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The Correlation Between Nucleated Red Blood Cells and Creatine Kinase Level with Hypoxic-Ischemic Encephalopathy in Asphyxiated Newborns

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ABSTRACT

Background & Objective: We aimed to compare the correlation between nucleated red blood cell (NRBC) and creatine kinase (CK) levels with hypoxic-ischemic encephalopathy (HIE) in asphyxiated newborns.

Materials & Methods: This case-control study included 30 asphyxiated (case) and 30 healthy neonates (control) born at Al-Zahra Hospital in Rasht, Iran. We collected the cord blood samples for CK level and NRBC at birth. The clinical stage of Sarnat indicated the severity of HIE. Data were analyzed in the two groups using IBM SPSS Statistics for Windows, version 22.

Results: The case group had a higher NRBC count (P=0.001). The CK level was significantly different between the two groups and was higher in the case group than in the control group (P= 0.002). Results demonstrated a significant association between NRBC count and the occurrence of HIE in neonates with asphyxia (P = 0.021). Besides, a positive correlation was found between HIE and CK levels in the case group (r = 0.7, P=0.001).

Conclusion: According to our results, NRBC count and umbilical cord CK level measurement are valuable predictors of asphyxia and HIE in neonates. In addition, measuring these parameters may help clinicians for faster diagnosis and better management.

Keywords: Asphyxia, Red Blood Cells, Creatine Kinase, Hypoxic-Ischemic Encephalopathy

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Introduction

The infant mortality rate is one of the most important health, socio-economic status, and development indicators of countries (1), which can be affected by preterm labor, congenital anomalies, infections, pregnancy, and childbirth problems (2).

Perinatal asphyxia, known as hypoxic-ischemic encephalopathy (HIE), is the leading cause of neurological mortality determined by clinical and laboratory evidence of brain injury (3, 4). As there is no consensus on defining asphyxia at birth, and it commonly occurs in the uterus, during childbirth, at birth, or in the postpartum period, perinatal asphyxia is the suitable term (5).

HIE is the most prevalent cause of neonatal encephalopathies. It accounts for approximately 23% of newborn deaths worldwide (6). The higher the severity of HIE, the higher occurrence of morbidity and mortality (7). Infants with HIE are at increased risk for severe cognitive and/or motor impairment, deafness, vision loss, learning difficulties, and seizure (8-10).

So far, several biomarkers of neurological damage due to asphyxia have been recognized and examined (11, 12). In neonates with asphyxia, there is an increased level of biomarkers, such as nucleated red blood cells (NRBC), that can help clinicians to estimate adverse neurological outcomes (3, 11-13). The NRBC count in the cord blood at birth is a novel indicator of the severity of asphyxia (14). The previous investigation mentioned the significant correlation between NRBC count with HIE severity and brain damage (15).

Evaluation of NRBC count and CK level at birth can help predict the severity of asphyxia and HIE **(16)**. Despite comprehensive efforts regarding its pathophysiology, the severity of asphyxia and its long-term outcomes remain a significant concern **(17, 18)**. Given the high prevalence of asphyxia and related complications, measuring NRBC count and CK level in neonates susceptible to asphyxia is necessary **(19)**. Therefore, this study aimed to compare the correlation between NRBC and CK level with HIE in asphyxiated newborns.

Methods

This is a case-control study on infants in the neonatal intensive care unit (NICU) of Al-Zahra Hospital Rasht, Iran, from January 2020 to January 2021. The case and control groups comprised 30 neonates with perinatal asphyxia and 30 non-asphyxiated neonates. The asphyxia criteria in this study included at least 2 of these items below:

1. Fetal distress symptoms.
2. Need for compulsory ventilation support >1 minute with positive pressure ventilation.
3. < four in the 1st and < seven 5th-minute Apgar scores.
4. The pH level of cord blood is < 7.20.
5. Thick meconium staining of amniotic fluid and respiratory depression, bradycardia, or hypotension.

We excluded premature newborns, newborns with congenital malformations, maternal drug addiction, preeclampsia, and diabetes, and infants with hemolytic disease and bleeding. A checklist was used to obtain demographic, clinical, and laboratory results. We collected sex, Apgar score, HIE stage, birth weight, and amniotic fluid status. The case group was examined for seizures and hypotonia because they can be associated with the severity of HIE. HIE stages were defined according to the Sarnat clinical staging. We defined first-degree HIE as hyperactivity, hyperreflexia, and no seizures for ≤ 24 hours. Lethargy and hypotonia indicated moderate HIE or grade II HIE, and paralysis, severe seizures, or coma prove severe HIE or grade III HEI.

Two ml of clamped cord blood of all newborns were taken to measure the complete count of blood cells, NRBCs, and CK levels. NRBC count was measured manually using a light microscope under 40Ò magnification. We measured it per cubic millimeter of blood and 100 white blood cells (WBC). Spectrophotometric kinetics was the method for measuring CK levels.

Ethical considerations:

We obtained informed consent from the parents. Moreover, the Ethics committee of the Guilan University of Medical Sciences approved this study (code: IR.GUMS.REC.1399.366).

Statistical analysis:

Statistical analysis was performed by (SPSS software, version 22, IBM corp. USA). The quantitative variables were reported as mean+SD. Kolmogorov–Smirnov test was used to assess the normality of quantitative data. We used the Pearson correlation coefficient, T-test, and Mann–Whitney U test to compare the two groups. A P-value less than 0.05 indicated statistical significance.

Results

The current case-control study consists of 60 eligible infants consisting of 30 infants in each group. According to Table 1, no considerable difference was noted regarding sex (P=0.12), type of delivery (P=108), maternal age (P=0.603), and gestational age (P=0.82) between the two groups. Although birth weight was higher in the control group than in the case group, it was insignificant (P=0.55). The mean PH was 7.19 ± 0.09 and 7.24± ± 0.09 in the case and control groups, respectively, with significant differences (P-value: 0.03).

Furthermore, the CK level was statistically different between the two groups (P= 0.002). It was found that the mean of NRBC count, amniotic fluid status, and first and fifth-minute Apgarhad significant differences in the groups (P<0.05). Table 1 shows the clinical and laboratory characteristics of the groups.

As Table 2 shows, we found a significant correlation between HIE with first and fifth- minute Apgar, NRBC count, and CK (P<0.05).

Table 3 shows that 15 case-patients did not have HIE. There was a significant association between the HEI stages with NRBC, CK level, 1st, and 5th -minute Apgar scores (P<0.05).

Table1. Clinical and laboratory characteristics of groups

Variables	Control (n=30)	Cases (n=30)	*P-value*
Sex (Girl, %)	18 (60%)	12 (40%)	0.12
Delivery kind (Natural, %)	14 (46.7%)	8 (26.7%)	0.108
Amniotic fluid (Normal, %)	30 (100%)	19(63.3%)	P< 0.001
Mother's age (year)	30.7 ± 60.11	29.5 ± 73.68	0.603
Gestational age(weeks)	38.30±1.23	38.36±1.32	0.82
Birth weight(Kg)	3.0 ± 6.38	2.0 ± 99.54	0.55
First Apgar (Minute)	8.13 ± 1.33	6.96 ± 1.18	P<0.001
Fifth Apgar (Minute)	9.40 ± 0.89	8.46 ± 1.22	P<0.001
Blood PH	7.24± ± 0.09	7.19 ± 0.09	0.03
WBC	11.2 ± 57.7	11.93 ± 4.17	0.69
Creatine kinase	14.83 ± 6.19	23.18 ± 12.17	0.002
NRBC	7.5 ± 6.01	17.54±2.12	0.001

Table2. Correlation of clinical characteristics and HIE

Variables	r (Pearson correlation)	*P-value*
PH	-0.305	0.67
NRBC	0.91	P< 0.001
Creatine kinase	0.82	P< 0.001
WBC	-0.305	0.101
First- minute Apgar	-0.61	P< 0.001
Fift- minute Apgar	-0.48	0.007

Table3. The characteristics of the case group

HIE Stage	NRBC	Creatinine Kinase	PH	WBC	first-minute Apgar	fifth-minute Apgar
No HIE (n=15)	7.32 ± 0.38	20.40 ± 3.37	7.21 ± 0.11	12.57± 3.64	7.80 0.56	9.06 1.18
Stage 1 (n=13)	10.59± 0.49	26.92 ± 3.68	7.16 ± 0.062	12.07 ± 4.53	6.23 0.92	8.00± 1.29
Stage 2 (n=1)	18.00± 0.00	33± 0.00	7.25 ± 0.00	5.90 ± 0.00	4 ±0.00	6.00 ±0.00
Stage 3 (n=1)	31.00± 0.00	42 ± 0.00	7.24 ± 0.00	6.40 ± 0.00	7 ±0.00	8.00 ±0.00
P – value	<0. 001	<0. 001	0.46	0. 24	<0. 001	0. 01

Discussion

We measured the NRBC in cord blood and neonatal CK level to find a correlation between these parameters and the occurrence of HIE**.** Our finding showed a higher NRBC count in the case compared to the control group. We demonstrated a significant correlation between CK level and NRBC count with HIE and its stages in the case group.

NRBCs are erythropoietic series immature cells. Hypoxia increases circulating erythropoietin secretion, and it consequently increases NRBC count. Cord blood NRBC count at birth is a good predictor of congenital asphyxia **(20)**. NRBC is present in the peripheral blood of normal healthy newborns and clears from the bloodstream shortly after birth **(21)**. Many acute and chronic circulatory stimuli increase the number of

NRBCs, which may be due to hematopoietic activity rise or the rapid depletion of bone marrow storage resources. The elevation in NRBC count is often due to prematurity, increased hematopoiesis following chronic illness, and acute stress leading to the release of NRBCs from the bone marrow. When an increase in the number of NRBCs is due to acute or subacute asphyxia, the amount of this increase indicates the severity and duration of asphyxia **(22)**. Previous investigations reported that the number of NRBC and CK levels in cord blood could help diagnose asphyxia. In a study of 69 high-risk infants, the number of primary NRBC in the control group was significantly lower than in the high-risk one.

Moreover, determining the number of NRBCs can be helpful for the short-term prediction of neonatal developmental status **(23)**. Also, the study by Ferns et al. showed that NRBC in asphyxiated infants was significantly higher than in the control group. They compared low Apgar scores, cord blood pH, and neonatal outcomes with the number of NRBCs **(24)**.

We found the number of NRBC at birth as a good predictor of determining the severity and short-term consequences of asphyxia during delivery. Similar to ours, Meena, et al. **(14)** reported that the mean NRBC count was higher in the asphyxia group than in the control group. Also, the findings in the study of Ghosh, et al. **(25)** are consistent with ours, and a significantly higher NRBC count was seen in those who developed HIE. Geetika **(26)** also observed that the NRBC count was statistically higher in newborns with birth asphyxia than in the control group. Similar reports were made by Korst, et al. **(27)** and Spencer et al. **(28)**. It was in line with previous statements that NRBC is released under the stressful situation of birth asphyxia due to hypoxia **(14, 29)**. Vandana, et al. **(30)** suggested that the increased production of NRBC in asphyxiated neonates primarily reflects hypoxic injury. In another study, Rai, et al. **(11)** showed that NRBC was higher in asphyxiated infants and associated with severe acidosis, low Apgar scores, and low platelet count. Minior, et al. **(31)** stated that an increase in NRBC alone could predict the poor prognosis of infants with developmental limitations during childbirth.

Our result revealed a significant negative correlation between HIE staging and Apgar score. Similar findings have been reported previously. For example, Meena, et al. **(14)** reported that as the HIE severity rises, the mean of Apgar scores decreases. Goel, et al. **(4)** showed that NRBCs/100 WBCs can be a good marker to assess the severity and initial outcome of asphyxia. They found a significant correlation between NRBCs / 100 WBCs and Apgar score and HIE. Similar to the previous studies, the blood pH was lower in the asphyxiated neonates than in non-asphyxiated neonates **(32, 33)**.

The total CK level increase in asphyxiated neonates compared to the control group and was consistent with the HIE. Similar to our study, Shivaprakash and Nigam **(16)** reported a significant difference between the two groups, and values for CK levels in neonates with asphyxia were higher than in neonates without asphyxia. Masaraddi Sanjay, et al. **(34)** found no significant relationship between CK level and the incidence of HIE. We also evaluated the relation between HIE and cord blood pH. No statistical correlation exists between HIE and blood pH (P=0.67). Another study by Meena, et al. **(14)** demonstrated a reverse correlation between HIE severity and cord blood pH. In other words, as the HIE severity increases, the cord blood pH decreases **(14)**, which is inconsistent with the results of the current study. This difference may be due to the different sample sizes.

The current investigation showed a positive correlation between the NRBC count and HIE. Mansour Ghanaie, et al. **(35)** reported that the increased NRBC in the neonatal circulation was related to relative hypoxia and unfavorable outcomes. This report is in line with the results of the current study.

In summary, our findings showed that NRBC count and umbilical cord CK level measurement are valuable predictors of asphyxia and HIE in infants. This current investigation had some limitations. These limitations include small sample size, no follow-up of patients, and the absence of other parameters such as magnetic resonance imaging and electroencephalography.

Conclusions

Our study showed that NRBC count in cord blood can be an early indicator of the occurrence and severity of birth asphyxia and may help estimate the neurological outcome in neonates with asphyxia. Also, measuring CK levels in the cord blood can help predict the occurrence of HIE. Evaluation of NRBC count and CK level, which are easy and non-expensive, can efficiently differentiate asphyxiated neonates from non-asphyxiated neonates. It may help in faster diagnosis and better management and outcome of these neonates. It seems that a multicenter study with larger sample size is necessary to determine the sensitivity and specificity of the absolute count and the NRBC count.

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Consent for publication

Not applicable.

Authors' Contribution

Marjaneh Zarkesh: Project leaders and responsible for the study conception. Marjan Malekifard: Involved in acquiring data and drafting the manuscript. Mandana Mansour Ghanaei and Sedighe Bab Eghbal: design and

critically revised the manuscript. Maryam Ghalandari: Contributed significantly to the analysis. All the authors provided their final approval for the completed manuscript.

Conflict of Interest

There were no Conflicts of interest to be declared.

Availability of data and materials

Not applicable.

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