Research Article

Assessment of Plasma FXII:C in pregnant women with severe preeclampsia

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Abstract

Background: Preeclampsia is a complication of pregnancy constituting a major cause of both maternal and fetal morbidity and mortality. Pregnancy is a hypercoagulable state with changes in procoagulant, anticoagulant, and fibrinolytic systems. In preeclampsia, there is a shift in the haemostatic balance towards a pro-thrombotic state, together with changes in endothelial function. It is a state of enhanced coagulation as evidenced by an increased amount of clotting factors in maternal circulation.

Aim of the study: • To study the changes in FXII:C activity in pregnant women suffering from severe preeclampsia, and compared with healthy normotensive pregnant women.

• To correlate its level with the severity of preeclampsia.

Patients and Methods: Thirty five pregnant women in the third trimester of pregnancy with severe preeclampsia were collected from the Department of Obstetrics and Gynaecology in AL-Yarmouk Teaching Hospital from July to October 2010. Their age range is 18-41 years. The criteria for severe preeclampsia included sustained blood pressure of 160/110 mmHg or higher with persistent proteinuria of 2+ or greater on urine dipstick. A total of thirty five healthy pregnant women who were not in labour, their age and gestational age matched with the patients and normotensive throughout gestation were included as a control group. Blood samples were taken from both groups. Four ml of citrated plasma was isolated for PTT, and FXII:C assay [by parallel line bioassay of coagulation factors].

Results: There was significant difference in mean PTT between patients with severe preeclampsia (27.17±4.11) and control group (31.40±4.53). (P value < 0.0001).There was significant difference in mean FXII:C between patients with severe preeclampsia (173.29±8.82) and control group (131.85±9.78). (P value < 0.0001).

Conclusions: Factor XII:C was significantly increased in patients with severe preeclampsia than control group and show significant direct linear correlation with the severity of preeclampsia. Activated Partial Thromboplastin Time was significantly shorter in patients with severe preeclampsia than control group.

Key words: factor XII, preeclampsia

INTRODUCTION

Preeclampsia is a gestational disorder that manifests in the second half of pregnancy with multi-organ disorder that causes both maternal and fetal morbidity and mortality. (1)

In normal pregnancy, there is a hypercoagulable state with increased concentration of blood coagulation factors , decreased number of circulating platelets , increased platelet adhesiveness , decreased concentration of blood coagulation inhibitors and impaired fibrinolysis. These changes increase with advancing gestation. (2,3)

In preeclampsia, these changes are exaggerated resulting in increased activation of the coagulation cascade associated with placental infarction and fibrin formation.(4,5) The state of enhanced coagulation in preeclampsia was evidenced by increased of certain clotting factors (6) and more enhancement of activation of platelets in maternal circulation. (7)

Since values of these analytes rise during pregnancy, the question arose as to whether a discernible difference would be observed in pregnant women with sever preeclampsia compared with values in women with a healthy normal pregnancy.

Clotting factors:

The central feature of the clotting cascade is its sequential activation of a series of proenzymes or inactive precursor proteins (zymogens) to active enzymes, resulting in significant stepwise response amplification, e.g. the generation of a small number of Factor VII molecules will activate many molecules for factor X which in turn generates even larger number of thrombin molecules. The local generation of fibrin enmeshes and reinforces the platelet plug. (8)The function of these enzymes is markedly facilitated by the formation of multiple component macromolecular complexes, such as the intrinsic tenases that activate factor X and the prothrombinase. (8)

Factor XII (Hageman Factor, Contact Factor):

Factor XII is the zymogen precursor of the serine protease factor XIIa. FXII is also known as contact factor for its role in the initiation of the contact or intrinsic pathway of coagulation on contact with substances such as glass or kaolin. (9)

FXII circulates in plasma at an average concentration of 40µg/ml with plasma half-life of 48 -52 hours. (10) It is produced by a single gene located on chromosome 5. (11) FXII, prekallikrein, and HMWK form a complex on anionic phospholipids of the cell membrane, and prekallikrein is cleaved, forming the enzyme kallikrein. Kallikrein then activates factor XII (plasmin activates factor XII as well). FXIIa can then bind negatively charged surfaces and activate factor XI and prekallikrein. (9) FXIIa activates FXI and prekallikrein by mechanisms dependent on anionic surfaces and the cofactor HMWK .Factor XIIa also activates the C1 component of the complement system. (9) In addition, factor XIIa down-regulates the Fc receptor on monocytes and macrophages, induces release of interleukin (IL)-1 and IL-6 from monocytes and macrophages, and stimulates neutrophils. (12) Factor XIIa also activates plasminogen to plasmin linking the contact pathway to fibrinolysis. C1 inhibitor is the major inhibitor of both factor XIIa and ß-factor XIIa and irreversibly inhibits both enzymes. Antithrombin-III and plasminogen activator inhibitor (PAI)-1 also inhibit factor XIIa. (9)

Coagulation changes in preeclampsia:

Blood coagulation consists of a complex series of events that give rise to insoluble fibrin. The fibrinolytic enzyme system is the physiological mechanism that removes this fibrin. Clearly an imbalance between these two processes may lead to inappropriate thrombosis. Activation of the coagulation cascade is usually associated with activation of the fibrinolytic system, and this is true for preeclampsia. (13)

It is known that normal pregnancy is a procoagulant status and that this tendency is increasing during the development of the pregnancy with the end-point of minimizing the blood loss intrapartum.

In preeclamptic pregnancies, the coagulation cascade is generally activated.(13) preeclampsia being by itself a highly thrombotic and procoagulant state with platelet activation and consumption, promoting of thrombin and fibrin formation with destruction. (14) In regarding to FXII levels were significantly higher in preeclampsia owing to its procoagulant effect in that it activates factor XI to become factor XIa which participates in the intrinsic coagulation pathway. (15)

Aims of the study:

- To study the changes in coagulation factor FXII:C activity in pregnant women suffering from severe preeclampsia, and compare it with healthy normotensive pregnant women.
- To correlate factor FXII:C level with the severity of preeclampsia.

PATIENTS AND METHODS

This study was carried out in the Laboratory Department of Al-Yarmuk Teaching Hospital from July to October 2010.

Thirty five pregnant women in the third trimester of pregnancy with severe preeclampsia were included in this study. They were just admitted to the Department of Obstetrics and Gynaecology. The criteria for severe preeclampsia included sustained blood pressure of at least 160/110 mmHg or higher with persistent proteinuria of 2+ or greater on urine dipstick (each 1+ is equal to 30mg/dl).

For each patient a questionnaire form was filled with the following information: age, gestational age, parity, history of previous preeclampsia, history of chronic hypertension, renal disease and diabetes.

The patients who had any confounding conditions that could have altered the coagulation tests such as placental abruption or previa, sepsis, stillborn or heavy vaginal bleeding were not included in the study and patients with a history of diabetes, renal disease, chronic hypertension, other cardiovascular illness and symptomatic infectious diseases or received anticoagulant drugs like heparin or aspirin were also excluded.

A total of thirty five healthy pregnant women who were not in labour, age and gestational age matched with the patients, normotensive throughout gestation were included as a control group.

Before receiving any medication blood samples were taken from each patient. Four milliliters of venous blood sample were collected using a clean aseptic venipuncture technique from each patient and divided as each 1.8 ml of venous blood was put into a separated clean disposable capped plastic tube containing 0.2 ml trisodium citrate dihydrate 32 g/L.

The two tubes with citrated blood were centrifuged without delay at room temp (20-25°C), 2500g for 15 minutes to prepare platelet poor plasma, one of them for measurement APTT without delay while plasma from the other tube was stored without delay in plastic tubes at- 40 C° to perform factor assay for factors XII:C later.

Fresh plasma was obtained from healthy normotensive pregnant women (35 women) to be used as control group and were processed in the same way as the patient,s sample.

Pooled fresh platelet poor plasma was obtained from 20 appearantly normal healthy donors (other than the control group) and separated in many plastic containers. They were stored without delay at -40°C. Each time one of them was used for drawing the calibration curve for factor assay.

Activated partial thromboplastin time (APTT):

This test measures the clotting time of plasma after the activation of contact factors but without added tissue thromboplastin, and so indicates the overall efficiency of the intrinsic pathway. The test depends not only on the contact factors and on factors VIII and IX, but also on the reactions with factors X, V prothrombin and fibrinogen. (16)

Reagents: APTT was determined using a commercially available kit (Biolabo/ Maziy/ France).cephalin (rabbit cerebral tissues). STA®-CaCl¬2¬ 0.025M.

Procedure:

Manual procedure was used. Each plasma sample was assayed in duplicate. Normal PTT is 30-40 seconds. (16)

Factor XII:C assay:

Principle: (parallel line bioassay of coagulation factors).

The assay consisted of the measurement of the clotting time, in the presence of cephalin and activator of a system in which all the factors are present, constant and in excess except factor XII which was derived from the sample being tested. (16)

Reagents:

The Factor XII assay was determined using a commercially available kit (Diagnostica Stago /France).

1. STA® - Deficient factor XII: freeze dried citrated human plasma from which factor XII has been removed by selective immuno-adsorption. (REF 00722)

2. STA® - PTT. (REF 00597)

3. Owren –Koller Buffer. (REF 00360)

4. STA®-CaCl2 0.025M. (REF 00367)

5. Frozen citrated platelet poor plasma stored at - 40°C for the patient and normal pooled plasma.

6. System control [N] + [P]: control plasma, normal and abnormal levels. (Quality control). (REF 00678)

Using log -log paper, the standard factor XII% was plotted on the X-axis, and their corresponding clotting times (seconds) on the Y-axis, then the best fit calibration line was drawn then interpolate the times were that the test patients' plasmas had taken to clot and those of the controls on the calibration line to determine their respective factor XII levels (%). Normal activity 60-150 %.(17)

In addition, Mean arterial pressure (MAP) was used as an indicator of the severity of the preeclampsia.

MAP was calculated using the following formula:

MAP = [(2 X diastolic blood pressure) + systolic blood pressure] / 3.(18)

RESULT

Thirty five pregnant women with sever preeclampsia were included in this study. Information about history of chronic hypertension, previous preeclampsia, diabetes, renal and infectious diseases were taken from each patient. Their ages were ranging from 18 years to 41 years.

Thirty five healthy normotensive pregnant women age and gestational age matched with patients were included as a control groups. Table 3 shows the clinical data on the preeclamptic women and healthy controls.

Maternal age (29.40±6.60) in PE (preeclamptic patients) and (30.80±6.54) in control and gestational age (35.97±2.79) in PE and (35.74±2.90) in control were not significantly different between the two groups.

There was significant difference in systolic, diastolic, and mean arterial pressure between PE (174.42±15.84, 114.571±5.19 and 134.80±6.65 in respect) and control group (112.00±9.0, 66.71±7.56 and 81.800±6.12 in respect). P value for all < 0.0001 as shown in table 3.

There was significant difference in mean APTT between patients with severe preeclampsia (mean 27.17±4.11) and control group (mean31.40±4.53). P value < 0.0001 as shown in table 3.

Mean FXII: C is significantly higher (P value < 0.0001) in patients with severe preeclampsia (173.29±8.82) than control group (131.85±9.78) as shown in table 3.

Table 1: The age distribution of the preeclamptic patients.

Age groups (years)	*No.*	*%*
18 - 20	4	11.4 %
21 – 25	5	14.3 %
26 – 30	11	31.4 %
31 – 35	8	22.9 %
36 – 41	7	20 %

Table 2: The parity distribution of preeclamptic patients.

Parity	*No.*	*%*
Para 0	16	45.7 %
Para 1	8	22.9 %
Para 2	5	14.3 %
Para 3	4	11.4%
Para 4 and more	2	5.7 %

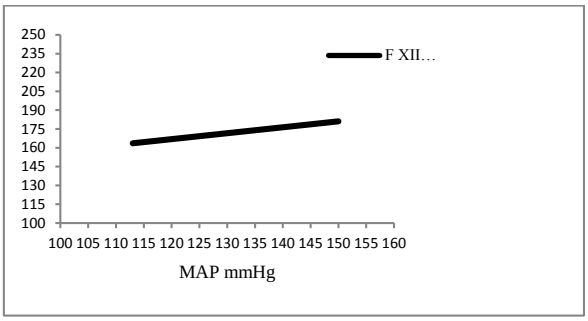

Figure 1: **The correlation between MAP and FXII% (P <0.003, r=0.490) of preeclamptic patients included in the study.**

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Table 3: showing mean, standard deviation, range and P value of different parameters in pregnant women with severe preeclampsia and control subjects

	Preeclampsia		Control		P value
	Mean±SD	**Range**	**Mean±SD**	**Range**	<
Age (years)	29.40±6.60	18-41	30.80±6.54	19-41	0.376
Gestational age (weeks)	35.97±2.79	30-40	35.74±2.90	30-40	0.738
Systolic blood pressure (mmHg)	174.42±15.84	160-210	112.00±9.0	100-130	0.0001*
Diastolic blood pressure (mmHg)	114.57±5.19	110-125	66.71±7.56	60-80	0.0001*
Mean arterial pressure (mmHg)	134.80±6.65	127-150	81.80±6.12	73-93	0.0001*
PTT (seconds)	27.17±4.11	21-36	31.40±4.53	23-38	0.0001*
Factor XII%	173.29±8.82	155-185	131.85±9.78	110-145	0.0001*

***:** Statistically significant difference ($p < 0.05$) from the controls

DISCUSSION

Preeclampsia is associated with changes in the hemostatic system and endothelial status.[19] Preeclampsia has been identified as a risk factor for venous thromboembolism in several widely available practice guidelines and is used as an identifier of patients requiring prophylactic heparin treatment .[20]

Coagulation factors in pregnancy associated hypertensive disorders are critically important in determining severity and prognosis of the pathology. However, the haematological parameters that may be involved in preeclampsia have very complex relations. As in preeclamptic pregnancies, the coagulation cascade is generally activated [13] and the results of this study showed elevated level of factor XII this may explain the results in regard to APTT which was significantly shorter in severe preeclampsia compared to healthy pregnant control. These results are similar to the results obtained by Zhang L.[21]

In regarding to FXII, its precise role is not well defined as FXIIa may have both pro- and anticoagulant effects.

It has a pro-coagulant effect in that it activates factor XI to become factor XIa which participates in the intrinsic coagulation pathway.[15]

Coppola founded that in the third trimester of pregnancy, higher levels of FXIIa in comparsion to the first trimester.[22]

A longitudinal study of FXIIa in normal pregnancy showed a progressive increase of FXIIa in pregnancy which decreased in postpartum period ,While some studies on factor XII showed no consistent changes during normal pregnancy in the third trimester. [23] This is in contrast to the results of this study which founded FXII levels were significantly higher in preeclampsia compared to healthy pregnant controls. Similar results are found by Condie RG. [24] and another recent study by Tham KM.[15]

Conclusions:

1) Plasma XII:C was significantly increased in patients with severe preeclampsia than control group and show significant direct linear correlation with the severity of preeclampsia (MAP).

2) Activated Partial Thromboplastin Time was significantly shorter in patients with severe preeclampsia than control group.

Recommendations:

1) Further studies for the identification of the hypercoagulable state in pregnant women with severe preeclampsia are required include: -
- Prothrombin fragment I and II assays.
- Protein C and S assay.
- Antithrombin assay.
- Thrombin antithrombin complex.
- β-Thromboglobulin.
-plasminogen activators and inhibitors

2) Routine haemostatic screeing tests for each preeclamptic pregnant women to detect prothrombotic state.

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