

## ANGIOTENSIN I-CONVERTING ENZYME GENE POLYMORPHISM IN PATIENTS WITH CHRONIC RENAL FAILURE

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### ABSTRACT

**Background:** The Renin-Angiotensin system (RAS) is a key regulator of both blood pressure and kidney functions and their interaction. In such situation, genetic variability in the genes of different components of RAS is likely to contribute for its heterogeneous association in the renal disease patients. Angiotensin converting enzyme-1 (ACE-1) is an important component of RAS which determines the vasoactive peptide Angiotensin-II. **Methods:** In the present study, we have investigated 100 ESRD patients and 50 normal healthy controls to deduce the association between ACE gene polymorphism and ESRD. A total of 50 normal healthy controls were also genotyped for ACE I/D polymorphism. The criterion of defining control sample as normal was

totally based on the absence of any kidney disease determined from the serum creatinine level. Genotyping of ACE I/D was assayed by polymerase chain reaction (PCR) based DNA amplification using specific flanking primers. **Results:** of 100 patients with chronic renal failure with different causes, the DD genotype was 56%, II genotype 13%, ID was 28% and negative results was 3%. The control group results was DD 26%, II 26%, ID 22%, and negative results was 26%. **Conclusion:** the results in this study of patients with chronic renal disease indicate that presence of the D allele in the ACE genotype may be of particular importance as a predictor of high rate of progression to ESRD.

**KEYWORDS:** chronic renal failure, ACE, polymorphism.

### 1. INTRODUCTION

Hypertension is the major contributor in progression of renal failure in patients with renal disease both with and without proteinuria. <sup>[1]</sup> Moreover, hypertension per se is a risk factor

for the development of ESRD. <sup>[2, 3]</sup> On the other hand, the prevalence of hypertension increases with decreasing renal function. These results from both decreased sodium excretion and an activation of the renin-angiotensin-aldosterone system (RAS). Therefore, it is possible that a genetic predisposition to salt-dependent hypertension or overactivation of the RAS may predispose to the development of renal failure. <sup>[1]</sup> and promote a more rapid loss of glomerular filtration rate in patients suffering from renal diseases. <sup>[2]</sup> Thus, genes that regulate renal sodium reabsorption or genes of the RAS may be extremely important in patients suffering from ESRD. Among the candidate genes of the of renal disease , the angiotensinogen gene (*AGT*), the angiotensin converting enzyme gene (*ACE*) and the aldosterone synthase gene (*CYP11B2*) are of particular interest. <sup>[4]</sup>

It is now recognized that the activity of RAS is regulated not only by renin and by angiotensin receptors, but also by other components of the system, specifically, availability of angiotensinogen (Atg) and the activity of the angiotensin converting enzyme (ACE). <sup>[5]</sup> In this regard, human genetic studies have revealed that the genes of RAS are highly polymorphic, raising the possibility that, in addition to environmental factors, the genetic make-up of RAS affects the status of RAS in individuals. Such polymorphism is the insertion/deletion polymorphism of the ACE gene. The ACE gene consists of 26 exons and spans 21 kb on chromosome 17. Within intron 16, a polymorphism exists, consisting of the presence or absence of a 287 base pair fragment. While this deletion polymorphism is associated with elevated serum and cellular ACE levels. <sup>[6, 7]</sup> its association with blood pressure levels or ischemic heart disease varies among populations of different genetic and environmental backgrounds. <sup>[8, 9, 10]</sup> Only a third to a quarter of all patients with IDDM develop advanced diabetic nephropathy. <sup>[11]</sup> Observations of a high concordance for nephropathy in families with multiple IDDM siblings indicate that hereditary factors may be important in its pathogenesis. <sup>[12]</sup> A clear candidate for this role is the diallelic (insertion/deletion [I/D]) polymorphism in the ACE gene. This polymorphism is correlated to plasma ACE concentration. <sup>[13]</sup> And ACE activity is significantly elevated in diabetic subjects with nephropathy. <sup>[14]</sup> Also other studies indicate that the deletion polymorphism in the ACE gene is significantly associated with the progressive loss of renal function in patients with IgA nephropathy, it appeared possible that ATI and Atg gene polymorphisms may, synergistically with the ACE gene affect the prognosis of renal diseases. <sup>[15]</sup> The Renin-Angiotensin system (RAS) is a key regulator of both blood pressure and kidney functions and may play a role in their interaction. 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 <sup>[16]</sup> It has been seen that RAS blockers i.e. both angiotensin converting enzyme (ACE) inhibitors and angiotensin receptor blockers lower blood pressure and can also attenuate or prevent renal damage. <sup>[17]</sup> However, major inter individual treatment responses to RAS inhibitors have been noted. <sup>[18]</sup> And it remains difficult to predict responders based on known pathophysiological characteristics. <sup>[19]</sup> In such a situation, genetic variability in the genes of different components of RAS is likely to contribute for its heterogeneous association in the renal disease patients. Angiotensin converting enzyme-1 (ACE-1) is an important component of RAS and it determines the vasoactive peptide Angiotensin-II. Its inhibition reduces the pace of progression of majority of chronic nephropathies. <sup>[20]</sup>

The present study is an attempt to investigate the association between ACE gene polymorphism and the causation of renal disease in end stage renal disease patients. This study aims to determine the genotype of this enzyme and any one of genotypes DD, ID and II is more related to renal disease.

## 2. MATERIALS AND METHODS

**2.1. Patients:** this study involved 100 patients with chronic renal failure. The patients were 42% females and 58% males. Patients aged between 18 to 80 years. The control group included 50 individuals aged (45.48±15.05).

**2.2. Method:** Blood samples were obtained from 100 patients and 50 control groups. Blood was collected in tube containing EDTA; DNA was extracted from the samples by wizard genomic DNA purification kit, (Promega, UK) 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.

### 2.3. DNA Quantification and Estimation of purity

DNA samples were quantified by Ultraviolet spectrophotometer (Unico, USA) reading at 260 and 280 nm <sup>[21]</sup> All samples were stored at -20 °C.

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 D.W.

PCR tube were closed and transferred into the thermal-cycler when reach temperature reach 95 C° and start the amplification program. The amplification program include: The reaction were 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 extention.

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

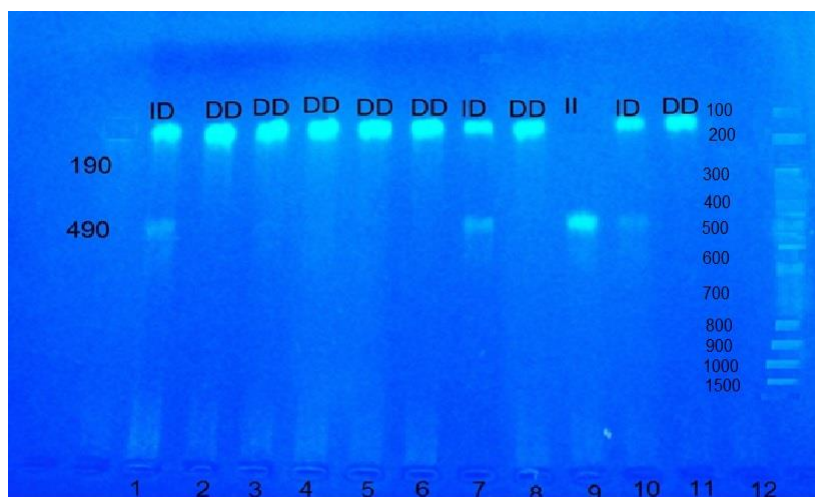
**2.4. Statistical analysis:** The statistical analysis used in this research include descriptive statistical analysis, mean and standard deviation and chi-square test.

### 3. RESULTS

100 blood samples were obtained from patients with ESRD, which caused by different causes. The patients group include 42% female and 58% male , their age between 18 and 80 years(mean=50.35±16.51) . The questionnaires contain informations like gender, diseases like diabetes, hypertension, stone, and of unknown causes. The control group included 50 individuls whom their ages matched those of patients.

The PCR result showed that, the homozygous individuals for the D allele (DD genotype) were identified by the presence of a single 190 bp PCR product.

The homozygous for I allele (II genotype) were identified by the presence of a single 490 bp PCR product while the heterozygous individuals (ID genotype) were identified by the presence of both 190 and 490 bp PCR products as showed in Figure 1.

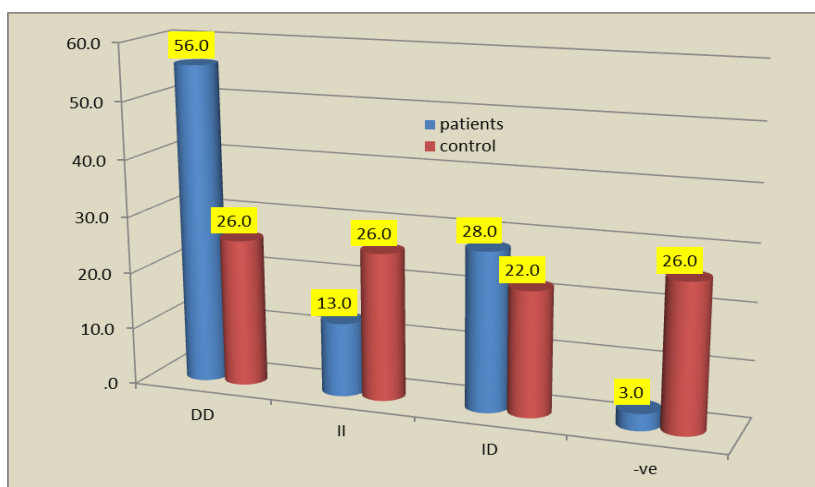


**Figure1:** Figure illustrating homozygous DD, homozygous II and heterozygous ID genotype, lane 1, 7, 10: heterozygous ID, lane 2, 3, 4, 5, 6, 11: homozygous DD, lane 9: homozygous II, lane 12: DNA ladder.

The DD genotype show high percent among patients group was 56%, II genotype 13%, ID was 28% and negative results was 3%. While The control group results was DD 26%, II 26%, ID 22%, and negative results was 26%. There is significance difference between patients and control group in DD genotype ratio. (Table 1, figure 2).

**Table 1:** show the percent of each genotype in patients and control group. DD (deletion/deletion), II (insertion/insertion), ID (insertion/deletion).

genotype	% of patients	% of control
DD	56.0	26.0
II	13.0	26.0
ID	28.0	22.0
-ve	3.0	26.0



**Figure 2:** The frequency of patients and controls represented in y-axis, polymorphism type represented in x-axis with reference to the percent of each genotype in patients and control group.

The present results showed that there was no relation between the types of disease might lead to renal failure and the polymorphism of the ACE. There was no relation between each of diabetes ( $p$  value=0.975, hypertension  $p$  value=0.284, stone ( $p$  value=0.295) and unknown( $p$  value=0.636) causes and with the sex of patients ( $p$  value=0.975).

#### 4. DISCUSSION

The data presented in this study were the first to be reported in Iraq regarding the role of genetic variants of ACE gene in causation and progression of renal diseases. These findings clearly established the association of ACE I/D gene polymorphism with the renal failure. The DD genotype was found to be a major risk determinants of ESRD among patients. Yoshida et al. found that patients with biopsy-documented IgA nephropathy was the most common type of glomerulonephritis.<sup>[22]</sup> They took advantage of experimental and clinical evidence that an activated renin system is involved in the pathogenesis of glomerular damage and that there is an individual variability in angiotensin-converting enzyme (ACE) activity which is linked to a polymorphism in the ACE gene. They then used molecular techniques to evaluate whether there is a link between the genotype of ACE and progressive loss of creatinine clearance in IgA nephropathy. There was a higher frequency of the D allele of the ACE gene in patients with proteinuria and a progressive loss of renal function, and this association was present even in patients with a normal blood pressure. Furthermore, Rigat et al. noted that the interindividual variation in plasma ACE activity was linked 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) expressed intermediate serum ACE activities. It was revealed that the D allele and a higher ACE activity could be associated with more extensive kidney damage<sup>[23]</sup>

Angiotensin II has a potentially important role in the development of glomerulosclerosis.<sup>[24]</sup> Through its action as a growth factor and regulator of the cell growth and matrix production.<sup>[25]</sup> It has also been implicated that the inhibition of its production attenuates the progression of diabetic and non -diabetic nephropathies [26]. In this regard the importance of ACE and its genetic variants becomes more apparent. Although most of the studies on ACE I/D polymorphism have been very encouraging with regard to the role of DD genotype in the pathophysiology and treatment of diabetic nephropathies. Similar studies in other types of nephropathies have yielded inconsistent results. For examples, studies on autosomal



dominant polycystic kidney patients have reported adverse effects of the D allele of the ACE gene in some cases. <sup>[27,28]</sup> whereas number of other studies did not confirmed such association. <sup>[29]</sup> Similarly, an adverse effect of D allele was also found in some studies in IgA nephropathy or ESRD in general. <sup>[30]</sup> In a study of 80 family trios (proband and parents) with interstitial nephritis, the D allele was transmitted significantly and more frequently than would have been expected if no association existed. Furthermore, the ID and the DD genotypes were associated with a faster rate of renal function decline. <sup>[31]</sup>

The results presented in this study showed high percent of DD genotype among patients of ESRD and these findings were almost similar to those of Gaurav et al. <sup>[32]</sup> Ola Samuelsson et al <sup>[33]</sup> Yoshida <sup>[34]</sup> and Zhou et al <sup>[35]</sup> Moreover, the results presented here showed no relation of hypertension, diabetic with the percent of DD as it was found by Gaurav et al [32] and Yoshida, <sup>[34]</sup> Chowdhury. <sup>[36]</sup> Also there was no relation with other diseases like stone or unknown causes. this findings might be due to inclusion of patients with different forms of disease create in homogeneous group with the consequent risk of "diluting out" minor gene effects in one disease group by including "uninformative patients. Other cause may be due to small number of cases (100 patients) that consider inadequate to assess the role of the ACE genotype in individual disease groups. The result reported here showed that no relation of polymorphism with gender and the same phenomenon did not confirm by neither Al-Awadi et al. <sup>[37]</sup> nor Gaurav et al. <sup>[32]</sup> However, these authors found that DD genotype is more prevalent among males than females and this due to that ACE gene may be related to X chromosome. The present result did not show difference between male and female in DD genotype as in previous two studies. This conclusion might be due to that most male patients studied were old aged with cardiovascular diseases and the D allele might be depleted in older patients or population with high incidence of cardiovascular diseases In conclusion, results of association studies may be interpreted as the ACE genes simply being close to a "true" candidate gene for renal-cardiovascular disease progression.

## 5. CONCLUSION

The present results suggested that the I/D polymorphism of the ACE gene may be related to the development and rate of progression of renal failure. These results call for further studies to establish the role and relative contribution of individual candidate genes. Moreover, the present findings suggest that I/D polymorphism of the ACE gene may be related to the development and rate of progression of glomerular disease.

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