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Does the Umbilical Cord Nucleated Red Blood Cellcan Help Predict Intraventricular Hemorrhage?

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ABSTRACT

Background: Intraventricular hemorrhage (IVH) is one of the most serious complications of premature deliveries, especially in very low birth weight (VLBW) newborns. The current study compared the number of nucleated red blood cells (NRBCs) in newborns with and without IVH.
Methods: This cross-sectional study was carried out on 109 VLBW neonates who were referred to the Neonatal Intensive Care Unit (NICU) Department of Ghaem Hospital in Mashhad, Iran. They were investigated to evaluate the predictive value of cord NRBC in the diagnosis of IVH. To this end, 77 neonates who had normal brain ultrasonographywere assigned to the control group and 32 newborns with IVH were allocated to the case group. The percentage of NRBC per 100 white blood cells was determined by a blood sample. These neonates underwent brain ultrasonography3-5 days after the birth; thereafter, mean NRBC/100 WBC and the absolute number of NRBC swere compared in both groups.
Results: In the current study, the mean scores of NRBC/100 WBC and the absolute number of NRBCs in neonates with IVH were obtained as 15.19 and 2272.38/µl. In the group without IVH, these values were reported as 40.95% and 5459.17/µl, respectively (P>0.05). Based on receiver operating characteristic (ROC) curve, the NRBC value does not have a diagnostic value in predicting the incidence of IVH.
Conclusion: As illustrated by the obtained results, the number of absolute NRBCs and the percentage of NRBC/100 WBC cannot help to predict the probability of neonatal IVH. Nonetheless, further studies are recommended in this regard.

Keywords: Brain ultrasonography, Intraventricular hemorrhage, Nucleated red blood cells, Premature infants, Very low birth weight

Introduction

The most common underlying cause of neonatal death in the world is prematurity and one of the major complications of prematurity is intraventricular hemorrhage (IVH). Severe IVH is one of the leading causes of mortality and morbidity in very low birth weight (VLBW) and extremely low gestational age newborns (1-4). Despite the technological advances, there is no essential treatment for IVH in VLBW neonates, and existing recommendations focus on prevention (5). Premature neonates are prone to IVH which leads to ventricular dilatation or unilateral parenchymal hemorrhage with increased risk of neurological complications (6).

IVH is widely detected in newborns less than 32 weeks' gestation or less than 1,500 grams due to their lower gestational age or lower birth weight (5). Since the late 1990s, the studies on IVH have demonstrated that 20% of VLBW newborns and 45% of extremely low birth weight (ELBW)neonates (less than 1000g) were affected by this problem (7-9).Over the past few decades, the incidence of IVH has decreased due to improved neonatal care; nonetheless, 15-25% of very premature neonates suffer from IVH (10).In a study conducted on 9575 neonates within 22-28 weeks and birth weight of 401-1500 grams, IVH was 36% in general, and the incidence of severe

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IVH (Grade III and IV) was also associated with a decrease in gestational age(GA) (9).

Apart from prematurity, low birth weight, and low GA, IVH is increased by some other factors, including respiratory distress, pneumothorax, early sepsis, hypoxia, duration of oxygen therapy, long-term mechanical ventilation, normal vaginal delivery, and five-minute low Apgar score. On the contrary, the incidence of IVH drops significantly with the use of steroids before delivery, Cesarean section delivery, as well as higher birth weight and GA (10-13).

Nucleated red blood cells (NRBCs) are the precursors of mature red blood cells that are commonly observed in the bone marrow. These cells are found in fetuses and newborns, as well as in some diseases in the peripheral blood (14, 15). Until weeks 6 and 7 of gestation, all fetal RBCs are nucleated (NRBC); nonetheless, they gradually decrease from week 12 in a way that their presence is uncommon in the newborn (16).The increase in the number of NRBCs at birth can be attributed to prematurity, ABO or RH blood incompatibility, intrauterine growth restriction (IUGR), fetal anomalies, maternal diabetes, acute and chronic hypoxia and asphyxia(14, 17-20), fetal hemorrhages (21), early pregnancy (16), and neonatal stress due to mother preeclampsia(22-26).

Since the number of NRBCs is one of the simple, available, and fast-response hematologic markers, it can play a peculiar role in facilitating diagnosis if it is predictive of IVH. In the current cross-sectional study, we evaluated the role of the number ofNRBCs in the prediction of IVH in premature newborns.

Methods

Study design and population

The present cross-sectional study was conducted on 217 neonates weighing less than 1500 grams and five-minute Apgar score more than 5 who were admitted on the first day in a neonatal intensive care unit(NICU), Ghaem Hospital, Mashhad, Iran, within February 2014-September 2017. The case group consisted of VLBW neonates who had IVH based on their brain ultrasound 3-5 days after their birth. On the other hand, the control group included VLBW newborns with normal ultrasonography.

Methods

On the first day after birth, an umbilical cord blood sample (containing 2 cc of blood in EDTA blood tube) was taken from neonates less than 1500 gr, and these samples were then sent to the laboratory. The blood smear was observed by a hematologist, and complete blood count (CBC) was performed with Sysmex Automatic Counters. In the next step, the blood smear was prepared to control the results of the device, and the cells were morphologically examined and separated; thereafter, the number of NRBCs was reported per 100 white blood cells (WBC).

Neonatal brain sonography was performed within 3-5 days after birth. Ultrasonography was carried out in case of seizure, apnea, increased head circumference, decreased hemoglobin, increased oxygen demand, or any major change in the clinical situation of patients. Finally, the two groups of neonates (with and without intraventricular hemorrhage in brain sonography) were compared in terms of some variables, including gender,weight, mode of delivery, gestational age,one and five-minute Apgar score, as well as laboratory information of newborns, such as blood culture, WBC, Platelet count, and NRBC.

Measuring tools: validity and reliability

At the time of birth, all newborns were examined and checked for weight, Apgar score, asphyxia, and other excluding factors. The neonates were weighted by German Seca digital scale with 100 gr calibration weight, and the study subjects were physically examined by a neonatologist. Ballard score was used to determine gestational age. According to ultrasonography criteria, Grade I IVH is defined as subependymal bleeding or less than 10% of the ventricle, is, in Grade II hemorrhage, there is bleeding between 10% and 50% of the ventricles, Grade III involves more than 50% of the ventricle with ventricular dilatation, and bleeding inside the parenchyma, in addition to the ventricles, is regarded as Grade IV.

Intervention

We did no new or annoying intervention or treatment on neonates in the present study and we used the same sample that is routinely taken for VLBW neonate's admission.

Ethical consideration

Ethics approval was obtained from the Ethics Committee of the Mashhad University of Medical Sciences (approved by the Ethics Committee: IR.MUMS.REC.1393.77). In all steps, information about the patient and his/her family was kept confidential and secured.

Inclusion and exclusion criteria

The inclusion criteria entailed: any live birth, weighting less than 1500 gram, and five-minute Apgar score more than 5, at the Ghaem hospital of Mashhad, Iran.On the other hand, the exclusion criteria included neonatal factors, namely asphyxia, IUGR, early sepsis, ABO or RH blood incompatibility, congenital malformations, as well as maternal factors, including history of diabetes and preeclampsia

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Laboratory measurements

Biochemical parameters, including CBC, were measured bySysmex Automatic Counters and reported per microliter (μL). A blood smear was observed by a hematologist and NRBCs were reported per 100 WBC, and the absolute number of NRBCs was then calculated.

Data Analysis

The obtained data were analyzed in SPSS software (version 24). Mean, standard deviation, and frequency distribution were separately used to describe the research sample. Mann-Whitney test was applied for quantitative variables, while the chi-square test was used for qualitative variables to determine the diagnostic value of the number of NRBCs in the differentiation of neonates with IVH. The receiver operating characteristic curve (ROC) curve was considered for the evaluation of the sensitivity and specificity of NRBC count in the diagnosis of IVH. A p-value less than 0.05 was considered statistically significant.

Results

A total of 108 newborns were excluded (30 cases due to death before ultrasonography, 28: preeclampsia, 19: asphyxia, 16: IUGR, 11: maternal diabetes, 3: early-onset infection, and 1: disseminated intravascular coagulopathy).

A number of 77 neonates were without IVH as the control group and 32 newborns with IVH were assigned to the case group (Figure 1). In the brain ultrasound, 77 newborns were normal, 13 with IVH grade I 11 with IVH grade II, 4 with IVH grade III, and 4 neonates with IVH grade IV.

Mean of gestational age was 29.47 weeks and weight was 1178.38 grams. The most common reason for neonates' hospitalization was prematurity (60.8%), RDS (33.6%) and feeding problem (2%), respectively. Other characteristics of neonates were compared between the two groups in Table 1.

Following items were studied within both groups in which there was no statically significant difference (P<0.05): mode of delivery (P=0.190), gender (P=0.148), five-minute Apgar score (P=0.229), positive second blood culture (P=0.728), RDS (P=0.807), White Blood Cell (P=0.732), platate (P=0.627), NRBC per 100WBCs (P=0.473), and total NRBC (P=0.663). There was a significant difference in both groups between birth weight (P=0.043) and gestational age (P=0.002). This implies that birth weight and GA were lower in neonates with IVH (Table 2).

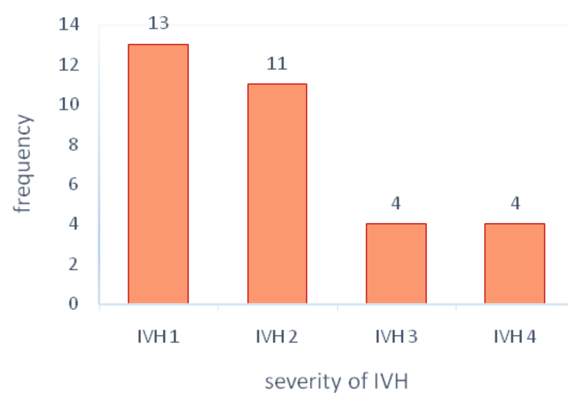

Figure 1. Frequency of intraventricular hemorrhage grades

Table 1. Comparison of some neonatal variables in both groups with/without intraventricular hemorrhage

Group variables	without IVH	with IVH	P-value
Delivery type			
Normal delivery	37 (48.1)	11 (34.4)	0.190
Cesarean/Section	40 (51.9)	21 (65.6)	
Blood culture (second)			
Negative	70 (90.91)	28 (87.5)	0.148
Positive	7 (9.09)	4 (12.5)	
Sex			
Male	34 (44.2)	19 (59.4)	0.728
Female	43(55.8)	13 (40.6)	
Respiratory distress			
Negative	21 (27.3)	8 (25)	0.807
positive	56 (72.7)	24 (75)	

Data presented as n (%).
P-values<0.05 are considered significant. Chi-square test.
IVH: Intraventricular hemorrhage

Table 2. Comparison of neonatal variables mean in both groups with/without intraventricular hemorrhage

variable	With IVH n=32	Without IVH n=77	P-value
Gestational age(week)	28.44±2.30	29.90±2.37	0.002
Weight(gr)	1114.53±213.54	1204.94±237.98	0.043
Five-minute Apgar	7.53	7.92	0.229
WBC / ìl	12762.5±11146	11627.3±7355	0.732
NRBC/100 WBC	15.19±21.52	40.95±101.64	0.473
Total NRBC	2272.38±3542.26	5495.17±15243.32	0.663
Platate / ìl	199218.8±110120	199233.8±89314	0.627

Data presented as Mean±SD and Mann-Whitney
WBC: White blood cells, NRBC: Nucleated red blood cell

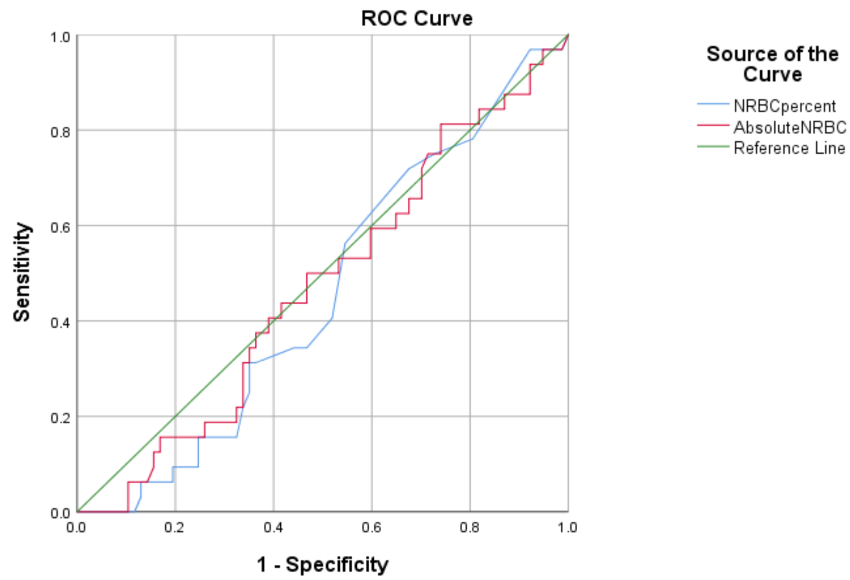

Figure 2. Receiver operating characteristic curve

The mean NRBC per 100 WBCs and total NRBC in IVH group and in control group are illustrated in Table 2. There was no significant difference in both groups (P= 0.473)

According to the Kruskal-Wallis test, no significant difference was detected between severity of IVH with NRBC percentage and the total NRBC (P=0.354; P=0.473). Based on the ROC curveand its sensitivity and specificity, the NRBC value does not have a diagnostic value in predicting the incidence of IVH.

Discussion

According to the present study, the gestational age of newborns with IVH was 1.5 weeks lower than the control group; moreover, the birth weight of neonates with IVH was also 100 g lower than the control group.In fact, in line with other related studies, the newborns with IVH had significantly lower weight and gestational age than control neonates (10,12,13). Prematurity and low birth weight are the most important risk factor for IVH which can be attributed to an increase in the susceptibility of immature vessels in the germline matrix (10).

In the present study, the mean NRBC per 100 WBCs in the control group was 2.7 times higher than that of the case group and the mean absolute NRBC in the control group was 2.4 times higher than that of the case group. Nevertheless, no significant difference was detected between the control and case groups. Moreover, IVH severity showed no significant relationship with these two variables.

The normal value of NRBC/100 WBC based on gestational age and birth weight has not been established. Furthermore, it is difficult for the NRBC count to reflect hematopoiesis due to the broad range of normal WBC counts in newborns. The ratio of NRBC to WBC is clinically wide-ranging, and the increase or decrease in the number of WBC decreases or increases this ratio, respectively. In a study, Hermansen estimated the average number of NRBC cells in the first few hours of life at about 500 and regarded NRBC>1000 as elevated. The NRBC/100WBC ratio within 0-10was considered to be normal and within 10-20 was regarded as elevated, although it is highly dependent on the total number of leukocytes (19).

In the current study, the mean NRBC scores in the control group and case group were reported as 40.95 and 15.19 per 100 WBCs, respectively. Nevertheless, in some studies, normal NRBC values for 100 WBC rarely reached 10 (16,18). This difference can be ascribed to the fact that the neonates in our control group were not completely healthy.

In addition, the mean percentage of NRBC and total NRBC value in the IVH group was lower than the control group. However, a study conducted in South Korean (27) on 112 VLBW neonates indicated that the mean NRBC count had a significant relationship with the incidence of perinatal death, necrotizing enterocolitis, and severe IVH (Grade III and Higher). This difference can be justified on the ground that in korean study, several variables were compared at the same time and may have contributed to the increase or decrease of NRBC. Moreover, Green et al. (28) conducted a study on 149 newborns less than 32 weeks of gestation to investigate the effect of intrauterine hypoxemia. The results of the mentioned study revealed that the absolute number of NRBCs on the first day after birth was higher in IVH group than normal group. However, this study was carried out on neonates with intrauterine hypoxia, and chronic hypoxia increases the production of erythropoietin and NRBCs according to the study of Hermansen (19). Therefore, it could itself be an independent factor for increased NRBC which was detected in the study of Green.

In another study carried out by Linder (11) on 36 neonates with VLBW and severe IVH, the early onset sepsis was a component of increased IVH. Along the same lines, in a study conducted by Poryo, primary sepsis, and patent ductus arteriosus (PDA) were associated with a higher rate of IVH (10). Nonetheless, in the present study, primary congenital infection was an outbreak factor and was not present in neonates under study. Furthermore, there was no significant difference between the two groups regarding the second blood culture.

In the current study, there was no significant difference in the type of delivery and respiratory distress syndrome (RDS) between two groups. However, in the study of Poryo (10), the cesarean section was associated with a low IVH level, while RDS was associated with an increased risk of IVH. In a study performed by Badiei 12), natural delivery was a major risk factor for IVH. Although it seems that passing through the delivery canal and its pressures in premature neonates due to vascular brittlenessincreases the likelihood of IVH in normal delivery, the current study demonstrated no significant differences.

Furthermore, there was no significant difference between the two groups in the five-minute Apgar score. This finding is contrary to the results of the studies conducted by Poryo (10), Badiei (12), and Khodapanahandeh (13) who concluded that a five-minute low Apgar score increases the risk of severe IVH. It can be ascribed to the fact that neonates with low Apgar scores were not included in our study from the beginning.

As noted above, unlike other previous studies, the present study demonstrated that NRBC levels in IVH neonates were not higher than the control group, and there was no significant difference between the two groups. This difference can be attributed to the small sample size in the current study.

On the other hand, the final sample size was smaller than the determined number in the study design since the exclusion criteria in our study which were based on the factors influencing IVH and the increase of NRBC were detected in many VLBW neonates (especially Low Apgar, Asphyxia, and IUGR). Therefore, more studies are needed to eliminate all the factors involved in the incidence of this disorder.

Conclusion

As evidenced by the obtained results, NRBC as an available indicator cannot yet be used along with other indicators for the early detection of IVH in neonates. It is worthy to note that the percentage of NRBC per 100 WBC and the absolute number of NRBCs cannot be used to diagnose neonates with a suspicion of IVH due to the fact that many variables affect it. Accordingly, it is recommended that more studies be conducted to obtain a more accurate judgment in this field.

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Conflicts of interest

There are no conflicts of interest.

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