Fetal Diagn Ther 1992;7:21–25

Fetal and Maternal Erythropoietin Levels in Normal Pregnancy

R. Ireland[c]
A. Abbas[a]
B. Thilaganathan[a]
O. Melbye[c]
R. Snijders[a]
M. Layton[b]
K.H. Nicolaides[a]

[a] Harris Birthright Research Centre for Fetal Medicine and
[b] Department of Haematological Medicine, King's College School of Medicine, and
[c] Department of Haematology, Brook Hospital, London, UK

Abstract

In a cross-sectional study of 120 pregnancies undergoing cordocentesis for prenatal diagnosis (n = 90) or elective caesarean section (n = 30), the umbilical cord and maternal venous plasma erythropoietin (Epo) concentrations were measured. Fetal Epo levels increased from a mean of 4 mU/ml at 16 weeks to 13 mU/ml at 40 weeks' gestation. There were no significant associations between fetal plasma Epo concentration and fetal blood gases, haemoglobin concentration, oxygen content or erythroblast count. The maternal plasma Epo concentration (mean = 14 mU/ml, range 1–77 mU/ml) did not change with gestation but was significantly higher than levels in non-pregnant females (mean = 6.6 mU/ml, range 1–25 mU/ml).

Key Words
Cordocentesis
Fetal haematology
Erythropoietin

Introduction

In normal fetuses, the erythrocyte count increases linearly with gestation whilst the erythroblast count decreases exponentially to reach a plateau at 24–26 weeks [1]. It has been previously suggested that the erythroblast count may be considered an indirect measure of extramedullary haemopoiesis whereas erythrocytes are the product of both medullary and extramedullary haemopoiesis. The rate of medullary haemopoiesis increases exponentially after 16 weeks and becomes the predominant site of erythropoiesis at 22–24 weeks' gestation [2]. It is uncertain whether the switch from hepatic to medullary erythropoiesis and haemopoietic maturation are influenced by environmental factors, such as tissue oxygenation and subsequent alteration in erythropoietin (Epo) levels, or whether it is controlled by a genetically determined 'developmental clock'.

In normal pregnancy, the fetal umbilical venous and umbilical arterial pO_2 and pH

Received:
September 19, 1991
Accepted:
November 4, 1991

Dr. Kypros Nicolaides
Harris Birthright Research
Centre for Fetal Medicine
King's College School of Medicine
Denmark Hill, London SE5 8RX (UK)

1015–3837/92/
0071–0021$2.75/0

decrease with gestation [3]. However, the oxygen content of fetal blood does not change because the haemoglobin concentration is increased [4]. It is uncertain whether the increase in haemoglobin concentration is a result of genetically determined maturation of haemopoiesis or if it represents an adaptive response to the decrease in pO_2 with gestation.

This study examines alterations in fetal plasma Epo concentration with gestation and its association with fetal blood gases, haemoglobin concentration and erythroblast count. Associated changes in maternal Epo concentration were also studied.

Patients and Methods

Plasma Epo concentration was measured in fetal blood samples obtained by cordocentesis from 90 pregnancies undergoing prenatal diagnosis at 16–38 weeks' gestation. The indication for fetal blood sampling included: (i) fetal karyotyping for women of advanced age that booked late, where amniocyte culture had failed or for low maternal serum alpha fetoprotein (n = 20); (ii) fetal blood grouping in red cell-isoimmunized pregnancies and where the fetus was subsequently found to be Coombs'-negative (n = 11); (iii) karyotyping for minor fetal malformations such as choroid plexus cysts or hydronephrosis (n = 53), and (iv) investigation of maternal primary rubella infection or toxoplasmosis where the fetus was subsequently found not to be infected (n = 6). In all cases, the fetal abdominal circumference, blood gas values, haemoglobin concentration and erythroblast counts were within the appropriate reference range for gestation and the fetal karyotype was normal.

Additionally, cord blood samples were collected from normal pregnancies at elective caesarean section (n = 30), which was performed either because of previous caesarean section and suspected cephalopelvic disproportion of for breech presentation. In all cases the infants were normal and their birth weight was above the 5th centile for gestational age.

The study was cross-sectional and in each case gestation was determined from the maternal menstrual history and confirmed by an ultrasound scan in early pregnancy. Cordocentesis was performed without maternal sedation or fetal paralysis and in all cases umbilical venous blood was obtained. Kleihauer-Betke testing confirmed that all cord samples contained only fetal blood.

Matched samples of maternal blood were collected from the antecubital fossa before fetal blood sampling. Additionally, plasma Epo was measured in 33 male and 87 non-pregnant female adult patients who had normal blood count indices.

Fetal blood samples (180 µl) were collected into 20 µl of isotonic edetic acid solution (0.5 mmol/l in 0.15 mmol/l sodium chloride) and the full blood count determined using a Coulter S-Plus counter (Coulter Electronics, Luton, England). Blood films were stained by the May-Grünwald-Giemsa method for the differential cell count. Blood samples (250 µl) were collected into heparinized syringes for measurement of oxygen tension (Radiometer ABL, Copenhagen, Denmark) and calculation of oxygen content from the pO_2, pH and haemoglobin concentration. Blood samples (0.5 ml) were also collected in heparinized syringes and both adult and fetal plasma Epo concentrations were measured.

Samples for Epo estimation were stored at –20 °C until thawed for assay. An Epo enzyme immunoassay kit (Clinigen TM, Amgen Diagnostics, USA) was used for quantitative determination of Epo levels. The reference curve was standardized against World Health Organization, second International Reference Preparation of human urinary Epo. The limit of sensitivity of the assay was determined to be 2 mU/ml (limit at three standard deviations from the zero Epo standard).

Statistical Analysis

Regression analysis was used to determine the significance of any association between plasma Epo concentration and gestational age. The data or residuals from linear regression was tested for normality. The distribution of fetal plasma Epo concentration with gestation was skewed, thus it was made Gaussian by logarithmic transformation. Regression data were used to calculate the adjusted means and residual standard deviations. To determine the reference range with gestation in the original units (mean and individual 90% confidence intervals), the limits of the calculated reference range in logarithms were subjected to antilogarithmic transformation. The distribution of adult plasma Epo concentration was also skewed and a logarithmic transformation was performed to allow derivation of the reference ranges.

Regression analysis was used to determine the significance of any associations between fetal plasma Epo concentration and haemoglobin concentration, ery-

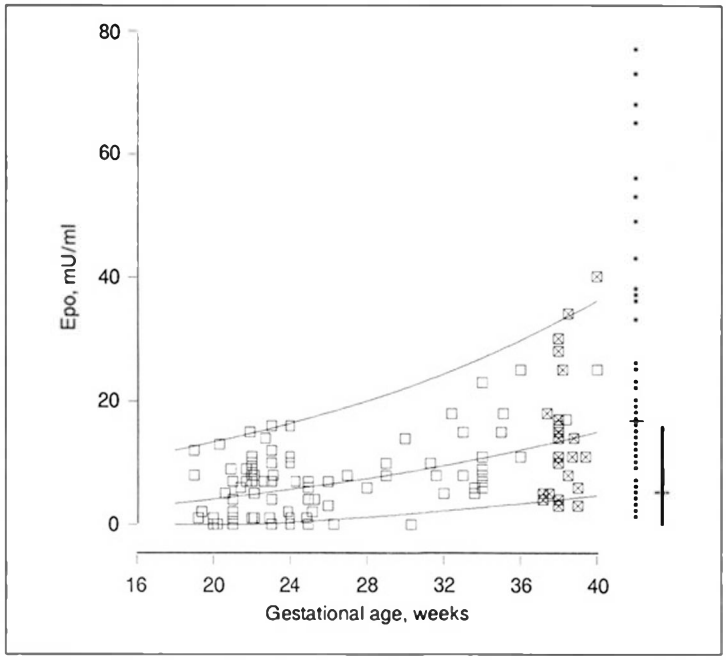

Fig. 1. Individual fetal (□ = cordocentesis, ⊠ = ceasarean section) plasma Epo concentrations plotted as a function of length of gestation. Individual maternal (·) Epo concentrations plotted on the right. The vertical bar represents the range of values for the non-pregnant population. The sloping lines are the mean, 5th and 95th percentile values for fetal plasma Epo concentration.

throblast count, fetal blood pO_2, pH and oxygen content. The relationship of maternal serum Epo levels to gestational age and its association with fetal plasma Epo concentration was also examined. Multiple regression analysis was used to determine the extent to which these variables contributed to alterations in fetal plasma Epo concentration in addition to the effect of gestation and to see whether knowledge of the mode of fetal sampling significantly changed the relationship between fetal plasma Epo concentration and gestational age. Non-parametric tests were used to measure the significance of any difference in the Epo concentrations between the mixed adult, non-pregnant female and maternal populations.

Results

The plasma Epo concentration (geometric mean = 6 mU/ml, range = 1–25 mU/ml) fo the mixed adult population had a log-normal distribution as described by previous studies [5, 6]. The plasma Epo concentration in non-pregnant women (geometric mean = 6.6 mU/ml, range = 1–25 mU/ml) was not significantly different from the mixed population ($z = 0.30$, $p = 0.7662$).

The mean maternal venous plasma Epo concentration (geometric mean = 14 mU/ml, range = 1–66 mU/ml) did not change significantly with gestation ($r = 0.074$) neither was it significantly associated with the fetal plasma Epo concentration ($r = -0.070$). However, it was significantly higher than the mean Epo concentration in nonpregnant women ($z = 5.58$, $p < 0.0001$).

The fetal pH, pO_2 and haemoglobin concentration in all cases were within two standard deviations of our reference range for normal pregnancies [1, 3, 4]. The fetal plasma Epo concentration increased significantly with gestation from a mean of 4 mU/ml at 16 weeks to 13 mU/ml at 40 weeks [fig. 1; $\log_{10}$ (erythropoietin + 5) = 0.691 + 0.014 × gestation, residual SD = 0.186, $r = 0.459$, $n = 120$, $p < 0.0001$]. Multiple regression analysis

demonstrated that mode of fetal blood sampling (cordoncentesis or elective caesarean section) did not contribute significantly in explaining the variance in fetal plasma Epo concentration after correcting for gestational age (F to remove = 0.02). Therefore, the data from elective caesarean sections were included in the reference range.

There were significant associations between fetal plasma Epo concentration and fetal blood pO_2 ($r = -0.245$, $p < 0.05$), pH ($r = -0.293$, $p < 0.01$) and haemoglobin concentration ($r = 0.322$, $p < 0.001$). However, multiple regression analysis demonstrated that none of these variables had an additional significant contribution in explaining the variance in Epo levels after correction for gestation (pO_2 F to remove = 2.56; pH F to remove = 1.68; haemoglobin F to remove = 1.28).

Discussion

The mean maternal plasma Epo concentration (14 mU/ml) is significantly higher than in non-pregnant women. This was also reported in previous studies that used either bioassay or radioimmunoassay methods to measure Epo. In our study, maternal Epo concentration did not change significantly with gestational age. In contrast, Coates and Canning [7] and Widness et al. [8] in longitudinal studies of 11 and 6 women, respectively, showed that there was a two- to threefold increase in Epo concentration from 8 to 40 weeks' gestation. Similarly, Beguin et al. [9], in a cross-sectional study of 142 pregnancies, showed an elevation in Epo concentration from 18 mU/ml in the first trimester to 35 mU/ml in the third trimester. Although, Coates and Canning [7] showed a significant association between maternal Epo and human placental lactogen concentration, Widness et al. [8] demonstrated that this association was due to coincidental changes in these variables with gestation. Goltner [10] postulated that the elevation of Epo levels may be secondary to hypoxaemia induced by the physiological anaemia of pregnancy. However, none of the above studies demonstrated a significant association between Epo and haemoglobin concentration.

The absence of a significant association between fetal and maternal levels suggests that Epo does not cross the placenta. Although in animal studies, maternal hypoxia is associated with an increase in both maternal and fetal Epo concentrations, the latter has been demonstrated to be a consequence of the accompanying fetal hypoxia as only the rise in maternal Epo is abolished by bilateral nephrectomy of the mother [11]. Furthermore, Epo administration to the mother stimulates only maternal haemopoiesis, and in animal studies where the fetus was rendered either hypoxic or anaemic, the increase in fetal Epo concentration was not accompanied by alterations in maternal Epo [12, 13].

This study has demonstrated that in normal pregnancy, fetal Epo is detectable as early as 16 weeks' gestation and that fetal plasma Epo concentration increases with gestation. Although Thomas et al. [14], using samples obtained antenatally by fetoscopy (n = 3) and after premature delivery (n = 40), also found an increase in Epo concentration with gestation, their levels were much higher than in the present study. However, the hypoxia endured by the fetus in 'normal' labour may be sufficient to stimulate Epo production [15]. Furthermore, the condition causing premature delivery itself could influence fetal Epo concentration, since it is unlikely that pregnancies ending before 37 weeks of gestation can truly be described as normal. These factors may account for the disparity in results and question the validity of their quoted 'normal range'.

The lack of a significant association between fetal plasma Epo concentration and erythroblast count suggests that the switch from hepatic to medullary haemopoiesis is not influenced by Epo. Furthermore, the lack of a significant contribution by markers of fetal oxygenation in explaining the increase in Epo concentration with gestation suggests that in normal fetal development, Epo production is not secondary to physiological changes in tissue oxygenation. Similarly, the lack of a significant association between fetal plasma Epo and haemoglobin concentration suggests that both the increase in haemoglobin and Epo concentration with gestation involve independent mechanisms.

Acknowledgement

Funding from the South East Thames Health Authority.

References

1 Nicolaides KH, Thilaganathan B, Mibashan RS: Cordocentesis in the investigation of fetal erythropoiesis. Am J Obstet Gynecol 1989;161:1197–1200.

2 Lipton JM, Nathan DG: The anatomy and physiology of hematopoiesis; in Nathan DG, Oski FA (eds): Hematology of Infancy and Childhood, ed 3. Philadelphia, Saunders, 1987, pp 128–158.

3 Nicolaides KH, Economides DC, Soothill PW: Blood gases, pH and lactate in appropriate and small for gestational age fetuses. Am J Obstet Gynecol 1989;161:996–1001.

4 Nicolaides KH, Soothill PW, Clewell WH, Rodeck CH, Mibashan RS, Campbell S: Fetal haemoglobin measurement in the assessment of red cell isoimmunization. Lancet 1988;i:1073–1075.

5 Rege AB, Brookins J, Fisher J: A radioimmunoassay for erythropoietin: Serum levels in normal human subjects and patients with haemopoietic disorders. J Lab Clin Med 1982;100:829–843.

6 Coates PM: The estimation of erythropoietin: Principles, problems and progress; in Rich IN (ed): Molecular and Cellular Aspects of Erythropoietin and Erythropoiesis. NATO ASI series. Berlin, Springer, 1987, pp 377–387.

7 Coates PM, Canning CE: Changes in serum immunoreactive erythropoietin during the menstrual cycle and normal pregnancy. Br J Obstet Gynaecol 1983;90:304–311.

8 Widness JA, Clemons GK, Garcia JF, Schwartz R: Plasma immunoreactive erythropoietin in normal women studied sequentially during and after pregnancy. Am J Obstet Gynecol 1984;149:646–650.

9 Beguin Y, Lipscei G, Oris R, Thoumsin H, Fillet G: Serum immunoreactive erythropoietin during pregnancy and in early postpartum. Br J Haematol 1990;76:545–549.

10 Goltner E: Erythropoietin in der Schwangerschaft und nach der Geburt. Arch Gynaekol 1964;200:60–87.

11 Zanjani ED, Peterson EN, Gordon AS, Wasserman LR: Erythropoietin production in the fetus: Role of the kidney and maternal anaemia. J Lab Clin Med 1974;83:281–287.

12 Matoth Y, Zaizov R: Regulation of erythropoiesis in the fetal rat. Isr J Med Sci 1971;7:839–842.

13 Gordon AS, Zanjani ED, Peterson EN: Studies on fetal erythropoietin; in Gordon AS, Condorelli M, Peschle C (eds): Regulation of Erythropoiesis. Milano, Il Ponte, 1973, pp 188–201.

14 Thomas RM, Canning CE, Coates PM, Linch DC, Rodeck CH, Rossiter CE, Huehns ER: Erythropoietin and cord blood haemoglobin in the regulation of human fetal erythropoiesis. Br J Obstet Gynaecol 1983;90:795–800.

15 Widness JA, Clemons GK, Garcia JF, Williams OH, Schwartz R: Increased immunoreactive erythropoietin in cord serum after labour. Am J Obstet Gynecol 1984;148:194–197.