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Plasma Levels of Tissue Factor Antigen in Patients with Non-Insulin-Dependent Diabetes Mellitus

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Patients with diabetes mellitus (DM) are associated with an increased risk of thrombosis, and are susceptible to a series of complications including nephropathy. It has also been known that plasma tissue factor (TF) antigen levels increase significantly in certain disease states. To investigate the clinical significance of an association with the various complications in patients with type 2 non-insulin-dependent DM (NIDDM), we measured the plasma levels of TF antigen in 63 patients (35 males and 28 females, mean age 60.8 yrs) with NIDDM and in 22 normal subjects (14 males and 8 females, mean age 56.0 yrs). The mean concentrations of TF were higher for patients with NIDDM (253.7 ± 144.9 pg/ml) than in normal subjects (187.3 ± 108.7 pg/ml with marginal statistical significance (p= 0.0530). The TF levels were higher for patients with a nephropathy than for patients without a nephropathy (p=0.0402). There was a significant positive correlation between levels of TF and BUN (r=0.84, $p<0.0001$) or creatinine (r=0.93, $p<0.0001$). However, TF levels were found to be similar for both groups with and without thrombosis, neuropathy, retinopathy, or infection. These results suggest that plasma TF antigen levels may be associated with nephropathy and they may reflect a renal dysfunction in NIDDM.

Key words: Diabetes mellitus, tissue factor, nephropathy, thrombosis

INTRODUCTION

It is widely accepted that a hemostatic system is triggered when the cell-surface protein, tissue factor (TF), is exposed to the blood following a vessel injury.[1] TF has been implicated in a wide variety of coagulopathies associated with sepsis and disseminated intravascular coagulation.[2,3] It has also been known that the plasma TF antigen levels increase significantly in certain disease states such as coronary artery disease, thrombotic thrombocytopenic purpura, vasculitis, chronic renal failure, diabetic microangiopathy and glomerulonephritis.[4]

Patients with diabetes mellitus (DM) are associated with an increased risk of thrombosis,[5] and they are susceptible to a series of complications including nephropathy.[6] In this study, we measured plasma TF antigen levels to assess their clinical significances in association with various complications for those patients with a type 2 non-insulin-dependent DM (NIDDM).

MATERIALS AND METHODS

Venous blood was collected from consecutive fasting patients (n=63, 35 men, 28 women, mean age 60.8 years) suffering from NIDDM with or without symptomatic complications, and healthy subjects (n=22, 14 men, 8 women, mean age 56.0 years), into 3.2% sodium citrate (9 parts blood : 1 part anticoagulant) in the early morning. The plasma was then obtained by centrifuging the blood at 1500g for 15 min. The plasma samples were stored at -70℃. The levels of plasma TF antigen were measured by a sandwich enzyme immunoassay using a reagent kit (IMUBIND Tissue Factor) from American Diagnostica Inc. (Greenwich, CT, USA). Briefly, each plasma sample was added to microplate- wells precoated with a capture antibody (murine anti-human TF

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monoclonal antibody). Once captured, the TF is detected using a biotinylated antibody fragment that specifically recognizes the bound TF. The subsequent binding of the streptavidin conjugated horseradish peroxidase (HRP) completes the formation of the antibody-enzyme detection complex. The addition of a tetramethylbenzidine (TMB) substrate and its subsequent reaction with HRP creates a blue colored solution. Sensitivity is increased by the addition of a 0.5 M sulfuric acid stop solution and this yields a yellow color. TF levels are determined by measuring absorbances at 450 nm and by comparing these measurements with those of a standard curve. The statistical significance between the groups was determined with a Student's t analysis with a two-tailed test. The Pearson correlation coefficient was used for correlation analysis. A p value of < 0.05 was considered to represent a statistical significance.

RESULTS

We measured plasma levels of the TF antigen in 63 patients (35 males and 28 females, mean age 60.8 yrs) with NIDDM and in 22 normal subjects (14 males and 8 females, mean age 56.0 yrs). The mean concentrations of TF were higher for patients with NIDDM (253.7 ± 144.9 pg/ml) than for the normal subjects (187.3 ± 108.7 pg/ml, n= 22); this displayed a weak statistical significance (p=0.053, Table 1).

The TF levels for patients with various complications including neuropathy, retinopathy, thrombosis or infection, were similar to those patients without complications. However, the TF levels were higher for patients with nephropathy than for patients without nephropathy (p=0.0402, Table 2). Both groups with or without nephropathy did not differ significantly with respect to

Table 1. Plasma Levels of Tissue Factor (TF) in Normal Subjects and Patients with Diabetes Mellitus

Group	n	TF antigen (pg/ml)	Significance
Normal subjects	22	187.3 ± 108.7	p=0.053*
Diabetes mellitus	63	253.7 ± 144.9	

Values are expressed as mean ± SD.
*Significance between normal subjects and diabetes mellitus.

Table 2. Plasma Levels of Tissue Factor (TF) According to the Subgroups in Patients with Diabetes Mellitus

Subgroup	n	TF antigen (pg/ml) Mean ± SD	Significance
Nephropathy			p=0.0402
Yes	25	304.6 ± 212.2	
No	38	219.7 ± 50.0	
Neuropathy			p=0.1889
Yes	18	225.5 ± 69.8	
No	45	264.6 ± 163.7	
Retinopathy			p=0.5082
Yes	13	237.6 ± 71.0	
No	50	257.5 ± 157.6	
Thrombosis			p=0.3267
Yes	23	278.6 ± 163.9	
No	40	238.9 ± 130.9	
Infection			p=0.7265
Yes	5	241.4 ± 67.8	
No	58	254.4 ± 148.8	

SD, standard deviation.

Table 3. Correlation of Various Parameters with Tissue Factor (TF) in Diabetes Mellitus

Parameters	Coefficient (r)	Significance
Age	0.1024	NS
BMI	0.0184	NS
Glucose	-0.0938	NS
HbA1C	-0.1	NS
Insulin	-0.0917	NS
C-peptide	-0.1251	NS
Cholesterol	-0.1492	NS
HDL-cholesterol	0.0359	NS
LDL-cholesterol	0.4025	NS
Triglyceride	-0.1046	NS
Lp (a)	0.238	NS
Free fatty acid	-0.0338	NS
Aspartic transaminase (AST)	-0.1512	NS
Alanine transaminase (ALT)	-0.1222	NS
Total bilirubin	-0.1461	NS
Blood urea nitrogen (BUN)	0.8396	$p<0.0001$
Creatinine	0.9317	$p<0.0001$
Prothrombin time (PT)	0.1942	NS
APTT	0.0376	NS

NS, not significant (p value>0.05).
APTT: activated partial thromboplastin time.

the type of DM, gender, lipid status or HbA1C levels.

There was a significant positive correlation between levels of TF and BUN (r=0.84, $p<0.0001$) or creatinine (r=0.93, $p<0.0001$) (Table 3). However, there was no significant correlation between the TF levels and the other parameters including age, body mass index, glucose, HbA1c, insulin, C-peptide, cholesterol, HDL-cholesterol, LDL-cholesterol, triglyceride, Lp(a), free fatty acid (FFA), aspartic transaminase (AST), alanine transaminase (ALT), total bilirubin, prothrombin time (PT) and activated partial thromboplastin time (APTT).

DISCUSSION

Diabetes mellitus (DM) is associated with a hypercoagulable state that contributes to vascular complications that include cardiovascular events.[5] The close association between TF and thrombogenesis makes it reasonable to ask whether TF is related to the development of complications that include a thrombosis. However, TF levels were found to be similar in both groups with and without a thrombosis. Instead, patients with nephropathy showed a tendency to have higher TF levels, compared to those patients without this complication. These findings may be consistent with other studies that report the plasma TF levels were significantly higher for diabetic patients with nephropathy as compared to healthy subjects[4] and to patients with microvascular complications.[7] In addition to nephropathy, Saito et al.[8] has demonstrated that TF levels were also increased for patients with retinopathy as compared to DM patients with no complications. This is not consistent with findings from the present study. Galajda et al. have also demonstrated that the diabetic patients without vascular complications didn't have significantly elevated values of tissue

factor as compared with normal controls.[9] Interestingly, TF antigen levels were reported to be higher in cases of both disseminated intravascular coagulation (DIC) with renal failure and chronic renal failure without DIC when comparing its levels in those patients without renal failure.[3] This finding may be consistent with strong correlation of TF levels with BUN or creatinine in the present study and it suggests that soluble TF *in vivo* is excreted primarily in the urine.[4] Interestingly, the urine TF antigen levels in DM patients were reported to be significantly lower than those in healthy subjects.[10] In contrast, urine TF activity determined by using kinetic chromogenic assay was reportedly increased in patients with immune complex glomerulonephritis. This may reflect the aetiopathogenesis of glomerulonephritis.[11] In addition, Koyama et al. demonstrated that TF levels in patients with diabetic nephropathy were much higher than in patients with chronic glomerulonephritis or polycystic kidney disease without correlation with serum creatinine levels.[4] So it was suggested that the level of vasculopathy associated with an underlying disease may be responsible for the elevated levels of circulating TF.[4] Contrary to this finding, there was a strong correlation with TF levels and serum BUN or creatinine concentration in the present study, suggesting that TF levels may reflect a renal dysfunction.

It has recently become evident that TF has an additional biological function apart from hemostasis. One of non-hemostatic functions is the characterization of TF as an immediate early gene that is induced during cell division[12] and upregulated during monocytic differentiation.[13] As an immediate early gene, TF is rapidly induced in response to pathophysiologically relevant stimuli such as cytokines, growth factors (including vascular endothelial growth factor), endotoxin and advanced glycation products from a variety of cells including endothelial cells and monocytes.[14-17] The glycation reaction is a consequence of chronic hyperglycemia and it has been implicated in the pathogenesis of diabetic complications.[17] Glycated proteins also have receptors on monocytes/macrophages and the glycated albumin-induced blood monocyte expression of the procoagulant protein TF at the mRNA level has been reported.[18] These observations may suggest that TF may play a role in pathogenesis of diabetic complications.

One limitation of this study is that we could not measure TF levels in urine from patients with DM. Further studies are required to evaluate the relationship between plasma and urine levels as well as their use as markers of glomerular injury.

In conclusion, although the origins and removal of plasma TF are not yet clear, plasma TF levels may be associated with diabetic microangiopathy. This is just one of the non-hemostatic roles of plasma TF and plasma TF may also be used as a potential marker for endothelial injury or renal dysfunction.

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