Thrombin-Activatable Fibrinolysis Inhibitor in Breast Cancer Patients

O. Kaftan^a B. Kasapoglu^a M. Koroglu^a A. Kosar^b S.K. Yalcin^a

Departments of ^aInternal Medicine and ^bHematology, Fatih University Medical School, Ankara, Turkey

Key Words

Thrombin-activatable fibrinolysis inhibitor • Breast cancer

Abstract

Objective: To evaluate the levels of thrombin-activatable fibrinolysis inhibitor (TAFI) activity and also its relationship with other homeostasis markers in breast cancer patients.

Subjects and Methods: Forty-two female patients with breast cancer and 24 healthy women (controls) were enrolled in the study and fasting blood samples of all cases were drawn from a large antecubital vein for assay of TAFI and other homeostasis tests. **Results:** The TAFI levels were 79.5 ± 15.5 and 39.3 ± 12.1 in patients and controls, respectively, and the difference was statistically significant ($p < 0.001$). In the patient group, the serum fibrinogen level was 504.9 ± 224.8 , while in the control group it was 393.9 ± 100.5 , and the difference was also statistically significant ($p < 0.001$). **Conclusion:** The data showed that increased levels of TAFI are a contributing factor of thrombotic disorders in breast cancer patients.

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Introduction

In the United States, more than 180,000 women are diagnosed with breast cancer each year, the second most lethal cancer type in women after lung cancer [1]; breast cancer among women is very prominent as well in both the Middle East and Asia [2]. Unfortunately, a large number of these tumors and some treatment modalities are compounded by risks that increase the mortality and morbidity associated with atherosclerotic complications. In patients with malignant disease, induction of coagulation mechanisms causes increased fibrinolytic function, so such patients show a tendency to coagulation and fibrinolytic disorders [3]. Many factors can affect the coagulation system, including thrombocytosis, elevated D-dimer levels or increased tissue factor levels in cancer patients [4]. Thrombin-activatable fibrinolysis inhibitor (TAFI) is a procarboxypeptidase that is synthesized in the liver and activated by thrombin and the thrombin-thrombomodulin complex that suppresses fibrinolysis by removing carboxy-terminal lysine residues from partially degraded fibrin [5]. TAFI is a proenzyme of the enzyme TAFIa which has a carboxypeptidase activity and a positive feedback mediator at the fibrinolytic cascades [6–8].

However, the importance of TAFI in homeostasis, fibrinolysis and the mechanisms regulated by TAFI are not

completely clarified. TAFI has a thrombotic tendency and can also contribute to atherogenesis as it is involved in the regulation of fibrin stability in the intravascular space [8]. Increased TAFI levels have been associated with several thrombotic conditions including venous thromboembolism [9, 10] and ischemic stroke [11–13]. In addition, increased TAFI has been reported to be a marker of small cell lung cancer (SCC) and distinguishes SCC from non-small cell lung cancer including adenocarcinoma [14]. Evaluation of the levels of TAFI in breast cancer patients may reflect the fibrinolytic capacity of the patients and thus identify those who are prone to cardiovascular disease.

Therefore, the aim of our study was to evaluate the levels of TAFI activity and its relationship with other homeostasis markers in breast cancer patients.

Subjects and Methods

Forty-two female subjects with breast cancer and 24 healthy control subjects matched for body mass index were recruited. Informed consent was obtained from each participant. Breast cancer was diagnosed by pathology. The study was approved by the Fatih University School of Medicine Ethics Committee and conducted in accordance with the ethical principles described by the Declaration of Helsinki. Written informed consent was obtained from all participants.

Study Protocol

This prospective study was carried out between 2007 and 2008. Fasting blood samples were drawn from large antecubital veins of the forearm, without interruption of venous flow for TAFI. Other homeostasis tests performed were: prothrombin time (PT); activated partial thromboplastin time (aPTT); fibrinogen; complete blood count, blood urea nitrogen, calcium, γ -glutamyl transferase (GGT), lactate dehydrogenase (LDH), aspartate amino transferase (AST) and alanine amino transferase (ALT).

Assessments

Blood samples for TAFI, PT, aPTT, fibrinogen and D-dimer were centrifuged within 30 min of collection, at 4°C for 20 min at 3,000 rpm. The supernatant plasma samples were separated and transferred into polypropylene tubes and stored up to 1 month at –30°C. Blood urea nitrogen, calcium, GGT, LDH, AST and ALT were determined using an autoanalyzer (Hitachi 912; Roche) with the company's original kits. PT and aPTT were assessed with a coagulometer, and fibrinogen levels were assessed with a nephelometer. Plasma TAFI activity was measured by a chromogenic assay (Imuclone TAFI, American Diagnostica Inc., Stamford, Conn., USA). Complete blood counts were measured with Coulter MaxM (Philadelphia, Pa., USA). Blood pressure was measured using a mercury sphygmomanometer after giving each participant a 5-min rest period. The means of two measurements of systolic and diastolic blood pressures were taken and recorded before withdrawing blood.

Table 1. TAFI, PT, aPTT, D-dimer, fibrinogen and LDH values

Parameter	Patients	Controls	p value
TAFI	79.5 ± 15.5	39.3 ± 12.1	<0.001
PT	11.2 ± 1.2	11.4 ± 1.6	>0.05
aPTT	31.2 ± 5.5	32.4 ± 5.6	>0.05
D-dimer, ng/ml	531 ± 199	141 ± 91	<0.001
Fibrinogen, mg/dl	504 ± 224	293 ± 100	<0.001
LDH, U/l	184.4 ± 30.2	153.1 ± 36.3	>0.05

PT = Prothrombin time; aPTT = activated partial thromboplastin time; LDH = lactate dehydrogenase.

The patients were also investigated for congenital and acquired coagulation disorders including platelet counts, protein C, activated protein C resistance, protein S and homocysteine levels; the only procoagulant risk factor was obesity.

Statistical Analysis

All statistical analyses were performed using the SPSS program, version 11.5 (SPSS Inc., Chicago, Ill., USA). Results are given as means ± standard deviation. Within and between group differences were analyzed by Student's paired and unpaired t tests. Analysis of variance was used to compare multiple-group means. A p value <0.05 was considered statistically significant.

Results

The TAFI levels were 79.5 ± 15.5 and 39.3 ± 12.1, in patient and control groups, respectively (table 1); the difference was statistically significant (p < 0.001). In the breast cancer patient group, the serum fibrinogen levels were 504.9 ± 224.8 and 393.9 ± 100.5 in the control group; this difference was also significant (p < 0.001). The D-dimer values were 531 ± 199 and 141 ± 91 ng/ml in the patient and control groups, respectively (p < 0.001). The hemoglobin levels were 12.5 ± 12.8 and 13.4 ± 0.9 in the patient and control groups, respectively (p > 0.05). In the patient group, serum LDH level was 184.4 ± 30.2, while in the control group it was 153.1 ± 36.3 (p > 0.05).

Discussion

In our study, TAFI and fibrinogen levels were significantly higher in the patient group, which may reflect the thrombotic state. Florian-Kujawski et al. [15] showed increases in both PAI-1 and TAFI levels in cancer patients, which may cause the fibrinolytic deficiency. Similarly, in

a study by Hataji et al. [14], TAFI levels were found to be increased in SCC patients. On the other hand, among patients with acute promyelocytic leukemia, reduced TAFI activity has been observed [16]. Interestingly, Reijerkerk et al. [17] showed that TAFI deficiency did not affect the formation and growth of tumor and tumor metastasis in mice in any tumor model. Overall, these data indicate that TAFI levels may be cancer type specific.

Cancer is a well-described prothrombotic state [18–20] and thromboembolic events are the second leading cause of death in patients with malignancies who have an 11% lifetime risk of thromboembolism [18, 20]. Because of the high prevalence of breast cancer, its complications are of high clinical relevance. Patients with malignancies display a wide range of coagulation disorders from asymptomatic laboratory changes to massive thromboembolism and disseminated intravascular coagulation. Approximately 50% of all cancer patients exhibit abnormalities in coagulation tests and 90% of these patients have metastatic disease [21–24]. Thrombocytosis and increased plasma fibrinogen levels are the most common abnormalities. Also, in patients with malignancies, immobilization, surgical operations, infections, vascular endothelial injury induced by chemotherapeutic agents and abnormalities of the blood coagulation system contribute to thrombophilic and hypercoagulable states. Up to 15% of patients with cancer present with venous thromboembolism during the course of their disease. Approximately 5% of breast cancer patients have thrombotic complications while on chemotherapy [3]. The increased levels of TAFI and fibrinogen found in our study may explain the thrombotic state of breast cancer patients.

Upon activation by thrombin or plasma, TAFI is converted to an enzyme (TAFIa), which acts as an inhibitor of tPA-dependent fibrinolysis. The most physiological activator of TAFI is thrombin, which increases its efficiency 1,000-fold. TAFI can also work alone or as a complex with

thrombomodulin. Interestingly, it is believed that TAFI activation by the thrombin-thrombomodulin complex occurs on endothelial surfaces adjacent to the hemostatic plug and protects from fibrinolysis outside of the fibrin matrix. On the other hand, TAFI activation by thrombin alone takes place within the hemostatic plug and serves to downregulate fibrinolysis induced by tPA incorporated into the fibrin plug [8].

In addition, the D-dimer level of the patient group in our study was statistically significantly higher than that of the control group. This finding is also compatible with our other findings since plasma D-dimer level is a marker of hypercoagulability and the fibrinolytic system. Similarly, Yigit et al. [25] found increased plasma D-dimer and fibrinogen levels in newly diagnosed breast cancer patients compared with controls. Moreover in a recent study, the plasma D-dimer level was found to be statistically significantly higher in 32 patients with breast cancer than in 43 healthy women [26].

One of the limitations of this study is that the patients were not grouped according to disease stage or the presence of metastases. That would be a goal of future works since this study focused on determining TAFI levels in breast cancer patients independent of tumor stage.

Conclusion

We demonstrated that TAFI levels were higher in breast cancer patients than in healthy women. Since TAFI is associated with a thrombotic tendency and may play a role in hypercoagulability states, it may be an important complicating factor in breast malignancies that should be evaluated during patient follow-up. Further studies are necessary to elucidate TAFI levels and their effects on metastatic disease and patient prognosis.

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