Thrombus Precursor Protein is Significantly Elevated in Asymptomatic Diabetic Patients

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Abstract: Background: Elevated levels of TpP are indicatives of a prothrombotic state and active thrombogenesis. Common conditions that lead to hypercoagulable state and constitute major risk factors for thrombogenesis are diabetes mellitus, and hypertension. Screening diabetic patients and early detection of patients vulnerable to thrombogenesis is essentially needed. **Aim:** In this study we tested the serum levels of TpP in a group of asymptomatic type 2 diabetic patients. **Patients and Methods:** 43 subjects without symptoms suggestive of cardiovascular events were included in the present study, they classified into; type II diabetic patients (n=34) and healthy controls (n=9). The plasma levels of thrombus precursor protein (TpP), Troponin I measured in both groups using ELISA & sandwich immuno-assay respectively. **Results:** There were no significant difference between groups regarding, age and sex ($P > 0.05$). Although the plasma levels of TpP was significantly elevated in type II diabetic patients ($P \leq 0.05$), there were no significant difference regarding Troponin I levels ($P > 0.05$). Diabetic hypertensive patients had significantly elevated TpP levels compared with diabetic normotensives. **Conclusion:** TpP may be a promising marker for detection of asymptomatic diabetic patients vulnerable to cardiovascular events.
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1. Introduction

In recent years, it has become increasingly evident that components of the coagulation and fibrinolytic pathways are predictors of cardiovascular events (1). As long as 150 years ago, Virchow suggested 3 components that should be fulfilled for thrombogenesis. These are referred as Virchow triad: abnormalities of blood flow, the vessel wall (endothelial dysfunction), blood constituents (abnormal levels of hemostatic, fibrinolytic factors and platelets activation) (2).

The third and final component of Virchow's triad refers to abnormalities in blood constituents, such as clotting or hemostatic and platelet activation. The process of thrombogenesis is a fine balance between the coagulation and fibrinolytic pathways. Many of the blood constituents in diabetic and hypertensive patients are components of coagulation and fibrinolytic systems (2).

One of the key events in the thrombogenesis is the conversion of circulating soluble plasma fibrinogen to insoluble cross-linked fibrin polymer. Thrombin claves fibrinopeptide A from fibrinogen molecule exposing polymerization sites on the newly formed desAA fibrin monomer units. As the polymerization of desAA proceeds, thrombin removes fibrinopeptide B from the fibrinogen molecule which result in the formation of a molecule known as desAABB fibrin (3). These soluble polymers are the immediate precursors of insoluble fibrin and are thus referred to as thrombus precursor protein (TpP). Elevated levels of TpP are indicatives of a prothrombotic state and active thrombogenesis (4, 5).

Common conditions that lead to hypercoagulable state and constitute major risk factors for thrombogenesis are diabetes mellitus, and hypertension. Screening diabetic patients and early detection of patients vulnerable to thrombogenesis is essentially needed. The ideal method for screening vulnerable patients should be relatively noninvasive and applicable to asymptomatic populations (6). In this study we tested the serum levels of TpP in asymptomatic type 2 diabetic patients.

2. Patients and methods

Thirty three patients with type II diabetes mellitus, and 9 healthy controls were included in the present study. Among 34 patients 19 were hypertensive. Patients with symptoms suggestive of acute cardiac events, or ongoing infection were excluded. In addition those with history of thromboembolic diseases, liver failure, chronic kidney disease and macro-albuminuria as well as

those with ECG changes consistent with ischemic heart disease or antithrombotic therapy, excluded.

All included persons (n=43) were subjected to full clinical assessment with special reference to age sex, body mass index (BMI) , symptoms and signs of thrombovascular diseases , BMI and ECG. Furthermore the fasting blood sugar, serum TpP and Troponin I were measured for all persons.

All procedures followed were in accordance with the ethical standards of ethical committee of hospitals of Al-azhar school of medicine on human experimentation all patients submitted an informed consent.

Blood sampling

Blood was drawn into sodium citrate vacutainer tubes, using a light tourniquet and 21 gauge needles. The first 2 ml blood was discarded. After centrifugation, the plasma was separated and stored at – 70 C^{o} until required.

Detection of TpP and Troponin I :

TpP was measured using monoclonal antibody that binds to specific neoepitopes of soluble cross linked and none cross linked desAABB fibrin polymers. The assay system is designed to exclude detection of fibrinogen, fibrinogen-degradation products, desAA fibrin monomers, desAA fibrin polymers, desAABB fibrin monomer, cross linked fibrinogen, desAA fibrin monomer-fibrinogen complexes, fibrin degradation products, and cross linked fibrin degradation products. The assay range of TpP was 0.05 µg /ml - 15 µg / ml, with 0.02 µg / ml sensitivity.

Cardiac troponin I (cTnI) was assayed using a two-site sandwich time-resolved immunofluorometric assay using two antibodies against epitopes in the central stable part of cTnI.

Statistical analysis of data

SPPSS version 17 program was used for analysis. Differences in frequency between groups were compared with the chi-square test or the Fisher exact test. A *P* value < 0.05 was considered significant.

3. Results

Among 34 patients with type II diabetes mellitus, they were 20 (58.8%) males, and 14 (41.2%) females, their mean age ± SD was 55±15.1 y. There were no statistically significant difference between type II diabetic patients and control groups regarding age and sex (P > 0.05). However type II diabetic patients had significantly higher body mass index (P ≤ 0.05), (table 1). Out of 34 patients with type II diabetes mellitus, 19 patients were hypertensive.

The mean fasting blood sugar of diabetic patients was 190 ± 62 mg/dl, compared with 89 ±17 mg/dl in control group. Although the mean TpP was significantly higher among diabetic patients (P ≤ 0.05), there were no significant difference between diabetic patients and control group regarding Troponin I levels (P > 0.05), (table 1).

Diabetic hypertensive patients were significantly older than diabetic normotensives (P ≤ 0.05). In contrast there were no significant difference between both groups regarding sex and MBI (able 2). The fasting blood sugar and TpP levels were significantly higher in diabetic hypertensive patients (P ≤ 0.05). Yet there were no significant difference between both groups regarding Troponin levels (P > 0.05), (table 2). Diabetic patients older than 55 years had significantly higher TpP levels, compared with diabetics younger than 55 years (P ≤ 0.05), (table 3).

Table 1: Basics characteristics of studied patients

	All patients (n= 34)	Control (n=9)	*X2*	*t*	*P*
Age	55±15.1 y	48±10.2 y		1.78	0.08
Sex Male Female	 20 (58.8%) 14 (41.2%)	 6 (66.7%) 3 (33.3 %)	0.18		0.66
BMI (Mean)	23±2.9	18±2.2		6.50	**> 0.0001**
Hypertension Yes no	 19 (55.9 %) 15 (44.1%)	 0 9 (100 %)	9.01		**0.002**
Fasting blood sugar	190 ± 62 mg/dl	89 ±17 mg/dl		4.80	**> 0.0001**
TpP (Mean ± µg/ml)	4.3 ± 2.1 µg/ml	2.8 ± 0.6 µg/ml		2.96	**0.004**
Troponin I (Mean)	5.8 ± 2.3	4.9 ± 1.6		1.50	0.13

BMI= Body mass index; TpP=Thrombus precursor Protein

Table 2: Diabetic hypertensive patients have increased Thrombus precursor Protein levels

	Diabetic patients (n= 15)	Diabetic hypertensive patients (n= 19)	*X2*	*t*	*P*
Age (Mean)	48.8 ± 19.3 y	59.7 ± 9.5 y		2.15	0.03*
Sex Male *n* (%) Female *n* (%)	 8 (53.3%) 7 (46.7%)	 13 (68.4%) 6 (31.6%)	0.80		0.36
BMI (Mean)	23 ± 2.8	22.3 ± 2.6		0.75	0.45
Fasting blood sugar	133 ± 17 mg/dl	222 ± 54 mg/dl		6.13	> 0.0001*
TpP (Mean)	3.96 ± 0.46	4.49 ± 0.45		3.34	0.002*
Troponin I (Mean)	5.1 ± 0.9	5.6 ± 1.1		1.42	0.16

*Significant; BMI= Body mass index; TpP=Thrombus precursor Protein

Table 3: Diabetic elderly patients have increased thrombus precursor protein levels

	Diabetics < 55 y (*n*=15)	Diabetics ≥ 55 y (*n*=19)	*X2*	*t*	*P*
Sex Male Female	 9(60%) 6(40%)	 11(57.9%) 8(42.1%)	0.01		0.90
BMI (Mean)	22.8 ± 2.5	22.4 ± 2.8		0.43	0.66
Fasting blood sugar	181 ± 72 mg/dl	196 ± 55 mg/dl		0.68	0.49
TpP Mean (Mean)	3.2 ± 0.7	4.96 ± 0.49		8.60	> 0.0001*
Troponin I (Mean)	4.4 ± 0.8	5.3 ± 1.7		2.03	0.052

*Significant; BMI= Body mass index; TpP=Thrombus precursor Protein

4. Discussion

In type 2 diabetes mellitus; endothelial dysfunction is markedly enhanced providing a significant pathphysiological basis for atherosclerotic heart diseases. Atherosclerotic cardiovascular disease results in 19 million deaths annually and coronary heart disease account for the majority of this toll. Despite major advances in treatment of coronary heart disease patients, a large number of victims of the disease who are apparently healthy die suddenly without prior symptoms. Available screening and diagnostic methods are insufficient to identify the victims before the event occurs (7, 8). The recognition of the vulnerable patients plays an important role in the outcome. The term vulnerable patient is proposed for the identification of subjects with high likelihood of developing cardiac events in the near future (6). A quantitative method including variables based on blood vulnerability is needed to develop, for cumulative risk assessment of vulnerable patients.

DM is association with pro-inflammatory and pro-thrombotic state (9), that involve a prolonged increase in the activity and levels of coagulation/fibrinolytic markers. TpP serve as particularly attractive marker of active thrombosis because it involves the step of the activated coagulation cascade immediately preceding the formation of fibrin (4, 5). Previous studies suggest that TpP is highly specific to active coronary thrombosis, can predict the initial occlusive process before ischemic stages of the disease and may have important role in risk stratification of vulnerable patients with ongoing thrombogenesis (10-12). In addition increased levels of TpP were associated with an increased risk of death or ischemic complications in patients with acute coronary syndrome (13). Elevated TpP levels in diabetic patients without evidence of acute cardiac events, in the current study, may reflect enhanced systemic activation of the coagulation system. The concept of the “vulnerable patients” with thrombogenic blood was highlighted by an expert panel report suggesting that transient alterations in the coagulation and fibrinolytic system are likely important factors contributing to acute cardiac events (6). Measuring TpP may be one way of quantifying an individual’s degree of thrombogenic activity in diabetic patients. In addition it may help in identifying patient who may benefit from antithrombotic prophylaxis and those on need for close supervision and intensive cardiac workup for early detection of vulnerable patients.

Hypertension per se may confer a prothrombotic or hypercoagulable state (14). Despite the exposure of the blood vessels to high pressures, the main complications of hypertension are paradoxically thrombotic in nature rather than hemorrhagic (2). Previous studies showed that increased both fibrinogen and D-dimer might contribute to the development and progression of atherosclerotic

vascular disease in hypertension. The link between hypertension and thrombogenesis may also involve other metabolic risk factors like hyperglycemia, insulin resistance and hyperinsulinemia (15-17). Subjects with higher blood pressures and other metabolic disturbances are more likely to exhibit greater abnormalities of hemostasis and fibrinolysis (2). This was supported by our results where diabetic hypertensive patients exhibited higher levels of TpP compared with diabetic non hypertensive patients.

The limitation of this study comes from the small number of patients, and another study including large number of patients is needed for more clarification and confirmation of our study.

In conclusion

In this study we showed that diabetic patients had elevated levels of TpP. TpP may be a promising marker for detection of asymptomatic diabetic patients vulnerable to cardiovascular events.

References

1. Ridker PM, Hennekens CH, Stampfer MJ, Manson JE, Vaughan DE. Prospective study of endogenous tissue plasminogen activator and risk of stroke.*Lancet*. 1994;343:940 –943.
2. Lip G.Y.H., Blann A.D. Does hypertension confer a prothromboticstate ? Virchow's triad revisited .*Circulation*. 2000;101:218-220
3. Van der Putten RF, Glatz JF, Hermens WT. Plasma markers of activated hemostasis in the early diagnosis of acute coronary syndromes. Clin Chim Acta 2006;371:37–54.
4. Fuster V, Verstraete M. Thrombosis in Cardiovascular Disorders. Philadelphia, PA: W. B. Saunders Company, 1992:9 –11.
5. Loscalzo J, Schafer A. Thrombosis and Hemorrhage. Boston, MA: Blackwell Scientific Publications, 1994:10726.
6. Naghavi M, Libby P, Falk E, et al. From Vulnerable Plaque to Vulnerable Patient: A Call for New Definitions and Risk Assessment Strategies: Part II. *Circulation.*2003;108:1772-1778
7. Douglas H, Michaelides K, Gorog DA, et al. Platelet membrane glycoprotein Ib- gene –5T/C Kozak sequence polymorphism as an independent risk factor for the occurrence of coronary thrombosis. *Heart*. 2002;87:70 –74.
8. Redondo M, Watzke HH, Stucki B, et al. Coagulation factors II, V, VII, and X, prothrombin gene 20210G3A transition, and factor V Leiden in coronary artery disease: high factor V clotting activity is an independent risk factor for myocardial infarction. *Arterioscler Thromb Vasc Biol*. 1999;19:1020 –1025.
9. Sambola A, Osende J, Hathcock J, et al. Role of risk factors in the modulation of tissue factor activity and blood thrombogenicity. *Circulation*. 2003;107:973–977.
10. Hermens WT, Pelsers MM, Mullers-Boumans ML, de Zwaan C, Glatz JF. Combined use of markers of muscle necrosis and fibrinogen conversion in the early differentiation of myocardial infarction and unstable angina. Clin Chem 1998;44:890 –2.
11. Nieuwenhuizen W. Soluble fibrin as a molecular marker for a prethrombotic state: a mini-review. Blood Coagul Fibrinolysis 1993;4:93–6.
12. Carville DG, Dimitrijevic N, Walsh M, et al. Thrombus precursor protein (TpP): marker of thrombosis early in the pathogenesis of myocardial infarction. Clin Chem 1996;42:1537– 41.
13. Mega JL, Morrow DA, de Lemos JA. et al. Thrombus Precursor Protein and Clinical Outcomes in Patients With Acute Coronary Syndromes. Journal of the American College of Cardiology 2008 ; 51:2422-2429
14. Lee AJ. The role of rheology and haemostatic factors in hypertension. *J Hum Hypertens*. 1997;11:767–776.
15. Glen SK, Elliot HL, Curzio JL, Lees KR, Reid JL. White-coat hypertension as a cause of cardiovascular dysfunction.*Lancet*. 1996;348:654–657.
16. Lip GYH, Blann A. Von Willebrand factor: a marker of endothelial dysfunction in vascular disorders? *Cardiovasc Res* 1997;34:255–265.
17. Boffa MC. Considering cellular thrombomodulin distribution and its modulating factors can facilitate the use of plasma thrombomodulin as a reliable endothelial marker. *Haemostasis*. 1996;26 (suppl 4):233–243.

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