Tissue factor and tissue factor pathway inhibitor in women with a past history of preeclampsia: implication for a hypercoagulable state postpregnancy!

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Preeclampsia (P-EC) is a multisystem disorder of pregnancy whose cause and pathogenesis remain poorly understood. However, abnormal haemostasis and endothelial dysfunction are thought to be implicated. Women with a past medical history of P-EC have a baseline hypercoagulable state postpregnancy. The aim of this study is to examine the relationship between tissue factor (TF) and TF pathway inhibitor (TFPI) in women who have had P-EC within the last 3 years (more than 6 months postpartum) and their normal counterparts. Blood specimens were collected from women known to have had P-EC within the last 3 years ($n = 26$) and aged-matched healthy women without past history of P-EC in previous pregnancy ($n = 26$). Plasma TF and TFPI levels were measured using ELISAs. Women who have had P-EC showed increased TF levels compared with their normal counterparts, whereas TFPI levels were reduced. Neither parameter differed significantly when the groups were tested against each other. Interestingly, the TF/TFPI ratio was significantly increased ($P = 0.024$) when the two groups were compared. In summary, there was a trend towards increased TF and reduced TFPI levels in the P-EC group. Such a tendency was not statistically significant. However, the TF/TFPI ratio was significantly increased when the groups were compared. Our findings suggest an imbalance between TF/TFPI levels in women with past history of P-EC postpregnancy. This may contribute to the development of maternal hypercoagulable states and may predispose women with a history of P-EC to cardiovascular risks later in life. *Blood Coagul Fibrinolysis* 25:000–000 © 2014 Wolters Kluwer Health | Lippincott Williams & Wilkins.

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Introduction

Tissue factor (TF) initiates blood coagulation by forming a complex with circulating factor VII (FVII) and activated FVII (FVIIa) [1]. This activity is regulated by TF pathway inhibitor (TFPI) [2,3]. TF is important in pregnancy, particularly in the first trimester, as it is required for embryogenesis [4] as well as angiogenesis [5].

Normal pregnancy is associated with haemostatic changes, tipping the balance towards hypercoagulability [6]. This reverts back to prepregnancy levels 4–6 weeks after delivery [7]. Preeclampsia (P-EC) is a major contributor to perinatal morbidity and mortality. Approximately 5–7% of pregnancies are complicated by P-EC [8], often necessitating premature delivery of the baby. It is characterized clinically by high blood pressure and proteinuria occurring after the 20th week of pregnancy [9].

Several studies have demonstrated changes in haemostatic parameters in women with P-EC [10–12]. Recently, it has been highlighted that changes in the maternal vasculature and coagulation profile may in turn predispose women with P-EC to subsequent deleterious cardiovascular consequences [13]. In this study, we examine individual levels of and relationship between TF and TFPI in women who have had P-EC compared with normal counterparts.

Materials and methods

Participants

This was a case–control study comparing TF and TFPI levels in a group of 26 women known to have had P-EC between January 2008 and October 2011; a group of 26 healthy age-matched women who have not had P-EC were also assessed (termed 'control group hereafter'). Ethical committee approval was granted for the study by the Southampton and South West Hampshire Research Ethics Committee. The participants were asked to complete a general medical questionnaire to confirm inclusion and exclusion criteria.

Exclusion criteria common for the two groups were current pregnancy and women who had given birth less than 6 months before testing. Comorbidities, including chronic hypertension, obesity, presence of cardiovascular, autoimmune/hepatic diseases, connective tissue disorders, diabetes, coagulation disturbances and cancer, women on anticoagulants or corticosteroids therapy were also excluded from the study.

Sample size

A sample size calculation was performed, based on a 0.6 correlation coefficient between the TF, TFPI levels and P-EC. The *P* value to assess this association was set to 5%, two-tailed. The power was 0.95. Given these criteria, 30 participants per arm would need to be recruited if the drop-out rate reached 25%.

Specimen collection

A 5 ml sample of venous blood was collected using a 21-gauge needle, into vacutainer tubes containing 3.8% trisodium citrate. The bloods were then centrifuged at 3000 rpm for 10 min at room temperature. Plasma samples were then immediately isolated and transferred into 250 μl aliquots, which were then stored at −86°C until used for batch wise analysis. For each assay, a previously unthawed aliquot was used.

Assays

Commercially available ELISA assays were used to measure TF and TFPI (Quantikine Human coagulation factor III/TF and TFPI; R&D Systems, UK). The intra-assay and interassay coefficient of variations for the TF and TFPI assays were 3.4 and 5.7%, and 3.6 and 5.9%, respectively. Assays were performed according to the manufacturers' instructions.

Statistical analysis

Statistical analyses were performed using SPSS (Statistical Analysis System, Chicago, Illinois, USA, version 19 for Windows). Data proved parametric, so summary statistics were calculated as means and SD. However, the ratios are not normally distributed, and thus expressed as a box and whisker plot, with outliers additionally identified. Differences between ratios were assessed by Mann–Whitney testing. The level for statistical significance was set at $P < 0.05$. Assay results were recorded as ng/ml in original sample.

Results

Demographic and clinical data

Characteristics of the groups are summarized in Table 1. No significant difference was found in women's age, BMI, smoking status and alcohol consumption. However, a number of women with P-EC had a family history of P-EC (six out of 26) and hypertension (eight out of 26). The control group was also sampled a year after last delivery.

Plasma tissue factor and plasma tissue factor pathway inhibitor

Women with history of P-EC postpregnancy showed slightly higher but statistically insignificant plasma TF levels (0.075 ± 0.01 ng/ml) when compared with the control group (0.056 ± 0.007 ng/ml). Conversely, plasma TFPI levels were slightly reduced in the P-EC group (356 ± 94.2 ng/ml) compared with controls (388 ± 72.8 ng/ml), but this was not significant.

Plasma tissue factor/tissue factor pathway inhibitor ratio

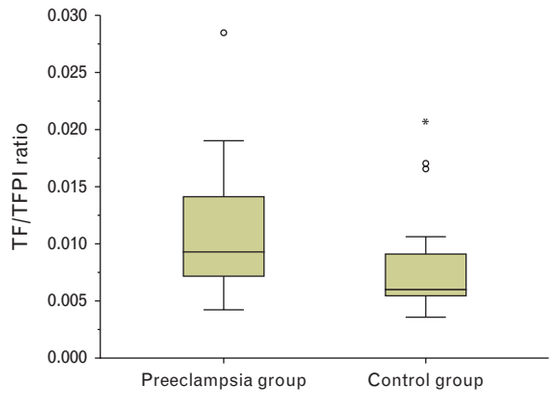
The TF/TFPI ratio was significantly raised when women with history of P-EC postpregnancy were compared with controls ($P = 0.024$; Fig. 1).

Table 1 Results of the general medical questionnaire completed by the study population

	Women with P-EC	Non-P-EC (controls)
Number of participants	26	26
Mean age of participants	33.6 years	30.5 years
Minimum to maximum age	24–47 years	22–43 years
Mean BMI of participants	27.1	24.9
Family history of P-EC	6	None
Family history of hypertension	8	4
Family history of type II diabetes	4	7
Family history of myocardial infarction	5	5
Family history of deep vein thrombosis	1	None
Ethnic group	24 participants were white British; 1 white European; and 1 black African	22 participants were white British; 1 white European; 1 Mexican Latino; 1 black African; and 1 Indian
Current smokers	4	2
Regular exercise (moderate activity for 30 min at least 3 times a week)	5	3
Alcohol consumers	22 – average units consumed is 3.3 units/week	18 – average units consumed is 3.2 units/week
Personal history of anaemia either during or after pregnancy	3	None
Currently on contraception	6	9

P-EC, preeclampsia.

Fig. 1



Plasma TF/TFPI ratio in the groups studied. Raw data were in ng/ml. Results are shown as box and whisker plot ($P = 0.024$, Mann–Whitney).

Discussion

P-EC is a complex multisystemic disorder and a main cause of perinatal morbidity and mortality. There is evidence suggesting that intravascular coagulation activation may be involved in the pathogenesis of P-EC [14]. Indeed, the process of intravascular coagulation activation may explain some of the clinical features associated with P-EC [15]. Coagulation indices may be of value in monitoring P-EC progress [16]. Recent studies suggested that a past history of P-EC may increase the risk of cardiovascular disease later in life [13,17].

This study investigates the relationship between TF and TFPI in women with a past history of P-EC and aged-matched healthy women with no past history of P-EC. The literature indicates that coagulation parameters normalize within 2 months following birth [7]. In the present study, both TF and TFPI measurements were made at least 6 months postpartum. Almost all of our study populations were in their 30s. Their family histories of cardiovascular disease are detailed in Table 1. Cardiovascular disease and P-EC share many risk factors. In this respect, the results that were particularly important were the number of current smokers and women currently on contraceptive medications. Although clinical and experimental studies have documented that either active or passive exposure to cigarette smoke promotes vasomotor dysfunction and thrombosis in multiple vascular beds [18], the analysis of plasma samples in those women who smoked showed no significant change in TF and TFPI levels. Likewise, women on contraception, including oral, injection, transplant and intrauterine devices, (12 out of 40) showed no significant differences in TF and TFPI levels. Thus, our findings are not in agreement with studies linking the use of oral contraceptives to an increased incidence of thrombovascular disease. It also challenges a previous report with respect to increased coagulation factors VII, X and fibrinogen during oral contraception usage [19].

During the course of pregnancy, preeclamptic women showed significant increase in TF and TFPI levels [12,20,21]. In the present study, we found no significant difference in TF and TFPI levels in women who had experienced P-EC within the last 3 years and their normal counterparts, although the trend was towards raised TF and reduced TFPI levels compared with controls. Increasing cohort sizes may make the differences statistically significant, but not necessarily make them any more practically important. However, when the results were expressed as TF/TFPI ratio, we found a significant increase in TF/TFPI ratio in women with a past history of P-EC compared with controls ($P = 0.024$). Our results showed no significant association between plasma TF and plasma TFPI levels in the P-EC group. This may be due to TFPI-1 being secreted by activated or damaged vascular endothelial cells, including cells of the microvasculature.

Several factors, such as hormonal or immune reaction, could influence studies of coagulation proteins, which are known to play an important role in the development of P-EC [15,22]. Upon answering the questionnaire, women were asked about medications in general without any further stratification. Some particular medications, such as oral contraceptive pills may affect the results. Therefore, more selective criteria could enhance discrimination. Clinically, P-EC is a very heterogeneous condition with varying degrees of severity. We only studied women who were diagnosed with P-EC generally, and these were not classified further. Correlating results with severity of P-EC, further details about gestational age at delivery, baby weight, placental disease and other pregnancy complications might add extra benefit to future studies. The assays reported here were all based on measurement of antigen; no functional tests were undertaken. In the context of the antibodies involved, it also meant that all of the target protein present was measured, irrespective of complexity.

In summary, our study has examined the relationship between plasma TF and TFPI in women who had P-EC compared with their normal counterparts. The study showed no significant changes in TF and TFPI levels. However, the TF/TFPI ratio was significantly increased in women with P-EC. The present result strongly supports the need for further work to examine the relationship between plasma TF and TFPI postpartum levels. It must be stated that this work should be seen as a pilot study, which might add beneficial information to more focused studies in the future.

Acknowledgements

Conflicts of interest

We have no conflict of interest that we need to declare.

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