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The Relationship between Evolutionary Premature Infants at the Age of Two and the Number of Nucleated Red Blood Cell Peripheral Blood

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ABSTRACT

BACKGROUND AND OBJECTIVE: Despite the increased chance of premature survived infants, the morbidity of these infants is still significant. The number of NRBCs in the cord of these neonates may be effective in predicting the long-term complications of these neonates. The purpose of this study is to investigate the relationship between the number of nucleated red blood cells in the peripheral blood of preterm infants and the same prognosis about children at the age of two.

METHODS: The present study is a cohort study, has been performed on 290 preterm infants younger than 32 weeks old admitted in neonatal ward of Ghaem hospital of Mashhad during years of 93 to 96 with available sampling. Cord blood samples were taken to determine NRBC/100WBC count. Data have been collected by a researcher-made checklist including neonatal, maternal, and laboratory characteristics. Infants' development up to two years of age has been studied. NRBC/100WBC numbers of infants were compared with normal prognosis (Denver 2 normal test) and abnormal prognosis (death or developmental delay).

FINDINGS: Of 290 neonates, 160 of them (55.2%) had normal development, 61 cases (21%) died and 69 neonates (23.8%) had abnormal development. The mean number of mm2 /NRBC and NWBC/100WBC in neonates with abnormal prognosis were 4025.11±210.04 and 31.83±15.14 and in normal neonates were 367.47±76.91 and 5.5±5.29, respectively ($p<0.001$). An absolute NRBC count> 300/mm2 suggests a poor prognosis at the age of two with a sensitivity of 75% and a specificity of 63%.

CONCLUSION: According to these results the mean of NRBC2/mm2 and NRBC/100WBC can be helpful in determining neonatal prognosis at the age of two.

KEY WORDS: *NRBC (nucleated red blood cells), prognosis, Premature infant, Developmental Disabilities, death.*

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Introduction

Preterm delivery is a traumatic physiological event in which neonatal neural and emotional development is threatened (1). In recent decades, the care and survival of premature infants has significantly increased. Infertility treatments have led to an increased likelihood of infertile mothers having children, and increased in incidence of prematurity, On the other hand, the expansion of the quality and quantity of neonatal units and the development of specialized neonatal staff and new methods of preserving premature infants have increased the chances of survival of premature and very low birth weight infants (2). Premature risk factors such as premature rupture of membranes can be effective in exacerbating these neonatal problems (3,4).

In Iran, infant mortality has declined in recent decades, with infant mortality reported at 8.2 per 1000 in 1396 and approximately 10%-28% of neonatal deaths due to prematurity and complications, but nerve damage is still high and about 20% of premature infants have some degree of pre-maturity (5). Predicting the problems of premature infants and their control may alleviate these problems. There have been many reports of predicting the problems of preterm infants, but reported laboratory factors are low and non-specific. Nucleated erythrocytes are actually immature erythrocytes that increase in response to increased erythropoietin in peripheral blood (6).

In healthy infants the NRBC count is less than 10 per 100 white blood cells and decreases rapidly after birth so that it is not usually seen on day 4, but in premature infants, NRBC levels are usually higher in peripheral blood and up to one week after birth (7). Increased NRBC counts are often due to pre-maturity, fetal anemia or neonatal hemolytic diseases, increased hematopoiesis following chronic disease, cyanotic heart disease, intrauterine growth retardation, maternal diabetes, preeclampsia, smoking , Intrauterine infections, chorioamnionitis or acute or chronic asphyxia, necrotizing enterocolitis, severe intraventricular hemorrhage , cerebral palsy, pre-mature retinopathy, and sepsis or death (8). Increased NRBC has been reported in inflammation, cerebral hemorrhage, and retinopathy (9,10). Increased NRBC counts not only indicate perinatal asphyxia, but also predict the risk of neurodevelopmental consequences (7,11). In the study of Li et al., who looked at primary predictors of brain injury and 2-year outcome in neonates with hypoxic-ischemic encephalopathy (HIE) based on the number of NRBCs at birth, neonates who had higher NRBC levels during the first 6 hours after birth had abnormal MRI and abnormal development at the age of two (12). In one study, cord NRBCs had acceptable sensitivity and high specificity in predicting complications in neonates with perinatal asphyxia (7).

Numerous studies around the world have evaluated the developmental status of preterm infants, but one study did not measure the number of NRBs in peripheral blood with prognosis of preterm infants, whereas the number of NRBCs may have been helpful in predicting the developmental status of preterm infants and used as a simple, low-cost method and as a warning sign for physicians and caregivers of these infants; Therefore, in this prospective study, the developmental prognosis of preterm infants at the age of two years was assessed using peripheral blood counts at birth.

Methods

This prospective study was approved by the Ethics Committee of the Vice Chancellor for Research of Mashhad University of Medical Sciences under the code of ethics IR.MUMS.fm.REC.1394.492 on 290 premature neonates less than 32 weeks of gestation born in the maternity ward of the Ghaem hospital which hospitalized in Mashhad during the years 1393-96 with available sampling. Infants with diabetic mother, preeclampsia, intrauterine growth retardation, smoking, intrauterine infections, chorioamnonitis, haemolytic icter, positive coombs, cyanotic heart disease, severe intraventricular hemorrhage (IVH) and congenital asphyxia, BPD, seizure, cerebral anomaly and chromosomal problems were excluded. A sample containing 1.5 cc of whole blood from the neonatal cord that was usually discarded was collected to evaluate NRBC levels and collected in EDTA anticoagulant vials. Peripheral blood samples were obtained and stained with Leishman's. NRBC count was calculated for 100 white blood cells on peripheral blood sample. Then, in a researcher-made checklist, neonatal characteristics (sex, gestational age, first minute apgar

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score, fifth minute apgar score, birth weight) and test results (urea, sodium, potassium, calcium, phosphorus, alkaline phosphatase, bilirubin, Direct bilirubin, PT, PTT, WBC, CRP, creatinine, hematocrit, platelets, neonatal NRBC) were recorded. The gestational age was based on the Ballard, LMP, or ultrasound charts for the first three months (13). Subsequently, infants were followed up until discharge and their developmental status was evaluated at 6, 12, 18 and 24 months using Denver II Evaluation Questionnaire. In the Denver II Evolutionary Test, children are assessed in four personal-social domains, subtle-adaptive movements, language (speech), and violent movements. If the infant has difficulty in any of the above groups (ie one of the obvious movements, subtle-adaptive, personal-social movements or speech), it is considered as developmental delay. If only one column had problems, considered as mild evolutionary delay, if two columns had problems, considered as intermediate evolutionary delays, and if 3 columns and above had problems, considered as severe evolutionary delays (7).

Children with developmental delay or death were considered as abnormal prognosis and other children were considered as normal development and finally the two groups were compared in terms of NRBC at birth. Data were analyzed using T-test, Chi-square and SPSS software (version: 23). Covariance statistical method was used to control for confounding variables. The ROC curve was used to check the sensitivity and specificity of NRBC/100WBC and the absolute number of NRBC. To control for confounding factors, covariance tests were used and $p<0.05$ was considered significant.

Results

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Table1. Comparison of mean variables of neonates with normal prognosis and neonates with abnormal prognosis

Groups / Variables	Natural prognosis160 newborns (55.2%) (Mean±SD) or number(%)	Abnormal prognosis 130 newborns (44.8%) (Mean±SD) or number(%)	P-value (T-Test)
First minute Apgar score	6.2±0.6	5.2±0.7	0.850
Fifth minute Apgar score	7.1±0.54	7.1±0.75	0.156
Gestational age (weeks)	30.2±1.29	29.2±1.41	0.000
Birth Weight (g)	1354.330±84.72	1142.267±11.42	0.000
Urea (mg/dl)	44.19±43.10	54.33±29.92	0.104
Creatinine (mg/dl)	0.75±0.27	0.87±0.38	0.058
Sodium (mEq/L)	139.5±92.65	140.5±22.64	0.776
Potassium (mEq/L)	4.01±0.68	4.07±0.92	0.716
(PT) seconds	21.9±7.31	21.7±13.50	0.984
(PTT) seconds	63.32±23.40	76.37±23.37	0.347
Platelet (thousand per millimeter)	196.83±88.53	172.77±27.76	0.055
WBC (thousand per millimeter)	11.7±03.48	12.8±30.60	0.221
NRBC Absolute Number (in mm)	367.364±76.91	4025.131±21.04	0.000
NRBC%	5.3±8.29	31.13±15.14	0.000
Sex			
Male	63(38.9)	57(60.9)	0.038
female	85(61.1)	45(39.1)	
Type of delivery			
normal	33(59.5)	32(45.8)	0.866
Cesarean section	38(40.5)	39(54.2)	

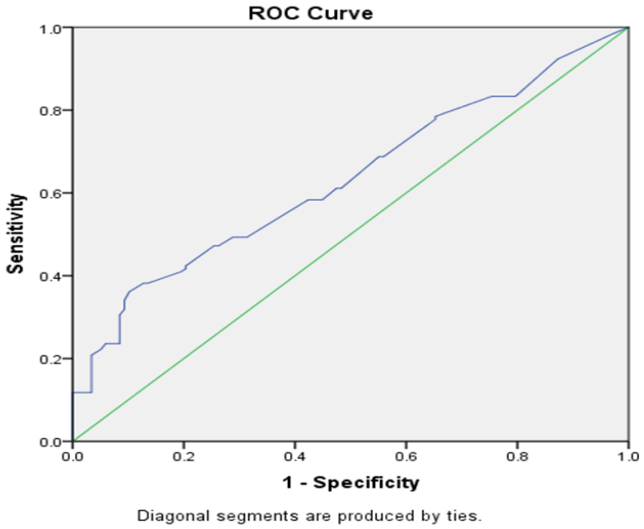

Figure 1: ROC curve to investigate the sensitivity and specificity of NRBC/100 WBC>3/5 to predict the control group and case (p=0.000, AUC=0.640, CI-95%=0/570-0/710)

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Discussion

The results of the study showed that the absolute count of cord NRBC level in infants who have problems at two years of age is 11 times more than that of normal infants, and the average number of NRBC/100WBC in infants with poor prognosis is about 5 times more than that of normal infants. After controlling for weight and gestational age as confounding factors again, there was a significant difference between the two groups. A similar study investigating such a relationship was not found in the available literature. In Cremer et al.'s study, there was a significant relationship between NRBC and mortality in the two groups of deceased and alive neonates (14).

In the study of Kil et al., the mean NRBC count was significantly associated with perinatal death, necrozan enterocolitis, and intraventricular hemorrhage (8). In the study of Baschat et al., infants with severe bronchopulmonary dysplasia and intraventricular dysplasia who died had a higher NRBC mean and had a poorer prognosis (15). In our study an absolute number of NRBC> 300/mm2 suggests a poor prognosis at age two with a sensitivity of 75% and a specificity of 63%. In a study 100WBC/NRBC> 11 with a sensitivity of 85% and a specificity of 90% expressed the predictive value of complications in neonates with asphyxia (8). In the study of Li et al., Neonates who had an NRBC greater than 6 and an absolute NRBC count greater than 1324 during the first 6 hours after birth had a higher risk of abnormal MRI and abnormal development at age 2 (12).

In one study, NRBC/100WBC≥ 70 predicted 82% of serious complications on the fourth day after birth (15). In one study, the sensitivity and specificity of more than 10 nucleated erythrocytes in differentiating neonates in the case and control groups were 33.3% and 100%, respectively (16). 23% of neonates discharged from neonatal ward in this study had developmental delay; most of which (76%) had mild to moderate developmental delay and 24% of neonates had severe developmental delay. According to the results of a study on 270 preterm infants, 49 of them (18%) at 24 months had some degree of developmental delay, 20% with severe developmental delay, and 80% with mild to moderate developmental delay (4). In the study of Krestjens et al. in preterm infants of 34-36 weeks, the most common complication was developmental delay (17).

In the study of Vatansever et al., the number of NRBCs in high-risk infants was higher than that in the control group and there was a significant difference in the number of NRBCs with prognosis. They also found that a number of NRBCs could be useful in short-term neonatal developmental status (18). Some recent studies also show that the number and length of staying high of NRBC stays with short-term and long-term unproductive consequences and also neural evolutionary disorders (19).

In the present study, the prognosis of male infants is worse than that of girls, which is similar to other studies (19,20). However, there is no specific reason for this difference (21,22). 23% of infants discharged from our neonatal ward had developmental delays, with three-quarters of mild to moderate developmental delays and one-fourth of neonates with severe developmental delays. Absolute cord NRBC levels in infants with developmental delays at two years were reported to be 11 times more than normal infants, and the mean NRBC/100WBC in infants with poor prognosis was about 5 times more than normal infants. An absolute NRBC count> 300/mm2 suggests a probability of poor prognosis at age two with sensitivity of 75% and specificity of 63%. Therefore, cord NRBC counting along with other methods can be helpful in predicting adverse neonatal complications.

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