Evaluation of plasma fibrinogen and plasminogen activator inhibitor- 1 in type 2 diabetes mellitus patients with coronary artery disease

Zeinab H. El Sayed[a], Sahar Mohamed Ismail[a], Heba A. ELhakeem[b]

Introduction Coronary artery disease (CAD) remains the main cause of death in patients with type 2 diabetes mellitus (T2DM). It is more extensive and diffuse in diabetics in spite of antiplatelet therapy. Hence, the prevention and the early diagnosis of CAD among patients with T2DM are very important. There is a variety of hemostasis abnormalities in T2DM. Therefore, fibrinogen and plasminogen activator inhibitor-1 (PAI-1) may have a link between T2DM and CAD.

Aim The aim was to evaluate the plasma fibrinogen and PAI-1 levels in some Egyptian patients with T2DM with and without CAD.

Patients and methods A total of 30 Egyptian patients with T2DM attending the Internal Medicine Department of Al Zahraa Hospital were included in this study. Their ages ranged between 31 and 54 years. Based on ECG changes and echocardiography, the patients were divided into the following: 15 who had CAD and 15 without. Another 15 apparently healthy participants were enrolled as a control group.
Blood samples were analyzed for routine blood tests, fasting lipid profile, renal function, liver function, glycosylated hemoglobin, plasma fibrinogen, and PAI-1, which was measured by enzyme-linked immunosorbent assay.

Results Plasma fibrinogen and PAI-1 were significantly higher in Egyptian patients with T2DM with CAD than those who had only T2DM, and both markers were higher in Egyptian patients with T2DM than healthy controls. Fibrinogen and PAI-1 levels were positively correlated with glycosylated hemoglobin in Egyptian patients with T2DM.

Conclusion Egyptian type 2 diabetic patients with T2DM are prone to develop CAD more often than not owing to increase in plasma fibrinogen and PAI-1 levels; therefore, we can give a small dose of anticoagulant for all patients with T2DM.
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Keywords: coronary artery disease, plasma fibrinogen, plasminogen activator inhibitor-1, type 2 diabetes mellitus

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Introduction

Type 2 diabetes mellitus (T2DM) is a real problem all over the world [1]. It is considered a strong risk factor for coronary artery disease (CAD). The incidence of CAD is greater in diabetics than nondiabetics and represents the most common cause of mortality among them [2,3].

T2DM is accompanied by changes in homeostasis mechanisms, which can contribute to the development of diabetic vascular disease. So, the association between CAD and T2DM may be via that defect of homeostasis. Moreover, the risk of CAD still remains high in T2DM despite the advances in antiplatelet therapies and control of hyperglycemia [4].

Many studies have reported that the elevation of plasma fibrinogen and plasminogen activator inhibitor-1 (PAI-1) levels is associated with increased risk of CAD development [5–7]. Therefore, the objective of this study is to evaluate the plasma fibrinogen and PAI-1 levels in some Egyptian patients with T2DM with and without CAD.

Patients and methods

Patients

The current study enrolled 30 Egyptian patients who had T2DM admitted to the ward of Internal Medicine Department of AL-Zahraa University Teaching Hospital from April 2017 to June 2017. The patients were divided into two subgroups (15 patients in each group) according to the presence or absence of CAD. Patients with T2DM were diagnosed as having CAD if they had ischemic changes on a resting electrocardiogram (e.g. abnormal Q waves, ST segment depression, or inverted T waves), echocardiography, a history of angina, or coronary angiography. Another 15 individuals apparently

healthy were included as a healthy control group. T2DM was diagnosed according to American Diabetes Association (ADA) guidelines [8].

Ethical approval

The research was approved by local ethics committee at Al Azhar University, and it was conducted in accordance with the Declaration of Helsinki, and consent was obtained from all participants of the study.

All participants were divided into three groups:

(1) Group A included 15 apparently healthy individuals as the control group.
(2) Group B included 15 patients with T2DM with CAD.
(3) Group C included 15 patients with T2DM without CAD.

Exclusion criteria

We excluded any cause that could affect the levels of plasma fibrinogen and PAI-1. We excluded patients on anticoagulants or antiplatelet agents, patients on hypolipidemic drugs, female who received or contraceptive, hypertension patients, patients with acute or previous myocardial infarction, patients with chronic liver disease, patients with chronic kidney disease, and patients with peripheral vascular disease.

Relevant history was taken, and detailed clinical examination was done in all groups.

All the patients and control underwent the following routine investigations:

(1) Resting ECG.
(2) Complete blood count.
(3) Fasting and postprandial blood glucose levels.
(4) Glycosylated hemoglobin (HbA1c) (%).
(5) Fasting lipid profile.
(6) Renal function tests.
(7) Liver function tests which included serum albumin, prothrombin time, and international normalized ratio.
(8) Plasma fibrinogen levels (normal range: 160–350 mg/dl).
(9) Plasminogen levels (normal range: 5–40 ng/ml).

Methods

Sample collection

Venous blood samples were taken from fasting diabetic patients. Samples were taken from anticubital vein using routine precautions observed in venipunctures and divided into two parts:

(1) Two milliliter was added to sterile EDTA vacutainers and centrifuged for 20 min at the speed of 2000–3000 rpm. Plasma was separated, aliquoted, and stored at −20°C for analysis of PAI-1. All samples were measured in a single assay to avoid repeated freeze-thaw cycles.
(2) For analysis of fibrinogen, 1.8 ml of blood was taken in a tube containing 0.2 Na citrate as an anticoagulant (dilution must be 1 : 9 Na citrate : venous blood). The citrated blood samples were centrifuged at 1500*g* for 15 min at room temperature, and the supernatant plasma was removed and stored at 15–25°C.

Analysis of PAI-1 concentration in plasma by ELISA

Plasma level of PAI-1 was analyzed according to manufacturer's instructions using ELISA technique, using a complete set of ELISA reader model Stat fax 2100, with human PAI ELISA kit, supplied by Glory Science (catalog number A1033; Texas, USA).

Quantitative determination of fibrinogen in plasma

Fibrinogen level was analyzed by modified by Clauss methods (citrated plasma is brought to coagulation by a large excess of thrombin. Here coagulation time depends largely on the fibrinogen content of the specimen), using CSS000100 semen coagulation automated analyzer, and with Multifibrin kit supplied by Semens Healthcare Diagnostics, Egypt, Cairo.

Statistical analyses

Statistical analyses were performed using SPSS software version 16.0 (SPSS Inc., Chicago, Illinois, USA), and results were expressed as mean±SD. We conducted the analysis of variance model and analysis of variance for repeated measurements. To compare the means between two groups, Student's *t*-test was used for normally distributed data and the Mann–Whitney test was used for non-normally distributed data. For categorical comparisons, the χ^2-test was used. A result with *P* value less than or equal to 0.05 was considered to be statistically significant. *P* value less than or equal to 0.01 was considered highly statistically significant.

Results

Baseline characteristics and the comparison between all diabetics and control

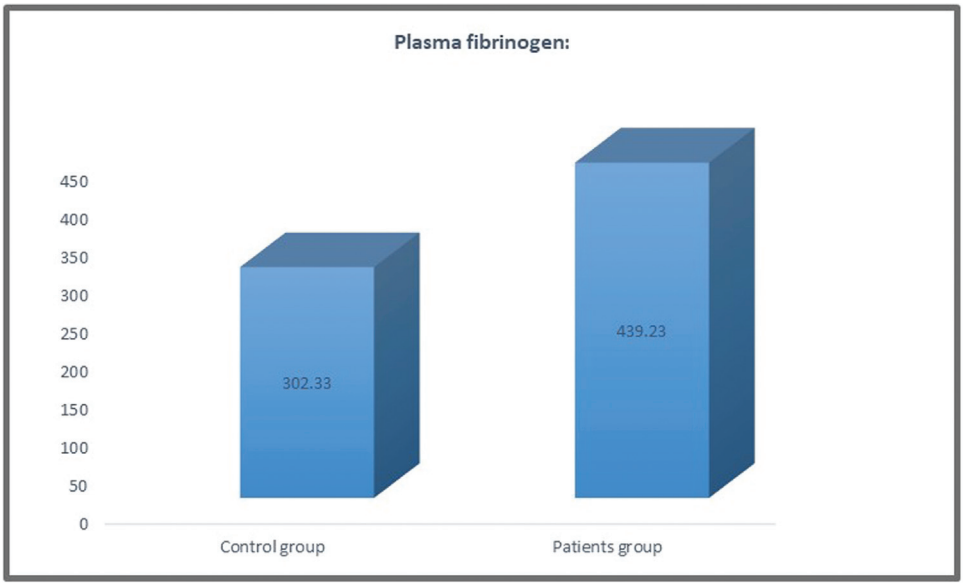
A total of 30 patients with T2DM, including 20 (66.70%) females and 10 (33.30%) males, were included in this study. Another 15 apparently healthy individuals, including 11 (73.33%) females

Table 1 Clinical and laboratory features in the control group and all patients with type 2 diabetes mellitus

Variables	Control group *N*=15	Patients with T2DM *N*=30	*P* value	Significance
Number	15	30		
Age				
Mean±SD	39.07±8.21	56.90±9.94	0.000	HS
Range	27–52	31–54		
Sex [*n* (%)]				
Females	11 (73.33)	20 (66.70)	0.649	NS
Males	4 (26.67)	10 (33.30)		
Smoking [*n* (%)]				
No	11 (73.33)	22 (73.33)	1.000	NS
Yes	4 (26.67)	8 (26.67)		
BMI (kg/m^2)				
Mean±SD	26.47±1.85	31.53±2.17	0.000	HS
Range	24–30	28–36		
Systolic blood pressure				
Mean±SD	125.27±9.62	130.27±22.21	0.411	NS
Range	110–140	100–190		
Diastolic blood pressure				
Mean±SD	78.67±6.55	80.00±9.47	0.628	NS
Range	70–90	70–100		
HbA1c (%)				
Mean±SD	4.93±0.29	8.82±1.54	0.000	HS
Range	4.50–5.50	5.8–11.10		
FBS (mg/dl)				
Mean±SD	101.60±10.57	203.43±77.25	0.000	HS
Range	90–120	67–415		
TG (mg/dl)				
Mean±SD	120.73±24.76	184.20±87.54	0.009	HS
Range	90–170	70–507		
TC (mg/dl)				
Mean±SD	176.00±17.93	190.17±51.30	0.307	NS
Range	150–200	100–322		
LDL (mg/dl)				
Mean±SD	101.73±11.63	123.73±32.07	0.014	NS
Range	90–130	82–210		
HDL (mg/dl)				
Mean±SD	50.12±11.08	44.74±14.60	0.216	NS
Range	30–66.8	18.4–79.5		
Platelet count				
Mean±SD	345.40±64.40	330.33±65. 73	0.457	NS
Range	250–450	208–450		
INR				
Mean±SD	1.10±0.11	1.15±0.14	0.326	NS
Range	1–1.4	0.9–1.7		
PT				
Mean±SD	13.27±1.10	14.40±2.43	0.094	NS
Range	11–15	10.4 –19		
Plasma fibrinogen (mg/dl)				
Mean±SD	302.33±76.54	439.23±158.51	0.003	HS
Range	170–469	206–858		
PAI-1 (ng/ml)				
Mean±SD	23.37±10.18	78.82±53.04	0.000	HS
Range	5–35.6	36–200		

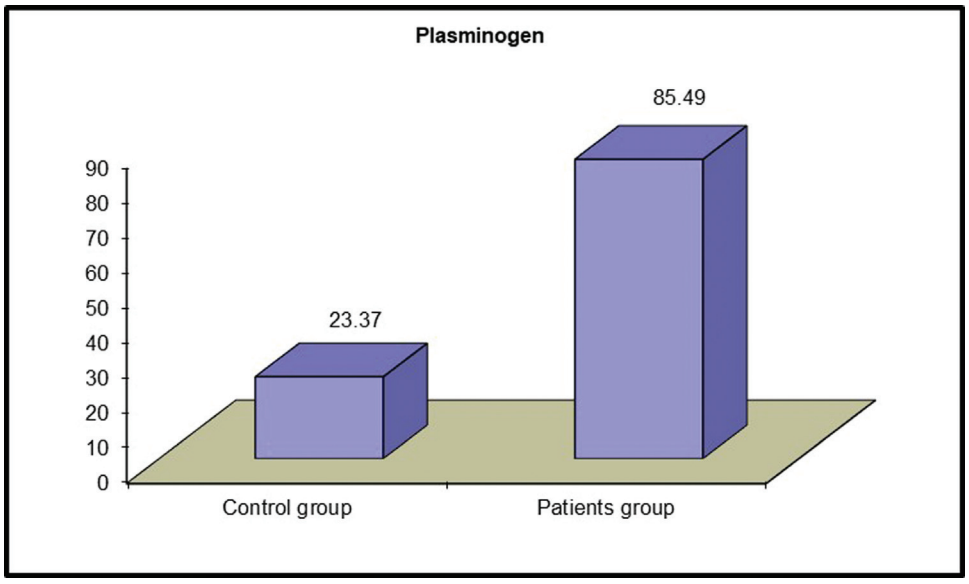
FBS, fasting blood sugar; HA1c, glycosylated hemoglobin; HDL, high-density lipoprotein; HS, highly significant; INR, international normalized ratio; LDL, low-density lipoprotein; PAI-1, plasminogen activator inhibitor-1; PT, prothrombin; S, significant; TC, total cholesterol; TG, triglycerides.

Figure 1



Comparison of mean plasma fibrinogen levels among control and all patients with type 2 diabetes mellitus.

Figure 2



Comparison of mean plasminogen activator inhibitor-1 levels among control and all patients with type 2 diabetes mellitus.

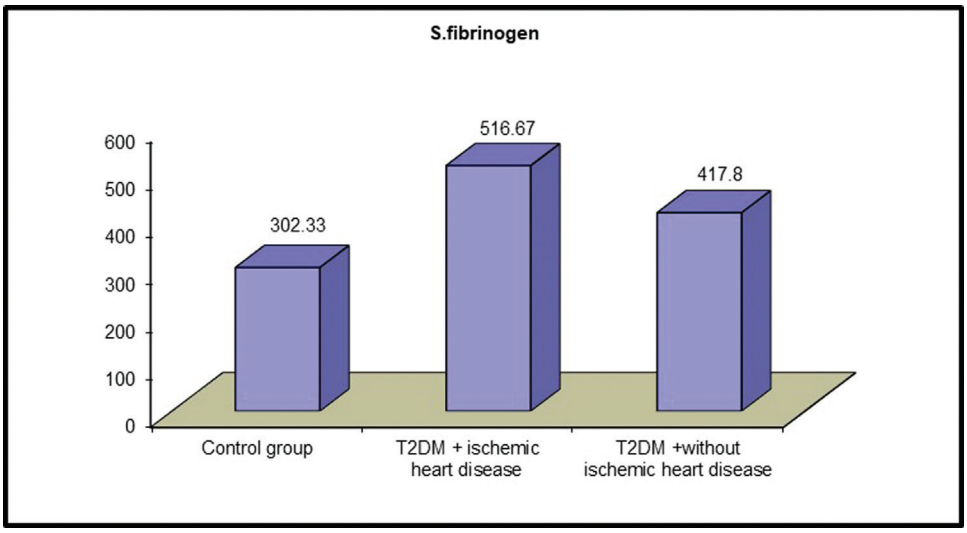
and four (26.67%) males, were enrolled as a control group. The mean±SD of age was 56.90±9.94 years for patients and 39.07±8.21 years for control. There was a significant increase in BMI (kg/m^2), plasma fibrinogen, and PAI-1 levels in patients when compared with control group, as shown in Table 1 and Fig. 1 for plasma fibrinogen and Figs 2 and 3 for PAI-1.

Table 2 shows that the maximum elevation of mean plasma fibrinogen level was found in group B (516.67 ±77.17 mg/dl) but the lowest elevation was seen in group A (302.33±76.54 mg/dl). The mean plasma fibrinogen level in group C (417.80±196.02 mg/dl) was that found between groups A and B.

Likewise, the maximum elevation level of mean PAI-1 was found in group B, but the lowest level was found in group A, and the mean level in group C was that observed between group A and group C, as shown in Table 2 and Fig. 4.

Table 3 shows there was a highly significant difference (P=0.001) between group B and group C regarding the

Figure 3



Comparison of mean plasma fibrinogen levels among the three study groups.

Table 2 Comparison of all groups according to type 2 diabetes mellitus duration, mean plasma fibrinogen, and plasminogen activator inhibitor-1 levels

Parameter	Group A	Group B	Group C	*P* value	Significance
Number of participants	15	15	15	–	–
Duration of T2DM	–	>5 years	<5 years	–	–
Fibrinogen level	302.33 ±76.54	516.67 ±77.17	417.80 ±196.02	0.000	HS
PAI-1 (ng/ml)	23.37 ±10.18	106.51 ±72.91	63.47 ±27.98	0.000	HS

HS, highly significant; PAI-1, plasminogen activator inhibitor-1; T2DM, type 2 diabetes mellitus.

mean fasting blood sugar. On comparing the mean level of plasma fibrinogen between group B and group C, we found a significant increase in the group B (*P*=0.000).

Moreover, when comparing the mean level of PAI-1 between group B and group C, we found a significant increase in group B (*P*=0.000).

In group B, all 15 patients had plasma fibrinogen levels above the maximum level of normal value (350 mg/dl) (Table 4), but only 13 patients in the same group had PAI-1 above the maximum level of normal value (40 ng/ml), and two patients had a normal value of PAI-1, as shown in Table 5.

Among group C, there were nine patients who had normal levels of plasma fibrinogen but at the upper normal level, and the rest six patients had plasma fibrinogen above the maximum normal levels (Table 4). All the 15 patients had elevated PAI-1 level in the same group (Table 5).

In the control group, 11 participants had normal levels of plasma fibrinogen and 4 had above the normal level (Table 4), but the same 15 participants had a normal level of PAI-1 (Table 5).

Among group B, 11 patients had elevated both plasma fibrinogen and PAI-1 levels above normal versus six patients in group C who had an elevation of both (Table 6).

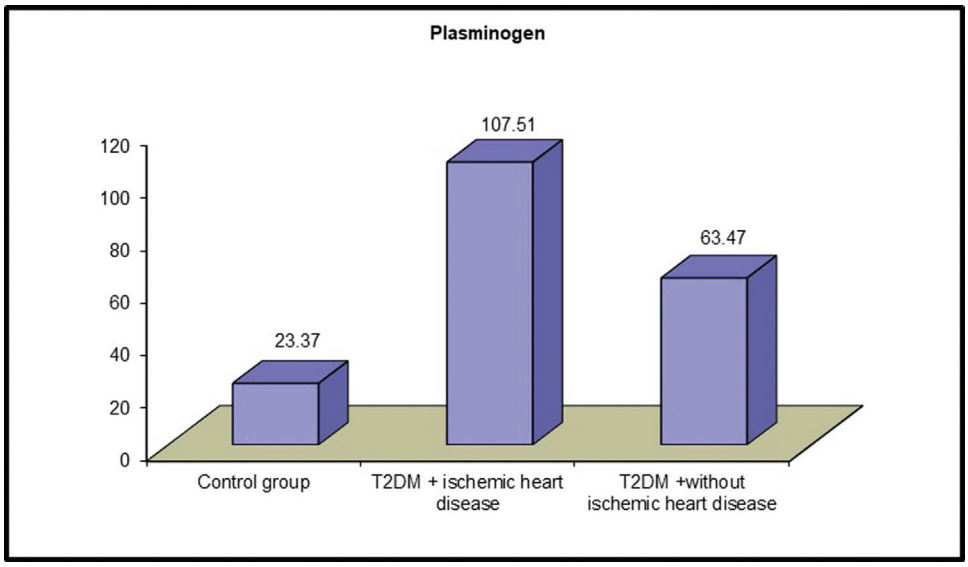
Table 7 shows there was a significant positive correlation between plasma fibrinogen level and HbA1c in all patients with T2DM (*r*=0.576, *P*=0.001) (Fig. 5), in group B (*r*=0.572, *P*=0.026) (Fig. 6), and in group C (*r*=0.630, *P*=0.012) (Fig. 7).

Table 8 shows the correlation coefficient study of PAI-1 in relation to HbA1c in all patients with T2DM, in group B, and in group C. We found a positive correlation between PAI-1 and HbA1c in all patients with T2DM (*r*=0.662, *P*=0.000) (Fig. 8), in group B (*r*=0.764, *P*=0.001) (Fig. 9), and in group C (*r*=0.630, *P*=0.012) (Fig. 10).

Discussion

In the current study, we found that the mean fibrinogen level was significantly higher in all diabetic patients in comparison with healthy control. A similar observation has been shown in many studies, where a significant elevation of fibrinogen level in diabetic patients compared with healthy non-diabetic control was reported [9,10]. Moreover, Madhu *et al.* [11] found ascending levels of plasma fibrinogen in patients with T2DM without macrovascular disease

Figure 4



Comparison of mean plasminogen activator inhibitor-1 levels among the three study groups.

Table 3 Comparative study between patients with type 2 diabetes mellitus with and without coronary artery disease

Parameters	Group B	Group C	*P* value	Significant
Number of patients	15	15	–	–
Duration of T2DM	>5 years	<5 years	–	–
FBS (mg/dl)	225.47±85.07	181.40±63.91	0.001	HS
HbA1c				
Mean±SD	8.75±1.72	8.89±1.39	0.000	HS
Range	6.5–11.1	5.8–11		
Plasma fibrinogen (mg/dl)				
Mean±SD	516.67±77.17	417.80±196.02	0.000	HS
Range	345–765	206–858		
PAI-1 (ng/ml)				
Mean±SD	94.17±67.37	63.47±27.98	0.000	HS
Range	36–200	44.4–150		

FBS, fasting blood sugar; HA1c, glycosylated hemoglobin; HS, highly significant; PAI-1, plasminogen activator inhibitor-1; S, significant; T2DM, type 2 diabetes mellitus.

Table 4 Plasma fibrinogen distribution in the different study groups

Parameters	Participants	No who had a normal level of fibrinogen (160–350 mg/dl)	No who had elevated level of fibrinogen (? 350 mg/dl)
Group A (number)	15	11	4
Group B (number)	15	–	15
Group C (number)	15	9 (at high normal)	6

Table 5 Plasminogen activator inhibitor-1 distribution in the different study groups

Parameter	Participants	Normal PAI-1 level (5–40 ng/ml)	Elevated PAI-1 level (? 40 ng/ml)
Group A (number)	15	15	–
Group B (number)	15	2	13
Group B (number)	15	–	15

PAI-1, plasminogen activator inhibitor-1.

compared with healthy controls, and the study by Saini *et al.* [12] concluded that patients with T2DM had a higher plasma fibrinogen level, and this elevation was significantly associated with microalbuminuria.

In contrast, a study by Lyer and Desai [13] reported no significant variation in the values of fibrinogen between the healthy normal and stable diabetic patients; this may be because they had cases with controlled mild diabetes and normal BMI. Furthermore, our study showed fibrinogen level to be significantly higher in patients with T2DM who also had CAD than those who had only diabetes. This result is in agreement with study by Khalid and Abdalla [14] who found

fibrinogen level was significantly increased among diabetic patients with CAD than patients without CAD. Kafle and Shresth [10] also found circulating fibrinogen levels were significantly higher in diabetic patients with CAD than those who had only CAD or only diabetes. In parallel, Sarangi *et al.* [15] demonstrated significantly higher fibrinogen levels among diabetic patients with complications than those without complications and in diabetic patients with metabolic syndrome than in those without [16].

In the current study, we found HbA1c was a highly significant elevated in patients with T2DM who had CAD when compared with patients with T2DM without CAD. As mentioned before, patients (T2DM with CAD) who had a higher significant fibrinogen level also had a higher significant HbA1c in our study. Our results also showed that the plasma fibrinogen to be significantly positively correlated with the HbA1c in patients with T2DM with or without CAD. Similar studies were done by Gupta and Dhawale [9], Mohan *et al.* [17], and Gupta *et al.* [18], which found serum fibrinogen level was higher in patients having uncontrolled diabetes, and there was a positive correlation between fibrinogen and HbA1c. In addition, the higher levels of fibrinogen and HbA1c are independent risk factors of multivessel CAD [19]. Hence, fibrinogen level increased as the value of HbA1c became higher.

Table 6 Plasminogen activator inhibitor-1 distribution in the different study groups

	Number of patients	No who had elevated level of fibrinogen and plasminogen activator inhibitor-1 (ng/ml)
T2DM with CAD	15	11
T2DM without CAD	15	6
Healthy control	15	–

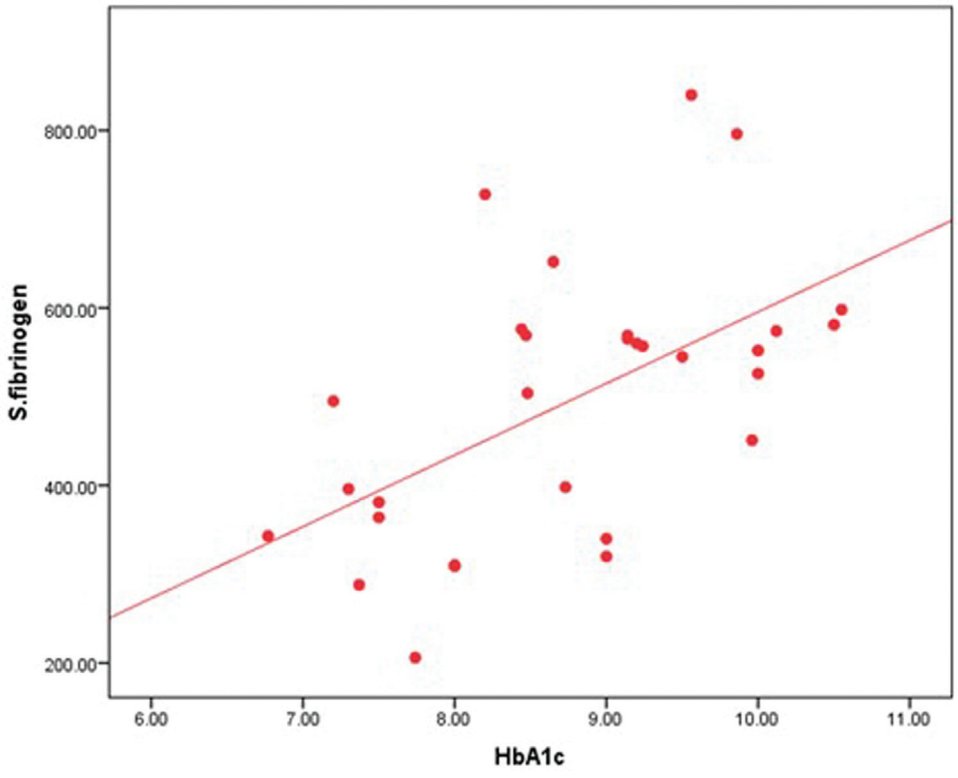
CAD, coronary artery disease; T2DM, type 2 diabetes mellitus.

Table 7 The correlation study between plasma fibrinogen and glycosylated hemoglobin in the three groups

Parameters	Plasma fibrinogen (mg/dl)		
	All T2DM	Group B	Group C
HA1c %			
r	0.576	0.572	0.630
P value	0.001	0.026	0.012
Significance	HS	S	S

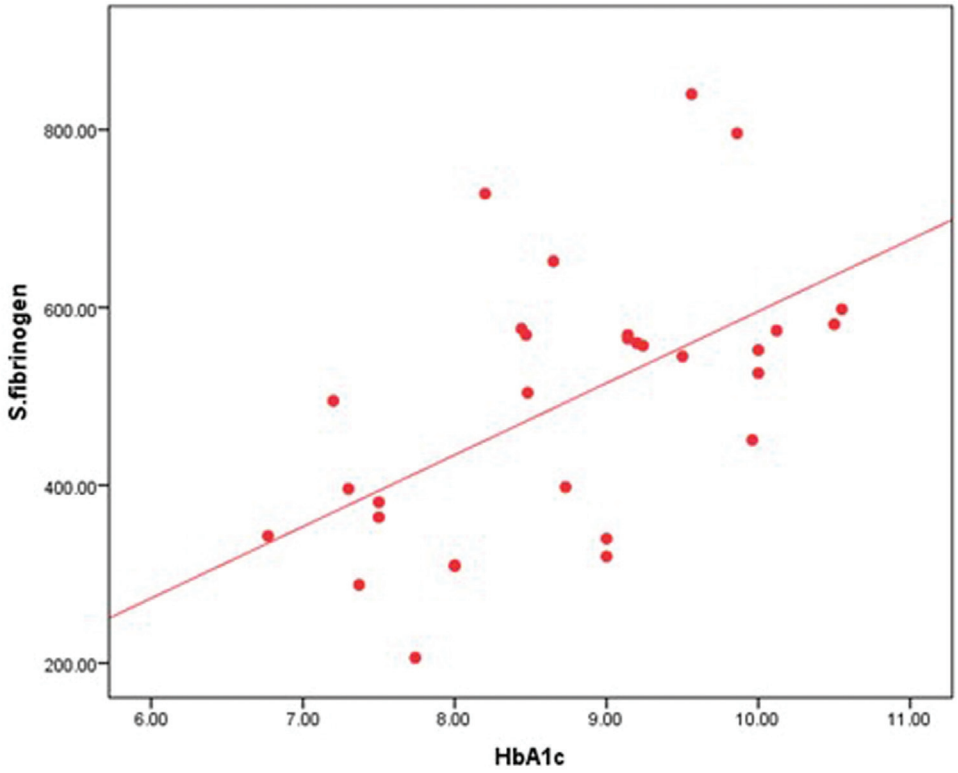
HA1c, glycosylated hemoglobin; HS, highly significant; PAI-1, plasminogen activator inhibitor-1; S, significant; T2DM, type 2 diabetes mellitus.

Figure 5



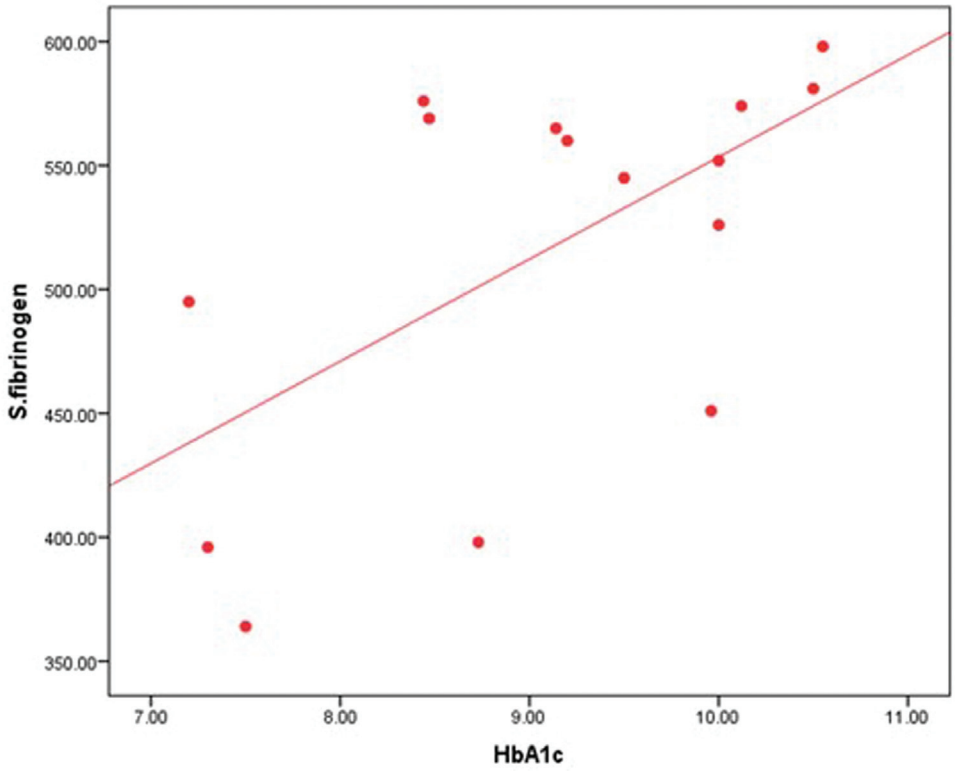
Correlation study between plasma fibrinogen and glycosylated hemoglobin in all patients with type 2 diabetes mellitus.

Figure 6



A positive correlative study between fibrinogen and HA1c in group B.

Figure 7



A positive correlative study between fibrinogen and HA1c in group C.

Regarding the PAI-1 levels, we found a significant elevation of PAI-1 among patients with T2DM compared with healthy controls. Our result is in accordance with previous studies which reported significantly elevated PAI-1 levels among individuals with T2DM when compared with controls [20–22]. In subgroup analyses, significant elevation of PAI-1 was reported in participants who have T2DM with CAD, as compared with those with T2DM only. In accordance with those data, the results from Brazionis *et al.* [23] observed that higher plasma PAI-1 activity was associated with a higher risk of CAD. Moreover, Schneider and Sobel [24] reported that the increased expression of PAI-1 typical of T2DM was a factor contributing to the increased incidence of MI and to premature CAD. Elevated PAI-1 is an independent risk factor for cardiovascular disease, especially those of atherosclerotic. Elevated levels of PAI-1 not only positively correlated with the severity of stroke but also greatly increased the failure rate of thrombolytic therapy. Ischemic heart disease was analogously reported. Swedish British and Polish reports indicated that elevated levels of PAI-1 should be considered an independent risk factor for CAD [25–27]. Furthermore, it has been clearly revealed that overexpression of PAI-1 in the vessel wall leads to the development of vulnerable plaques development and ACS. Many previous studies found a significantly increased level of PAI-1 in patients with myocardial infarction, stable or unstable CAD [28], or even endothelial dysfunction. In addition, increased concentration of PAI-1 in blood and the arterial wall was also found in patients with obesity, metabolic syndrome, and T2DM. Those results implied that increased expression of PAI-1 possibly induced by insulin resistance and hyperinsulinemia as major metabolic impairments underlying these diseases could be a factor contributing to premature CAD frequently seen in patients with T2DM [29].

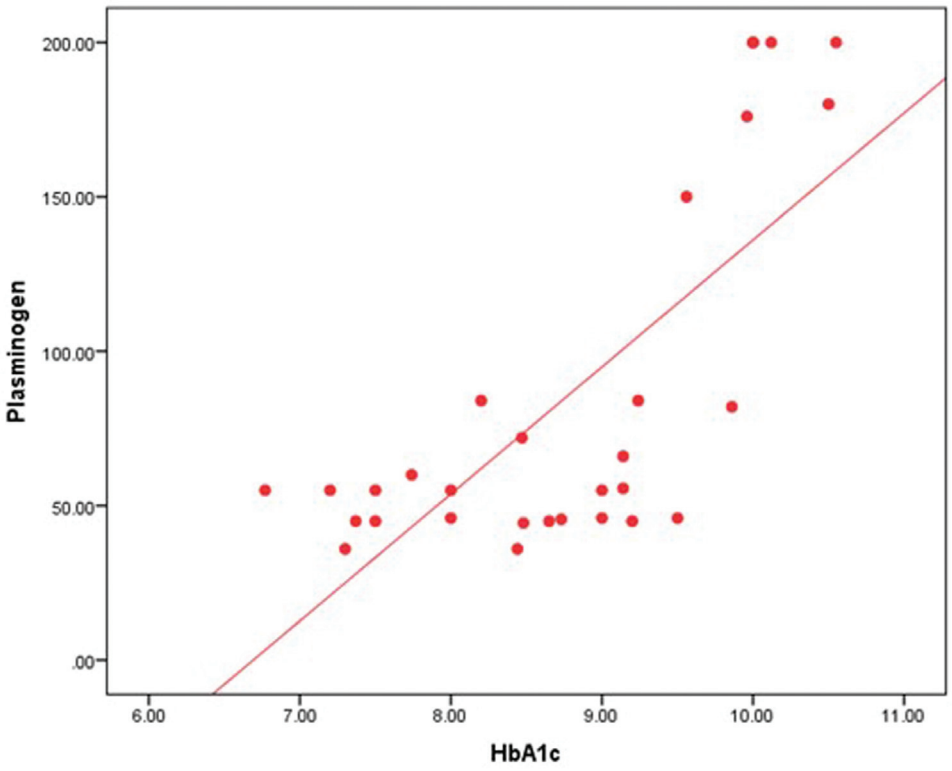
Table 8 The correlation study between plasminogen activator inhibitor-1 and glycosylated hemoglobin in the three groups

Parameter	PAI-1		
	All T2DM	Group B	Group C
HA1c %			
r	0.662	0.764	0.630
P value	0.000	0.001	0.012
Significant	HS	HS	S

HA1c, glycosylated hemoglobin; HS, highly significance; PAI-1, plasminogen activator inhibitor-1; S, significant; T2DM, type 2 diabetes mellitus.

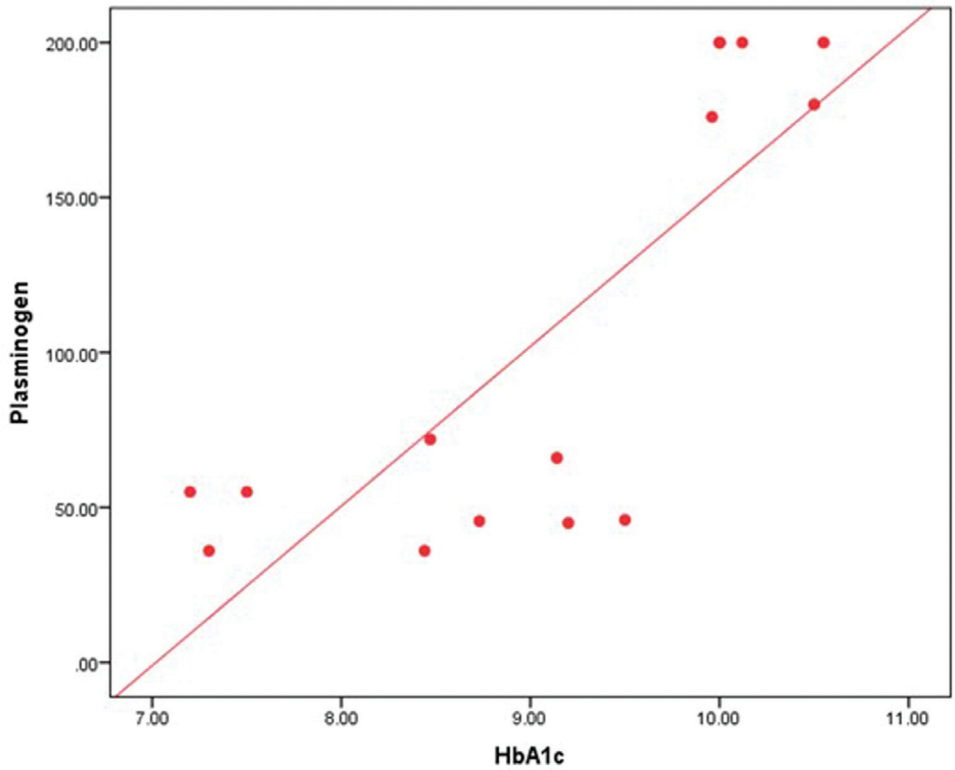
There are a number of mechanisms to explain increased fibrinogen levels in diabetes: the first may be related to the association; interleukin-6 levels are elevated in

Figure 8



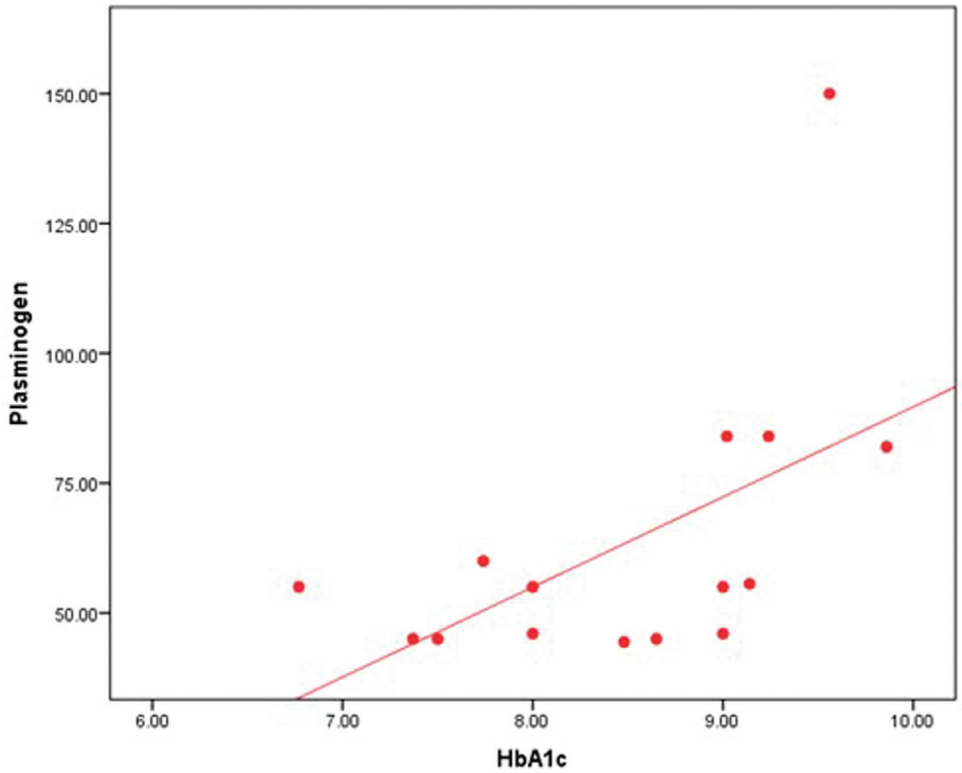
A correlative study between plasminogen activator inhibitor-1 and HA1c in all patients.

Figure 9



A correlative study between plasminogen activator inhibitor-1 and HA1c in group B.

Figure 10



A Correlative study between plasminogen activator inhibitor-1 and HA1c in the group C.

diabetes, and this cytokine has been shown to stimulate hepatocytes to produce fibrinogen, representing an important link between inflammation and hypercoagulation [30,31]. A second mechanism implicates insulin resistance, a crucial pathogenic mechanism in T2DM, which is associated with increased hepatic fibrinogen production in response to insulin, in contrast to healthy control. Insulin resistance also increases PAI-1 and reduces tissue PAI levels. The major increase in PAI-1 has been described in diabetic patients with poor glycemic control, and treatment with oral hypoglycemic agents glipizide or metformin comparably decreased PAI-1 [30].Increased fibrinogen synthesis has also been demonstrated postprandial in patients with T2DM but not in healthy controls, further suggesting hepatic deregulation of fibrinogen synthesis in this condition. Moreover, prolonged exposure to hyperglycemia was causing endothelial dysfunction, accelerated atherosclerosis, and increased oxidative stress, which was seen in T2DM [32], and fibrinogen plays an important role in the development of atherosclerosis starting from the stage of plaque formation till the formation of occlusive thrombus over a ruptured atherosclerotic plaque [33]. Moreover, alterations in fibrin structure in patients with T2DM owing to nonenzymatic glycation of fibrinogen. Glycated fibrinogen increases thrombogenicity by reducing tissue PAI and reducing plasminogen to plasmin transformation [34].

Conclusion

Our observations suggested that elevated level of plasma fibrinogen in the T2DM leads to increase in the coronary thrombosis risk and the elevated PAI-1 leads to delay in lysis of thrombus formed, so these patients enter a closed circle. Therefore, elevated levels of plasma fibrinogen and PAI-1 level in T2DM could be a link between T2DM and CAD.

Recommendation

We recommended that we can give patients with T2DM a small dose of anticoagulant as a preventive treatment in T2DM before complaining of coronary symptoms.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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