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Comparison of the NRBC/100 WBC Ratio in Blood Samples at Birth between Normal Premature Newborns and Deceased Infants

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Abstract

Background: This study aimed to compare the Nucleated red blood cells (NRBC)/100 White blood cells (WBC) ratio in blood samples collected at birth between normal premature newborns and deceased infants.

Methods: This cross-sectional descriptive study was conducted on preterm infants younger than 32 weeks who were admitted to the neonatal ward of Ghaem Hospital in Mashhad, Iran, from 2017 to 2024 using available sampling. Deceased infants were included in the case group, whereas those with normal development until discharge from the hospital were included in the control group. The NRBC results were compared between the two groups. All data were recorded and analyzed using SPSS version 25.

Results: The study included 277 children; 172 (62.1%) had a normal prognosis (control group) and 105 (37.9%) died (case group). The mean absolute NRBC count in deceased infants was 1718.59 ± 3693.77, approximately 13 times higher than that in infants with a normal prognosis. The NRBC/100 WBC ratio was five times higher in deceased infants than in those with normal prognosis. An absolute NRBC count >300/mm² indicated poor prognosis, with 75% sensitivity and 63% specificity. An NRBC/100 WBC ratio >11 predicted infant death with 88.6% sensitivity and 71.2% specificity (P=0.000). An absolute NRBC >190/mm² predicted infant death with 86% sensitivity and 82.2% specificity (P=0.000).

Conclusion: This study highlighted the prognostic value of NRBC counts in newborns and showed that the NRBC/100 WBC ratio was five times higher in deceased infants. Regular NRBC monitoring can guide early intervention and improve neonatal care and survival rates by identifying at-risk infants and tailoring medical treatment to enhance outcomes.

Key Words: Infants, Nucleated red blood cell, Premature, Prognosis, White blood cells.

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1- INTRODUCTION

A premature birth is a traumatic physiological event that can compromise the neurological and emotional development of infants (1). Common causes of premature birth include multiple pregnancies, chronic health conditions (e.g., diabetes, high blood pressure, and heart or kidney disease in the mother), infections of the urinary tract or amniotic membrane, lifestyle factors (smoking, illegal drug use, or excessive alcohol consumption during pregnancy), uterine abnormalities, premature birth, and socioeconomic factors (2). Infertility treatment also increases the likelihood of conception in infertile mothers, which in turn increases the incidence of prematurity (3). The survival rate of premature babies has significantly improved because of advances in neonatal care. Anticipating and controlling the potential problems of premature babies can help reduce these issues. Although many concerns have been raised about predicting problems in premature babies, the identified laboratory factors are few and non-specific (4).

Nucleated red blood cells (NRBCs) are immature blood cells that still contain nuclei. Although NRBCs are rarely observed in the bloodstream of older children, they frequently appear in the blood of newborns, especially those born prematurely (5). In healthy babies, the number of NRBCs is less than 10 per 100 white blood cells (WBC), and it decreases rapidly after birth, usually becoming undetectable by the fourth day (6). However, in premature babies, the NRBC count is typically higher and can remain detectable in peripheral blood for up to a week after birth. The fetal bone marrow primarily produces NRBCs in response to erythropoietin and stores them as precursors for reticulocytes and mature erythrocytes (7). Chronic stimuli can increase the number of circulating NRBCs due to heightened erythropoietic activity or sudden discharge of cells from marrow storage pools. As NRBCs mature, they typically lose their nuclei, making their presence in older children and adults unusual and indicative of stress or anemia (8). The NRBC/100 WBC ratio is a vital diagnostic tool for newborns. This ratio is significant because it can indicate stress or hypoxia (low oxygen levels) experienced by newborns before or during birth (5). An increased NRBC/100 WBC ratio has been reported in inflammation, cerebral hemorrhage (9), retinopathy (10), and perinatal asphyxia (11).

Given that no studies have measured the NRBC count in the peripheral blood at birth in relation to the prognosis of preterm infants, monitoring the NRBC/100 WBC ratio is crucial for understanding the infant's condition, guiding treatments, and improving outcomes (12). Identifying and addressing these factors early can significantly affect the health and growth of infants, making this ratio a valuable and cost-effective indicator of infant care (13). Therefore, this study aimed to compare the NRBC/100 WBC ratio in blood samples collected at birth between normal premature newborns and deceased infants.

2- MATERIALS AND METHODS

2-1. Study Population

This cross-sectional descriptive study was conducted on preterm infants younger than 32 weeks who were admitted to the neonatal ward of Ghaem Hospital in Mashhad, Iran, from 2017 to 2024 using available sampling. All premature babies born during this period whose parents consented to participate were included in the study. The exclusion criteria were infants born to mothers with diabetes, preeclampsia, intrauterine growth retardation, exposure to smoking or intrauterine infections, chorioamnionitis, hemolytic jaundice, Coombs-positive status, cyanotic heart disease, severe intraventricular hemorrhage (IVH), birth

asphyxia, bronchopulmonary dysplasia (BPD), seizures, brain anomalies, and chromosomal abnormalities.

2-2. Study Protocol

A 1.5-cc whole blood sample from the infant at admission time, typically discarded, was collected in ethylenediaminetetraacetic acid (EDTA) anticoagulant vials to evaluate the NRBC count. Peripheral blood samples were prepared, and Leishman staining was performed. The number of NRBCs was calculated for each 100 blood cells. The researcher recorded the characteristics of the babies (gestational age, Apgar scores at one and five minutes, birth weight) and the test results (WBC, platelets, NRBC count, Neutrophils, Lymphocyte, Hematocrit). Gestational age was determined using the Ballard score, last menstrual period (LMP), and first trimester ultrasound. Infants were followed until discharge or death. Deceased infants constituted the case group, whereas those with normal development constituted the control group. The NRBC results were compared between the two groups.

2-3. Statistical Analysis

Data analysis was performed using t-tests, chi-square tests, and SPSS software (version 25; Chicago, IL, USA). The results were initially described using statistical tables and graphs. The two groups of infants, those with normal and abnormal prognosis, were then compared using the chi-square test and t-test. The receiver operating characteristic (ROC) curve was used to evaluate the sensitivity and specificity of the NRBC/100 WBC count and absolute NRBC count. The significance level was set at $p \leq 0.05$.

2-4. Ethical Considerations

The study protocol was reviewed and approved by the Ethics Committee of Mashhad University of Medical Sciences, Mashhad, Iran IR.MUMS.MEDICAL.REC.1397.088961620.

3- RESULTS

This study involved 400 infants. However, 5 babies with congenital anomalies, 6 with evidence of hemolytic anemia, 26 born to diabetic mothers, 17 with incomplete follow-up, and 69 with abnormal development were excluded. Ultimately, 277 children were successfully followed up, with 172 (62.1%) being normal (control group) and 105 (37.9%) deceased (case group). The clinical and laboratory characteristics of the two groups are summarized in Table 1.

According to the statistical analysis, the Apgar score at the fifth minute, gestational age, baby weight, neutrophil count, and lymphocyte count were significantly higher in the control group than in the case group ($p<0.05$). Additionally, the WBC count, NRBCs/100 WBCs, and absolute number of NRBCs were significantly lower in the control group than in the case group ($P <0.05$).

In this study, the mean absolute number of NRBC/mm² in deceased infants was 1718.59 ± 3693.77, which is approximately 13 times the mean absolute NRBC in infants with a normal prognosis. After controlling for the confounding factor of gestational age with the regression model, the number of NRBCs/100 WBCs still had a significant difference between the two groups(P=0.009;OR:2.494, 95%CI: 1.393-4.464).

Furthermore, the NRBC/100 WBC ratio in deceased infants was significantly 5-fold higher than that in infants with a normal prognosis. An absolute NRBC count > 300/mm² indicated a poor prognosis, with a sensitivity of 75% and specificity of 63%.

Table-1. Comparison of mean infant variables according to group.

Variable	Control group (n=172) Mean ± SD	Case group (n=105) Mean ± SD	t*	P-value
Apgar score at the first minute	6.34 ± 1.95	4.69 ± 2.11	6.257	0.584
The Apgar score at the fifth minute	7.95 ± 1.44	6.58 ± 1.63	6.942	0.015
Gestational age (weeks)	31.98 ± 2.67	28.91 ± 2.45	9.264	<0.001
Baby weight (g)	1639.26 ± 613.57	1176.84 ± 375.27	6.499	<0.001
Platelet (thou/mm^3)	24281.19 ± 71243.30	18353.80 ± 51872.45	0.661	0.509
Hematocrit (%)	45.39 ± 7.28	40.81 ± 8.85	3.962	0.076
Neutrophils (%)	44.32 ± 14.67	36.50 ± 18.89	3.292	0.001
Lymphocyte (/µl)	50.45 ± 15.82	57.51 ± 21.84	-2.730	0.007
White blood cells (WBC) (thou/mm^3)	10.44 ± 6.71	14.11 ± 12.72	-3.109	<0.001
Absolute number of NRBCs (mm^2)	124.69 ± 228.27	1718.59 ± 3693.77	-5.599	<0.001
NRBCs/100 WBCs	11.83 ± 15.34	58.43 ± 105.30	-5.662	<0.001
*** T-Test**				

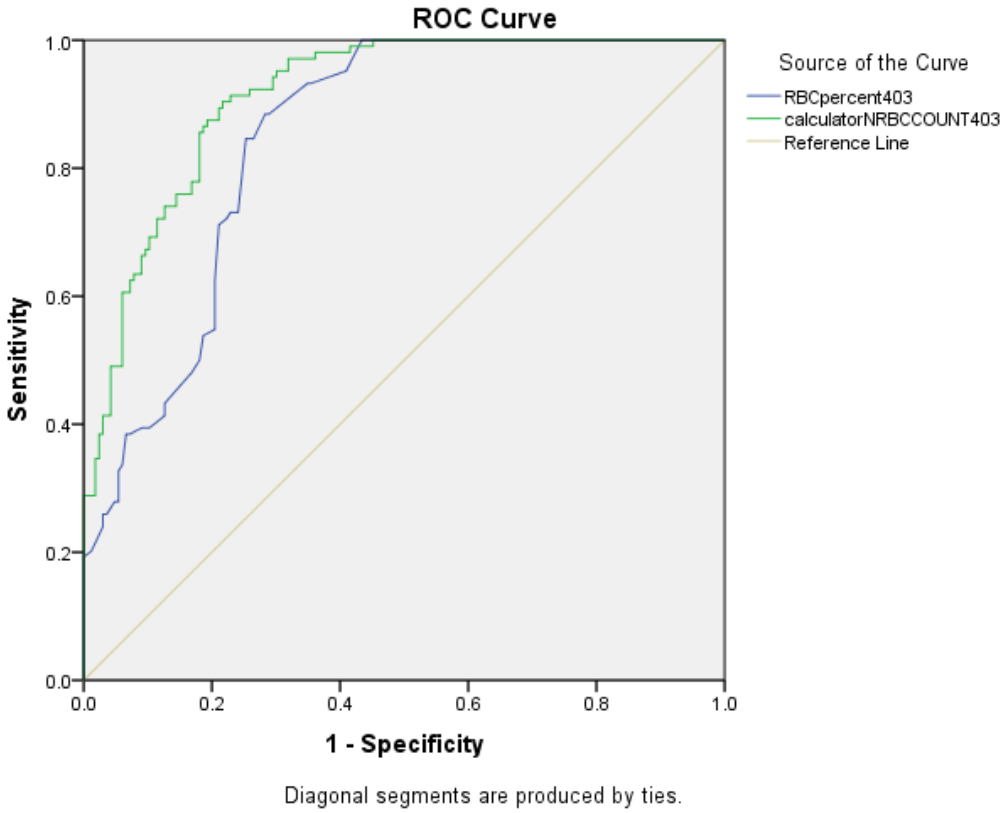

Figure-1. ROC Curve for Sensitivity and Specificity of NRBC/100 WBC and Absolute NRBC to Predict Infant Death [NRBC/100 WBC >11 (P=0.000); NRBC >190/mm² (P=0.000)].

Examination of the sensitivity and specificity of NRBC/100 WBC and absolute NRBC in predicting infant death showed that an NRBC/100 WBC ratio >11 suggests the possibility of infant death with 88.6% sensitivity and 71.2% specificity (P=0.000). Additionally, an absolute NRBC >190/mm² suggested the possibility of infant death with 86% sensitivity and 82.2% specificity (P=0.000) (Figure 1).

4- DISCUSSION

Considering that the NRBC/100 WBC ratio is a valuable tool for assessing health status, diagnosing hypoxia, and predicting outcomes in premature infants, regular monitoring of this ratio can facilitate early intervention and enhance the care of vulnerable infants (14). The study compared the NRBC/100 WBC ratio in blood samples at birth between normal premature babies and deceased infants. Results showed that the Apgar score, gestational age, baby weight, neutrophil and lymphocyte counts were significantly higher in normal premature babies. Deceased infants had a mean absolute NRBC count approximately 13 times higher than infants with a normal prognosis, and the NRBC/100 WBC ratio was 5 times higher in deceased infants. An NRBC count >300/mm² indicated a poor prognosis, with a sensitivity of 75% and a specificity of 63%.

The study results showed that 5 minutes after birth, normal premature infants had higher Apgar scores, were older in terms of gestational age, and weighed more compared to infants who died. The NRBC/100 WBC ratio exhibited significant variation, indicating underlying hematological processes. Premature delivery significantly influences the NRBC/100 WBC ratio (15). Premature infants born before 37 weeks of gestation often exhibit higher NRBC/100 WBC ratios than full-term infants. This elevated ratio reflects increased erythropoietic activity in response to intrauterine stressors, such as hypoxia and infection, which are more common in preterm deliveries (16). The immature bone marrow of premature infants produces more NRBCs, leading to higher blood NRBC counts (17). Medical experts can use Apgar scoring, including NRBC metrics, to evaluate neonatal adjustment immediately after birth, providing essential insights into an infant's health and survival prognosis (13). Greif et al. reported that although the evaluation started one minute after birth, more accurate readings were obtained at the fifth minute. To date, the NRBC/100 WBC ratio in stillbirths has likely remained >5. Thus, every neonate's fifth-minute Apgar score can help determine their NRBC status and assist in correlating NRBC with the pulmonary and systemic circulation (18). Monitoring the NRBC/100 WBC ratio in relation to gestational age and Apgar score helps healthcare providers assess the severity of stress and potential complications, enabling timely and appropriate medical interventions to improve outcomes in vulnerable infants (19).

Premature infants born before 37 weeks of gestation often have higher NRBC counts than full-term infants. A high NRBC count reflects the body's attempt to produce more red blood cells to compensate for critical conditions (20). Cremer et al. observed a significant relationship between increased NRBC counts and mortality in both deceased and surviving infants (21). Sokou et al. showed that the mean NRBC count was significantly associated with perinatal death, necrotizing enterocolitis, and intraventricular hemorrhage (8). El Mashad et al. reported that infants with bronchopulmonary dysplasia and severe intraventricular hemorrhage who died had a higher mean NRBC count and a poorer prognosis (22). The results of the current study showed that the mean absolute number of NRBC/mm² in deceased infants

was approximately 13 times higher than that in infants with a normal prognosis. Additionally, the NRBC/100 WBC ratio in deceased infants was significantly 5-fold higher than that in infants with a normal prognosis. Most studies have reported that premature infants with elevated NRBC counts have an increased risk of complications, such as hypoxic-ischemic encephalopathy, and higher mortality rates (23-25). Consistent evaluation of NRBC levels can facilitate early identification of these issues, thereby enabling prompt and appropriate medical interventions. By examining NRBC counts along with other clinical indicators, medical professionals can gain a more comprehensive understanding of premature infants' overall health status and potential outcomes, ensuring that they receive appropriate care to support their growth and development (26).

Our study demonstrated a strong correlation between elevated NRBC counts and poor prognosis in infants. Specifically, we found that the NRBC/100 WBC ratio was five times higher in deceased infants than in those with a normal prognosis. Additionally, an NRBC count exceeding 300/mm² was identified as a significant predictor of poor outcomes, with a sensitivity of 75% and specificity of 63%. These results align with those of Boskabadi et al., who reported similar predictive values for poor prognosis, albeit with higher sensitivity (85%) and lower specificity (23%) (23). Furthermore, our study contributes to a growing body of evidence suggesting that elevated NRBC counts can serve as a prognostic marker for various complications in infants. This is supported by the work of Kil et al., who found that an NRBC count >100/11 WBC had predictive value for complications in infants with asphyxia, although with lower sensitivity (45%) and specificity (12%) compared with our findings (27). Poryo et al. also provided valuable insights into the predictive power of NRBC counts in newborns. Their findings suggest that an NRBC count of 70 per 100 WBCs on the fourth day after birth can effectively predict serious complications with a high sensitivity of 82%. This information is particularly useful for neonatologists and pediatricians to identify infants at risk of developing severe health issues, thereby allowing for early intervention and appropriate management strategies (28). In contrast, another study examined the diagnostic utility of NRBC counts in differentiating between infants in the patient and control groups. The researchers found that using a threshold of more than 10 nucleated red blood cells yielded a very low sensitivity of 33.3% but an exceptionally high specificity of 100% (29). This stark difference in results compared with the study by Poryo et al. underscores the potential utility of NRBC count as a noninvasive and readily available biomarker for assessing infant health and predicting long-term outcomes. However, further research is needed to establish standardized cutoff values and to investigate the underlying mechanisms linking elevated NRBC counts to poor prognosis in infants.

The small sample size is a significant limitation, potentially affecting the generalizability and statistical power of the findings. While this constraint was unavoidable owing to the preliminary nature of the research, it underscores the need for larger-scale investigations to validate and expand upon the results. Future studies with more extensive participant pools could provide more robust and representative data, allowing for more definitive conclusions and potentially uncovering additional insights that may have been overlooked during this initial exploration. Another notable limitation of this study was the lack of consideration of the clearance time of NRBCs from neonatal venous blood. This

oversight precludes the examination of crucial parameters related to NRBC clearance and their potential significance in neonatal health assessments. Understanding the dynamics of NRBC clearance could offer valuable insights into neonatal physiology and could serve as an indicator of various neonatal conditions. Future research should incorporate time-based measurements of NRBC levels to elucidate the clearance process and its clinical implications, thereby addressing this gap in the current study and enhancing our understanding of neonatal hematology.

5- CONCLUSION

The findings of this study highlight the significant prognostic importance of NRBC count in assessing newborn health outcomes. Deceased infants had higher absolute NRBC counts compare to those with normal prognoses, and the NRBC/100 WBC ratio was notably elevated in infants who did not survive. These results emphasize the crucial role of NRBC monitoring in early intervention strategies, potentially leading to improved neonatal care and increased survival rates. Furthermore, integrating NRBC monitoring into standard neonatal care protocols could contribute to more comprehensive risk assessments, enabling a proactive approach to managing infant health and reducing mortality rates in neonatal intensive care units.

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7-CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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