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CLINICAL ARTICLE

Total and free tissue factor pathway inhibitor in pregnancy hypertension

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Abstract

Objective: To clarify the role played by tissue factor pathway inhibitor (TFPI) in pregnancy hypertension. *Methods:* Using enzyme-linked immunosorbent assays, hemostatic measurements were obtained for women with pre-eclampsia ($n=51$), nonproteinuric hypertension of pregnancy ($n=62$), postpartum pre-eclampsia 24 h after childbirth ($n=31$), and no hypertension (healthy pregnant controls, $n=100$). *Results:* There was a significant increase in circulating free TFPI levels in women with pre-eclampsia (9.7±6.2 ng/mL) or nonproteinuric hypertension of pregnancy (8.3± 5.3 ng/mL) compared with healthy controls (5.3±2.1 ng/mL). In women with pre-eclampsia the levels remained elevated after placental delivery (10.6±4.0 ng/mL). Free protein S levels were significantly higher in women with pre-eclampsia (40.0%± 10.7%), nonproteinuric hypertension of pregnancy (37.1%±12.5%), or postpartum pre-eclampsia (39.3%±9.1%) than in healthy pregnant controls (32.2%±8.5%). *Conclusion:* Increased levels of the physiologically active free forms of TFPI and free protein S, 2 coagulation inhibitors, may protect women with pregnancy-induced hypertension from the risks of hemostatic activation.

1. Introduction

Pregnancy hypertension (PH, traditionally called *toxemia of pregnancy*), the most prevalent pregnancy complication [1], is divided into 3 categories:

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gestational hypertension alone (nonproteinuric), pre-eclampsia, and eclampsia. Gestational hypertension is not associated with adverse maternal or perinatal effects but pre-eclampsia affects the health of both mother and fetus and accounts for 200,000 maternal deaths per year worldwide [1,2].

Pre-eclampsia is a pregnancy-specific multisystem disorder characterized by reduced organ perfusion related to vasospasm and activation of the coagulation system. It is believed to originate in the placenta and cause placental insufficiency, with secondary placental damage in the form of fibrin deposition and thrombosis. Maternal response follows as endothelial damage and multisystem disorders occur, with rise in blood pressure and disturbed renal, hepatic, and clotting functions [2].

A wide variety of hemostatic disturbances have been described in pre-eclampsia. They include thrombocytopenia as well as consumption of coagulation factors and inhibitors [2–5], with evidence of thrombin generation [3–5] and inhibited fibrinolysis [5–7]. However, the changes in the circulating levels of tissue factor pathway inhibitor (TFPI) in pre-eclampsia need to be clarified. Because most studies of pre-eclampsia have been conducted during pregnancy, distinguishing maternal from placental contribution to the increases in the values of hemostatic variables has not been possible. Moreover, it may be useful to compare hemostatic changes in pre-eclampsia and in nonproteinuric hypertension to see whether the assumed chronological progression from nonproteinuric hypertension to pre-eclampsia is accompanied by further increases in hemostatic values.

The aims of this study were to measure changes in the plasma levels of total and free TFPI and other natural coagulation inhibitors in women with PH (nonproteinuric hypertension or pre-eclampsia), and assess the effect of placental delivery on the plasma levels of these inhibitors.

2. Patients and methods

This prospective, cross-sectional case-control study was conducted at the Antenatal Clinic and the Obstetric Ward of King Khalid University Hospital, Riyadh, Saudi Arabia.

2.1. Study participants

Of the 113 women with PH, 51 had pre-eclampsia and 62 had nonproteinuric hypertension. There were 9 (17.6%) primigravidas among the former, 5 (9.8%) among the latter, and 11 (11%) among the 100 healthy pregnant controls. The participants were randomly selected according to the clinical and laboratory criteria proposed by Walker [2]. Those with PH received combinations of the following as indicated by their clinical state: methyldopa (Aldomet; Merck/Schering-Plough, Whitehouse Station, NJ, USA), hydralazine (Apresoline; Ciba Pharmaceuticals, Basel, Switzerland), atenolol (Tenormin; AstraZeneca Pharmaceuticals, Wilmington, DE, USA), labetalol (Normodyne; Schering-Plough, Kenilworth, NJ, USA), and magnesium sulfate.

All patients and controls were in the third trimester of pregnancy and their characteristics are shown in Table 1. Laboratory reference values were obtained from 100 healthy men and women aged 20 to 60 years.

Table 1 Characteristics of women with pregnancy hypertension and healthy pregnant controls in third trimester of normal pregnancy*

Characteristic	Pregnancy hypertension		Postpartum pre-eclampsia ($n=3$)	Normal pregnancy ($n=100$)
	Pre-eclampsia ($n=51$)	Nonproteinuric hypertension ($n=62$)		
Age, years	30.3±5.8 (20–41)	31.6±5.8[a] (19–44)	30.7±5.1 (22–39)	28.1±6 (18–43)
Weight, kg	87.3±18.5[a] (58–161)	89.3±14.6[a] (64–124)	88.4±15.1[a] (64.9–114)	74.1±14.4 (42–124)
Blood pressure, mm Hg				
Systolic	139±13[a] (110–170)	138±17[a] (100–192)	131±10[a] (113–149)	112±8 (90–130)
Diastolic	90±10[a] (60–110)	89.8±15.3[a] (60–110)	74±11 (59–98)	70±9 (45–97)
Circulating level of				
Creatinine, μmol/L	60.3±16	58.4±21.3	59.2±16	53–115[b]
Uric acid, μmol/L	316.4±95.7	285.0±77.5	298.2±95.9	120–420[b]
Albumin, g/L	28.2±6.2	28.3±6.6	28.4±5.8	34–50[b]
Blood urea nitrogen, mmol/L	3.6±1.9	3.2±1.4	3.1±1.1	2.5–6.5[b]

[a] Significant comparing nonproteinuric hypertension and pre-eclampsia levels with normal pregnancy levels.
[b] Normal laboratory range.
* Results are given as mean±S.D. (range); $P \leq 0.05$ was considered significant.

Among the women who had pre-eclampsia, 31 were tested 24 h after a noneventful vaginal delivery.

2.2. Blood sampling and processing

Venous blood was collected with a minimum of venous stasis directly into citrate tubes containing 0.11 M sodium citrate (Vacutainer; Terumo, Tokyo, Japan), for a blood–citrate ratio of 9:1. After gentle mixing, the tubes were transported without delay to the College of Medicine's Coagulation Laboratory. Platelet-rich plasma was separated by double centrifugation at 4 °C and 3000 rpm for 15 min each time. Plasma was then carefully removed using plastic pipettes and stored in aliquots at −80 °C to be assayed in batches at a later date.

2.3. Coagulation screening tests

Screening kits were used for prothrombin time (PT) (Manchester Comparative Reagents, Manchester, UK); activated partial thromboplastin time (APTT) (Manchester Comparative Reagents); and thrombin time (TT) (Parke Davis Topical Thrombin, Morris Plains, NJ, USA). The coefficient of variation (CV) of the screening tests varied from 5% for APTT to 2% for PT and TT. Plasma fibrinogen was measured by the turbidometric method of Ellis and Stransky [8] and the CV varied between 6% and 8%.

The following variables were assayed using reagents supplied by Diagnostica Stago, Asnières-sur-Seine, France: TFPI (Asserachrom enzyme-linked immunosorbent assay [ELISA] kit); protein S (Asserachrom ELISA kit); protein C (Asserachrom ELISA kit); antithrombin III (ATIII) (Stachrom kit); tissue plasminogen activator (tPA) (Asserachrom tPA kit); and plasminogen activator inhibitor (PAI) (Asserachrom ELISA kit).

Coefficients of variation were 5% or less for total TFPI, free TFPI, total protein S, free protein S, and protein C; they were 4% for ATIII and 8% for tPA; and they ranged between 4% and 8% for PAI.

2.4. Statistical methods

The data on hemostatic measurements were entered into a spreadsheet (Microsoft Excel; Microsoft Corporation, Redmond, WA, USA). The descriptive statistics of mean±S.D. and range for the measured values were calculated for normal pregnancies and used for comparisons between the study groups. Logarithm transformation was applied to the raw data to reduce variance. Comparisons between groups were performed by one-way analysis of variance followed by the Bonferroni multiple-comparison test. $P<0.05$ indicated statistical significance.

3. Results

3.1. Tissue factor pathway inhibitor

Changes in total plasma TFPI levels were not remarkable in any of the study groups. However, compared with values calculated for controls (5.3± 2.1 ng/mL), there were significant increases in free TFPI levels in women with nonproteinuric hypertension (8.3±5.3 ng/mL; $P<0.05$) and in women with pre-eclampsia both before (9.7±6.2; $P<0.05$) and after delivery (10.6±4.0; $P<0.05$).

3.2. Natural coagulation inhibitors

Compared with values calculated for controls (32.2%±8.5%), plasma levels of free protein S were significantly higher in women with nonproteinuric hypertension (37.1%±12.5%; $P<0.05$) and in women with pre-eclampsia (40.0%±10.7%; $P<0.05$). Elevated levels were maintained after delivery in women who had pre-eclampsia during pregnancy (39.3%±9.1%; $P<0.05$). Total protein S levels, however, were significantly elevated in women with pre-eclampsia but dropped after delivery to levels similar to those measured in both women who had nonproteinuric hypertension during pregnancy and controls. In all study groups values for total protein S levels were significantly lower than laboratory reference values ($P<0.05$).

Plasma levels of ATIII were significantly lower in women with pre-eclampsia (88.8%±19.0%; $P<0.05$) and returned to normal pregnancy levels (96.5%± 15.6%) after delivery (95.9%±15.4%).

Compared with the third-trimester levels (100.8%± 25.7%), postpartum plasma levels of protein C were elevated in women who had pre-eclampsia during pregnancy (108.4%±19.9%; $P<0.05$).

These results are shown in Table 2.

3.3. Fibrinolytic variables

Compared with mean plasma levels measured for controls, mean tPA levels were significantly higher during pregnancy, in women with nonproteinuric hypertension, and both before and after delivery in women with pre-eclampsia. On the other hand, compared with laboratory reference values, PAI levels were similarly elevated in all study groups; but although they dropped significantly in women

Table 2 Summary of hemostatic variables in pre-eclampsia, nonproteinuric hypertension, and normal pregnancy, as well as in pre-eclampsia, 24 h postpartum

Variable	Nonproteinuric hypertension	Pre-eclampsia	Pre-eclampsia, 24 h postpartum	Normal pregnancy	Reference value
Prothrombin time, s	13.0±1.5	14.0±6.4	13.6±0.94	13.1±1.1	13.5±1.5
Activated partial thromboplastin time, s	41.3±8.8	41.2±8.4	39.6±4.8	42.4±8.3	39.1±3.1
Thrombin time, s	13.5±4.3	12.4±2.5	13.8±2.3	12.8±2.6	12.8±2.5
Plasma fibrinogen, g/dL	5.0±1.7	5.5±1.2	6.1±1.2[a]	5.8±1.4	2.9±7.7
Antithrombin III, %	92.9±16.1	88.8±19.0[b]	95.9±15.4	96.5±15.6	101.0±12.3
Protein C, %	101.8±25.7	100.8±25.7	108.4±19.9[a]	98.7±25.8	103.5±21.5
Protein S, %					
Total	57.0±14.7	67.6±21.8[b]	59.4±15.4	59.7±22.8	94.5±20.1
Free	37.1±12.5[b]	40.0±10.7[b]	39.3±9.1	32.2±8.5	69.7±13.4
TFPI, ng/mL					
Total	56.6±22.2	57.2±15.2	60.9±10.2	58.8±11.3	60.0±13.9
Free	8.3±5.3[b]	9.7±6.2[b]	10.6±4.0	5.3±2.1	7.6±2.3
tPA, ng/mL	9.8±8.3[b]	12.4±8.9[b]	13.1±4.3[b]	5.8±2.2	7.1±2.8
PAI, ng/mL	77.1±30.05	75.1±30.6	42.8±20.8[a]	73.7±30.9	20.4±11.1

Abbreviations: PAI, plasminogen activator inhibitor; TFPI, tissue factor pathway inhibitor; tPA, tissue plasminogen activator.
Results are given as mean±S.D. (range); $P \leq 0.05$ was considered significant.
[a] Significant comparing pre-eclampsia levels before delivery and 24 h postpartum.
[b] Significant comparing nonproteinuric hypertension and pre-eclampsia levels with normal pregnancy levels.

with pre-eclampsia after placental delivery, they remained well above laboratory reference values in this group.

Hyperfibrinogenemia was noted in all study patients during pregnancy, and it was most pronounced postpartum in women who had pre-eclampsia.

4. Discussion

The current concept of blood coagulation activation is based on the triggering role played by tissue factor (TF) in the activation of the coagulation system and its major role as a regulator of coagulation, hemostasis, and thrombosis [9]. Its activity is controlled by TFPI, which is synthesized primarily by the vascular endothelium. Tissue factor pathway inhibitor inhibits the initial reaction in the extrinsic, tissue factor-mediated coagulation pathway.

Approximately 85% of TFPI is bound to the glucosaminoglycans of the vascular endothelial cell surface, with only 15% circulating; and since 80% of the circulating TFPI is bound to lipoproteins, 20% of the circulating TFPI is the free, physiologically active TFPI [9]. A role for TFPI was proposed in several diseases that are characterized by activation of the coagulation system and thrombus formation, such as stroke, ischemic heart disease, deep vein thrombosis, sepsis, and disseminated intravascular coagulation [9,10].

In one recent study, elevated plasma levels of free TFPI antigen were recorded in women with pre-eclampsia but not in women with nonproteinuric hypertensive disease of pregnancy, and the authors concluded that high plasma levels of free TFPI antigen could be predictive of the presence of pre-eclampsia [11]. In contrast, an earlier study found the activity of the extrinsic coagulation pathway inhibitor (presumably before it was named *TFPI*) to fluctuate in a similar manner and magnitude in PH and normal pregnancy over the 3 trimesters [12].

The present study, which used the more precise ELISA assay, is the first, so far the investigators know, to report on plasma levels of both total and free TFPI in pre-eclampsia and in nonproteinuric pregnancy hypertension before and after placental delivery. The finding of significantly higher levels of free TFPI in both forms of PH will allow to further characterize the thrombophilia associated with these abnormalities of pregnancy. Ballart et al. [13] reported elevated levels of tissue factor in pre-eclamptic women and concluded that tissue factor contributes in a major way to the hypercoagulable state of pre-eclampsia. In the present study, elevated levels of free TFPI in women with pre-eclampsia indicated that the extrinsic, tissue factor pathway activation of the coagulation system is balanced by a simultaneous increase in TFPI levels. The inhibition of the prime trigger of blood coagulation thus occurs upstream—which is of special physiologic significance given that ATIII levels are markedly reduced in pre-eclampsia, and that the prime ATIII location of inhibition occurs downstream in the coagulation cascade, at the level of thrombin.

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