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Study of angiotensin-converting enzyme insertion/deletion polymorphism in Egyptians with Chronic Kidney Diseases: A case-control study

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Abstract

Kidney disease is clinically characterized by increasing rates of urinary albumin excretion, starting from normo-albuminuria, which progresses to microalbuminuria, macro albuminuria nephropathy, and eventually to end stage renal disease. This study aimed to estimate the frequencies of different polymorphisms of ACE gene in chronic kidney disease, and Healthy control. A case-control study design was carried out on 205 Subjects they were classified into 103 chronic kidney injury patients in addition to 102 healthy subjects. The I/D polymorphism of the ACE gene was determined sequences of the sense and antisense primers PCR was performed in a final volume of 50 μl. Results: Genotype frequencies 2nd PCR (II, ID and DD-genotypes) in control group were found to be 12, 74 and 58; respectively while genotype frequencies 2nd PCR (II, ID and DD-genotypes) in CKD group were 0, 45 and 58; respectively. In Chronic Kidney Disease (CKD) patients, there were significant increase in [Creatinine (mg/dL), Cholesterol (mg/dL), T.G (mg/dL), LDL (mg/dL), Uric Acid (mg/dL)], 7.70 ± 2.98, 236.45 ± 42.19, 256.41 ± 43.11, 149.78 ± 12.78, 6.68 ± 0.82; respectively compared to control group ($p<0.01$). But there was a significant decrease in albumin 3.02 ± 0.419, compared to control group ($p<0.01$). From this study we conclude that there is a correlation between polymorphism of angiotensin converting enzyme gene and chronic kidney disease, especially DD genotype, which increases the chances of development to the stage of renal failure.

Keywords: Ace; Gene Polymorphism; Microalbuminuria; Macro Albuminuria; Chronic Kidney disease

1. Introduction

Angiotensinogen is produced in the liver and is found continuously circulating in the plasma. Renin then acts to cleave angiotensinogen into angiotensin I. Angiotensin I is physiologically inactive but acts as a precursor for angiotensin II. The conversion of angiotensin I to angiotensin II is catalyzed by an enzyme called angiotensin converting enzyme (ACE). ACE is found primarily in the vascular endothelium of the lungs and kidneys. After angiotensin I is converted to angiotensin II, it has effects on the kidney, adrenal cortex, arterioles, and brain by binding to angiotensin II type I (AT) and type II (AT) receptors [1]. Angiotensin converting enzyme is encoded by the ACE gene, which is found in 17q23 and encoded by a 21 kb gene that consists of 28 exons and 25 introns. Single nucleotide polymorphisms (SNPs) frequently occur in the ACE gene, it has been identified 6 polymorphism markers of ACE, and Alu insertion/deletion (I/D) fragment in the 16th intron is the most investigated, ACE gene polymorphism could be divided into DD, ID, II genotype based on this I/D polymorphic marker locus. (1) Although the I/D polymorphism is located on a non-coding region (i.e., intron) of the ACE gene, several investigators found that the D allele is related to increased activity of ACE in serum [2].

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2. Material and methods

A case-control study design was carried out on 205 subjects they were classified 103 chronic kidney injury patients in addition to 102 healthy subjects. They were age and gender matched, and all groups were recruited from Urology and Nephrology Centre, Mansoura University. The Study was approved by local ethical committee of Medical Experimental Research Centre, faculty of medicine, Mansoura University, Egypt. Written conformed approval consent was obtained from all study subjects.

2.1. Sample Collection

Whole blood samples (5 ml) were collected from the subjects under investigation by vein puncture using sterile disposable plastic syringes a tube containing EDTA solution, pH 8.0 as an anticoagulant. The blood was divided into two parts, one-part delivered to plain tubes and allowed to clot for 10-15 minutes, centrifuged and the separated serum is used to analyse the level of, cholesterol, triglycerides, HDL-cholesterol, albumin, creatinine, uric acid by Enzymatic Colorimetric Method. The second part of blood was taken in heparin coated tubes for separating white blood cells for extraction of DNA.

2.2. DNA extraction

(DNA) was obtained from the peripheral blood leucocytes by DNA Blood Extraction Kit, (Promega, USA). (Nanodrop™ 2000) was used to measure concentration and purity. Values less than 1.7 indicate protein contamination of DNA in early extraction steps while values more than 1.9, indicate DNA contamination with ethanol or other buffer remnants used in the extraction steps.

2.3. First PCR amplification

The I/D polymorphism of the ACE gene was determined sequences of the sense and antisense primers were (5'-CTG GAG ACC ACT CCC ATC CTT TCT-3' and 5'-GAT GTG GCC ATC ACA TTC GTC AGA T-3'), respectively. PCR was performed in a final volume of 50 µl that contained 25 µl master mix, ≈500 ng of genomic DNA, 12.5 pmol of each primer and 5% dimethyl sulfoxide (DMSO). Amplification was performed using a Gene Amp PCR system G-storm. Samples were denatured for 1 minute at 94°C and then cycled 30 times through the following steps: 45 seconds at 94°C, 1 minute at 62°C, and 1 minute at 72°C [3]. PCR products were electrophoresed in 2% agarose gel and visualized directly with ethidium bromide staining. The I allele was detected as a 490-bp band, and the D allele was detected as a 190-bp band. DMSO was included in the PCR to prevent underestimation of heterozygotes and overestimation of the DD genotype.

2.4. Second PCR amplification

A second PCR amplification was performed for each DD type with a primer pair that recognizes an insertion-specific sequence (5'- TGG GAC CAC AGC GCC CGC CAC TAC-3'; 5'-TCG CCA GCC CTC CCA TGC CCA TAA-3'), the absence of 5% DMSO.PCR was per-formed in a final volume of 50 µl that contained 25 µl master mix, ≈500 ng of genomic DNA, Samples were denatured for 1 minute at 94°C and then cycled 30 times through the following steps: 45 seconds at 94°C, 1 minute at 67°C, and 1 minute at 72°C [4]. PCR products were electrophoresed in 2% agarose gel and visualized directly with ethidium bromide staining.

2.5. Statistical Analysis

Data were analysed using SPSS (Statistical Package for Social Science, Inc., Chicago, IL, USA), was used in the analysis. For quantitative variables, mean and median, standard deviation. Frequency and percentage are presented for qualitative variables. Chi square test used to estimate differences in qualitative variables. Significance probability "p-value": $P<0.05$ is considered for statistical significance, while $P>0.05$ is considered for statistical non-significance.

3. Results

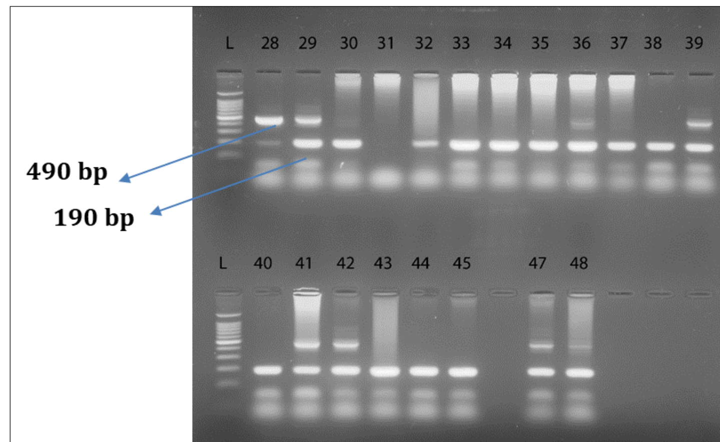

Figure 1 ACE polymorphism genotype 1st PCR: Lanes 29, 39, 41, 42, 47 showed heterozygous ID, 30, 32-38, 40, 43, 44, 45, 48 homozygous DD cases, 28 homozygous II cases (I allele 490 bp, D allele 190 bp)

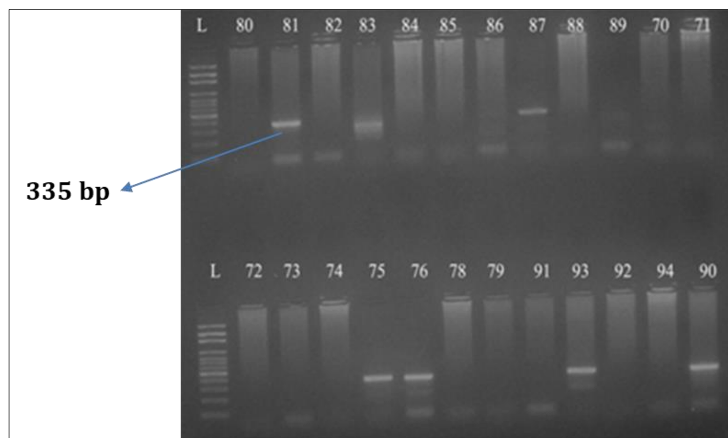

Figure 2 ACE polymorphism genotype 2nd PCR: Lanes 81, 87, 93, 75, 76. 93, 90 represent ID polymorphism. Lanes 80, 82, 83, 84, 85, 86, 88, 89, 70, 71, 72, 73, 74, 78, 79, 91, 92, and 94 represents DD polymorphism. I allele appear at 335 bp

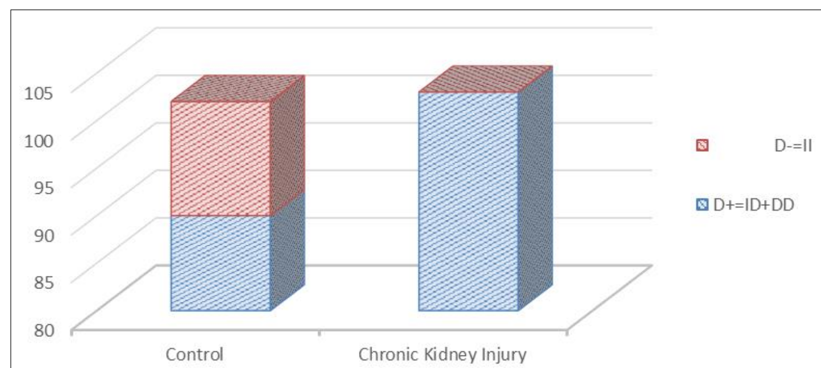

Figure 3 D+/D- in both chronic kidney disease patients and control, x axis represent groups while Y axis is frequency of D+ and D-

Table 1 Characters and Biochemical Parameters of Control and Chronic kidney disease groups

Characters	Control	Chronic Kidney Disease	
No.	102	103	
Age (year) Mean± S.D.	27.89 ± 5.39	38.99 ± 8.2	
range	20- 45	19- 55	
Male	59 (57.8 %)	63 (61.2 %)	
Female	43 (42.2 %)	40 (38.8 %)	
Creatinine (mg/dL)	1.27 ± 2.06	7.70 ± 2.98	P = 0.000*
Albumin (g/dL)	3.98 ± 0.83	3.02 ± 0.419	P = 0.000*
Cholesterol (mg/dL)	171.38 ± 17.95	236.45 ± 42.19	P = 0.000*
T.G (mg/dL)	73.25 ± 18.76	256.41 ± 43.11	P = 0.000*
LDL (mg/dL)	70.81 ± 12.62	149.78 ± 12.78	P = 0.000*
Uric Acid (mg/dL)	5.08 ± 0.35	6.68 ± 0.82	P = 0.000*

*P value ≤ 0.05 sig, P value > 0.05 non sig. Creatinine (mg/dL), Albumin (g/dL), Cholesterol (mg/dL), Triglycerides (mg/dL), Low Density Lipoprotein LDL (mg/dL), Uric Acid (mg/dL)

Table 2 Prevalence of ACE I and D polymorphism genotype 1st PCR in Chronic Kidney Disease patients and control groups

Genotype	Control	Chronic Kidney Disease	p-value
II	12	0	P = 0.000*
ID	66	21	
DD	24	82	

*P value ≤ 0.05 sig, P value > 0.05 non sig. II (Insertion/ Insertion), ID (Insertion/ Deletion), DD (Deletion/ Deletion)

Table 3 Prevalence of ACE I and D polymorphism genotype 2nd PCR in Chronic Kidney Injury patients and control groups

Genotype	Control	Chronic Kidney Disease	p-value
II	12	0	P =0.000*
ID	74	45	
DD	16	58	

*P value ≤ 0.05 sig, P value > 0.05 non sig. II (Insertion/ Insertion), ID (Insertion/ Deletion), DD (Deletion/ Deletion)

Table 4 Prevalence of ACE I / D alleles in Chronic Kidney Injury patients and control

Allele	Control	Chronic Kidney Disease	p-value
I allele	98	45	P=0.000*
D allele	106	161	

*P value ≤ 0.05 sig, P value > 0.05 non sig. I (Insertion Allele), D (Deletion Allele)

Table 5 D+/D- in both Chronic Kidney Injury patients and control

Allele	Control	Chronic Kidney Disease	p-value
D+=ID+DD	90	103	P=0.000*
D-=II	12	0	

*P value ≤ 0.05 sig, P value > 0.05 non sig

4. Discussion

A polymorphism of the ACE gene, consisting of a 287-base pair fragment within intron 16 defined by insertion [I] or deletion (D), has been shown to influence the circulating and cellular ACE concentration. The ACE gene I/D polymorphism are reportedly associated with the progression of several renal diseases, including diabetic nephropathy, IgA nephropathy, autosomal dominant polycystic kidney disease, and graft failure in renal transplant recipients. The D allele has a dominant effect and is associated with higher plasma ACE and angiotensin II levels [3]. Our study aims to estimate the frequencies of different polymorphisms of ACE gene in chronic kidney disease, and Healthy control group. The genetic origin of kidney diseases has been a focus of research in the past few years. There is significant evidence showing that the RAAS is involved in the pathogenesis of progressive renal disorders. In recent studies the association between disease progression and the ID/DD genotype of the ACE gene has been well described by [5].

It has been demonstrated that DD genotype is associated with high activity of ACE compared to ID and II genotype not only in plasma but also in several tissues such as heart and kidney. The deletion polymorphism is associated with elevated serum and cellular ACE levels. DD genotype of ACE gene leads to the development of diabetic nephropathy (DN), probably due to high level of angiotensin II, a vasoactive peptide that increased Glomerular Filtration Rate (GFR) and intra-glomerular pressure and promoted proliferation of mesangial cells matrix. DD genotype or D allele strongly associated with increased serum ACE activity leads to higher ACE expression and its activity and may predispose individuals to diabetic complication. Experimental data suggest that elevated ACE activity is genetically associated with pathogenesis of diabetic renal damage [6]. The patients with D allele showed higher values of serum ACE activity than II allele. Increased ACE activity promotes alterations in renal hemodynamic that contribute to increased intraglomerular pressure and hyperfiltration and hence, lead to glomerulosclerosis and ESRD [7]. The presence of DD genotype operates at cellular level leading to hypertensive state and renal diseases. Caucasians with DD genotype have serum ACE levels and intra-cellular ACE activity twice than those of II genotype [8]. In Chronic Kidney Disease (CKD) patients, there were significant increase in [Creatinine (mg/dL), Cholesterol (mg/dL), T.G (mg/dL), LDL (mg/dL), Uric Acid (mg/dL)], 7.70 ± 2.98, 236.45 ± 42.19, 256.41 ± 43.11, 149.78 ± 12.78, 6.68 ± 0.82; respectively compared to control group ($p<0.01$). But there was a significant decrease in albumin 3.02 ± 0.419, compared to control group ($p<0.01$), Table (1). Prevalence of ACE polymorphism genotypes 1st PCR (II, ID and DD-genotypes) in control, and chronic kidney disease (CKD) groups; were illustrated in. Genotype frequencies 1st PCR (II, ID and DD-genotypes) in control group were found to be 12, 66 and 24; respectively while genotype frequencies 1st PCR (II, ID and DD-genotypes) in CKD group were 0, 21 and 82; respectively. Table (2). Prevalence of ACE polymorphism genotypes 2nd PCR (II, ID and DD-genotypes) in control, and chronic kidney disease (CKD) groups; were illustrated in. Genotype frequencies 2nd PCR (II, ID and DD-genotypes) in control group were found to be 12, 74 and 58; respectively while genotype frequencies 2nd PCR (II, ID and DD-genotypes) in CKD group were 0, 45 and 58; respectively. Table (3). Prevalence of ACE alleles for insertion (I) allele and deletion (D) allele were estimated. For (I) allele frequencies were 98 and 45 for control and chronic kidney disease patients; respectively indicating higher frequency of (I) allele in control group compared to chronic kidney disease patients. For (D) allele the frequencies were 106 and 161 for control and chronic kidney disease patients; respectively indicating higher frequency of (D) allele in chronic kidney disease patients compared to control group. Tables (4). Prevalence of D+/D- in both Chronic Kidney Injury patients and control, for D+=(DD+ID) frequencies were 90 and 103 for control and chronic kidney disease patients; respectively indicating lower frequency of D+=(DD+ID) in control group compared to chronic kidney disease patients. For D-=II the frequencies were 12 and 10 for control and chronic kidney disease patients; respectively indicating lower frequency of (D-) in chronic kidney disease patients compared to control group. Tables (5), Figure (3). However, the meta-analyses conducted from 1994 to 2004 which included 8,663 cases and 6,064 controls demonstrated that the II genotype reduced the risk of DN compared to the D-allele carriers [9]. Another comprehensive meta-analysis in 2012 which included 14,108 cases and 12,472 controls from 63 published studies also found a significant association between the ACES I/D polymorphism and the risk of diabetic nephropathy [10].

5. Conclusion

The results recorded a marked increase in the level of [Creatinine (mg/dL), Cholesterol (mg/dL), T.G (mg/dL), LDL (mg/dL), Uric Acid (mg/dL)] in patients with chronic kidney disease compared with control group in the case of DD genotype compared genotype ID, II. The results also showed a significant reduction in the level of albumin in patients with chronic kidney disease compared with control group in the case of DD genotype compared genotype ID, II. From this study we conclude that there is a correlation between polymorphism of angiotensin converting enzyme gene and chronic kidney disease, especially DD genotype, which increases the chances of development to the stage of renal failure. Patients with chronic kidney disease are the most vulnerable to disease, hardening of the arteries due to a significant increase in the proportion of triglycerides and cholesterol in the blood. DD genotype was represented in chronic kidney disease compared to the healthy group, while the results showed that genotype II was not present in patients with chronic kidney disease compared to the healthy group.

Compliance with ethical standards

Acknowledgments

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Statement of informed consent

The protocol for this study was reviewed and approved by Research and Ethics Committee. All methods and analysis of data was approved by Research and Ethics Committee, and carried out in accordance with local and international guidelines and regulations. All data used in this study was collected as part of routine medical procedures. Informed consent was waived by Research and Ethical Committee for this study, absence of any patient identifying information.

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