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REPORT

Association between increased FVIIa-antithrombin complex/FVIIa ratio and pre-eclampsia

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Abstract

Objective: Activated factor VII-antithrombin (FVIIa-AT) complexes can be used to reflect the degree of intravascular exposure of tissue factor (TF). The aim of the present case-control study was to evaluate FVIIa-AT plasma levels during normal pregnancy and in pre-eclampsia (PE).
Methods: One hundred and five pregnant women were enrolled and namely $n = 30$ in the first (T1), $n = 30$ in the second (T2), $n = 30$ in the third (T3) trimester of pregnancy and $n = 15$ with PE. FVIIa-AT complexes were determined using a specific ELISA (Diagnostica Stago, Asnieres, France).
Results: FVIIa-AT complexes were significantly higher in pregnant (119 ± 24 pM) than in healthy (102 ± 12 pM, $p = 0.001$) women. No difference in FVIIa-AT levels between T3 women and with PE was observed. Interestingly, women with PE had significantly higher FVIIa-AT/FVIIa ratio than women during T3 (2.01 ± 0.44 versus 1.50 ± 0.29, $p = 0.001$).
Conclusion: FVIIa-AT complexes plasma levels differed significantly between normal pregnancy and non-pregnant women. Moreover, FVIIa-AT/FVIIa ratio was higher in patients with PE than in normal pregnant women.

Keywords

Hypercoagulability, pregnancy complications, tissue factor

History

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Introduction

During normal pregnancy, complex changes in the coagulation cascade occur. Increases in many pro-coagulant factors (i.e. FIX, FVIII, FVII and fibrinogen) and a decrease in protein S (PS) plasma levels have been previously reported [1]. Concomitantly an impairment of fibrinolytic system, due to the increase of plasminogen activator inhibitor-1 and -2 (PAI-1 and -2), has been described [2]. Taken together, these changes lead to an imbalance of the coagulation cascade toward a hypercoagulable state [3]. This condition is thought to be a normal response and is important in preventing severe bleeding during labor. Nevertheless, the hypercoagulability may contribute, in addition with pre-disposing factors, to the development of thrombotic and/or pregnancy complications like pre-eclampsia (PE). PE is a pregnancy syndrome characterized by high blood pressure and significant amounts of protein in the urine [4]. In this medical condition, a hypercoagulable state [5] and small placental thrombi are frequently observed [6]. Girardi [7] showed, using an animal model, that the microthrombi that are found in the placenta of mice with PE can be caused by a hypercoagulable state due to an increased placental tissue factor (TF) expression [7]. Moreover, in other obstetrical syndromes (i.e. preterm labor and fetal death) a higher TF activity has been reported [8–10]. The plasma levels of the complex between activated factor VII (FVIIa) and antithrombin (AT) was reported to reflect the degree of intravascular exposure of TF to the blood and consequently the activation of the clotting cascade [11]. The aim of the present retrospective case-control study was to evaluate if changes in factor VIIa-AT are observed in PE compared to normal pregnancy.

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Methods

Patients

Pregnant women referred to the Section of Maternal Foetal Medicine of University Hospital of Padua between February and March 2011 were enrolled into this study. Exclusion criteria were age $\leq$18 years, ongoing anticoagulant treatment at therapeutic or prophylactic dosage, active cancer and previous arterial or venous thrombosis. Normal pregnant women and women with PE were also included. PE was defined by high blood pressure (two separate readings taken at least 6 h apart of 140 or more in systolic blood pressure and/or 90 or more in diastolic blood pressure) and 300 mg of protein in a 24-h urine sample occurring after 20th week of pregnancy. A group of healthy women, friends of cases, age match ($\pm$3 years) with cases, without a personal history of

thrombosis and without pharmacological therapy acted as controls. None of the women enrolled in the control group were on hormonal contraceptive therapy. The study was performed according to the Declaration of Helsinki and informed consent obtained from all patients according to the University Hospital of Padua policy.

Blood samples

After informed consent, 9 ml of blood was drawn from an antecubital vein into a syringe pre-filled with 1 ml of Na-Citrate 109 mMol. In each women FVIIa-AT complex (pM), FVIIa activity (FVIIa:act, mU/ml) and AT (%) plasma levels were determined. FVIIa-AT complex levels were measured in plasma with a ''sandwich'' ELISA using a capture monoclonal antibody to FVIIa and a monoclonal anti-AT detection antibody, according to protocol supplied by the manufacturer (DiagnosticaStago, Asnieres, France). FVIIa:act was determined in plasma by a clotting assay (STAClot® VIIa-rTF, DiagnosticaStago) performed on ACL 3000 Research (IL, Milan, Italy) according to the protocol supplied by the manufacturer. AT activity assay was performed on a BCT-Analyser (Dade Behring, Marburg, Germany) with standard procedures.

Statistical analysis

All analyses were performed with SPSS version 11.5 software (Chicago, IL). Continuous variables were expressed as mean ± standard deviation (SD). Comparisons between cases and controls were made using an unpaired parametric Student's *t*-test. ''FVIIa-AT/FVIIa ratio'' was calculated as the ratio, for each subjects enrolled, between FVIIa-AT plasma levels and FVIIa plasma levels. Statistical significance was considered as a 2-tailed probability <0.05.

Results

Of the 135 eligible pregnant women, 105 were enrolled (age range 19–43 years). Thirteen patients were excluded because of ongoing, either therapeutic or prophylactic dosage, anticoagulant treatment; nine were excluded because of a previously thrombotic event; seven patients refused to give informed consent and six were younger than 18 years. Out of the patients admitted, 30 were in the first trimester of pregnancy (T1), 30 in the second trimester (T2) and 30 in the third trimester (T3). Finally, 15 women were affected by PE. Fifty healthy women without personal or family history of thrombosis and without pharmacological therapy acted as controls. The main demographic and laboratory characteristics of patients and controls were reported in Table 1. Overall, the 90 healthy pregnant women showed significantly higher FVIIa-AT complex plasma levels than healthy women (119 ± 24 versus 102 ± 12 pM, respectively; $p = 0.001$). No statistically significant difference in FVIIa was seen between these two groups of women (79 ± 31 versus 86 ± 18 mU/ml, $p = 0.3$). Considering the three trimesters separately, a slight increase in both FVIIa-AT and FVIIa was seen, but these increases were not significant (Table 1). Women with PE had higher FVIIa-AT complex plasma levels than the women during T3 (138 ± 33 versus 121 ± 27 pM), but the difference was not statistically significant ($p = 0.08$). On the contrary, FVIIa plasma levels were lower in PE women than in women during T3 (73 ± 13 versus 83 ± 20 mU/ml), although the difference was not statistically significant ($p = 0.06$). Considering the FVIIa-AT/FVIIa ratio, PE women had significantly higher ratio (2.01 ± 0.44) than women during T3 (1.50 ± 0.29, $p = 0.001$). No significant difference in mean AT plasma levels was seen between the normal pregnant PE and control groups.

Discussion

During normal pregnancy modifications of blood clotting cascade towards a hypercoagulable state have been extensively described. Both increased plasma levels of clotting factors and impaired fibrinolysis have been reported [1,2,5]. This pregnancy-associated tendency towards hypercoagulability can trigger hemostatic abnormalities during pregnancy and may be associated with pregnancy complications [12]. Increased TF activity/expression was previously reported both in PE [13] and in other obstetrical syndromes [8–10]. TF is the main physiological initiator of blood coagulation [14]. It is normally encrypted within cells. Circulating or ''soluble TF'' is absent or found at extremely low concentrations [15]. Subsequent to vascular wall damage, extra-vascular TF is exposed to blood and binds plasma factor VII with high affinity [16]. Once bound to TF, FVII is rapidly converted to FVIIa. The best characterized inhibitor of TF-FVIIa complex is TF pathway inhibitor (TFPI), a Kunitz-type protease that regulates TF-FVIIa activity by forming a stable quaternary complex (TF-FVIIa-FXa-TFPI) [17]. AT has also been found to inactivate FVIIa when it is bound to TF causing the accelerated dissociation of FVIIa-AT complex from TF [18,19]. Recently, we reported an increase in FVIIa-AT plasma levels in adults who had experienced a previous venous thrombotic episode (i.e. deep vein thrombosis and/or pulmonary embolism) or cerebral sinovenous accident [20,21]. The resulting circulating level of FVIIa-AT complex reflects the degree of intravascular exposure of TF (both the active and latent forms) to the blood or increased synthesis of TF. In the present study, pregnant women had higher FVIIa-AT complex plasma levels than healthy non-pregnant women. Similarly, women with PE had higher levels of the complex than normal pregnancy women in the third trimester, but the difference was not statistically significant. Due to the fact,

Table 1. Demographic and laboratory characteristics of the study population.

	Normal pregnant				
	I trimester	II trimester	III trimester	PE	Healthy women
Subjects (*n*)	30	30	30	15	105
Age (years)	29 ± 6	32 ± 4	32 ± 6	31 ± 3	31 ± 4
FVIIa-AT (pM)	118 ± 19*	118 ± 26*	121 ± 27*†	138 ± 33*	102 ± 12
FVIIa (mU/ml)	70 ± 26	78 ± 33	83 ± 20	73 ± 13	86 ± 18
AT (%)	99 ± 12	100 ± 11	101 ± 23	103 ± 26	102 ± 9

Plus-minus values are means ± standard deviation.
*$p < 0.01$ versus healthy women.
†$p = 0.08$ versus PE.

as we previously reported [20], that FVIIa plasma levels are the main determinant of FVIIa-AT complex and considering the mild reduction of FVIIa plasma levels in women with PE [22], we chose, as previously reported by Silvera et al. in a different setting [23], to consider the proportion of FVIIa-AT complexes relative to the total FVIIa amount (FVIIa-AT/FVIIa ratio) as the most appropriate parameter for the evaluation of TF expression. Doing so, a statistically significant higher ratio was found between normal pregnancy and PE. In conclusion, the dosage of FVIIa-AT complexes could be considered an indicator of TF-dependent hypercoagulability and therefore its increase in pregnancy as well as preeclampsia may reflect this aspect. The clinical relevance of FVIIa-AT still need to be fully clarified, but it is interesting to note that FVIIa-AT complex/FVIIa ratio might be useful for assessing and monitoring hypercoagulability in pregnancy and possibly for an early detection of PE.

Declaration of interest

The authors declare that they have no conflicts of interest with the exception of Mr Barry Woodhams, who is the Scientific Director of Diagnostica Stago – the kits of this company were used in this study.

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