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Hrombin Activatable Fibrinolysis Inhibitor (TAFI) Relationship with Insulin Resistance and Other Risk Factors of Cardiovascular Disease in Patients with Type 2 Diabetes Mellitus (DM)

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Abstract: Type 2 Diabetes Mellitus (DM), is a global public health crisis that threatens the economies of all nations, particularly developing countries. Diabetes affects at least 285 million people worldwide and the number is expected to reach 438 million by the year 2030. Egypt is an African country with a population (82 million). By the 2025, Egypt is expected to be among the top ten countries that have the highest prevalence rates of diabetes in the world, notably type 2 DM. Cardiovascular complications are the main cause of mortality in patients with diabetes. Premature atherosclerosis, increased platelet reactivity and activation of coagulation factors with associated hypo fibrinolysis all contribute to increased cardiovascular risk in this population. In fact, type 2 DM is considered the most frequent acquired thrombophilic state. Under physiological conditions, fibrinolytic system is responsible for the lysis of fibrin clot, thus maintaining the blood fluidity. Recently, a new potent inhibitor of fibrinolysis, TAFI was isolated from human plasma. To determine plasma concentrations of TAFI in patients with type 2 DM and its relation to insulin resistance and other risk factors for cardiovascular disease like duration of type 2 DM and serum cholesterol (chol.), triglyceride (TG), uric acid and body mass index (BMI). Eighty individuals were included in the study, classified into three groups Group1: 30 patients with type 2 DM with duration of disease less than one year. Group2: 30 patients with type 2 DM with duration of disease more than ten years. DM was diagnosed according to the criteria of the American Diabetes Association (ADA), 2009. Group3: control group, consists of 20 healthy volunteers who are age and sex matched to the patients. All groups were subjected to the following: (1) Full medical history (2) Full clinical examination. (3) Laboratory investigations: Serum fasting and postprandial blood glucose, urea, creatinine, uric acid, sodium, potassium, calcium, phosphorous, bilirubin, ALT, AST, albumin, triglyceride, total cholesterol, TAFI and insulin were done. Also, urine analysis, ECG, fundus examinations were done. In each subject, the degree of insulin resistance was estimated by Homeostasis Model Assessment Insulin Resistance (HOMA- IR). Low HOMA-IR values indicate high insulin sensitivity, whereas high HOMA-IR values indicate insulin resistance. There were highly statistical significant increases in TAFI, fasting and postprandial blood glucose, serum cholesterol, triglyceride, uric acid, insulin and HOMA-IR value for groups 1, 2 compared with group 3. In both groups 1 and 2, TAFI showed a significant positive correlation with FBG and HOMA-IR but it showed non-significant correlation with the postprandial blood glucose, cholesterol, triglyceride, BMI and uric acid. On the other hand, there was highly statistical significant increase in TAFI, insulin, serum cholesterol but non statistical significant increase in HOMA-IR value for group 2 compared with group1. In this study, we found increased level of TAFI in type 2 D.M patients in both groups compared to healthy subjects. The increased TAFI level in type 2 D.M. was more in group 2 than group 1. The increased level of TAFI was correlated positively with FBG and HOMA-IR value. Coexistence of the high fasting blood glucose, increased HOMA-IR value and duration of disease synergistically accelerates impairment of fibrinolysis via elevated concentrations of TAFI which is a risk factor for the occurrence of cardiovascular complication in patients with type 2 DM.

Key words: Type 2 DM · TAFI · Insulin resistance · HOMA-IR.

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INTRODUCTION

Type 2 diabetic patients showed enhanced activation of the coagulation system. This increased procoagulant activity is believed to be one of the factors that contributes to the high incidence of premature macro and microangiopathy and increased morbidity and mortality, attributable to myocardial infarction, nephropathy and retinopathy observed in diabetic patients. Thrombus formation results from disruption of the equilibrium between prethrombotic and antithrombotic factors that control clotting homeostasis; this imbalance may occur due to an ongoing stimulus to thrombogenesis, a defect of the natural anticoagulant or fibrinolytic system. Perturbance of homeostasis has also been implicated in the development of micro vascular complications [1]. However, the mechanism by which increased activation of the clotting system occurs in diabetic patients is not clear. Hypo fibrinolysis, a common finding in diabetes may also be an important cause of vascular thrombosis; hypo fibrinolysis alone is sufficient for extended fibrin deposition, even without preceding enhanced coagulation [2]. In the present study, we focused our attention on the relation of TAFI to insulin resistance and other risk factors in patients with type 2 DM like BMI, cholesterol, triglyceride, uric acid and duration of disease. Recently, a new potent inhibitor of fibrinolysis, TAFI or carboxypeptidase U, has been isolated and characterized from human plasma. TAFI is secreted in the liver and activated by thrombin-catalyzed proteolysis to a carboxypeptidase B-like enzyme that inhibits fibrinolysis [3].

Aim of the Work: To determine plasma concentrations of TAFI and its relation to insulin resistance and other risk factors of cardiovascular disease in patients with type 2 DM like BMI, cholesterol, triglyceride, uric acid and duration of disease.

Patients and Method: Eighty individuals were included in the study, classified into three groups:

Group 1: 30 patients with type 2 DM with duration of disease less than one year. Mean age (46.27±17.44) year with a range from 35-66 years. 20 patients were males (66.7%) and 10 were females (33.3%).

Group 2: 30 patients with type 2 DM with duration of disease more than ten years with mean age (59.13±9.42) year with a range from 45-70years. 18 patients (60%) were males and 12 patients (40%) were females.

Criteria for the diagnosis of diabetes in non-pregnant adults according to American Diabetes Association [4] included:

- Fasting plasma glucose (FPG) ≥ 126 mg/dl (7.0 mmol/l). Fasting is defined as no caloric intake for at least 8 h. OR
- Symptoms of hyperglycemia and random plasma glucose ≥ 200 mg/dl (11.1mmol/l). Random is defined as any time of day without regard to time since last meal. The classic symptoms of hyperglycemia include polyuria, polydipsia and unexplained weight loss. OR
- 2-h plasma glucose ≥200 mg/dl (11.1mmol/l) during an oral glucose tolerance test (OGTT) the test should be performed as described by the World Health Organization using a glucose load containing the equivalent of 75g anhydrous glucose dissolved in water.

In the absence of unequivocal hyperglycemia, these criteria should be confirmed by repeat testing on a different days [4].

Group 3: Control group, consists of 20 healthy volunteers with mean age (40.00±3.59) year with range from 22-62years. (12 individuals were men (60%) and 8 individuals were women (40%). The study was performed at AL-Zahraa University Hospital (Internal Medicine and Endocrinology Departments) from June 2011 to December 2011. The study was approved by the local ethical committee in university hospital and informed consents were obtained from Exclusion criteria: patients with known liver disease, because TAFI is produced mainly by the liver. Patients with medications that could affect the coagulation or fibrinolytic systems (such as anticoagulants and antiplatelets agents)were excluded the patients.

All groups were subjected to the following: Full medical history, full clinical examination and laboratory investigations: about 7 ml of fasting (overnight fast, 12-14 hours) venous blood samples were taken from each subject participating in the study and divided into aliquots: The 1st aliquot was about 3.2 ml of blood, was left to clot then the serum was separated by centrifugation and fasting blood glucose was measured immediately on Hitachi auto analyzer (Hitachi 736, Hitachi, Japan). The rest of the serum was stored at -20°C for determination of the followings: urea, creatinine, uric acid, sodium, potassium, calcium, phosphorous, bilirubin, ALT, AST, albumin, triglyceride, total cholesterol and insulin. The 2nd aliquot was about 2 ml of blood, was added to a tube

containing EDTA for determination of complete blood picture on Coulter Counter T890 (Coulter Counter, Harpenden, UK). The 3rd aliquot was about 1.8 ml of blood, added to a tube containing 0.2 ml of 3.2% (0.109 M) trisodium citrate then centrifuged at 1,500 x g for 15 minutes and stored at -20°C for determination of TAFI. About 2 ml of venous blood samples were withdrawn from each subject participating in the study two hours after meal and put in a tube containing fluoride for determination of postprandial blood glucose on Hitachi 736.

Plasma TAFI was determined using ELISA kit [5] and the kit was supplied from IMUBIND (American Diagnostica Inc.West Avenue, Stamford, USA). Serum insulin was determined using radio immunoassay [6]. Insulin resistance was calculated as Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) using the following equation:

HOMA-IR = fasting blood glucose (mg/dl) x fasting serum insulin (μIU/ml)/405 [7].

Urine analysis was performed for diabetic patients for albumin by reagent strip. ECG and fundus examinations were also done.

Statistical Analysis: Results were tabulated and analyzed by Microsoft Excel 2003, SPSS16 statistical package for social science. The value was given as mean ± SD. P value < 0.05 was considered significant. Correlation coefficients (r) were calculated using the Pearson's correlation analysis.

RESULTS

Comparative Statistics: Fasting blood glucose (FBG): Group 1 had a mean level of (260.13± 76.40) mg/dl; group 2 had a mean level of (190.20± 47.70) mg/dl, while group 3 had a mean level of (96.00 ±14.30) mg/dl. There was highly statistical significant increase in the mean level of group 1 than group 3 ($p<0.001$) (Table 3). Also, highly statistical significant increase in the mean level of group 2 than group 3 ($p<0.001$) was found in Table 5. The same when comparing group 1 versus group 2, highly statistical significant increase in the mean level of group 1 than group 2 ($p<0.000$) was found in Table 7.

Analysis of the Results: In our study, the mean TAFI level was measured for group1, 2 and 3 showing (40.67±10.72 Vs. 190.00±47.7 Vs. 16.84±3.03) ng/ml, respectively with highly significant difference between group1 and 3 ($p<0.001$) was found in Table 3, between group2 and 3 ($p<0.001$) (Table 5) and between group 1 and 2 ($p<0.001$) (Table 7 and Fig. 1). The mean HOMA-IR level was measured for group 1, 2 and 3 showing (18.99 ± 6.42Vs, 19.04 ± 4.68 Vs. 2.50 ± 0.52), respectively with highly significant difference between group1 and 3 ($p<0.001$) (Table 3), between group2 and 3 (Table 5) ($p<0.001$) and non-significant difference between group1 and 2 was found in Table 7 and Fig. 2. Correlation

Table 1: Important clinical data in both groups.

Items	Group 1 No. of patients	Group II No. of patients
Hypertension	1	2
Peripheral neuropathy	1	2
Retinopathy	0	1
Nephropathy (Micro albuminuria)	1	1
Peripheral vascular disease	0	1
Ischemic heart disease	1	2

Table 2: Demographic data in patients group 1and group 3(control)

	Group 1				Control				
Items	Mean	SD	Min	Max	Mean	SD	Min	Max	P value
Age (years)	46.27	17.44	35	66	40.00	3.59	35	45	> 0.05
Sex	Males: 20 (66.7%)		Females: 10 (33.3%)		Males: 12 (60%)		Females: 8 (40%)		--
BMI (kg/m^2)	28.27	4.70	248	37.11	24.89	1.57	22.04	27.68	< 0.01

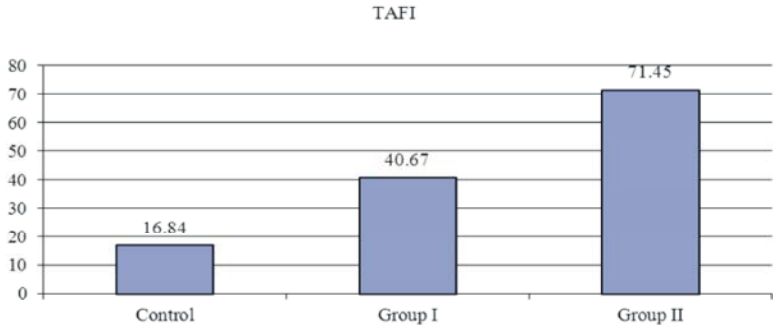

Fig. 1: Comparison of mean TAFI between group1, group2 and group3 (control)

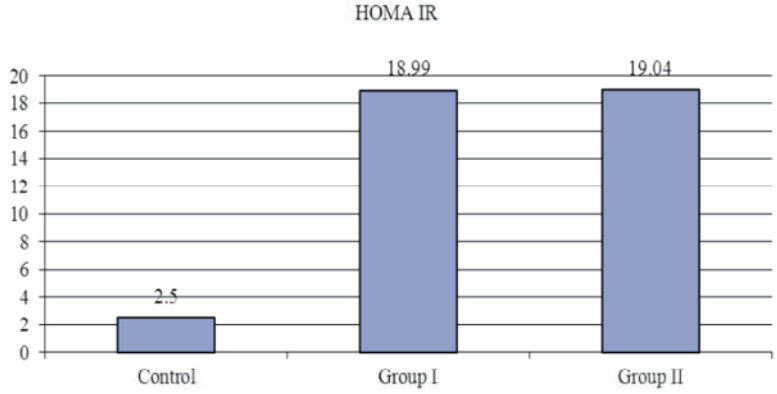

Fig. 2: Comparison of mean HOMA-IR between group 1, group 2 and group 3(control)

Table 3: Laboratory data in patients group 1 and group 3 (control)

	Group I		Control		
Items	Mean	SD	Mean	SD	P value
FBG (mg/dl)	260.13	76.40	96.00	14.30	< 0.01
2h PP (mg/dl)	262.27	76.34	139.20	5.98	< 0.01
WBC(10^3/mm^3)	8.95	2.03	6.39	1.01	< 0.01
Hemoglobin (gm %)	13.33	1.03	14.22	0.52	< 0.01
Platelets (10^3/mm^3)	281.73	93.61	319.40	57.42	> 0.05
Urea (mg/dl)	61.93	36.06	27.80	6.16	< 0.01
Creat. (mg/dl)	1.57	0.87	0.87	0.25	< 0.01
Na (mg/dl)	141.00	3.57	139.60	3.44	> 0.05
K (mg/dl)	4.44	0.60	4.36	0.56	> 0.05
Uric acid (mg/dl)	6.38	1.11	5.20	0.68	< 0.01
ALT (U/l)	27.47	15.24	27.00	6.86	> 0.05
AST (U/l)	21.80	10.42	22.50	4.62	> 0.05
ALB (g/dl)	4.53	0.56	4.84	0.62	> 0.05
Bilirubin (mg/dl)	0.88	0.19	0.84	0.22	> 0.05
Cholest (mg/dl)	140.20	33.54	101.70	25.31	< 0.01
TG (mg/dl)	113.40	47.97	122.68	35.66	> 0.05
Ca (mg/dl)	7.47	0.73	7.83	0.35	> 0.05
Ph (mg/dl)	4.22	1.06	3.61	0.61	> 0.05
TAFI (ng/ml)	40.67	10.72	16.84	3.03	< 0.01
Insulin (μIU/ml)	29.51	4.70	10.61	2.22	< 0.01
HOMA- IR	18.99	6.42	2.50	0.52	< 0.01

Table 4: Demographic data in patients group 2 and group 3 (control)

	Group 2				Control				
Items	Mean	SD	Min	Max	Mean	SD	Min	Max	P value
Age (years)	59.13	9.42	46	84	40.00	3.59	35	45	< 0.01
Sex	Males: 18 (60%)		Females: 12 (40%)		Males: 12 (60%)		Females: 8 (40%)		--
BMI (kg/m^2)	27.38	3.10	21.48	31.70	24.89	1.57	22.04	27.68	< 0.01

Table 5: Laboratory data in patients group 2 and group 3 (control)

	Group 2		Control		
Items	Mean	SD	Mean	SD	P value
FBG (mg/dl)	190.20	47.70	96.00	14.30	< 0.01
2h PP (mg/dl)	225.47	65.90	139.20	5.98	< 0.01
WBC(10^3/mm^3)	9.14	2.96	6.39	1.01	< 0.01
Hemoglobin (gm %)	12.61	1.20	14.22	0.52	< 0.01
Platelets (10^3/mm^3)	249.27	63.90	319.40	57.42	< 0.01
Urea (mg/dl)	77.93	37.60	27.80	6.16	< 0.01
Creat. (mg/dl)	1.63	0.83	0.87	0.25	< 0.01
Na (mg/dl)	143.13	4.87	139.60	3.44	< 0.05
K (mg/dl)	4.71	0.59	4.36	0.56	> 0.05
Uric acid (mg/dl)	5.99	1.01	5.20	0.68	< 0.05
ALT (U/l)	31.27	14.20	27.00	6.86	> 0.05
AST (U/l)	24.27	8.65	22.50	4.62	> 0.05
ALB (g/dl)	4.28	0.40	4.84	0.62	< 0.05
Bilirubin (mg/dl)	0.93	0.19	0.84	0.22	> 0.05
Cholest (mg/dl)	210.73	52.93	101.70	25.31	< 0.01
TG (mg/dl)	128.07	52.26	122.68	35.66	> 0.05
Ca (mg/dl)	8.30	0.70	7.83	0.35	< 0.05
Ph (mg/dl)	4.10	0.72	3.61	0.61	> 0.05
TAFI(ng/ml)	71.45	15.06	16.84	3.03	< 0.01
Insulin (μIU/ml)	40.79	4.00	10.61	2.22	< 0.01
HOMA- IR	19.04	4.68	2.50	0.52	< 0.01

Table 6: Demographic data in group 1 and group 2

	Group I				Group 2				
Items	Mean	SD	Min	Max	Mean	SD	Min	Max	P value
Age (years)	46.27	17.44	35	66	59.13	9.42	46	84	< 0.05
Sex	Males: 20 (66.7%)		Females: 10 (33.3%)		Males: 18 (60%)		Females: 12 (40%)		--
BMI (kg/m^2)	28.27	4.70	21.48	37.11	27.38	3.10	21.48	31.70	> 0.05

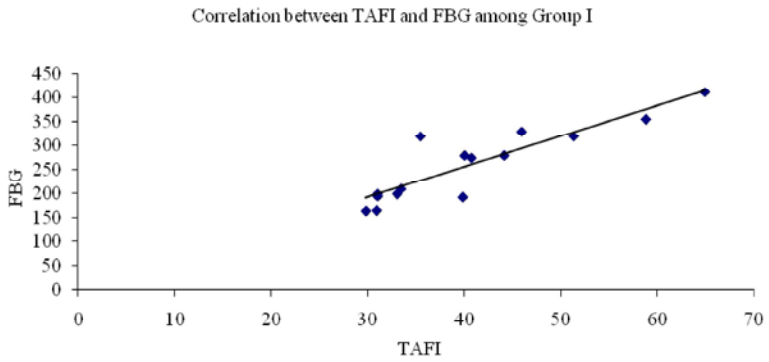

Fig. 3: Correlation between TAFI and FBG among group 1

Table 7: Laboratory data in group 1 and group 2

	Group I		Group 2		
Items	Mean	SD	Mean	SD	P value
FBG (mg/dl)	260.13	76.40	190.20	47.70	< 0.01
2h PP (mg/dl)	262.27	76.34	225.47	65.90	> 0.05
WBC(10^3/mm^3)	8.95	2.03	9.14	2.96	> 0.05
Hemoglobin (gm %)	13.33	1.03	12.61	1.20	> 0.05
Platelets (10^3/mm^3)	281.73	93.61	249.27	63.90	> 0.05
Urea (mg/dl)	61.93	36.06	77.93	37.60	> 0.05
Creat. (mg/dl)	1.57	0.87	1.63	0.83	> 0.05
Na (mg/dl)	141.00	3.57	143.13	4.87	> 0.05
K (mg/dl)	4.44	0.60	4.71	0.59	> 0.05
Uric acid (mg/dl)	6.38	1.11	5.99	1.01	> 0.05
ALT (U/l)	27.47	15.24	31.27	14.20	> 0.05
AST (U/l)	21.80	10.42	24.27	8.65	> 0.05
ALB (g/dl)	4.53	0.56	4.28	0.40	> 0.05
Bilirubin (mg/dl)	0.88	0.19	0.93	0.19	> 0.05
Cholest (mg/dl)	140.20	33.54	210.73	52.93	< 0.01
TG (mg/dl)	113.40	47.97	128.07	52.26	> 0.05
Ca (mg/dl)	7.47	0.73	8.30	0.70	< 0.01
Ph (mg/dl)	4.22	1.06	4.10	0.72	> 0.05
TAFI (ng/ml)	40.67	10.72	71.45	15.06	< 0.01
Insulin (µIU/ml)	29.51	4.70	40.79	4.00	< 0.01
HOMA- IR	18.99	6.42	19.04	4.68	> 0.05

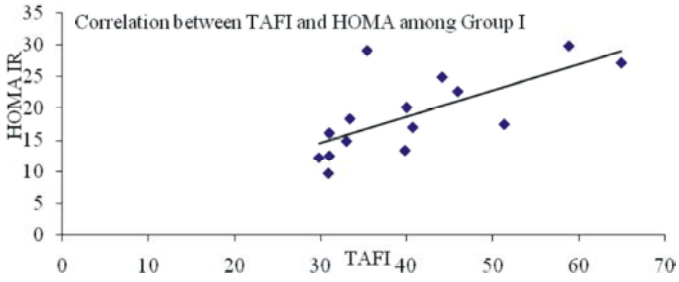

Fig. 4: Correlation between TAFI and HOMA-IR among group 1

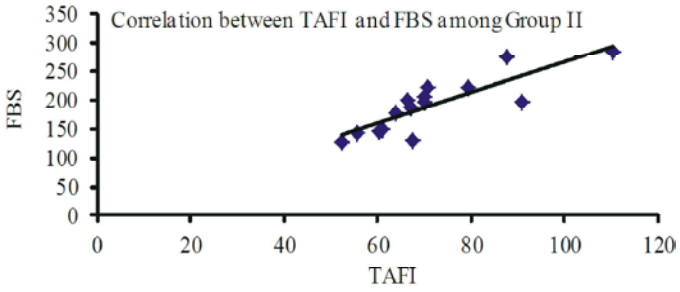

Fig. 5: Correlation between TAFI and FBG among group2

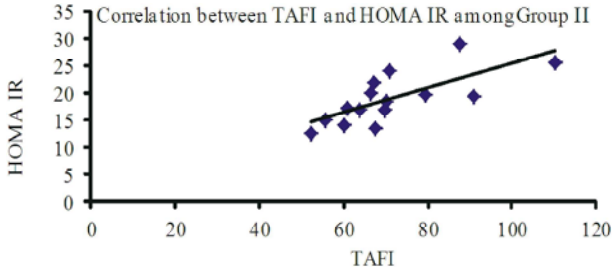

Fig. 6: Correlation between TAFI and HOMA-IR among group2

Table 8: Correlation between TAFI and other parameter in group1

Group I	TAFI (r value)	P value
TAFI	1.000	--
Age	-0.394	> 0.05
Wt	-0.301	> 0.05
Ht	-0.332	> 0.05
BMI	-0.180	> 0.05
FBG	0.890	< 0.01
2h PP	-0.068	> 0.05
WBC	-0.098	> 0.05
Hemoglobin	0.327	> 0.05
Platelets.	0.160	> 0.05
Urea	-0.196	> 0.05
Creat.	-0.217	> 0.05
Na	-0.405	> 0.05
K	-0.218	> 0.05
Uric acid	-0.130	> 0.05
ALT	0.098	> 0.05
AST	0.013	> 0.05
ALB	-0.220	> 0.05
Bilirubin.	-0.009	> 0.05
Cholesterol	0.051	> 0.05
TG	0.109	> 0.05
Ca	0.256	> 0.05
Ph	-0.253	> 0.05
Insulin	-0.109	> 0.05
Homa- IR	0.687	< 0.01

Table 9: Correlation between TAFI and other parameters in group 2

Group II	TAFI(r value)	P value
TAFI	1.000	
Age	0.347	> 0.05
Wt	-0.372	> 0.05
Ht	-0.262	> 0.05
BMI	-0.378	> 0.05
FBG	0.843	< 0.01
2h PP	0.477	> 0.05
WBC	0.080	> 0.05
Hemoglobin	0.039	> 0.05
Platelets	0.152	> 0.05
Urea	0.086	> 0.05
Creat.	-0.123	> 0.05
Na	-0.240	> 0.05
K	0.062	> 0.05
Uric acid	-0.309	> 0.05
ALT	-0.321	> 0.05
AST	0.227	> 0.05
ALB	0.155	> 0.05
Bilirubin	0.002	> 0.05
Cholesterol	0.253	> 0.05
TG	-0.218	> 0.05
Ca	0.057	> 0.05
Ph	-0.055	> 0.05
Insulin	-0.299	> 0.05
Homa- IR	0.720	< 0.01

between serum TAFI and other parameters in both groups (1 and 2): was shown in Tables 8 and 9. TAFI had non-significant correlation with BMI, postprandial blood glucose, urea, creatinine, uric acid, sodium, potassium, calcium, phosphorous, bilirubin, ALT, AST, albumin, triglyceride, total cholesterol and insulin. But it showed a high significant positive correlation with fasting blood glucose and HOMA-IR in both groups (Tables 8 and 9) and Figures 3, 4, 5 and 6.

DISCUSSION

Diabetes mellitus (DM) is a high risk of atherothrombotic disorders affecting the coronary, cerebral and peripheral arterial trees. The risk of myocardial infarction (MI) is 3-5 fold higher in type 2 DM, around 70% of deaths are vascular with poorer outcomes to both acute events and cardiological interventions [8]. Mortality in diabetic patient had an almost three-fold increased risk to die within the first 15 years after onset of diabetes compared with healthy men as reported by Torn *et al.* [9]. The increased cardiovascular risk in type 2 DM could be related to premature atherosclerosis, increased platelet reactivity and activation of coagulation factors associated with hypo fibrinolysis all contribute to increased cardiovascular risk in type 2 D.M patients as shown by Alzahrani and Ajjan [3]. HOMA-IR, has been shown to correlate with index of insulin sensitivity derived simply from basal glucose and insulin concentrations [10]. HOMA-IR was reported by Bonora *et al.* [11] as an independent predictor of cardiovascular disease (CVD) in type 2 diabetes. The improvement of insulin resistance might have beneficial effects not only on glucose control but also on CVD in patients with type 2 DM.TAFI is a procarboxypeptidase and member of the family called metallocarboxypeptidases. Activation of TAFI occurs by trypsin, plasmin and thrombin. The catalytic efficiency of thrombin to activate TAFI is increased about three orders of magnitude in the presence of thrombomodulin. Thus, the thrombin-thrombomodulin complex is most likely the physiologic activator of TAFI. TAFI removes C-terminal lysine residues from partially degraded fibrin, thus inhibiting further plasminogen activation. It could be stated that TAFI acts as an important link between coagulation and fibrinolysis [12, 13].

In the present study, the plasma concentration of TAFI was highly significant increase in type 2 D.M patients in both groups compared with healthy subjects

(control). These results suggested the involvement of TAFI in the mechanism of hypo fibrinolysis in diabetes. Our results agreed with finding of Yano *et al.* [14], who reported that the plasma levels of TAFI were significantly higher in diabetic patients than in normal subjects which may be involved in the mechanism of vascular endothelial damage in patients with type 2 DM. In another study, Hori *et al.* [15] found that TAFI levels and activity were significantly higher in plasma of type 2 DM subjects compared to healthy controls. The plasma levels of TAFI antigen and D-dimers were significantly and inversely correlated in all type 2 DM subjects. These observations support the role of TAFI in the mechanism of diabetes-associated hypo fibrinolysis. Many researches have demonstrated the relationship between TAFI and metabolic syndrome components as hypertension, cholesterol, triglycerides, BMI and insulin resistance in patients with type 2 DM. But, few researches had shown the relationship of TAFI and duration of disease. Also, Yano *et al*. [2] reported that the plasma levels of TAFI were significantly increased in type2 DM subjects with micro albuminuria (MAU) compared with those in type 2 DM subjects with normal albuminuria (NAU) and in normal subjects. Yoshimasa *et al.* [16] could not find any significant associations between plasma TAFI and components of the metabolic syndrome (MS) such as MI, triglyceride and HDL cholesterol in type 2 diabetic patients. Contrary to our result, Blaszkowski *et al.* [17] suggested that, there were no significant differences in the mean levels of TAFI in diabetics with coronary heart disease, hypertension and retinopathy and those without these complications. No significant correlations between TAFI levels and BMI and lipids were found. So, TAFI has no important role in the impairment of fibrinolysis and the development of cardiovascular complications in patients with type 2 diabetes mellitus. Yener *et al.* [18] observed in normotensive type 2 DM subjects without diabetes-related complications that PAI-1 level was significantly elevated, but level of TAFI antigen did not differ from healthy controls. In more recent studies, Chudý *et al.* [19] showed that TAFI was significantly increased only in the macro albuminuria (MAU) group compared to the controls. Neither the difference in TAFI levels between normal albuminuria (NAU) and controls, nor the difference between diabetic subgroups MAU and NAU were statistically significant. These results indicate that the disease progression in type 2 DM subjects leads to more profound TAFI mediated inhibition of fibrinolysis.

In the present study, the plasma concentration of TAFI, insulin and total cholesterol were highly significant increase in group 2 compared with group 1type 2 DM patients with positive correlation of TAFI with FBG and HOMA-IR in group1and 2, but no correlation was found between TAFI and BMI, cholesterol, TG and uric acid. Kural *et al.* [20] showed that TAFI levels were found to be significantly higher in patients with metabolic syndrome, when insulin resistance was calculated using the HOMA-IR formula but found no significant relation between fasting blood glucose and plasma TAFI levels in patients with coronary disease who underwent percutaneous coronary intervention (PCI) the presence of known diabetes and the insulin resistance were predictors of major cardiac events (MACE) that included death, non-fatal AMI and stroke at 12 months. Prediabetes and newly detected diabetes did not increase the risk of MACE in comparison with the normoglycemics. Hori *et al.* [15] showed that triglyceride, insulin resistance and serum LDL cholesterol to have an independent influence on plasma TAFI in type 2 diabetic patients. Juhan *et al.* [21] investigated cardiovascular risk factors and found a positive correlation between TAFI level and hypercholesterolemia. Similarly, Santamaria *et al.* [22] in their study on patients with coronary artery disease, found a positive correlation between TAFI levels and hypercholesterolemia.

CONCLUSION

In this study, it was found highly significantly increased level of TAFI and HOMA-IR in group1and 2 type 2 DM patients compared to healthy subjects. There was highly significantly increased TAFI level in type 2 DM in group 2 (duration of disease more than ten years) compared to group 1(duration of disease less than one year). But no significant difference in HOMA-IR was detected between group 1 and 2. These results showed that the disease progression in type 2DM subjects leads to more profound TAFI mediated inhibition of fibrinolysis. This could be explained by increased fasting blood glucose, hyper cholestrolemia, hyperinsulinemia, prolonged duration of the disease all contributed to more increment in TAFI level in group 2. However, the elevated level of TAFI in group1 indicating that hypo fibrinolysis state is present early in the course of the type 2DM which could explain the occurrence of cardiovascular complication in the beginning or even as presenting manifestation of patients with type 2 DM. Also, there was

positive correlation between TAFI and HOMA-IR and FBG in group 1and 2, so the tight control of blood glucose, improving insulin resistance can be obtained by diet control, exercise, drug therapy which may delay the cardiovascular complication of patients with type 2 DM.

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