Assessment of Cord Blood Hepcidin levels in Preeclampsia and Eclampsia

Thesis

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By

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Introduction

Preeclampsia is a pregnancy-specific disease characterized by hypertension and proteinuria. It is a significant cause of maternal and fetal morbidity and mortality worldwide ***(Kathleen et al., 2012)***. During embryogenesis and development, the fetus obtains oxygen and nutrients from the mother via placental microcirculation. The placenta is a unique organ that develops and differentiates per se, and that regulates fetal growth and maternal condition in the entire course of gestation. Several life-threatening diseases during pregnancy, as preeclampsia and eclampsia, are closely associated with placental dysfunction. Cumulative studies have suggested that preeclampsia is associated with hypoxic micromilieu of fetoplacental site with decreased utero-placental blood flow, ***Furuya et al. (2008)*** thus producing a state of chronic fetal hypoxia in utero ***(Hu et al., 2012)***.

Hepcidin, a small polypeptide produced by hepatocytes is mainly expressed in the liver ***(Liu et al., 2012)***. Other possible sites of hepcidin are heart, brain, choroid plexus, lung and placenta ***(Muehlenbachs et al., 2007; Ganz, 2004)***. Hepcidin is the main regulator of iron

absorption and its tissue distribution. Pathologically increased hepcidin levels cause or contribute to iron-restrictive anemia including anemia associated with some cancers, inflammation in hereditary hemochromatosis and ineffective erythropoisis **(*Ganz T and Nemeth, 2011).*** Hypoxia suppresses hepcidin, thereby promoting intestinal iron uptake and release from internal stores. While hypoxia-inducible factor (HIF), a central mediator of cellular adaptation to hypoxia, directly regulates renal and hepatic erythropoietin (EPO) synthesis under hypoxia, the molecular basis of hypoxia/HIF-mediated hepcidin suppression in the liver remains unclear ***(Nicolas et al., 2002)***.

Hypoxia caused by respiratory insufficiency in preterm infants was also associated with decreased hepcidin production ***(Gun et al., 2013)***. The presence of placental hepcidin has been documented ***(Lindheimer et al., 2008)***. However, knowledge about the other functions of hepcidin in neonates is limited ***(O'Brien et al., 2005)***. Compared hepcidin and erythropoietin levels in the cord blood of neonates with meconium-stained amniotic fluid (MSAF) to levels obtained from age-, body mass index- and gravidity-matched neonates with clear amniotic fluid and concluded that meconium release in low-risk

pregnancies may not be alarming in terms of neonatal outcome, they demonstrated a significant relationship between erythropoietin levels and meconium passage, but failed to show the existence of a relationship between hepcidin levels and meconium passage ***(ACOG, 2008)***. Recommended performing studies on high-risk pregnancies and asphyxiated neonates to assess the possible relation between hepcidin cord blood level and chronic neonatal hypoxia ***(Philip Lanzkowsky, 2011)***.

Aim of the Work

The aim of the current study is to compare hepcidin levels in cord blood of neonates in pregnant women with preeclampsia during labor to the levels of in cord blood of neonates with normal pregnant women.

Research question:

Is there a significant relationship between hepcidin cord blood level and preeclampsia

Patients and Methods

Recruitment and eligibility:

Site: This study will be recruited from women attending Ain Shams university maternity hospital and El Galaa teaching Hospital.

Study Population:

Women will be recruited according to the following inclusion and exclusion criteria:

Inclusion criteria:

1. Age between 20-40 years old.
2. Primigravida or multipara.
3. Gestational age ≥ 37 weeks' gestation.
4. BMI ≥ 25.
5. A systolic blood pressure of 140 mmHg or greater, and/or a diastolic blood pressure of 90 mmHg or greater.
6. Proteinuria of 300 mg or greater per 24 hours after 20 weeks of pregnancy, or urine dipstick 1+ or greater on 2 occasions for at least 4 hours.
7. All deliveries will performed by vaginal route.

Exclusion criteria:

1. Women with other medical disorders.
2. Congenital fetal malformation.
3. Intrauterine growth restriction due to any cause other than preeclampsia.
4. Meconium stained liquor.
5. Rhesus isoimmunization (Coombs positive).
6. Smoking.

Ethical issues:

The protocol of the study will be presented for approval by the ethical committee of the department of gynecology and obstetrics of Ain shams maternity hospital.

Consent process:

The population sample under study will be instructed about research protocol and informed consent are granted from each participant before randomization.

Study design

Type of study:

Case control study.

Methodology:

1. A detailed history and thorough physical examination of all participants will be carried out the degree of preeclampsia will be assessed.
2. A complete laboratory investigation for preeclampsia will be done, i.e
   - A complete blood picture with platelet count.
   - Liver functions test.
   - Renal function test.
   - 24 hours urine protein collection.
   - Coagulation profile.
3. The umbilical cord will double clamped and blood samples will collected from by needle puncture just after delivery.
4. All deliveries will performed by the vaginal route.

Intervention:

Cord blood samples of 67 neonates following term gestations (≥37 weeks' gestation) with preeclampsia and eclampsia (group 1) and 67 maternal age-, body mass index- and gravidity-matched controls with no evidence of preeclampsia or eclampsia (group 2) will analyzed in this study.

Laboratory method:

Umbilical cord blood sample 50-100 µL will be taken into vacutainer tubes and centrifuged. Serum samples will then be stored in aliquots at −70°C until the testing period. Serum hepcidin levels will be measured by enzyme-linked immunosorbent assay.

The hepcidin kit was a sandwich enzyme immunoassay for the in vitro quantitative measurement of hepcidin in human serum plasma and other biological fluids. The minimal detectable concentrations of hepcidin (sensitivity of the assays) is 1.17 ng/ml for Detection range of 4.69-300ng/ml.

Data collection:

- Demographic data, delivery outcomes and laboratory evaluations will be recorded and compared in both groups.
- Data or results which are collected after arrangement in suitable manner by a process known as processing of data may be manual or computerized .
- These data should be confidentially protected.

Presentation of data:

Data will be collected, tabulated, then analyzed on a personal commuter using IBM© SPSS© Statistics version 21 (IBM© Corp., Armonk, NY).

The Kolmogorov-Smirnov goodness of fit test will be used to test the normality of numerical data distribution. Normally distributed numerical data will be presented as mean and SD and differences between the two groups will be compared with the independent-samples t test.

Skewed numerical data will be presented as median and interquartile range and inter-group differences will be compared non-parametrically using the Mann-Whitney U test.

Qualitative data will be presented as number and percentage and the chi square test or Fisher's exact test, when appropriate, will be applied for comparison of the two groups.

All P values will be two-tailed. $P < 0.05$ will be considered statistically significant.

Sample size calculation:

The required sample size has been calculated using the G*Power version 3.1.7 software (Heinrich Heine

Universität, Institutfür Experimentelle Psychologie, Düsseldorf, Germany).

The primary outcome measure will be the difference between the two study groups as regards the level of hepcidin in cord blood.

Two recent studies reported that the level of hepcidin in cord blood is not normally distributed in the population ***(Briana et al., 2013).*** The former study reported that the median (range) level of hepcidin in the cord blood of appropriate-for-age (AFA) neonates was 17.85 (4.75–69.2) ng/ml compared with 20.2 (2.82–50.91) ng/ml in neonates with intrauterine growth retardation (IUGR*)* ***(Briana et al., 2013).***.

The latter study reported that the median (range) level of hepcidin in the cord blood of neonates with clear amniotic fluid was 27.4 (4.3–82.5) ng/ml compared with 40.2 (2.3–115.7) ng/ml in neonates with meconium-stained amniotic fluid (MSAF) ***(Gun Eryilmaz et al., 2013).***

Thus, it is estimated that a sample size of 67 patients in each study group would achieve a power of 80% (type II error = 0.2) to detect a medium effect size (d) of 0.5 as regards the primary outcome measure.

The required sample size has been estimated non-parametrically using the two-sided Wilcoxon rank sum test and significance has been targeted at a confidence level of 95% (type I error = 0.05).

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