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Plasminogen activator inhibitor-1 gene polymorphism as a risk factor for vascular complications in type 2 diabetes mellitus

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Abstract

Background: Diabetes mellitus (DM) can lead to microvascular and macrovascular damages through hyperglycemia that is the main cause of diabetic complications. Other factors such as hypertension, obesity, and hyperlipidemia may worsen or accelerate the others. Several studies have revealed definitive genetic predispositions to the development of type 2 diabetes mellitus (T2DM) and development of vascular complications. This study aimed to address the association between plasminogen activator inhibitor-1 (PAI-1) gene polymorphism and T2DM, and if this gene polymorphism may have a possible role in the development of vascular complications in T2DM. This study is a case control; it included 200 patients with T2DM, 117 patients had no vascular complications, and 83 had previous vascular complications (VCs). One hundred eighty volunteer blood donors were selected as a healthy control group. All patients and controls were subjected to clinical examination, and laboratory investigations included lipid profile, fasting and 2 h blood glucose, complete blood cell count, D-dimer, PAI-1, thrombin activatable fibrinolysis inhibitor (TAFI), and detection of PAI-1 gene polymorphism by real-time polymerase chain reaction (PCR).

Results: The most prevalent genotype of PAI-1 gene polymorphism in all studied groups, including controls, was 4G/5G with the highest allele frequency as 4G. The 4G/5G and 4G/4G genotypes were associated with increased risk of DM development as compared to 5G/5G genotype. The 4G/5G and 4G/4G genotypes also had a highly significant increased risk of VCs among diabetic patients, as compared to 5G/5G. The 4G allele also was highly associated with DM with VCs. The D-dimer TAFI, PAI-1 showed the highest levels in 4G/5G genotype followed by 4G/4G genotype. The lowest level was expressed in 5G/5G genotype in diabetic patients with and without VCs. The univariable analysis showed that genotypes 4G/5G and 4G/4G were potentially risk factors for development of VCs with T2DM patients.

Conclusion: This study concludes that the PAI-1 4G/5G polymorphism may be associated with T2DM and may be considered as a risk factor for development of thrombotic events. It may also help in selection and dosing of patients being treated with anticoagulant and fibrinolytic agents. Further large-scale studies are recommended to assess the possible role of environmental factors and gene interactions in the development of T2DM vascular risks.

Keywords: Plasminogen activator inhibitor-1, Polymorphism, Diabetes mellitus

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Background

Type 2 diabetes mellitus (T2DM) is a common metabolic disease in developing countries, in which combined insulin resistance and beta-cell impairment lead to hyperglycemia [1]. The long-term complications of diabetes mellitus (DM) are the major causes of morbidity, mortality, and high healthcare costs [2]. Vascular complications of DM represent a leading health problem worldwide [3]. Most of the complications caused by hyperglycemia involve damage to small vessels which leads to neuropathy, nephropathy and retinopathy, and large blood vessels, as in cardiovascular diseases. Risk factors such as hypertension, dyslipidemia, and obesity can also increase the risk of type 2 DM. Insulin resistance and hyperglycemia are associated with low-grade inflammation as well as chronic enhancement of oxidative stress, triggering endothelial dysfunction and promoting atherogenesis [4].

Plasminogen activator inhibitor-1 (PAI-1) is a 50-kDa glycoprotein that belongs to the serine protease inhibitor (serpins) family. PAI-1 is the main regulator of the endogenous fibrinolytic system [5]. PAI-1 is composed of three beta sheets and nine alpha helices. PAI-1 can bind to the somatomedin B domain, interact with the proteasome, and interfere with cell adhesion to the extracellular matrix [6]. PAI-1 is expressed and secreted in a variety of tissues, including the liver and spleen. The synthesis of PAI-1 is regulated by insulin, very-low-density lipoprotein (VLDL), low-density lipoprotein (LDL), and glucose [7].

PAI-1 is the main physiological inhibitor of tissue-type plasminogen activator (tPA) in the fibrinolytic system, which produces active plasmin from plasminogen that then cleaves fibrin. Impaired fibrinolytic function induced by increased PAI-1 expression is commonly observed in patients with thrombotic disease [8].

The *PAI-1* gene is located on human chromosome 7q21.3-22, spans 12.3 kb and contains nine exons and eight introns [9]. The genetic expression and polymorphisms of *PAI-1* are still incompletely understood. The main polymorphism of *PAI-1* consists of a common single insertion/deletion of a guanine (G) base at position 675 in the promoter region of *PAI-1* and can cause changes in the rate of gene transcription [10]. This polymorphism produces two alleles that contain either four or five sequential guanosines (4G and 5G) that differ in their regulation of the concentration of PAI-1 [11].

Individuals who are homozygous for the 4G allele (4G/4G) have higher levels of gene transcription and a higher PAI-1 plasma concentration than those who are homozygous for 5G (5G/5G) and therefore possibly have an increased risk for intravascular thrombosis [12]. Individuals who are heterozygous (4G/5G) have intermediate levels of PAI-1. The 4G allele produces up to six times more messenger ribonucleic acid (mRNA) than the 5G and is associated with increased PAI-1 activity [13]. An increase in the plasma concentration of PAI-1 is, therefore, associated with increased thrombotic events [14], recurrent myocardial infarction (MI) in young patients [15], and ischemic events in individuals with pre-existing atherosclerosis [16].

The role of the *PAI-1* polymorphism as a risk factor for thrombotic events in many other pathological conditions has been disused in previous studies. However, the relationship between the *PAI-1* gene polymorphism and the development of vascular complications in T2DM is still a matter of debate. Therefore, the present study investigated the possible association between the *PAI-1* gene polymorphism and the development of T2DM in Egyptian patients and identified the possible relationship of this gene polymorphism with vascular complications in these patients.

Methods

Our study included 200 patients with T2 DM (123 males and 77 females, with ages ranging from 37–68 years). The patients were from the Internal Medicine Departments of Menoufia, Al Zahraa and Helwan University Hospitals. Diagnosis of diabetes was performed according to American Diabetes Association (ADA) 2018, with either fasting blood glucose greater than 126 mg/dl, 2–h post-prandial blood sugar greater than 200 mg/dl during oral glucose tolerance test (OGTT), or hemoglobin A1C (HbA1C) greater than 6.5%. We also included patients who received an anti-diabetic treatment.

Hypertension was defined as systolic blood pressure (SBP) greater than 130 mmHg or diastolic blood pressure (DBP) greater than 80 according to American Heart Association (AHA) 2017 or patients receiving an anti-hypertensive treatment.

Inclusion criteria

All 200 included patients had T2DM, and 117 of them had T2DM without any vascular complications with a median age of 48 years. The remaining 83 patients had previous vascular complications with a median age of 49 years (17 patients had a proven myocardial infarction (MI), 15 had cerebral strokes (CSs), 17 had proven diabetic nephropathy (DN), 20 had coronary artery disease (CAD), and 12 had proven deep venous thrombosis (DVT)).

Exclusion criteria

Non-diabetic patients or patients with type 1 diabetes mellitus (T1DM), cardiogenic shock, risk factors of vascular complications due to causes other than T2DM, or acute renal failure due to causes other than DM; patients who were overt obese; patients who refused to participate

in the study; patients who were very debilitated; and patients above 70 years of age.

One hundred eighty-one blood donor volunteers were selected as the healthy control group (137 males and 44 females, with ages ranging from 32 to 58 and a median age of 46 years).

Written consent was obtained from all participants, and the study was approved by the ethical committee of National Liver Institute-Menoufia University. All procedures performed in this work were carried out in accordance with the 1964 Helsinki declaration and its later amendment.

All patients and controls were subjected to the following:

I. Complete history and clinical examination.
II. Laboratory investigations:
    - Blood samples were collected after overnight fasting from patients and controls into plain tubes for determination of their lipid profile [total cholesterol (TC), triglycerides (TG), low-density lipoprotein (LDL), high-density lipoprotein (HDL)] and fasting and postprandial blood glucose by an auto analyser Integra-800 (Roche-Diagnostics, Mannheim, Germany).
    - Ethylene-diamine-tetraacetic acid (EDTA) blood samples were used for complete blood cell counts and were measured by a Sysmix XT-1800 Haematology automatic cell counter (Germany).
    - Plasma samples were isolated from EDTA samples and stored at − 80 °C until used for detection of D-dimers by latex agglutination turbidimetry with a normal range of < 0.5 μg/ml.
    - A sodium citrate sample was used to determine the PAI-1 antigen using a commercial kit from Asserachrom PAI-1(Stago Diagnostic-France).
    - Human thrombin activatable fibrinolysis inhibitor (TAFI) was detected in EDTA plasma samples by a Sandwich enzyme-linked immunosorbent assay (ELISA) Kit (Catalog No: MBS2500580, MyBio-source). Briefly, standards or samples were added to the wells of a micro ELISA plate that was pre-coated with an antibody specific to human TAFI, followed by incubation with a biotinylated detection antibody specific for TAFI conjugated to avidin-horseradish peroxidase (HRP). After washing, the substrate solution was added to each well. Then, a blue color was developed, and the reaction was terminated by the addition of stop solution. The color turned yellow. The optical density (OD) was spectrophotometrically measured at a wavelength of 450 nm ± 2 nm. The OD value was proportional to the concentration of TAFI in the sample. The final concentration of TAFI in the samples was obtained from the standard curve. The detection range was 5–320 ng/ml, with a coefficient of variation of < 10%.
    - Detection of *PAI-1* genotyping by real-time PCR:

1. DNA extraction:
   Genomic DNA was extracted from whole EDTA blood samples using the Invitrogen DNA Blood Mini Kit according to the manufacturer's instructions. Briefly, genomic wash buffers 1 and 2 were placed in micro-centrifuge tubes. Then, 200 μl of whole blood was added, followed by the addition of 20 μl proteinase k and 20 μl RNase to each sample. The mixture was vortexed and incubated at room temperature for 2 min. After the addition of 200 μl buffer, vortexing was performed, and the mixture was incubated at 55 °C for 10 min to promote protein digestion. Two hundred microliters of ethanol (96–100%) was added to the sample and vortexed for 5 s. The mixture was applied to a Pure Link mini spin column and centrifuged at 8000 rpm for 1 min and filtered. Five hundred microlitres of buffer was added to the DNA pellet and centrifuged at full speed (13,000 rpm) for 3 min, followed by the addition of 100 μl Pure Link elution buffer, incubation at room temperature for 1 min, and centrifugation at maximum speed for 1 min. The extracted DNA was stored at − 20 °C until used for PAI-1genotyping by real-time polymerase chain reaction (PCR).
2. TaqMan genotyping assay:
   The *PAI-1* polymorphism was genotyped by real-time PCR fluorescence detection on a Rotor Gene Real Time PCR System (QIAGEN, GmbH-Germany) using fluorescent-labelled probes. TaqMan probes are sequence-specific oligonucleotides containing a fluorophore and a quencher. TaqMan minor groove binder (MGB) probes additionally include a MGB moiety, which helps to increase allelic discrimination using two probes that only differ by one nucleotide. PCR included two TaqMan probes; one was specific for the 4G allele and the other was specific for the 5G allele. These probes specifically annealed to the target region between the two primers. The fluorescent dye VIC was used for the homozygous 4G allele, and the fluorescence dye FAM was used for the homozygous 5G allele; the fluorescence signals for both dyes were heterozygous for both alleles (4G/5G). During PCR, DNA polymerase causes primer extension, cleaves the probe at the 5′ end, and separates the fluorophore dye from the

quencher dye. The probe then perfectly hybridizes to the target DNA. The fluorescence signal results from this cleavage were monitored by a real-time PCR detection system. The increase in the fluorescence signal was proportional to the amount of the specific released fluorophore, indicating which alleles are present in the sample. PCR was performed according to the Duggan et al.'s [17] protocol: 95 °C for 10 min, 95 °C for 15 s and 60 °C for 1 min and then repeating steps of 95 °C for 15 s and 60 °C for 1 min for 40 cycles (for amplification); the post-read step was performed when the PCR was completed. The results of the allelic discrimination run were plotted by the real-time PCR instrument software on a scatter plot for the 4G allele versus the 5G allele, and each well of the rotor was represented as an individual point on the plot. The allelic discrimination plate read was analyzed, and the allele types were documented and verified.

Statistical methods

The results were statistically analysed using the statistical package of social sciences (SPSS 22.0, IBM/SPSS, Inc., and Chicago, IL). Normally distributed variables are expressed as the mean and standard deviation (mean ± SD), while non-normally distributed variables are expressed as the median and interquartile range (IQR). The categorical test results are expressed as the frequency and percentage. For comparing continuous variables, ANOVA was used when normality and homogeneity assumptions were met; if not, then on-parametric equivalent Kruskal-Wallis or Mann-Whitney tests were applied. The Chi-square test was used to compare categorical variables or, alternatively, Fisher's exact test was used when the Chi-square assumptions were violated.

The association of the genotypes and clinical data with vascular complications in DM patients was explained by odds ratios (ORs) and 95% confidence intervals (95% CI). After univariable analysis was used to identify the potential risk factors for vascular complications, multivariable logistic regression analysis was conducted to estimate the adjusted ORs and 95% CIs of the independently associated risk factors. A P value of 0.1 was set for the variables included in the multivariable model, and highly correlated variables were excluded to avoid multicolinearity between entered variables. Statistical significance was set at a P value < 0.05.

Results

The baseline demographics and clinical characteristics of the studied groups were shown in Table 1. The majority of the studied groups were men (75.7% of the healthy controls, 71.8% of the DM without VCs patients' and 73.5% of DM with VCs patients). A history of smoking was recorded in 24.8% of diabetic patients without VCs, 28.9% in diabetic patients with VCs, and 18.2% of the healthy controls. Dyslipidemia was observed more frequently in diabetic group with VCs. The comparison of between patients groups and the control showed a matched age and gender with no statistically significant differences regarding smoking, diabetes disease duration, or hypertension.

The mean levels of body mass index (BMI), SBP, DBP, fasting blood sugar (FBS), 2-hr PBS, hemoglobin A1C (HbA1c %), total cholesterol, and LDL among the studied groups were significantly higher in diabetic patients (with or without VCs) compared to control group, but were not significantly different between both patient groups. Triglycerides level was significantly higher in diabetic patients with VCs compared to control group. HDL level was significantly lower in diabetic patients without VCs compared to control group.

There were significant difference in the level of PAI-1, TAFI, and D-dimer between studied groups, with the highest levels observed in DM with VCs more than DM without VCs and the lowest levels observed in the control group. PAI-1 median levels were (59, 43, and 32 respectively), while TAFI median levels were (89, 61, and 6.5 respectively) and D-dimer median levels were 2.4, 1.5, and 0.7 respectively).

In Table 2, the most prevalent genotype among the diabetic patients with vascular complications (VCs) was 4G/5G (56.6%), followed by 4G/4G (33.7%), and finally 5G/5G (9.6%). The allelic frequencies of the 4G and 5G alleles were 62.0% and 38% respectively. In diabetic patients without VCs, it was 41.0% with 4G/5G, 32.2% with 4G/4G, and 24.8% with 5G/5G comprising 54.7% with 4G allele and 45.3% with 5G allele. In the control group, 38.1% of the subjects had the 4G/5G genotype, 32.6% had the 4G/4G genotype, and 29.3% had the 5G/5G genotype. In this group, there were 51.7% with 4G allele and 48.3% with 5G allele. The genotype distribution among the three studied groups revealed a highly significant difference (P = 0.007), but there was no statistical difference regarding the allelic frequencies among the studied groups (P = 0.084). On adding the genotypes 4G/4G to 4G/5G in the genetic dominant model of 4G allele (4G/4G + 4G/5G), the distribution among the studied groups revealed more significant difference (P = 0.002) while it revealed non-significant difference (P = 0.957) in the genetic recessive model (4G/4G).

The analysis of genotype distribution, allelic frequencies, dominant, and recessive models of PAI-1 polymorphism in solely DM (without VCs) and control groups indicated that 4G/5G and 4G/4G genotypes were significantly associated (P < 0.05) with increased risk of DM as compared to 5G/5G genotype (OR = 1.27, 95% CI 0.71–2.28; OR = 1.24, 95% CI 0.68–2.27, respectively). Similar results were obtained for 4G allele compared to 5G allele (OR = 1.13,

Table 1 Comparison between control, DM without vascular complications, and DM with vascular complications groups regarding demographic and clinical data

Parameters	Control (*n* = 181)	DM without VCs (*n* = 117)	DM with VCs (*n* = 83)	*P* value
Age (year)				
Median (IQR)	46 (11)	48 (10)	49 (11)	0.089 $^{NS, 1}$
Range (min–max)	34–59	37–61	38–66	
Gender [*n* (%)]				
Male	137 (75.7)	84 (71.8)	61 (73.5)	0.750 $^{NS, 2}$
Female	44 (24.3)	33 (28.2)	22 (26.5)	
Smoking [*n* (%)]				
No	148 (81.8)	88 (75.2)	59 (71.1)	0.123 $^{NS, 2}$
Yes	33 (18.2)	29 (24.8)	24 (28.9)	
Dyslipidemia [*n* (%)]				
Absent	–	5 (4.3)	1 (1.2)	0.014 $^{S, 2}$
Present	–	112 (95.7) $_{a, b}$	82 (98.8) $_{b}$	
Hypertension [*n* (%)]				
Absent	–	18 (15.4)	11 (13.3)	0.287 $^{NS, 2}$
Present	–	99 (84.6)	72 (86.7)	
Duration of DM (year)				
Median (IQR)	–	3.6 (2.05)	3.8 (2.9)	0.539 $^{NS, 3}$
Range (min–max)	–	1.6–6.80	1.2–7.8	
PAI-1				
Median (IQR)	32.0 (16.5)$_{a}$	43.0 (19.5)$_{b}$	59.0 (23.0) $_{c}$	< 0.001$^{HS, 1}$
Range (min–max)	16.0–48.0	20.0–66.0	22.0–77.0	
TAFI				
Median (IQR)	6.5 (2.85) $_{a}$	61.0 (38.5) $_{b}$	89.0 (58.0) $_{c}$	< 0.001$^{HS, 1}$
Range (min–max)	1.1–10.4	22.0–112.0	34.0–164.0	
D-dimer				
Median (IQR)	0.7 (0.5) $_{a}$	1.5 (2.0) $_{b}$	2.4 (1.9) $_{c}$	< 0.001$^{HS, 1}$
Range (min–max)	0.3–1.5	0.4–3.5	0.5–4.5	
BMI (kg/m^3)				
Median (IQR)	25.5 (2.65)$_{a}$	28.3 (5.15) $_{b}$	28.1 (5.1) $_{b}$	< 0.001$^{HS, 1}$
Range (min–max)	22.0–28.4	24.2–37.0	23.7–35.1	
SBP (mmHg)				
Median (IQR)	120.0 (0.00) $_{a}$	120.0 (35.0) $_{b}$	142.0 (38.0) $_{b}$	< 0.001$^{HS, 1}$
Range (min–max)	110.0–130.0	110.0–168.0	110.0–170.0	
DBP (mmHg)				
Median (IQR)	80.0 (0.00) $_{a}$	88.0 (15.0) $_{b}$	90.0 (15.0) $_{b}$	< 0.001$^{HS, 1}$
Range (min–max)	70.0–90.0	70.0–112.0	70.0–120.0	
FBS (mg/dl)				
Median (IQR)	78.0 (8.0) $_{a}$	125.0 (26.5) $_{b}$	127.0 (40.0) $_{b}$	< 0.001$^{HS, 1}$
Range (min–max)	70.0–88.0	95.0–186.0	97.0–198.0	
2Hr PBS (mg/dl)				
Median (IQR)	90.0 (16.0) $_{a}$	142.0 (32.0) $_{b}$	155.0 (50.0) $_{b}$	< 0.001$^{HS, 1}$
Range (min–max)	76.0–115.0	102.0–225.0	112.0–227.0	
HbA1c (%)				

Table 1 Comparison between control, DM without vascular complications, and DM with vascular complications groups regarding demographic and clinical data *(Continued)*

Parameters	Control (n = 181)	DM without VCs (n = 117)	DM with VCs (n = 83)	P value
Median (IQR)	4.9 (0.6) $_a$	7.2 (1.7) $_b$	7.5 (2.1) $_b$	< 0.001 [HS, 1]
Range (min–max)	4.1–5.7	6.0–11.2	6.0–11.8	
T.C (mg/dl)				
Median (IQR)	176.0 (17.0) $_a$	195.0 (22.0) $_b$	198.0 (24.0) $_b$	< 0.001 [HS, 1]
Range (min–max)	143.0–204.0	153.0–232.0	153.0–240.0	
Triglycerides (mg/dl)				
Median (IQR)	156.0 (24.0) $_a$	160.0 (27.0) $_a$	166.0 (36.0) $_b$	< 0.001 [HS, 1]
Range (min–max)	126.0–206.0	126.0–210.0	142.0–210.0	
LDL (mg/dl)				
Median (IQR)	108.6 (16.9) $_a$	127.2 (22.9) $_b$	125.6 (27.2) $_b$	< 0.001 [HS, 1]
Range (min–max)	68.6–146.8	85.4–165.2	79.6–165.8	
HDL (mg/dl)				
Median (IQR)	36.0 (9.0) $_a$	33.0 (7.0) $_b$	38.0 (8.0) $_b$	< 0.001 [HS, 1]
Range (min–max)	22.0–48.0	22.0–47.0	23.0–48.0	

DM diabetes mellitus, *VCs* vascular complications, % percent within group, *IQR* interquartile range
[1]Kruskal-Wallis test; [2] Pearson chi Square test; [3] Mann-Whitney test
($_{a, b, c}$): Each subscript letter denotes the groups whose parameter do not differ significantly from each other using multiple pairwise comparisons adjusted by Bonferroni post hoc test at the level of 0.05
NS non-significant at P value ≥ 0.05; *S* significant at P value < 0.05; *HS* highly significant at P value < 0.01

95% CI 0.81–1.57), dominant model [4G/4G + 4G/5G vs. 5G/5G, (OR = 1.26, 95% CI 0.74–2.13)], and recessive model [4G/4G vs. 5G/5G + 4G/5G, (OR = 1.07 95% CI 0.66–1.76)].

Additionally, in DM with VCs and control group, both 4G/5G and 4G/4G genotypes had a highly significant increased risk of VCs associated DM as compared to 5G/5G (OR = 4.51, 95% CI 1.91–10.36, and P < 0.001; OR = 3.14, 95% CI 1.32–7.50, and P = 0.008, respectively). The subjects with the 4G allele also were associated significantly with increased risk of VCs of DM as compared with those carrying the 5G allele (OR = 1.53,

Table 2 Comparisons of genotype distribution and allele frequencies of *PAI-1* gene polymorphism between healthy control, DM without vascular complications, and DM with vascular complications groups

	Control (n = 181) n(%)	DM without VCs (n = 117) n(%)	DM with VCs (n = 83) n(%)	P value[a]	DM without VCS vs. control		DM with VCs vs. control		DM with VCs vs. DM without VCs	
					OR (95% CI)	P value[a]	OR (95% CI)	P value[a]	OR (95% CI)	P value[a]
PAI-1 genotypes										
5G/5G	53 (29.3)	29 (24.8)	8 (9.6)		Ref	–	Ref	–	Ref	–
4G/5G	69 (38.1)	48 (41.0)	47 (56.6)	0.007 [HS]	1.27 (0.71–2.28)	0.420[NS]	4.51 (1.97–10.36)	< 0.001[HS]	3.55 (1.47–8.56)	0.004[HS]
4G/4G	59 (32.6)	40 (34.2)	28 (33.7)		1.24 (0.68–2.27)	0.487 [NS]	3.14 (1.32–7.50)	0.008 [HS]	2.54 (1.01–6.37)	0.044 [S]
Dominant model										
5G/5G	53 (29.3)	29 (24.8)	8 (9.6)		Ref	–	Ref	–	Ref	–
4G/4G+ 4G/5G	128 (70.7)	88 (75.2)	75 (90.4)	0.002 [HS]	1.26 (0.74–2.13)	0.396 [NS]	3.88 (1.75–8.61)	< 0.001 [HS]	3.09 (1.33–7.16)	0.007 [HS]
Recessive model										
5G/5G + 4G/5G	122 (67.4)	77 (65.8)	55 (66.3)	0.957 [NS]	Ref	–	Ref	–	Ref	–
4G/4G	59 (32.6)	40 (34.2)	28 (33.7)		1.07 (0.66–1.76)	0.776 [NS]	1.05 (0.61–1.83)	0.855[NS]	0.98 (0.54–1.78)	0.947 [NS]
Alleles										
5G	175 (48.3)	106 (45.3)	63 (38.0)		Ref	–	Ref	–	Ref	–
4G	187 (51.7)	128 (54.7)	103(62.0)	0.084 [NS]	1.13 (0.81–1.57)	0.467 [NS]	1.53 (1.05–2.23)	0.026 [S]	1.35 (0.90–2.03)	0.143 [NS]

DM diabetes mellitus, *VCs* vascular complications, *OR* (95% CI) odd ratio with 95% confidence interval, % percent of genotype or allele within group
[a]Pearson Chi-square test; *NS* non-significant at P value ≥ 0.05; *S* significant at P value < 0.05; *HS* highly significant at P value < 0.01

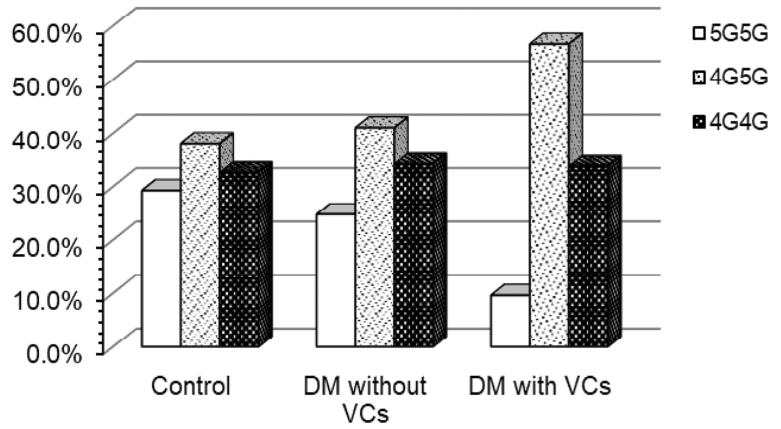

Fig. 1 Genotype distribution of PAI-1 gene polymorphism in the studied goups

95% CI 1.05–2.23, and P = 0.026). The 4G allele also showed a highly significant association with the risk of VCs of DM in dominant model (OR = 3.88, 95% CI 1.75–8.61, and P < 0.001) but different results were found in recessive model that showed non-significant association (OR = 1.05, 95% CI 0.61–1.83, and P = 0.855) (Table 2).

In the same way, considering both diabetic groups (with and without VCs), the 4G/5G genotype had a highly significant increased risk of VCs among diabetic patients, as compared to 5G/5G (OR = 3.55, 95% CI 1.47–8.56, and P = 0.004), whereas patients with 4G/4G genotype hardly had a significant risk (OR = 2.54, 95% CI 1.01–6.37, and P = 0.044). Patients carrying the 4G allele missed the significant association (OR = 1.35, 95% CI 0.90–2.03, and P = 0.143). However, the 4G allele in dominant model was associated highly significant with the increased risk of VCs (OR = 3.09, 95% CI 1.33–7.16, and P = 0.007) but the association was not significant in the recessive model (OR = 0.98, 95% CI 0.54–1.78), and P = 0.94.

Figure 1 showed that the most prevalent genotype among the diabetic patients with vascular complications (VCs) was 4G/5G (56.6%), followed by 4G/4G (33.7%), and finally 5G/5G (9.6%). In diabetic patients without VCs, it was 41.0% with 4G/5G, 32.2% with 4G/4G, and 24.8% with 5G/5G. In the control group, there were 38.1% of the subjects had the 4G/5G genotype, 32.6% had the 4G/4G genotype, and 29.3% had the 5G/5G genotype.

Figure 2 showed that among the diabetic patients with vascular complications (VCs) allelic frequencies of the 4G and 5G alleles were 62.0% and 38.%, respectively and the dominant model (4G/5G + 4G/4G) is more prevalent 90.4 % compared with the recessive model 4G/4G; 33.7%. In diabetic patients without VCs, comprising 54.7% with 4G allele and 45.3% with 5G allele and the dominant model (4G/5G + 4G/4G) is more prevalent 75.2 % compared with the recessive model 4G/4G; 34.2%. In the control group, there were 51.7% with 4G allele and 48.3% with 5G allele and the dominant model (4G/5G + 4G/4G) is more

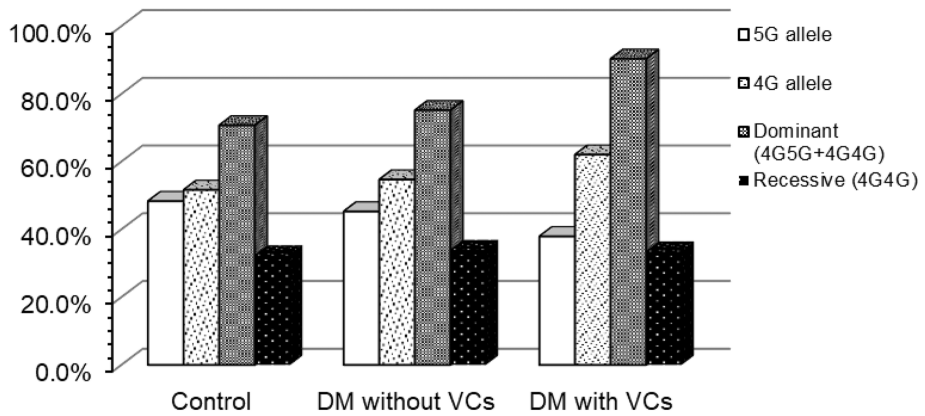

Fig. 2 Allelic frequencies, dominant and recessive genotypes of PAI-1 gene polymorphism in the studid groups

prevalent 77.7 % compared with the recessive model 4G/4G; 32.6%.

Table 3 showed comparison of clinical and biochemical data of the diabetes without vascular complication patient group and the genotypes of *PAI-1*. There were significant differences between the genotypes of *PAI-1* regarding the number of smokers and BMI of the patients with the 4G/5G genotype having the highest prevalence of smokers and highest BMI in the patients. In addition, significant differences were detected between the *PAI-1* genotypes regarding the levels of SBP, DBP, PAI-1, TAFI, D-dimer, dyslipidemia, FBS, 2Hrs BS, HbA1c %, total cholesterol, and LDL with the highest levels being expressed in 4G/5G genotype followed by 4G/4G genotype and the lowest level being expressed in 5G/5G genotype. Meanwhile, the level of triglycerides was the highest in 4G/4G genotype followed by 4G/5G genotype with the lowest level being expressed in 5G/5G genotype.

In contrast, no significant differences were observed between the *PAI-1* genotypes regarding age, gender, the diabetes duration nor the HDL level.

Table 4 showed comparison of clinical and biochemical data of the diabetes with vascular complication patients groups and the genotypes of *PAI-1*. There were significant differences between the *PAI-1* genotypes regarding the

Table 3 Comparison between *PAI-1* genotypes regarding demographic and clinical data in diabetes without vascular complication group

Parameters	*PAI-1* genotypes in DM without VCs			*P* value
	5G/5G (*n* = 29)	4G/5G (*n* = 48)	4G/4G (*n* = 40)	
Age (years)	48.0 (12.0)	47.0 (10.5)	48.0 (10.0)	0.890 [NS, 1]
Gender [*n* (%)]				0.498 [NS, 2]
Male	22 (75.9)	36 (75.0)	26 (65.0)	
Female	7 (24.1)	12 (25.0)	14 (35.0)	
Smoking [*n* (%)]				0.002 [HS, 2]
No	25 (86.2)	28 (58.3)	35 (87.5)	
Yes	4 (13.8) $_{a}$	20 (41.7) $_{b}$	5 (12.5) $_{a}$	
Dyslipidemia [*n* (%)]				0.017 [HS, 3]
Absent	4 (13.8)	1 (2.1)	0 (0.0)	
Present	25 (86.2) $_{a}$	47 (97.9) $_{a, b}$	40 (100.0) $_{b}$	
Hypertension [*n* (%)]				< 0.001 [HS, 3]
Absent	12 (41.4)	2 (4.2)	4(10.0)	
Present	17 (58.6) $_{a}$	46 (95.8) $_{b}$	36 (90.0) $_{b}$	
Duration of DM (years)	3.20 (1.65)	3.80 (2.10)	3.6 (2.03)	0.221 [NS]
PAI-1	27.0 (7.50)$_{a}$	55.0 (10.75)$_{b}$	41.5 (9.0)$_{c}$	< 0.001 [HS, 1]
TAFI	31.0 (13.50)$_{a}$	78.5 (24.0)$_{b}$	50.0 (21.50)$_{c}$	< 0.001 [HS, 1]
D-dimer (mg/dl)	0.60 (0.25)$_{a}$	2.80 (1.25)$_{b}$	1.25 (1.08)$_{c}$	< 0.001 [HS, 1]
BMI (Kg/m^3)	27.4 (2.80)$_{a}$	31.45 (6.38)$_{b}$	27.80 (3.73)$_{a}$	< 0.001 [HS, 1]
SBP (mmHg)	120.0 (8.0)$_{a}$	155.0 (40.0)$_{b}$	120.0 (29.5)$_{c}$	< 0.001 [HS, 1]
DBP (mmHg)	80.0 (10.5)$_{a}$	95.0 (21.0)$_{b}$	86.0 (11.5)$_{a}$	< 0.001 [HS, 1]
FBS (mg/dl)	117.0 (23.0)$_{a}$	132.0 (23.0)$_{b}$	124.50 (28.25)$_{a, b}$	< 0.001 [HS, 1]
2Hr BS (mg/dl)	132.0 (21.50)$_{a}$	155.5 (33.25)$_{b}$	140.0 (32.75)$_{a, b}$	0.003 [HS]
HbA1c (%)	6.7 (0.75)$_{a}$	7.7 (2.55)$_{b}$	7.15 (1.15)$_{a, b}$	< 0.001 [HS, 1]
T.C (mg/dl)	186.0 (17.5)$_{a}$	200.0 (23.25)$_{b}$	194.5 (25.25)$_{b}$	0.002 [HS, 1]
Triglycerides (mg/dl)	152.0 (46.5)$_{a}$	156.0 (22.5)$_{a, b}$	165.0 (37.25)$_{b}$	0.013 [S, 1]
LDL (mg/dl) (mean ± SD)	118.91 ± 12.49$_{a}$	130.78 ± 18.46$_{b}$	128.80 ± 17.71$_{b}$	0.011 [S, 4]
HDL (mg/dl)	34.0 (8.0)	32.0 (8.5)	33.0 (5.75)	0.528 [NS, 1]

Values are expressed as median (inter quartile range (IQR)) unless otherwise indicated; % percent within genotype subgroup
[1]Kruskal-Wallis test; [2] Pearson Chi-square test; and [3] Fisher's Exact test, adjusted for multiple pairwise comparisons by Bonferroni post hoc test; [4] ANOVA test, adjusted for multiple comparisons by Tukey HSD post hoc test
($_{a, b, c}$): Each subscript letter denotes the groups whose parameter do not differ significantly from each other after adjustment for multiple comparisons by post hoc test at the level of 0.05
NS non-significant at *P* value ≥ 0.05; *S* significant at *P* value < 0.05; *HS* highly significant at *P* value < 0.01

Table 4 Comparison between the genotypes of *PAI-1* regarding demographic and clinical data in diabetes with vascular complication group

Parameters	*PAI-1* genotypes in DM with VCs			*P* value
	5G/5G (*n* = 8)	4G/5G (*n* = 47)	4G/4G (*n* = 28)	
Age (years) (mean ± SD)	48.00 ± 5.76	49.06 ± 6.89	48.43 ± 5.95	0.867 [NS, 1]
Gender [*n* (%)]				0.933 [NS, 2]
Male	6 (75.0)	35 (74.5)	20 (71.4)	
Female	2 (25.0)	12 (25.5)	8 (28.6)	
Smoking [*n* (%)]				< 0.001 [HS,2]
No	7 (87.5)	25 (53.2)	27 (96.4)	
Yes	1 (12.5) $_{a, b}$	22 (46.8) $_{b}$	1 (3.6) $_{a}$	
Dyslipidemia [*n* (%)]				0.096 [NS, 2]
Absent	1 (12.5)	0 (0.0)	0 (0.0)	
Present	7 (87.5)	47 (100.0)	28 (100.0)	
Hypertension [*n* (%)]				0.385 [NS, 2]
Absent	2 (25.0)	5 (10.6)	4 (14.3)	
Present	6 (75.0)	42 (89.4)	24 (85.7)	
Duration of DM (years)	4.0 (2.9)	3.8 (3.8)	3.60 (2.23)	0.729 [NS, 3]
PAI-1	26.5 (6.25)$_{a}$	65.0 (10.0)$_{b}$	48.5 (11)$_{c}$	< 0.001 [HS, 3]
TAFI	46.5 (14.5)$_{a}$	115.0 (42.0)$_{b}$	74 (24)$_{a}$	< 0.001 [HS, 3]
D-dimer (mg/dl)	0.70 (0.20)$_{a}$	2.90 (0.80)$_{b}$	1.40 (0.98)$_{c}$	< 0.001 [HS, 3]
BMI (kg/m^3)	27.20 (2.15)$_{a,b}$	31.20 (5.40)$_{a}$	26.70 (2.65)$_{b}$	< 0.001 [HS, 3]
SBP (mmHg)	120.0 (32.50)$_{a, b}$	155.0 (40.0)$_{a}$	120 (17.0)$_{b}$	0.001 [HS, 3]
DBP (mmHg)	80 (20.50)$_{a,b}$	94 (26.00)$_{a}$	82 (10.0) $_{b}$	0.004 [HS, 3]
FBS (mg/dl)	122.5 (17.25)$_{a, b}$	145 (47)$_{a}$	121.50 (21.25)$_{b}$	0.027 [S, 3]
2Hr BS (mg/dl)	146.0 (18.5)	167.0 (55.0)	148.50 (34.75)	0.078 [NS, 3]
HbA1c (%)	7.0 (1.30)	8.0 (2.40)	7.20 (0.98)	0.055 [NS, 3]
T. C (mg/dl)	187.5 (21.75)$_{a}$	198.0 (22.0)$_{b}$	197.50 (35.00)$_{a, b}$	0.024 [S, 3]
Triglycerides (mg/dl)	151.5 (12.5)$_{a}$	170.0 (35.0)$_{b}$	166.0 (29.25)$_{b}$	< 0.001 [HS, 3]
LDL (mg/dl) (mean ± SD)	116.83 ± 8.55	130.11 ± 18.38	121.93 ± 21.33	0.084 [NS, 1]
HDL (mg/dl) (mean ± SD)	36.25 ± 5.18	37.09 ± 5.42	39.57 ± 4.03	0.077 [NS, 1]

Values are expressed as median (IQR) unless otherwise indicated; % percent within genotype subgroup
[1]ANOVA test, adjusted for multiple pairwise comparisons by Tukey HSD post hoc test, [2] Fisher's exact test, and [3] Kruskal-Wallis test, adjusted for multiple comparisons by Bonferroni post hoc test.
($_{a, b, c}$): Each subscript letter denotes the groups whose parameter do not differ significantly from each other after adjustment for multiple comparisons by post hoc test at the level of 0.05
NS non-significant at *P* value ≥ 0.05; *S* significant at *P* value < 0.05; *HS* highly significant at *P* value < 0.01

levels of PAI-1, TAFI, D-dimer, total cholesterol, and triglycerides with the highest levels being expressed in 4G/5G genotype followed by 4G/4G genotype and the lowest level being expressed in 5G/5G genotype.

There was no significant difference between the genotypes of *PAI-1* genotypes regarding age, gender, number of smokers, hypertension, diabetes duration, dyslipidemia, BMI, 2-Hrs PBS, HbA1c %, LDL nor the HDL levels.

In Table 5, univariable analysis was used to identify the potential risk factors associated with VCs among diabetic patients. The genotypes 4G/5G and 4G/4G were significantly associated risk factors meaning that carrying one 4G allele in dominant model (4G/4G + 4G/5G) had a significant risk (P = 0.009) for VCs (OR = 3.09, 95% CI 1.33–7.16). Additional risk factors significantly (P < 0.05) associated with VCs included PAI-1 (OR = 1.06, 95% CI 1.04–1.08), TAFI (OR = 1.04, 95% CI 1.03–1.05), D-dimer (OR = 1.58, 95% CI 1.20–2.07), FBS (OR = 1.01, 95% CI 1.00–1.03), 2hr BS (OR = 1.01, 95% CI 1.00–1.03), triglycerides (OR = 1.02, 95% CI 1.00–1.03), and HDL(OR = 1.16, 95% CI 1.09–1.24).

Many of these factors were highly correlated to each other and to gene polymorphism (data not shown) so the most relevant variable was selected to enter the

Table 5 Potential risk factors independently associated with vascular complications in diabetic patients

Variables	Univariable analysis		Multivariable analysis	
	OR (95% CI)	*P* value[a]	Adjusted OR (95% CI)	Adjusted*P* value[a]
Age (years)	1.03 (0.99–1.08)	0.147 ^{NS}	–	–
Sex (male)	1.09 (0.58–2.05)	0.791 ^{NS}	–	–
Smoking	1.23 (0.66–2.33)	0.515 ^{NS}	–	–
Hypertension	1.19 (0.53–2.67)	0.673 ^{NS}	–	–
Dyslipidemia	3.66 (0.42–31.93)	0.240 ^{NS}	–	–
Duration of DM(years)	1.11 (0.93–1.33)	0.242 ^{NS}	–	–
PAI-1 genotypes:	–	0.018 ^{S}	–	–
Genotype (5G/5G)	Ref. (1.00)	–	–	–
Genotype (4G/5G)	3.55 (1.47–8.56)	0.005 ^{HS}	–	–
Genotype (4G/4G)	2.54 (1.01–6.37)	0.047 ^{S}	–	–
Dominant: (4G/4G + 4G/5G)	3.09 (1.33–7.17)	0.009 ^{HS}	–	–
PAI-1	1.06 (1.04–1.08)	< 0.001 ^{HS}	1.06 (1.03–1.09)	< 0.001 ^{HS}
TAFI	1.04 (1.03–1.05)	< 0.001 ^{HS}	–	–
D-dimer (mg/dl)	1.58 (1.20-2.07)	< 0.001 ^{HS}	–	–
BMI (kg/m^3)	0.97 (0.89-1.06)	0.483 ^{NS}	–	–
FBS (mg/dl)	1.01 (1.00-1.03)	0.034 ^{S}	1.00 (0.99–1.02)	0.871 ^{NS}
2Hr PBS (mg/dl)	1.01 (1.00-1.03)	0.007 ^{S}	–	–
T.C (mg/dl)	1.01 (1.00-1.03)	0.083 ^{NS}	1.00 (0.98–1.01)	0.646 ^{NS}
Triglycerides (mg/dl)	1.02 (1.01-1.03)	0.006 ^{S}	1.01 (1.00–1.03)	0.107 ^{NS}
LDL (mg/dl)	1.00 (0.98-1.01)	0.680 ^{NS}	–	–
HDL (mg/dl)	1.16 (1.09-1.24)	< 0.001 ^{HS}	1.18 (1.11–1.26)	< 0.001 ^{HS}

DM diabetes mellitus, *OR* (95%CI) odd ratio with 95% confidence interval
NS non-significant at *P* value ≥ 0.05; *S* significant at *P* value < 0.05; *HS* highly significant at *P* value < 0.01
[a]Wald test

multivariable logistic regression model to avoid multicolinearity. Other conventional risk factors and confounders did not associate significantly ($P \geq 0.05$) with VCs, those included age, sex, smoking, hypertension, dyslipidemia, LDL, and duration of diabetes (Table 5). The *P* value at 0.1 was set for variable inclusion in multivariable model. Five variables, PAI-1, FBS, total cholesterol, triglycerides, and HDL, were included into the model where PAI-1 (OR = 1.06, 95% CI 1.03–1.09, and $P < 0.001$) and HDL OR = 1.18, 95% CI 1.11–1.26 and $P < 0.001$) were the only variables to be independent risk factors significantly associated with VCs in diabetic patients of the study population.

Discussion

The major causes of disability and death in patients with diabetes mellitus are vascular diseases, particularly atherosclerosis. Diabetes mellitus substantially increases the risk of developing coronary, cerebrovascular, and peripheral arterial diseases. A better understanding of the mechanisms leading to vascular dysfunction may help develop new strategies to reduce cardiovascular morbidity and mortality in patients with diabetes [18].

The *PAI*-1 gene, also known as serpin E1, is located on human chromosome 7q21.3-q22, spans 12.3 kb, and contains nine exons [19]. PAI-1 is a fast-acting fibrinolytic inhibitor. Increased plasma levels of PAI-1 are associated with increased incidences of thrombophilia and osteonecrosis [20]. The *PAI-1* 4G/5G polymorphism is associated with high levels of PAI-1, which is induced by the suppression of fibrinolysis by inhibition of the Plasminogen activator and promotion of thrombosis [21, 22].

In our study, there was an association between high levels of TAFI and D-dimers and other risk factors involved in vascular complications in T2DM. Those risk factors included smoking (in 28.9% of diabetic patients with VCs), dyslipidemia, and triglycerides (the level of which was significantly higher in diabetic patients with VCs). These findings agree with the study of Nilsson et al. [23] 2008, who stated that the etiology of cardiovascular disease is multifactorial but strongly involves genetic and environmental factors. An increase in PAI-1 in vulnerable atherosclerotic plaques associated with an increased inflammatory response might provide the necessary conditions for an atherothrombotic event [23].

Concerning the lipid profile, there were no statistically significant differences in the levels of HDL, LDL, total cholesterol, or triglycerides between diabetic patients without VCs and diabetic patients with VCs. These results were in agreement with those of Wijesuriya et al. [24] and Elnaggar et al. [25]. The HbA1c levels among the studied groups were significantly higher in both diabetic patients groups compared to the control group but were not significantly different between both patient groups. This result agreed with Elnaggar et al. [25], Rahimiet al. [26], and Mtiraoui et al. [27]. A study conducted by Eroglu et al. [28], however, found that the mean value of HbA1c was significantly higher in patients with diabetic nephropathy.

In the present study, there were significant differences in the plasma level of PAI-1 among the studied groups. The highest levels were observed in DM patients with VCs followed by DM patients without VCs. This result was in agreement with the study conducted by Madan et al. [29], who reported that PAI-1 was significantly increased in type 2 patients with microvascular complications, such as diabetic nephropathy. By contrast, Elnaggar et al. [25] reported no statistically significant association between the PAI-1 level and diabetic nephropathy [25].

In our study, the most prevalent genotype among all of the studied groups was 4G/5G (38.1% of controls, 41% of DM patients without VCs, and 56.6% of DM patients with VCs). The allelic frequency of the 4G allele was the highest (51.7% of controls, 54.7% of DM patients without VCs, and 62% of DM patients with VCs). In agreement with our results, Salas et al. [30] found that in the majority of populations around the world, the 4G allele appears at a greater frequency than the 5G allele [30].

Analyses of the genotype distribution, allelic frequencies, and dominant and recessive models of the *PAI-1* polymorphism in the solely DM (without VCs) and control groups indicated that the 4G/5G and 4G/4G genotypes were significantly associated with an increased risk of DM development compared to the 5G/5G genotype. Similar results were obtained for the 4G allele compared to the 5G allele, dominant model (4G/4G+4G/5G vs. 5G/5G), and recessive model (4G/4G vs. 5G/5G + 4G/5G). Similarly, Zhao and Huang [19] found that the *PAI-1* 4G/5G polymorphism was significantly associated with type 2 DM risk and that circulating PAI-1 levels could predict the development of type 2 DM [19].

In the same way, considering both diabetic groups (with and without VCs), the 4G/5G genotype was found to be a highly significant risk factor for VCs among diabetic patients compared to the 5G/5Ggenotype. Patients with the 4G/4G genotype did not have a significant risk of developing type2 DM. However, the 4G allele in the dominant model was highly significantly associated with an increased risk of VCs, but the association was not significant in the recessive model. Similar to our results, Abdel Rasol et al. [31] reported that the 4G/4G genotype contributed to the genetic susceptibility to diabetic retinopathy. A high level of PAI-1 was also independently associated with an increased risk of retinopathy among Egyptians. Additionally, in agreement with our findings, Salas et al. [30] showed that the 4G allele was a risk factor for myocardial infarction in diabetic patients.

In contrast to our findings, a meta-analysis performed by Kuanfeng et al. [32] showed that the *PAI-1* (4G/5G) polymorphism might not be a risk factor for DM, diabetic nephropathy, diabetic retinopathy, or diabetic coronary artery disease (CAD).

In the current study, diabetic patients without vascular complications with the *PAI-1* 4G/5G genotype had the highest prevalence of smoking, high BMI, high SBP and DBP, dyslipidemia, FBS, 2Hrs PBS, HbA1c %, total cholesterol, and LDL. Additionally, the highest levels of D-dimer, TAFI, and PAI-1 were found in patients with the 4G/5G genotype, followed by those with the 4G/4G genotype, and the lowest levels were expressed in those with the 5G/5G genotype. In diabetic patients with vascular complications, nearly the same results were reported, indicating that these risk factors are closely associated with the occurrence of thrombotic complications accompanying DM. The most commonly studied functional variant of the *PAI-1* gene is the guanine deletion polymorphism at position 675 relative to the transcription start site (rs1799889). The 4G/5G polymorphism is located in the *PAI-1* gene promoter region. The *PAI-1* -675 4G allele has higher transcriptional activity than the *PAI-1* -675 5G allele, and homozygous 675 4G is associated with higher plasma PAI-1 levels [9], which may explain our findings. In partial agreement with our finding, Salas et al. [30] found that 4G/4G homozygous subjects had the highest plasma concentrations of PAI-1; the lowest plasma concentrations were observed in subjects with the 5G/5G genotype, and intermediate concentrations were recorded in heterozygote subjects (4G/5G) [30]. The differences between our results and those of other studies may be explained by differences in the distribution of the 4G/5G polymorphism as a determining factor for the plasma concentration of PAI-1.

The *PAI-1* 4G/5G polymorphism is a DNA sequence variation that plays a key role in regulating *PAI-1* gene expression. Studies have shown that the PAI-1 activity of the 4G allele promoter is higher than that of the 5G allele promoter in a cytokine-stimulated state. The *PAI-1* 4G/5G polymorphism also influences *PAI-1* gene transcription similarly in non-stimulated cells [19]. Unlike

the 5G allele, which binds a transcription repressor protein, resulting in low *PAI-1* expression, the 4G allele does not bind a transcription repressor and those confers high *PAI-1* expression [33, 34].

Univariable analysis was used to identify the potential risk factors associated with VCs among diabetic patients. The 4G/5G and 4G/4G genotypes were significantly associated risk factors, meaning that carrying one 4G allele in the dominant model (4G/4G+4G/5G) had a significant risk for VCs. Additional risk factors significantly associated with VCs included PAI-1, TAFI, d-dimer, FBS, 2 hr PBS, triglycerides, and HDL. Other conventional risk factors and confounders did not significantly associate with VCs. The other conventional risk factors and confounders included age, sex, smoking, hypertension, dyslipidemia, LDL, and duration of diabetes. In the multivariable model, five variables, PAI-1, FBS, total cholesterol, triglycerides, and HDL, were included, and PAI-1 and HDL were the only variables that were independent risk factors significantly associated with VCs in diabetic patients in the study population.

Variability of PAI-1 plasma concentrations has been reported in different ethnic groups around the world [25]. In some cases, this variability appears to be governed by the 4G/5G polymorphism, while in others, environmental factors, such as smoking, are involved along with certain components of metabolic syndrome, such as dyslipidemia, obesity, and insulin concentration or the interaction between smoking and this syndrome, which increase the risk of cardiovascular disease [26]. The possibility that PAI-1 plays a role in the development of vascular diseases is supported by the biological characteristics of PAI-1 and the association between high plasma PAI-1 and other vascular disease risk factors [26].

Conclusion

In conclusion, the present study suggests that the *PAI-1* polymorphism can be considered a risk factor that influences thrombotic events in T2DM. The *PAI-1* polymorphism might be useful in further research to provide new strategies for the prevention and treatment of these complications in their early stages.

Abbreviations

2 Hr PBS: 2-Hours post brandial sugar; ADA: American Diabetes Association; AHA: American Heart Association; BMI: Body mass index; CAD: Coronary artery disease; CI: Confidence intervals; CSs: Cerebral strokes; DBP: Diastolic blood pressure; DM: Diabetes mellitus; DN: Diabetic nephropathy; DNA: Deoxy ribonucleic acid; DVT: Deep venous thrombosis; EDTA: Ethylene-diamine-tetraacetic acid; ELISA: Enzyme-linked immunosorbent assay; FBS: fasting blood sugar; HbA1c: Hemoglobin A1C; HDL: High-density lipoprotein; HRP: Horseradish peroxidase; IQR: Interquartile range; LDL: Low-density lipoprotein; MI: Myocardial infarction; mRNA: Messenger ribonucleic acid; OD: Optical density; OGTT: Oral glucose tolerance test; ORs: Odds ratios; PAI-1: Plasminogen activator inhibitor-1; PCR: Polymerase chain reaction; SBP: Systolic blood pressure; SD: Standard deviation; SPSS: Statistical package of social sciences; T1DM: Type 1 diabetes mellitus; T2DM: Type 2 diabetes mellitus; TAFI: Thrombin activatable fibrinolysis inhibitor; TC: Total cholesterol; TG: Triglycerides; tPA: Tissue-type plasminogen activator; VCs: Vascular complications; VLDL: Very-low-density lipoprotein

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Authors' contributions

FAK participated in study design and coordination and helped to draft the manuscript. HR carried out the detection of PAI-1 genotyping by real-time PCR. HMB carried out the laboratory investigation and the immunoassays. MMA participated in study design and coordination and helped to draft the manuscript. AAE choose the patients attending Internal Medicine Departments of Helwan University Hospitals. STA choose the patients attending Internal Medicine Department o Al Zahraa hospital, AL-Azhar University. WM performed the statistical analysis. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

Written consent was obtained from all participants. The study was approved by the ethical committee of National Liver Institute-Menoufia University "The Institution Review Board (IRB) of the National Liver Institute (NLI) , Menoufia University," NLI IRB Protocol Number: 00164/2018. Name of the IRB: NLI IRB 00003413. All procedures performed in this work were carried out in accordance with the 1964 Helsinki declaration and its later amendment.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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References

1. Stumvoll M, Goldstein BJ, van Haeften TW (2005) Type 2-diabetes: principles of pathogenesis and therapy. Lancet 9467:1333–1346. https://doi.org/10.1016/S0140-6736(05)61032-X
2. Guariguata L, Whiting DR, Hambleton I, Beagley J, Linnenkamp U, Shaw JE (2014) Global estimates of diabetes prevalence for 2013 and projections for 2035. Diabetes Res ClinPract 103:137–149. https://doi.org/10.1016/j.diabres.2013.11.002
3. Benjamin EJ, Blaha MJ, Chiuve SE, Cushman M, Das SR, Deo R et al (2017) Heart disease and stroke statistics-2017 update: a report from the American Heart Association. Circulation 135:e146–e603. https://doi.org/10.1161/CIR.0000000000000485
4. Low Wang CC, Hess CN, Goldfine AB (2016) Clinical update: cardiovascular disease in diabetes mellitus: atherosclerotic cardiovascular disease and heart failure in type 2 diabetes mellitus – mechanisms, management, and clinical considerations. Circulation 133:24. https://doi.org/10.1161/CIRCULATIONAHA.116.022194
5. Johansson L, Jansson JH, Boman K, Nilsson TK, Stegmayr B, Hallmans G (2000) Tissue plasminogen activator, plasminogen activator inhibitor-1, andtissue plasminogen activator/plasminogen activator inhibitor-1 complexas risk factors for the development of a first stroke. Stroke 31:26–32

6. Rabieian R, Boshtam M, Zareei M, Kouhpayeh S, Masoudifar A, Mirzaei H (2018) Plasminogen Activator Inhibitor Type-1 as a Regulator of Fibrosis. J Cell Biochem 119:17–27
7. Crandall DL, Quinent EM, Morgan GA, Busler DE, Mchendry-Rinde B, Kral AG (1999) Synthesis and Secretion of Plasminogen Activator Inhibitor-1 by Human Preadipocytes. J Clin Endocrinol Metab 84(9):3222–3227
8. Chevilley A, Lesept F, Lenoir S, Ali C, Parcq J, Vivien D (2015) Impacts of tissue-type plasminogen activator (tPA) on neuronal survival. Front Cell Neurosci. 9:415
9. Eriksson P, Kallin B, van't Hooft FM, Båvenholm P, Hamsten A (1995) Allele-specific increase in basal transcription of the plasminogen-activator inhibitor 1 gene is associated with myocardial infarction. Proc Natl Acad Sci U S A 92: 1851–1855
10. Nauck M, Wieland H, Marz M (1999) Rapid Homogeneous Genotyping of the 4G/5GPolymorphism in the Promoter Region of the PAI1Gene by Fluorescence Resonance Energy Transfer and Probe Melting Curves. Clinical Chemistry 45(8):1141–1147
11. Nordt TK, Lohrmann J, Bode C (2001) Regulation of PAI-1expression by genetic polymorphism. Impact on atherogenesis. Thromb Res 103(Suppl 1):S1–S5
12. Serrano Rios M (2007) The 4G/4G PAI-1 genotype is associated with elevated plasma PAI-1 levels regardless of variables of the metabolic syndrome and smoking status. A population-based study in Spanish population. Diab Obes Metab 9:134–136
13. Boeckoldt SM, Bijsterveld NR, Moon AH, Levi M, Buller HR, Peters RJ (2001) Genetic variation in coagulation and fibrinolyticprotein and their relation with acute myocardial infarction: asystematic review. Circulation 104:3064–3068
14. Kholer HP, Grant PJ (2000) Plasminogen-activator inhibitor type1 and coronary artery disease. N Engl J Med. 342:1792–1801
15. Hindorff LA, Schwartz SM, Siscovick DS, Psaty BM, Longstreth WT Jr, Reiner AP (2002) The association of PAI-1 promoter 4G/5Ginsertion/deletion polymorphism with myocardial infarction and stroke in young women. J Cardiovasc Risk 9:131–137
16. Vaughan DE, Rai R, Khan SS, Eren M, Ghosh AK (2017) Plasminogen Activator Inhibitor-1 Is a Marker and a Mediator of Senescence. Arterioscler Thromb Vasc Biol 37:1446–1452
17. Duggan E, O'Dwyer MJ, Caraher E, Diviney D, McGovern E, Kelleher D, McManus R and Ryan T (2007) Coagulopathy After Cardiac Surgery May Be Influenced by a Functional Plasminogen Activator Inhibitor Polymorphism. AnesthAnalg 104(6):1343–1347
18. Paneni F, Beckman JA, Creager MA, Cosentio F (2013) Diabetes and Vascular Disease Pathophysiology, Clinical Consequences, and Medical Therapy: Part I. Eur Hear J 30(31):2436–2443
19. Zhao L, Huang P (2013) Plasminogen activator inhibitor-1 4G/5G polymorphism is associated with type 2 diabetes risk. Int J ClinExp Med. 6 (8):632–640
20. Liang XN, Xie L, Cheng JW, Tan Z, Yao J, Liu Q et al (2013) Association between PAI-1 4G/5GPolymorphisms and osteonecrosis of femoral head: a meta-analysis. Thromb Res. 132(2):158–163
21. Cesari M, Pahor M, Incalzi RA (2010) Plasminogen activator inhibitor-1 (PAI-1): A key factor linking fibrinolysis and age-related subclinical and clinical conditions. CardiovascTher. 28(5):e72–e79
22. Xie X, Shi X, Xun X, Rao L (2017) Endothelial nitric oxide synthase gene single nucleotide polymorphisms and the risk of hypertension: a meta-analysis involving 63,258 subjects. Clin Exp Hypertens 39(2):175–182
23. Nilsson JB, Boman K, Jansson JH, Nilsson T, Näslund U (2008) The influence of acute-phase levels of haemostatic factors on reperfusion and mortality in patients with acute myocardial infarction treated with streptokinase. J Thromb Thrombolysis 26:188–195
24. Wijesuriya MA, De-Arbew WK, Weerathunga A et al (2012) Association of chronic complications of type 2 diabetes with the biochemical and physical estimations in subjects attending single visit screening for complications. J Diabetol:1–3
25. Elnaggar AA, Fawzy MM, Nabawy EL, Kamal MM, Ibrahim NM (2017) The Association of Plasminogen Activator Inhibitor (PAI-1) Level and 4G/5G Gene Polymorphism with Diabetic Nephropathy in Type 2 Diabetes Mellitus. J Am Sci 13(11)
26. Rahimi M, HasanVand A, Rahimi Z et al (2010) Synergestic effects of MTHFR C677Tpolymorphisms on the increased risk of microand macro-albuminuria and progression ofdiabetic nephropathy among Iranians with type IIdiabetes mellitus. Clin Biochem 43:1333–1339
27. Mtiraoui N, Ezzidi I, Chaib M et al (2017) MTHFR C677T and A1298C gene polymorphism and hyperhomocysteinemia as a risk factor for diabetic nephropathy in diabetic patients. Diabet Res Clin Pract. 75:99–106
28. Eroglu Z, Erdogan M, Tetik A, Xilmaz C (2007) The relationship of MTHFR C677T gene polymorphism in Turkish type 2 diabetic patients with and without nephropathy. Diabet Metab Res Rev 23:621–624
29. Madan R, Gupta B, Saluja S et al (2010) Coagulation Profile in Diabetes and itsAssociation with Diabetic MicrovascularComplications. JAPI 58:481–484
30. Salas II, Miranda AL, Sainz IM, Maldonado RE, Sánchez GB (2009) Association of the Plasminogen Activator Inhibitor-1 Gene 4G/5G Polymorphism with ST Elevation Acute Myocardial Infarction in Young Patients. Rev Esp Cardiol 62 (4):365–372
31. Abdel Rasol HA, Attia FM, Ismail S, Abdel Azeem AA, Nowier SR, Aziz MA, Osman ZM (2012) Association between 4G/4G plasminogen activator inhibitor-1 polymorphism, PAI-1 activity, and diabetic retinopathy. Egypt J Haematol [serial online] [cited 2019 Apr 21] 37:81–87
32. Xu K, Xiaoyun L, Yang F, Cui D, Yun S, Chong S, Tang W, Yang T (2013) PAI-1 -675 4G/5G Polymorphism in association with diabetes and diabetic complications susceptibility: a meta-analysis study. PLoS One 8(11):e79150. https://doi.org/10.1371/journal.pone.0079150
33. Franco RF, Reitsma PH (2011) Gene polymorphisms of the haemostatic system and the risk of arterial thrombotic disease. Br J Haematol 115:491–506
34. Festa A, Williams K, Tracy RP, Wagenknecht LE, Haffner SM (2006) Progression of plasminogen activator inhibitor-1 and fibrinogen levels in relation to incident type 2 diabetes. Circulation 113:1753-1759

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