Original Article

Early Diagnosis of Perinatal Asphyxia by Nucleated Red Blood Cell Count: A Case-control Study

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Abstract:

Background: Perinatal asphyxia is a major cause of neurologic morbidity and mortality. The purpose of this study was to investigate variations in nucleated red blood cell (NRBC) count per 100 white blood cells (WBC) and absolute NRBC/mm^3 in blood associated with perinatal asphyxia and its relationship to both the severity and short term prognosis of asphyxia.

Methods: A prospective (case-control) study was undertaken between October 2006 and December 2008, in the Neonatal Intensive Care Unit, Ghaem Hospital, Mashhad, Iran. A total of 91 infants completed the study. Levels of nucleated red blood cell per 100 white blood cells and absolute nucleated red blood cell counts in venous blood were compared for 42 asphyxiated (case group) and 49 normal neonates (control group). These parameters were also related to the severity of asphyxia and clinical outcome.

Results: The NRBC/100 WBC and absolute nucleated red blood cell levels in the blood of newborns in the control group were 3.87±5.06 and 58.21±87.57/mm^3, respectively; whereas the corresponding values in the cases were 18.63±16.63 and 634.04±1002/mm^3, respectively (P<0.001). A statistically significant negative correlation existed between nucleated red blood cell level and indicators of the severity of perinatal asphyxia, first minute Apgar score and blood pH (P<0.001), respectively. A positive correlation was demonstrated between these parameters and severity of asphyxia, acidosis, and poor outcome (P<0.05).

Conclusions: The NRBC/100 WBC and/or absolute nucleated red blood cell are simple markers for assessment of severity and early outcomes of perinatal asphyxia.

Keyword: asphyxia, diagnosis, hypoxic ischemic encephalopathy, nucleated red blood cells (NRBC)

Introduction

Asphyxia is a major cause of acute mortality and chronic neurologic disability amongst survivors, and is a complication that occurs between 2 – 10% of deliveries.[1] Parameters that have been used to predict or define perinatal asphyxia include: intrapartum electronic fetal monitoring, fetal or umbilical cord pH measurement, meconium-stained amniotic fluid, Apgar score, hypoxic ischemic encephalopathy (HIE), and major organ disorder. However, no single marker of perinatal asphyxia has shown good predictive efficiency and only a combination of various indices can help in the early diagnosis of perinatal asphyxia.[2,3]

Nucleated red blood cells (NRBC) are commonly seen in the blood of neonates. NRBC counts in umbilical venous blood of neonates has been reported as a possible marker of perinatal asphyxia.[4] The hypoxic event induces a compensatory response in the form of exaggerated erythropoesis, resulting in the release of immature red blood cells into the fetal circulation. The levels of NRBC may be correlated with the presence of perinatal asphyxia.[2,5] The number of NRBC/100 WBC is variable but is rarely greater than 10 in normal neonates.[4,6–8]

Because the present indices of asphyxia are un-

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helpful in the diagnosis and prediction of the severity of asphyxia, we wished to investigate the relationship between the absolute NRBC count, NRBC count/100 WBC, the severity of perinatal asphyxia and clinical outcomes in neonates, and to compare these with other established indices such as Apgar score and blood gas pH value.

Materials and Methods

A prospective, case control study was conducted between October 2006 and December 2008, in Ghaem Hospital, Mashhad, Iran. Informed parental consent was obtained for every neonate, before recruitment into the study. The study was approved by the Ethical Committee of Mashhad University of Medical Sciences. Of 4376 infants born during the duration of the study, 59 cases (1.35%) were originally eligible for inclusion, however, 17 were excluded for the following reasons: prematurity (n=5), intrauterine growth retardation (n=3), congenital or perinatal infection (n=3), hemolytic anemia (n=1), congenital malformation (n=1), mothers' pre-eclampsia (n=3), and maternal diabetes (n=1). Originally, 55 neonates were recruited as controls, but 6 were excluded for the following reasons: hemolytic jaundice (n=2), anemia (n=1), cyanotic heart disease (n=1), maternal smoking (n=1), and respiratory distress (n=1).

Inclusion criteria

Perinatal asphyxia was defined as the presence of at least two of the following conditions:

1) Signs of fetal distress (heart rate of less than 100 beats per minute, late decelerations, or an absence of heart rate variability)

2) Thick, meconium stained amniotic fluid and respiratory depression, hypotonia, or bradycardia

3) Apgar score of 4 or less at one minute or 6 or less at five minutes

4) A need for resuscitation for more than 1 minute with positive pressure ventilation and oxygen immediately after birth.

5) Blood pH value of less than 7.20 or a base deficit of at least 12 mmol/L within the first hour after birth.

There were 49 healthy neonates, defined as those who were free of any of the above inclusion criteria and an uneventful postnatal clinical course during the first week of life which were recruited as the control group. The entry criteria for these normal nonasphyxiated newborns were as follows: appropriate for gestational age neonate at more than 37 weeks gestation, birth weight >2500 g, Apgar score >7 at both one and five minutes, normal intrapartum fetal heart rate (FHR) pattern, clear amniotic fluid, normal neurologic evaluation at the first week, and hematocrit >40%.

Clinical assessment

The neurologic examination used to evaluate neurologic function of the term neonates at birth, 2 and 7 days of life, included: a systematic assessment of mental status (level of alertness), cranial nerve function and the motor and sensory systems. In particular, the motor examination included an assessment of spontaneous movement and muscle tone. Posture and resistance of muscles to passive movement were used to assess active tone. Newborn neurologic examination was performed by a single neonatalogist without knowledge of the NRBC level.

According to the criteria of Sarnat and Sarnat, HIE was classified as mild (Grade 1) if hyper-excitability, hyper-alertness, or hyper-reflexia persisted without seizures for at least 24 hours after birth; as moderate if the infant was lethargic, had hypotonia, weak primitive reflexes, pupil miosis, and seizures; and as severe if the infant had apnea, flaccid weakness, frequent seizures, decelerated posture, or coma. The outcome was classified as favorable or adverse. A favorable outcome was defined as normal neurological development and good general condition at the end of the first month. Adverse outcome was defined as the presence of at least one of the following conditions: death, hemiplegia, hypertonicity or significant hypotonia, unreliable sucking, seizures resistant to Phenobarbital, and sensory neural hearing loss.

Laboratory measurement

Blood culture, cerebrospinal fluid culture, urine culture, serum creatinine, sodium, potassium, calcium, and arterial blood gas were determined at the request of the clinicians at the initial evaluations. For complete blood cell count (CBC) and NRBC counts, 2 mL of umbilical blood were collected and delivered in sterile tubes that contained K3-EDTA anticoagulant. Initially, CBCs were measured us-

Table 1. Clinical characteristics of the studied population

Group	Asphyxia	Control	*P*-value
Number	42	49	—
Birth weight (gr)	2920±405	3070±416	0.397
Gestational age (weeks)	37.9±1.5	39.1±1.6	0.068
First minute Apgar score	4.0±1.6	8.4±0.7	<0.001
Fifth minute Apgar score	5.6±1.8	8.8±0.6	<0.001
Mode of delivery (ND/CS)	34/14	13/29	<0.001
Maternal age	27.6±6.0	27.3±5.3	0.853
Sex (male/female)	25/24	26/16	0.403
Values expressed as mean±SD or number (%)			

Table 2. Laboratory characteristics of the studied population

	Healthy newborn	Asphyxia newborn	*P*-value
Hematocrit	48.41±8.21	46.65±6.61	0.975
Platelet count ($\times 10^3/mm^3$)	200.7±69	156.0±69	<0.001
pH	7.33±0.05	7.11±0.16	<0.001
Base excess	-3.24±4.81	-11.58±10.12	<0.001
WBC ($\times 10^3/mm^3$)	14.13±8.34	21.93±15.06	<0.001
NRBC/100 WBC	3.87±5.06	18.63±16.63	<0.001
Absolute NRBC/mm^3	58.21±87.58	634.04±1002	<0.001
Values expressed as mean±SD. WBC= white blood cell; NRBC= nucleated red blood cell			

ing a Kx-21 calibrated electronic counter (obtained from Sysmex Company, Model Kx-21, Japan). Subsequently, for all subjects, blood smears with Geimsa staining prepared from the drawn blood and NRBCs were examined. The number of NRBCs was counted during the differential count.

Statistical analysis

Statistical comparisons were made by Mann-Whitney rank–sum tests, unpaired *t*-tests, ANOVA, Kruskal Wallis, and Chi-square tests as required. Pearson correlation coefficient and Spearman rank correlation coefficient were performed using SPSS 11.5 software. Receiver-operating characteristic (ROC) curves were also constructed allowing the calculation of positive and negative predictive values. A *P* value of <0.05 was considered statistically significant.

Results

Among 114 neonates who were originally recruited into the study, 91 (83.4%) completed the study [cases (n=42) and controls (n=49)]. There was no statistically significant difference (*P*<0.05) between the two groups regarding weight, gender, gestational age, maternal age, and maternal parity (Table 1). In comparison with the controls, the cases had a significantly lower Apgar score during the first minute and five minutes post partum, likelihood of cesarean section and complications during delivery (*P*<0.001 for all, Table 1).

Among 42 infants with perinatal asphyxia, 6 infants had asphyxia without HIE, 17 had an HIE grade 1, 8 had grade 2, and 11 had grade 3. All infants in the asphyxia group had negative body fluid cultures, and received antibiotic treatment for five days or less. CT brain scans were normal in infants with no HIE as well as in the infants with stage 1 HIE. Of the 18 infants with HIE stages 2 or 3 who had CT scans; 8 were found to have diffuse brain edema, 7 had homogenous hyper-echogenicity, and 3 had subarachnoid hemorrhage.

The NRBC counts were found to be 3.81±5.06/100 leukocytes for healthy infants and 18.63±16.62 for asphyxiated infants (*P*<0.001). The absolute NRBC counts were 58.21±87.57 for the control group and 634.04±1002 for cases, respectively (*P*<0.001). Mean pH was 7.33±0.05 for the control group and 7.11±0.7 for cases (*P*<0.001). The Base excess was -3.24±4.81 for the control group and -11.58±10.12 for cases (*P*<0.05, Table 2). The mean hematocrit was not different between the two group (*P*>0.05).

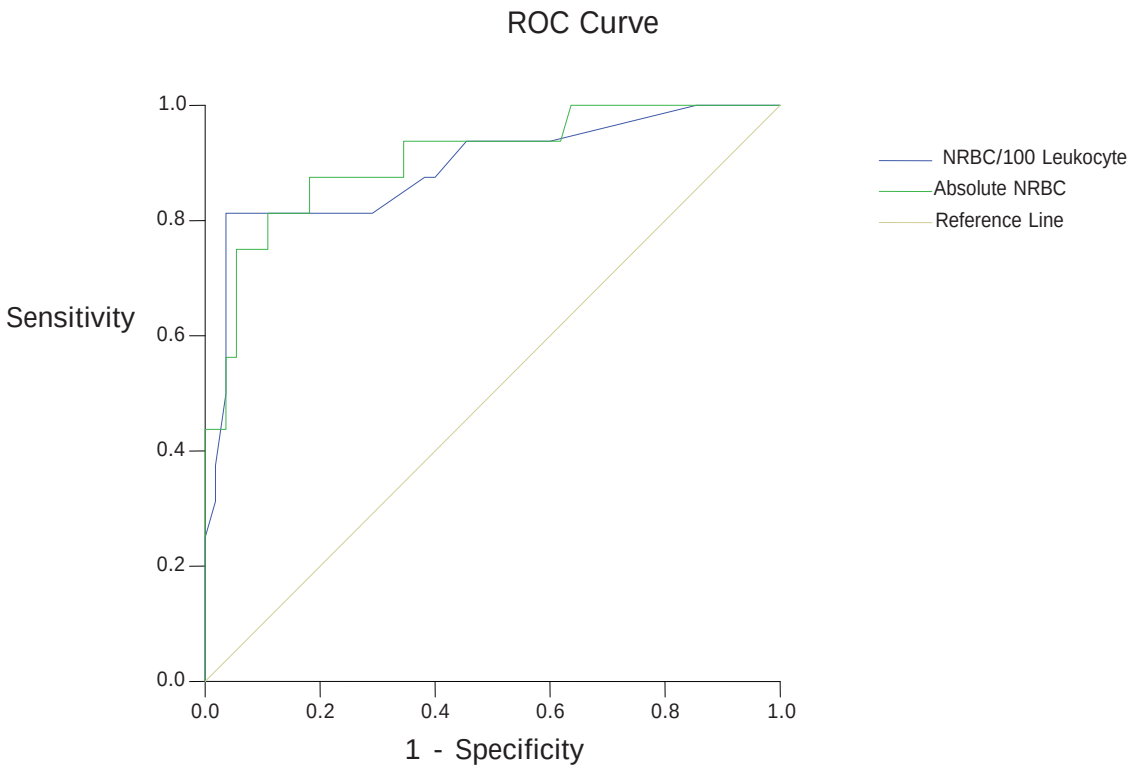

Figure 1. Receiver operating characteristics (ROC) graph to discriminate the sensitivity and specificity of NRBC/100 WBC count or absolute NRBC count in the diagnosis of perinatal infants

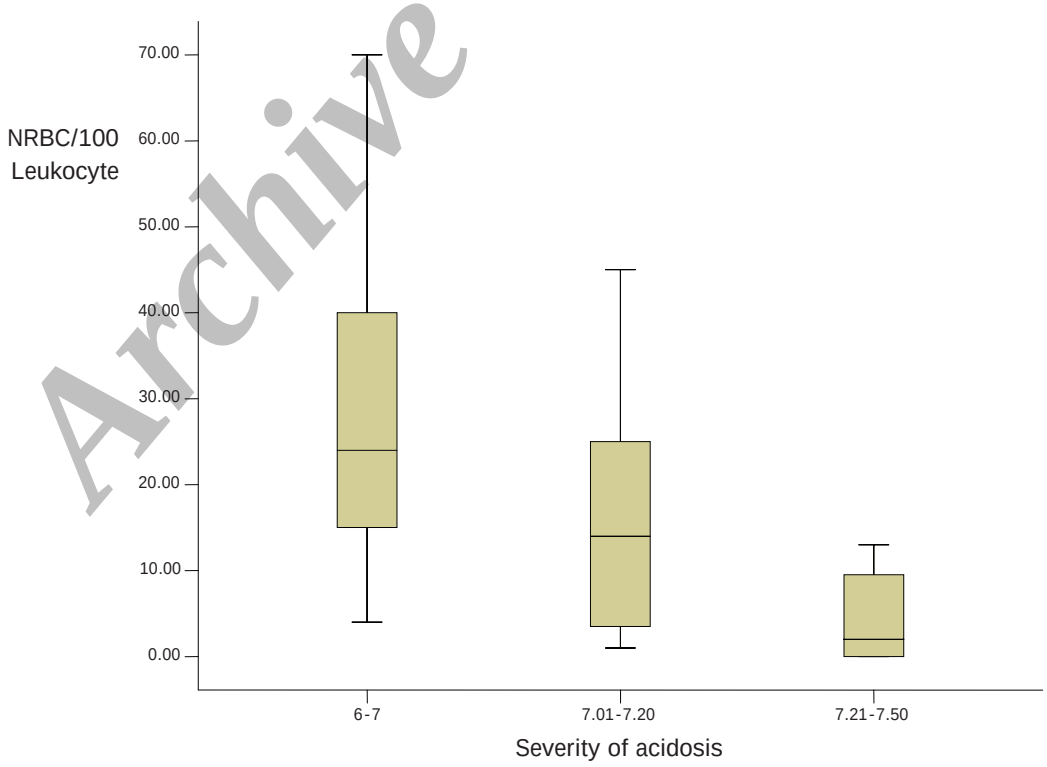

Figure 2. The relationship between nucleated red blood cell (NRBC) count/100 WBC to the severity of acidosis

For a NRBC count of >70, the sensitivity and specificity for the diagnosis of perinatal asphyxia were 83.4% and 73.5%, respectively (Figure 1). The NRBC count was found to be significantly related to the first blood pH and first minute Apgar score (P<0.001). The NRBC/100 leukocyte increased with progressive increases in neonatal acidosis and in progressive decrease in first minute of Apgar scores (Figure 2).

NRBC counts were found to be 3.87/100 leukocytes for healthy infants, 9.75 for asphyxiated neonates without HIE, 11.94 for HIE grade 1, 21.08 for HIE grade 2 and 29.18 for HIE grade 3. There was a significant relationship between NRBC/100 leukocytes and the degree of encephalopathy (P<0.001). Among 36 infants who had HIE, 22 had favorable outcomes (normal neurologic development), and 16 had an adverse outcome (10 died within the first month of life, and 6 developed neurodevelopment sequelae). The NRBC/100 leukocyte and absolute NRBC were significantly higher in neonates with adverse outcomes than in those with favorable outcomes (4.90 vs. 24.25 and 74.38 vs. 899.92, P<0.001) (Figure 3).

Discussion

In the present study, we have determined NRBC count/100 leukocytes and absolute NRBC in neonates with asphyxia and healthy controls. NRBC count/100 leukocytes and absolute NRBC were significantly higher in neonates with birth asphyxia, and high levels of NRBC were associated with a more severe acidosis, low Apgar score, low platelet count, and poorer short-term outcome.

Although NRBCs rarely circulate in older children, they are commonly seen in the blood of neonates. They are primarily produced in the fetal bone marrow in response to erythropoietin and are stored in the marrow as precursors to reticulocytes and mature erythrocytes. Many acute and chronic stimuli cause increases in the number of circulating nRBCs from either increased erythropoietic activity or a sudden release from the marrow storage pools.[5]

Previously reported causes of a high NRBC count include: prematurity, ABO or Rh incapability, maternal diabetes, intrauterine growth retardation,[9,10] acute asphyxia,[11–13] congenital infection, cyanotic heart disease, pre-eclampsia, maternal smoking, and

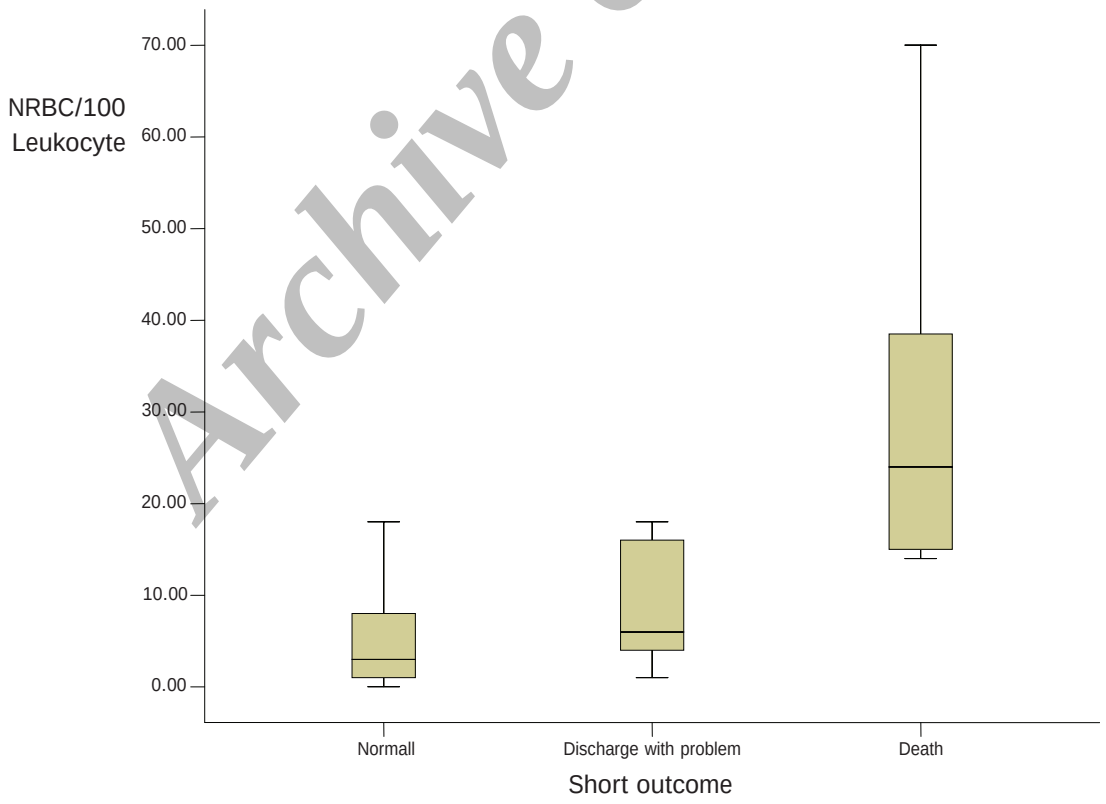

Figure 3. The relationship between nucleated red blood cell (NRBC) count/100 WBC to short outcome

chorioamnionitis.[5,14] However, neonates with these conditions were excluded from the current study.

Association between asphyxia and NRBC count

In the present study, the NRBC count for normal neonates was found to be 3.87±5.06, which was consistent with previous reports.[15,16] NRBC count/100 leukocytes and absolute NRBC/mm^3 increased during the first hours of life in perinatal asphyxia as compared with healthy control subjects. The mechanism causing the rapid release of NRBC following perinatal asphyxia is not known, although increased erythropoietin results from hypoxia and probably has a major role in the process.[6,15] NRBCs are immature erythrocytes whose production is thought to be driven primarily by the interplay of hypoxia and erythropoietin (EPO) synthesis.[17] Previous studies suggest that EPO increases erythroid production and releases immature forms of erythrocytes into the peripheral circulation in response to hypoxia. It is possible that increased NRBC production in the immediate neonatal state primarily reflects hypoxic injury.[18] Several studies have reported an increased NRBC in neonatal blood following perinatal asphyxia.[5–7,11,19,20] However, previous studies have not defined the cut-off value for NRBC count in predicting perinatal asphyxia and clinical outcome. The results of the present study give additional support to previous reports, but also define the cut-off value for NRBC as >70/mm^3 with a sensitivity of 83.4% and a specificity of 73.5% in predicting the development of asphyxia

Association between severity of asphyxia and NRBC count

Neonates diagnosed with HIE were found to have higher NRBC counts, when compared with control infants. NRBC count was significantly related to the Sarnat's grading of encephalopathy and also elevated in infants who subsequently died when compared to those who survived. Some authors have previously evaluated the relation between the severity of asphyxia and cord NRBC count.[5,16,19] Hanlon-Lundberg and Kirby evaluated the relation between the severity of asphyxia and increased NRBCs by comparing cord NRBCs with cord pH and Apgar scores. The NRBC counts increased with progressive increases in cord acidosis and with progressive decreases in the Apgar scores.[21]

The diagnosis of perinatal asphyxia requires a blood gas and acid base assessment demonstrating a significant mixed (especially metabolic) acidosis.[22] We found a significant negative correlation between NRBC counts and neonatal PH (Figure 2, P<001). Thilaganathan et al. found a significant association between the erythroblast count and umbilical cord blood pH (P<0.0001).[20] Saracoglu et al. also evaluated this relationship and found a significant correlation (P<0.001).[15] Other investigators have also found increased NRBCs associated with a decrease in cord pH.[23] However, to our knowledge, this is the first report to describe a relationship between NRBC count and base excess. The base excess ±SD were -3.42±4.81 for the control group and -11.5±10.12 for the case group, respectively. We found a significant negative correlation between NRBC counts and base excess in neonatal arterial blood gas (P<001). This simple test maybe helpful in the rapid assessment of perinatal asphyxia.

Association between short-term outcome, asphyxia and NRBC count

We found a considerable increase in NRBC count for asphyxiated neonates which were predictive of short-term outcome. Our data were consistent with the report of Ferns et al. who reported that the rate of erythropoiesis was related to the degree of asphyxia and the probability of neurological impairment. [24]

Naeye and Localio compared 16 term and preterm infants who developed cerebral palsy following acute asphyxia with 7 newborns that had long-standing developmental disorders unrelated to a perinatal event, and with 84 normal controls. Few normal controls had NRBC values that exceeded 2000 NRBCs/mm^3. All infants with cerebral palsy caused by developmental events unrelated to birth had less than 2000 NRBCs/mm^3. NRBCs increased to 2000/mm^3 or more in 15 of the 16 infants injured from acute ischemia and hypoxemia.[25]

In our study, a NRBC count of greater than 13/100 leukocytes had a sensitivity of 81.3% and a specificity of 94.4% in predicting adverse outcomes.

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