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CORRELATION OF CORD BLOOD PH AND NUCLEATED RBC IN NEONATES WITH BIRTH ASPHYXIA: A PROSPECTIVE OBSERVATIONAL STUDY

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ABSTRACT

Background*: Birth asphyxia is a leading cause of neonatal morbidity and mortality. Cord blood pH reflects acute hypoxia, while nucleated red blood cells (NRBCs) reflect prolonged or antenatal hypoxic stress.*

Objectives*: To assess the association between cord blood pH, NRBC count, fetal distress, and short-term neonatal outcome among term neonates with birth asphyxia.*

Methods*: A prospective observational study was conducted over 18 months among 40 term neonates with birth asphyxia. Cord blood pH was measured using a blood gas analyser, and NRBC counts were determined from peripheral smears. Statistical analysis included correlation tests and ROC curve analysis.*

Results*: A significant negative correlation was observed between cord blood pH and NRBC count ($r = -0.384$, $p = 0.014$). Cord blood pH demonstrated better predictive ability (AUC 0.760) compared to NRBC count (AUC 0.577).*

Conclusion*: Cord blood pH is a reliable marker of acute hypoxia, whereas NRBC count reflect prolonged or antenatal hypoxic stress.*

Keywords*: Birth asphyxia, cord blood pH, nucleated RBC, perinatal asphyxia*

INTRODUCTION

Birth asphyxia is a major contributor to neonatal mortality worldwide, particularly in low-resource settings, accounting for a significant proportion of neonatal deaths[1]. It is defined as the inability of the newborn to initiate and sustain adequate respiration, resulting in hypoxia and metabolic acidosis[2]. The severity of hypoxic insult determines the risk of complications such as hypoxic-ischemic encephalopathy (HIE), multiorgan dysfunction, and long-term neurological sequelae[3]. Umbilical cord blood pH is an objective and widely used indicator of neonatal acid-base status at birth. A low cord blood pH reflects metabolic acidosis due to anaerobic metabolism and lactic acid accumulation during hypoxia[4]. Several studies have demonstrated its strong association with adverse neonatal outcomes, including HIE and mortality[5]. Nucleated red blood cells (NRBCs) are immature erythroid precursors that are normally absent or minimally present in peripheral blood. Their increased presence in cord blood is a response to intrauterine hypoxia mediated by erythropoietin stimulation[6]. Elevated NRBC counts have been linked to fetal distress and prolonged hypoxic exposure[7]. Since cord blood pH reflects acute hypoxia and NRBC count reflects chronic hypoxia, their combined assessment may provide a more comprehensive evaluation of perinatal asphyxia. This study aims to correlate these two parameters and assess their clinical utility in predicting severity and outcomes.

Objectives:

1. To estimate NRBC in cord blood of asphyxiated neonates
2. To assess the association between cord blood pH, NRBC count, fetal distress, and short-term neonatal outcome among term neonates with birth asphyxia.

METHODS

This prospective observational study was conducted over 18 months at a tertiary care centre in Kolar, Karnataka. A total of 40 term neonates diagnosed with birth asphyxia were included after obtaining ethical clearance from the institution.

Inclusion Criteria

All neonates with birth asphyxia admitted to the NICU of RL Jalappa Hospital, Kolar, during the study period, whose parents or guardians provided informed consent, were included in the study.

Exclusion Criteria

1. Neonates with Rh isoimmunization
2. Neonates with severe congenital malformations

Sample Size

The sample size was estimated based on the specificity of nucleated red blood cells (nRBCs), reported as 95.2% for a cut-off value >27/100 in predicting adverse outcome in a previous study by Kumar Jena P et al. The sample size was calculated using the formula:

$n = (Z\alpha/2)^2 \times P(1 - P) / d^2$

Where:

- (P) = anticipated specificity (0.952)
- ($Z_{\alpha/2}$) = 1.96 for 95% confidence level
- (d) = absolute precision (0.07)

$P = 0.952$

$Z_{\alpha/2} = 1.96$

$d = 0.07$

$n = (1.96)^2 \times 0.952 \times (1 - 0.952) / (0.07)^2$

$n \approx 36$

"The calculated sample size was 36 neonates. To account for potential exclusions, the target sample size was rounded to 40 neonates."

Data Collection

Umbilical cord arterial blood samples were collected immediately after birth under aseptic precautions. Cord blood pH was measured using an automated blood gas analyser. Peripheral blood smears were prepared, stained, and examined manually to determine NRBC counts per 100 white blood cells.

Statistical Analysis

Data were analysed using SPSS software. Pearson correlation coefficient was used to assess the relationship between cord blood pH and nRBC count. Independent t-test and Chi-square test were applied for comparison between groups. ROC curve analysis was performed to evaluate predictive accuracy. A p-value <0.05 was considered statistically significant.

RESULTS

Table 1: Association between fetal distress and mean umbilical cord blood pH.

	N	Mean	Std. Deviation	95% Confidence Interval for Mean		Minimum	Maximum	P value
				Lower Bound	Upper Bound			
Yes	36	6.94433	.136162	6.89826	6.99040	6.700	7.200	0.152
No	4	7.05250	.180993	6.76450	7.34050	6.800	7.230	
Total	40	6.95515	.142262	6.90965	7.00065	6.700	7.230	

Neonates with fetal distress had a slightly lower mean umbilical cord blood pH (6.94 ± 0.14) compared to those without fetal distress (7.05 ± 0.18). However, this difference was not statistically significant (p = 0.152). Overall, the findings suggest that the presence of fetal distress was not significantly associated with changes in umbilical cord blood pH in this study population.

Table 2: Association between fetal distress and nucleated RBCs.

	N	Mean	Std. Deviation	95% Confidence Interval for Mean		Minimum	Maximum	P value
				Lower Bound	Upper Bound			
Yes	36	12.78	6.419	10.61	14.95	4	30	0.022*
No	4	5.00	1.155	3.16	6.84	4	6	
Total	40	12.00	6.532	9.91	14.09	4	30	

*p value <0.05; Hence statistically significant

Neonates with fetal distress had a markedly higher mean nucleated RBC count (12.78 ± 6.42) compared to those without fetal distress (5.00 ± 1.16). This difference was statistically significant (p = 0.022). The findings indicate a significant association between fetal distress and elevated nucleated RBC levels, suggesting increased fetal hypoxic stress among affected neonates.

Table 3: Correlation between umbilical cord pH and no of NRBCs.

	Mean	Std. Deviation	N	Pearson correlation	P value
Umbilical Cord Blood pH	6.95	.142	40	-0.384	0.014*
No of nucleated RBCs	12.00	6.532	40		

*p value <0.05; Hence statistically significant

There was a moderate negative correlation between umbilical cord blood pH and nucleated RBC count ($r = -0.384$), which was statistically significant ($p = 0.014$). This indicates that lower cord blood pH (greater acidosis) was associated with higher numbers of nucleated RBCs. The findings suggest that increasing fetal hypoxia and acidemia are accompanied by a rise in circulating nucleated RBCs in the neonate.

A total of 40 neonates with birth asphyxia were studied. There was a statistically significant negative correlation between cord blood pH and nRBC count ($r = -0.384$, $p = 0.014$), indicating that increasing acidosis was associated with higher nRBC counts.

Neonates with fetal distress had significantly higher nRBC counts compared to those without fetal distress (12.78 ± 6.42 vs. 5.00 ± 1.16, $p = 0.022$). Cord blood pH showed fair predictive ability for adverse outcomes with an area under the curve (AUC) of 0.760, sensitivity of 77.1%, and specificity of 80% at a cutoff value of 6.81.

In contrast, nRBC count demonstrated lower predictive accuracy with an AUC of 0.577, indicating limited standalone prognostic value.

DISCUSSION

Table 4: Comparison of the present study findings with selected previous studies evaluating cord blood pH and NRBC count in neonatal asphyxia

Parameter	**Present study**	**Previous studies**
Study population	40 term neonates with birth asphyxia	Zakerihamidi et al.: preterm neonates; Chauhan et al.: term neonates with and without birth asphyxia; Ghosh et al.: term neonates with birth asphyxia and healthy controls
Cord blood pH	Mean: **6.95 ± 0.14**	Zakerihamidi et al.: cord pH **<7.2** used as part of the inclusion criteria; Ghosh et al.: **7.06 ± 0.12** in asphyxiated neonates vs **7.29 ± 0.039** in controls
NRBC count	**12.00 ± 6.53 per 100 WBCs**	Chauhan et al.: **10.79 ± 9.58 per 100 WBCs** in cases vs **4.03 ± 2.87 per 100 WBCs** in controls; Ghosh et al.: **16.5 ± 6.4 per 100 WBCs** in cases vs **8.6 ± 7.0 per 100 WBCs** in controls

Parameter	Present study	Previous studies
NRBC count according to fetal distress	Fetal distress: **12.78 ± 6.42** vs no fetal distress: **5.00 ± 1.16 per 100 WBCs**; *p* = **0.022**	Previous studies have reported increased NRBC counts among neonates exposed to fetal hypoxia, birth asphyxia, and perinatal distress
Correlation between cord pH and NRBC count	**r = −0.384, p = 0.014**	Ghosh et al.: significant inverse correlation between cord pH and NRBC count (**r ≈ −0.48, p <0.001**)
Cord pH and adverse outcome	Cord pH: **AUC ≈ 0.760** for mortality	Previous studies have reported an association between lower cord blood pH and adverse neonatal or neurological outcomes
NRBC and adverse outcome	NRBC: **AUC ≈ 0.577** for mortality	Zakerihamidi et al.: elevated NRBC associated with adverse neonatal outcome; Chauhan et al.: higher NRBC associated with abnormal neurological outcome; Ghosh et al.: higher NRBC associated with HIE and adverse early neonatal outcome
Clinical outcome	**35 (87.5%)** discharged; **5 (12.5%)** deaths	Previous studies have reported associations between elevated NRBC levels, HIE, neurological abnormalities, and adverse neonatal outcomes
NRBC cutoff	No clinically validated cutoff established in the present study	Zakerihamidi et al.: **NRBC >370** reported as predictive; Chauhan et al.: **>3 NRBC/100 WBCs** associated with asphyxia; Ghosh et al.: **NRBC >18/100 WBCs** associated with abnormal neurological outcome
Overall biomarker findings	Cord pH showed better discrimination for mortality than NRBC; NRBC was significantly associated with fetal distress and inversely correlated with cord pH	Previous studies have demonstrated associations of cord blood pH and/or NRBC count with perinatal hypoxia, birth asphyxia, HIE, and adverse neonatal or neurological outcomes

The findings of the present study are broadly consistent with previous studies demonstrating an association between perinatal hypoxia, umbilical cord blood pH, and NRBC count. In the present cohort, lower cord blood pH was significantly associated with higher NRBC counts, and neonates with documented fetal distress had significantly higher NRBC counts than those without fetal distress. This inverse relationship between cord blood pH and NRBC count is comparable to findings reported in previous studies and may reflect the complementary biological responses to hypoxic stress, with cord blood pH representing the metabolic consequences of hypoxia at birth and NRBC elevation reflecting the fetal erythropoietic response to hypoxic exposure. However, the prognostic performance of NRBC count in the present study was lower than that reported in some previous studies. This variation may be attributable to differences in gestational age, sample size, study design, definitions of birth asphyxia and adverse outcomes, timing of blood sampling, and methods of NRBC quantification. In the present study, cord blood pH demonstrated better discrimination for mortality than NRBC count, suggesting that cord blood pH may have greater utility for immediate risk assessment, while NRBC count may provide complementary information regarding fetal hypoxic stress. Further larger prospective studies using standardised outcome definitions and long-term neurodevelopmental follow-up are required to determine the independent and combined prognostic value of these biomarkers.

CONCLUSION

This study demonstrated a significant inverse correlation between umbilical cord blood pH and NRBC count among term neonates with birth asphyxia. Neonates with documented fetal distress had significantly higher NRBC counts, whereas cord blood pH did not differ significantly according to fetal distress status. Cord blood pH demonstrated better discriminative ability for mortality than NRBC count, while the prognostic value of NRBC count alone was limited. These findings suggest that cord blood pH may be a more useful marker of immediate severity and short-term outcome, whereas NRBC elevation may reflect the degree of fetal hypoxic stress. Larger prospective studies with standardised definitions of perinatal asphyxia and clinically relevant long-term neurological outcomes are required to determine the independent and combined prognostic value of cord blood pH and NRBC count.

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