

Umbilical Cord Blood CD33 and Erythropoietin Levels of Pregnants with Abnormal Doppler Waveforms and to Compare with Normal Pregnancies

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Abstract

Objective: In this study, we aimed to investigate umbilical cord blood CD33 and erythropoietin (EPO) levels of pregnant women with abnormal umbilical and uterine artery doppler waveforms and to compare with normal pregnancies. **Materials and Methods:** Total 40 pregnant women were included in this study. Of these 40 women, while 20 patients had abnormal umbilical and uterine artery doppler waveforms, the other 20 patients had normal umbilical and uterine artery doppler waveforms. After the delivery, blood samples were taken from umbilical artery of double clamped umbilical cord for blood gas parameters, EPO and CD33 levels. Sociodemographic findings, antepartum, intrapartum test results, labor and delivery characteristics and newborn examination results were recorded. Blood gas parameters, EPO and CD33 levels between groups were analyzed. Mann-Whitney U test and t-test were used as statistical methods. **Results:** There were no differences between parity, gestational ages and newborn weights of the groups. Cord blood CD33 and EPO levels of group with abnormal umbilical and uterine artery doppler waveforms were significantly higher than group with normal umbilical and uterine artery doppler waveforms ($p < 0.01$). **Conclusion:** Pathology on doppler screen shows to us a connection between chronic hypoxemia and abnormal on doppler screen. Preference of high blood CD33 levels for cord blood transplantation especially during last years can also be used with preference of cord blood with abnormal doppler findings.

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Keywords

Antenatal Care, CD33, Doppler Ultrasonography, Erythropoietin, Perinatology, Umbilical Cord Blood

1. Introduction

At this time, the role of ultrasonography for detection and evaluation the pregnancy has been gradually increased beside clinic and laboratory examination. Especially with the color doppler ultrasonography, uteroplacental, umbilical and fetal blood flow has been evaluated very well. Doppler screening of the uterine circulation has positive predictive value for estimating uteroplacental perfusion. Also, examination of the fetal circulation with the doppler ultrasonography would be a better predictor of physiopathology of high risk pregnancies [1]-[3]. Hypoxia is associated with an increased risk of perinatal mortality, morbidity and impaired neurodevelopment. The exposure of the fetus to hypoxia has been a concern since the first report of an association between perinatal events and the mental and physical condition of the child. The umbilical cord blood gas analysis, the fetal heart rate and the doppler ultrasonography can be used in determining fetal hypoxia [4].

Erythropoietin (EPO) is considered to be the primary hormone controlling erythropoiesis in both the adult and the fetus. The tissue hypoxemia is resulted in increasing EPO production which is reflected by an increase in the plasma EPO levels. Since EPO does not cross the placenta, increasing the fetal plasma level of EPO is indicative of fetal hypoxemia. Although there is a good correlation between the cord blood and the amniotic fluid EPO levels, however, there is no correlation with the maternal EPO levels. Studies have shown that cord blood EPO levels are increased by some conditions (intrauterine growth retardation, post-term pregnancy, smoking and diabetes) associated with impaired oxygen supply at birth [5] [6].

CD33 (SIGLEC-3) is a member of the immunoglobulin superfamily which is restricted to cells of the myelomonocytic lineage but its functions and binding properties are still unknown. Especially, the cord blood with high CD33 level is preferred for cord blood transplantation during last years [7]-[9]. For this reason, during last periods more studies and researches were done about this subject.

In this study, we aimed to investigate umbilical cord blood CD33 and erythropoietin (EPO) levels of pregnant with abnormal umbilical and uterine artery doppler waveforms and to compare with normal pregnancies.

2. Materials and Methods

This prospective cohort study was conducted in 40 pregnant patients recruited from the labor room of the department of obstetrics and gynecology, Yuzuncu Yıl University, Van, Turkey over a 6-months period. Women including in this study have pregnancy between 28 - 40 weeks gestation. Multiple pregnancies, pregnancies with recognized fetal abnormalities and in utero ex fetus were excluded in the study. The study population was divided into two groups. Group I (study group) consisted of 20 cases with abnormal umbilical and uterine artery doppler waveforms, Group II (control group) consisted of 20 cases with no maternal or fetal risk factors and normal umbilical and uterine artery doppler waveforms.

Informed consent form was obtained from all of the cases. A detailed history was taken from all patients. The physical and the obstetrical examination were evaluated by the same physician. The placental site, the amniotic fluid index, the fetal gestational age, the number of fetus, the fetal anatomy, the fetal presentation and the fetal heart activity were examined with Toshiba 270 machine (Toshiba Medical Systems, Tokyo, Japan). Three arteries (umbilical, uterine and middle cerebral artery) were researched with doppler screening and the doppler indexes are followed as systolic/diastolic ratio (S/D), pulsatility index (PI) and resistance index (RI). All doppler examinations were performed by an obstetrician and recordings were made using a Toshiba 270 machine (Toshiba Medical Systems, Tokyo, Japan) real-time, color doppler ultrasound system. All ultrasound examinations were performed in the semirecumbent position. Three to five consecutive waveforms from each artery were obtained and the images frozen and the indexes were calculated. Measurements taken when a clear waveform was acquired in the absence of fetal breathing or body movement. On the basis of reference ranges reported in the past studies, high doppler indexes or the presence of notch or absent or reverse flow were used as abnormal doppler ultrasound criterion. Time interval between doppler examination and delivery was as far as minimal in

two groups.

After delivery, umbilical artery blood (10 ml) was drawn by a needle puncture after double clamping of the cord for estimation of CD33, EPO levels and umbilical cord blood acid-base state. The determination of the EPO concentration was conducted with the ELISA technique. CD33 was measured with flow-cytometry. These results were recorded. Mann-Whitney U test and t-test were used as statistical methods.

3. Results

A total of 40 patients with singleton pregnancies were entered into the study; there were no refusals to entry into the study and these 40 patients were divided into 2 groups as control and study group. The mean age of mothers in the study group was 27.4 years, and in the control group 27.5 years. Mean parity of study group was 2.9 and 1.8 in control group. The results about gestational age was 35.35 weeks and 35.55 in order. In control group, no response was seen to tokolytic therapy. There was no statistically significant difference among groups about mean age of mothers, gestational age and parity ($p > 0.05$). Mean birth weight of newborns were 2505 g and 2680 g in groups and there was no statistically significant difference about birthweight between groups ($p > 0.05$) (Table 1).

The study group consisted of 20 cases of which 8 (40%) cases were delivered by cesarean section, Whereas in the control group only 2 (10%) cases were delivered by cesarean section. There was statistically significant difference between groups according to delivery state ($p < 0.01$). Time interval between last doppler examination and delivery was 1 - 6 hour in the study group and 1 - 8 hour in the control group. After analysis of umbilical cord arterial blood for acid-base and blood gas parameters, values were different between groups. According to pH values and HCO_3 , there was statistically significant difference between groups ($p < 0.01$) but there was no statistically significant difference between groups according to PCO_2 and PO_2 ($p > 0.05$).

The values of BE (Base excess) of groups were -16.4 ± 0.62 mEq/L in the study group and -4.95 ± 0.38 mEq/L in the control group and this difference was statistically significant ($p < 0.01$). The EPO levels of groups were 96.1 mIU/ml in the study group and 12.95 mIU/ml in the control group. This difference was statistically significant ($p < 0.01$). The values of CD33 of groups were 67.60 in the study group and 14.35 in the control group and this difference was statistically significant ($p < 0.01$).

4. Discussion

The introduction of doppler ultrasound has allowed the fetal circulation to be examined. The value of doppler ultrasound screening is very important to predict complications of impaired uteroplacental blood flow in high-risk women. It is possible to monitor the response of the fetal circulation to hypoxia and acidemia. Because

Table 1. Characteristics of groups and results of the study.

	Group 1 Study (n = 20)	Group 2 Control (n = 20)	p value
Age (years)	27.4 \pm 1.71	27.5 \pm 1.54	$p > 0.05$
Parity	2.9 (0 - 9)	1.8 (0 - 6)	$p > 0.05$
Gestational age (week)	35.35 \pm 0.97	35.55 \pm 0.59	$p > 0.05$
Birth weight (g)	2505 (650 - 4400)	2680 (1300 - 3500)	$p > 0.05$
pH	7.21 \pm 0.02	7.31 \pm 0.02	$p < 0.01$
PCO_2 (mmHg)	45.7 \pm 1.82	42.9 \pm 2.5	$p > 0.05$
PO_2 (mmHg)	24.05 \pm 1.36	27.1 \pm 3.38	$p > 0.05$
HCO_3	17.15 \pm 0.70	20.4 \pm 0.48	$p < 0.01$
BE (mEq/L)	-16.4 \pm 0.62	-4.95 \pm 0.38	$p < 0.01$
CD33	67.60 \pm 4.69	14.35 \pm 1.51	$p < 0.01$
EPO (mIU/ml)	96.10 \pm 17.19	12.95 \pm 1.73	$p < 0.01$

Power of Hydrogen (pH), Partial Pressure of Carbon Dioxide (PCO_2), Partial Pressure of Oxygen (PO_2), Bicarbonate (HCO_3), Base Excess (BE), CD33 (SIGLEC-3), Erythropoietin (EPO).

of the potential of this technique, reference ranges for fetal doppler indices have been published for several vessels [10]-[12]. In this study, we used this reference ranges.

This study is one of the first clinical studies to report on the significance of doppler ultrasonography and EPO. A secondary aim of this study was to assess the agreement between EPO and CD33. Previous studies have established that erythropoiesis in the fetal and adult mammal is regulated by EPO. It has also been shown that fetal EPO production responds and increases during periods of fetal hypoxemia. The changing blood level of EPO following hypoxemia has been revealed in experiments on animals [13]-[15]. This studies showed a correlation between EPO concentration in cord blood and the grade of intrauterine hypoxemia.

In this study, the EPO level (12.95 mIU/ml) in the control group was generally consistent with the literature. Jazayeri *et al.* proposed their EPO level in control group as 26.1 mIU/ml in a study about pregnancies complicated by meconium passage [16]. Richey *et al.* reported as 26 mIU/ml in their 28 cases study in the control group [17]. Eckardt *et al.* stated as 35.6 mIU/ml [14], Ruth *et al.* stated 40 mIU/ml in their series with 122 cases [15]. In the other studies, this level was between 10 - 60 mIU/ml with a mean level of 30 mIU/ml. Values > 50 mIU/ml were considered elevated. Jazayeri *et al.* reported that meconium passage can be associated with chronic fetal hypoxia as demonstrated by elevated fetal EPO levels and EPO can be a marker that can help to differentiate acute and chronic hypoxemia [16].

In this study, we reported elevated EPO levels (mean 96.1 mIU/ml) in the study group which have anormal doppler ultrasound findings than the control group (mean 12.95 mIU/ml). This finding may better correlate with chronic hypoxemia and anormal doppler findings. Jazayeri *et al.* reported that there was negative correlation between umbilical cord EPO levels and cord blood pH and base excess [16]. We found the same finding in our study group.

Birth asphyxia is not a well-defined term. It implies a dysfunction resulting from a lack of oxygen supply to the baby's tissues during the birth process. The most important and reliable method to make the diagnosis is cord blood acid-base analysis [18] [19]. In our study, umbilical arterial blood was collected for gas analysis. We found low pH value (7.21 ± 0.02) and high base excess level (-16.4 ± 0.62) in the study group. Also low HCO_3 (17.15 ± 0.70) value was recorded in the study group. These levels were consistent with chronic asphyxia. In the control group, our findings supported the results presented in previous studies [19] [20].

The transplantation of unmanipulated cord blood cells has a major disadvantage of the low number of hematopoietic stem cell. For this reason, especially recent studies have reported about this subject. CD33 with an unknown properties and functions is a member of Ig superfamily that is restricted to cells of the myelomonocytic lineage. Recent studies reported that cord blood with high CD33 level was consistent with also high stem cell number [7]-[9]. Desplat *et al.* reported in 2002 year that 8-day liquid cultures of cord blood hematopoietic stem cells at 1% O_2 maintain proliferation better than at 20% O_2 . In the same study, it was reported that CD33 levels on the myelomonocytic surface were higher at hypoxic state [21]. In our study, in the study group, we reported higher level (mean 67.6) of CD33 than the control group (mean 14.35). These results suggested that a lineage between high CD33 levels in cord blood with anormal doppler ultrasound screening pregnancies but not with normal doppler ultrasound screening and the cord blood with high CD33 levels can be used for transplantation. This idea could be of paramount interest in cord blood transplantation.

5. Conclusion

Our study was designed to determine whether elevated levels of CD33 and EPO in cord blood in high-risk pregnancies were associated with pregnancies with anormal doppler ultrasound findings. In our study, we reported this association and also cord blood of pregnancies with abnormal doppler ultrasound findings can be used in cord blood transplantation like cord blood with high CD33 levels.

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