Huda Rafaa Sabbar et al /J. Pharm. Sci. & Res. Vol. 10(8), 2018, 1983-1985

ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

# Polymorphism Pattern of Angiotensin Converting Enzyme (ACE) Gene in the Chronic Renal Failure Patients

*** Huda Rafaa Sabbar ; **Omar Abdulkareem Ali ; ***Fazea Oleiwi Naser**

** Ph.D. Medical Microbiology/ College of Medicine/ University of Anbar*
*** MSc. Medical Microbiology/ College of Medicine/ University of Anbar*
****Higher deploma. Internal Medicine / Ministry of Health/ Iraq*

**Abstract**

Genetic variability in the genes of different components of renin system (RAS) is likely to contribute for its heterogeneous association in the renal disease patients.The angiotensin converting enzyme (ACE) gene consists of either an insertion (I) allele or a deletion (D) allele that form three possible genotypes: II, ID or DD. This study was aimed to detect if there is a relation of genetic factors changes as ACE polymorphism in the progression of patients to the End stage renal disease (ESRD.):

Blood samples were obtained from 100 Patients suffering and 50 samples as control groups. Blood was collected in tube containing EDTA; DNA was extracted from the samples by wizard genomic DNA purification kit, (Promega) according to the "Isolating Genomic DNA from whole blood protocol". The volume of the extracted DNA solution was usually 100 µl were stored at -20 °C.

The resultsshow the DD genotype percent was found to be high in the patients group 56%, followed by ID 28% then II genotype 16% .While in the control group the results were DD 34%, II 30%, ID 36%. There was a significant difference between patients and control group in DD( *P* value was 0.0019 and odd ratio 2.47) while II genotype the( *P* value 0.02 and Odd ratio 0.44), in the ID genotype there was no significant difference ( *P* value >0.05). There was no relation between the cofactors of chronic renal failure disease and ACE polymorphism *P* value for all was > 0.18 Also there was no relation between gender and the ACE polymorphism ( *P* value was > 0.05).

In most studies in spite of the percent of alleles distribution, they found that DD allele was mostly related to the progression of patients to ESRD. Although all our patients in ESRD, the large proportion of present patients had DD, which may be related to progression of patients to the end stage renal disease (ESRD).

**Key words:** ACE, Polymorphism, ESRD

## INTRODUCTION

The angiotensin converting enzyme (ACE) was encoded by a 21 Kb gene that consists of 26 exons and located on chromosome 17q23. A polymorphism of the ACE gene engross the insertion (I) or deletion (D) of a 287 bp AluYa5 repeat sequence inside intron (1). Though I/D polymorphism is located in a non-coding region of the ACE gene it is not quiet and that the D allele was related with increased activity of ACE in serum (the highest serum ACE activity was seen in the DD genotype while the lowest seen in II genotype) (2).ACE gene consists of either an insertion (I) allele or a deletion (D) allele that form three possible genotypes: II, ID or DD (3).

Rigat *et al*. (4) noted that the inter individual difference in plasma ACE activity was related to an insertion/ deletion polymorphism in an intron of the ACE gene; individuals homozygous for the shorter or deleted (DD) gene had the highest values of serum ACE activity compared with subjects with the longer or inserted (II) gene. Heterozygous individuals (ID) articulated intermediate serum ACE activities. It was reasoned that the D allele and a higher ACE activity could be associated with more extensive kidney damage (5).

DD homozygotes also have higher tissue levels??? of ACE. The ACE I/D gene polymorphism, correlate with circulating ACE concentration, has been concerned in the etiology of ESRD and has been investigated in several epidemiologic studies at present (6). Development of renal disease in the ESRD population was manipulated by the ACE polymorphism. The time from diagnosis to the beginning of ESRD was shorter in the existence of the ACE-DD than ID or II alleles.

The ACE gene I/D polymorphism may be important determinant of the reno or renal protective efficiency of ACE inhibitor treatment in independent diabetes mellitus (IDDM) patients. Those with the II genotype had a greater rate of progression of albumin excretion rate (AER) in the absence of ACE inhibitor treatment, patients in this group also had the greatest response to ACE inhibitors in terms of slowing the progression of AER, and those with the DD genotype were the most resistant (7). Thus, a concern must be taken during the treatment of these patients with these drugs. In the present study, we had not been capable to assume the association of ACE inhibition and DD genotype due to non-availability of the information of anti-hypertensive therapy consumed by the patients in this study. The Renin-Angiotensin system (RAS) is a key regulator of both blood pressure and kidney functions and may play a role in their relations. Its role in the pathogenesis of hypertension is well documented but its contribution to chronic renal failure and progression of kidney nephropathy is still debated (8, 9).

## MATERIAL AND METHODS

**1- Samples Collection & DNA Extraction:** Blood samples were obtained from 100 patients with ESRD and 50 as control groups. Blood was collected in tube containing EDTA; DNA was extracted from the samples by wizard genomic DNA purification kit, (Promega) according to the "Isolating Genomic DNA from whole blood protocol". The volume of the extracted DNA solution was usually 100 µl were stored at -20 °C.

**2- Estimation of Purity**

DNA samples were quantified by ultraviolet spectrophotometer (Unico,USA) reading at 260 and 280 nm (10). All samples were stored at -20 $^{o}$C until use.

**3- Amplification** :

The specific segment of ACE gene was amplified by polymerase chain reaction

(PCR) using the specific primers :

ACE-F (5-TGGAGACCACTCCC ATCCTTTC-3) and

ACE-R (5-GATGTGGCCATCACATTCGTCAGAT-3).

The PCR amplification was performed in a total volume of 25 µl containing:

- 5µl DNA (conc. 20 ng), 12.5 µl of 2X Go Taq green master mix. , 2.5 µl of ACE-F primer., 2.5 µl of ACE-R primer., 2.5 µl of Nuclease free water .

PCR tubes were closed and transferred into the thermal-cycler when reach temperature reach 95 C$^{o}$ and start the amplification program.The reaction was performed in: 4 min of initial denaturation at 94°C, followed by 32 cycles of 30 s at 94°C, 30 s

at 57°C and 1 min at 72°C and one cycle of 10 min at 72°C as a final extension.

**4- Result Analysis:**

Analysis of PCR results were based on the presence of specific bands of DD, ID and II genotypes. These identified by the presence of a single 190 bp, this represent the DD homozygous. The homozygous for I allele (II genotype) was identified by the presence of single 490 bp PCR product while the heterozygous individuals (ID genotype) were identified by the presence of both 190bp and 490bp PCR products as showed in post PCR gel electrophoresis.

## RESULTS:

**1- Pre-PCR Result**

After the extraction of DNA, the purity of all samples were assessed and mean levels were found to be (1.4) .The gel electrophoresis for extracted DNA was done to ensure the presence of DNA in the extracted samples and the result was shown in Figure 1.

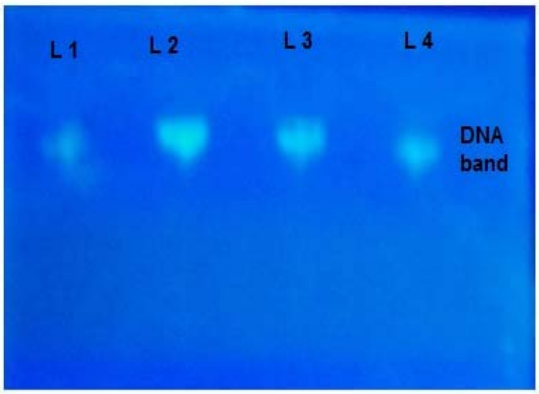


**Figure 1. Pre-PCR bands of extracted DNA.**

**2. Polymerase Chain Reaction**

The PCR result showed that, the homozygous individuals for the D allele (DD genotype) was identified by the presence of a single 190 bp PCR product. The homozygous for I allele (II genotype) was identified by the presence of a single 490 bp PCR product while the heterozygous individuals (ID genotype) was identified by the presence of both 190 and 490 bp PCR products as shown in Figure (2).

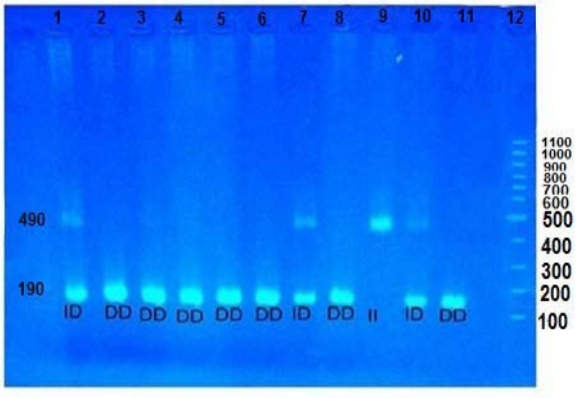


**Figure 2: Homozygous DD, homozygous II and heterozygous ID genotype, lane 1,7,10: heterozygous ID, lane 2,3,4,5,6,11: homozygous DD, lane 9: homozygous II, lane 12: DNA ladder.**

**3. Distribution of Patients According to Angiotensin converting enzyme Polymorphism**

The DD genotype percent was found to be high in the patients group56%, followed by ID 28% then II genotype 16% .While in the control group the results were DD 34%, II 30%, ID 36%. There was a significant difference between patients and control group in DD(P value was 0.0019 and odd ratio 2.47) while II genotype the( P value 0.02 and Odd ratio 0.44), in the ID genotype there was no significant difference (P value >0.05) as shown in Table 1 and Figure 3.

**Table 1. The distribution of ACE genotype frequency in the patients and controls group**

| Genotypes frequency percent | Patients % N (100) | Control % N (50) | P *value* | OR |
|---|---|---|---|---|
| DD | 56% | 34% | 0.0019 | 2.47 |
| ID | 28% | 36% | >0.05 | 0.69 |
| II | 16% | 30% | 0.02 | 0.44 |

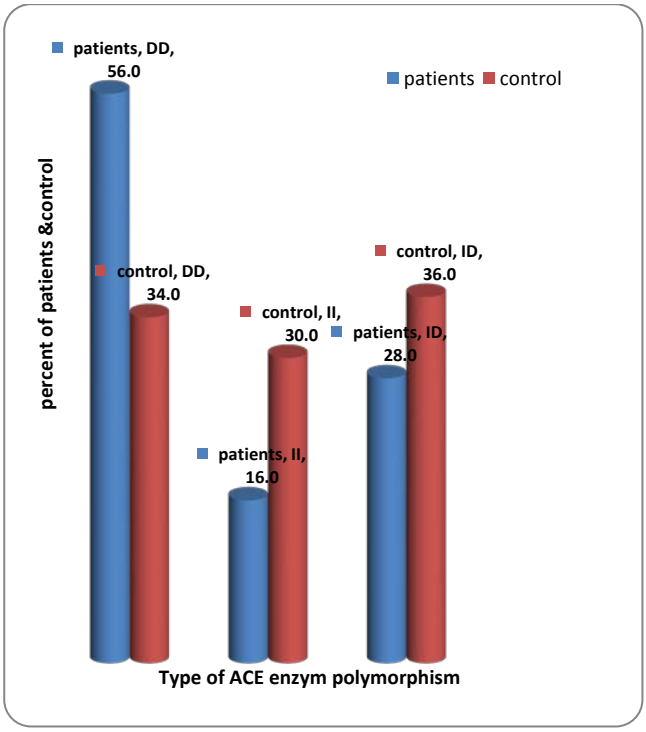


**Figure 3. Distribution of patients according to ACE polymorphism.**

**4. Relation between Angiotensin Converting Enzyme Polymorphism and Cofactors of Disease and the Gender.**

There was no relation between the cofactors of chronic renal failure disease and ACE polymorphism P value for all was > 0.18 as shown in Table 2. Also there was no relation between gender and the ACE polymorphism (P value was > 0.05).

**Table 2. Relation of ACE polymorphism with cofactors of disease**

| Cofactors | Chi- square value | P value |
|---|---|---|
| Hypertension | 4.900 | 0.179 |
| Diabetes mellitus | 0.655 | 0.884 |
| Urolithiasis | 0.77 | 0.81 |
| Other causes | 1.704 | 0.636 |

## DISCUSSION:

The present study showed that DD genotype was (56%) more frequent in the patients than other, followed by ID (28%) and II (16%). There was significant difference between patients and control in DD and II genotypes. This finding was in agreement

with Al-Awadi *et al* (11), who found that DD was predominant between population within other different diseases. Also agreed with Tripathin *et al* (9) in the presence of significant difference between patients and control in DD and II but not in percent of genotypes frequency. Also agreed with Mclaaughlin *et al* (12) who found that high prevalence of DD among patients with renal disease. The present study not consistent with Samuelsson *et al* (13) and Choudhry *et al* (14) in the distribution of DD, ID and II percent in patients of renal disease. This inconsistency could be in part due to the genetic and environmental heterogeneity among different ethnic groups, or may be caused by methodological differences or may be due to small sample sizes of population in the study.

In most studies in spite of the percent of alleles distribution, they found that DD allele was mostly related to the progression of patients to ESRD (9, 11, 12). Although all our patients in ESRD, the large proportion of present patients had DD, which may be related to progression of patients to the end stage renal disease (ESRD). This progression was indicated by many studies (9, 11, 12). However, disagreed with Choudhry et al (14). This progression may occur because the DD homozygotes had higher tissue levels of ACE and the ACE I/D gene polymorphism, correlating with circulating ACE concentration, has been implicated in the etiology of ESRD and has been investigated in numerous epidemiologic studies (15).

The phrase is repeated in the introduction). Those with the II genotype had a greater rate of progression of albumin excretion rate (AER) in the absence of ACE inhibitor treatment, patients in this group also had the greatest response to ACE inhibitors in terms of slowing the progression of AER, and those with the DD genotype were the most resistant (16). Therefore, a consideration must be taken during the treatment of these patients with these drugs. In the present study, we had not been able to deduce the association of ACE inhibition and DD genotype due to non-availability of the information of anti-hypertensive therapy consumed by the patients in this study.

Also ACE DD genotype was a known risk factor of cardiovascular diseases, including coronary heart disease and left ventricular hypertrophy, and that the latter was a strong predictor of the mortality in dialysis patients (17).

There was no significance difference between male and female in ACE polymorphism, this not confirm with Al-Awadi *et al* (11) and Samuelsson *et al* (13). This disagreement may be due to that the male patients in this study were older than female. DD allele may be depleted in older patients with cardiovascular disease (12). There was no relation between gene polymorphism and the type of disease, even with patients having the same allele frequency, in-spite of that patients had the DD and hypertension were more than the hypertensive patients without DD allele (35:21) ,

This difference was not significance. This result was not consistent with Al-Awadi *et al* (11) and Samuelsson *et al* ( 13) who found that most hypertensive patients had DD allele and this may be due to the inhomogeneous population, therefore, the large number of each type of disease must be involved to determine the presence of such relationship.

## REFERENCES


1- Michel MC, Bohner H, Koster J, et al(2004). Safety of telmisartan in patients with arterial hypetension: an open label, observational study. Drug Safety, ; 27:334-335.

2- Sakuma T, Hirata R D and Hirata M H.( 2004) Five polymorphisms in gene candidates for cardiovascular disease in Afro-Brazilian individuals. J Clin Lab Anal, ;18: 309-316.

3- Rigoli L, Chimenz R, di Bella C, et al.( 2004) Angiotensin-converting enzyme and angiotensin type 2 receptor gene genotype distributions in Italian children with congenital uropathies. *Pediatr Res*, ;56: 988–993.

4- Rigat B.; Hubert P. and Corvol P.(2009) PCR detection of the insertion/deletion polymorphism of the human angiotensin converting enzyme gene (DCP1) (dipeptidy 1 carboxy peptidase. Nucleic Acids Res, 20:1433-1439

5- Yu J, Kuznetsova T, Thijs L, et al.( 2011) Association of echocardiographic left ventricular structure with the ACE D/I polymorphism: A meta-analysis. J RAS, ; 12: 243–253.

6- Yoshida H, Kon V, Ichikawa I. Polymorphism of the renin-angiotensin system genes in progressive renal disease. Kidney Int., 1996;50:732-744.

7- Penno G, Chaturvedi N, Talmud Ph J, et al.( 1998) Effect of Angiotensin-Converting Enzyme (ACE) Gene Polymorphism on Progression of Renal Disease and the Influence of ACE Inhibition in IDDM Patients. Diabetes, ; 47: 1507-1510.

8- Mondry A, Loh M, Liu P, et al .( 2005) Polymorphism of the insertion/deletion ACE and M235T AGT genes and hypertension: surprising new finding and meta-analysis of data. BMC Nephrol, ; 6:1.

9- Tripathi G , Dharmani P, Khan F, et al.( 2006) High prevalence of ACE DD genotype among north Indian end stage renal disease patients.BMC Nephrol, , 7:15

10- Sambrook, J, Fritsch, EF and Maniatis, T.(1989) Molecular cloning : a laboratory manual. 2nd ed., cold spring harbor laboratory press, cold spring harbor, New York 1 .

11- Al-Awadi SJ, Ghareeb AM, Oleiwi AA et al.( 2006) Genotype Distribution of Angiotensin I- Converting Enzyme in Iraqi Arab Population. Iraqi J of Can and Med Genet, ; 4(2): 24-29.

12- Mclaaughlin KJ, Harden PN, Ueda Sh, et al.(1996) The Role of Genetic Polymorphisms of Angiotensin-Converting Enzyme in the Progression of Renal Diseases.Hyper,1 ; 28 (5): 912-915.

13- Samuelsson O, Attman P, Larsson R, et al.(2000) Angiotensin I-converting enzyme gene polymorphism in non-diabetic renal disease. Nephrol Dial Transpant, ;15: 481-486.

14- Choudhry N, Nagra SA, Shafi T et al.(2012) Lack of association of insertion/deletion polymorphism in angiotensin converting enzyme gene with nephropathy in type 2 diabetic patients in Punjabi population of Pakistan. African J of Biotech, ; 11(6): 1484-1489.

15- Zhou T , Yin Sh and Qin Y.(2014) Association between angiotensin converting enzyme insertion/deletion gene polymorphism and end stage renal disease susceptibility. J Re-Ang- Ald Sys, ;15(1): 22–31.

16- Penno G, Chaturvedi N,Talmud PJ et al.(1998) Effect of Angiotensin-Converting Enzyme (ACE)Gene Polymorphism on Progression of Renal Disease and the Influence of ACE Inhibition inIDDM Patients. Diabetes, ; 47: 1507-1510.

17- Yoshida H, Kuriyama S, Atsumi Y et al.(1996) Angiotensin I converting enzyme gene polymorphism in non-insulin dependent diabetes mellitus. Kidney Inter, ; 50: 657-664.