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Original Article

Comparison of the umbilical artery blood gas, nucleated red blood cell, C-reactive protein, and white blood cell differential counts between neonates of diabetic and nondiabetic mothers

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Abstract

Objective: The aim of this study was to compare the neonatal umbilical artery blood gas values, C-reactive protein (CRP) levels, nucleated red blood cells (NRBCs), and white blood cells (WBCs) differential counts between offspring's of the diabetic mothers who needed insulin during pregnancy and normal mothers after cesarean delivery.
Materials and Methods: A prospective study was performed involving 68 pregnant diabetic women who needed insulin during pregnancy and 410 healthy pregnant women and their neonates with gestational ages between 35 weeks and 41 weeks. Arterial blood was analyzed for pH and blood gas values and venous blood was analyzed for CRP level, NRBC, and WBC differential counts.
Results: The mean NRBC count in the neonates of diabetic mothers and healthy mothers was $560 \pm 985/\mu L$ and $202 \pm 281/\mu L$, respectively ($p < 0.001$). The umbilical arterial blood gas showed a lower pH (7.22 ± 0.07 *vs.* 7.24 ± 0.04, $p = 0.004$) and a higher pCO_2 (49.33 ± 10.08 *vs.* 47 ± 8.67, $p = 0.045$) in neonates of diabetic mothers compared with the controls. Values of pO_2, HCO_3^-, base excess, WBC differential counts, and CRP levels were almost similar in the two groups.
Conclusion: Lower pH, higher pCO_2, and elevated NRBC counts were found in the neonates of diabetic mothers that may be suggestive of chronic intrauterine acidosis.

Keywords: C-reactive protein; Diabetes; Nucleated red blood cells; Pregnancy; Umbilical arterial blood gas; White blood cell counts

Introduction

The increasing prevalence of Type 2 diabetes in general, and in younger people in particular, has led to an increasing number of pregnancies with this complication [1]. Diabetic women in pregnancy can be separated in two groups: those who were known to have diabetes before pregnancy (pre-gestational or overt) and those diagnosed during pregnancy (gestational) [2]. The fetus of a diabetic mother is exposed to unexplained fetal death. It has been suggested that hypoxia and acidosis may at least partially account for the increased incidence of intrauterine fetal deaths in diabetic pregnancies [2]. During the past decade, umbilical cord blood gas analysis has increasingly been recognized as the most reliable indication of fetal oxygenation and acid-base condition at birth. Umbilical arterial blood most accurately reflects fetal status because it flows directly from the fetus [3]. In the neonates, increasing of circulating nucleated red blood cells (NRBC) is reported in states, such as hemolysis [4]; intrauterine growth restriction; and preeclampsia [5]. Few studies with small sample sizes reported NRBC values and hematological data in the neonates of diabetic mothers who were born by different routes of delivery [5,6].

On the other hand inflammation may play a role in the pathogenesis of hypoxia-related neonatal complications. Moderately raised C-reactive protein (CRP) levels have been

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found in the subjects at risk of developing cardiovascular diseases [7] and Type 2 diabetes [8]. Total white blood cell (WBC) counts and lymphocyte counts have been suggested to be as possible markers of fetal hypoxic injury [9]. The levels of hemoglobin A1c (HbA1c) is positively correlated with the long-term variations in maternal blood glucose levels in the preceding 2 months [10].

The aim of this study was to compare CRP levels, WBC, and NRBC counts in the umbilical vein and umbilical arterial blood gas values between the neonates of the diabetic mothers who needed insulin and neonates of normal mothers who were born by cesarean delivery. Also HbA1c levels were measured in the diabetic mothers at delivery to evaluate the long-term maternal control of blood sugars.

Materials and methods

Sixty-eight diabetic mothers who needed insulin therapy and cesarean deliveries were selected as the study group and 410 normal pregnant women who had elective cesarean deliveries because of previous cesarean sections were selected as the control group.

Gestational ages were calculated by the last menstrual period and confirmed by ultrasound. All participants were screened for diabetes during pregnancy and gestational diabetes was diagnosed according to the guidelines of the American College of Obstetricians and Gynecologists [11]. Blood samples were collected from the diabetic mothers at the time of delivery and HbA1c levels were measured by ion exchange high-performance liquid chromatography (Jamea Co., Tehran, Iran) to evaluate the effect of long-term blood sugar control on the fetal outcomes.

After delivery blood samples were collected from neonatal umbilical veins for hematological analyses. Hematological analysis was performed by an automated cell counts analyzer (Sysmex Kx 21N, Kobe, Japan) and the peripheral smears evaluated by a hematologist. Heparinized blood was taken from the umbilical artery for blood gas analysis (COMPACT 3 Blood Gas Analyser, Roche Diagnostics; Graz, Austria). Respiratory, metabolic, and mixed acidemia were defined according to the guidelines of American College of Obstetricians and Gynecologists [12].

The CRP level was measured by qualitative and semi-quantitative method of latex agglutination test (Kimia Pajouhan, Iran). We followed the maternal and neonatal admission charts and medical records for the information and outcomes. This study was approved by the medical ethics committee of Shiraz University of Medical Sciences, and written consents were provided by all the participants. Statistical analysis was made by SPSS Version 15 software (SPSS Inc., Chicago, IL, USA). Statistical t test and χ^2 test were used to evaluate the significance of differences in individual groups. A p value less than 0.05 was considered significant.

Results

There were 68 diabetic mothers who needed insulin during pregnancy, whose ages were between 20 years and 43 years (mean 31.13 ± 5.02) and 410 normal pregnant women with the age of 15–43 years (mean 27.7 ± 4.2). The gestational ages at deliveries were between 35 weeks and 41 weeks in the diabetic group and 38–41 weeks in the control group. The mean gravidity for the diabetic mothers was 2.83 ± 1.76 and for the normal group was 2.38 ± 0.97 ($p =$ not significant). The mean abortion times for the diabetic mothers and the normal group were 0.58 ± 0.86 and 0.25 ± 0.53 ($p = 0.001$), respectively.

All of these women had cesarean deliveries. Diabetic patients had cesarean deliveries because of severe preeclampsia, fetal macrosomia, previous cesarean sections, or signs of fetal distress and the control group had elective cesarean deliveries because of previous abdominal deliveries. The characteristics of the neonates of the study groups are shown in Table 1.

Table 2 shows the hematological and umbilical blood gas analyses of the neonates of the diabetic mothers compared with the control mothers. Absolute NRBC count in the neonates of diabetic mothers was significantly higher than neonates of healthy mothers (mean $560 \pm 985/\mu L$ *vs.* $202 \pm 281/\mu L$, $p < 0.001$). CRP levels that were measured in the serum derived from the cords of all neonates of diabetic and control groups were exclusively negative (Table 2). The blood gas analysis showed significantly lower pH and higher pCO_2 values in the neonates of the diabetic mothers compared with the control group. However, HCO_3^-, O_2 saturation, WBC differential counts, and CRP levels were not statistically significant between the two groups.

Diabetic women were divided into two subgroups, namely pregestational diabetics with 21 cases and gestational diabetics with 47 cases. Hematologic parameters and blood gas values were all compared between these two groups but there was no significant statistical difference between them. The maternal and neonatal data of these two groups are compared in Table 3. All of the diabetic women had HbA1c less than 10 mg/dL.

Table 1
Comparison between the characteristics of the neonates born from the diabetic and normal mothers

Variables	Diabetic mothers ($n = 68$)	Control mothers ($n = 410$)	Significance (p)
Birth weight (g)	$3,400 \pm 524$	$3,190 \pm 393$	<0.001
Gestational age (wk)	38.07 ± 1.05	38.64 ± 0.65	<0.001
First minute Apgar score <7	6	0	<0.001
Fifth minute Apgar score <7	0	0	NS
Birth weight ≥4,000 g, n (%) (range)	9 (13.23); 4,000–4,500	14(3.4); 4,120–4,350	0.002

Data are mean values ± SD.
NS = not significant; SD = standard deviation.

Table 2
Hematologic data and umbilical artery blood gas in the infants of diabetic mothers and in the control infants

Variables	Diabetics ($n = 68$)	Controls ($n = 410$)	Significance (p)
pH	7.22 ± 0.07	7.24 ± 0.04	0.004
pO_2	28.62 ± 13.03	28.05 ± 11.99	NS
pCO_2	49.33 ± 10.08	47.00 ± 8.67	0.045
HCO_3^-	20.25 ± 4.16	20.01 ± 2.81	NS
Base excess	−7.29 ± 3.6	−7.16 ± 2.7	NS
O_2 saturation	49.39 ± 22.51	53.26 ± 20.86	NS
WBC counts/μL	10,306.47 ± 3,152	9,930.73 ± 2,199	NS
Neutrophil count/μL	5,076 ± 1,923	4,925 ± 1,674	NS
Lymphocyte count/μL	4,290 ± 1,366	4,303 ± 1,058	NS
NRBCs/100 leukocytes	4.54 ± 6.3	1.98 ± 2.63	<0.001
Absolute count of NRBC/μL	560 ± 985	202 ± 281	<0.001
Hb (g/dL)	14.82 ± 1.71	14.18 ± 1.44	0.001
CRP	Negative	Negative	NS

Data are expressed as mean ± SD.
CRP = C-reactive protein; Hb = hemoglobin; NRBC = nucleated red blood cell; SD = standard deviation; WBC = white blood cell.

There were seven neonates with maternal diabetes who passed meconium with WBC counts of 12,557.14 ± 3116.54/μL compared with 61 neonates who did not pass meconium with WBC counts of 10,048.2 ± 3076.49/μL ($p = 0.03$). Although NRBC/100 WBCs were higher in the presence of meconium (5.85 ± 9.51 *vs.* 4.39 ± 5.92) but difference was not statistically significant.

Discussion

This study was designed to compare the blood gas values and hematological parameters between the neonates born from the diabetic mothers who needed insulin during pregnancy and normal women. The measurements in this study were performed on the umbilical cord blood, which were collected immediately after cesarean deliveries compared with the other reports, which were performed on the blood collected from the neonates on varying periods of time after birth and different delivery methods [5,6,13] and this may have helped to have more accurate results.

Before tests of fetal health and maturity became available, preterm delivery was considered to avoid unexplained fetal deaths in the diabetic mothers. Although this practice has been abandoned, there is still an increased frequency of preterm delivery in diabetic mothers [14]. Investigations using cordocenthesis have provided new insights into acid-base metabolism in the fetuses of diabetic mothers. Hyperglycemia-mediated chronic aberrations in transport of oxygen and fetal metabolites may account for unexplained fetal deaths. It was hypothesized that osmotically induced placental villous edema can lead to impaired fetal oxygen transport [2].

The neonates born from the diabetic mothers in this study had lower pH and higher pCO_2 values. The pO_2 and O_2 saturations were not different between the neonates of diabetic and healthy mothers. Although O_2 saturations were lower among the infants of diabetic mothers, the difference was not statistically significant. A decrease in pH and increase in pCO_2 values can affect the dissociation of oxyhemoglobin favorably and accounts for normal pO_2 in neonates of diabetic mothers. Also, an increase in hemoglobin concentrations in the neonates of diabetic mothers may help to normalize pO_2 saturation. There are other studies that confirm these results [15]. In another study, it was found that the mean pH was significantly lower and pCO_2, hemoglobin, and erythroblast counts were significantly higher in the neonates born from diabetic mothers than the appropriate normal mean for the gestation [16]. This is in favor of a chronic acidosis and hypercapnia in diabetic pregnancies. Recent studies also suggest that in diabetic patients the fetuses are exposed to increased oxidative stress [15].

In this study, the neonates of diabetic mothers had higher NRBC counts than neonates of healthy mothers. We believe that neonates of diabetic mothers have elevated NRBC counts because of chronic acidosis. Diabetes is associated with increased levels of erythropoietin [16,17]. Erythropoietin is produced by kidneys in response to hypoxia and, in turn, increases erythrocytosis and releases immature forms of

Table 3
Characteristics of the mothers and neonates in the groups with pregestational and gestational diabetes

Variables	Mothers with pregestational diabetes ($n = 21$)	Mothers with gestational diabetes ($n = 47$)	Significance (p)
Age (yr)	31.9 ± 5.83	30.78 ± 4.63	NS
Birth weight (g)	3,354.28 ± 542.63	3,420.42 ± 520.9	NS
Gestational age (wk)	37.7 ± 1.19	38.24 ± 0.94	0.013
Gestational age, <37 wk, *n* (%)	5 (24.28)	5 (10.63)	NS
Hypertensive disorders, *n* (%)	2 (9.52)	7 (14.89)	NS
Meconium stain, *n* (%)	3 (14.28)	4 (8.51)	NS
HbA1c, %; (mean ± SD, range)	7.89 ± 1.6 (5.6–12.6)	7.14 ± 1.28 (4.3–11.6)	0.044
Birth weight ≥4,000 g, *n* (%); range	2 (9.52); 4,300–4,450	7 (14.89); 4,000–4,500	NS
Duration of diabetes (mo)	63.71 ± 75.26	2.9 ± 1.24	<0.001
NPH insulin (IU/d)	52.19 ± 19.81	18.46 ± 17.61	<0.001
Regular insulin (IU/d)	27.33 ± 17.14	11.53 ± 13.25	<0.001
Maximum blood sugar 48 hr before delivery	144.65 ± 48.22	123.82 ± 27.06	0.042

Data are mean values ± SD.
HbA1c = hemoglobin A1c; NPH = neutral protamine Hagedorn; NS = not significant; SD = standard deviation.

erythrocytes into the circulation. The purported mechanism is that the disturbed metabolic state of diabetes, including hyperglycemia, produces relative hypoxia with activation of the erythropoietin-hematopoietic system [18]. The reticulocyte response to hypoxia-induced erythropoietin release is generally not seen until the 2nd day or 3rd day after hypoxia [6]. In this case, elevated NRBC counts would be seen in cord blood only if the hypoxic event occurred or the hypoxic process began at least several days before delivery. Recent studies have reported an association between elevated umbilical cord NRBC counts and abnormal fetal heart rate patterns [19], intrauterine acidemia [9], neonatal cerebral white matter injury [20], preeclampsia [5], and adverse perinatal outcome in growth-restricted fetuses [21]. These data suggest that rising of NRBCs serve as a marker of chronic intrauterine hypoxia [22], acidemia [9], and fetal asphyxia [22,23].

The NRBC values are somehow lower in this study (6.3 ± 4.54) in the neonates of diabetic mothers compared with other studies that had the values 8.3 ± 17.8 [5] and 14.26 ± 12.24 [4]. The differences in the population demographics, risk factors, inclusion and exclusion criteria, sample sizes, and the most importantly the different status of maternal blood sugar control may account for these discrepancies. Our patients had well-controlled diabetes evidenced by low HbA1c levels and maximum blood sugar levels (Table 3).

Passage of meconium as a marker of intrauterine stress is also related to the gestational age. Neonates born from diabetic mothers who passed meconium had higher NRBC counts than those who did not pass meconium but the difference was not significant. WBC counts were significantly higher and base excess was significantly lower in the infants who passed meconium. Because of the study design, we did not have any infant with meconium passage in the healthy control group to be compared. However, acute hypoxia at delivery would not be anticipated to produce elevated NRBC counts in the cord blood but might result in elevated counts in the neonatal period. This idea has been supported by other studies [6]. Absence of statistical significance of NRBC counts between neonates of diabetic mothers with and without meconium may be because of the relatively small number of cases that were evaluated in this study compared with other studies [6].

Mean WBC, neutrophil, and lymphocyte counts were similar among the infants of diabetic and nondiabetic mothers in our study, which may be because of relatively good blood sugar control in the diabetic mothers. Both lymphocyte and neutrophil counts have been demonstrated to increase in response to hypoxia in the human adults and animal models [24,25] and are considered to be related to respiratory and metabolic acidemia [9].

Studying the effect of maternal diabetes on inflammatory markers in the fetus is important in two aspects. First, inflammation may play a role in the pathogenesis of hypoxia-related neonatal complications. Second, maternal diabetes has long-term effects on the health of the offspring [13]. In the present study, we checked serum CRP in the cord blood that was negative in all neonates of diabetic and healthy mothers as reported by other studies [13]. This indicates that an intrauterine inflammatory process may not be responsible for the increased complications in the offspring of diabetic mothers.

Conclusion

Neonates of diabetic mothers have lower pH and higher pCO_2 values and elevated NRBC counts suggestive of intrauterine chronic acidosis.

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