Nucleated Red Blood Cells Count in Pregnancies with Idiopathic Intra-Uterine Growth Restriction

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Abstract

Objective: Elevated nucleated red blood cell (NRBC) count is introduced as a potential marker of intra-uterine growth restriction (IUGR). To investigate the probable association regardless of any known underlying disease, we aimed to study disturbances in NRBC count in infants experiencing idiopathic IUGR.

Materials and methods: Twenty three infants regarded IUGR without any known cause were chosen to be compared to 48 normal neonates. Blood samples were collected instantly after birth and the same measurements were done in both groups.

Results: NRBC count/100 white blood cells was significantly higher in the IUGR group (P value<0.001). pH measurements did not reveal any significant difference.

Conclusion: Increased NRBC count in cases of idiopathic IUGR in absence of chronic hypoxia could strengthen its predictive value suggested in previous studies. It could help early IUGR detection and beneficial intervention.

Keywords: NRBC, IUGR, Peripheral blood smear

Introduction

Intra-uterine growth restriction (IUGR) is known as a serious complication in pregnancy that could dramatically affect the morbidity and mortality rate. Several conditions are known to be in association with IUGR and the causes are not exactly clarified (1). Functional disturbance of placenta could be the underlying pathology (2).

There are many efforts to reach non-invasive diagnostic methods to detect IUGR prior to birth (1). Ultrasound evaluations are among the most common techniques (3,4). There are investigations studying the changes occur in the cells type or their distributive pattern under circumstances leading to IUGR (1). Disordered shift of nucleated red blood cells (NRBCs) has been noticed in specific pregnancy complications (5-9).

Elevated NRBC count is in relation with complications like hypoxia (5), hypoxemia (8), asphyxia, maternal diabetes, prenatal brain damage, preterm infants (6), RDS (7), and preeclampsia (9). Increased NRBCs could itself provoke progressive growth restrictions (10). Therefore, NRBC count is introduced as a marker that shows disturbed fetal status and could help to detect and manage obstetric

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and neonatal complications prior to birth (11).

This study intends to investigate the disturbances of NRBC count in IUGR infants who did not experience any known underlying disease. This could help to withdraw any other known causes leading to IUGR and elevated NRBC count.

Materials and methods

Twenty three newborns known to have experienced IUGR, born between the 30 and 36 weeks of pregnancy, were chosen to be studied. Our study was carried out at the high risk ward of an Obstetrics and Gynecology specialized center, affiliated to the Tehran University of Medical sciences. The methodology was ethically confirmed and patients were asked to fill out the informed consent form before any corporation. Forty eight cases were considered as the control group. Those were selected from neonates that experienced normal pregnancy. All cases were born by cesarean section.

Cases experiencing maternal hypertension, anemia, diabetes, cardiovascular diseases, or fetal abnormal karyotype, infection, and chronic hypoxia during pregnancy were omitted due to their known effect on IUGR (12). The gestational age was calculated referring to the last menstrual period (LMP) and the 1st trimester ultrasound evaluation. The IUGR cases were chosen regarding the 10th percentile for the gestational age (12).

Blood samples were collected instantly after birth from the umbilical vessels. Weight of the neonates and maternal age were registered. Fetal NRBC count, fetal total RBC and WBC count, and pH were measured all in the same laboratory in which kits and protocols were reliable. NRBCs were counted per 100 WBCs in peripheral blood smear (PBS) after staining the samples with Wright protocol.

SPSS software version 14 (SPSS Inc. Chicago, IL, USA) was used for statistical analysis. Independent-samples T test was utilized to compare differences between parametric data groups and chisquare test was employed wherever needed. We considered p value< 0.05 as significant.

Results

Weight of the neonates showed significant difference between the IUGR and control group. The mean value of the IUGR group was 1626± 249 grams versus 2553± 423 grams in the control group (p value< 0.001). Of those in the IUGR group (n=23) one (4%) was < 1kg, 5 (21%) were 1-1.5 kg, 17 (74%) weighed 1.5-2 kg and none of them was above 2 kg. While 47 (98%) of the cases in control group (n=48) weighed above 2 kg, 1 (2%) 1.5-2 kg and none under 1.5 kg.

The average of the maternal age did not demonstrate any significant contrast between two groups, 23.32± 2.85 and 24.13± 3.57 of years in IUGR and control group respectively (p value= 0.3). Nine of IUGR pregnancies (39.1%) had one gravidity, 5 (21.7%) had two, remaining 9 cases (39.1%) had three or more. In the control group, 20 (41.7%) experienced the 1st gravidity, 16 (33.3%) the 2nd and 12 (25%) the 3rd or more. No significant association was found between IUGR and gravidity numbers (p value=0.41).

NRBC count/100 WBCs was compared between two groups. The average of NRBC count in IUGR group was 35.8± 11.7 versus 7.4± 3 NRBCs/100WBCs in the control group. Those values revealed a significant difference between two groups (p value<0.001).

pH was measured to investigate chronic hypoxia in newborns. We did not find any significant difference in the number of cases who experienced chronic hypoxia in both groups. In IUGR infants, 8.7% (2 cases) had pH measurements lower than 7.2 whereas 4.2% (2 cases) in control group had the same condition (p= 0.39).

A summary of our results is brought in Table 1.

Table1. Comparison between Perinatal characteristics and NRBC count

	Control group	IUGR group	p value
Maternal Age (years)	24.13± 3.57	23.32± 2.85	NS
Birth Weight (grams)	2553± 423	1626± 249	<0.001
Gravidity	1.83± 0.8	2± 0.9	NS
NRBC/100 WBC Count	7.4± 3	35.8± 11.7	<0.001
pH	7.33± 0.06	7.33± 0.07	NS

Values are mean± SD, NS: Not Significant

Discussion

Neonates weight was expectedly lower in the IUGR group (p< 0.001). We found that the NRBC count was significantly higher in the IUGR group (p< 0.001). No other difference was found comparing the other variables including maternal age, gravidity numbers, and pH levels.

During pregnancy, NRBC count elevation is reflected in the maternal blood (13). Villous trophobalstic layers separate the maternal and fetal

blood circulation in placenta (2). Placental villus development is disturbed in pregnancies with IUGR which could be due to inconvenient oxygenation (14). In IUGR condition the cytotrophoblasts of villus are decreased and the exchange of extra cellular matrix is intensified between the maternal and fetal circulation. That results in increased NRBC count in maternal blood (15). Maternal NRBC count is not reliable in the first trimester due to insufficient villous invasion process which is accomplished in the second trimester (1). However, a significant increase in erythroblast proportion of transferred fetal cells into maternal blood in pregnancies with early onset IUGR was reported by Al-Mufti et al in 2002 (16).

Diseases causing severe IUGR elevate the NRBC count in the second trimester which may remain high days after birth (7). Some percentage of NRBCs is rooted from maternal origin (17). Those should be distinguished from the fetal hemoglobin expressing NRBCs (FHE-NRBC). It has been reported that almost 23% of NRBCs are from the mother in normal pregnancies and the rest are produced in fetus (18). Increased NRBCs in pregnancies with IUGR shows strong relation to positive Kleihauer-Betke stained cells indicating true fetal NRBCs (19). According to those findings, elevated NRBC count could be remarkably referred to fetal erythropoiesis.

To investigate if NRBC count is a hypoxia maker or not, Ravishankar V et al studied the effect of hypoxia and NO synthase inhibition (by L-Name) on NRBC count in a rat model of IUGR. Interestingly, they indicated that NRBC count could not serve as a hypoxia or erythropoietin elevation marker in IUGR while it is related to nitric oxide inhibition (20). However, Vatansever U et al reported significant relation between NRBC count and neonatal relative hypoxia in IUGR, asphyxia, diabetic mothers, and preterm infants. There was not such an association in case the serum erythropoietin was measured. They suggested that NRBC count could have predictive value in short term neurodevelopmental outcome (6).

There are many investigations confirming the elevation of NRBC count in IUGR and other circumstances ending in IUGR or several other fetal abnormalities. Those data is always said to be due to the increased erythroblasts in fetal circulation even if placenta has normal perfusion (2). A significant difference of NRBC count in umbilical artery samples of normal pregnancies versus preeclamptic ones with and without IUGR was reported in 2010 (9). Kovalak suggested the significant relation between NRBC count and nonreasuring fetal heart rates during labour (21). In 2003, Baschat also introduced the association between NRBC and prenatal complications in IUGR. It was showed that the presence of NRBCs in neonates is related to antenatal abnormal umbilical artery Doppler evaluations. They also mentioned that the prolonger the NRBCs present, the more abnormalities has occurred. Those may introduce the diagnostic value of NRBC count in IUGR fetuses (7). Their result is in congruency with our and the aforementioned studies. Therefore, NRBC count could have a potential role in predicting IUGR and its complications.

In case of asphyxia, NRBC count is correlated with umbilical vessels pH at birth. Chronic fetal distress causes higher counts compared to acute condition. It is reported that the cut-off value of 14NRBC/100 WBC could be the predictor of severity and duration of acidosis. (Sensitivity 89%, Specificity 81%) (5). However we could not find such association. Since we omitted neonates having IUGR due to any known asphyxia, it may have influenced our results. According to our study, in idiopathic IUGR, no association exists between acidosis and NRBC count. That could strengthen the association found between increased NRBC count and IUGR under different conditions resulting in fetal growth retardation. It is showed that several patterns of underlying diseases causing preterm delivery lead to different results of NRBC count. This could be due to different mediators of NRBC release, cytokines versus erythropoietin (22).

Other studies concluded that the increased NRBC count in IUGR neonates is significantly higher than small for gestation but healthy neonates (23). In 1989, there was a comparison made between appropriate for age (AGA) and small for age (SGA) infants with very low birth weight (500-1500 g). The NRBC to WBC count in SGA was significantly higher in evaluations in their first hours. Compared to findings associating hypoxemia with increased NRBCs in umbilical blood samples of growth retarded fetuses, they proposed NRBC count elevation is suggestive for chronic intrauterine insults (24). In 1997, Bernstein et al concluded that the neonates small for gestation who had abnormal Doppler studies in their gestational period had higher NRBC counts along with lower platelet count (3). Those findings may indicate the reason of our findings in IUGR cases without any known underlying cause. Since they suggested there could be

antenatal thrombotic events due to fetal response that results in such changes.

Predictive value

NRBC count could have predictive value in short term neurodevelopmental outcome (6) or in IUGR complications (23). Axt-fleidner et al showed the potential predictive role of NRBC count in SGA infants, to distinguish healthy infants from IUGR ones who may have complications (25). The high sensitivity (94.4%) and specificity (80%) of NRBC count to predict asphyxia in preeclampsia is also reported (9). However since its weak relation with pH and O2 pressure of umbilical artery, it is believed that its predictive role is restricted (21). In 2007 Baschat et al, studied the predictive value of daily NRBC count in several neonatal complications. They concluded that elevated NRBCs beyond day 3 of birth could independently predict poor outcomes in preterm growth restricted neonates. They believed NRBC count provides information equivalent to some more complicate methods (11). However the same group mentioned its limitations due to other prenatal variables affecting and triggering those complications (7).

Conclusion

We found the average number of NRBC count/100 WBCs was 35.8±11.7. This value is higher than the cut-off levels other studies suggested (5, 11), and is significantly higher than the normal infants considered as the control group. As previously described this number could be reliably considered as fetal NRBC count (19). Increased NRBC count could have a predictive role in IUGR diagnosis and may help the clinicians to detect fetal distress in an appropriate time before the birth and even before ultrasonically diagnosis. The outcome of a pregnancy could improve in case of early intervention.

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