

Original Article:

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 in this pathway has been described in Type 2 diabetes mellitus (T2DM). The aim of the work was 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:** Study was conducted on 60 T2DM patients divided into group I : 30 patients without history or clinically detected heart disease and group II : 30 patients with a history of myocardial infarction compared to 20 controls. Patients were recruited from Ain Shams University diabetes clinic from September 2007 to May 2009 after their informed consent. 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 compared to controls ($p < 0.001$). TF and TFPI were significantly higher in group II (236.50 ± 79.23), (242.33 ± 85.84) 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. They were significantly higher in hypertensives and dyslipidemics ($P = 0.001$), ($P = 0.006$) but not in smokers ($p = 0.64$), ($p = 0.11$). **Conclusion:** There was correlation between high TF, TFPI plasma levels, FVIIa activity and cardiothrombotic complications in T2DM especially in the presence of high risk factors as poor glycemic control, dyslipidemia and obesity. Future targeted therapy against TF may be beneficial for T2DM patients.

Keywords: tissue factor, tissue factor pathway inhibitor, diabetes.

Introduction:

Type 2 diabetes mellitus (T2DM) is associated with accelerated atherosclerosis, endothelial damage [1] and a high tendency to thrombotic complications including peripheral vascular disease, [2] cardiovascular events [3] and strokes [4].

The T2DM procoagulant state can be contributed to abnormalities in several plasma proteins involved in blood coagulation and platelets activation [5]. Thrombotic myocardial infarction may be secondary to complicated or ruptured atherosclerotic plaques with further exposure of procoagulant proteins that initiate blood coagulation [6] or due to contact between blood and damaged endothelium [7].

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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 blood [8]. Activation of extrinsic coagulation pathway is mediated via binding of FVII to TF with formation of TF-FVIIa complex, further activation of factor IX, X, XI, formation of prothrombinase complex and thrombin generation [9].

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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), and then TFPI-Xa complex binds

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 glycated end products to their specific receptors induces an intravascular oxidative stress response, leading to TF expression in vitro [16]. TF was found to be 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]. Increased TFPI reflects endothelial dysfunction or altered binding of TFPI to the endothelium by glucosaminoglycans because TFPI is mainly produced by vascular endothelium [19].

Plasma FVII level depends 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 was to assess plasma levels TF and TFPI together with FVII coagulant activity (FVIIa) in T2DM with and without cardiovascular complications and correlated these with metabolic and clinical behavior of the patients.

Subjects and Methods

This study included 60 patients with T2DM and 20 age and sex matched health controls. The patients were subdivided into 30 patients without history or clinical findings suggesting the presence of heart disease (group I), 30 patients with history of myocardial infarction (Group II).

Exclusion criteria included DM less than 10 years, history of myocardial infarction within the last three months, congestive heart failure, surgery or trauma within the prior three months, known

malignant, renal or hepatic diseases, heparin or oral contraceptive pills intake.

Patients were recruited from the Diabetes Clinic of Internal Medicine at Ain Shams university hospital from September/2007 to May/2009. All studied subjects gave their informed consent and the study was approved by the Ain Shams University Hospital Ethics Committee.

All the studied subjects were subjected to the following:

- Full detailed history laying stress on diabetes history, vascular complications, hypertension, smoking and history of drug intake including heparin, oral anticoagulants and antiplatelet drugs and available cardiological investigations (ECG, ECHO, Angiography).
- Careful clinical and cardiological examination including blood pressure measurement [diabetics with systolic blood pressure >130 mmHg or diastolic >80 mmHg were considered hypertensive].
- Body mass index calculation.
- The following laboratory investigations:
 1. Prothrombin time (PT) and activated partial thromboplastin (APTT) time using (SYSMEX CA-1500, Dade Behring, Germany).
 2. Fasting and postprandial serum glucose level, serum cholesterol, Low density lipoprotein (LDL) and high density lipoprotein (HDL) levels using Synchron CX-7 (Beckman, Switzerland). [Patients were considered dyslipidemic when triglycerides level exceeds 150 mg/dl, LDL >100 mg/dl, HDL<40 mg/dl in men or <50 mg/dl in women and cholesterol level exceeds 200mg/dl].
 3. Glycated hemoglobin (HBA1C) using Stanbio Glycohemoglobin (STANBIO, Italy).
 4. Electrocardiogram (ECG), Echocardiography (ECHO).
 5. Measurement of plasma TF and TFPI using enzyme linked immunosorbant assay (ELISA) technique.
 6. Measurement of FVII coagulation activity (FVIIa) in the plasma using FVII deficient plasma.

Sampling:

Two ml of venous blood was collected from patients and controls. It was then anticoagulated with buffered sodium citrate (3.2%) in the proportion of 9:1. Plasma was separated and kept at -70 until analyzed.

Methods:

The plasma level of the 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 has been pre-coated onto well microplate. TF in standards and samples is 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 wave length 450 nm and related to standard curve. TF level 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 has been pre-coated onto well microplate. TFPI in standards and samples is 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 related to standard curve. TFPI level expressed in pg/dl.

Quantitative FVII coagulant activity was determined based on prothrombin time assay using coagulation FVII-deficient plasma (Dade Behring, Germany). Frozen citrated plasma was diluted 1: 20 and the assay procedure was done according to manufacturer's instructions. 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 expressed in percentage (%).

Statistical methods:

Data was analyzed statistically using SPSS statistical package version 12. The following tests were done: descriptive statistics (including mean and SD), Chi-square test: χ^2 (Fisher exact test was performed in tables containing value less than 5), t-test to compare independent samples and correlation study.

Probability or *p* value of <0.05 was considered statistically significant, *p* value =0.001 statistically highly significant, while *P* >0.05 was considered non significant.

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

Parameter	Controls (no=20)	Type 2DM Patients (no=60)
	Number (%)	
Males (%)	11 (55%)	32 (54%)
Smokers (%)	-	8 (13%)
Hypertensive (%)	-	23 (38%)
Dyslipidemics (%)	-	35 (58%)
	Mean \pm SD	
Age (years)	47.9 \pm 6.1	49.5 \pm 8.6
BMI (Kg/m ²)	23.68 \pm 1.76	32.78 \pm 3.91
FBS (mg/dl)	82.68 \pm 8.41	197.40 \pm 62.00
2pp (mg/dl)	118.05 \pm 8.79	284.90 \pm 87.49
HBA1C	5.15 \pm 0.64	9.85 \pm 1.98
Cholesterol (mg/dl)	151.52 \pm 9.25	218.88 \pm 53.41
Triglycerides (mg/dl)	116.57 \pm 14.75	165.33 \pm 49.52
HDL (mg/dl)	45.73 \pm 4.91	41.61 \pm 5.72
LDL (mg/dl)	91.10 \pm 19.06	130.10 \pm 41.57
TF (ng/ml)	193.41 \pm 90.61	72.89 \pm 31.28
TFPI (pg/ml)	197.56 \pm 94.88	40.11 \pm 13.16
FVIIa (%)	108.25 \pm 26.72	75.79 \pm 11.21

Table 2- Comparison between complicated and non complicated patients regarding demographic data and serum TF, TFPI & FVIIa levels.

Parameters	Complicated DM (Mean ± SD)	Non Complicated DM (Mean ± SD)	p value
Males (%)	18(60%)	14(46.6%)	0.43
	X ² =1.071		
Age (years)	51 ± 8.6	49 ± 8.5	0.18
	t=1.38		
TF (ng/ml)	236.50±79.23	150.33± 81.16	**<0.001
	t=4.16		
TFPI (pg/ml)	242.33± 85.84	152.8± 82.46	**<0.001
	t=4.12		
FVIIa (%)	109.83± 25.55	106.67± 28.2	0.65
	t=0.46		

P >0.05: non significant,* p=0.05:significant, **p=0.001:highly significant.

Results:

Table 1 shows the Clinical and metabolic parameters of patients and controls: The study was carried out on 60 T2DM patients; 32 males and 28 females with males to females ratio 1.1:1, with mean age (years) (49.5±8.6) and 20 controls, 11 males and 9 females with male to female ratio 1.2:1 with age (years) (47.9±6.1) and they were matched as regard to age and sex.

Out of the 60 patients, there were 23 (38%) hypertensives, 8 (13.3%) smokers, and 35 dyslipidemics.

Comparison between patients and controls regarding TF, TFPI and FVIIa. TF and TFPI plasma levels as well as FVIIa were statistically significantly higher in patient groups (193.41±90.61 ng/ml, 197.56±94.88 pg/ml, 108.25±26.72 %) respectively compared to

controls (72.89 ± 31.283 ng/ml, 40.11 ± 13.16 pg/ml, 75.79 ± 11.2 %) respectively; t (5.670, 7.181, 5.135), (p<0.001).

Table 2 shows the comparison between group I and II as regards to plasma levels of TF, TFPI, and FVIIa. Both groups I and II were matched as regard to 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 (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 without statistically significant difference (p=0.65).

Correlations between plasma levels of TF, TFPI and FVIIa among studied subjects: TF plasma level was significantly correlated to TFPI plasma level (r 0.611, p<0.001) and FVIIa (r 0.828, p<0.001). Moreover, a significant correlation between TFPI plasma level and FVIIa was found among all the studied subjects (r 0.457, p<0.001).

Table 3 shows correlations between plasma levels of TF, TFPI, FVIIa and different studied parameters among T2DM. There were significant 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).

FVIIa was statistically significantly correlated to BMI (p=0.006), FBS (p=0.003), 2hPP (p=0.04), and HBA1C (p<0.001).

Table 4 shows impact of Smoking, hypertension and dyslipidemia on plasma levels of TF, TFPI and FVIIa. 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) as well as TFPI (225.71± 79.49 pg/dl) vs. (158.16± 102.02 pg/dl),(p=0.006).

Although FVIIa was higher among dyslipidemics compared to non dyslipidemics, the difference was not statistically significant (p=0.184). Moreover, diabetic hypertensive patients also had 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).

Discussion

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

The circulating TF considered as a marker for vascular injury [21,22] and a source of procoagulant activity, when expressed on the surface of circulating microparticles [9, 23]

In this study we demonstrated not only plasma levels of TF but also TFPI and FVIIa were higher in T2DM patients compared to healthy controls, which matches previous studies [10,24-28] The heightened TF and TFPI levels probably reflects the increase of TF and TFPI activity in diabetic patients.

TFPI is mostly bound to vascular endothelium and only 20-30% is free [29] and its level shows diurnal variation that parallels to FVII activity to maintain homeostatic coagulation mechanism in healthy subjects [30]. Higher morning levels of TFPI are demonstrated in cardiac patients following coronary spasm that can be a counter balance mechanism to prevent coagulation [31].

Table 3- Correlations between TF and TFPI plasma levels, FVIIa and different studied parameters among type 2 diabetic patients

Parameters		TF	TFPI	FVIIa
BMI (kg/m ²)	r	0.258	-0.06	0.34
	p	0.04*	0.65	0.006**
FBS (mg/dl)	r	0.32	0.34	0.37
	p	0.01*	0.007**	0.003**
2hPP (mg/dl)	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 (mg/dl)	r	-0.16	-0.31	-0.11
	p	0.23	0.02*	0.39
LDL (mg/dl)	r	0.48	0.37	0.23
	p	<0.001**	0.003**	0.08

Table 4- Impact of smoking, hypertension and dyslipidemia on TF, TFPI plasma levels and FVIIa.

Parameter (Mean ± SD)	TF (ng/ml)	TFPI (pg/ml)	FVIIa (%)
Smoking:			
Yes (8)	179±107.51	246.87±133.86	102.5±30.58
No (52)	195.57±88.74	189.98±86.68	109.13±26.3
	t=0.468	t=1.600	t=0.650
	P=0.64	P=0.11	P=0.51
Hypertension:			
Yes (23)	262.82±69.49	239.56±59.86	118.04±24.24
No (37)	150.27±74.25	171.45±103.61	102.16±26.68
	t=5.848	t=2.864	t=2.320
	P<0.001	P=0.006	P=0.02
Dyslipidemia:			
Yes (35)	225.43 ± 92.11	225.71± 79.49	112.14± 28.06
No (25)	148.6 ± 67.66	158.16± 102.02	102.80± 24.24
	t=3.540	t=2.882	t=1.344
	P=0.001	P=0.006	P=0.184

In this work we demonstrated significant positive relation between TFPI plasma level and FVIIa among all the studied subjects.

Moreover significant positive relation between TF and TFPI plasma level was noted in our studied subjects. This is in line with Falciani et al., who observed positive correlation between TF and TFPI levels in ischemic heart disease patients [32]. Moreover, 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 an attempt to compensate for hypercoagulable state but was not sufficient to overcome elevated TF [32].

In our work cardiovascular complicated T2DM patients had significantly higher TF and TFPI plasma levels compared to uncomplicated patients. This finding was confirmed by Boden et al. [34] and Krupinski et al. [35] Moreover Sommeijer et al., noticed higher soluble TF level in type 2 DM with microvascular complications [17].

Activated FVII was significantly higher among complicated T2DM patients [10,24,28]. However in our 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]. Confirming a preliminary report [10]. In our 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 morbid 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 risk of thrombotic complications [39,40]. Raised blood glucose with hyperinsulinemia induce 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 procoagulant state [34,41]. In our study, as previously recorded 25,28, high plasma levels of TF, TFPI and FVIIa were significantly correlated with fasting blood glucose and HbA1c.

T2DM patients frequently present with dyslipidemia. Dyslipidemia alters TF and TFPI expression in atheromatous plaque to favour 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 correlation with previous results [8,28].

In addition, our hypertensive diabetic patients had higher TF and TFPI levels than non hypertensive. Similar results were reported by Sommeijer et al., [17]. Therefore, proper control of blood pressure may reduce TF level and further reduction in cardiovascular complications.

The effect of smoking is contradictory, contrary to Hölschermann et al. [45] and Barua et al. [46]. Smoking did not significantly relate to TF, FVII nor TFPI levels among our patients which in line with previous study by Sobol et al. [47].

Summary and Conclusion: plasma levels of TF, TFPI and FVIIa were significantly higher in T2DM. In addition, TF and TFPI were significantly higher in patients with cardiovascular complications. The positive correlation of the procoagulant markers (TF/FVIIa) and blood glucose, HbA1c, lipid profile and BMI reinforce the importance of their optimal control in T2DM patients. Furthermore, pharmacological control of plasma TF activity or augmenting TFPI activity might reduce thrombotic cardiovascular complications.

Further studies with larger number of patients on other ischemic vascular diseases are be needed.

References:

1. Paoletti R, Bolego C, Poli A, Cignarella A. Metabolic syndrome, inflammation and atherosclerosis. *Vasc Health Risk Manag; Vasc Health Risk Manag.* 2006;2(2):145-52.
2. Laakso M, Lehto S. Epidemiology of risk factors for cardiovascular disease in

- diabetes and impaired glucose tolerance. *Atherosclerosis*. 1998 Apr;137 Suppl:S65-73.
3. Sowers JR, Lester MA. Diabetes and cardiovascular disease. *Diabetes Care*;1999; 22:14-20.
 4. Gentile NT, Vaidyula VR, Kanamalla U, DeAngelis M, Gaughan J, Rao AK. Factor VIIa and tissue factor procoagulant activity in diabetes mellitus after acute ischemic stroke: Impact of hyperglycemia. *Thromb Haemost*; 2007 Nov;98(5):1007-1013.
 5. Boden G, Rao AK. Effects of hyperglycemia and hyperinsulinemia on the tissue factor pathway of blood coagulation. *Curr Diab Rep*. 2007 Jun;7(3):223-7.
 6. Gutstein DE, Fuster V. Pathophysiology and clinical significance of atherosclerotic plaque rupture. *Cardiovasc Res* 1999 Feb;41(2):323-33.
 7. Koenig W. Haemostatic risk factors for cardiovascular diseases. *Eur Heart J* 1998 Apr;19 Suppl C:C39-43.
 8. Sambola A, Osende J, Hathcock J, Degen M, Nemerson Y, Fuster V, et al. Role of risk factors in the modulation of tissue factor activity and blood thrombogenicity. *Circulation*. 2003 Feb 25;107(7):973-7.
 9. Mackman N. Role of tissue factor in hemostasis, thrombosis, and vascular development. *Arterioscler Thromb Vasc Biol*. 2004 Jun;24(6):1015-22.
 10. Vambergue A, Rugeri L, Gaveriaux V, Devos P, Martin A, Fermon C, et al. Factor VII, tissue factor pathway inhibitor, and monocyte tissue factor in diabetes mellitus: influence of type of diabetes, obesity index, and age. *Thromb Res*. 2001 Mar 1;101(5):367-75.
 11. Panteleev MA, Zarnitsina VI, Ataulakhanov FI. Tissue factor pathway inhibitor: a possible mechanism of action. *Eur J Biochem* 2002;269:2016-31.
 12. Ardissino D, Merlini PA, Ariens R, Coppola R, Bramucci E, Mannucci PM. Tissue-factor antigen and activity in human coronary atherosclerotic plaques. *Lancet* 1997 Mar 15;349(9054):769-71.
 13. Creasey AA, Reinhart K. Tissue factor pathway inhibitor activity in severe sepsis. *Crit Care Med* 2001 Jul;29(7 Suppl):S126-9.
 14. Eckel RH, Wassef M, Chait A, Sobel B, Barrett E, King G, et al. Prevention Conference VI: Diabetes and Cardiovascular Disease: Writing Group II: pathogenesis of atherosclerosis in diabetes. *Circulation* 2002 May 7;105(18):e138-43.
 15. Grundy SM, Hansen B, Smith SC Jr, Cleeman JI, Kahn RA. Clinical management of metabolic syndrome: report of the American Heart Association/National Heart, Lung, and Blood Institute/American Diabetes Association conference on scientific issues related to management. *Circulation* 2004 Feb 3;109(4):551-6.
 16. Bierhaus A., Illmer T, Kasper M, Luther T, Quehenberger P, Tritschler H, et al. Advanced glycation end products-mediated induction of tissue factor in cultured endothelial cells is dependent on RAGE. *Circulation*.1997, 96:2262-71.
 17. Sommeijer DW, Hansen HR, van Oerle R, Hamulyak K, van Zanten AP, Meesters E, et al. Soluble tissue factor is a candidate marker for progression of microvascular disease in patients with Type 2 diabetes. *J Thromb Haemost*. 2006 Mar;4(3):574-80
 18. Leurs PB, Van Oerle R, Wbattel BH, Hamulyak K. Increased tissue factor pathway inhibitor (TFPI) and coagulation in patients with insulin-dependent diabetes mellitus. *Thromb Haemost*. 1997 Mar;77(3):472-6.
 19. Lindahl AK, Sandset PM, Abildgaard U. The present status of tissue factor pathway inhibitor. *Blood Coagul Fibrinolysis*. 1992 Aug;3(4):439-49.
 20. Lam KS, Ma OC, Bourke C, Chan LC, Janus ED. Genetic influence of the R/Q353 genotype on factor VII activity is overwhelmed by environmental factors in Chinese patients with Type II (non-insulin-dependent) diabetes mellitus. *Diabetologia*. 1998 Jul;41(7):760-6.
 21. Koyama T, Ohdama S, Aoki N. Plasma tissue factor reflects endothelial cell injury rather than upregulation of tissue factor expression. *Thromb Haemost* 1997 Aug; 78(2): 972.

22. Makin AJ, Blann AD, Chung NA, Silverman SH, Lip GY. Assessment of endothelial damage in atherosclerotic vascular disease by quantification of circulating endothelial cells. Relationship with von Willebrand factor and tissue factor. *Eur Heart J*. 2004 Mar;25(5):371-6.
23. Falati S, Gross P, Merrill-Skoloff G, Furie BC, Furie B. Real-time in vivo imaging of platelets, tissue factor and fibrin during arterial thrombus formation in the mouse. *Nat Med* 2002 Oct; 8(10): 1175–81.
24. Rao AKL, Chouhan v, Chen X, Sun I, Boden G. Activation of the tissue factor pathway of blood coagulation during prolonged hyperglycemia in young healthy men. *Diabetes*. 1999 May;48(5):1156-61.
25. Leurs PB, Stolk RP, Hamulyak K, Van Oerle R, Grobbee DE, Wolffenbuttel BH. Tissue factor pathway inhibitor and other endothelium-dependent hemostatic factors in elderly individuals with normal or impaired glucose tolerance and type 2 diabetes. *Diabet Care* 2002 Aug;25(8):1340-5.
26. Song KS, kim Hk. Plasma levels of tissue factor antigen in patients with non-insulin - dependent diabetes mellitus. *Yonsei Med J*. 2004 Feb 29;45(1):38-42.
27. Ludwig S, Dharmalingam S, Erickson-Nesmith S, Ren S, Zhu F, Ma GM, et al. Impact of simvastatin on hemostatic and fibrinolytic regulators in type 2 diabetes mellitus. *Diabetes Res Clin Pract* 2005; 70:110–118.
28. El-Ghoroury EA, Gamal El-Din H, Abdel-Kader M, Ragab S. Study of factor VII, tissue factor pathway inhibitor and monocyte tissue factor in noninsulin-dependent diabetes mellitus. *Blood Coagulation and Fibrinolysis* 2008, 19:7–13
29. Kato H. Regulation of functions of vascular wall cells by tissue factor pathway inhibitor: basic and clinical aspects. *Arterioscler Thromb Vasc Biol*. 2002 Apr 1;22(4):539-48.
30. Pinotti M, Bertolucci C, Portaluppi F, Colognesi I, Frigato E, Foà A, et al. Daily and Circadian Rhythms of Tissue Factor Pathway Inhibitor and Factor VII Activity. *Arterioscler. Thromb. Vasc. Biol*. 2005;25:646-649.
31. Misumi K, Ogawa H, Yasue H, Soejima H, Suefuji H, Nishiyama K, et al. Circadian variation in plasma levels of free-form tissue factor pathway inhibitor antigen in patients with coronary spastic angina. *Jpn Circ J*. 1998 Jun;62(6):419-24.
32. Falciani M, Gori AM, Fedi S, Chiarugi L, Simonetti I, Dabizzi RP, et al. Elevated tissue factor and tissue factor pathway inhibitor circulating levels in ischaemic heart disease patients. *Thromb Haemost* 1998 Mar;79(3):495-9.
33. Gosk-Bierska I, Wysokin Ski W, Karnicki K, Adamiec R. Tissue factor, tissue pathway factor inhibitor and risk factors of atherosclerosis in patients with chronic limbs ischemia: preliminary study. *Int Angiol*. 2008 Aug;27(4):296-301.
34. Boden G, Vaidyula VR, Homko C, Cheung P, Rao AK. Circulating tissue factor procoagulant activity and thrombin generation in patients with type II diabetes: effects of insulin and glucose. *J Clin Endocrinol Metabol*. 2007, 92:4352-58.
35. Krupinski J, Turu MM, Font MA, Ahmed N, Sullivan M, Luque A, et al. Increased tissue factor, MMP-8, and D-dimer expression in diabetic patients with unstable advanced carotid atherosclerosis. *Vasc Health Risk Manag*. 2007;3(4):405-12.
36. Bastard JP, Maachi M, Lagathu C, Kim MJ, Caron M, Vidal H, et al. Recent advances in the relationship between obesity, inflammation, and insulin resistance. *Eur Cytokine Netw*. 2006 Mar;17(1):4-12.
37. Pi-sunyer FX. The relation of adipose tissue to cardiometabolic risk. *Clin Cornerstone*. 2006;8 Suppl 4:S14-23.
38. Kopp CW, Kopp HP, Steiner S, Kriwanek S, Krzyzanowska K, Bartok A, et al. Weight loss reduces tissue factor in morbidly obese patients. *Obes Res*. 2003;11:950 –956.
39. Derosa G, D'Angelo A, Tinelli C, Devangelio E, Consoli A, Miccoli R, et al. Evaluation of metalloproteinase 2 and 9 levels and their inhibitors in diabetes and healthy subjects. *Diabetes Metab*. 2007, Apr;33(2):129-34.

40. Turu MM, Krupinski J, Catena E, Rosell A, Montaner J, Rubio F, et al. Intraplaque MMP-8 levels are increased in asymptomatic patients with carotid plaque progression on ultrasound. *Atherosclerosis*. 2006 Jul;187(1):161-9
41. Abdel-Hafiz E, Vaidyula VR, Bagga S, London FS, Boden G, Rao AK. Elevated whole blood tissue factor procoagulant activity in diabetes mellitus. Vitamin E inhibits glucose induced tissue factor activity in vitro. *Blood*. 2002. 100:496a.
42. Zawadzki C, Susen S, Richard F, Haulon S, Corseaux D, Jeanpierre E, et al. Dyslipidemia shifts the tissue factor/tissue factor pathway inhibitor balance toward increased thrombogenicity in atherosclerotic plaques: evidence for a corrective effect of statins. *Atherosclerosis*. 2007 Dec;195(2):e117-25.
43. Kaneko T, Wada H, Wakita Y, Minamikawa K, Nakase T, Mori Y, et al. Enhanced tissue factor activity and plasminogen activator inhibitor-1 antigen in human umbilical vein endothelial cells incubated with lipoproteins. *Blood Coagul Fibrinolysis* 1994 Jun;5(3):385-92.
44. McGee MP, Foster S, Wang X. Simultaneous expression of tissue factor and tissue factor pathway inhibitor by human monocytes. A potential mechanism for localized control of blood coagulation. *J Exp Med* 1994 Jun 1;179(6):1847-54.
45. Hölschermann H, Terhalle HM, Zakel U, Maus U, Parviz B, Tillmanns H, et al. Monocyte Tissue Factor Expression Is Enhanced in Women who Smoke and Use Oral Contraceptives. *Thrombosis and Haemostasis* 1999 Dec;82(6):1614-20.
46. Barua RS, Ambrose JA, Saha DC, Eales-Reynolds LJ. Smoking is associated with altered endothelial-derived fibrinolytic and antithrombotic factors: an in vitro demonstration. *Circulation* 2002 Aug 20;106(8):905-8.
47. Sobol AB, Galar M, Mochecka A, Stanczyk L, Kloczko J. Tissue factor and its inhibitor in patients up to 50 years of age with ischemic stroke. *Pol Merkur Lekarski*. 2001 Feb;10(56):92-5.