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CORD BLOOD NUCLEATED RED BLOOD CELL COUNT AS A MARKER OF PERINATAL ASPHYXIA AND ITS SEVERITY AMONG TERM NEONATES: A COMPARATIVE CROSS SECTIONAL STUDY

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ABSTRACT

Introduction: *Introduction: Perinatal asphyxia remains one of the leading causes of neonatal morbidity, neurological disability and mortality worldwide. The present study was conducted to evaluate the association between cord blood nucleated red blood cell (NRBC) count per 100 white blood cells (WBCs) in healthy and asphyxiated neonates and to determine its relationship with the severity of asphyxia and short-term neonatal outcomes.*

Materials and Methods: *This study was a comparative cross-sectional study conducted in Department of Pediatrics at National Medical College. The study was carried out over a period of one year, from October 2022 to September 2023. Immediately after delivery, 2 ml of umbilical cord blood was collected in EDTA vial and sent for nucleated red blood cell (NRBC) analysis.*

Results: *The mean NRBCs per 100 WBCs in the case and control groups was 20±12.176 and 4.70±3.344, respectively, which was statistically significant (p<0.001). The mean NRBCs per 100 WBCs in HIE 1, 2 and 3 was 13.30±4.923, 21.54±13.90 and 28±11.22, respectively, which was statistically significant (p=0.028), implying a positive correlation between NRBC count and HIE stages.*

Conclusion: *Cord blood NRBC count is significantly elevated in asphyxiated neonates and correlates with the severity of hypoxic-ischemic encephalopathy.*

Keywords: *Asphyxiated Newborns, HIE, NRBC*

INTRODUCTION

Perinatal asphyxia is a serious health issue among newborns across the globe, particularly in developing countries. It is one of the leading causes of neonatal death and long-term complications. Perinatal asphyxia occurs during pregnancy or labor when the baby experiences impaired gas exchange, resulting in low oxygen levels (hypoxemia), buildup of carbon dioxide (hypercarbia), and increased acidity in the blood (fetal acidosis), as confirmed with umbilical arterial blood pH<7.[1] The World Health Organization has defined birth asphyxia as "failure to initiate and sustain breathing at birth".[2] National Neonatal Perinatal Database (NNPD) defines perinatal asphyxia as Apgar score of less than seven at one minute of life. NNPD also defined moderate asphyxia as slow gasping breathing or an Apgar score of 4-6 and severe asphyxia as no breathing or an Apgar score of 0-3 at one minute of life[3].

The American Academy of Pediatrics and American College of Obstetricians and Gynecologists has proposed the term perinatal asphyxia should be reserved to describe an infant who manifests with all of the following criteria: [4]

- Profound metabolic or mixed acidemia (pH<7) in umbilical artery blood.
- Persistence of an Apgar score of 0-3 for longer than 5 minutes.
- Neonatal neurologic manifestation (e.g., seizures, encephalopathy, tone abnormalities).

- Multiple organ involvement (e.g., kidney, lungs, liver, heart, intestines).

It quantitates clinical signs of neonatal depression, such as cyanosis or pallor, bradycardia, depressed reflex response to stimulation, hypotonia and apnea or gasping respirations. The score is noted at 1 minute and 5 minutes after birth for all infants and 5-minute intervals after that until 20 minutes for infants with a score less than 7. If Apgar score at 5 minutes is seven or greater, it is unlikely that peripartum hypoxia-ischemia caused neonatal encephalopathy. Perinatal asphyxia is a common cause of neonatal mortality and causes 23% of neonatal death worldwide.[5] Among the leading underlying causes of neonatal death were complications arising from intrapartum events (31%), prematurity (28%), infectious diseases (17%), respiratory disorders (11%), and congenital malformations (8%).[6] NNPD suggest perinatal asphyxia contribute 45.1% of all still birth.[3] In most cases, deaths related to birth asphyxia happen within the first seven days after birth.[7] According to Nepal's 2024-25 Multiple Indicator Cluster Survey, the neonatal mortality rate was 17 deaths per 1,000 live births, with birth asphyxia causing 16% of those deaths.[8] Even though monitoring during pregnancy and labor, as well as newborn resuscitation techniques, have improved, the rate of perinatal asphyxia in developed countries is still about 1.5% of live births. In developing nations, however, the rate appears to be 10 to 15 times higher[1].While nucleated red blood cells (NRBCs) are rarely seen in older children, they are commonly found in newborns' blood. These cells are mainly produced in the fetal bone marrow in response to erythropoietin and are stored there as precursors to reticulocytes and mature red blood cells. Various sudden or long-term triggers can raise the number of circulating NRBC, either by increasing erythropoietin activity or by causing a rapid release from the bone marrow's storage pools.[9]

In chronic fetal hypoxia, often resulting from placental insufficiency, intrauterine growth restriction, or maternal comorbidities, tissue hypoxemia activates hypoxia-inducible factors (primarily HIF-2a), which transcriptionally upregulate erythropoietin (EPO) production predominantly in the fetal liver. Elevated circulating EPO binds to erythropoietin receptors on erythroid progenitor cells (BFU-E and CFU-E) in the bone marrow (and to a lesser extent in extramedullary sites such as the liver and spleen), thereby inhibiting apoptosis, promoting proliferation and accelerated differentiation of the erythroid lineage, and expanding the pool of erythroid precursors. This intense bone marrow stimulation disrupts the normal maturation sequence within the marrow microenvironment, leading to premature release of nucleated red blood cells (NRBCs, or normoblasts) into the fetal circulation before nuclear extrusion and full maturation. Consequently, elevated NRBC counts serve as a sensitive biomarker of the duration and severity of intrauterine hypoxic stress, reflecting both compensatory erythropoiesis to enhance oxygen-carrying capacity and stress-induced alterations in marrow barrier integrity. [9, 10, 11]

Term neonates with APGAR score <7 in one minute was diagnosed as Perinatal Asphyxia.[3] Various indicator is present that predict the severity of perinatal asphyxia: Umbilical cord blood pH, APGAR score, NST and neuroimaging of which cord blood pH remains the gold standard. However, cord blood pH is available in few selected tertiary care hospitals, are expensive and requires sophisticated equipment thus rendering them unreachable for most of the population. This problem is compounded in countries like Nepal where there is a wide gap between the need and accessibility of health services. Therefore, there is a need for simple test to identify perinatal asphyxia. Thus in this study we tried to determine whether cord blood NRBCs per 100 WBCs can be used for early detection and severity of Perinatal Asphyxia.

MATERIALS AND METHODS

This comparative cross-sectional study was conducted in Department of Pediatrics at National Medical College, Birgunj, Nepal from October 2022 to September 2023. The study was reviewed and granted approval by the Medical Research and Ethics Committee of NMCTH, Birgunj on 14th October 2022 (IRC Approval Registration Number: F-NMC/608/079-080). Written informed consent was obtained from parents of enrolled neonates.

All newborns who met the inclusion criteria were enrolled the study. Cases were term gestation neonates with perinatal asphyxia defined as a 1-minute Apgar score <7. Controls were term gestation neonates with a 1-minute Apgar score ≥7 and clear amniotic fluid. Exclusion criteria included multiple pregnancies; preterm, post-term and IUGR neonates; chorioamnionitis; diabetic mother; mother with pre-eclampsia/eclampsia; and TORCH infection.

The power of this study was 90%. The sample size for the case was determined by using the formula[12]

$n=2\sigma^2/(m_1-m_2)^2 x\ f(\alpha,\beta)$

n= required number of subjects in each study

m_1=Mean of expected NRBC count on cases

m_2=Mean of expected NRBC count on normal population

f (α, β) = 10·5

$n=2(19.55)^2/(17.82-1.42)^2 x10.$

n=8026·25/268·96

n = 30 in each group.

Immediately after delivery, 2 ml of umbilical cord blood was collected from the double-clamped cord into an EDTA vial and sent to the pathology laboratory for estimation of nucleated red blood cells (NRBC) per 100 white blood cells (WBC). The evaluating pathologist was blinded to the case/control status of the samples. A thin blood smear was prepared and stained with Leishman stain. NRBCs were counted under an Olympus microscope at 40X magnification and recorded as NRBC per 100 WBC. Asphyxiated newborns were transferred to the Neonatal Intensive Care Unit (NICU) after initial resuscitation, while healthy babies were shifted to the Obstetrics and Gynecology (OBG) ward. Hypoxic-ischemic encephalopathy (HIE) staging was performed using the Sarnat and Sarnat staging system.

Antenatal history of the mother and birth history of the child were obtained, and a detailed physical examination of the neonate was performed. Details of history and clinical examination were entered in a structured data collection form. Data were entered and analyzed using SPSS version 25. Appropriate statistical tests including Student's t-test for two group mean comparison, Chi-square test for categorical variables and ANOVA for HIE stages were used. The NRBC per 100 WBC was compared across different grades of asphyxiated neonates according to Sarnat staging for HIE.

RESULT

The study was performed between October 2022 and September 2023 in Department of Pediatrics at NMCTH, Birgunj, Nepal. A total of 60 term newborns were included, comprising 30 cases and 30 controls.

Table1: Distribution of NRBC in Different Groups

STUDY GROUPS	FREQUENCY	MEAN ±SD	P-VALUE
CASES	30	20±12.17	
CONTROL	30	4.70±3.34	<0.001

Table1 shows the distribution of study groups according to NRBC/100WBC. Among cases mean NRBC count was 20±12.17 and among control mean NRBC count was 4.70±3.34. The study groups showed a statistically significant difference in NRBC counts per 100 WBC.

Table 2: Apgar scores among study groups at 1minute

STUDY GROUP	APGAR SCORE AT ONE MINUTE			P-VALUE
	0-3	4-6	>6	
CASE	66.7%	33.3%	0	<0.001
CONTROL	0	0	100%	

Table 2 shows the distribution of two study groups in terms of Apgar score at 1 minute. Among the cases, 66.7% had severe asphyxia with a one-minute Apgar score of 0-3 and 33.3% had moderate asphyxia with Apgar score 4-6. Among control, all babies had a 1 minute Apgar score of more than 6. There was a statistically significant difference between the study groups in terms of Apgar score at 1 minute.

Table 3: Apgar scores among study groups at five minutes

STUDY GROUP	APGAR SCORE AT 5MINUTE			P-VALUE
	0-3	4-6	>6	
CASE	23.3%	76.7%	0	<0.001
CONTROL	0	0	100%	

Table 3 shows the distribution of two study groups in terms of Apgar score at 5 minutes. Among the cases, 23.3% had severe asphyxia with a five-minute Apgar score of 0-3 and 76.7% had moderate asphyxia with Apgar score 4-6. Among control, all babies had a five-minute Apgar score of more than 6. There was a statistically significant difference between the study groups in terms of Apgar score at five minutes.

Table 4: NRBC/100WBC in different HIE stages

STUDY GROUP	FREQUENCY	NRBC COUNT (MEAN ±SD)	P-VALUE
HIE 1	10	13.30±4.923	0.028
HIE 2	13	21.54±13.908	
HIE 3	7	28±11.221	
TOTAL	30		

Table 4 demonstrates a significant association between NRBC count and HIE severity. The mean NRBC count was highest in stage 3 (28±11.221), followed by stage 2 (21.54±13.908), and lowest in stage 1 (13.30±4.923). This indicates that the NRBC count increases progressively with the severity of HIE, and the difference was found to be statistically significant.

Table 5: Statistics for the diagnostic utility for NRBC/100WBC at a cutoff value of ≥10 NRBC/100 WBC

Sensitivity	93.5%
Specificity	96.6%
PPV	97.7%
NPV	93.3%
AUC FOR ROC	0.97 (95% CI: 0.93, 1.00; p < 0.001)

Table 5: shows the cutoff NRBC value of ≥ 10/100WBC found to have a sensitivity of 93.5%, specificity of 96.6%, PPV of 97.7%, and NPV of 93.3%. Significance and sensitive area for ROC curve was 0.97.

ROC CURVE

As NRBC count correlated with various established markers of perinatal asphyxia like Apgar score at one minute and five minute and development of HIE. The receiver operating characteristics (ROC) graph was plotted to determine the NRBC count cut-off value for predicting birth asphyxia. Birth asphyxia was defined as an Apgar score at 1 minute<7.

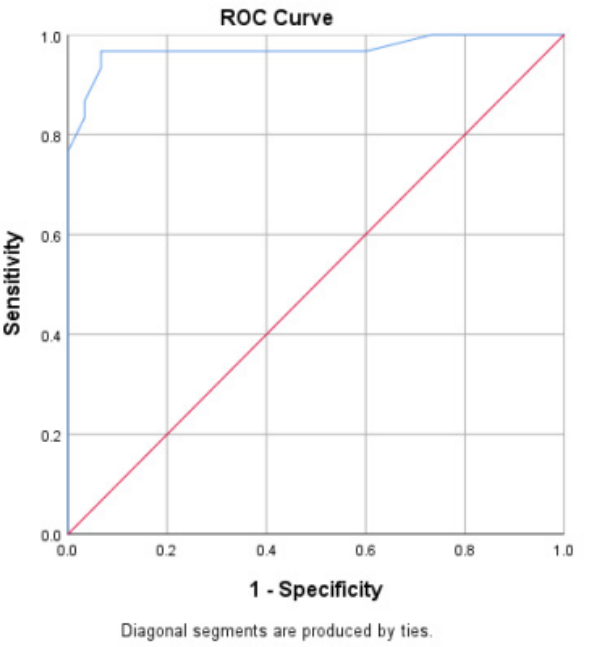

Figure 1: Receiver operating characteristics (ROC) curve to discriminate the sensitivity and specificity of NRBC/100WBC count in the diagnosis of perinatal asphyxia babies

DISCUSSION

In the present study, 60 neonates were studied over 12 months of which 30 were cases and 30 controls.

Nucleated red blood cells (NRBCs) are frequently present in the cord blood of healthy neonates at time of birth. In full-term, non-asphyxiated neonates, the number of NRBCs varies but is seldom higher than 10 per 100 white blood cells (WBCs). However, in babies with perinatal asphyxia, NRBC count per 100 WBCs typically rises during the first few hours of life compared to healthy newborns.[14, 15,16,17,18]

In our study, asphyxiated newborns showed a mean NRBC count of 20.47±12.176 per 100WBC. The results demonstrated that NRBC levels were significantly elevated in the asphyxiated group when compared to healthy babies (p-value < 0.001).

Ferns et al[19] found a statistically significant difference in nucleated red blood cell (NRBC) counts between cases and controls, as well as across different stages of hypoxic-ischemic encephalopathy (HIE), with a p-value of less than 0.001.

In another study conducted by Colaco et al[20], researchers examined 70 cases and 70 control subjects. The mean NRBC/100 WBC was 17.43±19.86 in the case group and 2.97±4.79 in the control group. The difference between the two groups was statistically significant ($p < 0.001$).

Study done by Boskabadi et al[21], 42 cases and 49 control group were studied where the mean NRBC/100WBC was 18.63±16.63 and 3.87±5.06 which was statistically significant ($p<0.001$).

Newborns diagnosed with HIE (hypoxic-ischemic encephalopathy), the number of NRBC (nucleated red blood cells) was higher than in healthy control babies. This NRBC count correlated strongly with the severity of encephalopathy as measured by Sarnat and Sarnat's grading system. Additionally, newborns who later died had elevated NRBC levels compared to those who survived.

Colaco et al[20] found that a rise in NRBC count could serve as an early indicator of future neurological damage.

Table 6: Comparison of mean NRBC count among different HIE stages in our study vs other studies.

	HIE stages	Frequency	NRBC/100WBC (MEAN±SD)
Present study	HIE 1	10	13.30±4.92
	HIE 2	13	21.54±13.90
	HIE 3	7	28.71±11.22
			p-value=0.028
Tungalag et al[22]	HIE 1	12	9.1±3.69
	HIE 2	11	8.25±2.14
	HIE 3	8	12.42±18.21
			P-value<0.001
Colaco et al[20]	HIE 1	10	11.1±9.03
	HIE 2	22	25.95±17.62
	HIE 3	9	45.55±25.88

Table 7: Comparison of Sn, Sp, PPV, and NPV of the test in our study vs other studies.

	Cut off value	Sn	Sp	PPV	NPV
Our study	≥10NRBC/100WBC	93.5%	96.6%	96.7%	93.3%
Hemalatha et al[23]	>6NRBC/100WBC	93.48%	98.15%	97.7%	94.64%
Kanodia et al[24]	≥10NRBC/100WBC	98.67%	97.75%	96.12%	84.47%

In our research, using a threshold of 10 or more NRBCs per 100 white blood cells gave a sensitivity of 93.5% and a specificity of 96.6% with PPV of 96.7% and NPV of 93.3%.

Our findings are consistent with Nepalese data reported by Kanodia et al.[24], who demonstrated high sensitivity and specificity of cord blood NRBC counts in predicting perinatal asphyxia.

In another study done by Rai et al[25] showed that neonates with abnormal neurological status at discharge had a high NRBC count (7.9±6.0) as compared to neonates with normal neurological status (4.4±6.6) with a p-value of 0.007.

In a similar study done by Hemalatha et al[23], an NRBC count of > 6/100 WBC yielded a sensitivity of 93.48% and a specificity of 98.15% with PPV of 97.7% and NPV of 94.64%.

Tungalag et al[22] found that newborns with neurological damage caused by birth asphyxia had markedly elevated NRBC levels relative to the control groups, with a statistically significant difference ($p < 0.001$).

Nucleated red blood cell (NRBC) count from umbilical cord blood represents a simple, cost-effective and feasible marker for perinatal asphyxia in low-resource settings, where it can be manually enumerated on a blood smear with basic microscopy and minimal training, facilitating early risk stratification, timely referral and prognostic assessment even without advanced equipment. Unlike cord blood pH, which provides an immediate indicator of acute fetal academia, NRBC elevation reflects a more prolonged erythropoietin response to hypoxia via erythropoietin stimulation, potentially offering complementary information on the duration and cumulative impact of the insult. Similarly, while cord lactate directly reflects acute anaerobic metabolism and metabolic acidosis, and base deficit quantifies the metabolic component of acid-base imbalance, NRBC counts correlate with asphyxia severity and hypoxic-ischemic encephalopathy staging but may better predict longer-term outcomes such as neurological sequelae or mortality. Integrating NRBC enumeration with these conventional cord gas parameters could enhance

diagnostic accuracy and prognostic value; however, its specificity may be influenced by other hypoxic conditions (e.g. intrauterine growth restriction or preeclampsia), underscoring the need for multimodal assessment and further comparative studies in diverse settings.

CONCLUSION

Nucleated red blood cell (NRBC) count in umbilical cord blood serves as a simple, low-cost, and widely available marker for detecting perinatal asphyxia. Higher NRBC counts were observed among neonates with lower Apgar scores and greater HIE severity. Using a cut-off of ≥10 NRBCs per 100 white blood cells offers high diagnostic accuracy, with a sensitivity of 93.5% and a specificity of 96.6%. Given its minimal infrastructure requirements, this test provides good predictability regarding the clinical course and outcome of perinatal asphyxia, making it highly feasible for any healthcare facility.

CONFLICT OF INTEREST: The authors declare no conflict of interest.

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