

The Association of Plasminogen Activator Inhibitor (PAI-1) Level and 4G/5G Gene Polymorphism with Diabetic Nephropathy in Type 2 Diabetes Mellitus

Ahmed Abdelmoaty Elnaggar¹, MD, Mai Mohsen Fawzy¹, MD, Waleed Elnabawy², MD, Manal Mohamed Kamal³, MD; and Nahed Mohamed Ibrahim³, MD.

¹Internal Medicine Department, Faculty of Medicine, Cairo University, Egypt

²Internal Medicine Department, Faculty of Medicine, Beni swef University, Egypt

³Clinical and Chemical Pathology Department, Faculty of Medicine, Cairo University, Egypt
aanaggar71@hotmail.com

Abstract: Background and purpose: Plasminogen activator inhibitor-1 (PAI-1) is a regulatory enzyme of fibrinolytic system point mutation. PLA-1 is implicated in the pathogenesis of diabetic nephropathy (DN) in many ethnic groups. The aim of the study was to detect the association of 4G/5G PAI-1 polymorphism as a risk factor for DN in type 2 diabetes mellitus (T2DM) patients. **Study design:** case control study. **Place:** Internal Medicine department, Chemical Pathology department, Kasr Al Ainy Hospital **Subjects & Method:** ninety patients with type 2 DM divided into two equal groups one without diabetic nephropathy and the other had diabetic nephropathy. **Assessment:** The assessment was carried out by obtaining full personal history and history of diabetes (onset, duration and complications). Laboratory investigations were done including (fasting blood sugar, lipid profile, glycosylated hemoglobin, albumin / Creatinine ratio, genomic DNA extraction and analysis of PAI gene polymorphism by single- specific primer (SSP)- PCR. Plasma level of PAI-1 was assayed by ELISA). The gained measures were analyzed by using SPSS program, t-test and chi-square were used to compare between groups. **Results:** The genomic DNA analysis of (PAI-1) 4G/5G gene polymorphism (rs 1799889) revealed no statistically significant difference in the distribution of different genotypes ($p= 1.0$). The frequency of 4G/4G genotype was (8.9% and 11.1%), 4G/5G genotype was (48.9% and 48.9%) and 5G/5G genotype was (42.2% and 40%) in group one (diabetic patient without nephropathy) and group two (diabetic patients with nephropathy) respectively. Plasma levels of PAI-1 were not associated with PAI-1 mutation, also there was no statistically significant difference between PAI-1 level and DN ($p= 0.548$). **Conclusion:** PAI-1 gene mutation was not associated with increased risk for diabetic nephropathy in type 2 diabetic patients and had no effect on plasma levels of PAI-1.

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Key words: diabetes mellitus type 2 / diabetic nephropathy / Plasminogen activator inhibitor-1.

1. Introduction:

Diabetes mellitus is a chronic disease that requires continuing medical care to support the patient and prevent complications. Characterized by decreased production of insulin together with insulin resistance [1]. With increase in the duration of diabetes many complications arise including diabetic nephropathy [2]. Diabetic nephropathy is defined as increased protein excretion in urine firstly it is microalbuminuria and finally macroalbuminuria and proteinuria [3]. The Plasminogen activator inhibitor-1 (PAI-1) gene variant is deeply studied overall. PAI-1 is important cytokine of the fibrinolytic system that regulates the degradation of the extracellular matrix and involved in many physiopathologic mechanisms [4]. The PAI-1 gene is located at chromosome 7 in human as the 4G/5G polymorphism is most studied. The association between the DN and 4G/5G variants has been reported in several studies [5].

2. Patients and Methods

This is a case control included ninety patients with type 2 DM divided into two equal groups one without diabetic nephropathy and the other has diabetic nephropathy, excluded from other causes of proteinuria (urinary tract infection, sever hypertension and hematuria) and non-diabetic kidney disease. The assessment was carried out by obtaining full personal history, history of diabetes (onset, duration and complications), clinical examination including abdominal examination and abdominal ultrasound and CT scan. Laboratory investigations were done including (fasting blood sugar, lipid profile, glycosylated hemoglobin, blood urea and Creatinine, albumin / creatinine ratio, Plasma level of PAI-1 was assayed by ELISA. Genomic DNA extraction and analysis of PAI gene polymorphism by single- specific primer (SSP)- PCR using the following primers [6].

4 G	5'-GTC TGG ACA CGT GGG GA 3'
5 G	5'-GTC TGG ACA CGT GGG GG 3'
COMMON REVERSE	5'- TGC AGC CAG CCA CGT GAT TGT CTA G 3'

The gained measures were analyzed by using SPSS program, t-test and chi-square were used to compare between groups.

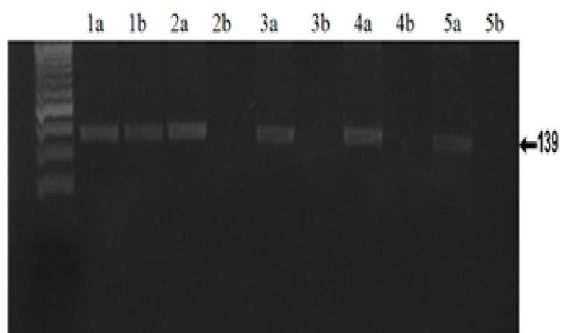


Figure (1) showing the identified bands after UV transillumination 1a, 1b are heterozygous genotype (4G/5G) 2a, 2b, 3a, 3b, 4a, 4b, 5a, 5b are homozygous genotype (4G/5G) a is 5G while b is 4G

3. Results:

This study was conducted on 90 type II diabetes patients who were recruited from diabetes outpatient clinic in internal medicine department and they were

divided into two equal groups first without diabetic nephropathy and second one with diabetic nephropathy.

The studied groups showed no statistically significant difference between two groups as regards age, weight and height and showed significant difference in the duration of diabetes as illustrated in table (1):

As regards to different laboratory investigations in the two studied groups, it was found that urea, creatinine urinary albumin and albumin/creatinine ratio were significantly higher group II (diabetic patient with nephropathy) while There are no statistically significant difference between the two groups as regards PAI-1 level as in table (2).

As regards to other measured biochemical parameters as fasting blood glucose, glycosylated hemoglobin, cholesterol, TG, LDL, HDL, urine creatinine and PAI-1 level showed no statistically significant difference between two studied groups as in figure (2) and (3).

As regards PAI-1 genotypes frequencies, there was no statistically significant difference between two groups, 4G/5G genotype was the most common genotype in the two groups as in table (3):

Table (1): Age, weight, height and duration of diabetes distribution

Studied parameters	Group I (n=45)		Group II (n=45)		P-value
	Mean	SD	Mean	SD	
Age (Year)	61.5	±8.07	62.8	±7	0.413
Weight (Kg)	85.8	±24	80.3	±4.9	0.140
Height (Cm)	162.1	±22.4	167.8	±2.9	0.101
Duration of diabetes in years	6.6	±3.2	8.8	±2.6	0.001*

Table (2): Urea, Creatinine, urinary albumin and albumin/creatinine ratio in both groups.

Biochemical parameters	Group I (n=45) Median	Group II (n=45) Median	P-value
Urea (mg/dl)	25 (5.6-6.6)	67(42.5-106.5)	0.000*
Creatinine (mg/dl)	0.9(0.6-1.3)	2.9(2.2-4.05)	0.000*
Urinary albumin (Ug/dl)	3.7(1.5-7)	186(91.6-432.5)	0.000*
Albumin/Creatinine (Ug/mg Cr.)	2.6(0.9-5.2)	121(64-315.9)	0.000*
PAI-1 level	14.3 (10.7 – 24)	14.6 (8.3 – 27.5)	0.548

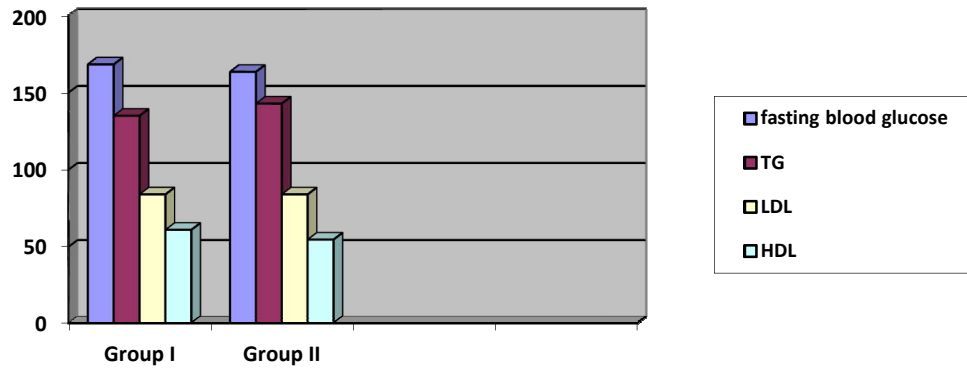


Figure (2): Fasting blood glucose, TG, LDL, HDL among both groups

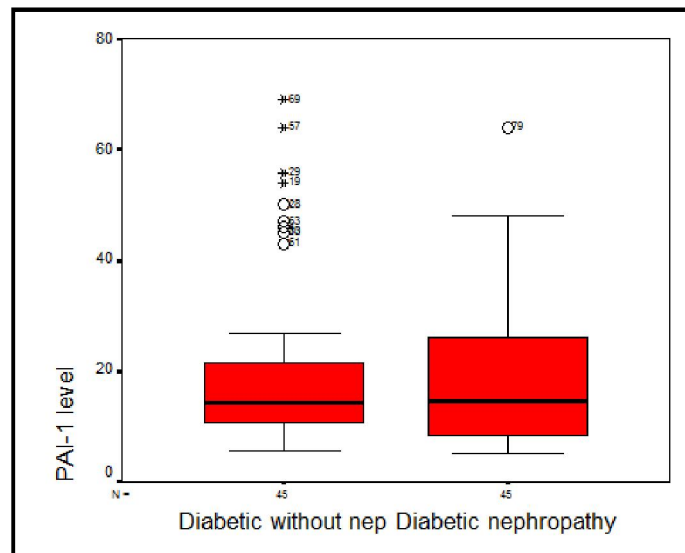


Figure (3): PAI-1 level among studied groups

PAI-1	Group I (n=45)	Group II (n=45)	P-value
4G/4G	4 (8.9%)	5 (11.1%)	1.0
4G/5G	22 (48.9%)	22 (48.9%)	
5G/5G	19 (42.2%)	18 (40%)	

4. Discussion

The present study is a case control study that examined the relationship between the Plasminogen activator inhibitor-1 (PAI-1) 4G/5G polymorphism and diabetic nephropathy. Serum level of PAI-1 were assessed from diabetic patients at the outpatient clinic at Cairo University hospitals. They were divided into two groups according to the presence of diabetic nephropathy.

In the current study, age, weight and height showed no statistically significant difference between the two groups with the (p value =0.413, 0.140 and

0.101) respectively. This was in agreement of the study of **Rahimi et al.**, [7] (p= 0.328).

In this study patients with diabetic nephropathy had longer disease duration than those without diabetic nephropathy (p=0.001). Disease duration is an established risk factor for occurrence of complications. This was in agreement of a Chinese study conducted by **Liu et al.** [8] which proved that chronic complications had significantly increased with the duration of the diabetes (p=0.007).

Concerning lipid profile there was no statistically significant difference in the levels of HDL, LDL, cholesterol and triglycerides between the two groups.

These results was in agreement with **Wijesuriya et al., [9]** (p=0.06). The mean value of HbA1c showed no statistically significant difference between the two groups (p=0.251) which was in agreement of **Rahimi et al., [6]** and **Mtiraoui et al [10]**. On the other hand the study of **Eroglu et al., [11]** found the mean value of HbA1c was significantly higher in patients with diabetic nephropathy (p=0.002).

In the present study there was no statistically significant association between PAI-1 level and diabetic nephropathy (p=0.548). This was not in agreement with the study of **Madan et al. [12]** who reported that PAI-1 was significantly increased in type 2 diabetic nephropathy with microvascular complications (p=0.001). In the current study the genomic DNA analysis of (PAI-1) 4G/5G gene polymorphism revealed no statistically significant difference in the distribution of different Phenotypes (p=1.0) and this was in agreement of the study of **AL-Hamodi et al. [13]**.

Conclusion

In conclusion, plasminogen activator inhibitor-1 gene mutation was not associated with increased risk for diabetic nephropathy in type 2 diabetic patients.

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