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Fetal Hemoglobin in Maternal Serum as A Predictor of Severe Preeclampsia

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Abstract: Introduction: About 10% of pregnancies are complicated by hypertension. Preeclampsia accounts for 70% of hypertension in pregnancy. preeclampsia is a disease associated with high maternal and perinatal morbidity and mortality. Fetal hemoglobin is structurally made up of 2 alpha and 2 non-alpha chains; in addition, Hb-F has 2 - gamma chains and the predominant adult blood HbA1 has 2 beta chains. **Aim of the Work:** The aim of the study was to assess the role of measuring free fetal haemoglobin of maternal serum as a predictor of severe preeclampsia in cases of mild preeclampsia. **Patients & Methods:** The study included fifty pregnant women admitted to tanta University Hospital. 50cases presented with signs and symptoms of mild preeclampsia as follows: arterial blood pressure $\geq$ 140/90 mm Hg and < 160/110 mm Hg (measured 2 times at least 6 hours apart and less than 1 week) and proteinuria: $\geq$ 0.3g & < 5.0 g/24 hours or 2+ on dipstick analysis of midstream or catheter urine. Methods: after history, examination & ultrasound assessment of fetal condition 5 ml of maternal venous blood were collected & Free fetal hemoglobin (Hb-F) was differentiated from the adult form (Hb-A) by Human fetal Hemoglobin ELISA Quantitation Set. **Results:** The study is comparative study which there was statistical significant higher level of free fetal hemoglobin in cases of severe preeclampsia terminated before 34 weeks more than cases terminated after 34 weeks including mild preeclampsia and severe preeclampsia and also there was statistical significant higher level of free fetal hemoglobin in cases ended by severe preeclampsia after 34 weeks more than cases ended by mild preeclampsia. **Conclusion:** Fetal hemoglobin level in maternal blood is considered as a good predictor of severe preeclampsia.

1. Introduction:

Incidence:

About 10% of pregnancies are complicated by hypertension. The incidence varies according to the studied population and the criteria used for diagnosis. Preeclampsia accounts for 70% of hypertension in pregnancy, and chronic hypertension accounts for the remaining 30%.[1-2]

Patients with hypertension in pregnancy have higher incidence of preeclampsia, placental abruption, preterm delivery, disseminated intravascular coagulation (DIC), hemorrhage, pulmonary edema, renal insufficiency, stroke, and death. Apart from being the most common medical complications of pregnancy, hypertensive disorders are associated with significant maternal, fetal, and neonatal morbidity and mortality.[3,4] African-American women have four fold increase in mortality, mortality rate is also increased in women over 35 years ofage.[5,6]

Definition & measurement of blood pressure:

The American College of Obstetricians and Gynecologists (ACOG) defines hypertension in pregnancy as either a systolic blood pressure of $\geq$140 mmHg or a diastolic blood pressure$\geq$ 90 mmHg observed on two occasions at least 6 hours apart.[7]

Direct intra-arterial blood pressure measurement is the gold standard for blood pressure monitoring and is the preferred method for critically ill patients. However, in clinical practice, the indirect (auscultatory) method is the more convenient and widely accepted technique in use.[8]

Classification of hypertensive disorders in pregnancy:

It is often difficult to differentiate between preeclampsia (hypertension with proteinuria diagnosed after 20 weeks of gestation), gestational hypertension (elevated blood pressures without symptoms or proteinuria after 20 weeks of gestation), chronic hypertension (elevated blood pressure prior to conception or prior to 20 weeks of gestation), and

chronic hypertension with superimposed preeclampsia.[9-10]

2. Patient and Methods:

The study included 50 pregnant women attending the antenatal care clinic of Tanta University Hospital from November 2017 to November 2018.

Inclusion criteria:
1- Primigravida.
2- Pregnant women with gestational age between 20-24 weeks gestation.
3- Maternal age< 35 y.
4- Singleton pregnancy.
5- Living fetus.

Exclusion criteria:
1- No history of threatened abortion, Chorionic villus sampling, amniocentesis or antepartum hemorrhage in the current pregnancy.
2- Pregestational hypertensive disorders.
3- Diabetes mellitus.
4- Hematological and coagulation disorders.
5- Liver and renal disorders.
6- Polyhydraminos.
7- Medically on thyroid treatment.

All cases were subjected to the following:
A) **History:**
1- **Personal data:** name, age, occupation, and address.
2- **Obstetric history:** gravidity, parity, course and fate of previous deliveries and abortions.

3- **Medical & Surgical history:** to exclude general and systemic diseases as diabetes mellitus, hypertension.
4- **Duration of study:** starting from November 2017.
5- All measures for privacy of participants and confidentially of the data were taken.
B) **Examination:**
1. General examination:
▪ Vital signs:

Statistical analysis of the data
- Data were fed to the computer using IBM *SPSS software package version 20.0.*
- Qualitative data were described using number and percent. Comparison between different groups regarding categorical variables was tested using Chi-square test.
- Quantitative data were described using mean and standard deviation for normally distributed data while abnormally distributed data was expressed using median, minimum and maximum.
- *For normally distributed data, comparison between two independent population were done using independent t-test while more than two population were analyzed F-test (ANOVA) to be used.*
- Significance test results are quoted as two-tailed probabilities. Significance of the obtained results was judged at the 5% level.

3. Results:

Table (1): Comparison between cases ended by mild preeclampsia and severe preeclampsia regarding free fetal hemoglobin (Fetal hemoglobin measurement at time of sampling).

	Mild Preeclampsia	Severe preeclampsia	t-test	p
Free fetal Hemoglobin (Hb-F) Mean S.D.	893.54 181.92	1019.57 223.81	3.333	0.004*
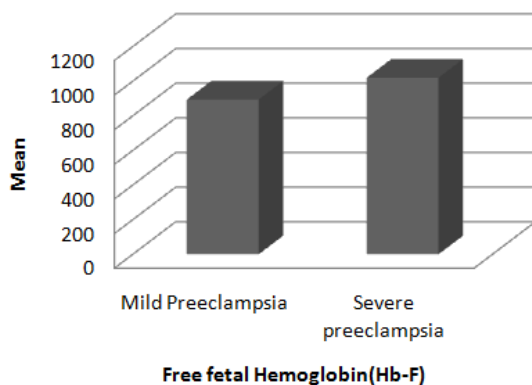
Fig. (1): Comparison between cases ended by mild preeclampsia and severe preeclampsia regarding free fetal hemoglobin (Fetal hemoglobin measurement at time of sampling).

Table (1) shows measure of fetal Hb in the cases subjected to this study and was classified according the fate of degree of preeclampsia either mild or severe preeclampsia at the time of termination. There was statistical significant higher level of free fetal Hb in cases ended by severe preeclampsia versus those case that was ended by mild preeclampsia (P < 0.05).
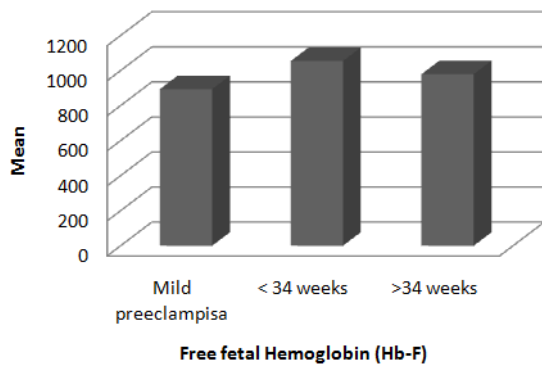
Table (2) shows the free fetal Hb level measured in patient with mild preeclampsia early onset severe preeclampsia that delivered before 34 weeks and late onset severe preeclampsia that delivered after 34 weeks. There was statistical significant difference between cases with mild preeclampsia, early onset severe preeclampsia that delivered before 34 weeks and late onset severe preeclampsia that delivered after 34 weeks regarding free fetal Hb (P < 0.05).

There was statistically significant higher level of free fetal Hb in cases of severe preeclampsia terminated before 34 weeks more than cases terminated after 34 weeks including mild and severe preeclampsia, also there was statistically significant higher level of free fetal Hb in cases ended by severe preeclampsia after 34 weeks more than cases ended by mild preeclampsia.

Table (2): Comparison of free fetal Hb between cases with mild preeclampsia, early onset severe preeclampsia that delivered before 34 weeks and late onset severe preeclampsia that delivered after 34 weeks.

|	Mild preeclampisa	Sever pre-eclampsia	
		< 34 weeks	>34 weeks
Free fetal Hemoglobin (Hb-F) Range Mean S.D.	542.0-1219.0 893.5 181.9	757-1555 1054.05 219.48	643-1248 979.0 228.62
ANOVA **p**	12.65 0.015*		

Fig. (2): Comparison of free fetal Hb between cases with mild preeclampsia, early onset severe

preeclampsia that delivered before 34 weeks and late onset severe preeclampsia that delivered after 34 weeks.

Receiver operating characteristics (ROC) curve was used to calculate different cut off of values of free fetal Hb with different specifities and sensitivities, the optimal cut off value was chosen depending on the area under the curve with as high sensitivity as possible for low false positive rate, at this high sensitivity point the area under the curve was 0.698 and the corresponding cut off value was 920 ng/ml.

Table (3): Sensitivity, specificity and accuracy of Free fetal Hemoglobin in predict the incidence of preeclampsia.

Area	P value	Cut off value	Sensitivity	Specificity	Accuracy	PPV	NPV	95%C.I.	
								Lower	Upper
0.698	0.037*	920	75.0	68.0	72.0	69.0	72.0	0.501	0.821
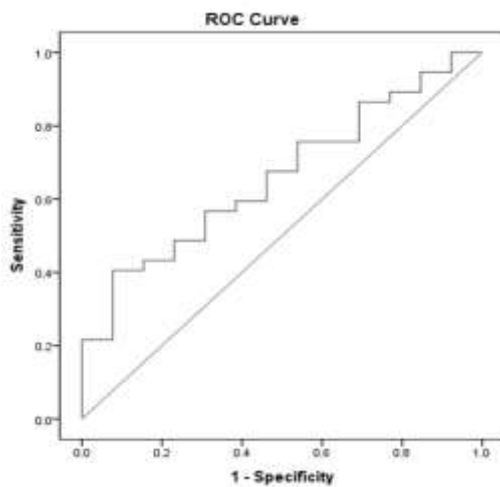
Fig. (3): ROC curve to predict the sensitivity and specificity and accuracy of free fetal hemoglobin in predict the severe preeclampsia.

4. Discussion:

In PE, there is an evidence that abnormal placental angiogenesis occurs in pregnancies complicated by preeclampsia. The repeated reperfusion leads to irregular oxygen tension which in turn results in formation of reactive oxygen species (ROS) and a state of oxidative stress occurs. [55,56] The oxidative stress damages the placental cells and thereby induces an inflammatory response. When the inflammation affects the vascular endothelium the placental barrier is broken allowing placental and fetal cells and cell debris to leak into the maternal circulation. [57-59]

Normally, Hb-F is present in very low levels in maternal blood. In PE, there was eight fold increase of free Hb F in the maternal plasma. [48,60]

The increased leak of Hb-F into the maternal circulation can mediate systemic effects. Free Hb is able to increase vascular contractility and thereby

increase blood pressure. Hb is a potent nitric oxide which in turn leads to endothelial dysfunction and increased vascular tone.[58-60] Thus, free Hb in the maternal circulation may play a role in the haemodynamic changes that are typical of PE.[60]

On the other hand, the increase in free Hb in the maternal serum may be a new important etiological factor in the progression of PE and a potentially important diagnostic biomarker, possibly also reflecting the severity of PE.[61-63]

If the levels of Hb-F in the maternal serum reflect the severity of PE, then in cases of severe preeclampsia it is expected to find higher levels of Hb-F than in mild PE. If this can be proved, the development of severe preeclampsia in cases of mild PE can be predicted through measurement of free Hb-F in maternal serum.[62,63]

In our study we assess the role of measuring free fetal haemoglobin of maternal serum as a predictor of severe preeclampsia in cases of mild preeclampsia.

The study included 50 pregnant women attending the antenatal care clinic of Tanta University Hospital. Those cases presented with signs and symptoms of mild preeclampsia as follows: Arterial blood pressure $\geq$ 140/90 mm Hg and < 160/110 mm Hg (measured 2 times at least 6 hours apart and less than 1 week) and proteinuria: $\geq$ 0.3g & < 5.0 g/24 hours or 2+ on dipstick analysis of midstream or catheter urine.

There were no statistical significant difference between cases ended with mild preeclampsia and severe preeclampsia regarding gravidity, parity, abortion.

Also there were no statistical significant difference regarding blood pressure andproteinuria.

There was statistical significant higher level of free fetal hemoglobin in cases ended by severe preeclampsia terminated before 34 weeks more than cases terminated after 34 weeks including cases ended by mild preeclampsia and severe preeclampsia and also there was statistical significant higher level of free fetal hemoglobin in cases ended by severe preeclampsia after 34 weeks more than cases ended by mild preeclampsia.

Receiver operating characteristics (ROC) curve was used to calculate different cut off of values of free fetal Hb with different specifities and sensitivities, the optimal cut off value was chosen depending on the area under the curve with as high sensitivity as possible for low false positive rate, at this high sensitivity point the area under the curve was 0.698 and the corresponding cut off value was 920 ng/ml.

In the present study the calculated specifity and sensitivity of free fetal hemoglobin in maternal blood were 68 % and 75 % respectively, the positive and negative predictive values were 69 % and 72 % respectively.

In the present study, the calculated mean was 893.54 ng/ml in mild preeclampsia group, 1054.05 ng/ml in cases ended by severe preeclampsia before 34 weeks, 979.0 ng/ml in cases ended by severe preeclampsia after 34 weeks and calculated P value was 0.004 in our study.

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