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ORIGINAL ARTICLE

Plasma thrombin-activatable fibrinolysis inhibitor (TAFI) antigen levels in diabetic foot ulcers

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Abstract Plasma TAFI may participate in arterial thrombosis in cardiovascular diseases (CVD) and may be involved in the mechanism of vascular endothelial damage in diabetic patients. The aim of this study was to investigate the association of plasma TAFI antigen level in the development of diabetic foot ulcer in Type 2 diabetes. The TAFI antigen levels were determined in 50 patients with diabetic foot ulcers and 34 patients without diabetic foot ulcers and 25 healthy individuals. We measured TAFIa/ai antigen in plasma samples with a commercially available ELISA Kit. Diabetic foot ulcer group and diabetic group were similar in terms of mean age and sex distribution. Diabetes duration, retinopathy, neuropathy, macrovascular disease and infection were related to diabetic foot ulcers. HbA1c, HDL-cholesterol and Folic Acid levels were decreased in the diabetic foot ulcer group. TAFI levels were 99.44 ± 55.94% in control group, 135.21 ± 61.05% in diabetic foot ulcer group, 136.75 ± 59.38% in diabetic group and was statistically different ($P < 0.05$). But no difference was seen in TAFI levels between the diabetic foot ulcer group and diabetic group ($P > 0.05$). No significant difference in plasma TAFI levels were seen between diabetic foot ulcer stages. TAFI antigen levels are increased in Type 2 diabetic patients, but are not related to diabetic foot ulcer development.

Keywords TAFI antigen · Diabetic foot ulcers · Type 2 diabetes

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Introduction

Since diabetes mellitus is growing at epidemic proportions worldwide, the prevalence of diabetes-related complications is bound to increase. Diabetic foot disorder, the major source of disability and morbidity, is a significant burden for the community and a true public health problem [1]. Diabetic foot ulcerations result from different pathophysiological mechanisms; a clear understanding of them is crucial to reduce their incidence, provide early care, and finally delay the amputation risk. The three main diabetes complications involved in foot ulcerations are neuropathy, peripheral artery disease, and infection [2].

Diabetic neuropathy is the most common complication of diabetes, affecting 50% of diabetic patients [3]. The most important factor related to the development of foot ulcer is peripheral neuropathy, associated with loss of pain sensation. Neuropathy can be associated with peripheral vascular disease and foot deformities [4]. There is no study investigating the relationship between TAFI levels and neuropathy.

Peripheral vascular disease is one of the components of the diabetic foot. Diabetic patients should be assessed for lower limb arterial disease. Medical management includes minimization of vascular risk factors, anti-thrombotic therapy, and walking rehabilitation. Vascular testing is required in the presence of a foot wound [5].

Foot infections are common in patients with diabetes and are associated with high morbidity [6]. Infection is always the consequence of a preexisting foot wound whose chronicity is facilitated by the diabetic peripheral neuropathy, whereas peripheral vascular disease is a factor of poor outcome, especially regarding the risk for leg amputation [7].

In recent years, a new plasma protein, that may play an important regulatory role in fibrinolysis, has been identified. It is a carboxypeptidase B-like proenzyme [8, 9] that can be activated by thrombin. Upon activation by thrombin or plasma, it is converted to an active enzyme thrombin-activatable fibrinolysis inhibitor (TAFIa) which acts as an inhibitor of tissue-type plasminogen activator (t-PA) dependent fibrinolysis. It decreases plasmin formation by removing lysine residues from the fibrin surface [10]. Fibrinolytic system abnormalities may play role in the development of micro- and macrovascular complications in diabetes. Plasma activator inhibitor (PAI-1) is considered an independent risk factor for cardiovascular disease in diabetics and found to be increased in diabetic patients [11]. Plasma TAFI may participate in arterial thrombosis in CVD [12] or in venous thrombosis [13]. Increased plasma TAFI levels were shown in obese and type 2 diabetic patients, and in insulin resistance [14, 15]. Plasma TAFI correlated independently with the components of metabolic syndrome components such as visceral fat, glucose infusion rate and insulin resistance and also is increased in diabetic patients [14].

Elucidation of TAFI antigen levels in diabetic foot ulcers may reflect fibrinolytic capacity of those patients who are prone to CVD. Abnormalities in the coagulation and fibrinolytic pathways may also contribute to the development of diabetic foot ulcers in type 2 diabetic patients. However, there is not enough information regarding the status of fibrinolytic system in diabetic foot ulcers, particularly TAFI activity. In this study, we aimed to evaluate the TAFI antigen levels and its relationship with other markers in a group of patients with diabetic foot ulcers in comparison with healthy controls.

Materials and methods

After getting approval for the Ethics Committee, 50 type 2 diabetic patients with diabetic foot ulcers, 34 type 2 diabetic patients without diabetic foot ulcers and a control group of 25 healthy individuals enrolled in the study. Informed consent was obtained from each participant. A detailed medical history of each patient was obtained. The phenotypic characteristics were determined. Age, sex, duration of diabetes, Body mass index (BMI) and blood pressure were recorded.

Blood samples were taken in the morning after overnight fasting. Serum total-cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides, uric acid, creatinine, vitamin-B12, folic acid, thyroid stimulating hormone, glycolizated hemoglobin (4.5–6%), high sensitive C-reactive protein (hs-CRP), erithrocyte sedimentation rate (ESR) and microalbuminuria levels were measured. Biochemical parameters were studied with Roche/Hitachi Moduler autoanalyzer, complete blood count was studied with Roche Symex autoanalyzer.

Foot ulcer is defined as full-thickness skin defect that did not heal within 14 days [16]. Wagner classification was used for diabetic foot ulcers. Infection was defined as at least two of the following; purulent discharge, local heat increment, local erythema, lymphangitis, edema, fever and bad odour [17, 18].

Retinopathy is diagnosed by ophthalmologic evaluation [19].

Microalbuminuria was diagnosed when albumin excretion rate (AER), measured by radioimmunoassay (RIA), was 30–300 mg/24-h in at least two out of three 24-h urine collections over a 3-month period. Creatinine clearance, is calculated by Cockroft-Gault formula [GFR = (140 − age) × weight (kg)/plasma creatinine × 72 (in women × 0.85)] [20].

Macrovascular disease is defined as; presence of ischemic heart disease, stroke, transient ischemic attack or peripheral artery disease [17, 19]. Peripheral artery disease is diagnosed by absence of both feet pulses and/or ankle-brachial pressure index [ABPI]) lower than 0,90 [16–18, 21]. ABPI measurement is made by a hand doppler (Hadeco ES-101EX, 8 MHz).

Peripheral sensory neuropathy is defined as insensitivity to the 5.07 (10 g) Semmes–Weinstein monofilament at any one of 10 sites on either foot [16, 21].

Since TAFI is produced mainly by the liver, patients with known liver disease were excluded from the study [22]. Patients with medications that may affect the blood coagulation or fibrinolytic systems (such as anticoagulants and antiplatelet agents) were also excluded from the study. Venous blood samples were taken from all groups. Vacutainer system and evacuated blood collection tubes containing trisodium citrate (0.109 mol/l, citrate/blood ratio was 1:9) were used. All samples were immediately centrifuged for 20 min (300 rpm). Sufficient volume of the plasma was stored at −30°C to establish concentrations of TAFI antigen. We measured TAFIa/ai antigen in plasma samples with a commercially available ELISA kit (Imuclone TAFI, American Diagnostica Inc. Stamford, CT, USA). Intra and interassay coefficients of variation were 6 and 10%, respectively.

Briefly, samples were added to microwells which were precoated with a TAFIa/ai antigen specific capture agent.

The TAFIa/ai antigen present in the samples was immobilized to the microwell. Following a washing step, anti-human TAFI monoclonal antibody was added to the microwells and bound to the captured TAFIa/ai antigen. After the enzymatic reaction had stopped, the absorbance of the solution was measured at 450 nm with a microplate reader.

Statistical analyses

The significance of the differences in averages between the groups was evaluated by using Student's *t* test and Mann–Whitney U test. The descriptive statistics were shown as standard deviation ± SD for continuous variables. Correlations between parameters were tested by Pearson's correlation analysis. The presence of directional relation between plasma TAFI levels and the levels of hs-CRP is evaluated by correlation test. "Statistical Package for Social Sciences 11.0" program was used for statistical analysis. $P < 0.05$ was considered as statistically significant.

Results

Anthropometric and clinical characteristics of diabetic patients are shown in Table 1; laboratory parameters are shown in Table 2.

After examining the anthropometric and clinical parameters of the groups, sex ($P < 0.05$), diabetes duration ($P < 0.05$), retinopathy ($P < 0.05$), neuropathy ($P < 0.05$) and infection ($P < 0.001$) were statistically different between patient groups and controls (Table 1). Systolic and diastolic blood pressures were significantly different between patients and controls ($P < 0.001$; Table 1). Ankle brachial index is measured but no difference was found between groups.

HbA1c ($P < 0.001$), HDL cholesterol ($P < 0.01$), CRP ($P < 0.001$), ESR ($P < 0.001$), Vitamin B_{12} ($P < 0.05$) and folic acid ($P < 0.001$) levels were different between diabetic patient groups. HDL cholesterol ($P < 0.01$) and TG ($P < 0.05$) levels were different between diabetic patients and controls (Table 2).

Plasma TAFI levels were 99.44 ± 55.94% in the control group, 136.75 ± 59.38% in the diabetic patient group without foot ulcers and 135.21 ± 65.05% in the diabetic foot ulcer group (Fig. 1). TAFI levels were not significant between diabetic patient groups but were significant between diabetic patients and controls ($P < 0.05$). There was no difference in plasma TAFI level according to diabetic foot ulcer stages ($P > 0.05$; Table 3).

Discussion

Diabetic foot ulcers are likely to occur in up to 25% of people with diabetes mellitus at some time in their life [23].

Table 1 Anthropometric and clinical features of diabetic patients and healthy controls

	Controls ($n = 25$)	Diabetic patients without foot ulcers ($n = 34$)	Diabetic patients with foot ulcers ($n = 50$)	*P* value
Numbers (M/F)	25 (10/15)	34 (10/24)	50 (26/24)	<0.05*
Age (years)	55.40 ± 4.50	57.41 ± 9.53	59.82 ± 10.55	>0.05
BMI (kg/m^2)	25.98 ± 5.32	29.82 ± 5.50	28.32 ± 5.81	>0.05
Diabetes duration (years)	–	10.02 ± 5.74	12.92 ± 6.52	<0.05*
Smoking (%)	20	22	14.7	>0.05
Systolic blood pressure (mm/Hg)	110.40 ± 12.06	124.12 ± 13.95	126.40 ± 15.18	<0.001***
Diastolic blood pressure (mm/Hg)	68.00 ± 8.66	75.00 ± 8.61	75.50 ± 7.30	<0.001***
Retinopathy (%)	–	20	41	<0.05*
Nephropathy (%)	–	76.5	86	>0.05
Macrovascular disease (%)	–	41.2	62	>0.05
Neuropathy (%)	–	61.8	96	<0.001***
Infection (%)	–	0	50	<0.001***

Sex, diabetes duration, retinopathy, neuropathy and infection were statistically different between diabetic patient groups. Systolic and diastolic blood pressures were significantly different between patients and controls

n number of individuals

* Statistically significant difference was determined between two groups ($P < 0.05$)

*** Statistically significant difference was determined between two groups ($P < 0.001$)

Table 2 Laboratory findings of diabetic patients and healthy controls

	Controls ($n = 25$)	Patients without diabetic foot ulcers ($n = 34$)	Patients with diabetic foot ulcers ($n = 50$)	P value
HbA1c (%)	–	12.1 ± 2,81	9.76 ± 0.31	<0.001***
Total cholesterol (mg/dl)	174.92 ± 7.37	182.24 ± 39.34	170.14 ± 51.08	>0.05
LDL-cholesterol (mg/dl)	101.00 ± 21.01	117.35 ± 34.77	106.32 ± 41.65	>0.05
HDL-cholesterol (mg/dl)	45.56 ± 2.54	40.97 ± 12.01	36.12 ± 13.90	<0.01**
Triglyceride (mg/dl)	127.96 ± 8.41	171.00 ± 89.11	165.72 ± 92.74	<0.05*
Creatinine (mg/dl)	0.71 ± 0.09	0.88 ± 0.26	0.98 ± 0.42	>0.05
TSH (mIU/ml)	1.13 ± 0.56	1.37 ± 1.01	1.28 ± 0.96	>0.05
CRP (mg/dl)	0.25 ± 0.18	1.14 ± 0.98	5.37 ± 3.22	<0.001***
ESR (mm/h)	12 ± 5.24	36.71 ± 25.55	69.52 ± 30.99	<0.001***
Vitamin B_{12} (pg/ml)	512.36 ± 156.32	482.29 ± 298.27	685.92 ± 468.02	<0.05*
Folic acid (ng/ml)	13.02 ± 2.31	12.41 ± 4.28	7.94 ± 5.35	<0.001***
TAFI (%)	99.44 ± 55.94	136.75 ± 59.38	135.21 ± 65.05	<0.05*

* Statistically significant difference was determined between two groups ($P < 0.05$)

** Statistically significant difference was determined between two groups ($P < 0.01$)

*** Statistically significant difference was determined between two groups ($P < 0.001$)

HbA1c, HDL-cholesterol, CRP, ESR, Vitamin B12 and folic acid levels were statistically different between diabetic patient groups. HDL-cholesterol, TG and TAFI levels were statistically different between controls and diabetic patients

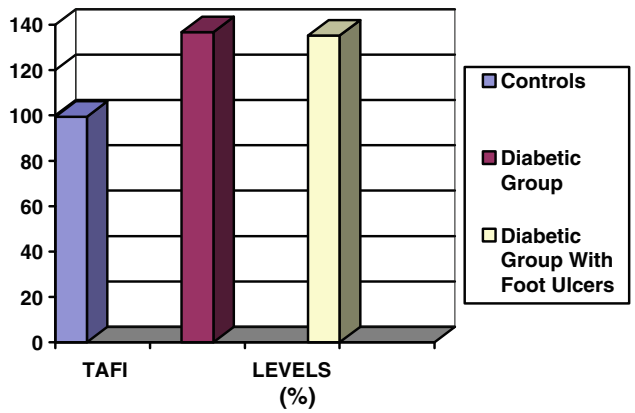

Fig. 1 TAFI levels in controls and patient groups

Table 3 Plasma TAFI levels according to diabetic foot ulcer grades

Grade	N	Plasma TAFI levels (%)
Grade 1	13	166.65 ± 58.69
Grade 2	14	138.98 ± 75.24
Grade 3	9	116.37 ± 47.26
Grade 4 + 5	14	114.36 ± 45.99

There was no difference in plasma TAFI levels between diabetic foot ulcer grades ($P > 0.05$)

Disease of the foot is one of the most fearful complications of diabetes. The ultimate endpoint of diabetic foot disease is amputation, which is associated with significant morbidity and mortality, besides having immense social, psychological and financial consequences [24, 25]. Diabetic foot ulcers constitute a major health problem and they are recalcitrant to healing due to a constellation of intrinsic and extrinsic factors [26].

Diabetic foot is a disease of the elderly men. Mean age was 59 ± 10 years in the diabetic patients with foot ulcers and male gender was 52% in our study which is consistent with other studies.

The predisposing factors include abnormal plantar pressure points, foot deformities, and minor trauma. Usually vulnerable feet already have vascular insufficiency and peripheral neuropathy [27, 28]. Clinically, 45–60% of ulcerations is pure neuropathic, 10% is pure ischemic and 25–45% is neuroischemic [29]. We determined the neuropathy frequency as 96% in diabetic foot ulcer patients which is consistent with other studies.

Retinopathy was seen (40%) higher in the diabetic foot ulcer group as expected.

The other major risk factor is peripheral artery disease and accompanies 60% of nonhealing diabetic foot ulcers [30]. Ischaemia due to peripheral arterial disease significantly contributes to its pathogenesis and natural history [31]. Peripheral artery disease incidence was not different between diabetic groups in our study.

Infection is one of the major risk factors for diabetic foot ulcer development. In the literature, infection is seen in 35–50% of diabetic foot ulcers [18]. We found infection in

50% of patients with foot ulcers and also high CRP and ESR levels.

HDL-cholesterol levels were significantly lower in the diabetic foot ulcer group. Lower HbA1c levels were due to the intensive therapy in this group.

Relation between cardiovascular disease and plasma TAFI level is well known. TAFI levels were found to be correlated with increased ischemic stroke [32, 33]. TAFI levels in plasma are reported to increase the risk of acute coronary artery disease (CAD) almost fourfold [34] and are reported to increase in patients going under coronary artery bypasses grafting (CABG) [35].

Patients with diabetic foot ulcers who are prone to CVD have elevated levels of TAFI antigen levels which may reflect fibrinolytic capacity. Abnormalities in the fibrinolytic and coagulation pathways may play role in the development of diabetic foot ulcers. It has been postulated that TAFI-thrombin activatable fibrinolysis inhibitor, which couples two opposite systems: coagulation and fibrinolysis, may be involved in the mechanism of vascular endothelial damage in diabetic patients [36]. In diabetes neuropathy, nephropathy, retinopathy and disturbed nutritive tissue perfusion result from reduced capillary microcirculation. Functional thromboresistance of the endothelial layer is reduced in the diabetic state. Increased intravascular thrombin generation, reduced fibrinolytic potential and hyperactive platelets lead to a prethrombotic state. This thrombotic diathesis raises the permanent danger of acute flow disruption. The relation between TAFI levels and diabetes complications is demonstrated [37]. Yano et al. showed that plasma level of TAFI in diabetic patients was significantly elevated, compared with normal subjects (147.4 ± 11.6 vs. $99.5 \pm 4.9\%$; $P < 0.05$). The plasma level of TAFI in diabetic patients with microalbuminuria was significantly higher than the level in diabetic patients with normoalbuminuria. Univariate analysis showed that the plasma TAFI levels are significantly and proportionally correlated with urinary albumin excretion rate ($r = 0.58$; $P < 0.005$) and with plasma soluble thrombomodulin level, a marker of endothelial cell damage, in all diabetic patients ($r = 0.42$; $P < 0.01$). These data suggest that increased plasma level of TAFI may be involved in the mechanism of vascular endothelial damage in patients with type 2 diabetes mellitus [38].

In our study, plasma TAFI level in diabetic patients was significantly elevated according to healthy controls as expected but this difference was not seen between diabetic foot ulcer group and the diabetic group. Although TAFI level was not statistically different between Wagner stages in diabetic foot ulcer group, the more severe is the ulcer the lower is TAFI. Higher Wagner stages may be related to more severe vasculopathy and inflammation so it can be hypothesized that increased consumption of TAFI may be seen in higher Wagner stages of foot ulcers.

As a result relation between TAFI level and diabetes was shown in this study, but was unable to show additional effect in the ulcer development. Further studies with larger number of patients are needed to show this relation.

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