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Original Article

Levels of thrombin-activatable fibrinolysis inhibitor and platelet-activating factor in recurrent pregnancy loss patients

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ABSTRACT

Objective: The aim of this study was to investigate factors associated with thrombosis that may contribute to recurrent pregnancy loss (habitual abortion), specifically differences in serum levels of platelet-activating factor and thrombin-activatable fibrinolysis inhibitor (carboxypeptidase B2) between women with a history of recurrent miscarriage and those with no recurrent miscarriage history.
Materials and methods: A case-controlled, prospective study design was adopted to compare women with a history of two or more first-trimester miscarriages ($n = 42$) with those with no history of recurrent miscarriage ($n = 36$). Participants were recruited from the Department of Obstetrics and Gynecology of Turgut Ozal University Hospital. Platelet-activating factor and thrombin-activatable fibrinolysis inhibitor levels in serum samples were measured by an enzyme-linked immunosorbent assay.
Results: Platelet-activating factor levels were significantly ($p = 0.018$) higher in the recurrent miscarriage group. There was no difference in levels of thrombin-activatable fibrinolysis inhibitor expression between the groups.
Conclusion: Platelet-activating factor is significantly higher in serum of patients with a history of recurrent miscarriage than in those without such a history, with potential implications for placental function and fetal growth, which could be relevant to miscarriage recurrence. Larger studies are indicated to further examine these findings.

Introduction

Loss of pregnancy in the first trimester is relatively common, occurring in 10–20% of clinically recognized pregnancies and in many more that are not yet clinically recognized [1–3]. Recurrent miscarriage (habitual abortion) is defined by the United Kingdom's Royal College of Obstetricians and Gynaecologists as three or more consecutive pregnancy losses [1]. Risk of subsequent miscarriage is estimated to be 30% after two pregnancy losses as opposed to 33% after three losses [2]. This suggests that an evaluation after two pregnancy losses is advisable in women who have not had previous live births, as recommended by the American College of Obstetricians and Gynecologists [4]. The incidence of recurrent miscarriage has been estimated at 0.5–3% among fertile couples of reproductive age [3]. However, while there are various possible causes, in more than 50% of cases no clear cause can be identified [1–3]. Among the accepted etiologies are parental chromosomal abnormalities, untreated hypothyroidism, diabetes, antiphospholipid antibody syndrome, and some congenital uterine abnormalities [2,5]. Other

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suggested etiologies include endocrine disorders, immunological causes, or infections. Both inherited thrombophilias and acquired thrombophilias such as hyperhomocysteinemia, or activated protein C resistance have also been implicated [2,5].

One hypothesis put forward to explain recurrent miscarriage is that it is the result of exaggerated hemostatic responses owing to the existence of a prothrombotic state prior to pregnancy [6]. Normal embryonic implantation involves elements of the coagulation and fibrinolytic pathways. Thus, pregnancy in itself is associated with higher levels of procoagulants, lower levels of anticoagulants, and a reduced fibrinolytic activity with an increased risk of thrombosis [7]. A current theory suggests that defects in hemostatic mechanisms contribute to placental microthrombi and placentation defects in recurrent miscarriage [6–8]. However, the subject of whether an underlying prothrombotic state contributes to recurrent miscarriage is controversial. Some studies dispute this link and the use of prophylactic heparin during pregnancy in women with idiopathic recurrent miscarriage without any known inherited thrombophilia [9,10].

Evidence from other studies, on the other hand, supports the idea of an underlying prothrombotic state, at least in a subgroup of women with recurrent miscarriage. For example, increased tissue factor activity, procoagulant phospholipids, and levels of thrombomodulin, an activator of both protein C and thrombin-activatable fibrinolysis inhibitor (TAFI), has been shown in patients with two or more miscarriages compared with in women with normal pregnancies or nonpregnant women [8]. Other studies have shown increased platelet aggregation in response to arachidonic acid [6], increased levels of procoagulant microparticles derived from platelets and other cell types [7,11], increased thrombin levels or endogenous thrombin potential [12,13], and increased clot strength and stability measured by thromboelastography in women with unexplained recurrent miscarriage [14].

Some of the controversies surrounding this subject are caused by differences in techniques between studies and poor control of some patient studies in terms of inclusion criteria and definition of recurrent miscarriage [10]. Given the implication of platelet reactivity and activation factors in thrombosis and recurrent pregnancy loss, the authors aim to determine the levels of platelet-activating factor (PAF) and of TAFI in women who had suffered two or more unexplained first-trimester miscarriages without any diagnosed thrombotic disorder in a well-designed, case-controlled prospective study. PAF is associated with maintenance of healthy pregnancy [15], while TAFI is a procarboxypeptidase (carboxypeptidase B2) involved in fibrinolysis inhibition and contributes to thrombosis [16].

Materials and methods

This case-controlled, prospective study was carried out with women recruited from the Department of Obstetrics and Gynecology of Turgut Ozal University Hospital in Ankara/Turkey.

This study was approved by Fatih University Ethical Committee and it complied with the Helsinki Declaration. All women provided written informed consent prior to the start of the study.

Participants

A total of 78 nonpregnant women were recruited in this study. Exclusion criteria included smoking and use of hormonal medication such as oral contraceptive pills. The study group consisted of 42 Caucasian patients with a history of two or more pregnancy losses prior to 12 weeks of gestation and normal thrombophilia panel tests. Three participants of the study group had a history of preeclampsia and two had chronic hypertension. The control group consisted of 36 healthy Caucasian women matched for age (Table 1) who had no history of miscarriage or obstetric morbidity. No patient or control had any aspirin, steroid, or anticoagulant intake. Participants were recruited a minimum of 6 months after the last miscarriage event.

Sample preparation

Venous blood samples were collected after overnight fasting using a 21-gauge butterfly needle and placed in no-additive-containing tubes. The serum fraction was obtained by centrifugation (2000*g*, 10 minutes, 4°C) after storing the whole blood at room temperature (approximately 10 minutes). All samples were stored at −80°C prior to assays.

Measurement of PAF and TAFI levels

Enzyme-linked immunosorbent assay kits were used to measure the levels of PAF and TAFI according to the manufacturer's instructions (USCN Life Science Inc., Houston, TX, USA). All samples were analyzed in duplicate. Serum TAFI and PAF levels were presented as ng/mL and pg/mL, respectively.

Statistical analysis

All statistical analyses were performed using the SPSS 16.0 (SPSS Inc., Chicago, IL, USA) statistical package. Distributions were evaluated using one-sample Kolmogorov–Smirnov test. Student *t* and Mann–Whitney *U* tests were used for testing differences between groups. The results were expressed as mean ± standard deviation. Spearman rho correlation test was used to indicate relationships between variables. A probability level of $p < 0.05$ was considered statistically significant. Correlation between body mass index (BMI) and PAF levels was evaluated using Pearson correlation. The results were evaluated within 95% confidence interval, and $p < 0.05$ was accepted as the level of significance. Logistic regression analysis was performed to discriminate between contributions of BMI and PAF for recurrent abortus.

Results

Table 1 shows the biochemical and clinical characteristics of the study participants. The BMI was significantly higher in the recurrent miscarriage group than in controls ($p = 0.019$). The number of gravidity and abortions was also higher in the study group than in the control group ($p < 0.001$ for both), while the parity was higher in the study group ($p < 0.001$).

With respect to the mediators tested, serum PAF levels were significantly higher in the recurrent miscarriage group than in the control group ($p = 0.018$) (Table 1). No difference was detected in serum TAFI levels between groups (Table 1).

Given the significantly higher BMI in the study group compared with that in the control group (Table 1), a correlation analysis was carried out to determine if there was any correlation between BMI and PAF levels (Table 2). The analysis did not indicate any correlation ($p = 0.829$; Table 2). This indicated that BMI and PAF levels were independent of each other. When using BMI as a covariate in a logistic regression analysis, the association with recurrent miscarriage group was attenuated to $p = 0.105$ for PAF level association and $p = 0.998$ for BMI association. This suggests a higher contribution of PAF than BMI to recurrent miscarriage.