Nucleated Red Blood Cell Count in Infants of Women With Well-controlled Gestational Diabetes Mellitus

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Abstract

Objective: To compare the absolute number of nucleated red blood cell (NRBC) counts within twelve hours of birth in healthy infants and infants of women with gestational diabetes mellitus (GDM).

Methods: We compared absolute NRBC counts in two groups of term infants, appropriate size for gestational age (AGA) infants of women with gestational diabetes (n=20) and AGA infants of nondiabetic women (n=20). To eliminate confounding variables that could affect absolute NRBC numbers, we excluded infants born to women with hypertension, smoking, alcohol abuse and those with intrapartum fetal heart rate abnormalities, low Apgar scores, hemolysis, blood loss, or chromosomal anomalies.

Results: There were no significant differences between two groups regarding maternal age, gravidity, parity, gestational age, newborn gender, cesarean rate, 1st and 5th minute Apgar scores and birth weight. The absolute NRBC counts were significantly higher in the GDM group than the control group ($p<0.001$).

Conclusion: We found higher absolute NRBC counts in infants of women with GDM which is close to the level of chronic hypoxia. This finding suggests that fetuses born to women with GDM were exposed to a relatively hypoxemic environment for a time period.

Key words: Gestational diabetes mellitus, hypoxia, neonatal nucleated red blood cell

Özet

İyi Kontrollü Gestasyonel Diabetes Mellitusta Neonatal Normoblast Sayısı

Amaç: Kontrollü gestasyonel diabetes mellituslu kadınların infantları ile sağlıklı kadınların infantlarında ilk on iki saat içinde bakılan mutlak normoblast (NRBC) sayısının karşılaştırılması.

Materyal ve Metot: Gestasyonel diabetes mellitusu olan (20) ve diyabeti olmayan anneden doğan normal doğum ağırlığına sahip (AGA) term infantların mutlak normoblast sayılarını karşılaştırdık. Mutlak normoblast sayısını etkileyebilecek değişkenleri elimine etmek amacı ile hipertansiyonu olan, sigara ve alkol kullanan annelerin infantları ile intrapartum fetal kalp hızı anormalliği, düşük Apgar skoru, hemoliz-kan kaybı ve kromozomal anomalisi olan infantlar çalışma dışı bırakıldı.

Sonuçlar: Her iki grup arasında anne yaşının, gebelik sayısı, doğum sayısı, gebelik haftası, cinsiyet, sezaryen hızı, 1. ve 5. dakika Apgar skorları ve doğum ağırlığı açısından bir fark yoktu. Gestasyonel diabetes mellitusu olan gruptaki mutlak normoblast sayısı kontrol grubuna göre anlamlı olarak yüksekti ($p<0.001$).

Tartışma: Gestasyonel diabetes mellitusu olan kadınların infantlarında mutlak normoblast sayısını kronik hipoksi seviyesine yakın derecede yüksek bulduk. Bu bulgular bize iyi kontrol edilmiş olsa bile gestasyonel diabetes mellituslu kadınların fetuslarının intrapartum herhangi bir dönemde görece hipoksemik bir ortam ile karşılaştıklarını düşündürmektedir.

Anahtar sözcükler: Gestasyonel diabetes mellitus, hipoksi, neonatal normoblast

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Table 1. Demographic data in control infants and in infants of women with gestational diabetes mellitus.

	GDM	Control	P Value
Birth Weight (g)	3415±401	3462±517	p>0.05
Gestational age (weeks)	38.4±1.1	38.7±0.9	p>0.05
Sex (males/females)	11/9	10/10	p>0.05
Maternal age	31.0±7.5	27.9±5.2	p>0.05
Gravidity	2.2±1.2	2.4±1.5	p>0.05
Parity	0.9±0.6	0.9±0.8	p>0.05
Cesarean rate (%)	30	25	p>0.05
1st min Apgar score	9 (9-10)	9 (8-10)	p>0.05
5th min Apgar score	10 (9-10)	10 (9-10)	p>0.05

All data are expressed as mean standard deviation (SD) or n (%), except Apgar scores which are expressed as median (range).
GDM= Gestational diabetes mellitus.

Table 2. Hematologic data in control infants and in infants of women with gestational diabetes mellitus.

	GDM	Control	P Value
pH	7.27±0.04	7.32±0.05	p>0.05
Hematocrit (%)	51.2±8.0	48.1±7.3	p>0.05
RBC (x10^6/mm^3)	4.8	4.5±	p>0.05
Platelets (x10^6/mm^3)	192.4±79.1	233.2±86.8	p>0.05
Corrected white blood cells(x10^3/mm^3)	18.0±6.8	15.6±5.6	p>0.05
Absolute lymphocyte count (x10^3/mm^3)	7.8±2.5	5.4±2.8	p>0.05
Absolute NRBC count (x10^3/mm^3)	0.68 (0.10-5.20)	0.1 (0.08-1.60)	p<.001

All data are expressed as mean standard deviation (SD) except absolute NRBC counts, which are expressed as median (range).
GDM= Gestational diabetes mellitus.
RBC= Red blood cell.
NRBC= Nucleated red blood cell.

Introduction

The clinical manifestations of the diabetic state in pregnancies have been attributed to fetal hyperglycemia, hyperlipidemia, hyperinsulinemia and reduced placental blood flow resulting in chronic fetal hypoxia [11]. A consequence of chronic intrauterine fetal hypoxia is increased erythropoiesis through erythropoietin stimulation which lead to increased numbers of circulating nucleated red blood cells, and increased hematocrit levels at birth [14]. Nucleated red blood cells (NRBCs) are immature erythrocytes and the presence of increased numbers of NRBCs in the circulation of term infants has been associated with states of relative hypoxia [3,6,7,12,13]. Several authors have demonstrated that insulin dependent diabetes is associated with elevated NRBC counts [3,5,11].
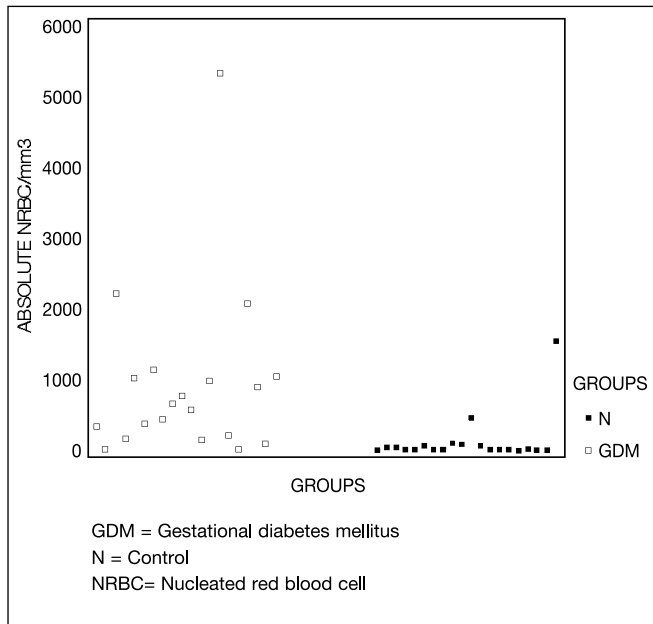
Gestational diabetes mellitus (GDM) is one of the most common clinical issues facing obstetricians and their patients and is associated with increased maternal and fetal-neonatal morbidity [1]. There is little information about the hematologic status of infants of women with GDM. The purpose of the current study was to compare the absolute number of nucleated red blood cells within twelve hours of birth in healthy infants and infants of women with GDM.

Materials and Methods

We prospectively studied two groups of term infants (37-41 weeks' gestational age by last menstrual period, confirmed by early ultrasonographic assessment), who were born at our Institution between January and November 31, 2001. The first group consisted of 20 infants of women with gestational diabetes who were born appropriate size for gestational age (AGA; between the 10th and 90th percentile,) according to the intrauterine growth curves of Hadlock [4], and the second group consisted of 20 AGA healthy infants of nondiabetic mothers.

In our clinic all pregnant women are routinely screened for gestational diabetes mellitus (GDM) between 24 and 28 weeks' of gestation by 50 g oral glucose load. When values were equal to or exceed 135 mg/dl, patients were given a 3-hour 100 g oral glucose tolerance test (OGTT) and GDM was diagnosed according to the criteria of Carpenter and Coustan [2].

Figure 1. Absolute NRBC counts in control infants and in infants of women with gestational diabetes mellitus.

All women with GDM in the first group were managed with diet alone (fasting glucose and 2-hour postprandial values were below 95 mg/dl and 120 mg/dl respectively) [1], and none required insulin. Glucose challenge screens were normal in the mothers of healthy infants (group 2).

To eliminate confounding variables that could affect absolute NRBC numbers, we excluded infants born to women with pregnancy-induced hypertension; placental abruption or placenta previa; any maternal systemic disease; maternal smoking, drug or alcohol abuse. We also excluded infants who had perinatal infections, abnormal electronic intrapartum monitoring, low Apgar scores (below 7 at one and five minutes), perinatal hemorrhage, hemolysis (blood group incompatibility with positive Coombs test), or chromosomal anomalies.

The study was approved by the institutional review board of the university and informed consent was obtained from each parent just before entering to the study.

Venous blood samples for hematologic analysis were obtained via venipuncture from the neonates into an ethylene diamine tetra-acetic acid tube within twelve hours of birth and analyzed with Cell Count Analyzer (Coulter-STKS, Luton, England). A slide was prepared with Wright stain and differential cell counts were done manually.

Clinically it is best to express NRBCs as an absolute number of cell per unit volume, either "NRBCs/mm^3" or "NRBCs/l" [7]. Unfortunately the extreme variability in the number of leucocytes after birth results in a wide range of values for NRBCs when they are expressed relative to the white blood cell (WBC) count [7]. Thus, we expressed the number of NRBC as an absolute number rather than per 100 WBC.

Blood was drawn from the umbilical artery into a heparinized syringe for blood pH analysis (GEM Premier 5300 blood gas analyzer; Mallinckrodt sensor Systems, Ann Arbor,USA).

Data were expressed as mean standard deviation (SD) or median (range) and statistical analysis was performed by SPSS for windows release 10.0 using Student's t test for normally distributed parameters and Mann-Whitney U-test for abnormally distributed ones. Differences were considered statistically significant at a level of $p<0.05$.

Results

Demographic data for the study population were shown in Table I. There were no significant differences between the two groups regarding maternal age, gravidity, parity, gestational age, newborn gender, 1st and 5th minute Apgar scores and birth weight (Table I). The mean gestational age at birth for the two groups were 38.4±1.1 and 38.7±0.9 weeks and the mean birth weights were 3415±401 g and 3462±517 g respectively. There were also no significant differences in cesarean rates between these two groups (p>0.05) (Table I).

Mean arterial cord blood pH were found to be 7.27±0.04 for GDM and 7.32±0.05 for control group and there were no significant difference between these two groups (p>0.05) (Table II).

The absolute NRBC counts were significantly higher in the GDM group than the control group (p<.001).(Figure 1) The white blood cell counts, absolute lymphocyte counts, red blood cell counts and hematocrit levels were higher in the GDM group than the control group however, no significant differences were found for any of these parameters between these two groups (p>0.05).(Table II) There were also no significant differences in platelet counts between the two groups (p>0.05).(Table II)

Conclusion

Although NRBCs are rarely found circulating in older children, they are commonly seen in the blood of newborns [7]. They are primarily produced in the fetal bone marrow in response to erythropoietin and are stored in the marrow as a precursor to reticulocytes and mature erythrocytes. Many acute and chronic stimuli cause increases in the number of circulating NRBCs from either increased erythropoietic activity or a sudden release from the marrow storage pools [7]. In 1924, Lippman [9] reported NRBCs in the blood of 41 of 42 newborns in the first day of life. These cells constituted about 500 NRBCs/mm^3 or 0.1% of the newborns circulating red blood cells. Since then, many investigators have reported similar values at and shortly after birth [3,5,7]. It is reasonable to conclude that the mean value of NRBCs in the first few hours of life in healthy term newborns is about 500 NRBCs/mm^3, and that a value above 1000 NRBCs/mm^3 can be considered elevated [5]. In our study the median (range)

of NRBC counts in normal population was found to be 100 (82-1600)/mm^3, concordant with the literature [3,5,7].

The presence of increased numbers of NRBC's in the circulation of term infants has been associated with diverse pathologic conditions such as hematologic disease of the newborn, intrauterine growth restriction, preeclampsia and perinatal brain damage [3,6,7,12,13]. An increase of NRBC's was also documented in infants of insulin dependent diabetic women, in whom a compensatory increase in erythropoiesis from chronic intrauterine hypoxia occurs as a result of poor glycemic control [11]. Indeed, maternal hyperglycemia triggers fetal hyperglycemia and hyperinsulinism, with subsequent fetal hypoxemia resulting from increased placental oxygen consumption and decreased fetal oxygen delivery [11]. Kamath et al. [8] has also shown that GDM induces oxidative stress in the fetus and in these pregnancies the risk for fetal hypoxia increases towards term. The hematopoietic system responds to hypoxia by increasing erythropoietin, which then increases erythroid production and releasing less mature form into circulation [5,7]. The reticulocyte response to hypoxia-induced erythropoietin release is generally not seen until the second or third day after hypoxia [10]. A similar increase was also documented in our study and infants of women with GDM had significantly higher absolute NRBC counts than the healthy infants (Figure1). This finding suggests that fetuses born to women with GDM were exposed to a relatively hypoxemic environment for a time period. In spite of a good glycemic control the infants of women with GDM in our study, had higher absolute NRBC counts. So, we can speculate that the maternal glucose fluctuations and hyperinsulinism or other metabolic changes that are not recognized by the standard monitoring criteria in GDM patients, may affect fetal oxidative status. So, the existing monitoring strategies for GDM such as the frequency and threshold of blood glucose needs to be refined and new metabolic- biochemical parameters should be used in addition to routine patient monitoring strategies.

In this study, the mean hematocrit did not differ significantly between infants of women with GDM and those of healthy non-diabetics. This might be due to our exclusion of infants who might have experienced the most severe hypoxia (ie, SGA, abnormal electronic intrapartum monitoring and low Apgar scores). In those cases, prolonged or pronounced hypoxia might sufficiently stimulate the bone marrow to cause a rise in hematocrit. However, the normal hematocrit

levels in the study patients can be explained by the absence of prolonged hypoxia.

In summary we found a higher absolute NRBC counts in infants of women with GDM which is close to the level of chronic hypoxia. Particular attention should perhaps be paid to infants of women with GDM in terms of fetal surveillance during pregnancy. Our results also suggest that in patients with GDM, the maintenance of maternal normoglycemia (fasting glucose and 2-hour postprandial values below 95 mg/dl and 120 mg/dl respectively) [1] solely, may not be sufficient to avoid the complications of fetal hypoxemia.

Before definite conclusion, regarding possible clinical applications of NRBC determinations is made further studies should determine the optimal NRBC threshold values and examine the validity of our results with a larger population of neonates.

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