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Angiotensin Converting Enzyme Insertion/Deletion Gene Polymorphism in Egyptian Population with Type 2 Diabetes and Its Relation to Diabetic Nephropathy

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ABSTRACT

Background: Angiotensin-converting enzyme insertion/deletion (ACE I/D) polymorphism has been linked to diabetes and the progression of diabetic comorbidities.

Objectives: Study the correlation between the ACE I/D gene polymorphism and: a) Risk of development of type 2 diabetes mellitus (T2DM) in Egyptian population. b) Development of diabetic nephropathy.

Patients and methods: we studied 80 participants: including 20 healthy participants, and 60 patients with diabetes. The following laboratory tests were performed: HA1C, lipid profile, estimated glomerular filteration rate (eGFR), serum creatinine, urine analysis, urine albumin creatinine ratio, DNA Isolation and Determination of ACE Genotype (GT) were performed to all patients and control subjects.

Results: In diabetic patients about 81 % had Genotypes II and 18.3% had Genotype ID, while in control group 95 % had genotype II. In diabetics without chronic kidney disease (CKD) 96 % had genotype II and about 3 % had genotype ID. In diabetics with CKD about 66 % had genotypes II while 33 % had genotype ID. There was a significant difference found in the studied groups, p was 0.033. the mean HbA1c, GFR and creatinine in diabetics with chronic kidney disease with genotype II was 7.81,22.57 and 4.08 respectively, while the mean HbA1c, GFR and creatinine in diabetic chronic kidney with genotype ID was 8.92, 13.44 and 5.02 respectively. There was significant difference found in the studied groups according to GFR, p was 0.005.

Conclusion The current study demonstrated that; ACE gene I/D polymorphism has potential link to DM and progression to diabetic nephropathy.

Keywords: Diabetic chronic kidney, ACE, Insertion/deletion polymorphism.

INTRODUCTION

Disturbances in the metabolism of carbohydrates, fats, and proteins are a hallmark of diabetes mellitus, a metabolic illness with numerous aetiologies caused by a complex interplay between hereditary and environmental variables. Long-term harm, malfunction, and organ failure are among the consequences of DM [(1, 2)].

With regard to both T2DM cases and non-diabetic ones, several earlier research found that those with the ACE gene DD genotype were more insulin sensitive and with a reduction in insulin response to oral glucose loading in comparison with cases with the ID/II genotype. Many investigations demonstrated that the DD genotype is substantially related with elevated plasma or serum ACE levels despite the fact that the I/D polymorphism resides in the intronic area of the ACE gene [(3-10)].

The study's objective is to assess the correlation between the ACE insertion/deletion gene polymorphism and: a) T2DM risk in the Egyptian population; and b) the onset of diabetic nephropathy (diabetic chronic kidney disease).

SUBJECTS AND METHODS

This was a case control cross-sectional prospective study which is carried on 60 Egyptian cases with T2DM (group A) their age ranges from (41to 84) years old and 20 subjects with age and sex matched appear healthy volunteers serving as control (group B).

Diabetic patients were recruited from those attended Endocrinology Unit in Banha University Hospital. The period of the study was from April 2020 to November 2021.

All patients were informed about their participations in the study and a signed a consent that was reviewed by the local ethical committee of Banha University.

Patients with T2DM sub groups: group A1 was 30 diabetics with diabetic nephropathy and the second group A2 was 30 patients with T2DM without diabetic nephropathy.

Diagnosis of diabetic nephropathy is based on eGFR less than 60 ml/hour \1.73 m^2 or urine albumin creatinine ratio more than 30mg/gm creatinine. Microalbuminuria (A2) was defined as 30-300 mg/gm creatinine in aspot urine collection and macroalbuminuria (A3) as more or equal 300mg per g cr**.**

Exclusion criteria were: patients with type 1 diabetes, Presence of other systemic disease which might cause chronic kidney disease and infection within the previous month.

Entire cases were subjected to a thorough history taking and examination.

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The following laboratory tests were performed: Heamoglobin A1c lipogram: total cholesterol. LDL, HDL, triglycerides serum creatinine, sGFR, urine analysis: normal urine microscopy, urine albumin creatinine ratio. Normo-albuminuria (A1) was defined on the basis of an albumin excretion rate less than or equal to 30g/g creatinine. Albuminuria was defined as more than or equal to30mg/gm creatinine in a spot urine collection. The reference range in this study was adopted by **American Diabetic Association** [11].

DNA isolation and determination of ACE genotype were performed to all patients and control subjects [8]**.** Leukocytes from peripheral blood were used to isolate genomic DNA. Genomic DNA was amplified using PCR to ascertain the ACE genotype. A flanking primer pair was used initially, followed by a primer pair that recognises the insertion-specific areas, to test the specificity of the amplification responses.

Ethical approval:

This study was approved by Banha Medical Ethics Committee of Benha Faculty of Medicine (number RS. 3-12-2019). After being fully informed, all participants provided written consent. The study was conducted out in line with the Helsinki Declaration.

Statistical analysis

The data was coded, processed, and analysed using SPSS version 24. (IBM SPSS Inc, Chicago, IL, USA). To ascertain if the data distribution was normal, the Shapiro Walk test was employed. To represent qualitative data, frequencies and relative percentages were employed. Chi square (X^2) test or Fisher's exact test (FET) was used to identify differences between two or more sets of qualitative variables. The numerical data was shown as mean±SD and range. The independent samples t-test was utilized in the context of comparison between 2 independent groups with normal distribution of the variables. P values below 0.05 were considered to be significant.

RESULTS

Table (1) shows demographic characters of the control and cases. There was no significant difference found in the studied groups in sex and age. BMI was significantly high in diabetic patients compared to control subjects.

Table (1): Sociodemographic characteristics of the studied subjects

Variable		Patients (n=60)		Controls (N=20)		Test of sig.	P
		No.	%	No.	%		
Sex	**Male**	22	36.7	8	40.0	χ^2= 0.071	0.79 (NS)
	Female	38	63.3	12	60.0		
Age (yrs)	**Mean±SD**	57.8±10.9		53.1±8.3		St. "t" =1.78	0.079 (NS)
	Range	41-84		41-65			
BMI (kg/m^2)	**Mean±SD**	30.5±4.2		25.1±3.3		St. "t" =5.2	<0.001 (HS)
	Range	22-39		20-32			

Tables (2, 3) show that there was no significant difference found between diabetic and control groups regarding the genotypes or allele polymorphism. There was a significant difference found between the 2 subgroups of the studied diabetic patients regarding the genotypes or allele polymorphism.

Table (2): Comparison between the studied groups regarding ACE insertion/deletion gene and allele polymorphism

ACE insertion/deletion gene and allele polymorphism.		Diabetic patients (n=60)		Controls (n=20)		OR (95%CI)	P
		No.	%	No.	%		
Genotypes (GT)	**II**	49	81.7	19	95.0	4.27 (0.51-35.7)	0.28 (NS)
	ID	11	18.3	1	5.0		
Allele	**I**	109	90.8	39	97.5	3.94 (0.49-31.5)	0.19 (NS)
	D	11	9.2	1	2.5		

Table (3): Comparison between the studied groups of patients based on ACE insertion/deletion gene and allele polymorphism (In comparison with the control).

Variable		Controls (n=20)		Diabetics without chronic kidney disease (n=30)		OR (95%CI)	P	Diabetics with chronic kidney disease (n=30)		OR (95%CI)	P
		No.	%	No.	%			No.	%		
Genotypes	**II**	19	95.0	29	96.7	**1.52 (0.09-25.9)**	1.0 (NS)	20	66.7	**9.5 (1.1-83.3)**	**0.033 (S)**
	ID	1	5.0	1	3.3			10	33.3		
Allele	**I**	39	97.5	59	98.3	**1.51 (0.091-24.9)**	0.77 (NS)	50	83.3	**7.8 (1.08-70.8)**	**0.042 (S)**
	D	1	2.5	1	1.7			10	16.7		

Table (4) shows that there was no significant difference found in the studied groups regarding the genotypes in both males and females. There was significant difference found in the studied groups according to BMI.

Table (4): Comparison of Genotypes according to demographic characteristics among diabetic patients and control group

	Diabetic chronic kidney disease(n=30)		Diabetics without chronic kidney disease (n=30)		Controls (n=20)		Test of significance (p)
	II (n=20)	**ID (n=10)**	**II (n= 29)**	**ID (n=1)**	**II (n= 19)**	**ID (n=1)**	
Age (ys)							F= 2.258 P= 0.089
Mean ± SD	57.5 ± 10.39	61.2 ± 7.33	55.2 ± 7.6	---	53.1 ± 8.3	---	
BMI (kg/m^2)							**F= 11.76, p<0.001***
Mean ± SD	31.4 ± 4.42	32.1 ± 4.06	31.3 ± 4.3	---	25.1 ± 3.3	---	
Multiple comparisons	p1= 0.97, p2= 0.999, p3<0.001*, p4= 0.95, p5= 0.002*, p6<0.001*						
Sex							χ^2= 5.712, p= 0.127
Male	7 (35.0)	1 (10.0)	4 (13.8)	0 (0.0)	7 (36.8)	0 (0.0)	
Female	13 (65.0)	9 (90.0)	25 (86.2)	1 (100)	12 (63.2)	1 (100)	
Multiple comparisons	p1= 0.57, p2= 0.876, p3<0.001, p4= 0.214, p5<0.001*, p6<0.001						

*: Significant.

Table 5 shows that there was no significant difference found in the studied groups regarding Albumin creatinine ratio grade according to genotype frequency.

Table (5): Albumin creatinine ratio grade according to genotype frequency.

			Genotype		P
			II	**ID**	
Albuminuria	Micro-albuminuria (A2)	Count	12	2	0.058 (NS)
		% within genotype	60.0%	20.0%	
	Macro-albuminuria (A3)	Count	8	8	
		% within genotype	40.0%	80.0%	
Total		Count	20	10	
		% within genotype	100.0%	100.0%	

FET was used

Table (6) shows laboratory findings according to genotype frequency among chronic kidney group. There was significant difference found in the studied groups according to GFR. There was no significant difference found in the studied groups according to HbA1c.

Table (6): Laboratory findings according to genotype frequency among nephropathy group.

Variable	II (n=20)			ID (n=10)			St. "t"	P
	Mean	±SD	Range	Mean	±SD	Range		
HbA1c %	7.81	1.12	6.5-10	8.29	1.02	7.4-11	1.13	0.27 (NS)
GFR (ml/min/1.73m^2)	22.57	8.75	10-40	13.44	4.50	10-22	**3.08**	**0.005 (S)**

DISCUSSION

The age of studied subjects ranged from (41 to 84) years. The current study aimed to demonstrate the risk of development of T2DM with ACE gene polymorphism and the role of the latter in development of diabetic CKD.

Regarding the relation between gene allele (I/D) polymorphism and diabetic chronic kidney disease, our study showed a significant relation between ACE gene I/D polymorphism and diabetic chronic kidney disease. In diabetics with chronic kidney disease 66.7% had genotype II while 33.3% had genotype ID.

Our results showed a significant difference found in the studied groups according to BMI, p was < 0.001.

Low mean GFR was associated with ID genotype. Among chronic kidney group: the mean GFR with genotype II was 22.57 ml/min/1.73m^2 ,while the mean GFR in diabetic chronic kidney disease with genotype ID was 13.44 ml/min/1.73m^2 There was significant difference found in the studied groups according to GFR, p was 0.005. The existence of the D allele is accompanied by greater circulation values of ACE, hypothesised to raise the RAS activities, which helps to explain the potential mechanism by which ACE D Allele influences diabetic chronic kidney disease. Increased RAS system activation is seen in diabetics with prolonged hyperglycemia that causes endothelial damage. Obesity increases insulin resistance and the difficulties of persistent hyperglycemia, which are risk factors for type 2 diabetes [(8)].

Our research is in agreement with **Deepashree *et al.*** [(9)], who studied that there were 104 controls and 253 diabetic patients with chronic renal disease. Patients underwent genotyping for NOS3 VNTR and ACE ID polymorphisms. The findings point to a connection between diabetic nephropathy and ACE ID and NOS3 VNTR polymorphism in the South Indian population. **Yousef *et al.*** [(8)] discovered that the DD and ID genotypes had considerably larger percentages of high ACE activity (>64 IU/l) than the II GT, and the D allele was linked to higher ACE activity in comparison to I allele in both groups.

Our study in agreement also with **Aggarwal *et al.*** [(5)], who investigated 94 healthy volunteers, 100 instances of type 2 diabetes without diabetic nephropathy, and 75 cases of type 2 diabetes with diabetic chronic kidney disease. The genotypes of the ACE gene were detected by SSP-PCR analysis. With regard diabetic cases with diabetic chronic kidney disease, the ACE (DD, ID, II) gene polymorphism was 44%, 52%, 4%, but in T2DM patients without diabetic chronic kidney disease, it was 23%, 72%, 5%.

Our research supports the results of **Akhundova *et al.*** [(10)] **and Tziastoudi *et al.*** [(11)] who found a link between the ACE I/D gene polymorphism and the possibility of developing diabetes mellitus and progressing to diabetic nephropathy.

However, we disagree with **Sharma *et al.*** [(12)] who found no changes with regard to the frequencies of ACE I/D GTs between 59 patients with diabetic nephropathy and controls from North India. In that research, there was no significant increase in the frequency of the D allele (69.1%) among diabetics cases with macroalbuminuria than it was in diabetic cases without nephropathy (58.3%). We also disagree with **Nikzamir *et al.*** [(13)] who claimed to have shown a non-significantly increased prevalence of hypertension in Iranian macroalbuminuric T2DM patients who had the DD genotype.

The ACE DD GT didn't, however, increase the incidence of hypertension in macroalbuminuric type 2 diabetes patients. Moreover, several investigations found no consistent correlation between the DD genotype or alleles and the occurrence of T2DM in the Lebanese and South Asian populations [(14)].

This gap may be the result of sampling bias or racial disparities. Second, the research participants' backgrounds, who were chosen from a diabetic clinic in our target demographic, may have had an impact. Race disparities, including other social or cultural variables, may potentially have influenced the outcomes.

About the association between the risk of developing diabetes and gene I/D polymorphism, we demonstrated that 81.75% of diabetic patients have genotype II and 18.3% have genotype ID. While in

control 95% have genotype II. The ACE genotype and risk of diabetes were not significantly associated. This finding may be explained by the ethnic makeup of the study population, gene-environment interactions, variations in the stage of nephropathy, sample size, and length of DM.

Our result is in agreement with in a cross-sectional investigation conducted in south London, **Sagnella *et al.*** [15] found no correlation between the I/D polymorphism and reduced blood glucose tolerance or the likelihood of developing type 2 diabetes. Several environmental variables, including diet and exercise, have been associated with changes in epigenetic status. As a result, this interplay between polymorphisms and epigenetic alterations illuminates the complex genetic architecture that may be responsible for the inconsistent results of correlation researches with the ACE I/D polymorphism among groups. We disagree with the study conducted in Iranian communities, which discovered that diabetes patients had considerably higher frequencies of the DD genotype and the D allele. The D-allele is a predisposing factor for diabetes and impaired glucose tolerance (IGT) in comparison to control groups, which is how this disagreement is explained [16].

In addition, we disagree with **Dhumad *et al.*** [17], who evaluated 142 T2DM patients, including 100 healthy volunteers and 62 male and 80 female patients. The notion that the D allele is substantially related with T2DM was supported by them. Compared to the control group (normal subjects), the DD genotype was linked to a threefold increased chance of developing DM.

Regarding the relation with gene I/D polymorphism and albuminuria we found that 60 % of diabetic chronic kidney disease with genotype II had micro albuminuria, while 40 % had macroalbuminuria. 20% of diabetic chronic kidney disease with genotype ID had microalbuminuria, while 80 % had macroalbuminuria. There was no significant difference found in the studied groups, p = 0.058. The underlying cause may be due to D allele cause increased expression of angiotensin two resulting in increased intraglomerular pressure and efferent vasoconstriction leading to albuminuria.

Our study is in agreement with **Sharma *et al.*** [12], they found no discernible variations in the frequency of ACE I/D genotypes between 59 patients with diabetic nephropathy from North India and controls. In that research, the frequency of the D allele was non-significantly greater (69.1%) in diabetic cases with macroalbuminuria than it was in diabetic individuals without nephropathy (58.3%). While the existence of the DD genotype was related with a 2.87-fold greater risk of macroalbuminuria, this polymorphism may not be strongly connected with the development of diabetic nephropathy in the current sample (P = 0.057) since it didn't reach a statistically significant level. Patients with the DD genotype had greater values of tissue and plasma ACE in comparison with subjects with the II genotype because the ACE I/D polymorphism impacts circulating and tissue values of ACE [12].

We disagree with **Yousef *et al.*** [8] who found that the D allele was linked to enhanced ACE activity in comparison with the I allele in both groups, and the % of high ACE activity (>64 IU/l) was considerably greater in the DD and ID genotypes in comparison with the II genotype. Macroalbuminuric patients with ID and ID + DD genotypes exhibited considerably greater ACE activity than normoalbuminuric cases with ID and ID + DD genotypes, according to a comparison of the two groups. Losartan has the most positive benefits on cases with DD and ID genotypes, and it has been hypothesised that higher internal ACE activity is the cause of the accelerated development of renal function loss in individuals with DD and ID genotypes.

Limitation of the study:

This is a cross sectional investigation. A bigger placebo controlled study will be necessary to properly evaluate the link between ACE gene polymorphism and the possibility of progression to diabetic nephropathy.

CONCLUSION

The current study found that ACE gene polymorphism especially ID genotype is linked with chronic kidney disease, ACE gene ID polymorphism is accompanied by low mean GFR and ACE gene polymorphism isn't accompanied by risk of diabetes in Egyptian population.

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