

## Plasma Factor VII and Histidine-Rich Glycoprotein as A Potential Markers in Pre Eclampsia

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### ABSTRACT

**Background:** Preeclampsia (PE) is a complex and serious multi-system disorder of pregnancy with a worldwide incidence of 5-7% and contributes significantly to maternal and perinatal morbidity and mortality. Normal pregnancy is associated with a local hypercoagulable state that becomes more profound in PE. Histidine-rich glycoprotein (HRG) is a protein interacting with angiogenesis, coagulation, and inflammatory responses, processes known to be altered in preeclamptic pregnancies.

**Aim of the work:** Is to analyze changes in the circulating levels of plasma factor VII (Plasma F VII) and HRG in women developing PE and to evaluate them as markers for early diagnosis of PE.

**Subjects and Methods:** This study was carried out on 80 pregnant women after 20 weeks of gestation. The subjects were divided into: Group I (G1): 40 cases with preeclampsia; Group II (G2): 40 normal pregnant women who were matched for age and gestational period. Plasma FVII and HRG were measured by ELISA

**Results:** Plasma FVII levels were significantly higher in G1 ( $206.22 \pm 46.25$  ng/ml) as compared to G2 ( $97.46 \pm 21.95$  ng/ml) ( $p < 0.001$ ). Significant positive correlation of plasma FVII with fibrinogen and significant negative correlation with platelet count and HRG were detected. At cut off 125.75 ng/ml plasma FVII levels shows high sensitivity and specificity (95%). HRG levels were significantly lower in G1 ( $37.1 \pm 7.52$  pg/ml) as compared with G2 ( $79.87 \pm 24.15$  pg/ml) ( $p < 0.001$ ). HRG shows significant positive correlation with platelet count and significant negative correlation with each of fibrinogen and plasma FVII. At cut off 46.45 pg/ml. HRG shows high sensitivity (92.5%) and specificity (87.5%).

**Conclusion:** Plasma FVII and HRG can be used as early marker for detection of PE.

**Key words:** PE: preeclampsia-HRG: histidine rich glycoprotein-plasma factor VII

### INTRODUCTION

Preeclampsia (PE) affects 5% to 7% of pregnant women each year all over the world, responsible for up to 18% of maternal deaths in the United States each year, and is the number one cause of premature births<sup>(1)</sup>.

In normal pregnancy, it is well established that, a local hypercoagulable state is essential for placental homeostasis. These physiological compensatory mechanisms thought to be important in preventing severe bleeding during pregnancy and delivery. However, small placental thrombi are frequently observed in women with pre-eclampsia (PE), which may compromise placental perfusion and fetal growth development and possible progression to intrauterine death. It could also account for many of the features' of PE, whose etiology is complex<sup>(2)</sup>.

The pathogenesis of preeclampsia is not fully understood, it occurs on a two-step model. The first one is an inappropriate trophoblast invasion of the maternal spiral arteries in early pregnancy, leading to inadequate implantation and placentation, thus causing relative hypoxia in the placenta. The second step involves general systemic inflammatory response

in which endothelial cell dysfunction is of major relevance<sup>(3)</sup>.

A number of circulating factors which contributes to maternal endothelial cell dysfunction have been known, including syncytiotrophoblast microparticles, cytokines, apoptotic factors, and antiangiogenic as well as proangiogenic factors. Moreover, endothelial cell dysfunction causes increased coagulability in the placental microvasculature and in the vasculature in general, which leads to disturbances of the coagulation and the fibrinolytic system<sup>(4)</sup>.

Blood coagulation begins when tissue factor (TF) is exposed to blood, binds plasma factor (F) VII, and change it to active form (FVIIa). In the presence of  $Ca^{2+}$  and lipid, this bimolecular complex (TF:FVII:FVIIa) activates FIX and FX, thus triggering coagulation pathways, leading to thrombin generation<sup>(2)</sup>.

Histidine-rich glycoprotein (HRG) is a multidomain protein involved in hemostasis as well as in the angiogenic pathway, and has both proangiogenic and antiangiogenic characteristics<sup>(5)</sup>.

HRG is a potent inhibitor of FXIIa procoagulant activity, and its plasma levels are relatively high and do not fluctuate widely, except

in pathologic conditions because HRG is a negative acute phase protein. Moreover, HRG is more likely to act as a reservoir for FXIIa formed in situ or for FXIIa that has escaped from the site of injury. In this way, HRG may compensate for the low rate of FXIIa inhibition by C1 inhibitor as HRG binds fibrin<sup>(6)</sup>. HRG is retained within the fibrin clot where it is poised to modulate FXIIa-mediated activation of coagulation. Thus, in addition to its role in fibrinolysis, HRG may also play a role in coagulation<sup>(5)</sup>.

Diagnosis of PE is currently dependent on the presence of clinical symptoms which occur at a relatively late stage and inadequate to provide a reliable guide for optimum timing of delivery to achieve the best chance of a viable fetus. Moreover, dependence on symptomology can lead to inappropriate under or over treatment<sup>(2)</sup>.

In the light of these data, changes in blood coagulation proteins and endothelial function are prominent features of PE. Hemostatic changes begin weeks to months before the clinical onset of PE, so coagulation indices including plasma factor VII and HRG may be of value in monitoring its progress<sup>(2)</sup>.

## AIM OF THE WORK

The aim of this study is to analyze changes in the circulating levels of plasma factor VII and HRG in women developing preeclampsia and to evaluate them as markers for early diagnosis of preeclampsia.

## SUBJECTS AND METHODS

This study involved 40 pregnant women with preeclampsia and 40 women with normal pregnancy (control group), all after 20 weeks of gestation, attending Department of Obstetrics and Gynecology, at Zagazig University Hospital from April 2016 to April 2017. All patients were subjected to thorough history taking with special emphasis on gestational age (in weeks), history of medical disease (as chronic hypertension, renal disease, diabetes mellitus, thyroid disorders, liver diseases) and clinical examination regarding blood pressure and body mass index. Routine investigations as liver enzymes, complete blood count, urine analysis, serum urea, uric acid, creatinine and coagulation profile were done. Estimation of serum plasma factor VII and HRG were done. Approval of the ethical committee and

a written informed consent from all the subjects were obtained.

Preeclampsia was defined by diastolic blood pressure  $\geq 110$  mmHg at admission, or  $\geq 90$  mmHg on two or more consecutive occasions, 4 hours apart; and proteinuria (either  $\geq 300$  mg protein per day or an urinary protein/creatinine ratio  $\geq 30$  mg/mmol) occurring after 20th week of pregnancy.

None of the studied women had chronic hypertension, coagulation disturbance, hemostatic abnormalities, cardiovascular diseases, renal disease, diabetes mellitus, or infections such as urinary tract infections.

## Sample collection

3 ml of peripheral venous blood sample on 3.8% trisodium citrate tubes in the proportion of 9 volumes of blood to 1 volume of anticoagulant solution and were drawn in standardized manner by trained nurses. Blood was centrifuged and plasma separation was performed within 30 minutes. Following centrifugation of whole blood in 1.5 ml Eppendorf tubes according to the manufacturer's instructions for each assay. Plasma samples were split and 100  $\mu$ l aliquots were stored at  $-20^{\circ}\text{C}$ . Frozen plasma specimens were thawed at  $37^{\circ}\text{C}$  for 15 or 30 minutes before testing.

## Measurements of plasma factor VII

Commercially available ELISA kits (Assaypro LLC 3400 Harry S Truman Blvd St. Charles, MO 63301, Catalog No. EF1007-1) were used according to the manufacturer's instructions to measure FVII (ng/ml).

## Measurements of HRG

Commercially available ELISA kits (Glory Science Co., Ltd, CATALOG #:A0993) were used according to the manufacturer's instructions to measure HRG (pg/ml)

## Statistical analysis

Background variables are presented as mean values  $\pm$  S.D. Comparisons between different continuous variables were made with Student's t-test. A receiver operator characteristic curve was constructed to determine the best cut-off values of HRG and plasma factor VII for prediction of preeclampsia. Two tailed P values  $< 0.05$  were considered as statistically significant. All statistical analyses were performed by SPSS 18.0 for Windows software pack (SPSS, Chicago, IL). Sensitivity, specificity, positive predictive value,

and negative predictive value were calculated. Pearson correlation was done to find correlation coefficient value (r) either positive (direct correlation) or negative (in verse correlation)

**RESULTS**

**Table (1):** Descriptive statistics and comparison between preeclampsia group and the control group regarding demographic data.

Variable	preeclampsia (n=40)	Control (n=40)	t	p
<b>Age: (year)</b>				
Mean ± SD	31.71 ± 8.24	29.58±5.65	1.35	>0.05
Range	21 - 40	18 - 35		NS
<b>BMI: (Kg/m<sup>2</sup>)</b>				
Mean ± SD	30.94 ± 2.78	29.35 ± 2.12	2.88	0.005*S
Range	24 - 36	24 - 33		
<b>GA: (week)</b>				
Mean ± SD	31.15 ± 3.7	31.15 ± 3.7	0	1
Range	22 - 36	22 - 36		NS

SD: Standard deviation; t: independent t test; \*: Significant (P<0.05)

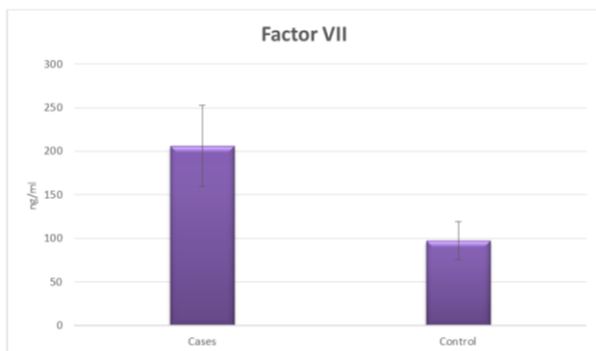
This table shows that there was statistical significant increase in BMI (body mass index) in preeclampsia cases compared to the control group and non-significant difference between the two groups as regard age and gestational age.

**Table (2):** Descriptive statistics and comparison between preeclampsia group and the control group regarding HRG and plasma factor VII.

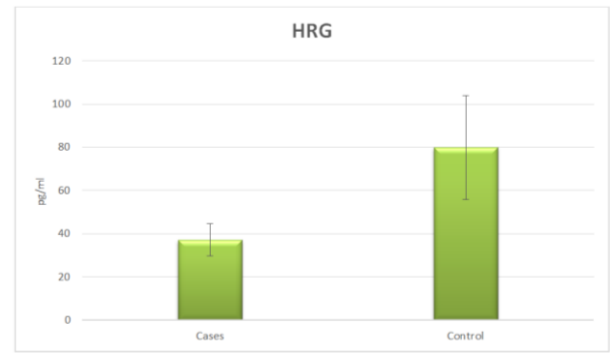
Variable	Cases (n=40)	Control (n=40)	t	p
<b>HRG: (pg/ml)</b>				
Mean ± SD	37.1 ± 7.52	79.87 ± 14.15	10.69	<0.001**
Range	24 - 58	41 - 117		
<b>Factor VII: (ng/ml)</b>				
Mean ± SD	206.22 ± 46.25	97.46 ± 21.95	13.44	<0.001**
Range	110 - 287.6	50.3 - 134.8		

SD: Standard deviation; t: independent t test; \*\*: Highly significant (P<0.001)

This table shows that there was high statistical significant increase in factor VII and high statistical significant decrease in HRG in preeclampsia cases compared to the control group (p<0.001).



**Figure (1):** Factor VII among the two studied groups.



**Figure (2):** HRG among the two studied groups.

**Table (3):** Pearson correlation between HRG and factor VII and age, gestational age, blood pressure and lab. investigations of the preeclampsia group

Variable	HRG (n=40)		Factor VII (n=40)	
	r	P	r	P
Platelets count: (x10 <sup>3</sup> /mm <sup>3</sup> )	0.34	0.03*	-0.32	0.04*
PT: (sec.)	-0.05	0.77	0.27	0.09
PTT: (sec.)	-0.08	0.64	0.16	0.32
Fibrinogen: (g/l)	-0.59	<0.001**	0.37	0.02*
HRG (pg/ml)	-----	-----	-0.39	0.01*
Factor VII (ng/ml)	-0.39	0.01*	-----	-----

r: Pearson's correlation coefficient \*: Significant (P<0.05) \*\*: Highly significant (P<0.001)

This table shows that there was statistical significant -ve correlation between HRG and platelet count and -ve significant correlation between HRG and both of fibrinogen and factor VII. Also there was -ve significance correlation between platelet count and Factor VII and +ve significant correlation between fibrinogen and factor VII in the preeclampsia group of patients.

**Table (4):** Validity of HRG and factor VII in prediction of pre-eclampsia.

Variable	Cutoff	AUC	95% CI	Sens. %	Spec. %	+ PV%	-PV%	Accuracy
HRG	≤ 46.45	0.97	0.94 - 0.99	92.5	87.5	88.1	92.1	90
Factor VII	≥125.75	0.99	0.97 - 1.01	95	95	90.5	94.7	92.5

AUC: Area under curve; CI: Confidence interval; Sens: Sensitivity Spec: Specificity; +PV: Positive predicted value; -PV: Negative predicted value.

This table shows validity values of HRG at cut off 46.45 pg/ml as follow: Sensitivity 92.5%, specificity 87.5% and accuracy 90%. While validity values of factor VII at cut off 125.75 ng/ml were as follows: sensitivity 95% specificity 90.5% and accuracy 92.5%

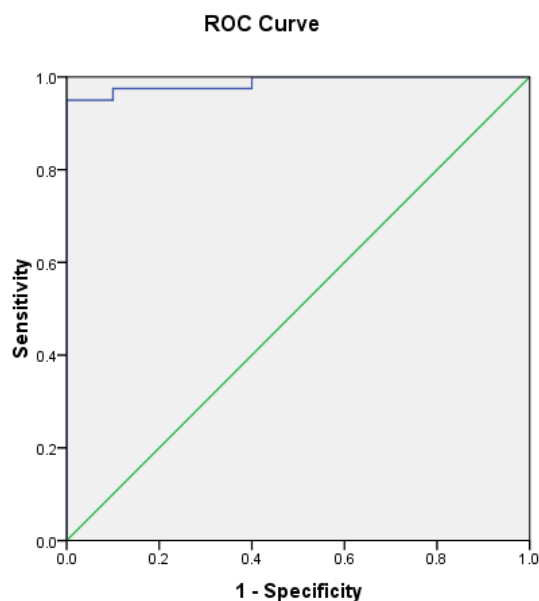


Figure (3): ROC curve for factor VII.

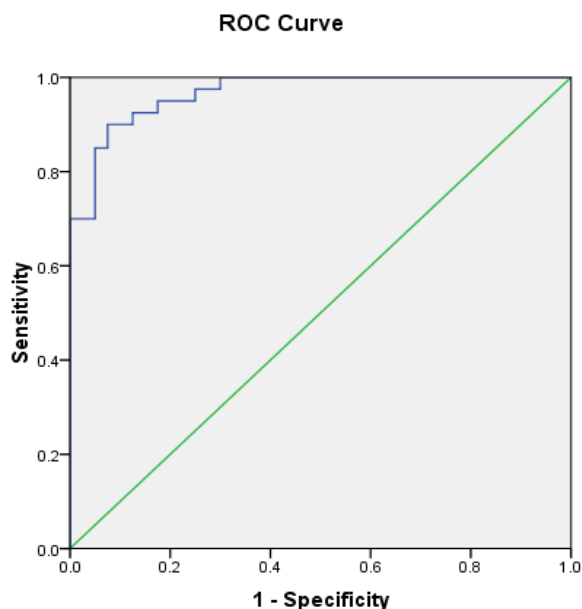


Figure (4): Receiver operator characteristic (ROC) curve for HRG.

## DISCUSSION

Diagnosis of PE is currently dependent on the presence of clinical manifestation i.e., hypertension, proteinuria and other related symptoms and may therefore occur at a relatively late stage. These indicators are not enough to provide a reliable guide for optimum timing of delivery to achieve the best chance of a healthy fetus and dependence on symptomology may lead to inappropriate treatment. Coagulation factors are involved in placental hemostasis and in placental

blood vessel differentiation. Changes in blood coagulation proteins and endothelial function are prominent features of PE. Hemostatic changes begin weeks to months before the clinical onset of PE, so coagulation indices may be of value in monitoring its progress <sup>(2)</sup>. Thus, this study has been designed to examine changes in plasma factor VII and HRG in preeclamptic patients.

The coagulation system as well as the angiogenic pathway are known to be dysfunctional in preeclampsia. HRG is a protein interacting with all these processes. It has an antiangiogenic role that has been suggested to be mediated by signal transduction targeting focal adhesions and thereby interrupting vascular endothelial growth factor-induced endothelial cell motility. Moreover, it has an angiogenic role by blocking the antiangiogenic effect of thrombospondin. HRG has also been reported to act as a negative acute phase reactant and plasma levels are consequently reduced in response to tissue injury. In the regulation of the coagulation system HRG is of great importance <sup>(7)</sup>.

The purpose of this study is to analyze whether there is a difference in circulating levels of plasma F VII and HRG during pregnancy in women developing preeclampsia compared to normal healthy pregnancies. Furthermore it is important to evaluate whether plasma factor VII and HRG have the potential of being an early biomarkers of preeclampsia.

The present study shows highly significant decrease of HRG in patients with preeclampsia ( $37.1 \pm 7.52$  pg/dl) when compared with normal pregnant women ( $79.87 \pm 24.15$  pg/dl),  $p < 0.001$ .

Similar to these results *Bolin et al.* observed that the levels of HRG decreased during pregnancy in all women, but the levels were significantly lower at gestational weeks 10, 25, and 28 in women who later developed preeclampsia than in normal pregnant women ( $P < 0.05$ ) <sup>(7)</sup>.

Against these results, *Aksornphusitaphong and Phupong* showed that pregnant women with preeclampsia did not have significantly lower serum HRG levels than the controls ( $6.0 \pm 1.3$   $\mu$ g/ml vs  $6.2 \pm 2.6$   $\mu$ g/ml,  $p = 0.46$ ). In addition, pregnant women with early-onset preeclampsia also did not have significantly lower serum HRG levels than the controls ( $5.5 \pm 2.0$   $\mu$ g/ml vs  $6.2 \pm 2.6$   $\mu$ g/ml,  $p = 0.568$ ) <sup>(8)</sup>.

The cutoff value for serum HRG level was established using the ROC curve and the value was 6.12 µg/ml. When using a serum HRG level below 6.12 µg/ml, the sensitivity, specificity, PPV, and NPV for predicting preeclampsia were 33.3%, 37.2%, 3%, and 90.6%, respectively. For predicting early-onset preeclampsia, the sensitivity, specificity, PPV, and NPV were 50%, 37.2%, 1%, and 98.3%, respectively.

In the present work, Pearson correlation analysis shows positive significant correlation of HRG with PC and negative significant correlation with each of Fbg and plasma factor VII.

HRG bound to the activated platelets and remained surface bound. TSP is a candidate for being an HRG receptor on the platelet surface. The activated platelets aggregate with each other through the complex formation between activated surface glycoprotein GPIIb/IIIa and Fbg. As described earlier, HRG can bind to Fbg and thus modulate this platelet aggregation <sup>(9)</sup>.

HRG is transported in the platelets and its release depends on the stimuli to which the platelets are exposed. The platelet degranulation response, as detected by CD63 expression, is enhanced in pregnancy, and to more extent in preeclampsia. It is possible that the generally activated endothelium in preeclamptic women stimulates the release of these hyper-reactive granules which may cause clot formation and adhesion of platelets to the vascular wall <sup>(7)</sup>.

These facts may explain the positive correlation of HRG with platelet count, as both may be consumed in the fibrin clots formation which is characteristic feature in preeclampsia.

ROC curves analysis for HRG shows that at cut off  $\leq 46.45$ , the area under the curve was 0.97 (95% confidence interval, 0.94 – 0.99; P = 0.001), 92.5% sensitivity, 87.5% specificity, 88.1% positive predictive value (PPV), 92.1% negative predictive value (NPV).

**Bolin et al.** observed that women who developed preterm preeclampsia had significantly lower levels of HRG compared with controls (45.0 vs. 82.7 µg/ml, P = 0.001). However, for the entire group of women who developed preeclampsia, no difference was found in comparison with controls (80.2 vs. 82.7 µg/ml). At cut off  $<67.8$  µg/ml, HRG had a sensitivity of 74% and a specificity of 66%, the area under the curve was 0.72 (95% confidence interval, 0.61–0.83; P = 0.001) <sup>(7)</sup>.

**Kårehed et al.** in their study measured the placental level of HRG and showed that the levels of HRG in association with the endothelium of early-onset preeclamptic placentas were significantly higher as compared to the placental levels in the control group (P=0.001), but there was no significant difference between decrease HRG levels late-onset pre-eclampsia and that of the controls in either endothelium or stroma <sup>(10)</sup>.

Several biological processes are associated with preeclampsia; these include the imbalance between angiogenesis and antiangiogenesis, the excessive inflammatory response, and the hypercoagulability seen in general, HRG is a protein known to interact with all these processes. HRG is known to interact with Fbg and these proteins are recognized as key regulators of the coagulation system. Moreover, HRG has been shown to be incorporated in fibrin clots, a process that decreases the amount of free HRG in plasma and which has been speculated to be associated with the microthrombi seen in placentas of preeclamptic patients <sup>(7)</sup>.

The reduced plasma level in preeclamptic group may be explained by compensatory mechanisms of increase angiogenesis attempting to overcome the hypoxia caused by the placental insufficiency characteristic of preeclampsia, a process that consumes and decreases the amount of free HRG in plasma.

In the present work, plasma factor VII was elevated in women with preeclampsia (206.22 ± 46.25 ng/ml) when compared with normotensive control (97.46 ± 21.95 ng/ml) with highly significant statistical difference (p<0.001). At cut off 125.75 ng/ml plasma factor VII showed 95% sensitivity, 90% specificity, 90.5% PPV, 94.7% NPV, 92.5% accuracy with AUC (0.99) 95% CI (0.97 - 1.01) P<0.001.

In agreement with these results, **Dusse et al.** <sup>(2)</sup> showed that FVII was higher in severe PE (median 551.4 ng/ml) compared with nonpregnant women (median 104.6 ng/ml; P<0.001) and normotensive pregnant women (median 258.3 ng/ml; P<0.003). FVII levels were also higher in normotensive pregnant women compared with the nonpregnant women group (P<0.001) <sup>(11)</sup>.

**Dusse et al.** <sup>(2)</sup> found that plasma factor VII showed high sensitivity (90%) and specificity (80%). PPV and NPV were 86%. The AUC and the 95% CI for the ROC curve against the healthy

nonpregnant women or normal pregnant women groups were (0.94; 90%CI: 0.87-1.0%;  $P < 0.001$ ). So, plasma FVII levels can distinguish women with PE from healthy non-pregnant women or normal pregnant women, at the third trimester, with high sensitivity (90%) and specificity (80%)<sup>(4)</sup>.

Plasma factor VII showed significant positive correlation with Fbg and significant negative correlation with PC and HRG. This suggests the presence of increased intravascular coagulation activation and possibly reflect the extent of pathophysiological processes in the placental vessels, which is the starting point for such changes in women with PE.

Based on the present findings, both Plasma factor VII and HRG can distinguish women with preeclampsia from normal pregnant women with high sensitivity and specificity at cut off 125.75 ng/ml for plasma factor VII and 46.45 pg/ml for HRG. Therefore, the obtained results indicate that these markers could be useful for assessing and monitoring hypercoagulability in pregnancy and possibly for an early detection of PE. Thus, the inclusion of plasma FVII levels and HRG into protocols for evaluating potential panels of markers for preeclampsia cases are recommended.

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