Is thrombin-activatable fibrinolysis inhibitor antigen (TAFIag) level significant in recurrent miscarriage?

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Summary

Objective: The aim of this study was to evaluate the plasma thrombin-tat fibrinolysis inhibitor antigen (TAFIag) levels in women with recurrent miscarriage (RM) and age-matched healthy parous women as controls. *Materials and Methods:* A total of 80 patients were enrolled in this study. As a study group (group 1), the authors evaluated 49 RM patients who had two or more consecutive abortions with unknown etiology before 12 weeks of gestation. The remaining 31 patients (group 2) were age-matched healthy parous women with no history of miscarriage and experienced at least one live baby. *Results:* Comparisons of blood TAFIag levels revealed no statistically significant difference between women with recurrent miscarriages and control group. *Conclusions:* The findings of the present study indicated that TAFIag level was not associated with recurrent miscarriages.

Key words: Thrombin-activatable fibrinolysis inhibitor; Recurrent miscarriage; Unknown etiology.

Introduction

Recurrent miscarriage (RM), defined as three consecutive miscarriages, affects approximately one to three percent of couples trying to have a child with the same partner [1]. Recently, RM is defined as two or more consecutive early pregnancy losses by many clinicians [2]. Genetic and uterine abnormalities, thrombophilias, environmental, endocrinologic, and immunologic factors have been proposed to play a role in the etiology of RM, and underlying pathology remains unidentified in approximately 30-50 % of the recurrent miscarriages [3].

A successful pregnancy requires cooperation between coagulation and fibrinolysis during placentation of embryo and trophoblastic invasion. The imbalance between coagulation and fibrinolysis creates a tendency to thrombosis. Thrombosis and hypofibrinolysis are considered to be the causes of RM [4].

Plasmin is important for the fibrinolysis and produced in the liver as an inactive plasminogen form and t-PA and urokinase convert plasminogen to plasmin which is the active form. Plasminogen cannot degrade fibrin without tissue plasminogen activator (t-PA) and urokinase. Also, plasmin stimulates its own formation by producing both t-PA and urokinase. The role of the plasmin is to break down the fibrin clots into soluble fibrin degradation products. Plasmin activity is reduced by thrombin activatable fibrinolysis inhibitor (TAFI) [5].

TAFI is a glycoprotein synthesized by the liver and megakaryocytes. Thrombin, thrombin-thrombomodulin complex, and plasmin cleavage TAFI at Arg 92, and provide activated TAFI (TAFIa). It is a well-known attenuator of the fibrinolytic rate and inhibits fibrinolysis by removing carboxy-terminal residues from partially degraded fibrin, thus decreasing plasminogen binding on the surface of fibrin [6]. In a previous study, TAFI has been shown to be involved in normal pregnancy. Plasma level of TAFI has been found to be unchanged or reduced during pregnancy [7] or on the contrary, increase during normal pregnancy and then return to baseline levels after delivery [8]. Increased TAFI level has been associated with low level of fibrin degradation and thrombotic conditions which is necessary for normal pregnancy [9]. TAFI may be a contributing factor in the development of thrombotic events and it may act as a link between coagulation and fibrinolysis [10]. The importance of TAFI for RM is undefined. Therefore the aim of this study was to assess TAFI levels in women with RM with unknown etiology.

Materials and Methods

This prospective case-control study was performed in the Zeynep Kamil Training and Research Hospital, Department of Obstetrics and Gynecology, between September 2010 and June 2011. The study protocol was conducted according to the revised Declaration of Helsinki and was approved by the local Research and Ethics Committee of the hospital. Written informed consent form was also obtained from all participants.

A total of 80 patients were included in our study. These patients were grouped as 49 patients with unknown etiology who had two or more consecutive miscarriage before the 12 weeks of gestation as group 1 (study group) and the remaining 31 patients with age-matched healthy parous women who had no history of miscarriage and had experienced at least one live baby as group 2 (control group).

There were no known etiological factors for RM in the study group. In other words; gynecological examination, transvaginal ultrasonography, endocrinologic analysis (ovarian hormones, adre-

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Table 1. — *Demographic characteristics and TAFIag levels of the study and control group.*

	Study group (women with RM) n = 49	Control group (healthy women) n = 31	*p*
*Maternal age (years)	29.5±5.6	31.2±6.1	0.208
*BMI (kg/m^2)	23.1±2.4	23.4±2.6	0.681
**Tobacco (%)	18.4	19.4	0.912
*TAFIag level (U/dl)	66.2±25.3	62.6±30.7	0.240

*Student's t-test; **Chi-square test; BMI: body mass index
TAFIag: thrombin-activable fibrinolysis inhibitor antigen

Table 2. — *Demographic characteristics and TAFIag levels of the study population subgroups.*

	Group 1A (2 losses) n = 20	Group 1B (3 losses) n = 17	Group 1C (≥ 4 losses) n = 12	*p*
*Maternal age (years)	29.3±5.7	28.9±5.2	30.7±6.4	0.691
*Pregnancy loss week	7.1±1.7	6.7±1.5	7.3±1.1	0.619
*BMI (kg/m²)	23.2±2.7	23.5±1.6	22.3±2.3	0.428
**Tabacco use (%)	15	17.6	25	0.775
*TAFIag level (U/dl)	68.4±19.9	68.1±28.4	59.7±29.4	0.603

*One way ANOVA test; **Chi-square test; BMI: body mass index
TAFIag: thrombin-activable fibrinolysis inhibitor antigen

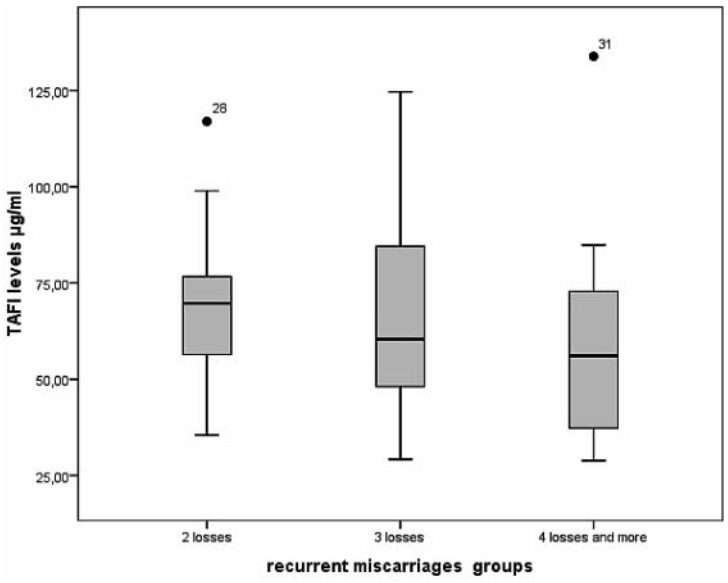

Figure 1. — Box plot showing thrombin activatable fibrinolysis inhibitor antigen (TAFIag) levels determined by an enzyme-linked immunosorbent assay (ELISA) technique and group 1A (two losses), 1B (three losses), 1C (≥ four losses). Each box represents the middle 50% of the data (25% to 75% range). The central horizontal line represents the median. Vertical lines represent the 10% to 90% range of data, as indicated by the small horizontal lines. Statistical differences between the subgroups are indicated.

nal androgens, thyroid function tests), blood glucose level, chromosomal analysis (maternal and paternal), Lupus anticoagulants, thrombophilia pattern (protein C, protein S, activated protein C resistance, antithrombin III, hyperhomocysteinemia), thrombophilic gene mutations (factor V, prothrombin G20210 A, methylene tetrahydrofolate reductase C677T), coagulation parameters (activated partial thromboplastin time, prothrombin time), hysterosalpingography, and hysteroscopy (if performed because of any suspicious finding) were normal.

The exclusion criteria were history of venous thromboembolism, hepatic diseases and renal diseases, known cancer, pregnancy and women with only terminated pregnancy, ectopic pregnancy or recent child birth, taking anticoagulant, antiplatelet medications, and oral contraceptives for at least six months

For TAFI analysis, blood samples were obtained from the antecubital vein into citrated tubes, centrifuged at 1,500 rpm for 15 minutes and stored at –80°C until analysis. Plasma TAFIag level were measured with an Imuclone TAFI enzyme linked immunosorbent assay (ELISA) kit. Frozen plasmas were thawed rapidly at 37°C. Thawed plasmas were stored at 2°–8°C and assayed within four hours.

Datas were given as mean ± standard deviation (sd) or percentage (%) as appropriate. The SPSS for Windows 17.0 software was used for the statistical analyses. Student's t and chi-square tests were performed as appropriate. A $p < 0.05$ was accepted as statistically significance.

Results

A total of 80 women were enrolled in this study as 49 women had recurrent miscarriage with unexplained etiology (group 1), whereas 31 women had at least one uncomplicated pregnancy without any miscarriage (group 2). The demographic characteristics and TAFIag levels of groups are presented in Table 1. There was no statistically significant difference between the study and control group for maternal age, body mass index (BMI), and tobacco use. Comparisons of blood TAFIag levels revealed no statistically significant difference between the groups (group 1; 66.2 ± 25.3, group 2; 62.6 ± 30.7, p = 0.240, Table 1).

Between the subgroups studied, a statistically significant difference in maternal age, BMI, tobacco use, pregnancy loss week were not observed (Table 2). TAFI was lower in group IC when compared with group IA and IB. However, this difference was not statistically significant (group IA; 68.4 ± 19.9 U/dl, group IB; 68.1 ± 28.4 U/dl, group IC; 59.7 ± 29.4 U/dl, p = 0.603, (Table 2, Figure 1).

Discussion

The relationship between thrombosis and recurrent miscarriage has been derived from numerous studies. Several maternal thrombophilic conditions such as protein C, protein S and anti-thrombin III deficiency, hyperhomocysteinemia, and thrombophilic gene mutations have been shown to be involved in RM [11]. Microthrombosis and necrosis were commonly found in placentas of patients with RM [12]. A successful pregnancy requires cooperation between coagulation and fibrinolysis and the fibrinolysis is important in this balance because of shown participation of fibrinolytic system in the regulation of early human trophoblastic invasion [13].

Previous studies have been focused on fibrinolytic defects and RM, but the role of TAFI is unclear in women with RM and there are conflicting results in the literature [14-17]. As a result of increased TAFI, hypofibrinolytic states have been thought to be associated with vascular thrombosis, uteroplacental thrombosis and finally miscarriage [16]. By contrast with this hypothesis, studies suggested that increasing levels of TAFIag during normal pregnancy may protect against early RM and also reported that high TAFIag levels were not associated with increased risk of early RM [5,14,15]. Hypofibrinolysis prevent formation of fibrin degradation product. Therefore fibrin degradation products which have negative effects on trophoblasts as trophoblastic apoptosis which leads to RM are not observed [18].

The studies in which TAFI is investigated contain heterogeneous groups in terms of etiology [5, 14-16]. When the researches about the effect of TAFI to the maternal hemostatic system at the patients having recurrent miscarriages, are designed, it is important to consider the false affects of the other factors at the etiology of the recurrent miscarriages. For example, in patients with antiphospholipid syndrome (APS), reduced fibrinolytic activity has been described which may be responsible for thrombotic events [18]. In same way, in thrombophilia patients, increased thrombin generation may enhance TAFI activation leading to a hypofibrinolytic state, which may further contribute to the thrombotic tendency. For these reasons, with the aim of preventing patient selection bias and obtaining more reliable data, the present authors created their study groups comprising of women with RM with unexplained etiology, so there were no proven thrombotic condition. In the present study, the authors did not demonstrate any difference between control group and RM group with unexplained etiology in terms of TAFIag level. This study was strengthened by the homogenous group of RM with unexplained etiology. None of subjects were with proven thrombotic condition. One of the limitations of this study was relatively small sample size, therefore studies which include homogen group of RM with larger sample sizes are needed.

In conclusion, there was no significant difference in terms of TAFIag levels between the women with recurrent miscarriages with unexplained etiology and control group.

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