2008;198:426.
18. Mannon A, Mimouni FB, Fink D, Sela H, Hammerman C. Elevated nucleated red blood cells at birth predict hemodynamically significant patent ductus arteriosus. J Pediatr 2016;177:313e5.
19. Swarnkar K, Swarnkar M. A Study Of Early Onset Neonatal Sepsis With Special Reference To Sepsis Screening Parameters In A Tertiary Care Centre Of Rural India. The Internet Journal of Infectious Diseases 2012; 10(1).

ISSN (O): 2687-5365; ISSN (P): 2753-6556