

Umbilical cord blood Erythropoietin as a biomarker of subacute and chronic hypoxia in deliveries complicated by meconium stained amniotic fluid

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Abstract

Background: The presence of meconium stained amniotic fluid (MSAF) during labour is often associated with a lot of adverse outcomes and historically has been considered to be a predictor of bad neonatal outcome. It is associated with fetal hypoxia and leads to emergency C/S, instrumental vaginal delivery for fetal distress, low Apgar score at birth, meconium aspiration syndrome and neuro-developmental handicaps. Umbilical cord serum erythropoietin levels have been claimed to be elevated in response to fetal hypoxia.

Objective: To evaluate the umbilical cord serum erythropoietin levels in neonates born through MSAF and to determine whether it is influenced by gestational age.

Study Design: Prospective case control study.

Materials & Methods: Women with term uncomplicated singleton pregnancies at gestational age between (37-41⁺⁶) weeks admitted to the labour room in the active phase of labour were enrolled to participate in this study. The studied population has been divided into two groups, women with thick meconium stained amniotic fluid (study group) and women with clear amniotic fluid (control group).

Umbilical cord serum Erythropoietin levels will be measured for both groups by ELISA, after delivery and Apgar score will be assessed for neonates of both groups then statistical analysis will be performed.

Results: A significant relationship was found between MSAF and elevated umbilical cord serum erythropoietin levels since the p value was $P < 0.001$. Apgar score was lower significantly after 1 minute in study group, but lower none significantly after 5 minutes, as $P < 0.001$ and 0.109 respectively.

Conclusions: The elevated umbilical cord serum Erythropoietin levels in neonates born through MSAF suggest subacute or chronic fetal Hypoxia, independent of gestational age.

Keywords: Erythropoietin, pregnancy, meconium stained amniotic fluid (MSAF), gestational age.

1. Introduction

“Meconium” is derived from the Greek word Mekonion, which means “poppy juice”. It represents the colonic content, and composed of swallowed amniotic fluid, bile pigments which give its green color, squamous cells and vernix.[1] MSAF had been identified in approximately 25% of women who delivered after the onset of labour.[2] There are many theories have been suggested for meconium passage by the fetus in utero, it may represent normal gastrointestinal tract maturation or reflects

fetal response to both acute and chronic hypoxia.[3] MSAF which is accompanied by hypoxic event predisposes to meconium aspiration syndrome (MAS).[4] It is also a risk factor for neonatal hypoxic-ischemic encephalopathy, seizures and cerebral palsy.[5] Therefore, the presence of MSAF alerts the obstetricians for the possibility of non-reassuring fetal status. The measurement of cord blood levels of biomarkers at time of birth may help in determining the risk of consequent neonatal insult from

hypoxia [6], one of suggested markers is erythropoietin also known as EPO, which is a glycoprotein hormone, it acts on late erythroid precursor cells in the bone marrow, stimulates their production and differentiation. [7] In addition it exhibits anti-apoptotic activity and plays a potential neuroprotective role against ischemia. [8] It is produced mainly by interstitial cells in the peritubular capillaries of the renal cortex, with small amounts being produced in the liver. [9] Liver is the predominant site of its production in the fetal and perinatal period, while renal production predominates during adulthood.[10] Production and secretion of EPO are regulated by tissue oxygen supply.[11] Mean cord plasma EPO levels from healthy term pregnancies prior to onset of labour range between 20 and 35 mU/ml. EPO has not been shown to cross the placenta which indicates it's fetal origin in the cord blood[12], it's half-life among neonates is 2-4 hours[8], furthermore it does not seem to be stored in the fetal tissues for later release. In adults plasma EPO levels start to rise about 90 min following the induction of acute hypoxia [13] reaching a peak 12 hours after the onset of hypoxic event.[14] These characteristics make EPO suitable marker of subacute hypoxia.

2. Patients and Method

A prospective case-control study was carried out in the department of Obstetrics and Gynecology in Al-Yarmouk Teaching Hospital, Baghdad- Iraq, after approval of the hospital ethical committee from January 2015 to January 2016. Verbal consent was obtained from all participant women before any blood samples collection.

One hundred three pregnant women with term singleton pregnancies who were admitted to the labour room in the active phase of labour participated in this study. The studied population was divided into two groups:

2.1 Study group

Included 44 women their deliveries complicated by thick meconium stained amniotic fluid, diagnosed after spontaneous or artificial rupture of amniotic membranes then delivered by vaginal route or caesarean section.

2.2 Control group

Included 59 women with clear amniotic fluid.

2.3 Inclusion criteria

Inclusion criteria include Singleton term pregnancy (37-41⁺⁶ weeks of gestation) at any women age and parity.

2.4 Exclusion Criteria

Women with multiple pregnancy, congenital abnormality of fetus were seen by Ultrasound, patients with any medical complication during pregnancy including anemia or those with chronic medical illness e.g. Diabetes mellitus, Renal disease, pregnancy with Rh iso-immunization or IUGR, smoker mothers, intrauterine death, antepartum hemorrhage and chorioamnionitis.

The demographic characteristics of each patient were assessed including maternal age, gravidity and parity. For both groups, the gestational age was assessed by the 1st day of the last menstrual period (LMP) and confirmed by early ultrasonography.

After delivery, 2ml of umbilical cord venous blood were obtained for serum erythropoietin concentration measurement.

Fetal outcome were assessed by Apgar score, one and five minutes after delivery.

2.5 Serum Erythropoietin Measurement:

Quantitative determination of erythropoietin levels were measured by enzyme-linked immunosorbent assay (ELISA), using Demeditec, Diagnostics EPO Eliza GmbH. Lise-Meitner-Strasse 2 .D-24145 Kiel (Germany). To assay the specimen in duplicate, 400 µml of serum was required. The specimens were collected between 7:30 am to 12:00 pm.

Two ml of whole blood were drawn from umbilical vein, immediately after delivery of the neonate via syringe then put into test tube without anticoagulant and transferred to the laboratory where allowed to clot between 2-8°C then the serum was promptly separated and stored at -15°C or lower. Prior to use, all specimens were left to thaw to room temperature (22°C to 28°C) and mixed by gentle inversion or swirling.

2.6 Statistical analysis

Anderson Darling test of normality was done to assess whether continuous variables follow normal distribution, mean and standard deviation were used for representing them if they follow normal distribution, median and interquartile range (IQR:25% - 75% percentile) were used if they follow non normal distribution. Discrete variables presented using their numbers and percentages

Indecent two samples t- test was used to analyze the differences in mean, while Mann Whitney U test was used to analyze the differences in median of the two groups.

MedCalc-version 14.8.1 program was used for sample size calculation which revealed a minimum 27 in the study group and 54 in the control group are required to detect a difference in the mean 20 m IU/ml and SD 30 m IU/ml in both groups and a ratio of control to study 2:1.

Univariate binary regression analysis was used to test the relationship between erythropoietin, MSAF, gestational age and Apgar score, the risk presenting using odd ratio (OR) and it's confidence interval (95%). Multivariate logistic regression analysis was used to test whether the relationship between erythropoietin, MSAF and gestational age are independent of the effect of each variable.

Receiver operator curve was used to see the validity of erythropoietin and gestational age in separating women in study group from normal control and area under the curve i.e. AUC and its p value prescribe this validity (if

AUC ≥ 0.9 mean excellent test, 0.8 – 0.89 means good test, 0.7– 0.79 fair test otherwise unacceptable). Trapezoidal method was used to calculate the curve.

3. Results

3.1 Demographic data

A total of 103 parturient women participated in this study (44 were with thick meconium stained liquor and 59 were normal control), there was no significant difference between both groups in terms of age of the mothers and number of parity as illustrated in table 1.

Table 1: Demographic data of the mothers

	Control	Study	P value
Number (%)	59 (57.3%)	44 (42.7%)	-
Age of the mother	25.47 \pm 3.0	25.61 \pm 2.94	0.815 ^a
Parity	2 (1 – 3)	2 (1 – 2.75)	0.119 ^b

^a Two sample t test, ^b Mann Whitney U test

Parity presented by median and IQR, age presented by mean and SD

3.2 Fetal characteristics

There were significantly higher gestational age, birth weight, and cord erythropoietin levels in study group. Apgar score was higher significantly after 1 minute, but higher none significantly after 5 minutes in control group as shown in table 2.

Table 2: Fetal characteristics

	Control	Study	P value
Number (%)	59 (57.3%)	44 (42.7%)	-
Gestational age (weeks)	38 (38 – 40)	40 (39 – 41)	<0.001 ^a
Birth weight (Gram)	3195 \pm 181.5	3460.9 \pm 179.9	<0.001 ^b
Erythropoietin (mIU/mL)	23.99 (15.41 – 47.59)	44.64 (25.32 – 58.87)	<0.001 ^a
Apgar 1 minute	9 (8 – 10)	7.5 (7 – 8)	<0.001 ^a
Apgar 5 minutes	9 (7 – 10)	8 (6 – 10)	0.109 ^a

^a Mann Whitney U test, ^b t test

Gestational age, erythropoietin, Apgar presented by median and IQR, birth weight presented by mean and SD

3.3 Relationship between gestational age and MSAF

The odd of having MSAF is significantly higher as gestational age is increased (odd ratio = 1.933, 95%CI: 1.409 – 2.651), P value <0.001. To determine at which week of gestational age the prediction of meconium is clinically significant, receiver operator curve analysis performed and revealed that above or equal to 39.5 weeks there is higher probability of presenting with meconium delivery (sensitivity: 65.9%, specificity: 69.5%), this prediction is fair in power since AUC of prediction is 0.760, as illustrated in table 3.

3.3.1. Validity analysis of gestational age in predicting MSAF

Table 3: Optimal cut off value of gestational age to predict MSAF

	AUC	95% CI	Cut off value	P value
Gestational age	0.760	0.668 – 0.852	≥ 39.5	<0.001

Sensitivity: 65.9%, specificity: 69.5%

ROC test

3.4. Relationship between gestational age, cord blood erythropoietin and MSAF

To determine whether the relationship between erythropoietin and gestational age with MSAF is dependent or independent multiple logistic regressions performed with forward selection methods were used as criteria, and revealed they both independently correlated with MSAF with adjusted odd ratio of 1.037 (slightly increased OR) and 1.986 (slightly decreased) for erythropoietin and gestational age respectively, also gestational age in this module performed suppressing effect on erythropoietin, as illustrated in table 4.

Table 4: Relationship between meconium passage and gestational age

	OR	95%CI	P value
Erythropoietin	1.037	1.014 – 1.06	0.002
Gestational age	1.986	1.409 – 2.798	<0.001

Forward multiple binary logistic regression analysis.

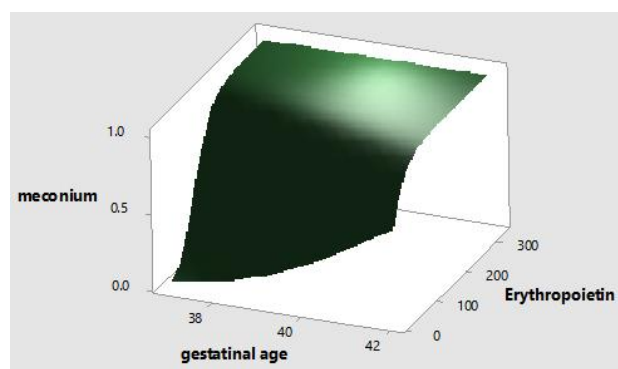


Figure 1: Surface plot demonstrating the combined relationship between increased probabilities of MSAF, increased gestational age, and increased cord erythropoietin (light area present higher rates of meconium passage).

Figure 1 illustrates the 3 dimensional relationships between all the 3 variables showing that as both gestational age and erythropoietin increase there is higher probability of meconium delivery (lighter area of the surface plot is more elevated).

3.5 Apgar score at 1 and 5 minutes

Only Apgar score after 1 minute associated inversely with meconium passage (i.e. lower Apgar score baby is more probable with meconium delivery).

Table 6: Relationship between MSAF and Apgar score

	OR	95%CI	P value
Apgar 1 minute	0.363	0.238 – 0.555	<0.001
Apgar 5 minutes	0.855	0.698 – 1.046	0.127

Univariate binary logistic regression analysis

4. Discussion

Many research works have examined the association between fetal hypoxia and elevated umbilical cord serum erythropoietin levels. [15-17]

The current study was designed to evaluate erythropoietin, a marker of subacute hypoxia, in neonates born through MSAF, which showed that there was a significantly higher cord serum erythropoietin level in meconium group. Our finding of a statistically significant higher gestational age in the study group let us perform Forward multiple binary logistic regression analysis which revealed that the increased erythropoietin was independent of gestational age in agreement with Jazeyeri *et al* and Richey *et al* findings.[18,19]

The higher birth weight in the meconium group is explained by their higher gestational age as we excluded the small for gestational age newborn from the study. While Morley *et al* found a negative correlation between cord blood EPO and birth weight after adjusting for gestational age and gender therefore they assumed that the correlation between birth weight and EPO is U-shaped.[20]

Sazak *et al* found a statistically significant higher cord blood Erythropoietin among neonates of smoker than nonsmoker mothers (61 ± 46 versus 24 ± 9 IU/L) at a comparable gestational age and birth weight, these observations suggest that factors other than gestational age and birth weight determine cord blood EPO level. [21]

Our findings that meconium passage is significantly higher as gestational age is advanced with the highest probability at 39.5 weeks and the median gestational age is 40 weeks was comparable to that of Oyelese *et al* who found that the mean gestational age in deliveries complicated by meconium stained liquor was 39.7 weeks.[22]

Maier *et al* Although they did not confirm a significant correlation between elevated EPO and meconium-stained amniotic fluid, they still reported a much greater EPO values in fetuses with MSAF compared with those with clear liquor (80.6 versus 28 mU/ml).[23]

Although the Apgar score at 5 min was lower in the study group, it is a poor clinical predictor of long term outcome. [24]

Manchanda *et al* showed an elevated cord blood EPO levels both in term and post-term fetuses who born through MSAF. [25]

Ostlund *et al* measured erythropoietin in fetal serum obtained by cordocentesis and in amniotic fluid from fetuses with signs of intrauterine growth restriction, concluded that EPO levels in amniotic fluid and in fetal cord serum are highly correlated, and thus both can be used as markers for chronic hypoxia prior to the onset of labour.[26]

Teramo *et al* reported that increased fetal plasma and amniotic fluid erythropoietin concentrations can serve as markers of intrauterine hypoxia. [27]

Elfaragy *et al* demonstrated a cut-off value of cord blood EPO > 34 mIU/ml is used as a predictor meconium aspiration syndrome.[28]

Ruth *et al* concluded that a high erythropoietin level after normal pregnancy indicates an increased risk for cerebral palsy.[29]

5. Conclusions

The elevated umbilical cord serum Erythropoietin levels in neonates born through MSAF suggest subacute or chronic fetal Hypoxia, independent of gestational age.

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