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Study of Angiotensin Converting Enzyme Gene Polymorphism in Egyptian Type 2 Diabetes Mellitus with Diabetic Kidney Disease

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Abstract

Objective: Diabetic kidney disease DKD (Diabetic nephropathy DN) is considered one of the chronic micro vascular complications of diabetes mellitus and considered the commonest cause leading to chronic renal failure and chronic renal dialysis. Genetic susceptibility has been implicated in DKD. The angiotensin converting enzyme (ACE) is one of the key roles in the renin angiotensin system cascade by converting angiotensin I to angiotensin II which plays a key role in regulation of blood pressure as well as electrolytes and fluid balance. This study addressed the association of (ACE) gene polymorphisms with DN in Egyptian (T2DM) patients. Methods: Our research comprised of 75 cases of T2DM with diabetic kidney disease, 100 cases of T2DM without DKD and 94 healthy volunteers. Different genotypes of ACE gene were determined by SSP-PCR analysis. Results: Gene polymorphism of ACE (DD, ID, II) in diabetic patient with DKD is 44%, 52%, 4% respectively and for T2DM individuals without DKD is 23%, 72%, 5% respectively. (DD) had significant higher frequencies in T2DM patients with DKD compared to those without DKD ($p < 0.005$) and (ID) had significant higher frequencies in T2DM without DKD ($p < 0.0001$). These results indicated that there is an association between ACE gene polymorphisms and susceptibility of diabetic patients to be affected by diabetic kidney disease. Conclusion: From our results, we can conclude that genotype of ACE in Egypt DD is the genotype of cases diabetic kidney disease. So the presence of D allele has a significant relation with diabetic kidney disease. Our data confirm the role of ACE in its relationship with diabetic kidney disease in Egyptian type 2 diabetic patients.

Keywords

ACE Gene Polymorphism, Insertion/Deletion, Type 2 Diabetes Mellitus, T2DM, Diabetic Kidney Disease, Diabetic Nephropathy, Microvascular Complications of Diabetes Mellitus

1. Introduction

Diabetic kidney disease DKD (Diabetic nephropathy DN) is a clinical syndrome characterized by persistent albuminuria (>300 mg/d or >200 μg/min) that is confirmed on at least 2 occasions 3 - 6 months apart, progressive decline in the glomerular filtration rate (GFR) and elevated arterial blood pressure [1].

Diabetic kidney disease is considered one of the most common causes for chronic renal failure and chronic hemodialysis [2]. Moreover, DKD also was considered one of the micro vascular complications in diabetic individuals and one of the leading causes of high mortality among patients with diabetes [3]. There are different etiologies that carry major and a key role that affects onset and progression of DKD, of these factors genetic predisposition and environmental circumstances. A genetic susceptibility, depends on familial clustering of DKD, has been implicated in different pathogenic background of DKD in T2DM individuals [4]. One possible genetic factor is the Angiotensin converting enzyme gene (ACE).

Gene insertion (I), deletion (D) polymorphism within the human ACE gene [5], shown to be associated with predisposition to emerging different T2DM complications, including diabetic eye disease [6] and DKD [7]. ACE possesses a crucial role in the regulation of conversion process of renin angiotensin system by controlling conversion angiotensin I to II [8].

Angiotensin II (Ang II) considered a very strong vasoconstriction factor of the systemic and the local blood pressure [9]. Ang II increases systemic and glomerular blood pressure, stimulates mesangial cell proliferation and tissue growth [10].

Several polymorphisms depend on the presence or absence of a 287 base pair sequence in intron 16, three main different genotypes homozygotes (DD, II) and heterozygote ID are found [11].

2. Methodology

2.1. Aim of the Work

The aim of our research was to check for the association of ACE gene polymorphisms with the susceptibility to Diabetic kidney disease in Egyptian individuals with T2DM.

2.2. Subjects

This research has included 175 subjects with type 2 Diabetes Mellitus. They were

recruited from the Internal Medicine Hospital (Diabetes clinic), Mansoura University, Egypt in the period between May and December 2017. The ethics committee approved the study protocol and the study was carried out in accordance with the Declaration of Helsinki. Written informed consent was obtained from all the patients included in the study.

An inclusion criterion includes type 2 Diabetic patients fulfilling criteria of diabetic nephropathy. **An exclusion criterion includes**, Type 1 diabetes mellitus, gestational diabetes, secondary diabetes, associated autoimmune diseases, non diabetic kidney diseases, patients suffering from hematuria, acute infections particularly urinary tract infections, and pregnant females were excluded from the study.

Selected Diabetic patients divided into 2 groups according to the presence of nephropathy consists of 75 subjects affected with diabetes type 2 associated with DKD compared to 94 healthy volunteers. In the Type 2 diabetic patients affected with Diabetic kidney disease, the mean ± SD age was 58.03 ± 6.34 ranges from 45 to 74 years. They were in the form of 38 (50.7%) males and 37 (49.3%) females. The other group was affected with diabetes type 2 without DKD (n = 100), their mean ± SD age was 52.1900 ± 8.31901 range from 34 to 75 years; the gender divided between 30 (30.0%) males and 70 (70.0%) (Table 1).

3. Method

All subjects were questioned about history of diabetes mellitus, hypertension, hypercholesterolemia, history of DKD in the first degree relatives. Regarding clinical examination, blood pressure, weight, heights were measured. Laboratory investigation was done and included detection of urinary albumin with the following cutoff values (microalbuminuria, (Albumin/creatinine ration (ACR) between 30 - 300 mg/g) and macroalbuminuria, (ACR more than 300) (according to national kidney foundation), detection of Glycated hemoglobin (HbA1c), lipid profile (Table 2).

Table 1. Descriptive data of all studied cases of (T2DM with nephropathy, T2DM without nephropathy and healthy people) regarding their characteristics.

Groups		N	Mean
Age	T2DM with neuropathy	75	58.03 ± 6.34
	T2DM without neuropathy	100	52.1900 ± 8.31901
	Health control	94	51.179 ± 9.217
		Male	**Female**
Sex	T2DM with neuropathy	50.7%	49.3%
	T2DM without neuropathy	30%	70%
	Health Control	46.8%	53.2%
Body mass index (kg/rn^2)	T2DM with neuropathy	75	28.3 ± 4.1
	T2DM without neuropathy	100	28.6 ± 5.2
	Health control	94	26.7 ± 3.6

Data are means and SD.

Table 2. Descriptive data of the two groups (T2DM with nephropathy and T2DM without nephropathy) regarding clinical findings.

Parameters	Groups		Mean ± SD
duration of diabetes (Years)	T2DM with neuropathy		14.85 ± 5.032
	T2DM without neuropathy		14.74 ± 4.15
HbAlc (%)	T2DM with neuropathy		8.4 ± 1.2
	T2DM without neuropathy		8.1 ± 1.6
Retinopathy	T2DM with neuropathy	Background	52/75 (69.33%)
		Proliferative	21/75 (28%)
	T2DM without neuropathy	Background	18/100 (18%)
		Proliferative	4/100 (4%)
Creatinine (mg/dl)	T2DM with neuropathy		1.2 ± 0.9
	T2DM without neuropathy		0.8 ± 0.2
Cholesterol (mg/dl)	T2DM with neuropathy		210.22 ± 69.16
	T2DM without neuropathy		155 ± 44.32
Triglyceride (TG, mg/dl)	T2DM with neuropathy		140.97 ± 88.60
	T2DM without neuropathy		133 ± 45.23
Blood Pressure	T2DM with neuropathy	Mild hypertension	35 (45.9%)
		Moderate hypertension	28 (37.9%)
		Severe hypertension	8 (10.8%)
	T2DM without neuropathy	Normal	4 (5.4%)
		Mild hypertension	31 (31%)
		Moderate hypertension	23 (23%)
		Severe hypertension	9 (9%)
		Normal	37 (37%)
High density lipoprotein (HDL, mg/dl)	T2DM with neuropathy		39.14±12.67
	T2DM without neuropathy		42.11 ± 6.32
Low density lipoprotein (LDL, mg/dl)	T2DM with neuropathy		144.35 ± 63.21
	T2DM without neuropathy		111.24 ± 24.105
Microalbuminuria/ Macroalbuminuria	T2DM with neuropathy		20/42
	T2DM without neuropathy		-

Data are means and SD.

3.1. DNA Extraction and Purification

At first taking informed consent from all diabetic individuals included in our research and healthy volunteers, venous blood samples (3 ml) were withdrawn and added on EDTA (ethylenediamine tetra acetate) containing tubes, DNA was extracted promptly using DNA extraction and purification kit (Gentra Systems, USA) according to manufacturer's instructions and then stored at −20˚C till use.

3.2. PCR Amplification

ACE genotype analysis was performed by PCR-RFLP analysis.

Genomic DNA was isolated from peripheral blood leukocytes according to a standard salting out method [12]. Amplification was carried out in a DNA thermocycler (Eppendorf Master Cycler). First, PCR was performed using 20 pmoles of each primer (flanking primer pair): Sense oligo 5'-CTG GAG ACC ACT CCC ATC CTT TCT 3' and anti-sense oligo: 5'-GAT GTG GCC ATC ACA TTC GTC AGA T-3' in a final volume of 25 µl, containing (0.5 µ g genomic DNA, 2 mM $Mgcl_2$, 50 mM kcl, 10 mM Tris-Hcl (pH = 8.3), 0.2 mM of each dNTP, and 0.5 unit of Taq polymerase. PCR was done with an initial denaturing time at 94˚C for l min. Then the DNA was amplified for 30 cycles with denaturation at 94˚C for 30 s, annealing at 58˚C for 30 s, and extension at 72˚C for l min. This was followed by final extension at 72˚C for 8 min. PCR products were directly visualized using ethidium bromide staining after electrophoresis in a 2% agarose gel [13]. The amplification product is a 190 bp fragment in the presence of the deletion (D) allele and a 490 bp fragment in the presence of the insertion (I) allele. Therefore, there were three genotypes after electrophoresis: A 490 bp band (genotype II), 190 bp band (genotype DD), or both 490 and 190 bp band (genotype ID). Mistyping of ID heterozygote as D homozygotes may occur. Thus, each sample that had the DD genotype was applied to PCR amplification using the forward: 5-TCG GAC CAC AGC GCC CGC CAC TAC-3'; and the reverse: 5'-TCG CCA GCC CTC CCA TGC CCA TAA-3' primers with identical PCR conditions except for an annealing temperature of 67˚C. The reaction yields a 335-bp amplicon only in the presence of an I allele, and no product for homozygous DD samples.

4. Statistical Analysis

Data were prepared and undergoing analysis through Statistical Package of Social Science (SPSS, version 10.0). The frequencies of different allelic polymorphisms of all studied individuals were compared between groups by using Fisher's exact test (modified Chi square test) and Odds ratio. A value of $p < 0.05$ was considered to be significant.

5. Results

Comparing studied cases of T2DM with nephropathy to that of T2DM without nephropathy regarding the gene polymorphism of ACE and alleles (**Table 3**, **Figure 1** and **Figure 2**) showed that cases of T2DM with DKD had a statistically significant lower frequencies of the ID genotype and I allele compared to cases of T2DM without DKD (52.0% vs. 72.0%, $p < 0.0001$ and 30.0% vs. 41.0%, $p < 0.045$ respectively) while they had a significantly very high frequency of the DD genotype and D allele (44.0% vs. 23.0%, $p < 0.005$ and 70.0% vs. 59.0%, $p < 0.045$ respectively). On the other hand, II genotype had no significant frequency in cases of T2DM with nephropathy or cases of diabetes type II without nephropa-

thy.

By comparing cases of T2DM without nephropathy versus healthy people (Table 4), had a significant lower frequency of genotype DD and allele D compared to healthy people (23.0% vs. 48.9%, $p < 0.0001$ and 59.0% vs. 71.8%, $p < 0.011$ respectively), while they had a statistically significant very high frequency of ID & I allele (72.0% vs. 45.7%, $p < 0.0001$, 41.0% vs. 28.2%, $p < 0.011$, respectively). But by Comparing cases of diabetes T2DM with nephropathy and healthy people had no significant frequency (Table 5).

Comparing cases albuminuria >300 with those <300 as regards the studied ACE gene polymorphisms, it is observed that cases >300 have high frequency of DD genotype (45.2% vs. 30%, OR = 1.93, p = 0.75). Also cases >300 had low frequency of ID genotype (50% vs. 65%, OR = 0.54, p = 0.403). Regarding the allele frequencies, the D allele showed higher level among cases with macroalbuminuria (70.24% vs. 62.5%, OR = 1.42, p = 0.51), while the I allele showed lower level (29.76% vs. 37.5%, OR = 0.71, p = 0.51) (Table 6).

Comparing cases with blood pressure groups regards that the studied ACE gene polymorphisms, it is observed that in DD genotype normal have high level then sever then mild and moderate is the lower one (75%, 50%, 44.1%, 39.3, p = 0.232). In addition, in ID genotype found that moderate have higher level then mild then sever and normal is lower one (57.1%, 52.9%, 50%, 0%, p = 0.232) (Table 7).

Comparing cases with hyperlipidemia with those without hyperlipidemia as regards the studied ACE gene polymorphisms, it is observed that cases with hyperlipidemia have high frequency of DD genotype (46.2% vs. 44.7%, OR = 1.06, p = 0.903). In addition, cases with hyperlipidemia had high frequency of ID genotype (53.8% vs. 48.9%, OR = 1.22, p = 0.874). Regarding the allele frequencies, the D allele showed higher level among cases with hyperlipidemia (46.2% vs. 44.7%, OR = 1.06, p = 0.903), while the I allele showed lower level (26.9% vs. 30.9%, OR = 0.83, p = 0.76) (Table 8).

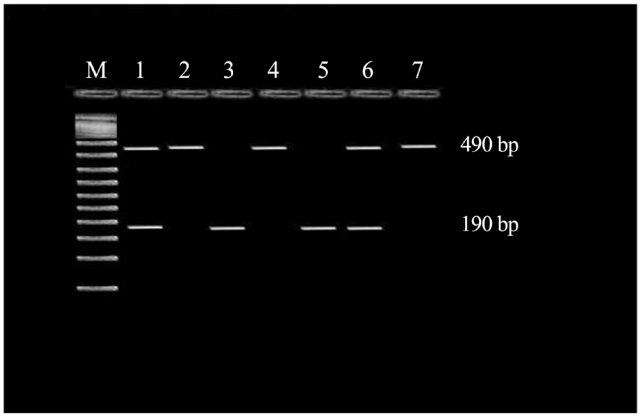

Figure 1. Detection of genetic polymorphism of ACE using PCR amplification: This figure showed 3 main different picture (DD, ID and II): DD homozygous (a single 190 base pair), ID heterozygous (190 and 490) Base pair, II homozygous (490 bp).

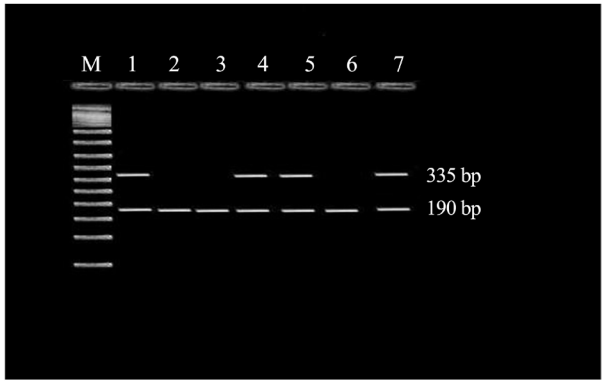

Figure 2. Second PCR amplification of ACE gene polymorphism: This figure shows, homozygous DD (a single 190 bp product) and heterozygous ID (190 and 335 bp product).

Table 3. Comparison between T2DM subjects with nephropathy and T2DM subjects without nephropathy regarding the frequency of their allele and gene polymorphism of ACE.

	Diabetic subjects with nephropathy n (%)	Diabetic subjects without nephropathy n (%)	χ^2 (P)	OR (95% CI)
	N = 75	N = 100		
DD	33 (44.0)	23 (23.0)	7.75 (0.005)*	2.6 (1.37 - 5.05)
ID	39 (52.0)	72 (72.0)	13.32 (<0.0001)**	0.27 (0.14 - 0.54)
II	3 (4.0)	5 (5.0)	0.003 (0.956)	0.792 (0.18 - 3.42)
Alleles	N = 150	N = 200		
D	105 (70)	118 (59)	4.023 (0.045)*	1.62 (1.035 - 2.54)
I	45 (30)	82 (41)	4.023 (0.045)*	0.62 (0.39 - 0.97)

Table 4. Comparison between cases T2DM without nephropathy and healthy volunteers regarding the frequency of their allele and gene polymorphism of ACE.

	Diabetic subjects without nephropathy n (%)	Healthy subjects n (%)	χ^2 (P)	OR (95% CI)
	N = 100	N = 94		
DD	23 (23.0)	46 (48.9)	13.11 (<0.0001)**	0.31 (0.17 - 0.58)
ID	72 (72.0)	43 (45.7)	12.77 (<0.0001)**	3.05 (1.7 - 5.54)
II	5 (5.0)	5 (5.3)	0.05 (0.823)	0.94 (0.26 - 3.35)
Alleles	N = 200	N = 188		
D	118 (59)	135 (71.8)	6.45 (0.011)*	0.57 (0.37 - 0.86)
I	82 (41)	53 (28.2)	6.45 (0.011)*	1.77 (1.16 - 2.71)

n = number of cases, (%) = percentage of cases, Odds ratio & 95% confidence interval = OR (95% CI). * $p < 0.05$ (significant) ** $p < 0.001$ (extremely significant): Significance using Fisher's exact test.

Table 5. Comparing between type 2 diabetes mellitus with nephropathy group and healthy control regarding their allele frequency and genotype distribution of ACE polymorphism.

	T2DM with Nephropathy n = 75 (%)	**Healthy Control n = 94 (%)**	X^2 (p)	OR 95% CI
DD	33 (44.0)	46 (48.9)	0.234 (0.629)	0.82 (0.45 - 1.51)
I D	39 (52.0)	43 (45.7)	0.43 (0.51)	1.29 (0.7 - 2.4)
I I	3 (4.0)	5 (5.3)	0.001 (0.98)	0.74 (0.17 - 3.21)
Allele	N = 150	N = 188		
D	105 (70)	135 (71.8)	0.059 (0.808)	0.92 (0.57 - 1.47)
I	45 (30)	53 (28.2)	0.059 (0.808)	1.092 (0.68 - 1.75)

Comparing cases with diabetic nephropathy with those healthy controls as regards the studied ACE gene polymorphisms, it is observed that cases with nephropathy lower frequency of DD genotype, which was not significant from that of healthy controls (44% vs. 48.9%, OR = 0.82, p = 0.629). Also cases of nephropathy had higher frequency of ID genotype (52% vs. 45.7%, OR = 1.29, p = 0.51). Regarding the allele frequencies the D allele showed lower level among cases diabetic nephropathy (70% vs. 71.8%, OR = 0.92, p = 0.808), while the I allele showed higher level (30% vs. 28.2%, OR = 1.09, p = 0.059).

Table 6. Comparison between macroalbuminuric T2DM with nephropathy group and microalbuminuric T2DM with nephropathy group regarding their allele frequency and gene polymorphism of ACE.

	Macroalbuminuria N = 42 (%)	Microalbuminuria N = 20 (%)	X^2 (p)	OR 95% CI
DD	19 (45.2)	6 (30.0)	0.75 (0.39)	1.93 (0.62 - 5.99)
I D	21 (50.0)	13 (65.0)	0.7 (0.403)	0.54 (0.18 - 1.62)
I I	2 (4.8)	1 (5.0)	0.35 (0.55)	0.95 (0.081 - 11.14)
Alleles	N = 84	N = 40		
D	59 (70.24)	25 (62.5)	0.43 (0.51)	1.42 (0.64 - 3.13)
I	25 (29.76)	15 (37.5)	0.43 (0.51)	0.71 (0.32 - 1.56)

Comparing cases albuminuria >300 with those <300 as regards the studied ACE gene polymorphisms, it is observed that cases >300 have high frequency of DD genotype (45.2% vs. 30%, OR = 1.93, p = 0.75). Also cases >300 had low frequency of ID genotype (50% vs. 65%, OR = 0.54, p = 0.403). Regarding the allele frequencies, the D allele showed higher level among cases with macroalbuminuria (70.24% vs. 62.5%, OR = 1.42, p = 0.51), while the I allele showed lower level (29.76% vs. 37.5%, OR = 0.71, p = 0.51).

Table 7. Comparing between blood pressure groups as regard ACE polymorphism in cases of diabetic nephropathy.

	Blood. p				X^2 (p)
	Mild N = 34 (%)	Moderate N = 28 (%)	Severe N = 8 (%)	Normal n = 4 (%)	
DD	15 (44.1)	11 (39.3)	4 (50.0)	3 (75.0)	
I D	18 (52.9)	16 (57.1)	4 (50.0)	0 (0)	8.090 (0.232)
I I	1 (2.9)	1 (3.6)	0 (0)	1 (25.0)	

Comparing cases with blood pressure groups regards that the studied ACE gene polymorphisms, it is observed that in DD genotype normal have high level then sever then mild and moderate is the lower one (75%, 50%, 44.1%, 39.3, p = 0.232). In addition, in ID genotype found that moderate have higher level then mild then sever and normal is lower one (57.1%, 52.9%, 50%, 0%, p = 0.232).

Table 8. Comparing between cases with hyperlipidemia and without hyperlipidemia as regard ACE polymorphism.

	With Hyperlipidemia N = 26 (%)	Without Hyperlipidemia N = 47 (%)	X^2 (p)	OR 95% CI
DD	12 (46.2)	21 (44.7)	0.015 (0.903)	1.06 (0.41 - 2.78)
I D	14 (53.8)	23 (48.9)	0.025 (0.874)	1.22 (0.47 - 3.18)
I I	0 (0)	3 (6.4)	0.22 (0.64)	0.24 (0.012 - 4.83)
Allele	N = 52	N = 94		
D	38 (73.1)	65 (69.1)	0.096 (0.76)	1.21 (0.57 - 2.57)
I	14 (26.9)	29 (30.9)	0.096 (0.76)	0.83 (0.39 - 1.75)

Comparing cases with hyperlipidemia with those without hyperlipidemia as regards the studied ACE gene polymorphisms, it is observed that cases with hyperlipidemia have high frequency of DD genotype (46.2% vs. 44.7%, OR = 1.06, p = 0.903). In addition, cases with hyperlipidemia had high frequency of ID genotype (53.8% vs. 48.9%, OR = 1.22, p = 0.874). Regarding the allele frequencies, the D allele showed higher level among cases with hyperlipidemia (46.2% vs. 44.7%, OR = 1.06, p = 0.903), while the I allele showed lower level (26.9% vs. 30.9%, OR = 0.83, p = 0.76).

6. Discussion

There are several researches indicated that development and progression of diabetic kidney disease are multifactorial including different pathophysiologic mechanisms especially environmental or genetic susceptibility. Epidemiological studies found familial clustering of diabetic kidney disease in diabetic siblings, supporting an important role of genetic defects in the pathogenesis of diabetic kidney disease [14]. ACE is an enzyme (zinc metallopeptidase enzyme) that found to be highly expressed on the epithelial and endothelial surfaces. The function of ACE is to convert angiotensin I to angiotensin II that is the highly active biochemical end product of the rennin-angiotensin system (RAS) [15] and this associated with an increased risk of vascular disease [16]. Clinical studies investigated association between diabetic kidney disease and ACE gene polymorphism showed contradictory results.

This study included (269) 75 patients with diabetic kidney disease (DKD), 100 patients T2DM patients without DKD and 94 healthy people. In selection of cases, we were keeping to have cases affected with T2DM associated with diabetic kidney diseases. Their mean age was 58.02 years, with a SD of ±6.34 years. Out of them, 94.6% having hypertension, and 35.6% with hyperlipidemia and 22.5% have consanguinity and 72% have family history to diabetes. For comparison 100 cases diabetic (T2DM) without nephropathy (mean age was 51.7 years, with a SD of ±9.4 years) (**Table 1** and **Table 2**).

This study showed that Egyptian cases of diabetic kidney disease had significantly higher frequency of genotype (DD) than cases diabetic with no DKD (44% versus 23%, p = 0.005). In addition, it's noticed that cases of diabetic nephropathy had significantly lower frequency of ID genotype than cases of diabetes without nephropathy (52% versus 72%, p < 0.0001) (**Table 3**).

Diabetic nephropathy cases showed low frequency of II genotype than diabetic without nephropathy 5.3% (4% vs. 5%). Meanwhile, total cases found to have statistically significant more frequent expression of D allele (70% vs. 59%, p = 0.045) with a significant lower level of I allele than cases of diabetic without nephropathy (30% vs. 41%, p = 0.045). Finally, in both group of cases and controls we observed that frequency D allele is higher than frequency of I allele.

In a previous study among Egyptian cases, reported that the II, ID and DD ACE genotypes was 4%, 52% and 44% in cases of diabetic nephropathy and 5%, 72% and 23% in cases without nephropathy. The ACE DD genotype shows significant association with diabetic nephropathy. We can speculate that the difference may be related to the ethnic background variations between our locations in Egypt and other countries.

Analyzing the results among Egyptian controls, this study showed that Egypt control cases (diabetic without nephropathy) had a higher frequency of ID than that of DD genotype (72% vs. 52%) with a higher frequency of II genotype (5% vs. 4%).

Also, our results showed that by comparing cases albuminuria >300 with those <300 as regards the studied ACE gene polymorphisms, it is observed that cases >300 have high frequency of DD genotype (45.2% vs. 30%, OR = 1.93, p = 0.75). Also cases >300 had low frequency of ID genotype (50% vs. 65%, OR = 0.54, p = 0.403).

Regarding the allele frequencies, the D allele showed higher level among cases with macroalbuminuria (70.24% vs. 62.5%, OR = 1.42, p = 0.51), while the I allele showed lower level (29.76% vs. 37.5%, OR = 0.71, p = 0.51) (**Table 6**). These results were in agreement with [17] who showed that females patients with abnormal excretion in urine (either micro or macro) found to have higher frequency of a genotype DD versus females with no albumin excreted in urine (DD = 27.9%, ID = 21.2% and II = 10.5%, respectively; p ≤ 0.044) in Mexico population.

These findings are supported by study carried out in India population by [18] who demonstrated that the analysis of different genotype of ACE showed the following findings: genotype DD found in (22.75%) cases with DKA, 15.42% in T2DM individuals, and 21.62% in healthy volunteers. Chi-square test between DKA group and healthy volunteers found to be non-significantly different in allele D. but, there is statistically significant difference (p < 0.05) between patients with DKD and diabetic patients.

Regarding other micro vascular complications related to diabetic kidney disease [19] demonstrated that there was highly significant correlation between diabetic retinopathy and genotype DD. Their prevalence was found to be higher in individuals affected by genotype DD (DD, ID, and II, 90.4%, 71.2%, and 70.6%; p < 0.05 respectively). individuals carry genotype DD reached the end point [s. creatinine levels more than 2.0 mg/dL (176.8 micromole/L)] more rapidly than individuals carry other different genotypes (DD, 11.38 ± 4.08 years; ID, 13.85 ±

4.04 years; II, 14.04 ± 4.06 years, respectively; $p < 0.05$) and those individuals (with DD) was progressively reach to chronic hemodialysis earlier than others (DD, 13.10 ± 4.45 years; ID, 16.21 ± 4.74 years; II, 15.13 ± 4.09 years, respectively; $p < 0.05$). Also, regarding hypertensive diabetic patients, there was a highly significant correlation between genotype DD and systolic blood pressure with progressive nature of Diabetic kidney diseases, in multiple logistic regression analysis.

Comparing cases with blood pressure groups regards that the studied ACE gene polymorphisms, it is observed that in DD genotype normal have high level then sever then mild and moderate is the lower one (75%, 50%, 44.1%, 39.3%, $p = 0.232$). In addition, in ID genotype found that moderate have higher level then mild then sever and normal is lower one (57.1%, 52.9%, 50%, 0%, $p = 0.232$) (**Table 7** and **Figure 3**).

In South Korea, subjects with genotype DD compared to others with genotype II, the OR was 3.881 (95% confidence interval, 1.564 9.628; $p = 0.003$ approximately), these results indicated that the DD genotyping of ACE gene may be considered a significant risk factor for the progressive nature of diabetic kidney disease.

Also, [20] found that diabetic individuals with a high levels of insulin resistant states, diabetic kidney disease was represented in 2/11 diabetic individuals with ACE gene (II genotype) versus 19/25 diabetic individuals represented by DD or ID genotype ($p = 0.002$). The prevalence of diabetic kidney disease was higher in the majority of individuals with both D allele plus significant insulin resistant states (19/25) compared to other patients (14. 37; OR, 5.20). These findings indicated that the ACE gene effectively can influence both onset and/or progressive nature of diabetic kidney disease in Japanese individuals with T2DM with high levels of insulin resistant state.

Also, in Tokyo, [21] concluded the following data, diabetic individuals with diabetic kidney disease have an excessive expression of genotype ID versus those individuals DKD ($p < 0.02$) and less of the genotype II versus with healthy volunteers ($p < 0.01$) and diabetic individuals without DKD ($p < 0.01$). T2DM individuals presented with genotype II have a low risk for the development of diabetic kidney disease.

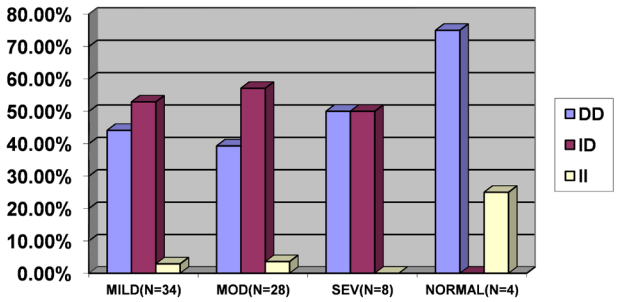

Figure 3. Comparing between blood pressure groups as regard ACE polymorphism in diabetic nephropathy cases.

Also these findings were in contrast with [22] who found that the presentation of different genotypes (II, DD and DI) did not differ significantly between T2DM individuals with or without DKD (46%; II: DD: 44%; ID: 10% vs., II: 12%, DD: 41%; ID: 47%; respectively). Also, no statistically any significantly different results between three main genotypes and allelic frequencies of the (I/D) polymorphism in all T2DM individuals versus healthy individuals with normal glycemic state (II: 11%, ID: 46%; DD: 43%; vs. II: 15%, DD: 37%; ID: 48%; respectively). So in Tunisian T2DM individuals, the (I/D) polymorphism within the ACE gene was found to be not associated with T2DM or DKD.

In addition, [23] showed that cases with DN incidence in T2DM individuals among three groups (II 45.8%, ID 52.3%, and DD 46.1%, respectively, $p > 0.05$). The increased degree of urinary excretion of albumin among the 3 groups was similar at the end-point of the study ($p > 0.05$). So, this study concluded that in china, genotype DD of ACE gene may not considered as genetic marker of clinical significance for prediction of the onset and progression of DKD in Type 2 diabetic patients in.

In addition, [24] found that there are no statistically differences in the mean eGFR according to different genotypes of ACE gene (ID: 99.5 ± 25.1 ml/min, II: 96.6 ± 19.6 ml/min, DD: 89.9 ± 28.1 ml/min). Also, they found no statistically significant changes in distribution of different genotypes diabetic individuals with different grades of albuminuria (DD:ID:II [%], normoalbuminuric patients-35:46:19, macro albuminuric patients-31:55:14, microalbuminuric patients-28:55:17). So, they concluded that, in Turkey population, ACE gene polymorphism (D/I) found to be not to be associated with eGFR in T2DM individuals.

In addition, In Poland, [25] studied (No.941) cases with renal complication of T2DM diabetic individuals. Of them 127 diabetic individuals with macroalbuminuria or end stage renal disease, 335 diabetic individuals with microalbuminuria, and a control group of 254 diabetic individuals without albuminuria with duration of diabetic state of 10 years and more. They concluded that, there were no any statistically significant differences in the different genotype D/I distribution of ACE gene or allelic frequency was found in-between different tested groups. The conclusion of this research strongly found that there was no associated link between the ACE gene D/I polymorphisms and DKD in T2DM individuals.

In addition, In Germany, [26] concluded that genetic polymorphism D/I related to ACE gene does not significantly have a major role in the onset and progression of DKD.

Comparing cases with hyperlipidemia with those without hyperlipidemia as regards the studied ACE gene polymorphisms, it is observed that cases with hyperlipidemia have high frequency of DD genotype (46.2% vs. 44.7%, OR = 1.06, $p = 0.903$). In addition, cases with hyperlipidemia had high frequency of ID genotype (53.8% vs. 48.9%, OR = 1.22, $p = 0.874$).

Regarding the allele frequencies, the D allele showed higher level among cases with hyperlipidemia (46.2% vs. 44.7%, OR= 1.06, p = 0.903), while the I allele showed lower level (26.9% vs. 30.9%, OR= 0.83, p = 0.76) (Table 8).

Possible explanation of this controversy in the results of different researches related to genetic polymorphisms may be due to multifactorial aspects, mainly the major differences in ethnic aspects of studied diabetic individuals and healthy volunteers. Other factors include the definition of nephropathy or Diabetic kidney disease and inclusion criteria of the diabetic control group without renal complication and small sample sizes in some studies.

7. Conclusion

Our findings indicated that there is a strong relation between diabetic kidney disease and genetic polymorphism of ACE gene and from our results we also found that genotype of ACE in Egypt is DD genotype of diabetic cases with diabetic kidney disease so the presence of D allele has a significant relation with diabetic kidney disease. Our data confirm the significant role of angiotensin converting enzyme gene in its relationship with diabetic kidney disease risk in Egyptian population.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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Abbreviations

T2DM: type 2 diabetes mellitus

DN: diabetic nephropathy

ACE: Angiotensin-converting enzyme

SSP-PCR: sequence specific primer-polymerase chain reaction