Original Article

Comparison of the Percentage of Umbilical Cord Nucleated Red Blood Cells in Preterm Neonates during Vaginal Delivery and Emergency Cesarean Section

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Abstract

Background: There are insufficient and contradictory reports regarding the effect of delivery method on the rate of umbilical cord Nucleated Red Blood Cell (NRBC). Therefore, the present study aimed to compare the percentages of umbilical cord NRBC in vaginal delivery and emergency cesarean section (C-section) in preterm neonates. **Materials and Methods:** The present cross-sectional study was performed on mothers with vaginal delivery and C-section, from 2020 to 2021. The samples (n = 221) were preterm neonates selected using the convenience sampling method. The percentages of NRBC in neonates born by natural childbirth and by emergency C-section were measured and compared in this research. A researcher-made checklist, which included maternal and neonatal characteristics and laboratory evaluation, was used as a data collection tool. **Results:** The statistical population of thisresearch included 93 (42.10%) and 128 (57.90%) neonates born by vaginal delivery and by C-section, respectively.The mean (SD) score of gestational ages at birth was 30.75 (2.81) weeks. The mean (SD) score of umbilical cord NRBC level were estimated at 8.01 (5.93) and 25.64 (22.61) for the neonates born by natural childbirth and by emergency C-section, respectively (t=-8.43, df = 150, p<0.001). Statistically significant differences were observed in the gestational age (t=-3.36, df = 218, p = 0.001), fifth-minute Apgar score (t=-2.32, df = 200, p = 0.021), umbilical cord NRBC (t=-8.43, df = 160, p<0.001), and short-term prognosis (p = 0.032) between the two groups. It was also revealed that the number of NRBCs in the dead neonates was about 1.5 times higher than that in the discharged neonates. **Conclusions:** Based on the results of the present study, emergency C-section increased the mean of umbilical cord NRBC by three times, compared to that of normal delivery. Since an increase in the NRBC raises the risk of infant death, it is advisable to take steps to maintain the health of children by identifying high-risk neonates through umbilical cord NRBC measurement immediately after delivery and special care.

Keywords: *Cesarean section, mothers, natural childbirth, neonate, nucleated erythrocytes, umbilical cord*

Introduction

Natural childbirth is the healthiest for extrauterine life. Hematological changes, many aspects of which are still unknown, occur in the newborn during childbirth.[1] NRBC, as the primary prerequisite for erythrocytes, is naturally found in fetal and neonatal circulation during the first week of life and is associated with gestational age and health status.[2] It is an erythropoietic progenitor cell not found in the peripheral blood of healthy neonates.[3] Numerous acute and chronic stimuli increase the number of NRBCs in circulation due to the enhanced activity of erythropoietic or the sudden release of bone marrow reserves. In healthy neonates, the number of NRBCs is less than 10/100 WBC and decreases rapidly after birth, so that NRBCs are not usually observed on the fourth day. However, in premature infants, NRBC levels are usually higher and are seen in the peripheral blood up to a week after birth. The reason for elevated NRBCs is often attributed to prematurity, fetal anemia or neonatal hemolytic disease, cyanotic heart disease, intrauterine growth retardation, maternal diabetes, preeclampsia, smoking, intrauterine infections, acute or chronic chorioamnionitis or asphyxia, necrotizing enterocolitis, severe Intraventricular

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Hemorrhage (IVH), retinopathy of prematurity, and infections or death.[4,5]

The increase of nucleated red blood cells in the peripheral blood occurs because of stillbirths, hypoxic conditions and antenatal stress,[6] erythroblastosis fetalis, maternal diabetes mellitus, acute fetal distress, intrauterine growth restriction, premature rupture of membranes, and chorioamnionitis. During pregnancy, fetal NRBCs are found in maternal blood circulation.[7] Anxiety about childbirth and the secretion of cortisol and catecholamines may prolong labor and cause the mother's blood vessels to constrict during labor, reduce placental blood flow, and reduce fetal oxygenation, leading to fetal hypoxia. Hypoxia, in turn, leads to an increase in the number of NRBCs in cord blood.[8,9] In Bedrick's study, an elevated NRBC count after childbirth indicated utero hypoxemia that likely occurred before delivery.[10] According to the results of a study performed by Boskabadi, absolute NRBC amounts and NRBC percentages could be used as markers for predicting the neonatal morbidity and mortality rate.[11] The results of research conducted by McCarthy showed that delivery in low-risk pregnant women, in the absence of an abnormal fetal heartbeat, had no effect on umbilical cord NRBC levels.[7] Due to the contradictory results of previous studies; the uncertain effect of the type of delivery on the number of umbilical cord NRBCs, especially in premature neonates; and the importance of identifying high-risk preterm infants based on NRBCs, the present study was performed to compare the percentages of umbilical cord NRBC in vaginal delivery and in emergency cesarean section.

Materials and Methods

This cross-sectional study was performed on 221 premature neonates born in the maternity ward of Hospital, from 2020 to 2021. The premature neonates born by vaginal delivery or emergency C-section were selected through the convenience sampling method. A sample containing 1.5 cc of whole blood from the neonate's umbilical cord, which is usually thrown away, was collected to evaluate the NRBC level. The samples were gathered in ethylenediaminetetraacetic acid anticoagulant tubes. Peripheral blood samples were prepared, and a Leishman stain was performed on them. In this study, the number of NRBCs per 100 white blood cells and the absolute number of NRBCs per cubic millimeter in the blood were calculated. The exclusion criteria were neonates with diabetic mothers, preeclampsia, hemolytic jaundice, positive Coombs test, infections, and acute and chronic hypoxia, as well as neonates born by elective cesarean section.

By using the test of two averages related to a quantitative trait in two communities and with alpha of 1% and beta of 1% (power of 99%), 92 premature births were obtained in each group of 92 babies.

The required data were collected using a researcher-made questionnaire, which contained information about maternal characteristics (e.g., maternal age, type of delivery, and parity), neonatal characteristics (e.g., gestational age, first-minute Apgar score, fifth-minute Apgar score, birthweight, gender, need for resuscitation, short-term prognosis) and neonatal laboratory specifications (e.g., umbilical cord NRBC). The gathered data were analyzed in SPSS software (version 26, IBM SPSS Statistics 26.0 IF006). Initially, statistical tables and graphs were used to describe the results. Next, the Chi-square and *t*-test tests were applied to compare the NRBC levels in newborns born by the two delivery methods. The significance level was considered $p \leq 0.05$ in all cases.

Ethical considerations

The parents' consents were obtained before entering the study. The approval to conduct this study was provided by the ethics committee of Mashhad University of Medical Sciences (IR.MUMS.MEDICAL.REC.1399.702).

Results

In this research, 300 mothers were entered into the study; however, 30 neonates with asphyxia, 26 newborns with preeclampsia mothers, 19 cases with diabetic mothers, 1 case with hemolytic jaundice, and 4 cases with infection were excluded from the study. Finally, 93 (42.10%) neonates born by vaginal delivery and 128 (57.90%) ones by C-section were studied. In this study, the mean (SD) scores of gestational ages at birth, umbilical cord NRBC, and neonatal weight were estimated at 30.75 (2.81) weeks, 18.22 (19.65) per 100 white blood cells (WBCs), and 1428.84 (494.66) g, respectively. Based on the findings of

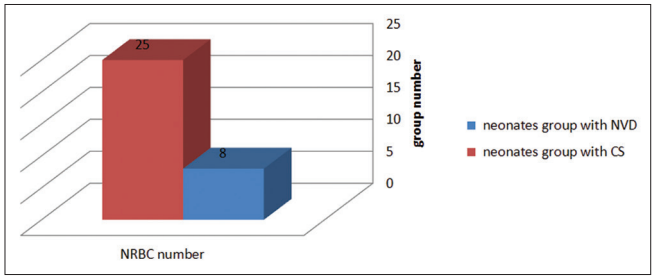

Figure 1: Comparison of nucleated red blood cell in two groups of neonates born by vaginal delivery and emergency cesarean section

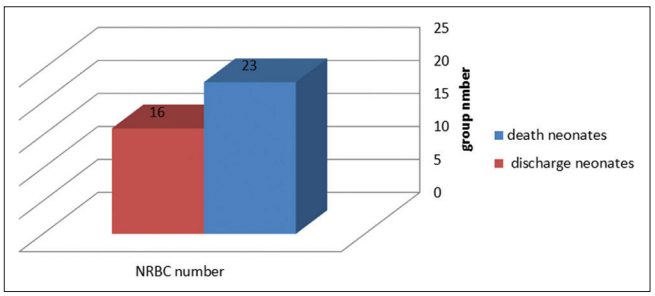

Figure 2: Comparison of nucleated red blood cell in two groups of dead neonates and discharged neonates

the present study, the first-minute Apgar score (p = 0.207), birthweight (p = 0.083), maternal age (p = 0.164), and parity (p = 0.232) were not significantly different between the two groups. Nevertheless, the difference between the two groups was statistically significant regarding the gestational age (p = 0.001), fifth-minute Apgar score (p = 0.021), and umbilical cord NRBC (p < 0.000), with the values being lower in the group of neonates born by natural childbirth [Table 1].

Also, another statistically significant difference was found regarding the short-term prognosis (p = 0.032), meaning that the mortality rate was about 1.5 times higher in the group of neonates born by C-section than in their counterparts born by vaginal delivery (36% vs. 21%). It was also revealed that discharges were more in the group of neonates born by natural childbirth than in the group of neonates born by cesarean delivery (78% vs. 63%). Nevertheless, no statistically significant difference was observed between the two groups in terms of neonatal gender (p = 0.354).

The number of NRBCs was more than three times higher in the group of neonates born by emergency C-section than in the group of their counterparts born by vaginal delivery [Figure 1]. According to the results of the multivariate logistic regression model, variables NRBC/100WBC and gestational age had a significant effect on the response variable. After adjusting for confounding factors such as gestational age and fifth-minute APGAR score, again the number of NRBCs in the emergency cesarean section was significantly higher than in normal delivery (p = 0.022).

Furthermore, the number of NRBCs was found to be about 1.5 times higher in the group of dead neonates than in the discharged ones [Figure 2].

Discussion

Based on the results of the present study, the umbilical cord NRBC/100WBC was more than 3 times higher in neonates born by emergency cesarean delivery than in neonates born by vaginal delivery. It was also found that the levels of umbilical cord NRBC varied according to the type of delivery in different studies. After controlling for confounding factors such as gestational age with multivariate regression analysis, NRBC levels in neonates born by emergency cesarean delivery were still significantly higher than in neonates born by vaginal delivery. In a study performed by Mital, the NRBC levels were significantly lower in the vaginal delivery group than in the cesarean delivery grou*p* 9.25(7.29) vs. 14.25 (6.51).[12] In another research carried out by McCarthy, the mean scores of NRBC/100WBC were calculated at 7.80 (7.40) and 9.3 (10.5) in the neonates born by elective C-section and natural childbirth groups, respectively, which was not significantly different.[7] According to the results of a study performed by Mansour Ghanaie, the mean umbilical cord NRBC count of neonates born by elective C/S was less than those delivered by natural childbirth, although this difference was not significant.[13]

In our study, since clear cases of asphyxia were excluded, mechanisms other than hypoxia might have been effective in increasing NRBC. Numerous mechanisms have been suggested to increase NRBC during labor among term newborns, most of which have been attributed to justifying the increase in NRBC following hypoxia during labor or pregnancy. There was also the possibility that mild hypoxia failing to result in birth asphyxia led to increased NRBC. Elevated NRBC has also been reported in important neonatal diseases, such as infections, retinopathy of prematurity, and IVH, which complicates the justification of the mechanism of changes. On the other hand, based on the findings of studies, NRBC can be a predictor of neonatal morbidity and mortality.[5]

In this study, the mean scores of gestational ages at birth and umbilical cord NRBC were estimated 30.75 (2.81) weeks and 18.22 (19.65) per 100 White Bood Cells (WBCs), respectively. It has been reported that the NRBC count at birth is higher in more premature neonates.[14] In premature newborns, erythropoiesis increases; therefore, a significant number of NRBCs can be observed for a longer period after birth. The increased number of NRBCs

Table 1: Comparison of the mean (SD) of some maternal and neonatal variables in the two groups of neonates born by natural childbirth and emergency cesarean section

Variables	Neonates born by natural childbirth 93 (42.10%) cases Mean (SD)	Neonates born by emergency cesarean delivery 128 (57.90%) cases Mean (SD)	*p*
Gestational age (weeks)	30.02 (3.00)	31.28 (2.54)	0.001
First-minute Apgar score	5.94 (2.32)	6.35 (2.26)	0.207
Fifth-minute Apgar score	7.48 (1.59)	8.01 (1.61)	0.021
Birthweight (g)	1360.77 (510.76)	1478.15 (478.64)	0.083
Mother age (years)	28.87 (6.30)	30.31 (6.73)	0.164
Parity	2.16 (1.00)	1.97 (1.06)	0.232
Umbilical cord NRBC* Per 100 WBC	8.01 (5.93)	25.64 (22.61)	p<0.001

*Nucleated Red Blood Cells

indicates an increase in erythropoiesis for a variety of reasons, including chronic hypoxia, anemia, acute stress, or postpartum hypoxia. The results of several studies were indicative of the existence of a relationship between an increase in the number of NRBCs and an increase in postnatal morbidity and mortality.[15] Valina *et al*. reported significant differences in the number of NRBCs between the group of term and preterm infants, especially on the first day of birth. It was revealed that in term newborns (relatively healthy and sick), the number of NRBCs decreased significantly between days 0 and 1 and to a lesser degree between days 1 and 4. They also found that in preterm infants, the number of NRBCs decreased dramatically between days 1 and days 2-4.[16]

It was found that NRBC was significantly higher in dead newborns than in discharged live neonates. Among patients admitted to the pediatric intensive care unit, NRBC levels, especially more than 200 per microliter, are associated with a mortality rate of post-admission in the intensive care unit and post-discharge.[17] It was revealed that increased NRBC on days 2-5 of birth is associated with high mortality.[15] Furthermore, increased adverse neonatal outcomes, such as white matter injury, necrotizing enterocolitis, and open arterial duct, are also associated with increased NRBC in preterm neonates.[18] According to the results of a study carried out by Morton, there was a significant relationship between the number of NRBCs and the increased risk of neonatal mortality among newborns hospitalized over 7 days in the ICU.[19] Christensen *et al*. found that an increase in the number of NRBCs in newborns at birth indicated the onset of hypoxia from at least 28 to 29 hours before birth. To date, limited data have been provided regarding the diagnostic and predictive value of NRBC in sick neonates.[14] In research conducted by Sokou, NRBCs showed significant predictive power for mortality in neonates with sepsis, especially in preterm newborns with sepsis, with a cutoff point of ≥1% and 81.60% sensitivity and 78.10% specificity.[20] Therefore, NRBC counting can be considered an early diagnostic and predictive marker for sick neonates.[4]

The most important limitation of our study involved not investigating the causes of emergency C-section and preterm labor. Furthermore, studies could be conducted taking these causes into account. It is also suggested to conduct a similar study with a larger sample size.

Conclusion

The findings of the present study suggest that umbilical cord NRBCs increase during emergency C-section and in cases of neonatal death. Therefore, the examination of NRBC in premature infants can serve as a prognostic factor for the newborn. On the other hand, it may be affected by the method of delivery, which had better be considered in the evaluation of NRBC of the newborn.

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Conflicts of interest

Nothing to declare.

References

1. Boskabadi H, Zakerihamidi M, Bagheri F. Outcomes of vaginal delivery and cesarean in Mashhad Ghaem University Hospital. Tehran Univ Med J 2014;71:807-15.
2. Christensen RD, Henry E, Andres RL, Bennett ST. Reference ranges for blood concentrations of nucleated red blood cells in neonates. Neonatology 2011;99:289-94.
3. Pedersen SJ, Chok R, McKillop S, Rojas-Vasquez M, Duff JP, Szkotak A, *et al*. Peripheral nucleated red blood cells and mortality in critically ill children. J Pediatr Hematol Oncol 2022;44:79-83.
4. Boskabadi H, Zakerihamidi M, Sadeghian MH, Avan A, Ghayour-Mobarhan M, Ferns GA. Nucleated red blood cells count as a prognostic biomarker in predicting the complications of asphyxia in neonates. J Matern Fetal Neonatal Med 2017;30:2551-6.
5. Boskabadi H, Sadeghian MH, Sadeghinasab J. Investigation of changes in nucleated red blood cells in neonatal infection. Iran J Neonatol 2017;8:26-32.
6. Cohen MC, Peres LC, Al-Adnani M, Zapata-Vázquez R. Increased number of fetal nucleated red blood cells in the placentas of term or near-term stillborn and neonates correlates with the presence of diffuse intradural hemorrhage in the perinatal period. Pediatr Dev Pathol 2014;17:1-9.
7. Hebbar S, Misha M, Rai L. Significance of maternal and cord blood nucleated red blood cell count in pregnancies complicated by preeclampsia. J Pregnancy 2014;2014:496416. doi: 10.1155/2014/496416.
8. Masoudi Z, Akbarzadeh M, Vaziri F, Zare N, Ramzi M. The effects of decreasing maternal anxiety on fetal oxygenation and nucleated red blood cells count in the cord blood. Iran J Pediatr 2014;24:285-92.
9. Reynolds F. The effects of maternal labour analgesia on the fetus. Best Pract Res Clin Obstetr Gynaecol 2010;24:289-302.
10. Bedrick AD. Nucleated red blood cells and fetal hypoxia: A biologic marker whose 'timing' has come?. J Perinatol 2014;34:85-6.
11. Boskabadi H, Rakhshanizadeh F, Zakerihamidi M. Assessment of umbilical cord nucleated red blood cell count in discharged and dead very low birth weight infants. Iran J Neonatol 2020;11:36-42.
12. Mital P, Mital P, Hooja N, Makkar P, Gupta D, Singhal S. Association of cord blood nucleated red blood cells with socio-demographic factors in term pregnancy. Age 2016;15:20-5.
13. Mansour Ghanaie M, Mansour Ghanaie R, Asgari Galebin SM, Sharami SH, Mohebalizade M. Does labor affect neonatal NRBC count?. J Obstet Gynecol Cancer Res 2022;7:25-31.

14. Christensen R, Lambert D, Richards D. Estimating the nucleated red blood cell 'emergence time' in neonates. J Perinatol 2014;34:116-9.
15. Cremer M, Roll S, Graf C, Weimann A, Buhrer C, Dame C. Nucleated red blood cells as marker for an increased risk of unfavorable outcome and mortality in very low birth weight infants. Early Hum Dev 2015;91:559-63.
16. Valina O, Linssen J, Rutsche J, Leibundgut K. Evaluation of the automated nucleated red blood cell (NRBC) enumeration on Sysmex XN analyser in preterm and term neonates. Sysmex J Int 2020;30:1-8.
17. Purtle SW, Horkan CM, Moromizato T, Gibbons FK, Christopher KB. Nucleated red blood cells, critical illness survivors and postdischarge outcomes: A cohort study. Crit Care 2017;21:1-9.
18. Bin-Nun A, Mimouni FB, Fink D, Sela H, Hammerman C. Elevated nucleated red blood cells at birth predict hemodynamically significant patent ductus arteriosus. J Pediatr 2016;177:313-5.
19. Morton SU, Brettin K, Feldman HA, Leeman KT. Association of nucleated red blood cell count with mortality among neonatal intensive care unit patients. Pediatr Neonatol 2020;61:592-7.
20. Sokou R, Ioakeimidis G, Lampridou M, Pouliakis A, Tsantes AG, Tsantes AE, *et al*. Nucleated red blood cells: Could they be indicator markers of illness severity for neonatal intensive care unit patients? Children 2020;7:197.