Thrombin activatable fibrinolysis inhibitor (TAFI) is not associated with recurrent miscarriage

S.D. Sezer[1], A. Baz[1], M. Küçük[2], A.R. Odabaşı[1], M. Serter[3], H. Yüksel[1]

[1]Department of Obstetrics and Gynecology, Adnan Menderes University, Aydın
[2]Department of Obstetrics and Gynecology, Cine State Hospital, Aydın
[3]Department of Biochemistry, Adnan Menderes University, Aydın (Turkey)

Summary

Aim(s): The present study aimed to discover whether there is an association between thrombin-activatable fibrinolysis inhibitor (TAFI) antigen levels and recurrent miscarriage (RM). In particular, TAFI antigen levels of women with RM were compared with those of a control group of women with previous uncomplicated pregnancies. *Method(s):* Group 1 comprised 48 women with RM, defined as the occurrence of two or more fetal losses before 20 weeks of gestation. Group 2 (the control group) was made up of 40 women who had undergone at least two healthy pregnancies and had no history of miscarriage. Group 1 was then stratified in to two groups according to the number of pregnancy losses and group 1A (2 pregnancy losses) consisted of 22 women whereas group 1B (three or more pregnancy losses) consisted of 26 women. *Results:* No difference was observed with regard to serum TAFI antigen levels between groups 1 and 2. There was also no statistical difference in serum TAFI antigen levels between group 1A and group 1B. *Conclusion:* The findings of the current study indicated that TAFI antigen levels are not associated with RM. Further multi-centric research with more subjects is needed to better evaluate the role of TAFI in RM.

Key words: Thrombin-activatable fibrinolysis inhibitor (TAFI) antigen; Recurrent miscarriage.

Introduction

Thrombin-activatable fibrinolysis inhibitor, also known as TAFI, is a recently discovered glycoprotein. TAFI is also described as procarboxypeptidase U (pro-CPU), plasma procarboxypeptidase B (pro-pCPB), or procarboxypeptidase R (pro- CPR) [1, 2].

TAFI is defined as a new pathway linking coagulation to fibrinolysis [3]. It is a single-chain plasma protein composed of 401 amino acids. Produced mainly by the liver [2], it is also synthesized by megakaryocytes [4]. Cleavage of TAFI at Arg 92 by action of thrombin, the thrombin-thrombomodulin complex and plasmin leads to activated TAFI (TAFIa) [5].

TAFI suppresses fibrinolysis. TAFIa removes C-terminal lysine and arginine residues from partially degraded fibrin and prevents high-affinity plasminogen binding [6]. When there is low level of high-affinity plasminogen binding, tissue-type plasminogen activator-mediated plasmin production remains at low levels leading to low plasma plasmin concentrations. This in turn results in a low level of fibrin degradation [7]. Thus, the degraded fibrin co-factor that is necessary for efficient tissue-type plasminogen activator-mediated plasminogen activation is rendered inactive by TAFI [8].

It has recently been claimed in increasing numbers of studies that TAFI not only has antifibrinolytic effects but also plays a role in modulating inflammation. Recent studies have shown that TAFIa plays a role in the degradation of the anaphylatoxins C3a and C5a and bradykinin [9].

Revised manuscript accepted for publication November 4, 2010

Currently, there is little knowledge available on the possible relationship between TAFI and recurrent miscarriage (RM). TAFI inhibits plasminogen activator-induced fibrinolysis and may cause the development of thrombosis [10]. Increased TAFI levels may be responsible for the development of thrombosis and may lead the way to placental thrombosis, resulting in fetal loss. There are a few clinical studies that have explored the association between TAFI levels and RM however the results are conflicting [11-13].

The present study aimed to discover whether there is an association between TAFI levels and RM. In particular, TAFI levels of women with RM were compared with those of a control group of women with previous uncomplicated pregnancies.

Material and Methods

This descriptive and cross-sectional study was conducted at the Obstetrics and Gynecology Outpatient Clinic of Adnan Menderes University Hospital in Turkey.

Patients who had no history of cardiovascular, renal-thyroid, pancreas disease and had suffered no myocardial infarction or any kind of malignancy or thromboembolic disease were recruited into the study. Patients who had taken aspirin, sex steroids, heparin or antiplatelet agents within the last six weeks were excluded. Those who did not or could not provide their informed consent were also excluded from the study.

TAFI is basically produced by the liver and therefore patients with liver disease were excluded from the sample. Care was taken in obtaining medical history and during the physical examination to rule out any inflammatory, autoimmune or other diseases that could affect hemostasis. All of the study subjects were of the Caucasian race.

The study defined RM as the occurrence of two or more fetal

losses before 20 weeks of gestation [14]. Medical histories were carefully recorded to determine RM diagnosis. The patients' history was verified by checking all available clinical documents and clinical reports including ultrasound examinations or hCG assay results.

The study accepted that fetal loss up until and including the 12th week as early abortion and accepted fetal loss after the 12th week and 20 weeks as late abortion.

Group 1 comprised 48 women with RM, defined as the occurrence of two or more fetal losses before 20 weeks of gestation. Group 2 (the control group) was made up of 40 women who had undergone at least two healthy pregnancies and had no history of miscarriage.

The ages indicated in the study were the ages of the subjects at the time they were recruited into the research.

At a later stage of the study, the patients in group 1 were stratified in two sub-groups according to the number of their pregnancy losses. Group 1A comprised patients who had had two consecutive abortions and group 1B were identified as women who had had three or more consecutive abortions. Group 1A consisted of 22 women and group 1B of 26 women.

At the early follicular phase (cycle day 2-5) in the morning after an overnight fast, venous blood was sampled for the measurement of serum concentrations of TAFI. Serum was separated by centrifugation and immediately stored at −80°C until the assay was carried out. Serum TAFI antigen (TAFIag) concentrations in plasma samples were measured with a commercially available ELISA (enzyme linked immunosorbent assay) kit (American Diagnostica, Stamford, CT). ELISA was performed according to the manufacturer's instructions.

Ethics of research

The study protocol was approved by the local ethics committee. Participation was on a voluntary basis. All women participating in the study provided their written informed consent. Women were not offered any incentives for their participation in the study.

Statistical analysis

Statistical analysis was carried out by using the Statistical Package for Social Sciences (SPSS Inc., Chicago, IL, USA), version 13.0. Significance was defined as $p < 0.05$. Data were presented as mean ± standard deviation (SD) Parametric continuous variables were analyzed with the t-test, and nonparametric data was analyzed by using the Mann-Whitney U test. Differences between categorical variables were analyzed using the chi-square test and Fisher's exact test.

Results

A total of 88 participants were enrolled in the study. Group 1 (women with RM) consisted of 48 women, whereas the control group (group 2) consisted of 40 age-matched women who had experienced at least two uncomplicated pregnancies and had no history of miscarriage. Group 1 was then stratified into two groups according to the number of pregnancy losses and group 1A (2 pregnancy losses) consisted of 22 women whereas group 1B (three or more pregnancy losses) consisted of 26 women.

The main characteristics of the sample and TAFI ag levels are presented in Table 1.

There was no significant difference between the groups for body mass index, and age (Table 1). No difference was observed with regard to TAFIag levels between groups 1 and 2 (group 1: 140.3 ± 34.2 U/dl; group 2: 147.1 ± 34.6 U/dl) (Table 1).

There was also no statistical difference in TAFIag levels between group 1A and group 1B (group 1A: 137.1 ± 31.9 U/dl; group 1B: 143.0 ± 36.5 U/dl) (Table 2).

Table 1. — *Characteristics of the participants*.

	Group 1 (RM*) n = 48	Group 2 (Control) n = 40	*p* value
Age (years)	30.7 ± 5.1	31.9 ± 3.8	0.233
BMI (kg/m²)	21.3 ± 1.5	21.1 ± 1.6	0.601
TAFIag** levels (U/dl)	140.3 ± 34.2	147.1 ± 34.6	0.359

*RM = Recurrent miscarriage; **TAFIag = thrombin-activatable fibrinolysis inhibitor antigen.

Table 2. — *Characteristics of the participants in group 1 stratified according to the number of abortions*.

	Group 1A (2 fetal losses) n = 22	Group 1B (≥ 3 fetal losses) n = 26	*p* value
Age (years)	29.4 ± 6.3	31.8 ± 3.5	0.975
BMI (kg/m²)	21.3 ± 1.6	21.2 ± 1.5	0.709
TAFI ag** levels (U/dl)	137.1 ± 31.9	143.0 ± 36.5	0.093
Pregnancy losses n (%)			
Early abortion	18 (81.8)	21 (82.1)	0.950
Late abortion	4 (18.2)	5 (17.9)	0.975

*RM = Recurrent miscarriage; **TAFIag = thrombin-activatable fibrinolysis inhibitor antigen.

Discussion

No significant difference was observed in plasma TAFI ag levels between the women in the RM group and the control group. Moreover, there was also no difference in TAFI ag levels between women in the sub-groups.

Effective uteroplacental circulation is a prerequisite for the healthy progress of pregnancy. This circulation may be affected by maternal hemostatic abnormalities. In pregnancy, the homeostatic balance between coagulation and fibrinolysis shifts toward hypercoagulation. This is caused by an increase in procoagulants, a decrease in anticoagulants, and a decline in fibrinolysis. The imbalance between coagulation and fibrinolysis may result in a series of early or late pregnancy complications, of which RM is one [14, 15]. An increase in stored fibrin or a reduction in fibrin degradation, leading to placental thrombosis and failure due to a decline in trophoblastic invasion has been suspected of causing these complications [13, 16].

After, the importance of the fibrinolytic system increased during early trophoblastic invasion was shown, research in recent years has concentrated on the fibrinolytic stage in achieving maternal hemostasis [17]. TAFI is a newly identified fibrinolytic inhibitor that plays an important role in maintaining the balance between coagulation and fibrinolysis [18].

TAFI turns into its active form, known as TAFIa and inhibits fibrinolysis by affecting the formation of plasma [3]. A decline in fibrinolysis may be associated with uteroplacental thrombosis leading to placental failure.

To the best of our knowledge there are three articles in the literature that explore the association between pregnancy loss and TAFI. In their retrospective research, Folkeringa *et al*. [11], investigated the association between TAFIag levels and sporadic pregnancy losses. Masini *et al*. conducted prospective research [13] on the association between recurrent pregnancy loss (RPL) and TAFI gene polymorphisms. Knol *et al*. in their retrospective research [12], reanalyzed previously published cases and making use of particularly the data of Folkeringa *et al*. [11] explored the correlation between RPL and plasma TAFIag levels.

Folkeringa *et al*. [11] have asserted that there is no association between plasma TAFIag levels and fetal loss. Other researchers, however, have reported that high TAFIag levels may protect against RPL [12, 13]. The present study too has found no relationship between RM and TAFIag levels.

In the study by Folkeringa *et al*. [11], the data of four previous studies on venous thromboembolism risk [19-22], were re-analyzed. From the perspective of fetal loss rates, there were no differences seen between the "high" TAFIag and the "normal" TAFIag groups.

Knol *et al*. [12] made a further analysis of the data of the four studies reanalyzed by Folkeringa *et al*. [11]. In this study, a lower threshold value was used for high TAFIag levels. This time, 213 out of 843 were stratified as the "high" TAFIag group. In the "high" TAFIag group, total RPL was 3.8%, but the early RPL rate was found to be 2.8%. On the other hand, in the "normal" TAFIag group, these rates were calculated as 7.9% and 7.0%, respectively. When Knol *et al*. [12] decreased the TAFIag level threshold value used in the Folkeringa *et al*. [11] series, they found a significant difference between total RPL and early RPL. Interestingly enough, Knol *et al*. [12] found that in cases with high levels of TAFIag, total RPLs were lower only in early cases of RPL. The researchers stressed that high levels of TAFI could prevent RPL.

In their investigation of TAFI gene polymorphisms, Masini *et al*. [13] analyzed 86 cases with two or more pregnancy losses. Several genetic polymorphisms were found to produce an increase in TAFIag levels and were found to be associated with a reduced risk of RPL thus leading the researchers to believe that the genotypes might be playing a role in preventing the development of RPL. As did the researchers in the present study, Masini *et al*. [13] stratified their study group into two subgroups: group 1A (n = 52) were cases with two pregnancy losses and group 1B (n = 34) with three or more pregnancy losses. No significant difference was seen between the sub-groups, however, in terms of allele frequencies.

The increase in TAFI levels and the drop in fibrinolysis may be expected to be associated with uteroplacental thrombosis resulting in placental failure. It would have been expected that TAFI would cause uteroplacental thrombosis and that rates of fetal loss would be high but the data did not support this expectation. To the contrary, no correlation could be found in the present study between TAFIag levels and RM.

Our finding was supported by the impact of TAFI seen on trophoblasts. Products of fibrin degradation may induce trophoblast apoptosis and lead to fetal loss [23]. High levels of TAFI inhibit fibrinolysis and so fibrin degradation products decrease, thereby possibly preventing trophoblast apoptosis and reducing the risk of RM.

TAFI's impact on the hemostatic system may be effective through different mechanisms in the venous and arterial systems [18]. Besides being a fibrinolytic inhibitor, TAFI is a plasma protein that plays a role in the regulation of the immune response, acting as a mediator in glucocorticoid actions as well as in the modulation of the proinflammatory effects of selected anaphlotoxins such as C3a and C5a [13]. Again, by deactivating bradykinin, TAFI is also indirectly effective in regulating vascular tonus and permeability in the control of vascular response to inflammation [24]. It has also been asserted that TAFI's impact on inflammation may protect against arterial thrombosis [13]. In short, TAFI's effects on inflammation may be more pronounced in pregnancy loss and it is believed that this may protect against pregnancy loss by preventing the development of arterial thrombosis in uteroplacental circulation.

One reason no association could be found between TAFI and RM could be because the different effects of TAFI on RM in the different mechanisms described above were in balance. Furthermore in a recent study neither aspirin combined with nadroparin nor aspirin alone were found to improve the live-birth rate when compared with placebo, among women with unexplained RM [25].

The first of the limitations in this study was about the definition of RM. While RM is defined as loss of three or more pregnancies, more and more clinicians are beginning to accept RM as the loss of two or more pregnancies. Other studies in the literature, too, define RM as two or more pregnancy losses [12, 13]. To overcome this limitation the present study group was divided into two subgroups according to the number of fetal losses.

The distribution of patients accepted into the research was heterogeneous in terms of their etiology. While TAFIag levels affecting the maternal hemostatic system were the subject being investigated, it is also possible that other etiologies were also playing a role in patients' conditions. In this respect, it is believed that if the study group would have been composed of cases of unexplained RM, more reliable data could have been obtained.

The small number of cases in the research was another limitation. To obtain a larger sample, it is necessary to conduct the research at multiple health centers.

When it was shown that TAFI activity had a correlation with plasma TAFIag levels, plasma TAFIag levels became the subject of various studies. TAFIag when confirmed with a hemostatic parameter that is defined as clot lysis time would have been assessed more reliably. That this parameter was not included in the study was another limitation of the present research.

Despite the limitations of the study we still believe that our key finding that TAFIag levels are not associated with RM is of value.

In conclusion the findings of the current study indicate that TAFIag levels are not associated with RM. Further multi-centric research with more subjects is needed to better evaluate the role of TAFI in RM.

References

[1] Eaton D.L., Malloy B.E., Tsai S.P., Henzel W., Drayna D.: "Isolation, molecular cloning, and partial characterization of a novel carboxypeptidase B from human plasma". *J. Biol. Chem.,* 1991, *266,* 21833.

[2] Bajzar L., Manuel R., Nesheim M.E.: "Purification and characterization of TAFI, a thrombin-activable fibrinolysis inhibitor". *J. Biol. Chem.,* 1995, *270,* 14477.

[3] Mosnier L.O., Bouma B.N.: "Regulation of fibrinolysis by thrombin activatable fibrinolysis inhibitor, an unstable carboxypeptidase B that unites the pathways of coagulation and fibrinolysis". *Arterioscler. Thromb. Vasc. Biol.*, 2006, *26,* 2445.

[4] Mosnier L.O., Buijtenhuijs P., Marx P.F., Meijers J.C., Bouma B.N.: "Identification of thrombin activatable fibrinolysis inhibitor (TAFI) in human platelets". *Blood,* 2003, *101,* 4844.

[5] Bajzar L., Morser J., Nesheim M.: "TAFI, or plasma procarboxypeptidase B, couples the coagulation and fibrinolytic cascades through the thrombin-thrombomodulin complex". *J. Biol. Chem.,* 1996, *271,* 16603.

[6] Sakharov D.V., Plow E.F., Rijken D.C.: "On the mechanism of the antifibrinolytic activity of plasma carboxypeptidase B". J. Biol. Chem., 1997, *272*, 14477.

[7] Wang W., Boffa M.B., Bajzar L., Walker J.B., Nesheim M.E.: "A study of the mechanism of inhibition of fibrinolysis by activated thrombin-activable fibrinolysis inhibitor". *J. Biol. Chem.,* 1998, *273*, 27176.

[8] Walker J.B., Nesheim M.E.: "A kinetic analysis of the tissue plasminogen activator and DSPAalpha1 cofactor activities of untreated and TAFIa-treated soluble fibrin degradation products of varying size". *J. Biol. Chem*., 2001, *276,* 3138.

[9] Campbell W.D., Lazoura E., Okada N., Okada H.: "Inactivation of C3a and C5a octapeptides by carboxypeptidase R and carboxypeptidase N". *Microbiol. Immunol*., 2002, *46*, 131.

[10] van Tilburg N.H., Rosendaal F.R., Bertina R.M.: "Thrombin activatable fibrinolysis inhibitor and the risk for deep vein thrombosis". *Blood,* 2000, *95*, 2855.

[11] Folkeringa N., Korteweg F.J., Veeger N.J., Middeldorp S., Hamulyak K., Prins M.H. *et al*.: "Thrombin activatable fibrinolysis inhibitor (TAFI) is not associated with fetal loss, a retrospective study". *Thromb. Res.,* 2009, *123,* 511.

[12] Knol H.M., Veeger N.J., Middeldorp S., Hamulyák K., Van Der Meer J.: "High thrombin-activatable fibrinolysis inhibitor levels may protect against recurrent fetal loss". *J. Thromb. Haemost.,* 2009, *7,* 903.

[13] Masini S., Ticconi C., Gravina P., Tomassini M., Pietropolli A., Forte V. *et al*.: "Thrombin-activatable fibrinolysis inhibitor polymorphisms and recurrent pregnancy loss". *Fertil. Steril.,* 2009, *92,* 694.

[14] Stirrat G.M.: "Recurrent miscarriage". *Lancet,* 1990, *336,* 673.

[15] Patnaik M.M., Haddad T., Morton C.T.: "Pregnancy and thrombophilia". *Expert Rev. Cardiovasc. Ther.,* 2007, *5,* 753.

[16] Blumenfeld Z., Brenner B.: "Thrombophilia-associated pregnancy wastage". *Fertil. Steril.,* 1999, *72,* 765.

[17] Lala P.K., Chakraborty C.: "Factors regulating trophoblast migration and invasiveness: possible derangements contributing to preeclampsia and fetal injury". *Placenta,* 2003, *24,* 575.

[18] Bouma B.N., Mosnier L.O.: "Thrombin activatable fibrinolysis inhibitor (TAFI) at the interface between coagulation and fibrinolysis". *Pathophysiol. Haemost. Thromb.,* 2003, *33,* 375.

[19] Bank I., Libourel E.J., Middeldorp S., Van Pampus E.C., Koopman M.M., Hamulyák K. *et al*.: "Prothrombin 20210A mutation: a mild risk factor for venous thromboembolism but not for arterial thrombotic disease and pregnancy-related complications in a family study". *Arch. Intern. Med.,* 2004, *164,* 1932.

[20] Bank I., Libourel E.J., Middeldorp S., Hamulyák K., van Pampus E.C., Koopman M.M. *et al*.: "Elevated levels of FVIII: C within families are associated with an increased risk for venous and arterial thrombosis". *J. Thromb. Haemost*., 2005, *3*, 79.

[21] Lijfering W.M., Coppens M., van de Poel M.H., Middeldorp S., Hamulyák K., Bank I. *et al*.: "The risk of venous and arterial thrombosis in hyperhomocysteinaemia is low and mainly depends on concomitant thrombophilic defects". *Thromb. Haemost.,* 2007, *98,* 457.

[22] Brouwer J.L., Veeger N.J., Kluin-Nelemans H.C., van der Meer J.: "The pathogenesis of venous thromboembolism: evidence for multiple interrelated causes". *Ann. Intern. Med.,* 2006, *145*, 807.

[23] Isermann B., Sood R., Pawlinski R., Zogg M., Kalloway S., Degen J.L. *et al*.: "The thrombomodulin-protein C system is essential for the maintenance of pregnancy". *Nat. Med.,* 2003, *9*, 331.

[24] Myles T., Nishimura T., Yun T.H., Nagashima M., Morser J., Patterson A.J. *et al*.: "Thrombin activatable fibrinolysis inhibitor, a potential regulator of vascular inflammation". *J. Biol. Chem.,* 2003, *278*, 51059.

[25] Kaandorp S.P., Goddijn M., van der Post J.A., Hutten B.A., Verhoeve H.R., Hamulyák K. *et al*.: "Aspirin plus heparin or aspirin alone in women with recurrent miscarriage". *N. Engl. J. Med.,* 2010, *362*, 1586.

Address reprint requests to:
S.D. SEZER, M.D.
Assistant Professor of Obstetrics and Gynecology
Department of Obstetrics and Gynecology
Adnan Menderes University
Aydın (Turkey)
e-mail: sdemircan@adu.edu.tr