Journal of Neonatal Surgery
ISSN(Online): 2226-0439
Vol. 14, Issue 29s (2025)
https://www.jneonatalsurg.com

OPEN ACCESS

The Role of Nucleated Rbc in the Diagnosis of Neonatal Asphyxia with Meconium-Stained and Clear Amniotic Fluid

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Cite this paper as: Dr. Janga. Tarun Siva Nagi Reddy, Dr. M. Thiyagarajan, Dr. Sudarshan.N. E, (2025) The Role of Nucleated Rbc in the Diagnosis of Neonatal Asphyxia with Meconium-Stained and Clear Amniotic Fluid, *Journal of Neonatal Surgery*, 14 (29s), 860-864

ABSTRACT

Introduction: Perinatal asphyxia remains a significant contributor to neonatal morbidity and mortality, particularly in low- and middle-income countries. Early identification of at-risk neonates is critical for initiating timely neuroprotective interventions. Nucleated red blood cells (NRBCs) and lactate levels in cord blood have emerged as promising markers of hypoxia. This study aims to evaluate the diagnostic value of NRBCs and lactate levels in neonates born through meconium-stained and clear amniotic fluid.

Materials & Methods:A comparative cross-sectional study was conducted at Meenakshi Medical College Hospital and Research Institute between January and June 2024. Fifty term neonates were enrolled and divided into Group 1 (meconium-stained amniotic fluid) and Group 2 (clear amniotic fluid). Umbilical cord blood was analysed for NRBC count and lactate levels. Statistical analysis was performed using SPSS, and significance was determined at $p < 0.05$.

Results: Group 1 neonates had significantly higher mean NRBC counts (18.63 ± 4.63) compared to Group 2 (5.87 ± 1.06; $p < 0.001$). Mean lactate levels were also elevated in Group 1 (5.63 ± 1.63 mmol/L) versus Group 2 (3.11 ± 0.6 mmol/L; $p < 0.001$). The diagnostic cut-off for NRBCs >6.5/100 WBCs showed 85% sensitivity and 95% specificity, while lactate >5.1 mmol/L demonstrated 88.9% sensitivity and 100% specificity for predicting neonatal asphyxia.

Conclusion: Elevated NRBC and lactate levels in umbilical cord blood are reliable early indicators of perinatal asphyxia. Their high sensitivity and specificity make them valuable, cost-effective tools for the timely identification and management of at-risk neonates, especially in resource-limited settings.

Keywords: *Perinatal asphyxia, nucleated red blood cells, Lactate, Meconium-stained amniotic fluid, Neonatal hypoxia, Cord blood biomarkers.*

1. INTRODUCTION

Perinatal asphyxia is a significant cause of neonatal morbidity and mortality, particularly in low- and middle-income countries. It occurs due to impaired oxygen delivery to the fetus before, during, or immediately after birth, leading to metabolic acidosis and potential multiorgan dysfunction [1]. One of the most feared consequences of severe perinatal asphyxia is hypoxic-ischemic encephalopathy (HIE), a condition responsible for a large proportion of neonatal neurological disability and death [2,3].

Early identification of neonates at risk for HIE is critical, as the effectiveness of neuroprotective strategies like therapeutic hypothermia diminishes significantly if not started within the first six hours of life [4]. Thus, there is a growing demand for accessible, sensitive, and cost-effective markers for early detection of asphyxia.

Nucleated red blood cells (NRBCs), normally present in small amounts during fetal life, are released in increased numbers into the fetal circulation in response to chronic or acute hypoxia [5]. Elevated NRBC counts in umbilical cord blood have therefore been proposed as an early haematological indicator of perinatal hypoxia and asphyxia [6].

Meconium-stained amniotic fluid (MSAF) is a common obstetric finding, particularly in post-term pregnancies. Its presence has long been associated with fetal distress and hypoxia [7]. Several studies have reported an association between MSAF and increased risk of adverse neonatal outcomes, including birth asphyxia, meconium aspiration syndrome, and neonatal intensive care unit (NICU) admission [8–10]. However, the relationship between MSAF and NRBC count as a marker of intrauterine hypoxia has not been fully elucidated.

In contrast, clear amniotic fluid typically reflects a normal intrauterine environment, but cases of asphyxia may still occur

without overt meconium passage. Thus, comparing NRBC counts in neonates born through meconium-stained versus clear amniotic fluid may help determine its diagnostic relevance across different clinical scenarios.

Blood lactate levels have also been recognized as useful biochemical markers of hypoxia in critically ill neonates, though they require specific biochemical assays and reflect more acute metabolic shifts [11]. In contrast, NRBC counts offer a simpler and cost-effective hematologic assessment, potentially suitable for use in resource-limited settings [12].

This research study aims to find out the relationship between meconium-stained amniotic fluid and nucleated red blood cells in the umbilical cord blood of neonates, and to assess the diagnostic value of NRBC count in identifying neonatal asphyxia in both meconium-stained and clear amniotic fluid scenarios

2. MATERIALS & METHODS

This comparative cross-sectional study was conducted at Meenakshi Medical College Hospital and Research Institute (MMCHRI) over a six-month period from January 2024 to June 2024, with a total sample size of 50 participants.

Inclusion criteria: The study included pregnant women who delivered singleton, live-born infants between 37 and 42 completed weeks of gestation, with either clear or turbid amniotic fluid.

Exclusion criteria: the exclusion criteria comprised preterm deliveries (<37 weeks of gestation), neonates with congenital heart disease, Rh isoimmunisation, chromosomal abnormalities, and those born to mothers with diabetes, preeclampsia, chorioamnionitis, or who had smoked during pregnancy.

Statistical analysis: Data were coded, processed, and analyzed using SPSS software. For qualitative variables, differences between two or more groups were assessed using the Chi-square test. Quantitative data were presented as mean ± standard deviation (SD), and comparisons between two independent groups with normally distributed variables were made using the independent samples t-test. A p-value of less than 0.05 was considered statistically significant.

Methodology: After obtaining informed consent, a thorough history was taken from the parents, including details of presenting complaints, maternal illness during pregnancy, and relevant family history. A complete clinical examination was performed on all neonates. For laboratory analysis, 2 mL of umbilical cord blood was collected in a sterile syringe during neonatal resuscitation and transferred to the clinical pathology department within 30 minutes in an EDTA tube. The sample was fixed with ethanol, and a peripheral smear was prepared using Leishman stain. The number of nucleated red blood cells (NRBCs) was then assessed under microscopy. Participants' confidentiality was maintained, and the study's objectives were clearly explained to parents to ensure informed participation and cooperation.

3. RESULTS

Table1: Basic Characteristics and Obstetric History of the Studied Groups

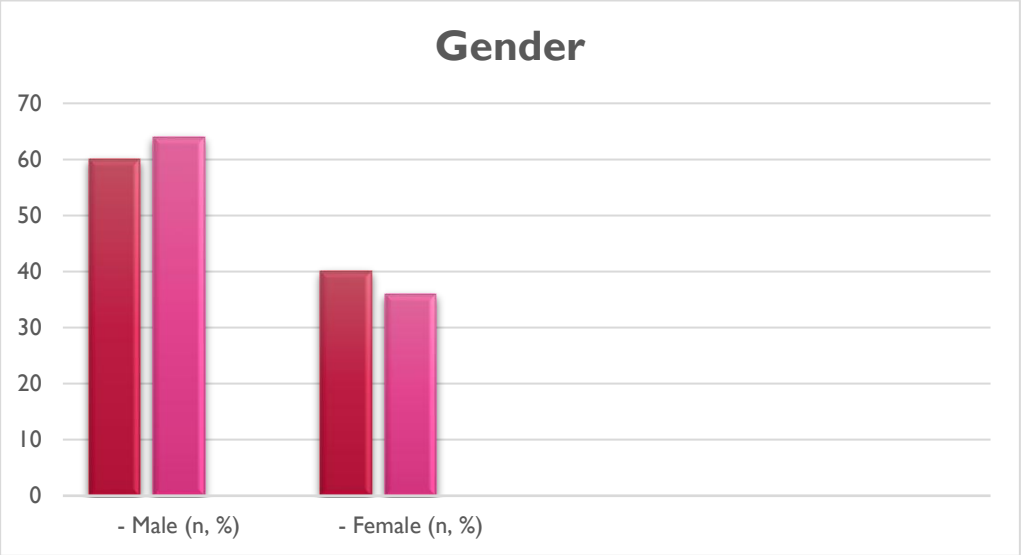
Variables	Group 1 (n=25)	Group 2 (n=25)	p-value
Age (years), Mean ± SD	27.4 ± 6.6	28.3 ± 4.9	0.4
GA at delivery (weeks), Mean ± SD	38.8 ± 1.2	39.0 ± 2.7	0.07
Gravidity			0.4
- Primigravida	3	4	
- Multigravida	22	21	
Parity			0.7
- Primipara	10	8	
- Multipara	15	17	

This table compares the demographic and obstetric parameters between Group 1 (n=25) and Group 2 (n=25). The mean age of participants in Group 1 was 27.4 ± 6.6 years, while in Group 2 it was 28.3 ± 4.9 years, showing no statistically significant difference (p = 0.4). The mean gestational age at delivery was 38.8 ± 1.2 weeks in Group 1 and 39.0 ± 2.7 weeks in Group 2, with a p-value of 0.07, indicating no significant difference. Regarding gravidity, Group 1 included 3 primigravida and 22

multigravida women, while Group 2 had 4 primigravida and 21 multigravida women (p = 0.4). Parity was also comparable between the groups, with Group 1 having 10 primipara and 15 multipara, and Group 2 having 8 primipara and 17 multipara (p = 0.7). Overall, there were no statistically significant differences in the basic characteristics and obstetric history between the two groups.

Table 2: Clinical characteristics of neonates between the studied groups

Variable	Group 1 (n = 25)	Group 2 (n = 25)	p-value
Birth Weight (mean ± SD)	2605 ± 310	2790 ± 278	0.055
Sex			
- Male (n, %)	15 (60%)	16 (64%)	0.74
- Female (n, %)	10 (40%)	9 (36%)	



The table compares birth weight and sex distribution between the two groups, each with 25 participants. The mean birth weight in Group 1 was 2605 ± 310 grams, while in Group 2 it was slightly higher at 2790 ± 278 grams; however, this difference was not statistically significant (p = 0.055). Regarding sex distribution, males made up 60% of Group 1 and 64% of Group 2, while females comprised 40% and 36% respectively, with no significant difference between the groups (p = 0.74). Overall, both groups were comparable in terms of sex distribution, and the slight difference in birth weight approached but did not reach statistical significance.

Table 3: NRBCs/100 WBCs distribution in the studied group

NRBC/100 WBCs	Group 1(n=25)	Group 2(n=25)	P value
Mean	18.63	5.87	<0.001
SD	4.63	1.06	<0.001

The mean nucleated red blood cell (NRBC) count per 100 white blood cells (WBCs) was significantly higher in Group 1 (18.63 ± 4.63) compared to Group 2 (5.87 ± 1.06), with a p-value < 0.001, indicating a statistically significant difference between the two groups. This suggests that elevated NRBC counts may be associated with the condition or exposure characteristic of Group 1.

Table 4: Lactate levels distribution in the studied group

Lactate (mmol/L)	Group 1(n=25)	Group 2(n=25)	P value
Mean	5.63	3.11	<0.001
SD	1.63	0.6	<0.001

The mean lactate level was significantly higher in Group 1 (5.63 ± 1.63 mmol/L) compared to Group 2 (3.11 ± 0.6 mmol/L), with a **p-value < 0.001**. This indicates a statistically significant difference, suggesting that elevated lactate levels may be associated with the condition observed in Group 1.

Table 5: Diagnostic criteria of NRBC's to predict HIE cases

	Cut off	Sensitivity	Specificity	PPV	NPV
NRBCs	>6.5 NRBC's/ 100 WBCs	85	95	96.0	92.3

The cutoff value for NRBCs was determined to be >6.5 NRBCs/100 WBCs, which showed a sensitivity of 85% and specificity of 95%. The positive predictive value (PPV) was 96.0%, and the negative predictive value (NPV) was 92.3%, indicating that NRBC count is a highly reliable marker for predicting the condition assessed in this study.

Table 6: Diagnostic criteria of Lactate to predict HIE cases

	Cut off	Sensitivity	Specificity	PPV	NPV
Lactate (mmol/L)	>5.1	88.9	100	93.3	98.0

For lactate levels, a cutoff value of >5.1 mmol/L demonstrated a sensitivity of 88.9% and a specificity of 100%. The positive predictive value (PPV) was 93.3%, while the negative predictive value (NPV) was 98.0%, indicating that elevated lactate levels are a highly accurate predictor for identifying the studied condition.

4. DISCUSSION

This comparative cross-sectional study evaluated the association between nucleated red blood cells (NRBCs) and lactate levels with neonatal asphyxia among term neonates. The results demonstrated a significantly higher mean NRBC count in neonates with asphyxia (Group 1) compared to those without (Group 2), with values of 18.63 ± 4.63 versus 5.87 ± 1.06 respectively ($p < 0.001$). Similarly, the mean lactate level was significantly elevated in the asphyxiated group (5.63 ± 1.63 mmol/L) compared to controls (3.11 ± 0.6 mmol/L), also with a p-value < 0.001. These findings indicate a strong correlation between elevated NRBCs and lactate with the presence of perinatal asphyxia.

Our findings align with those of Phelan et al., who reported that increased NRBCs serve as a marker of intrauterine hypoxia, often present in cases of birth asphyxia [Phelan JP et al., 1995, *Am J Obstet Gynecol*]. Furthermore, a study by Basu et al. also confirmed that elevated NRBC counts are associated with perinatal hypoxia and can serve as a useful early diagnostic marker [Basu P et al., 2009, *Pediatr Res*].

Regarding lactate levels, our findings are consistent with the study by Martin-Ancel et al., which showed that arterial lactate is a reliable early marker of neonatal hypoxia and correlates with the severity of asphyxia [Martin-Ancel A et al., 1995, *Acta Paediatr*]. Lactate, being a by-product of anaerobic metabolism, reflects tissue hypoxia and acidosis, both of which are hallmarks of perinatal asphyxia.

The sensitivity and specificity of NRBCs >6.5/100 WBCs in predicting neonatal asphyxia were 85% and 95%, respectively, while lactate >5.1 mmol/L demonstrated an even higher sensitivity of 88.9% and perfect specificity (100%). These diagnostic

values support the utility of both markers in early identification and risk stratification of neonates susceptible to hypoxic-ischemic injury. A study by Mehta et al. similarly found high diagnostic accuracy for NRBCs and lactate in identifying hypoxic neonates [Mehta S et al., 2010, *Indian Pediatr*].

Although our study did not find significant differences in maternal demographics or neonatal clinical characteristics (e.g., gestational age, birth weight, and sex distribution) between groups, the hematologic and biochemical parameters clearly distinguished the affected neonates, supporting their use as early biomarkers.

5. CONCLUSION

This study demonstrates that both elevated nucleated red blood cell (NRBC) counts and increased cord blood lactate levels are significant markers of perinatal asphyxia in term neonates. A cut-off value of >6.5 NRBCs/100 WBCs and lactate >5.1 mmol/L showed high sensitivity, specificity, and predictive values for detecting neonatal asphyxia. These findings support the clinical utility of NRBCs and lactate as early, reliable, and cost-effective indicators of hypoxic stress at birth. Incorporating these markers into routine neonatal assessments may help identify and manage at-risk newborns, thereby potentially improving neonatal outcomes

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