Abnormal Foetal Heart Rate Pattern and Their Relationship to Amniotic Fluid Erythropoietin Levels *

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OBJECTİVE: The aim of the study was to evaluate the relationship between abnormal foetal heart rate pattern and erythropoietin concentrations in amniotic fluid, umbilical cord, maternal plasma and 1 and 5 minutes Apgar scores.
STUDY DESİGN: Twenty-one patients with abnormal FHR patterns in the non-stress test were included to this study. All patients underwent caesarean section. Amniotic fluid, maternal and umbilical cord samples were obtained for erythropoietin levels. We measured amniotic fluid erythropoietin, maternal serum erythropoietin and umbilical cord blood erythropoietin by radio-immunoassay. Apgar scores at 1 and 5 minutes were recorded.
RESULTS: Umbilical cord, amniotic fluid and maternal serum erythropoietin levels were found 62.7±67.3 mU/ml, 26.8±22.1 mU/ml and 36.1±38.9 mU/ml respectively. UA-EPO levels were higher than AF-EPO (p=.016). MS-EPO and UA-EPO levels were similar (p=.070). Similarly, MS-EPO and AF-EPO levels were not different (p=.523). Four of the 21 infants had low Apgar scores at 1 and 5 minutes. Three of four patients MS-EPO, AF-EPO and UA-EPO levels were normal. In only one of four patients UA-EPO level was found elevated but AF-EPO and MS-EPO levels were normal. Seventeen infants had normal Apgar scores despite an abnormal foetal heart rate pattern.
CONCLUSİON: We conclude that abnormal foetal heart rate pattern may signal imminent foetal risk but do not confirm foetal hypoxia. We did not find any correlation between abnormal foetal heart rate pattern, AF-EPO, UA-EPO and MS-EPO levels, and Apgar scores of 1 and 5 minutes.
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Key Words: Erythropoietin, Foetal heart rate, Apgar scores

The glycoprotein erythropoietin (EPO), the primary hormone responsible for erythropoiesis in the foetus, neonate and adult, is produced in response to tissue hypoxia by an unknown pathway. As it does not cross the placenta, elevated cord blood concentrations should be a marker of foetal hypoxia. Erythropoietin has similar structure and signaling mechanisms to the family of type I cytokines and is markedly induced by hypoxia. Erythropoietin is synthesized by peritubular cells in the cortex-medullary border of the kidney and in the liver during fetal and neonatal development. A variety of other tissues have been reported to express erythropoietin including bone marrow macrophages, trophoblasts, breast glands, and astrocytes[1,2]. The plasma elimination half-life of erythropoietin in the foetus is probably similar to that in the premature human neonate, i.e. 1-2 h compared to 3-5 h in the adult. The half-life of amniotic fluid EPO is approximately 24 h. Increased cord blood EPO concentrations have been observed in growth-retarded infants, in infants with severe erythroblastosis, in maternal diabetes, maternal hypertension and acute foetal hypoxia[3] Abnormal foetal heart rate patterns are considered to indicate imminent foetal risk. Whether meconium-stained amniotic fluid (AF) is related to foetal distress is controversial. Elevated foetal plasma or amniotic fluid erythropoietin levels are associated with chronic foetal hypoxemia during the second half of pregnancy in humans. Studies by Maier[3] using a polycythemic mouse bioassay method for EPO measurements, demonstrated elevated amniotic fluid EPO levels in pregnancies complicated by severe Rh immunization. The best indirect markers of acute foetal distress are abnormal foetal heart rate (FHR) patterns, meconium-stained AF, and umbilical arterial blood pH. The physiological range of EPO for non-pregnant healthy women is 5-20 mU/mL. EPO levels increase during pregnancy, especially four-fold by 37 weeks. In healthy foetuses during normal term pregnancies, foetal plasma erythropoietin levels range from 10-60 mU/ml[3,4] We hypothesized that foetuses with abnormal FHR patterns would have elevated plasma EPO, which might be reflected by an elevated amniotic fluid EPO. The aim of this study was to determine the relationship between amniotic fluid erythropoietin (AF-EPO), maternal serum erythropoietin (MS-EPO) and umbilical cord blood erythropoietin (UA-EPO) and abnormal FHR pattern, and Apgar scores at 1 and 5 minutes.

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Table I. The clinical characteristics of the patients.

	Mean±S.D	95% CI for Mean	Parameters	Values (mean±s.d)
I-AF-EPO mU/ml	26.8±22.1	16.7-36.9	Maternal age	27.2±1.45
II-MS-EPO mU/ml	36.1±38.9	18.3-53.8	Fetal weight (kg)	3.07±133
III-UA-EPO mU/ml	62.7±67.3	32.0-93.4	Maternal Hb (%)	11.2±0.3
	I vs II	.523	First minute Apgar	5.33±1.46
P* value	I vs III	.016	Fifth minute Apgar	7.33±1.59
	II vs III	.070	*The mean difference is significant at the .05 level.	

Table 2. The relation among EPO levels, fetal weight, maternal Hb and gestational week

	AF-EPO	MS-EPO	UA-EPO	Fetal-weight	Maternal-Hb	Gestational week
MS-EPO	.127	.	.834	.021*	.001**	.035*
Fetal-weight	.141	.021*	.148	.	.027*	.000**
Maternal-Hb	.066	.001**	.262	.027*	.	.013*

*: Correlation is significant at the 0.05 level (2-tailed).
**: Correlation is significant at the 0.01 level (2-tailed).

Material and Methods

Twenty-one women with abnormal FHR patterns were included in this study. Patients with chronic anaemia, foetal growth restriction, post-term pregnancy, severe erythroblastosis, diabetes, chronic hypertension, pre-eclampsia and pregnancy with maternal smoking were excluded. The main indication for caesarean section in the 21 patients was suspected foetal asphyxia. FHR was recorded continuously during labour using a cardiotocograph (Spacelabs AM-67). FHR analysis was based on the description of heart rate patterns by Nijhuis et al.[5] The recordings were blinded and analyzed by one of us with respect to baseline heart rate, accelerations, decelerations, and FHR patterns. Clinical parameters such as duration of pregnancy, infant weight, infant sex and Apgar score at 1 and 5 minutes were recorded. EPO concentrations were measured in amniotic fluid following amniotomy at caesarean section. The umbilical cord was doubly clamped before the infant's first cry. For EPO measurements, we drew maternal and umbilical arterial blood by needle puncture into heparinized syringes, after cord clamping. Samples were centrifuged and the serum was stored at-20 ºC. Each EPO measurement required 50µl serum and amniotic fluid. Concentrations of EPO were analyzed in triplicate in the umbilical cord plasma, maternal plasma and amniotic fluid samples by radio-immunoassay, as described by Eckardt et al.[6] AF samples were made up in equal volumes of 5 g/100 mL bovine serum albumin in order to raise the protein content to the range used in the standards and in plasma. The intra-assay coefficient of variation was approximately 10% and the inter-assay coefficient of variation was about 13%.

Data analysis: All calculations were done with the SPSS for Windows version 10.0, using correlations and descriptive analysis (One way ANOVA). Post hoc testing was by Least Significant Difference. Data are presented as the mean±s.d. and, a value of $p<0.05$ was considered to be statistically significant.

Results

The clinical characteristics of the patients are summarized in Table 1. The AF-EPO, MS-EPO, UA-EPO levels and maternal age, mean±s.d., were 26.8±22.1, 36.1±38.9, 62.7±67.3 mU/ml and 27.2±1.45 years respectively. UA-EPO levels were greater than AF-EPO ($p<0.05$). There were no significant differences between MS-EPO and AF-EPO, and UA-EPO and MS-EPO levels. One and five minute Apgar scores were 5.33±1.46, 7.33±1.59 respectively. Five of the 21 patients had elevated UA-EPO levels, but normal levels of MS-EPO and AF-EPO. One of five infants had Apgar scores of 2 and 5 at 1 and 5 minutes respectively. The other four infants showed normal Apgar scores at 1 and 5 minutes. On the other hand, three of the remaining 16 patients had low Apgar scores at 1 and 5 minutes. As a result of, seventeen infants had normal Apgar scores despite an abnormal foetal heart rate pattern. There were negative correlations between maternal Hb levels and MS-EPO ($p<0.01$), gestational week and MS-EPO ($p<0.05$), foetal weight and MS-EPO levels ($p<0.05$, Table 2). No relationships were found between foetal weight, foetal sex, maternal Hb, gestational week and AF-EPO and UA-EPO levels.

Conclusion

Erythropoietin, a hematopoietic growth factor, binds with its receptor, EPOR (a member of the cytokine super family), to stimulate proliferation, differentiation, and maturation of erythroid progenitor cells during erythropoiesis. Foetal erythropoiesis is regulated by EPO, which is detectable as early as the 11th week of pregnancy, and increases thereafter. EPO is a large molecule, molecular weight 30400 Da, which does not cross the placenta[3] The close correlation between umbilical plasma EPO and AF-EPO levels both in normal pregnancies and those complicated by hypoxia without labour suggests that AF-EPO is of foetal origin. Foetal EPO in amniotic fluid probably comes from the foetal urine in the

second half of pregnancy, because elevated EPO concentrations have been found in foetal urine in infants with high umbilical plasma EPO concentrations[3] Whereas adults produce EPO in the kidney, the main foetal production site is probably the liver.[3,4] Widness et al[7] observed that cord blood EPO concentrations in infants with bilateral renal agenesis were similar to those in healthy term infants.

Neonatal hypoxia-ischemia is one of the major causes of morbidity and mortality in the perinatal period. There is no standard diagnosis of foetal hypoxia.[3,8] Apgar score, umbilical arterial blood pH or meconium-stained AF do not usually reflect the degree of acidosis at birth.[9,10] Our study shows that an abnormal FHR pattern does not correlate with high levels of AF-EPO, UA-EPO and MS-EPO. In the absence of acidosis, the relationship between elevated AF-EPO levels and abnormal FHR pattern was weak. In these conditions we assume that there is no clear association between acute/subacute hypoxia and cord blood and AF-EPO levels. Because of the patients with acute foetal distress underwent urgent caesarean section so that maternal serum, cord blood and amniotic fluid EPO levels may be determined as normal. Therefore we conclude that abnormal FHR patterns may signal imminent foetal risk but do not confirm foetal hypoxia. The finding of infants with high levels of UA-EPO but normal AF-EPO and MS-EPO levels support this.

This finding confirms those of several previous reports, which indicated that intrapartum hypoxia can elevate circulating UA-EPO levels.[3] However, controlled clinical trials should be performed to determine the clinical significance of EPO levels in the detection of foetal hypoxemia. We did not find any relationship between AF-EPO, UA-EPO and MS-EPO concentrations and abnormal FHR patterns. There were no relationships between abnormal FHR, AF-EPO, MS-EPO, UA-EPO and 1 and 5 minute Apgar scores.

Finally, despite some limitations, our results suggested that MS-EPO and AF-EPO do not significantly increase the incidence of subacute or labor-associated hypoxia. Because the study had relatively low power due to possible unexpected confounders, the conclusion that acute hypoxia did not elevate maternal or amniotic fluid erythropoietin levels may need further reevaluation with larger sample size and appropriate adjustment of confounding factors.

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