

ASSOCIATION OF ANGIOTENSIN CONVERTING ENZYME GENE POLYMORPHISM AND DIABETIC NEPHROPATHY IN TYPE 2 DIABETES MELLITUS IN SHARKIA GOVERNORATE

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ABSTRACT

Diabetic nephropathy (DN) is the leading cause of end – stage renal disease (ESRD) worldwide. The pathogenesis of this condition is not well understood clearly at present. The relationship between angiotensin- converting enzyme (ACE) insertion/ deletion (I/D) gene polymorphism and risk of DN is still conflicting. Therefore, we designed this study to investigate the association of ACE I/D gene polymorphism and development of diabetic nephropathy in a cohort of Egyptian population from Sharkia Governorate. Ninety patients with type 2 diabetes mellitus were included in the study. The first group included 45 without nephropathy while the second group included 45 with nephropathy. Both groups were subjected to full history taking, physical examination, routine laboratory investigations including fasting and 2 hours post prandial blood glucose, glycosylated hemoglobin, urine albumin excretion and urinary creatinine estimation. ACE I /D genotyping was carried out by polymerase chain reaction (PCR) amplification using allele specific primers. The frequencies of ACE DD, ID and II genotypes in group I were 11.1%, 55.6%, 33.3%. But in group II were 33.3%, 46.7%, 20% respectively. DD genotype was significantly more frequent in group II than group I (33.3% VI 11.1%), odd ratio 1.5; 95%, Confidence interval (1.049-2.145), $p < 0.01$). The ACE DD genotype is associated with diabetic nephropathy in Sharkia governorate.

Key words: Diabetic nephropathy, Angiotensin converting enzyme, insertion/deletion polymorphism

INTRODUCTION

Diabetes is a group of metabolic diseases characterized by hyperglycemia resulting from defects in either insulin secretion, insulin action, or both. The chronic hyperglycemia of diabetes is associated with long-term failure of different organs, especially the eyes, kidneys, nerves, heart, and blood vessels⁽¹⁾.

Diabetic nephropathy (DN) is the leading cause of renal failure in the world. It is represented by albuminuria and abnormal glomerular function and it is a hyperglycemic "micro vascular" complication of diabetes⁽²⁾.

The important risk factors for diabetic nephropathy are hyperglycemia and arterial hypertension, the genetic susceptibility in both type 1 and type 2 diabetes is of great importance. Other risk factors are smoking, dyslipidemia, proteinuria, glomerular hyper filtration and dietary factors⁽³⁾. Genes that seem to be of importance is angiotensin I converting enzyme (ACE) which involved in the pathogenesis of diabetic kidney disease⁽⁴⁾.

Renin Angiotensin system (RAS) plays an important role in physiology and pathophysiology of the kidney. ACE is the regulator enzyme in RAS. It produces angiotensin II from angiotensin I and inactivates bradykinin. Angiotensin II is a vasoconstrictor whereas bradykinin is vasodilator. The activation of angiotensin converting enzyme results in vasoconstriction⁽⁵⁾.

An insertion-deletion polymorphism of the gene encoding angiotensin converting enzyme is reported to be a candidate gene predisposing to

diabetic nephropathy⁽⁶⁾. ACE gene covers 21 kb on chromosome 17 carrying 26 exons. An insertion-deletion polymorphism of a 287 bp Alu repetitive sequence in the intron 16 of this gene has been reported which results in three genotypes DD&II homozygotes and ID heterozygotes⁽⁷⁾. This polymorphism determines the blood level ACE, DD genotype individuals have approximately double ACE plasma/serum levels than II genotype individuals and ID individuals have intermediate values⁽⁸⁾.

MATERIALS AND METHODS

Subjects

This study was carried out in Diabetes Mellitus (DM) Outpatient Clinic of Zagazig University Hospital and Medical Biochemistry and Internal Medicine Departments of Faculty of Medicine, Zagazig University.

The subjects were grouped into 2 categories based on the urinary albumin–creatinine ratio (ACR): Group I: 45 Subjects with type 2 DM without DN. Group II: 45 subjects with type 2 DM complicated with DN. Duration of diabetes should be more than or equal to 5 years, age between 30 and 70 years. Diabetic nephropathy was diagnosed according to American Diabetes criteria (ADA). Subjects should be of Sharkia origin. Type 1 diabetes and other kidney diseases were excluded from the study.

Demographic and clinical characteristic

Full history taking and complete physical examination with emphasis on age, sex, BMI, systolic and diastolic blood pressure , duration of

DM , symptoms and signs of peripheral neuropathy, retinopathy, IHD and cerebrovascular insufficiency , fundus examination and drug intake.

Albumin/Creatinine Ratio (ACR) was calculated based on the urine albumin excretion level in the urine sample measured by immunometric enzyme immunoassay (ORGENTEC) Diagnostika GmbH according to Ritzmann and Daniels⁽⁹⁾, and urine creatinine level measured by Jaffe colorimetric-End point (Diamond) according to Henry⁽¹⁰⁾. Quantitative colorimetric technique was used for determination of glycohemoglobin in whole blood (Stan bio) according to Abraham et al.⁽¹¹⁾. Fasting and postprandial blood glucose was measured by glucose oxidase method according to Trinder⁽¹²⁾.

Genotype assays

Determination of ACE genotype by PCR amplification according to Lee and Tsai⁽¹³⁾, DNA was isolated using the Genomic DNA Purification Kit purchased from Fermentus. Polymerase chain reaction (PCR) was employed for genotyping of the ACE I/D polymorphism. Reactions were performed with 10 pmol of each primer: 22 forward primer 5'-CTGGAGACCACTCCCATCCTTCT-3', reverse primer 5'-GATGTGGCCATCTTCGTCAGAT -3', in a final volume of 20 µl containing 3 mM MgCl₂, 50 mM KCl, 10 mM Tris-HCl (pH 8.4), 0.5 mM of each dNT and 2U Taq polymerase. PCR amplification was carried out under these conditions: initial denaturation at 94°C for 5 minutes, followed by 35 cycles of denaturation at 94°C for 45 seconds, annealing at 60°C for 1.15 minutes, extension at 72°C for 2.30 minutes and final extension at 72°C for 5 minutes. PCR products were separated on 2.0% ethidium bromide-stained agarose gel and visualized by a UVP BIOLMAGING gel doc system. The products were 490 bp for allele I and 190 bp for allele D.

Ethics statement

Informed written consent from participants in the study is a necessity prior to participation in the research and the study was approved by Zagazig Faculty of Medicine Institution Review Board.

Statistical analysis:

Results were collected, tabulated and interpreted using standard statistical techniques.

RESULTS

The patients with type 2 diabetes mellitus as well as diabetic nephropathy patients were compared for mean levels of biochemical parameters employing a student T test. The mean level of different parameters was significantly higher in DN patients compared to group I.

Mean values of fasting blood glucose in group I is 133.3±27.9 and 207±51.6 in group II, While 2 hours postprandial blood glucose in group I was 210 .7±26 and in group II it equal 297.9±74.1. Glycosylated hemoglobin was elevated in group II it was 9.2±1.2 but in group I it was 7.4±0.6.

Mean urine albumin excretion in group I was 17 ±4.1 and 141.3±60.1 in DN, Mean values of creatinine in urine was 0.7±0.1 in group I and 1±0.3 in DN.

There was strong positive correlation between glycosylated HB and urine albumin excretion and creatinine in group I and in group II.

The frequencies of ACE DD, ID and II genotypes in group I were 11.1%, 55.6%, 33.3%. But in group II were 33.3%, 46.7%, 20%.

Number of patients suffering from retinopathy in group I is 9/45 (20%) while the number of patients suffering from retinopathy in group II is 41/45 (91.1%). In the same time hypertensive patients in group I is 12/45 (26.7%) but in group II is 38/45 (84.4%). That is highly significantly increased prevalence of retinopathy and hypertension in group II than group I.

Table (1): Prevalence of diabetic retinopathy and hypertension & distribution of ACE genotype in both groups.

	Group (1) (No. &%)	Group (2) (No. &%)	χ ²	P value
Diabetic retinopathy	9(20.0)	41(91.1)	46.08	**<0.001
Hypertension	12(26.7)	38(84.4)	30.42	**<0.001
ACE genotype				
II	15(33.3)	9(20.0)	6.85	*0.033
ID	25(55.6)	21(46.7)		
DD	5(11.1)	15(33.3)		
Total	45(100.0)	45(100.0)		

Table (2): Correlation between glycosylated HB, and other parameters in group 1.

	Glycosylated HB	
	(r)	p. value
Urinary micro albumin	0.634	**<0.001
Urinary microalbumin /creatinine ratio	0.354	*0.017

*=-Significant

Table (3): Correlation between glycosylated HB, and other parameters in group 2.

	Glycosylated HB	
	(r)	p. value
Urinary micro albumin	0.867	**<0.001
Urinary microalbumin /creatinine ratio	0.448	*0.002

*=-Significant

This table shows that there was positive correlation between Glycosylated HB and Urinary micro albumin and creatinine in (group 2) with highly statistical significant difference as p. value <0.001.

Table (4):-Correlation between Hypertension and glycosylated Hb and Urinary micro albumin in group1.

	Hypertension in group1	
	(r)	p. value
Urinary micro albumin	0.623	<0.001**
Glycosylated Hb	0.161	0.29

*=-Significant

This table shows that there was strong positive correlation between **hypertension** and **Urinary micro albumin** in (group 1) with highly statistical significant difference as p. value <0.001.

Table (5): Distribution of ACE genotypes and alleles frequencies in both groups

	Group 1		Group 2		OR (95% CI)	X ²	P
	n=45		n=45				
	n(%)		n (%)				
ACE genotypes	II	15 (33.3)	9 (20.0)	0.167(0.08-0.346)	2.05	0.152	
	ID	25 (55.6)	21 (46.7)	0.167(0.068-.408)	0.71	0.398	
	DD	5 (11.1)	15 (33.3)	1.5(1.049-2.145)	6.43	*0.01	

* Statistically significant (P< 0.05)

OR: odd ratio; 95% CI: 95% confidence interval.

X² (chi square test)

DISCUSSION

Why some diabetics develop nephropathy, whereas others do not, despite having long term hyperglycaemia remains an unresolved question. Because known environmental factors do not fully explain this, researchers have sought the answer in the genetic background of the host⁽¹⁴⁾. Diabetic nephropathy (DN) is a major micro vascular complication

accounting for about 30% of End Stage Renal Disease (ESRD) cases⁽⁶⁾. Diabetic nephropathy leads to end stage renal disease if glycemic control and micro vascular protection is inadequate⁽¹⁵⁾.

The familial clustering of patients with diabetic nephropathy and beneficial effects of ACE Inhibition has favoured most researchers to investigate genetics of renin –angiotensin system

(RAS)⁽¹⁶⁾. Recently Kundu et al.⁽¹⁷⁾ reported that, impaired glycemic control is associated with significant elevations in urinary microalbumin levels.

It is known that ACE gene is one of the most important genes of RAS. The first study on ACE I/D gene polymorphism in diabetic nephropathy was that of Marre et al.⁽¹⁸⁾ who proved a protective role of II genotype against the development of diabetic Nephropathy. A considerable number of studies have investigated the possible role of ACE I/D polymorphism in the pathogenesis of diabetic nephropathy and most of them have reported relation of D allele as a risk factor⁽¹⁹⁾ However, some studies have claimed lack of D allele association^(20,21) with diabetic nephropathy and suggested a possible role of ACE -D allele in progression of nephropathy rather than susceptibility to nephropathy⁽²²⁾. An important factors contributing to discrepancies in the results reported by different studies is considerable ethnic variation in the distribution of ACEI/D genotypes⁽²⁰⁾.

In the present study, both groups were matched for age, sex and duration of diabetes. We observed the percentage of DD genotype was statistically significantly higher in patients with diabetic nephropathy compared with patients without nephropathy.

These results were reported also by Khan et al.⁽⁶⁾ whose results indicate that Type 2 diabetic patients with D allele have more than two fold risk of developing nephropathy. The same results reported by El-Bazz et al.⁽²³⁾ in study suggested that the DD genotype of ACE gene may be associated with development of diabetic nephropathy among Egyptian patients.

Haque et al.⁽²⁴⁾ found that patients with DD allele of the ACE gene are more likely to have progressive diabetic nephropathy with micro-vascular and macro-vascular complications. Study of Movva et al.⁽⁸⁾ have shown that in Asian Indians with D allele and Type 2 are at greater risk for developing DN. Wang et al.⁽²⁵⁾ reported that the ACE I/D polymorphism may contribute to DN development, especially in the Asian group with T2DM.

Contrary to our results, a study done by Moleda et al.⁽²⁶⁾ reported that ACE genotype was not associated with presence of micro vascular complications in T2DM. Also Arfa et al.⁽²⁷⁾ reported the I/D polymorphism within the ACE gene is likely not associated with diabetic nephropathy in type 1 nor type2 diabetes in the Tunisian population.

The I/D polymorphism of the ACE gene is not significantly associated with both T2DM and/or diabetic nephropathy in Malaysian population regardless of ethnicity and gender⁽²⁸⁾.

The possible mechanism by which ACE DD genotype affects DN explained by the DD genotype has been reported to be associated with high level of ACE⁽²⁹⁾. In diabetic patients with nephropathy there is higher ACE activity compared with diabetic patients without nephropathy⁽³⁰⁾. The increased protein expression of ACE is responsible for high level of angiotensin II. Angiotensin II increases the podocyte injury and loss of podocytes is a hallmark of progressive kidney diseases including DN⁽³¹⁾. In addition to its hemodynamic effects in diabetic kidney as it increases the intraglomerular pressure and glomerular filtration rate⁽³²⁾. Angiotensin II stimulates the release of several cytokine mediators of glomerulosclerosis, such as osteopontin, platelet-derived growth factors, fibonectin, and transforming growth factors B, finally leading to ESRD⁽³³⁾.

The observation of our study found that hypertensive patients in group I is 12/45 (26.7%) but in group II is 38/45 (84.4%). That is highly significantly increased prevalence of hypertension in group II than group I.

Our results indicating that, the ACE gene I/D polymorphism are not a risk factor for development of hypertension. These findings are in agreement with those of Kabadou et al⁽³⁴⁾ revealed that the ACE I/D polymorphism is not significant factor for hypertension in the Tunisian population. The study of Rasyid et al⁽³⁵⁾ did not support that the ACE I/D polymorphism associated with hypertension in a South Sulawesi Indonesian population. Also O'Donnell⁽³⁶⁾ failed to identify such associations.

However, Ali et al⁽³⁷⁾ whose noted that hypertensive cases showed a significantly higher frequency of the ACE mutant D allele carriage than I allele carriage. Also, Zarouk et al⁽³⁸⁾ reported that DD genotype and the D allele are significantly associated with hypertension in Egyptian patients.

This study also highlights that there was a positive correlation between hypertension and microalbumin in diabetic group, however in group II all the patients had micro albuminuria regardless having hypertension or not.

Hypertension is probably both a cause and an effect of diabetic nephropathy. In the glomerulus, an early effect of systemic hypertension is dilatation of the afferent arteriole, contributing to

intraglomerular hypertension, hyperfiltration, and hemodynamically mediated damage⁽³⁹⁾.

The role of genetic factors in diabetic retinopathy (DR) is unclear. We investigated the relationship between diabetic retinopathy and an insertion/deletion polymorphism in the angiotensin-converting enzyme (ACE) gene in our study. We found that, the number of patients suffering from retinopathy in group I is 9/45 (20%) while the number of patients suffering from retinopathy in group II is 41/45 (91.1%). the ACE gene I/D polymorphism is not a risk factor for development of retinopathy.

Our results are in agreement with Li et al⁽⁴⁰⁾ who proved, there is no association between ACE gene insertion/deletion (I/D) polymorphism and diabetic retinopathy in patients with type 2 diabetes mellitus.

Nikzimar et al⁽⁴¹⁾ reported, the D allele of the ACE gene is independently associated with DR in Iranian type 2 diabetic patients.

In contray, **Matsumoto et al**⁽⁴²⁾, reported relationship between the presence of the D allele polymorphism in ACE gene and advanced retinopathy in Japanese diabetic subjects with type 2 diabetes but not with overt nephropathy.

As the pathologic mechanism of diabetic retinopathy and diabetic nephropathy is similar, most of the patients with diabetic retinopathy have diabetic nephropathy⁽⁴³⁾.

Diabetic nephropathy and diabetic retinopathy are more likely to develop in patients with poor glycemic control. Previous studies have showed a positive correlation between severity of retinopathy and high levels of HbA1c⁽⁴⁴⁾.

In the present study showed that, in addition to the genetic factors, elevated Bp albuminuria, and haemoglobin A1C are independent risk factors for development of diabetic nephropathy as there a strong positive correlation between glycosylated HB and urinary micro albumin and creatinine. Because the above mentioned non genetic risk factors explain only approximately 30% to 50% OF the variation in loss of GFR^(45,46). There is evidence that blood sugar control and ACE inhibition therapy might prevent or at least delay the onset of DN and progressive renal failure in T2DM⁽⁴⁷⁾.

In conclusion: This study supports the view of association between the DD genotype of ACE gene and the risk of ESRD in patients with type 2 diabetes mellitus in Sharkia. More investigations are required to explain the pathogenic role of I/D

ACE gene polymorphism in initiation and propagation of nephropathy in patients with diabetes mellitus. As well, the role of I/D ACE gene polymorphism as a predictor of risk for ESRD in DM should be clarified.

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