

Angiotensin 1 Converting Enzyme Encoding Gene Polymorphism in Renal Patients

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ABSTRACT

Background: Angiotensin I is converted to angiotensin II by an angiotensin-converting enzyme, which is an important component of renin-angiotensin framework. Multifactorial chronic kidney disease includes risk factors such as hypertension, obesity, inherited factors, and diabetes. A genetic factor associated with premature signs of renal failure is predominantly increased arterial hypertension and albumin excretion, which add to the pathophysiological movement of disintegration in renal capacity. This enzymatic assay aimed to detect ACE levels in various renal patients compared with controls to confirm the relationship between ACE quality polymorphism and enzymatic ACE levels.

Materials and Methods: The study population of our study included 56 patients with chronic kidney disease. Who was confirmed to have chronic kidney disease after being diagnosed in the dialysis ward of the tertiary care hospital.

Results: A total of 56 chronic kidney disease were enrolled. Mean age of patients were 55.1±13.6. Among total recruited patients, 60% male patients and 40% were female patients. The HDL, and LDL were lower in chronic kidney disease patients than control group. While BMI, total cholesterol, triglyceride, systolic blood pressure, diastolic blood pressure, and ACE level were found higher in CKD patients than control group. The frequency of genotypes ACE II, ID, and DD in patients groups was 12 (12.43%), 28 (48.21%), and 17 (30.35%).

Conclusion: Our study result indicates that the D allele is involved in the progression of chronic diseases. When we look at the frequency of I and D alleles it clearly shows that the frequency of the D allele is significantly higher in patients with CKD than the frequency of the I allele.

Keywords: ACE, CKD, Renal patients, Polymorphism

INTRODUCTION

Multifactorial chronic kidney disease (CKD) includes risk factors such as hypertension, obesity, hereditary factors, and diabetes¹. A genetic factor associated with premature signs of renal failure is predominantly increased arterial hypertension and albumin excretion, which contribute to the pathophysiological chain of renal impairment². CKD can be explained in people due to the consolidated impacts of hereditary weakness and ecological presentation². Kidney capacity and pulse are constrained by a framework called the renin-angiotensin framework (RAS)³. Angiotensin I is converted to angiotensin II by an angiotensin-converting enzyme, which is an important component of RAS⁴. ANG-II has various effects on the kidney, such as vasoconstriction, power of glomerular filtration rate, the guideline of rounded vehicle, the arrival of aldosterone, management of nitric oxide discharge, and increased collagen synthesis⁵.

The ACE gene comprises 26 exons, 21 regions, and 21 kb on the long arm of chromosome 17. Polymorphism is characterized by a deletion/absence allele (allele D) or an insertion/presence allele (allele I) at 287bp⁶. Angiotensin II mediates the action of RAS and participates in sodium metabolism, the parameter of blood pressure, and renal hemodynamics⁷. Angiotensin II mediates vasoconstriction and salt-preserving actions of the renin system. Angiotensin has two subtypes: type I receptor (AT1R) and type 2 receptor⁸. ACE genes can take many forms, which increases the likelihood that the hereditary makeup of the ACE influences the status of people with ACE⁴. A carrier of the D allele has a higher risk of CKD than a carrier of the I allele⁹.

The prevalence of CKD is approximately 10% in several countries¹⁰. Genetic factors, including a family history of the disease and ethnicity, assume a significant function in the pathogenesis of CKD¹¹. RAS is composed of various components such as renin, angiotensin I, angiotensin II, and angiotensinogen, as well as its receptors¹². Kininase II or ACE is dictated by the ACE genes situated on chromosome 17q23.3; while ACE 2 is located on the Xp22 chromosome. Both dipeptidyl carboxypeptidase and ACE 2 antagonize some of the effects of ACE¹³. The lungs produce ACE, which plays a vital role in the hydrolysis of angiotensin I to

angiotensin II in the RAS. This product is an effective vasopressor and releases aldosterone. ACE also inactivates bradykinin, which is a potent vasodilator¹⁴. Genetic ACE metabolism has been associated with microvascular complications of diabetes, progression of Alzheimer's disease, severe acute respiratory syndrome, renal tubular dysgenesis, and susceptibility to myocardial infarction¹⁵. Angiotensin receptor type 1 and 2 act together with Angiotensin II, and are situated on chromosome 3q21 – q25 and Xq23 – 23 correspondingly. AT1 intercedes the key cardiovascular consequence of angiotensin II, and mainly affects arterial blood pressure, sodium, vasoconstriction, and water retention¹⁶.

ACE polymorphism gives the establishment for examining the relationship between hereditary variations and movement of vascular or renal harm¹⁷. The D allele has been related to a breakdown of the reno-defensive activity of ACE inhibitors to ruin the movement of ESRD¹⁸. The identification of a genetic abnormality of the ACE gene as a pathogenetic factor in CKD necessitates special attention in addition to the likely significance of polymorphism as a predictor of risk. This is because a variety of drugs that are targeted inhibitors of the i-e ACE are readily available. For this reason, we are conducting research to determine the relationship between CKD, serum ACE, and ACE gene polymorphism.

MATERIALS AND METHOD

The Health Biotechnology Laboratory, Department of Biotechnology, Garden Campus Abdul Wali Khan University Mardan, was used for the current study's experimental activity (AWKUM). Blood samples were taken from the dialysis unit of the LRH Hospital in Peshawar from renal patients. We first get permission from the MD (Medical Director) and the head of the dialysis unit at LRH Peshawar Hospital before taking blood. As a standard technique, educated composed assent was gotten from all patients as well as from the control group. These patients mainly belonged to the Khyber Pakhtunkhwa province. The Institutional Review Board of the AWKUM Ethics Committee has approved this

study, before participating in the study, we obtained permission from all subjects by the rules.

The study population included in our study included 56 patients with chronic kidney disease (37 men and 19 women; mean age for this study was 50.3 ± 9.7) who were confirmed to have chronic kidney disease after they were diagnosed in the dialysis unit of a lady reading hospital Peshawar. This study recruited 76 control group individuals of different ages, of which 45 were men and 31 were women. Diabetes, hypertension, and other common diseases were observed both in the control group and in the patient group. Subjects' basic demographics were recorded, including age and gender lipid profile. CKD was assessed by viewing angiograms by the dialysis patient.

After an overnight fast, blood samples were taken from patients and control subjects to measure glucose and lipid profiles. Using the Biotrol Kit (BIOTROL, USA) for measuring the plasma glucose concentration by the oxidase method. A commercial Biotrol kit was used to measure total serum cholesterol. HDL cholesterol was determined using a commercial Randox Kit (Randox Laboratories Ltd., UK), and LDL cholesterol was calculated using the Friedwald formula. Triglyceride willpower was determined by the UV lipase/glycerol kinase endpoint method on an operating analyzer. ACE levels in both patients and controls were determined using a commercially available human ACE (angiotensin-converting enzyme) enzyme-linked immunosorbent assay (ELISA) kit, available from Elabscience Biotechnology Incorporation (USA). This enzymatic assay aimed to detect ACE levels in various renal patients compared to controls to confirm the association between ACE gene polymorphism and ACE enzymatic levels. This method helped us find out that the ID, DD, or II allele is responsible for higher serum ACE levels. ACE Genetic polymorphism was investigated using conventional PCR. For this, the following steps were performed. Isolated DNA samples were quantified using a 2000 Thermo fisher Scientific nanodroplet, Wilmington, Delaware, USA. Quantification was carried out by optical density at 260 nm. The conditions of the polymerase chain reaction during thermal cycling are as follows.

RESULT

Out of the 56 patients, 37 (66.1%) were men and 19 (33.9%) were women. Of the 76 control groups, 60% and 40% were male and female patients respectively. The mean age reported in this study was 50.3 ± 9.7 in the case group and 55.1 ± 13.4 in the control group ($p = 0.000$). The mean BMI was significantly higher between the control group and the CKD group 24.7 ± 2.5 and 27.9 ± 3.5 , respectively. The mean SD for systolic and diastolic blood pressure was 157 ± 22 and 92 ± 13 mm Hg. Accordingly, in the group of cases, compared with 139 ± 19 and 85 ± 11 mm Hg. Art. Accordingly, in the control group. There was a significant difference in blood pressure between CKD and control subjects. The mean SD for total serum cholesterol during follow-up was 197.2 ± 5.3 mg/dL in the case group versus 180.5 ± 9.4 in the control group, and the mean serum triglyceride level was 160.2 ± 14.3 (mg/dl) in the comparison group. with 129 ± 8.4 (mg / dl) in the control group. LDL cholesterol values for patients with CKD and the control group were 139.3 ± 3.6 and 120.3 ± 7.5 , respectively. Total cholesterol, LDL cholesterol, and triglycerides were significantly higher among the patients compared with the control group (Table 1).

ACE I / D genotype and allele frequency The polymorphism identified by PCR were noted either as the presence of a 490 bp PCR product. (allele I), or as an allele with a deletion, that is, the presence of a 190 bp fragment. in the absence of an insert sequence (allele D). Thus, each DNA sample showed one of three probable patterns after electrophoresis: 490 bp band. genotype II), lane 190 bp (genotype DD) or both bands 490 and 190 bp (genotype ID).

The frequency of genotypes ACE II, ID, and DD in the control group was 28 (36.84%), 33 (43.42%), and 15 (19.74%) respectively. While in the group of cases, the frequency of genotypes was 12 (21.43%), 27 (48.21%), and 17 (30.35%), respectively. Genotype II

separation in control subjects was higher than in CKD patients (36.84% versus 21.43%), while CKD patients had a significantly higher frequency (48.21%) of DD genotype compared to controls (19.74%). Our result indicates that the D allele is involved in the progression of chronic kidney disease.

Table 1: Baseline characteristics of patient versus controls

Characteristic	Controls n=76	CKD group n=56	P-value
Age, years Mean \pm SD	55.1 \pm 13.6	50.8 \pm 9.7	0.000
Gender Male, % Female, %	45(60%) 31(40%)	37(66.1%) 19(33.9%)	
BMI kg/ m ²	24.7 \pm 2.5	29.9 \pm 8.6	0.000
HDL-c, mg/dl Mean \pm SD	42.5 \pm 2.3	31.8 \pm 1.2	0.005
LDL-c, mg/dl Mean \pm SD	120.3 \pm 7.5	139.3 \pm 3.6	0.032
T.Cholesterol (mg/dl)	180.5 \pm 9.4	197.2 \pm 5.3	0.014
Triglyceride (mg/dL)	129 \pm 8.4	160.2 \pm 14.3	0.005
Systolic blood pressure (mmHg)	139 \pm 19	157 \pm 22	0.245
Diastolic blood pressure (mmHg)	85 \pm 11	92 \pm 13	0.450
Ace level (ng/ml)	185.2	231.4	

Table 2: Genotype distribution of insertion/ deletion polymorphism of the ACE gene

Genotype	Groups		Test of association	
	Controls N (%)	CKD patients N (%)	P value	OR (95 % confidence interval)
II	28 (36.84%)	12 (21.43%)	0.0098	
ID	33 (43.42%)	27 (48.214%)	0.0546	1.23(0.78-1.75)
DD	15 (19.74%)	17 (30.35%)	0.0004	1.78(1.32-2.76)
Total	76	56		

There were no significant differences in the distribution of ID genotypes between patients with CKD and the control group. Logistic regression analysis of these data shows that the DD genotype was associated with a 1.7-fold increased risk of developing CKD in MMR residents (OR 1.7; 95% CI 1.32–2.76; $p = 0.002$) (Table 2).

Table 3: Allelic frequencies of ACE I/D polymorphism

Alleles	Groups		Total	Test of association P value
	Controls	CKD patients		
I	45 (0.59%)	25 (0.45%)	70(53.03%)	0.001
D	31 (0.41%)	31 (0.55%)	62(46.97%)	0.002
Total	76	56	132	

Plasma ACE level

The mean serum ACE level determined by the Elisa kit for all subjects according to genotype II, ID, and DD was 165 ± 108 , 231 ± 112 , and 283 ± 119 respectively as shown in table 3. This result illustrated a relation between ACE gene polymorphism and ACE level. There was a significant difference between II and DD genotype's serum ACE levels ($p < 0.001$). This suggests a probability of linkage of DD genotype with higher serum ACE level. the frequency of the D allele was higher in CKD patients compared with normal control subjects.

Table 4: Serum ACE level stratified by ACE genotype

Genotype	II	ID	DD	P-value
Ace level ng/ml (SD)	165 \pm 108	231 \pm 112	283 \pm 119	<0.012

Ace levels are shown as mean (SD)



Figure 1: Agarose gel electrophoresis picture of amplified PCR product of a human ACE gene. M corresponds to 100bp ladder, lanes 1-4 signify DD genotype (190bp), and lane 5-7 represents ID genotype(190bp and 490bp both), lane 8 and 9 represents II genotype (490bp) of ACE gene.

DISCUSSION

Every ethnic group has its environmental features so therefore we examined the relationship between I/D polymorphism in the gene of ACE and serum ACE level in Pakhtoon CKD patients belonging to the Khyber Pakhtunkhwa province of Pakistan. The group with have ACE DD genotype has the highest-circulating ACE concentration while the ACE concentration is lowest in the ACE II genotype. Their study concluded that the incidence of ACE D allele has been found elevated in the CKD group in contrast with the control group ($P < 0.01$).

The range of ACE gene alleles differed because of the insertion or deletion of 287 – bp DNA order at the 16th intron. In their study, the occurrence of the insertion (I) allele is 0.406 and the occurrence of the deletion (D) allele is 0.594. Both plasma and ACE genotypes showed a considerable association between the D allele. The genotype DD has the highest ACE levels concentration Crisan¹⁹. Similarly in our study, the frequency of the ACE D allele was higher in the CKD group than in control subjects ($P < 0.01$). In present study, the DD genotype of the ACE gene is connected with serum ACE levels and can be identified in CKD patients (association polymorphism). In our study, the frequency of the D allele was 0.58% and 0.41% for the I allele among CKD patients. On the other hand, there was a considerable relationship found between the DD genotype of the ACE gene and high ACE levels in plasma (p -value = <0.001). We examine the alliance linking circulating ACE levels and ACE gene polymorphisms. A family study was directed by Cambien et al., in which they proposed a model for hereditary control of plasma ACE levels²⁰. Their outcome demonstrated that these levels were influenced by the significant quality, assessed to represent 29% of the absolute phenotypic change of plasma ACE in adults. A clear difference in the serum level of ace was found in the three genotypes and the gene responsible for it was ACE. The ACE level was found to be high in patients with DD genotype and was the lowest in patients with II genotype and ID genotype patients. The insertion/deletion polymorphism in the ACE gene was also validated by studies from Korea, China, and Japan. According to Yoshida et al., the ACE DD genotype has an excellent predictive probability for the steadily declining quality of renal function²¹. The meta-analysis by Neugarten et al. looks at how gender affects the progression and development of non-diabetic renal disorders and finds that males with chronic renal disease from a range of etiologies are more likely than women to experience a more rapid decline in renal function over time²². Tripathi et al. compared genetic biomarkers detected in post-dialysis patients to healthy control participants in their study on hereditary polymorphisms in ESRD patients. They discovered that D allele recurrence was 50.5% among ESRD patients and I allele

recurrence was 49.5%³. Nonetheless, all of the CKD patients in our research had hypertension. The ACE-DD genotype, according to Tripathi et al., may be a risk factor for the advancement of an existing renal disease³. When clinical boundaries between DD and non-DD genotypes were explored, blood urea and serum creatinine barriers were factually significant. In the lipid profile, two boundaries HDL and total cholesterol were statistically significant for the DD genotype. LDL cholesterol was factually very high for the DD genotype among the individuals in the benchmark group, in addition to other clinical criteria. The initial potential research was available recently. In 310 people with type 1 diabetes, the chance of developing microalbuminuria and progressing to more advanced, complicated phases of the illness was shown to be higher in patients with the DD genotype²³. Some Malaysian populations²⁴ found a lower frequency of DD genotype²⁵.

CONCLUSION

It is concluded that the ACE gene polymorphism in the D allele is more like to develop chronic kidney disease. We also find that the D allele is a possible carrier of chronic kidney disease.

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