Association of Angiotensin-Converting Enzyme Insertion/Deletion Gene Polymorphism with Diabetic Nephropathy Patients: a Case-Control Study

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Abstract
The ACE (I/D) plays an important role in Diabetic nephropathy patients is affected by both genetic and non-genetic influences, Existing literature suggests that the renin-angiotensin system (RAS), which plays an important role in the regulation of blood pressure and fluid-electrolyte homeostasis, is an active mediator of progressive renal injury in DN. Our study involved (170) people aged (40-75) years who were distributed into two groups, the control group (G1) involved (90) healthy , and the second group (G2) included (80) Diabetic nephropathy patients .A PCR- based I/D polymorphism study in patients of the city of Diwaniyah in Iraq was performed to detect ACE polymorphism and its pathophysiological role in Diabetic nephropathy, and the relationship between them .The results revealed that the overall genotype of the ACE I/D gene was significantly different between the DN patients (G2) as compared with control (G1) for the genotype DD ($\chi2$ = 8.916, p-value = 0.012), I allele ($\chi2$ = 11.516, p-value = 0.001), and ID & II compared to the DD genotype ($\chi2$ = 8.887, p-value = 0.003). Genotype DD, I allele, and ID & II levels in the blood were substantially higher in DN patients, with a clear correlation with the increase in Diabetic nephropathy. In conclusion genotype DD, I, ID & II have a clear relationship with increased Diabetic nephropathy, so it can be a marker for early detection of Diabetic nephropathy disease (DN).
Keywords: Diabetic nephropathy, Angiotensin-converting enzyme, ACE.

1. Introduction

Diabetic nephropathy (DN) is a severe complication of diabetes, accounting for roughly 40% of end-stage renal impairment (ESRD) [1]. It directly causes ESRD and indirectly increases cardiovascular risk, resulting in considerable morbidity and mortality [2]. Is the leading cause of end-stage renal disease (ESRD) and chronic kidney disease (CKD) (ESRD) [3].There have been no new direct therapeutics for DN available on the market in the previous twenty years, save from new anti-diabetic medications with strong cardiovascular and renal protective benefits [4] Recently, well-designed clinical trials for the treatment of DN, such as bardoxolone and atrasentan, with appealing pathogenetic rationale, were abandoned or stopped because of safety concerns or failure to attain the endpoints, respectively [5]. While microalbuminuria (MA) is the first symptom of diabetes, detecting it early and controlling it early slows the progression of the disease [6] Inflammation plays a key role in the DN progression. Preclinical studies have identified several anti-inflammatory molecules that effectively decrease albuminuria and/or proteinuria [7]. The angiotensin-converting enzyme (ACE) is found on chromosome 17q23 and consists of 26 exons and 25 introns [8]. ACE is zinc metallopeptidase enzyme that converts angiotensin 1 (ACE1) (which is deficient in aldosterone) to angiotensin II and bradykinin [9]. ACE contributes to blood pressure regulation by constricting blood vessels and controlling liquid blood flow, as angiotensin II functions as a vasoconstrictor, stimulating aldosterone's action, which results in water reabsorption [10]. The polymorphism in the ACE gene (I / D) accounts for approximately half of the apparent difference in serum ACE levels and results in three genotypes (II homozygous, ID heterozygous, DD homozygous). The genotype (DD homozygous) has the largest plasma expression and has a detrimental impact on the pathways underlying different diseases, while the genotype (II homozygous) has the lowest plasma expression and has a protective effect [11].

2. Material and Methods

Study population

The study population consisted of 170 persons of related subjects, comprised 90 Control (G1), and 80 Diabetic nephropathy patients (G2) , their ages (40-75) years old, who attended the Diwaniyah Teaching Hospital in Diwaniyah ,AL-Sader Hospital and Al-Hakim Hospital in Najaf, Iraq.

DNA isolation

Genomic DNA was extracted from whole blood using a DNA Extraction Kit (AddBio/Korea), were equal or less than 20 µg/ml.

Estimation of the concentration and purity of DNA

After DNA was extracted, the concentration and purity of extracted DNA were estimated by measuring the absorbance at A 260 nm and A 280 nm by the Nanodrop device. And the results showed a concentration was between (10-65 ng/µl) and purity (1.7-1.9).

PCR Amplification

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Isolated DNA is amplified with specific primers, 5'CTGGAGACCACTCCCATCCTTTCT- 3' (Forward) and 5'-GATGTGGCCATCACATTCGTCACGAT-3' (Reverse). The PCR products were run on 1% agarose gel electrophoresis. The different fragments obtained were homozygous DD genotypes (490 bp); heterozygous ID genotypes (190 bp); homozygous II genotypes (190 & 490 bp).

Statistical Analysis

The statically analysis between the allele frequencies and genotype distributions of the four groups was confirmed by the descriptive statistics at (*P*-value < 0.05) by using SPSS 23 software by computing the odds ratio (OR) and their confidence intervals (95% CI) and χ^2 values. Comparison between groups for biochemical and immunochemical tests was conducted by paired sample T test at (p value < 0.05) as a significant difference.

3. Results and Discussion

Polymorphisms ACE (I/D)

When studying the Figure (3) of comparison of genotype distribution and allele frequencies that appeared of ACE gene, it was found that this gene has two types of alleles: I and D, where the percentage of allele D was greater than the percentage of allele I in all groups (Control, diabetic nephropathy patients), it was found that the percentage of allele D was 149 (82.7%), 107(66.8%), in Control, Diabetic nephropathy patients, respectively. While the percentage of allele I was 31 (17.2%), 53(33.1%) in control, diabetic nephropathy patients, respectively, as shown in Figure (1).

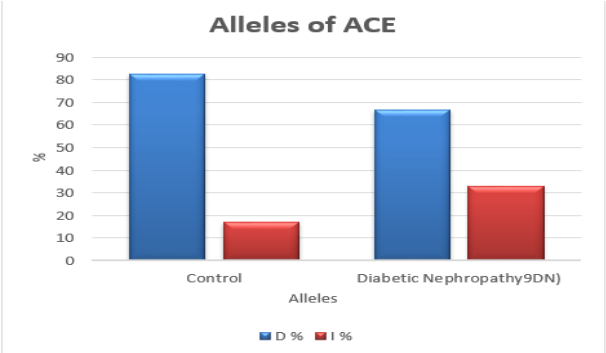

Figure 1: The percentage of alleles I/D of ACE in blood samples of Control and Diabetic nephropathy patients recovered.

The Figures of genotype distribution observed for ACE (I/D) (Intron 25 D > I) also showed that there are three types of polymorphisms: II, ID, and DD. The values of the percentages of the first polymorphism II were 10% and 20% for Control, and diabetic nephropathy patients. Respectively. The values of the percentages of the second polymorphism ID were 14.4%, and 26.3% for control, and diabetic nephropathy patients. Respectively. The values of the percentages of the third polymorphism DD were 75.5%, and 53.7% for control, and diabetic nephropathy patients. Respectively (Figure 2). The DD genotype was the major genotype at the control and diabetic Nephropathy patients in me /D (ACE).

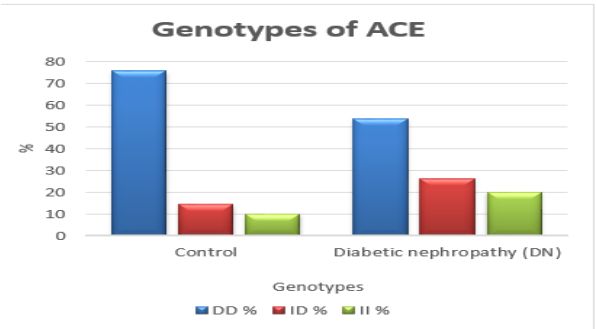

Figure 2: The percentage of II, ID, and DD genotypes of ACE in samples of Control and Diabetic nephropathy patients.

Investigation between the association of ACE (I / D) Gene Polymorphisms and risk of Diabetic nephropathy

These studies examined the association between ACE (I / D) gene polymorphism with risk of Diabetic nephropathy in patients recovered. The study was based on the results obtained from the genotyping. The statistical analysis between the allele frequencies and genotype distributions of ACE (I / D) Gene Polymorphisms in the three groups (control G1, Diabetic nephropathy patients (G2) was confirmed by the descriptive statistical at (p value < 0.05). The compression between groups as follows:

Control (G1) with Diabetic nephropathy Patients (G2) for ACE (I / D)

The genotype frequencies and allele distributions of I / D polymorphism of ACE (I / D) for (G1) and (G2) groups that showed in table 1 calculated from figure 3.

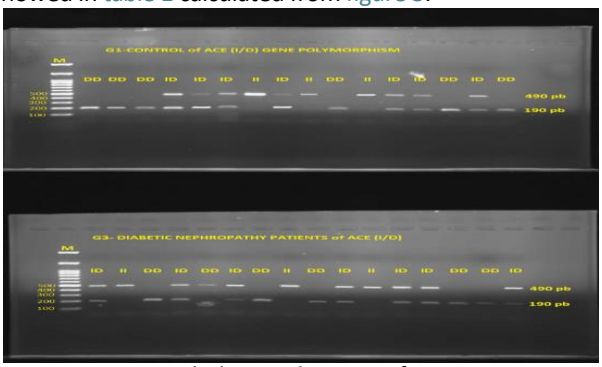

Figure 3: Gel electrophoresis of ACE ID gene polymorphism amplified with a specific pair of primers using conventional PCR. The PCR products were stained with safe stain.

The genotype frequencies and allele distributions of me/D polymorphism of ACE (I/D) for control (G1) and diabetic nephropathy patients (G2) groups are shown in Table (1.1). The results revealed a significant association between ACE (I/ D) I, D-alleles, and diabetic nephropathy where χ2 8.916 is and (P-value =0.012 < 0.05). This indicate that there was a significant relationship between this SNP and the risk of diabetic nephropathy. Also, the results show that the frequencies were 10% for II, 13% for ID, and 75.5% for DD in G1 (Control). The frequencies were 20% for II, 26.3% for ID, and 53.7% for DD in G2 (diabetic nephropathy patients).There was a significant association in I/D polymorphism of ACE between G1 and

G2 (P-value< 0.05) as shown in Table (1). In G1, the I allele frequency was 31 (17.2%) and the D allele was 149 (82.7%) and in G2 the frequencies were 53 (33.1%) and 107 (66.8%) for I and D alleles, respectively.

Descriptive statistics analyses revealed that the I/D polymorphism, ID, and DD genotypes were compared with the II genotype there was none a significant difference (P-value =0.066 > 0.05), while when compared between ID and II genotypes with DD genotype we found there was a significant difference (P-value = 0.003 < 0.05).There was also a statistically significant relationship between the D allele and the I allele (P-value = 0.001 < 0.05). The genotype frequencies and allele distributions of I/D polymorphism of ACE for Control (G1) and diabetic nephropathy patients (G3) groups that showed in table(1) calculated from Figure (3).

Table 1: The genotypes and allele distribution of ACE (I/D) polymorphism in G1 (Control) and G2 (DN).

Polymorphisms ACE (I/D)	G1 (Control) N=90(%)	G2 (DN) N=80(%)	X2	P value	OR (95%CI)	P-value
DD	68(75.5%)	43(53.7%)	8.916	0.012*	1.0ref (1.0ref)	
ID	13(14.4%)	21(26.3%)			2.555 (1.159-5.629)	0.018*
II	9(10%)	16(20%)			2.811(1.141-6.925)	0.021*
D allele	149(82.7%)	107(66.8%)			1.0ref (1.0ref)	
I allele	31(17.2%)	53(33.1%)	11.516	0.001*	2.381 (1.432-3.957)	
DD	68	43			1.0ref (1.0ref)	
ID&II	22	37	8.887	0.003*	2.660 (1.383-5.102)	
II	9	16			1.0ref (1.0ref)	
ID&DD	81	64	3.377	0.066	2.850(0.933-5.424)	

*Denotes the level of significant association between case and control, N - Number of individuals in study group, % - Genotype allele frequency and carriage rate expressed in percentage.

Based on the values in Table (1), we found that individuals carrying the ID genotype of I/D manifested effect on the increased risk of diabetic nephropathy in comparison with those carrying the DD genotype (OR = 2.555, 95%CI = 1.159-5.629, P-value = 0.018 < 0.05). These results are consistent with the finding by [12].Also, the II genotype of I/D manifested an increased risk of diabetic nephropathy compared with those carrying the DD genotype (OR = 2.811, 95%CI = 1.141-6.925, P-value = 0.012 < 0.05). These results are consistent with the finding by [13].

In the dominant model, observed that the ID & DD genotype of I/D showed no significant which it is not effect on increasing risk of diabetic nephropathy compared with the II genotype (OR = 2.850, 95%CI = 0.933-5.424 , P = 0.066 > 0.05) these results are consistent with the finding by [14].

While in the recessive model, there was showed a significant association found when compared ID & II genotype with DD genotype, where (OR = 2.660, 95%CI =1.383-5.102, P = 0.003 < 0.05). These results are consistent with the finding by [15].

4. Conclusions

In summary, the results in this study indicate a significant association between ACE gene polymorphisms in Iraqi patients with Diabetic nephropathy; we found that individuals carrying the DD genotype of I/D showed an increased risk of Diabetic nephropathy (OR = 2.555, 95%CI = 1.159-5.629, P-value = 0.018 < 0.05). also individuals carrying the I allele genotype of I/D showed an increased risk of Diabetic nephropathy (OR = 2.381, 95%CI = 1.432-3.957, p-value = 0.001 < 0.05),and that individuals carrying the ID&II genotype of I/D showed an increased risk of DN compared with the DD genotype (OR = 2.660, 95%CI = 1.383 – 5.102, P = 0.003 < 0.05).

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