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Assessment of Umbilical Cord Nucleated Red Blood Cell Count in Discharged and Dead Very Low Birth Weight Infants

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ABSTRACT

Background: Predictive value of the umbilical nucleated red blood cells (NRBCs) at birth for neonatal outcomes has been assessed. Hence, the present study was conducted to compare NRBC between discharged and dead neonates.

Methods: This cross-sectional study was performed on preterm newborns with a birth weight of < 1,500 g admitted to the Neonatal Intensive Care Unit of Ghaem Hospital, Mashhad, Iran, within 2012-2018. The subjects were divided into two groups of discharged and dead. Data collection tool was a researcher-made questionnaire containing three sections, namely maternal demographic information, neonatal data, and blood tests (measuring white blood cell [WBC], absolute NRBC, NRBC/100 WBC and blood gas). The collected data were analyzed in SPSS software (version 20), using t-test, Chi-square test, receiver operating characteristic curve, and regression models.

Results: A total of 205 neonates, including 136 discharged neonates (66.03%) and 69 dead neonates (33.7%), were examined in the present study. The results demonstrated a significant difference between the two groups in terms of the first minute Apgar score (P=0.023), fifth minute Apgar score (P=0.010), gestational age (P=0.000), birth weight (P=0.000), WBC (P=0.020), absolute NRBC (P=0.004), NRBC percentage (P=0.001), duration of mechanical ventilation (P=0.029), duration of oxygen therapy (P=0.012).Moreover, mechanical ventilation (P=0.036), type of oxygen therapy (P=0.000), NRBC percentage (P=0.001), and absolute NRBC count (P=0.001) showed a statistically significant relationship with neonatal survival rate.

Conclusion: As the findings indicated, mechanical ventilation, type of oxygen therapy, absolute NRBC count, and NRBC percentage can be used as markers for predicting neonatal mortality rate.

Keywords: Death, Discharged, Neonates, NRBC, Prediction

Introduction

Neonatal mortality index is one of the most important indicators of health level in every community. Generally, most cases of neonatal deaths (75%) occur in the first week of birth, and more than a quarter of the deaths happen in the first 24 h of life (1, 2). Neonatal mortality generally occurs due to premature birth and its complications, like asphyxia, infections, and severe abnormalities (3).The most common reported causes of neonatal deaths in areas with low and high death rates are prematurity/congenital anomalies and asphyxia/congenital infections, respectively. In a study conducted at Ghaem Hospital, Mashhad, Iran, the main underlying causes of neonatal mortality were reported as severe prematurity (gestational age of <32 weeks; 57.4%), asphyxia (5-minute Apgar score of <6; 30.86%), congenital anomalies (27.16%), infections (25.3%), and respiratory, blood, and cerebral disorders (24.7%, 6.8%, and 6.2%, respectively) (4).

Nucleated red blood cells (NRBCs) are actually premature erythrocytes in peripheral blood, which increase in response to elevated erythropoietin in

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peripheral blood. Erythropoietin is a glycoprotein hormone secreted in response to hypoxia by such organs as the kidney, liver, spleen, lung, and bone marrow (5, 6). Many acute and chronic stimulators increase the amount of NRBC in blood circulation by the elevation of the erythropoietin activity (7). Increase in the number of NRBCs in embryonic blood circulation may be secondary to hypoxia or inflammation (8).

Previous studies have shown that inflammatory response, characterized by the release of cytokines (especially IL-6), is associated with an increase in NRBC production (9, 10). Embryonic inflammatory response and embryonic stress may play a role in the production or release of NRBC in the peripheral circulation. Additionally, in pregnancies with histological chorioamnionitis, host inflammatory responses may lead to an increase in the fetal NRBC, independent of erythropoietin (5). Furthermore, NRBC is helpful in the diagnosis of neonatal infections. In a study, it was revealed that NRBC/100 white blood cell (WBC) and absolute NRBC count can be useful in the early detection of neonatal sepsis with acceptable sensitivity and specificity(11).

Fetal hypoxemia is a major risk factor for unfavorable perinatal outcomes leading to the augmentation of NRBC count (12). The high number of NRBCs in the neonatal circulation is associated with relative hypoxia and unfavorable outcomes (13). In a study, NRBCs more than 11 per 100 WBCs had 85% sensitivity and 90% specificity in predicting asphyxia complications (5). The NRBC and reticulocyte, produced in the embryonic blood circulation system, cannot be removed from the placenta and maternal system. Accordingly, they can be used as proper predictors for the expression of the fetal situation and related stress (7).

The predictive value of umbilical NRBC at birth for neonatal outcomes has been already investigated (12). In the first month of birth, an increase in NRBC count has a predicting value for mortality, ventilation, and inotrope support, predominantly among term neonates (14). During the first week of birth, NRBC count is an appropriate parameter to predict perinatal asphyxia. In addition, a higher NRBC count is associated with a higher mortality rate (13).

The available indicators are not sufficient for the diagnosis and determination of the prognosis of neonatal mortality. The NRBC increases in both asphyxia and infection, which are two major causes of neonatal mortality(5,11). Therefore, NRBC may be helpful in predicting neonatal complications and mortality. Neonatal survival and healthare issues are of fundamental importance. Regarding this and given the need for achieving the goals of 2,025 concerning the neonatal death reduction, this study was conducted to compare the NRBC/100 WBC and other tests (White Blood Cell, Absolute NRBC, urea, creatinine, sodium, potassium, calcium, direct and indirect bilirubin, PT, PTT, and blood gas) between discharged and dead neonates.

Methods

This cross-sectional study was conducted on 205 extremely premature neonates with a birth weight of < 1,500 g and/or gestational weeks of < 32, admitted to the Neonatal Intensive Care Unit of Ghaem Hospital, Mashhad, Iran, within 2012-2018. This study was approved by the Ethics Committee of Mashhad University of Medical Sciences, Mashhad, Iran. At first, blood samples were taken from the umbilical cord of all preterm neonates with a birth weight of < 1,500 g for measuring complete blood count and peripheral blood smear. Then, complete blood counting was carried out with an automatic Cysmax counter in a laboratory. In the next step, the peripheral blood smear was provided to control the results of the device, and the cells were examined and separated by considering their morphology. The number of NRBCs was reported per 100 WBCs observed.

The data were collected through a three-section researcher-made questionnaire. The first section included the mother's demographic data (e.g., maternal age and type of delivery). In addition, the second part entailed the neonatal data, including 1-minute Apgar score, 5-minute Apgar score, gestational age, birth weight, birth head circumference, birth height, duration of oxygen therapy, type of oxygen therapy, need for respiratory support, duration of mechanical ventilation, administration of surfactant, cause of hospitalization, and final diagnosis. Finally, the third part involved blood tests, such as WBC, absolute NRBC, NRBC/100 WBC, urea, creatinine, sodium, potassium, calcium, total and direct bilirubin, PT, PTT, and blood gas. These data were collected based on patients' medical records, interview with the mothers, and neonatal physical examination. The newborns were followed up until discharge or death.

Statistical analysis

The data were analyzed in SPSS software (version 20). First, the researchers used the

tables and statistical charts to describe the results. Then, a comparison was made between the two research groups in terms of NRBCs using Chi-square test and t-test. In addition, the role of intervening factors in the neonatal outcome was examined by means of a regression model. A p-value less than 0.05 was considered statistically significant.

Results

To assess the level of NRBC as a survival indicator for neonates, a total of 205 neonates, including 136 (66.3%) discharged cases and 69 (33.7%) dead neonates, were included in the study. Based on the results, the mean 1-minute and 5-minute Apgar scores were obtained as 6.01±2.03 and 7.56±1.49, respectively. In addition, the mean gestational age and birth weight were estimated at 29.82±2.48 weeks and 1163.22±350.80 g, respectively. Other features of the examined newborns are presented in Table 1.

There was no significant difference between the two groups in terms of maternal age (P=0.986), creatinine (P=0.218), sodium (P=0.947), potassium (P=0.923), calcium (P=0.098), total bilirubin (P=0.647), direct bilirubin (P=0.694), PT (P=0.573), blood pH at the first minute of birth (P=0.914), base excess at the first minute of birth (P=0.531), and arterial bicarbonate (P=0.320). In contrast, a significant difference was observed between the two groups in terms of such variables as 1-minute Apgar score (P=0.023), 5-minute Apgar score (P=0.010), gestational age (P=0.000), weight (P=0.000), WBC (P=0.020), absolute NRBC (P=0.004), NRBC percentage (P=0.001), duration of mechanical ventilation (P=0.029), duration of oxygen therapy (P=0.012), urea (P=0.029), and PTT (P=0.012).

Table 1. Mean scores of neonatal clinical parameters in discharged and dead groups

Groups Variables	SD±mean
First minute APGAR score	6.01±2.03
Fifth minute APGAR score	7.56±1.49
Gestational age (week)	29.82±2.48
Weight (g)	1163.22±350.80
headcircumference (cm)	28.22±2.39
High (cm)	39.57±2.87
WBC (CBC)	11.77±7.87
Absolut NRBC	2961.18±5654.54
NRBC/100WBC	21.06±33.48
Duration of mechanical ventilation (day)	9.12±9.76
Urea	52.36±28.30
Creatinine	0.81±0.29
Sodium	140.76±5.00
Potassium	4.79±0.92
Calcium	8.79±1.01
Bilirubin	8.65±3.73
Direct bilirubin	0.50±0.32
PT	21.36±9.07
PTT	67.41±31.66
Ph1	7.28±0.14
Be1	-1.85±8.25
Hco3a	20.36±4.53

WBC: white blood cell, NRBC: nucleated red blood cell, PT: prothrombin time, PTT: partial thromboplastin time, HCO_3a: arterial bicarbonate

In this regard, the mean 1- and 5-minute Apgar scores, gestational age, and birth weight were lower in the dead group than in the discharged group. On the other hand, the values of maternal age, WBC, absolute NRBC, NRBC percentage, duration of mechanical ventilation, duration of oxygen therapy, urea, and PTT were higher in the dead group (Table 2).

Based on the findings of the present study, mechanical ventilation was significantly associated with the survival of neonates (P=0.036). In other words, the newborns of the discharged group underwent mechanical ventilation more frequently than the dead group.

Discharged newborns received higher amounts of surfactant, yet there were no significant correlations between the use of surfactant and neonatal survival (P=0.140). In addition, the use of respiratory support was not significantly different between the two groups of neonates (P=0.216). While the dead group had a higher rate of natural delivery, the discharged group had a higher rate of cesarean section. However, there was no significant relationship between the type of delivery and neonatal survival (P=0.100).

The type of oxygen therapy was significantly different between the two groups (P=0.000). In this respect, the neonates of the discharged group were mostly subjected to nasal continuous positive airway pressure, whereas the dead group neonates frequently received endotracheal tube synchronized intermittent mandatory ventilation.

The NRBC percentage showed a significant relationship with the neonatal survival rate (P=0.001). In other words, the NRBC percentage was higher in the dead group than in the discharged group. Moreover, the absolute number of NRBC was significantly associated with the survival rate of neonates (P=0.001). In this regard, NRBC count was higher in the dead group as compared to that in the discharged group (P=0.000; Table 3).

Table 2. Comparison of the mean of neonatal variables between discharged and dead groups

Variables	Groups		
	Discharged neonates 136 (66.3%) cases	Dead neonates 69 (33.7%) cases	Significance level* T-test
First minute APGAR score	6.26±1.93	5.46±2.16	0.023
Fifth minute APGAR score	7.78±1.30	7.07±1.76	0.010
Gestational age (week)	30.60±2.25	28.15±2.11	0.000
Weight (g)	1338.06±243.24	1080.27±300.50	0.000
Age of mother (year)	28.87±5.94	28.89±7.33	0.986
WBC (CBC)	10.80±7.39	13.66±8.49	0.020
Absolut NRBC	2036.25±4644.52	4770.25±6922.87	0.004
NRBC/100WBC	14.37±24.68	34.26±43.44	0.001
Duration of mechanical ventilation (day)	7.76±9.54	14.28±9.16	0.029
Duration of oxygen therapy (day)	6.36±6.18	11.77±9.14	0.012
Urea	46.50±19.69	70.27±41.36	0.029
Creatinine	0.79±0.25	0.87±0.37	0.218
Sodium	140.73±4.75	140.81±5.69	0.947
Potassium	4.80±0.86	4.77±1.12	0.923
Calcium	8.89±0.92	8.45±1.23	0.098
Bilirubin	8.56±3.26	9.18±5.91	0.647
Direct bilirubin	0.50±0.33	0.55±0.30	0.694
PT	20.43±10.03	22.72±7.84	0.573
PTT	52.69±24.54	84.81±31.11	0.012
Ph1	7.28±0.10	7.28±0.20	0.914
Be1	-2.25±6.68	-11.47±0.84	0.531
HCO_3a	20.66±4.34	19.75±4.89	0.320

*Values are based on mean± standard deviation.
WBC: white blood cell, NRBC: nucleated red blood cell, PT: prothrombin time, PTT: partial thromboplastin time, HCO_3a: arterial bicarbonate

Table 3. Comparison of some neonatal variables between discharged newborns and dead cases

Variables		Groups		
		Discharged neonates 136 cases (66.3%)	Dead neonates 69 cases (33.7%)	Significance levels* Chi-square test
Gender	Male	67 (52.80%)	38 (64.40%)	0.0136
	Female	60 (47.20%)	21 (35.60%)	
Mechanical ventilation	Yes	77 (85.60%)	41 (97.60%)	0.036
	No	13 (14.40 %)	1 (2.40%)	
Respiratory support	Lack of oxygen therapy	6 (9.10%)	0 (0%)	0.216
	Nasal. Cpap	30 (45.45%)	16 (34.78%)	
	ETT	30 (45.45%)	30 (65.22%)	
Type of delivery	Natural	33 (37.10%)	13 (68.40%)	0.100
	Caesarian	37 (52.90%)	6 (31.60%)	
NRBC (%)	0-5	70 (51.50%)	18 (26.10%)	0.001
	6-150	66 (48.50%)	51 (73.90%)	
NRBC count	0-460	66 (49.30%)	14 (20.30%)	0.000
	461-2800	68 (50.70%)	55 (79.70%)	

*Values are based on standard deviation±mean.
NRBC: nucleated red blood cell

Discussion

As the results of the present study indicated, the neonates with lower 1- and 5-minute Apgar scores, gestational age, and birth weight were prone to a higher risk of mortality. In the dead newborns, the absolute numbers of NRBCs and NRBCs/100 WBC were twice as much as those in the discharged cases.

In this study, dead neonates had lower gestational age and birth weight in comparison with the discharged neonates. Prematurity and low birth weight are considered as the strongest predictors of neonatal mortality. The main causes of low birth weight are prematurity or intrauterine growth restriction (15).Mortality models and vital registration statistics indicate that around 28% of newborns die due to prematurity complications (16). Preterm labor plays a pivotal role in the morbidity of the

neonates. In 2015, the World Bank reported that 85% of neonatal mortality in the world was due to preterm labor and prematurity (17). Therefore, preterm delivery is directly responsible for one million estimated deaths per year (18).

In the present study, the dead infants had lower 1- and 5-minute Apgar scores. These scores are considered a good diagnostic tool for the prediction of neonatal mortality (19). Accordingly, this score is a neonatal assessment method in developing countries, where available laboratory tests are not available. Owing to the low cost of Apgar scoring, this test is used widely to identify infants who require further care, even in the absence of laboratory tests (1). Apgar scores, birth weight, and gestational age are highly correlated with the survival rate of a neonate. Consequently, a combination of these factors can be regarded as a good scale for the determination of neonatal health status, success of resuscitation, and growth and development of newborn (20). Undoubtedly, 1-minute Apgar score is an important scale for the prediction of neonatal mortality (21). In spite of the advances in technology and medical care for the preservation of premature neonates, their mortality rate is still very high. Accordingly, the chance of mortality in newborns weighing less than 1,500 g is around 200 times higher than that in term neonates. Although several methods have been proposed to predict the problems of premature newborns, the suggested laboratory factors are few with low sensitivity and specificity.

According to the findings of the present study, the number of the umbilical cord NRBCs at birth in the dead neonates was 2.5 times higher than that in the discharged newborns. Although the researchers could not find any study with similar findings, it has been shown in several studies that high levels of NRBC would increase the likelihood of some neonatal complications, such as asphyxia, severe cerebral hemorrhage, ROP, and early infection(3,5,6,11). Because these factors are among the most important causes of mortality in premature neonates, NRBC count may have a direct or indirect correlation with neonatal mortality (5).

The NRBCs are premature erythrocytes, which increase in response to erythropoietin elevation in peripheral blood (6). Inflammation increases the release of NRBC to peripheral blood, possibly by releasing some cytokines (22). Based on the evidence, inflammatory processes can potentially affect neonatal prognosis by increasing the risk of cerebral hemorrhage, retinopathy of prematurity (ROP), and even death (23).Increased count of NRBC and reticulocyte in the umbilical cord blood at birth shows extramedullary hematopoiesis in the liver and spleen which can be a sign of hemolytic disease in the fetus. Moreover, these markers are increased in the intracranial and intraventricular hemorrhage of immature embryos (24). Up to 12 h after birth, NRBC levels are reduced by 50%, and only 20-30 NRBCs per mm^3 are found up to 48 h later. In healthy term neonates, no NRBC is found after the third or fourth day of birth. However, there may be a small amount of NRBC in blood up to a week after birth in preterm infants (25).

In a study, it was revealed that the mean number of NRBCs at the first 12 h after birth was significantly higher in the blood of infected infants than in the blood of un-infected neonates. Therefore, NRBC could be considered a helpful factor in the prediction of neonatal infections (26).

High levels of NRBC in the umbilical cord blood strongly indicate the intrauterine hypoxemia during several hours before birth. Consequently, NRBC count can be used not only as a marker of perinatal brain damage but also as a time indicator of a hypoxic event, which is very important in legal issues (12). The high number of NRBC in the umbilical cord blood shows intrauterine hypoxia during several hours before birth. Hence, this indicator can be used not only to determine perinatal brain damage but also to estimate the time of hypoxic events (12).Based on the results of studies, total NRBC and NRBC per 100 WBCs are simple markers for assessing the severity and outcomes of perinatal asphyxia (5, 27, 28).

The results of this study revealed that most of the dead neonates were born by natural delivery. Acute and subacute stress can increase the NRBC. Even the relative hypoxia during normal labor without asphyxia may be associated with elevated levels of erythropoietin, compared with cesarean section (29).

Additionally, NRBC has a prognostic value in the first month of birth. It has been shown that most of the neonatal adverse outcomes are related to total NRBC count or NRBC/100 (14). In a study, birth weight, gestational age, and Apgar score were significantly lower in the group with an NRBC count of > 40. In the mentioned study, neonatal infection, ventilatory support, abnormal brain ultrasonographic findings, morbidity rate, and neonatal adverse outcomes were significantly higher in the case group than in the control group. Therefore, it seems that an increase in the NRBC count of preterm infants born from pregnancies

with severe preeclampsia is the first important marker to identify neonatal unfavorable outcomes (30, 31).

Conclusion

The results of this study indicated that neonatal death was correlated with several factors, including 1-minute Apgar score, 5-minute Apgar score, birth weight, gestational age, final WBC, NRBC, final NRBC percentage, mechanical ventilation, duration of oxygen therapy, type of oxygen therapy, urea, and PTT. Consequently, the total NRBC index and NRBC percentage can be used as markers for predicting neonatal mortality.

Acknowledgments

This study was approved by the Ethics Committee of Mashhad University of Medical Sciences, Mashhad, Iran.

Conflicts of interests

None declared.

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