

The Role of Fetal Hemoglobin in Maternal Blood in Determining the Severity of Pre-Eclampsia

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Abstract

Background: Preeclampsia is a disorder of widespread vascular endothelial malfunction and vasospasm that occurs after 20 weeks of gestation. At this time, preeclampsia still lacks early means of diagnosis and markers that determine the severity of the condition. Extracellular fetal hemoglobin is involved in the pathogenesis of preeclampsia and its concentration in the maternal blood can be used to determine the severity of preeclampsia.

Patients and Methods: The current work is a retrospective randomized study that was conducted on 60 pregnant women. Patients were classified into two groups. Group I (study group) included 40 patients diagnosed as preeclampsia and subdivided into group IA (included 20 patients diagnosed as mild preeclampsia) and group IB (included 20 patients diagnosed as severe preeclampsia). Group II (control group) included 20 healthy pregnant women with uncomplicated pregnancy.

Results: The study revealed as regard socio demographic data that there was no statistically significant difference of age, parity, gestational age between the study and the control group but there was statistically significant difference of gravidity between the two groups.

As regard the vital data; there was statistically significant difference of blood pressure between mild and severe preeclampsia groups.

As regard the investigations; there was statistically significant difference of proteinuria, fetal hemoglobin and uterine Doppler ultrasound between mild and severe preeclampsia groups.

Conclusion: Fetal hemoglobin level in maternal blood is an important, simple, cheap and available biomarker for preeclampsia. It can be used late in pregnancy for diagnosis of preeclampsia and for determining the severity of pre-eclampsia.

Key Words: Preeclampsia – Fetal hemoglobin – Eclampsia – HELLP Syndrome oxidative stress – Placenta – Vascular endothelial growth factor – Alpha 1 microglobulin.

Introduction

PREECLAMPSIA is a disorder of widespread vascular endothelial malfunction and vasospasm that occurs after 20 weeks of gestation and affecting 3-7% of pregnant women. Preeclampsia is one of the leading causes of maternal mortality and morbidity especially in the developing countries [1,2].

Preeclampsia is diagnosed when pregnant women develop hypertension (BP \geq 140/90 at two separate times) and proteinuria (presence of at least 300mg of protein in a 24-hour urine collection or a urine dipstick reading of \geq +1) with or without pathological edema [3,4].

Preeclampsia may be complicated by:

HELLP syndrome, eclampsia, hemorrhagic or ischemic stroke, liver damage and dysfunction, acute kidney injury, acute respiratory distress syndrome [5,6,7].

Preeclampsia is also associated with increased frequency of Caesarian section, preterm delivery, and placental abruption. Fetal complications include fetal growth restriction and potential fetal or perinatal death [7,8].

Prevention of preeclampsia includes aspirin, calcium supplementation and blood pressure medications such as labetalol and methyldopa that may be used to improve the mother's condition before delivery [9].

Magnesium sulfate may be used to prevent eclampsia in those with severe disease. Bed rest and salt intake restriction have not been found to be useful for either treatment or prevention [10].

Currently, only symptomatic blood pressure treatment is available for preeclampsia and the

only known cure to date is delivery of the fetus and placenta. When delivery becomes recommended depends on how severe the preeclampsia and how far along in pregnancy a person is (WHO, 2011).

The exact cause of preeclampsia remains unclear; there is a strong evidence that a major cause predisposing a susceptible woman to preeclampsia is an abnormally implanted placenta.

This abnormally implanted placenta may result in poor uterine and placental perfusion leading to a state of hypoxia and increased oxidative stress [11,12].

Free fetal hemoglobin (outside red blood cells) is a potential key factor in the pathogenesis of preeclampsia by aggravating oxidative stress causing damage to the placental barrier and leaking into the maternal blood stream. It causes endothelial damage and vasoconstriction [13,14].

Leakage of cell-free fetal hemoglobin (HbF) into the maternal circulation has been documented in women with preeclampsia. This leakage of HbF from the placenta into the maternal circulation with increased levels of cell-free HbF could be documented as early as the first trimester in women who subsequently developed preeclampsia [15,16].

Predictive tests such as Doppler Ultrasound of the uterine arteries to investigate signs of inadequate placental perfusion, elevations of serum uric acid and angiogenic proteins such as vascular endothelial growth factor (VEGF) and placental growth factor (PGF) [7].

Recent studies have been suggested that the presence of cell-free HbF in the maternal circulation is not only an important factor in the pathophysiology but also a potential and useful biomarker for prediction and diagnosis of preeclampsia [17].

Patients and Methods

A retrospective study was conducted on pregnant women diagnosed as preeclampsia and admitted at Tanta University Hospital in a duration of one year from September 2016 to September 2017. All patients were undergoing the standard procedures of the protocol.

Ethics of the study:

- An approval from the ethical committee and an informed written consent was taken from each patient after explanation of benefits and risks.

- Any unexpected risk appeared during the course of the study was cleared to the patients and the ethical committee on time and the proper measures were taken to overcome these risks.
- All patients' data were confidential with secret codes and were used for the current study only.

Study population:

Case control study was carried out in a duration of one year. It was conducted upon total number 60 pregnant women in Tanta University Hospitals classified into:

- *Group I (Study group):* This group includes 40 pregnant women diagnosed as Preeclampsia at gestational age (28-40 weeks) and subdivided into:

- a- *Group (IA):*

This group included 20 pregnant women diagnosed as mild Preeclampsia.

- b- *Group (IB):*

This group included 20 pregnant women diagnosed as severe preeclampsia.

- *Group II (Control group):* This group included 20 healthy pregnant women with uncomplicated pregnancy.

Inclusion criteria: Primigravida, multigravida, age (18-35 years old), gestational age (28-40 weeks), BP \geq 140/90 and Proteinuria \geq +1.

Exclusion criteria: Chronic hypertension, gestational hypertension, epilepsy, renal diseases, liver diseases, hemolytic uremic syndrome, thrombotic thrombocytopenic purpura, gall bladder disease, pancreatic disease, diabetes mellitus, twins and old primigravida \geq 35 years old.

All patients underwent the following:

Full history including: Maternal age, parity, number of gestations, gestational age, medical history (diabetes, hypertension, cardiac, chest diseases or epilepsy), special habits and drug history.

Full clinical examination: Vital data (blood pressure, heart rate, temperature, respiratory rate, random blood sugar), neurological, cardiovascular, chest, abdominal and extremities examination.

Laboratory investigations: Complete blood count, liver function test, serum creatinine, urine analysis, random blood sugar and fetal hemoglobin.

Radiological investigation: Uterine Doppler Ultrasound.

Blood sampling:

About 5ml venous blood sample was taken from each patient. 2ml blood was put into vacutainer EDTA tube for complete blood picture. The remaining 3ml was put in vacutainer tube without additives for estimation of liver function tests, renal function tests, fetal hemoglobin and random blood sugar.

Estimation of serum fetal hemoglobin in maternal blood by Enzyme Linked Immunosorbent Assay (ELISA) supplied by Shanghai Sunred Biological Technology Co., Ltd.

Principles:

Fetal hemoglobin in samples were added to monoclonal antibody Enzyme well; which is pre-coated with Human fetal hemoglobin monoclonal antibody and then incubated.

Fetal hemoglobin antibodies labeled with biotin, and Combined with Streptavidin-HRP were added to form immune complex; then carry out incubation and washing again to remove the uncombined enzyme.

Chromogen Solution A, B was added so, the color of the liquid changes into the blue, and at the effect of acid, the color finally becomes yellow.

The chroma of color and the concentration of the Human Substance fetal hemoglobin of sample were positively correlated.

Reference values: Serum level: <0.5ug/ml.

Statistical analysis:

In this study, data were organized and tabulated. SPSS version 23 (Statistical Package for Social Studies) created by IBM, Illinois, Chicago, USA was used to statistically analyze the collected data. The level of significant was adopted at $p < 0.05$.

Results

The results were summarized, tabulated and statistically analyzed in the following tables and figures. Sociodemographic data are presented in Table (1).

The study revealed as regard socio demographic data that there was no statistically significant difference of age, parity, gestational age between the study and the control group but there was statistically significant difference of gravidity between the two groups.

About 10% of group IA while 25% of group IB showed signs of inadequate placental perfusion.

Table (1): Comparison of baseline criteria between the study and the control groups.

Baseline criteria	Group IA	Group IB	Group II	p-value
Age (years)	29.2±3.607	30.1±3.410	27.8±2.913	0.106
Gestational age (weeks)	33.8±3.679	33.5±3.426	31.7±3.840	0.159
Gravidity (times)	2.3±1.324	4.7±2.477	2.3±1.218	<0.001
Parity (times)	1.1±1.252	1.95±1.432	1.2±1.240	0.090
Fetal hemoglobin	1.5±0.530	2.5±0.705	0.7±0.184	<0.001

Table (2): Correlation between HBF level and other measures.

	Fetal hemoglobin (HBF)	
	Pearson correlation	p-value
Gravidity	0.301	0.019*
SBP	0.772	<0.001*
DBP	0.726	<0.001*
Proteinuria	0.726	<0.001*

* Denotes Statistical Significance.

Table (3): Sensitivity and accuracy of fetal hemoglobin in diagnosis of preeclampsia.

Cut off	Accuracy of fetal hemoglobin				
	Sensitivity	Specificity	PPV	NPV	Accuracy
0.8	82.5%	85%	91.67%	70.83%	83.33%

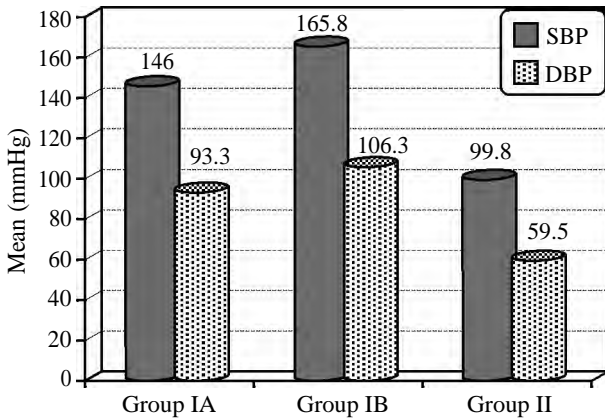


Fig. (1): Comparison of SBP and DBP between the study and the control group.

SBP: Systolic blood pressure. DBP: Diastolic blood pressure.

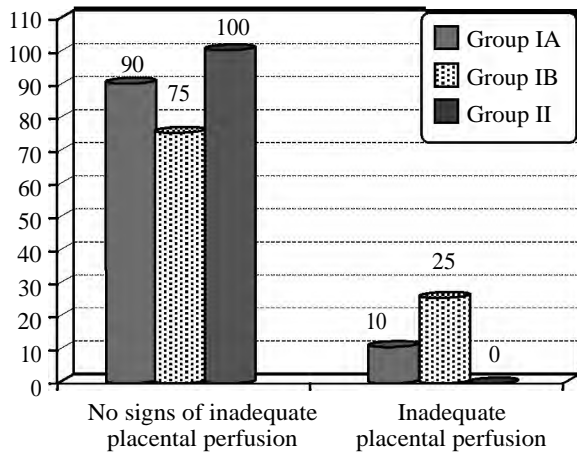


Fig. (2): Comparison of Doppler US finding between the PE and the control groups.

Discussion

Preeclampsia (PE) is one of the most serious complication in pregnancy. At this time, PE still lacks early means of diagnosis and markers that determine the severity of the condition.

Recent studies reported that extracellular fetal hemoglobin (HbF) in maternal blood is involved in the pathogenesis of preeclampsia and its concentration in the maternal serum can be used as early predictive biochemical marker for preeclampsia.

This study was performed to correlate between the level of fetal hemoglobin in the maternal serum and the severity of preeclampsia.

In the present study, the mean gestational age at sampling in the preeclampsia group (group I) was 33.6 weeks.

In Anderson et al. [18] and Anderson U et al. [14] studies, the mean gestational age at sampling

for the Preeclampsia group was 13.7 and 14.1 weeks.

This variation in gestational age can be explained by the difference in the aim of each study. In our study, cases were selected at late pregnancy till the manifestations of severe preeclampsia become well established so, we can correlate the level of fetal hemoglobin between mild and severe cases.

While in the other studies, cases were selected at early pregnancy as this studies were performed to prove that fetal hemoglobin is an early predictive biochemical marker for preeclampsia during the first trimester.

This study found that women with preeclampsia (group I) were older than patients in control group (group II) with a mean age was 29.7 and 27.8 respectively.

A study by Gram et al. [19] was in agreement with our study and found that the mean maternal age was 31 years in preeclampsia group.

Another study by Olsson et al. [20] agreed with our results in which the mean maternal age was 30 years in the Preeclampsia group.

Our study found that most preeclampsia patients showed a history of multiple pregnancy. The mean number of gravidity was 3.5 in preeclampsia group (2.3 and 4.7 in mild and severe preeclampsia respectively).

These findings agreed with Anderson et al. [18] who evaluated the maternal serum levels of HbF as predictive biomarkers for preeclampsia. They found that the mean number of gravidity was 2.7 in preeclampsia patients.

High blood pressure is important for diagnosis of preeclampsia. Severe preeclampsia is associated with higher blood pressure. The mean systolic blood pressure was 155.9mmHg in preeclampsia group (146mmHg and 165.8mmHg in mild and severe preeclampsia respectively).

The mean diastolic blood pressure was 99.3 mmHg in preeclampsia group (93.3mmHg and 106.3mmHg in mild and severe preeclampsia respectively).

Results of Gram et al. [19] study that investigated the levels of cell-free fetal hemoglobin in plasma of preeclampsia women were similar to our results.

They found that the mean systolic blood pressure was 161mmHg and the mean diastolic blood pressure was 101mmHg in preeclampsia group.

Also, Olsson et al. [20] study that analyzed fetal hemoglobin in women diagnosed as preeclampsia was in agreement with our results and found that the mean systolic blood pressure was 149mmHg and the mean diastolic blood pressure was 103mmHg in preeclampsia group.

Fetal hemoglobin level in the serum of maternal blood:

In the present study, fetal hemoglobin level was higher in preeclampsia patients. Severe preeclampsia cases showed higher level of fetal hemoglobin when compared to mild preeclampsia cases. The mean value was 0.6 γ g/ml, 1.7 γ g/ml and 2.7 γ g/ml in the control, mild PE and severe PE groups with sensitivity 82.5% and specificity 85%.

Fetal hemoglobin level in the serum of maternal blood increased three-folds in patients with mild preeclampsia and more than four-folds in patients with severe preeclampsia. So, HbF in our study was well correlated with the severity of PE.

Our study was in line with Anderson et al. [18] who found that the mean value of fetal hemoglobin was 5.6 γ g/ml in the control group and 10.1 γ g/ml in the preeclampsia group (two-fold increase) with sensitivity 65%. They also found that HbF level in early pregnancy in women who subsequently developed preeclampsia was elevated so, it can be used in prediction and early diagnosis of preeclampsia.

A study by Gram et al. [19] reported results similar to our findings with the mean fetal hemoglobin 3.85ng/ml and 14.6ng/ml in control group and preeclampsia. There was a four-fold increase in preeclampsia group compared with the control group.

Results found by Annalisa et al. [21] was in agreement with this study. The concentration of fetal hemoglobin in early pregnancy was significantly elevated in women who subsequently developed preeclampsia with sensitivity 69%.

In agreement with the present study, Anderson et al. [22] found that sensitivity of HbF in diagnosis of preeclampsia was 70%. They also reported that fetal hemoglobin plays a central role in the pathophysiology of preeclampsia.

A study by Anderson et al. [14] reported results similar to our findings. The mean value of fetal

hemoglobin level was 1.38 and 0.45 γ g/ml in preeclampsia group and control group respectively with three-fold increase in HbF in preeclampsia group compared with control group and sensitivity 78%. They concluded that the serum concentration of fetal hemoglobin has the potential of being used as first trimester biochemical marker for the subsequent development of preeclampsia.

Olsson et al. [20] reported that the fetal hemoglobin level in preeclampsia group (mean value 1.09 γ g/ml) increased nine-folds compared with control group (mean value 0.126 γ g/ml).

These variations in sensitivity may be due to small size of sample in our study. Also the gestational age at sampling was above 28 weeks (preeclampsia signs become well established and fetal hemoglobin level increased). While in the other studies, gestational age at sampling was early in the first trimester.

In conclusion, fetal hemoglobin concentration in the maternal serum is considered an important biomarker in preeclampsia for the following characteristics:

- HbF Plays a central role in the pathogenesis of preeclampsia.
- It can be used for early prediction of the disease in early pregnancy as it appears early or before the clinical manifestations.
- HbF is a simple test and is available to be measured in the maternal blood.
- It shows a high sensitivity (82.5%) and specificity (85%).
- It correlates with the severity of the condition in late pregnancy.
- Be expressed at very low levels in normal pregnancy.

Limitation of the study:

- The limited number of our patients in both groups.
- Women in this study were selected at gestational age older than other studies previously reported.

These limitations could have an impact on our findings.

Conclusion:

In conclusion, fetal hemoglobin level in the serum of maternal blood is an important, simple, cheap and available biomarker for pre-eclampsia.

It can be used late in pregnancy for diagnosis of preeclampsia and for determining the severity of pre-eclampsia.

Recommendation:

Serum fetal hemoglobin is a good prognostic marker for preeclampsia that present in higher level in severe cases than mild cases so, we recommend using it in follow-up of preeclampsia.

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دور الهيموجلوبين الجنيني في دم الأم في تحديد شدة تسمم الحمل

تظهر متلازمة ما قبل تسمم الحمل في النصف الثاني من الحمل ويصيب حوالي 7٪ من النساء الحوامل ومن أعراضه الصداع، زغلة بالعين، ارتشاح تحت الجلد وقد تحدث مضاعفات تصل إلى الفشل الكلوي والتشنجات.

في الأونة الأخيرة تبين أن الهيموجلوبين الجنيني في دم الأم هو عامل رئيسي محتمل في التسبب في المرض نتيجة لتفاقم الأكسدة مما يؤدي إلى تدمير الحاجز المشيمي وتسريه إلى دم الأم مما يؤدي إلى حدوث أضرار ببطانة الأوعية الدموية وانقباض الأوعية الدموية.

أثبتت دراسات عدة أن تسرب الهيموجلوبين الجنيني في الدورة يحدث في الأم التي تعاني من متلازمة ما قبل تسمم الحمل وأن تسرب الهيموجلوبين الجنين من المشيمة إلى الدورة الدموية للأم وارتفاع نسبة الهيموجلوبين الجنيني في دم الأم يمكن إكتشافه مبكراً في الفترة الأولى من الحمل في النساء اللاتي عانت من تسمم الحمل قبلاً.

يهدف البحث إلى دراسة العلاقة بين الهيموجلوبين الجنيني في دم الأم التي تعاني من تسمم الحمل وشدة المرض في مستشفيات طنطا الجامعية.

تم إجراء الدراسة على مرضى يعانين من تسمم الحمل في مستشفيات جامعة طنطا على مدار عام وقد اشتملت على عدد ستين (٦٠) من النساء الحوامل.

أظهرت الدراسة أنه لا يوجد أختلافاً إحصائياً كبيراً بين مجموعات الدراسة بالنسبة ل سن الأم وسن الجنين وعدد مرات الولادة غير أن هناك فرق إحصائي في عدد مرات الحمل.

وأن هناك فرقاً إحصائياً كبيراً بين مجموعتي الدراسة بالنسبة لضغط الدم ونسبة الزلال بالبول والسونار الرحمي ومعدل الهيموجلوبين الجنيني.

وأثبتت الدراسة أن معدل الهيموجلوبين الجنيني يزداد في حالات تسمم الحمل الشديدة عن الحالات المعتدلة.