

Tissue Factor, Tissue Factor Pathway Inhibitor and Factor VII Activity in Cardiovascular Complicated Type 2 Diabetes Mellitus

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Abstract:

Objectives: Tissue factor (TF) is the main initiator of the extrinsic coagulation pathway through factor VII (FVII) activation, which is physiologically inhibited by tissue factor pathway inhibitor (TFPI). Alteration of this pathway has been described in Type 2 diabetes mellitus (T2DM). The aim of this study is to assess TF and TFPI plasma levels and FVII coagulant activity (FVIIa) in T2DM in relation to cardiothrombotic disease and their correlation to metabolic and clinical behavior of the patients.

Methods: The study was conducted on 80 T2DM patients divided to accordingly; group I: 40 patients without a history or clinically detected heart disease, and group II: 40 patients with a history of myocardial infarction compared to 30 controls. The patients were recruited from Ain Shams University diabetes clinic from September 2007 to February 2009 after informed consent was obtained. Peripheral blood samples were taken for measurement of plasma TF and TFPI levels using ELISA technique and quantitative FVIIa using FVII deficient plasma.

Results: Plasma levels of TF, TFPI and FVIIa were significantly higher in T2DM patients compared to the controls ($p < 0.001$). TF (236.50 ± 79.23) and TFPI (242.33 ± 85.84) were significantly

higher in group II, compared to group I (150.33 ± 81.16), (152.8 ± 82.46), ($p < 0.001$). TF and TFPI were significantly correlated to body mass index and glycemic control. Also, TF and TFPI were significantly higher in hypertensives ($p = 0.001$) and dyslipidemics ($p = 0.006$) but not in smokers ($p = 0.64$), ($p = 0.11$) respectively.

Conclusion: There was a correlation between high TF, TFPI plasma levels, FVIIa activity and cardiothrombotic complications in T2DM especially in the presence of high risk factors such as poor glycemic control, dyslipidemia and obesity. Future target therapy against TF may be beneficial for T2DM patients.

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Introduction

Type 2 diabetes mellitus (T2DM) is associated with accelerated atherosclerosis, endothelial damage and a high tendency to thrombotic complications including peripheral vascular disease, cardiovascular events and stroke.^{1,2,3,4} The T2DM procoagulant state can be contributed to abnormalities of several plasma proteins involved in blood coagulation and platelet activation.⁵ Thrombotic myocardial infarction may be secondary to complicated or ruptured atherosclerotic plaques with further exposure of procoagulant proteins that initiate blood coagulation or due to contact between blood and damaged endothelium.^{6,7}

Tissue factor (TF) is a key enzyme in extrinsic coagulation pathway, present in the adventitia of normal blood vessels, atherosclerotic plaques, in addition to a circulating pool in the blood.⁸ The activation of extrinsic coagulation pathway is mediated via the binding of FVII to TF to form a TF-FVIIa complex, further activation of factor X, IX, XI, the formation of prothrombinase complex and thrombin generation.⁹

On the other hand, TFPI is a 40-kDa endogenous protein synthesized by the vascular endothelium and is present in plasma,

platelets and bound to endothelial heparan sulphates.¹⁰ TFPI inhibits TF-initiated coagulation by binding with activated factor X (FXa), then TFPI-FXa complex binds to the TF-FVIIa complex and modifies their activity.¹¹ Altered TF/TFPI ratio have been related to the development of atherosclerosis, acute coronary syndrome, disseminated intravascular coagulation, sepsis, or thrombotic complications of malignancies.^{12,13}

Although T2DM is associated with increased systemic blood coagulation activity, evidence that activated coagulation or inhibited fibrinolysis has clinical implications in patients with T2DM remains circumstantial.^{14,15}

In chronic hyperglycemia, the binding of advanced glycosylated end products to their specific receptors induces an intravascular oxidative stress response, leading to TF expression in vitro.¹⁶ TF was found to be an independent factor related to microvascular diabetic complications (microalbuminuria, retinopathy) and neuropathy, and it reflects endothelial dysfunction, rather than procoagulant activity.¹⁷

Also, increased TFPI activity was demonstrated in patients with diabetes particularly in patients with microalbuminuria.¹⁸ Increases in TFPI reflected endothelial dysfunction or altered binding of TFPI to the endothelium by glucosaminoglycans

because TFPI is mainly produced by vascular endothelium.¹⁹ Plasma FVII levels depend on serum lipids and obesity index, so it is mostly elevated among T2DM patients who are often obese, and frequently present with dyslipidemia.²⁰

The aim of the present work is to assess TF and TFPI plasma levels together with FVII coagulant activity (FVIIa) in T2DM with and without cardiovascular complications and correlate the results with metabolic and clinical behavior of the patients.

Methods

The study included 80 patients with T2DM and 30 healthy controls of matched age and sex who were recruited from the Diabetes Clinic of Internal Medicine of Ain Shams university hospital from September 2007 to February 2009. The patients were subdivided into two subsets. Group I: 40 patients without a history, clinical or electrocardiography (ECG) findings suggesting the presence of heart disease, and group II; 40 patients with a history of myocardial infarction as documented by their ECG, Echocardiography or coronary catheter. The exclusion criteria included DM of less than 10 years, history of myocardial infarction within the last three months, congestive heart failure, surgery or trauma within the previous three months, known malignant, renal or hepatic diseases, heparin or oral contraceptive pills intake. All studied subjects gave their informed consent and the study was approved by the Ain Shams University Hospital Ethical Committee.

A full detailed history regarding diabetes history, vascular complications, hypertension, smoking and history of drug intake including heparin, oral anticoagulants and antiplatelet drugs was obtained. Careful clinical and cardiological examinations including ECG and echocardiography, and revising previously done stress ECG and coronary angiography were performed. Body mass index was calculated as BMI=body weight in Kg divided by (height in meter).²

Laboratory investigations were conducted to determine prothrombin time (PT) and activated partial thromboplastin time using (SYSMEX CA-1500, Dade Behring, Germany), fasting and postprandial serum glucose level (using glucose oxidase method), serum cholesterol, triglycerides (TG), low density lipoprotein (LDL) and high density lipoprotein (HDL) levels using Synchron CX-7 (Beckman, Switzerland), glycated hemoglobin (HBA1C) using Stanbio Glycohemoglobin (STANBIO, Italy), measurement of plasma TF and TFPI using enzyme linked immunosorbant assay (ELISA) technique, and measurement of FVII coagulation activity (FVIIa) in the plasma using FVII deficient plasma. 2 ml of venous blood were collected from patients and controls and was anticoagulated with buffered sodium citrate (3.2%) in the proportion of 9:1. Plasma were separated and kept at -70 until the time of analysis.

The plasma level of TF was determined by means of the AssayMax Human TF ELISA Kit (GENTAUR, Belgium, catalogue number: ET1002-1). A quantitative sandwich enzyme immunoassay technique was employed. A polyclonal antibody specific for TF was pre-coated onto well microplate. The TF in standards and samples was sandwiched by the immobilized antibody and the biotinylated polyclonal antibody specific for TF, which is recognized by a streptavidin-peroxidase conjugate. All unbound material was then washed away and a peroxidase enzyme substrate was added. The color development was stopped and the intensity of the color was measured at wavelength 450 nm and it was related to the standard curve. The level of TF was expressed in ng/ml.

Plasma level of TFPI was measured by means of AssayMax Human TFPI ELISA kit (GENTAUR, Belgium, catalogue number: ET1005-1). This assay employs a quantitative sandwich enzyme immunoassay technique. A murine antibody specific for TFPI was pre-coated onto well microplate. TFPI from the standards and samples were sandwiched by the immobilized antibody and a polyclonal antibody specific for TFPI, which is recognized by a peroxidase conjugate. All unbound material was then washed away and a peroxidase conjugate substrate was added. The color development was stopped and the intensity of the color was measured at wave length 450 nm and was related to a standard curve. The level of TFPI was expressed in pg/dl.

Quantitative FVII coagulant activity was determined based on the prothrombin time assay using coagulation FVII-deficient plasma (Dade Behring, Germany). Frozen citrated plasma was diluted 1:20 and the assay procedure was performed according to the manufacturer instructions. A standard curve was prepared using 20 normal plasma pool mixed with the deficient plasma. The test was performed on SYSMEX CA-1500 (Dade Behring, Germany) Instrument and the results were related to the standard curve. FVIIa is expressed as a percentage (%).

The data was analyzed using SPSS statistical package version 12. Descriptive statistics including mean and SD were analyzed using the Chi-square test (X^2). The Fisher exact test was performed in tables containing values less than 5 and the Pearson's correlation test (t-test) performed to compare independent samples and correlation study. Probability or *p* value of <0.05 was considered statistically significant, while *p* value ≤ 0.001 was considered statistically highly significant.

Results

The study was conducted on 80 T2DM patients; 42 males and 38 females with a 1.1:1 male to female ratio, with mean age (years) of 49.5±8.6 against 30 controls, 16 males and 14 females with a 1.14:1

male to female ratio with mean age (years) of 47.9 ± 6.1 and they were matched in terms of age and sex. The clinical and metabolic parameters of the patients and controls are demonstrated in Table 1.

Out of the 80 patients, there were 33 (41.25%) hypertensive, 18 (22.5%) smokers, and 45 (56.25%) dyslipidemics (cholesterol >200 mg/dl, triglycerides >150 mg/dl).

A comparison between the patients and controls regarding TF, TFPI and FVIIa is demonstrated in Table 2.

TF and TFPI plasma levels as well as FVIIa were statistically significantly higher in the patient group (193.41 ± 90.61 ng/ml, 197.56 ± 94.88 pg/ml, 108.25 ± 26.72 %) respectively compared to the controls (72.89 ± 31.283 ng/ml, 40.11 ± 13.16 pg/ml, 75.79 ± 11.2 %) respectively ($p < 0.001$). A comparison between group I and II in terms of plasma levels of TF, TFPI, and FVIIa is highlighted in Table 3.

Table 1: Clinical and metabolic parameters of patients and control.

Parameter	Number (%)	
	Controls	Type 2DM Patients
Men	16 (53.3%)	42 (52.5%)
Smokers	-	18 (22.5%)
Hypertensive	-	33 (41.25%)
Dyslipidemics	-	45 (56.25%)
Mean \pm SD		
Age (years)	47.9 ± 6.1	49.5 ± 8.6
BMI (Kg/M ²)	23.68 ± 1.76	32.78 ± 3.91
FBS (mg/dl)	82.68 ± 8.41	197.40 ± 62.00
2pp (mg/dl)	118.05 ± 8.79	284.90 ± 87.49
HBA1C	5.15 ± 0.64	9.85 ± 1.98
Cholesterol (mg/dl)	151.52 ± 9.25	218.88 ± 53.41
Triglycerides (mg/dl)	116.57 ± 14.75	165.33 ± 49.52
HDL	45.73 ± 4.91	41.61 ± 5.72
LDL	91.10 ± 19.06	130.10 ± 41.57

Both groups I and II were matched in terms of gender ($p=0.43$) and age ($p=0.18$). TF and TFPI plasma levels were significantly higher in cardiovascular complicated patients (236.50 ± 79.23 ng/ml, 242.33 ± 85.84 pg/ml) compared to non complicated patients (150.33 ± 81.16 ng/ml, 152.8 ± 82.46 pg/ml), ($p < 0.001$). However FVIIa tended to be higher among complicated cases but there was no significant statistical difference ($p=0.65$).

A correlation between plasma levels of TF, TFPI and FVIIa among studied subjects is demonstrated in Table 4.

Table 2: Comparison between patients and controls as regards the TF, TFPI, and FVIIa.

	Cases (mean \pm SD) n=80	Control (mean \pm SD) n=30	t	p
TF (ng/ml)	193.41 ± 90.61	72.89 ± 31.28	5.670	**<0.001
TFPI (pg/ml)	197.56 ± 94.88	40.11 ± 13.16	7.181	**<0.001
FVIIa (%)	108.25 ± 26.72	75.79 ± 11.21	5.135	**<0.001

$p > 0.05$: non significant; * $p \leq 0.05$: significant; ** $p \leq 0.001$: highly significant.

TF plasma level was significantly correlated to TFPI plasma level and FVIIa. Moreover, a significant correlation between TFPI plasma level and FVIIa was found among all the studied subjects ($p < 0.001$). Correlations between plasma levels of TF, TFPI, FVIIa and different studied parameters among T2DM are highlighted in Table 5.

Table 3: Comparison between complicated and non complicated patients regarding demographic data and serum TF, TFPI & FVII levels.

Parameters	Complicated DM	Non Complicated DM	p value
Males No (%)	28(70%) $\chi^2=1.071$	24(60%)	0.43
Age (years) (mean \pm SD)	51 ± 8.6 $t=1.38$	49 ± 8.5	0.18
TF (ng/ml) (mean \pm SD)	236.50 ± 79.23 $t=4.16$	150.33 ± 81.16	**<0.001
TFPI (pg/ml) (mean \pm SD)	242.33 ± 85.84 $t=4.12$	152.8 ± 82.46	**<0.001
FVIIa (%) (mean \pm SD)	109.83 ± 25.55 $t=0.46$	106.67 ± 28.2	0.65

$p > 0.05$: non significant; * $p \leq 0.05$: significant; ** $p \leq 0.001$: highly significant.

There were significant positive correlations between TF plasma level and BMI ($p=0.04$), FBS ($p=0.01$), HBA1C ($p < 0.001$) and LDL ($p < 0.001$). On the other hand, TFPI plasma level showed significant correlation to FBS ($p=0.007$), 2hPP ($p=0.04$), HBA1C ($p=0.008$), LDL ($p=0.003$) and HDL ($p=0.02$).

Table 4: Correlations between TF, TFPI, and Factor VIIa among type 2 diabetic patients.

Correlation parameters	r	p
TF and TFPI	0.611	**<0.001
TF and FVIIa	0.828	**<0.001
TFPI and VIIa	0.457	**<0.001

**P<0.001:highly significant

FVIIa was statistically significantly correlated to BMI ($p=0.006$), FBS ($p=0.003$), 2hPP ($p=0.04$), and HBA1C ($p<0.001$). The impact of Smoking, hypertension and dyslipidemia on plasma levels of TF, TFPI and FVIIa is shown in Table 6.

Table 5: Correlations between TF and TFPI plasma levels, FVIIa and studied parameters among type 2 diabetic patients

Parameters		TF	TFPI	FVIIa
BMI	r	0.258	-0.06	0.34
	p	0.04*	0.65	0.006**
FBS	r	0.32	0.34	0.37
	p	0.01*	0.007**	0.003**
2hPP	r	0.23	0.26	0.27
	p	0.08	0.04*	0.04*
HBA1C	r	0.54	0.34	0.47
	p	<0.001**	0.008**	<0.001**
HDL	r	-0.16	-0.31	-0.11
	p	0.23	0.02*	0.39
LDL	r	0.48	0.37	0.23
	p	<0.001**	0.003**	0.08

$p>0.05$: non significant,* $p\leq 0.05$:significant,** $p\leq 0.001$:highly significant.

T2DM patients with dyslipidemia had significantly higher TF (225.43 ± 92.11 ng/dl) compared to non dyslipidemics (148.6 ± 67.66 ng/dl), ($p=0.001$). Also, TFPI was higher in patients with dyslipidemia (225.71 ± 79.49 pg/dl) compared to non-dyslipidemics (158.16 ± 102.02 pg/dl), but the difference was not statistically significant ($p=0.006$).

Although FVIIa was higher among dyslipidemic compared to non dyslipidemics, the difference was not statistically significant ($p=0.184$). Moreover, the diabetic hypertensive patients exhibited significantly higher plasma level of TF ($p<0.001$) and TFPI ($p=0.006$) as well as FVIIa ($p=0.02$) compared to non hypertensives. However, smoking did not significantly affect TF ($p=0.64$), TFPI ($p=0.11$) plasma levels or FVIIa ($p=0.51$).

Table 6: Impact of smoking, hypertension and dyslipidemia on TF, TFPI plasma levels and FVIIa.

Parameter (Mean \pm SD)	TF (ng/ml)	TFPI (pg/ml)	FVIIa (%)
Smoking: no			
Yes (18)	179 \pm 107.51	246.87 \pm 133.86	102.5 \pm 30.58
No (62)	195.57 \pm 88.74	189.98 \pm 86.68	109.13 \pm 26.3
	t=0.468	t=1.600	t=0.650
	P=0.64	P=0.11	P=0.51
Hypertension: no			
Yes (33)	262.82 \pm 69.49	239.56 \pm 59.86	118.04 \pm 24.24
No (47)	150.27 \pm 74.25	171.45 \pm 103.61	102.16 \pm 26.68
	t=5.848	t=2.864	t=2.320
	P<0.001	P=0.006	P=0.02
Dyslipidemia: no			
Yes (45)	225.43 \pm 92.11	225.71 \pm 79.49	112.14 \pm 28.06
No (35)	148.6 \pm 67.66	158.16 \pm 102.02	102.80 \pm 24.24
	t=3.540	t=2.882	t=1.344
	P=0.001	P=0.006	P=0.184

Discussion

Cardiovascular complications in T2DM patients related to several pathogenic mechanisms including the hypercoagulable state especially among poorly controlled DM patients.

The aim of the present work was to assess plasma levels TF and TFPI together with FVIIa in T2DM with and without cardiovascular complications and correlated the findings with metabolic and clinical behavior of the patients.

The circulating TF is considered a marker for vascular injury and a source of procoagulant activity, when expressed on the surface of circulating microparticles.^{21,22,23,9}

This study has demonstrated that not only plasma levels of TF but also TFPI and FVIIa were also higher in T2DM patients compared to the healthy controls. This is in agreement with previous studies.^{10,24-28} The heightened TF and TFPI levels probably reflect the increase of TF and TFPI activity in diabetic patients.

TFPI is mostly bound to vascular endothelium and only 20-30% is free.²⁹ However, its level shows diurnal variation which is parallel to FVII activity to maintain homeostatic coagulation mechanisms in healthy subjects.³⁰ Higher morning levels of TFPI demonstrated in cardiac patients following coronary spasm that can be a counter balance mechanism to prevent coagulation.³¹ This work demonstrated a significant positive correlation between TFPI plasma level and FVIIa among all the studied subjects.

Moreover, a significant positive relationship between TF and TFPI plasma level was noted in the studied subjects. This is in line with Falciani et al. who observed a positive correlation between TF

and TFPI levels in ischemic heart disease patients.³² While Gosk-Bierska et al. confirmed this correlation among atherosclerotic peripheral vascular disease.³³ Thus, increased TFPI levels in this condition may be related to activation of TF-dependent pathway in attempt to compensate for the hypercoagulable state but was not sufficient to overcome elevated TF.³²

In this study, cardiovascular complicated T2DM patients had significantly higher TF and TFPI plasma levels compared to uncomplicated patients. This finding was confirmed by Boden et al. and Krupinski et al.^{34,35} Moreover Sommeijer et al. noticed higher soluble TF levels in T2DM with microvascular complications.¹⁷ Activated FVII was significantly higher among complicated T2DM patients.^{10,24,28} However in this study, FVIIa tended to be elevated in cardiovascular complicated patients but the difference did not reach statistical significance.

Obesity is known to be a risk factor for heart diseases and is an obstacle in proper glycemic control in diabetics.³⁶ Obesity enhances thrombotic tendency through up regulation of TF, altered expression of proteins participating in the coagulation cascade as well as atherosclerosis.³⁷ Thus confirming a preliminary report.¹⁰ In this study, increased BMI was found to be significantly correlated with raised TF plasma level and FVIIa. Moreover, Kopp et al. found significantly high TF and VII but lower TFPI among morbidly obese diabetics.³⁸

The importance of hyperglycemia in the pathogenesis of diabetic complications and the role of proper glycemic control in reducing mortality and morbidity in diabetic patients have emerged over the past years. Derosa et al. and Turu et al. detected that hyperglycemia accelerates atherogenesis and potentially increases the risk of thrombotic complications.^{39,40} Raised blood glucose with hyperinsulinemia induce a marked rise in TF activity, TF expression, FVIIa T2DM.⁵ On the other hand, proper glycemic control reduces TF activity and FVIIa with further reduction in the procoagulant state.^{34,41} In this study, as previously recorded, high plasma levels of TF, TFPI and FVIIa were significantly correlated with fasting blood glucose and HBA1C.^{25,28} T2DM patients frequently present with dyslipidemia.

Dyslipidemia altered TF and TFPI expression in atheromatous plaque to favoring thrombosis.⁴² Previously, it has been shown that leukocyte and endothelial cell cultures incubated with triglyceride (TG)-rich chylomicrons and very low-density lipoproteins activates FVII, and TF expression.^{43,44} In this study, diabetic dyslipidemic patients had significantly higher TF and TFPI plasma level compared to non dyslipidemic patients, which echoes previous results.^{8,28}

In addition, the hypertensive diabetic patients in this study had higher TF and TFPI levels than non hypertensives. Similar

results were reported by Sommeijer et al. in 2006.¹⁷ Therefore, proper control of blood pressure may reduce TF levels, resulting in further reduction in cardiovascular complications.

The effect of smoking was contradictory. Contrary to Hölschermann et al.⁴⁵ and Barua et al.⁴⁶ smoking was not significantly correlated to TF, FVII nor TFPI levels among the studied patients, which is also in line with a previous study by Sobol et al.⁴⁷

Conclusion

Plasma levels of TF, TFPI and FVIIa were significantly higher in T2DM. In addition, TF and TFPI were significantly higher in cardiovascular complicated patients. The positive correlation of the procoagulant markers (TF/FVIIa) and blood glucose, HbA1c, lipid profile and BMI reinforces the importance of their optimal control in T2DM patients. Furthermore, pharmacological control of plasma TF activity or augmenting TFPI activity may reduce thrombotic cardiovascular complications. Further studies with larger number of patients are needed to confirm these findings.

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