Study of ACE gene and its allele polymorphism in Chronic Kidney Disease patients.

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Abstract

Introduction: Functionality of any organ depends on its vascular supply. Among plethora of factors affecting vascular tone, the most important contribution is of Rennin-Angiotensin system (RAS). This system has been implicated in pathological changes of organ damage through modulation of gene expression, proliferation and inflammatory response. Angiotensin converting enzyme (ACE) is a key component of RAS. ACE I/D polymorphism varies as per individual, Ethnicity, geography and is associated with common diseases like Hypertension, Coronary heart disease (CHD) and Nephropathy.The aim of this study is to identify the role of ACE gene alleles and also to find out the effect of these damage in chronic kidney disease (CKD) patients.

Material and Methods: The patients available in medicine department diagnosed as CKD for a period of 12-15 months were included in this study. The patients who have given consent were included. Patients with co existing other illnesses, cancer patients, patients on chemotherapy or drugs likely to cause kidney damage were excluded. The peripheral venous blood was used for DNA isolation as per kits available. The isolated DNA was amplified by PCR using primer for ACE gene as per protocols. The PCR products were subjected to 10% PAGE electrophoresis for identification of insertion and deletion.

Results: In this study it was found that frequency of D allele is more in case of CKD patients. 11 patients showed no insertion or deletion in ACE gene.

Conclusion: Comparability between the alleles of ACE gene with CKD patients showed strong relation with DD allele.

Keywords: DNA, Gene, Allele, kidney.

Introduction

Nephropathy (CKD) is an increasing burden on healthcare. The number of patients on waiting list for transplant of kidney is increasing day by day. Complications of nephropathy because of uraemic toxins causing damage to DNA is also on rise. Some patients proceed to end stage renal disease very fast. Genetic etiology in such cases needs to be evaluated. So that early aggressive treatment or preventive measures can be initiated with respect to genetic mutations enhancing nephropathy. There needs to be change in modality of treatment with drugs.[1] For example ACE inhibitor role in case of ACE gene mutations needs to be reviewed. Such patients may also be given preference in transplantation. This study will help to understand role of ACE gene mutations in CKD patients. The main objectivewas to study role of ACE gene polymorphism in CKD.

Materials and methods

5cc of venous blood sample was taken from all patients before starting dialysis with all aseptic precautions in EDTA and heparin vacutainers.

Inclusion criteria: Patients available in medicine department (Dialysis unit) and diagnosed as CKD for a period of 12-18 months was considered. Patients who have given informed consent were included for the study.

Exclusion criteria: Patients with critical illnesses, cancer, taking chemotherapy or drugs likely to cause DNA damage were excluded from the study.

Sampling criteria: Venous blood of 5 cc was taken in EDTA and heparin containing vacutainers from notified cases and control group with all precautions

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the sampling was done.

DNA Extraction and PCR

DNA was extracted from whole blood containing EDTA by Qiagen kit method. The quality and quantity of the DNA was analyzed by biospectrometer. To determine the ACE genotype of cases the genomic DNA fragments on the intron 16 of ACE gene was amplified by PCR. The conditions for amplification were, Initial denaturation: 94 C-5 min, Denaturation: 94 _C-30 s, Annealing: 58 _C-45 s, Extension: 72 _C-45 s, Cycling condition: 30 cycles, Final extension: 72 _C-7 min, Hold at 4 _C. Primers for ACE Polymorphism used in this study The flanking primer sequences as reported by Rigat et al[2] were;

Forward Primer: CTG GAG ACC ACT CCC ATC CTT TCT (50nmol)

Reverse Primer: GAT GTG GCC ATC ACA TTC GTC AGAT (50nmol)

Once the amplicons were obtained, they were subjected to 2% agarose horizontal gel electrophoresis with ethidium bromide and the bands were visualized under UV light. Further PCR products were subjected to 10% PAGE electrophoresis with the help of DNA ladder, Deletion (D allele) and Insertion (I allele), were identified at 191 and 478 bp fragment respectively. Statistical analysis was done by representing the data in mean ± SD and percentage.

Results

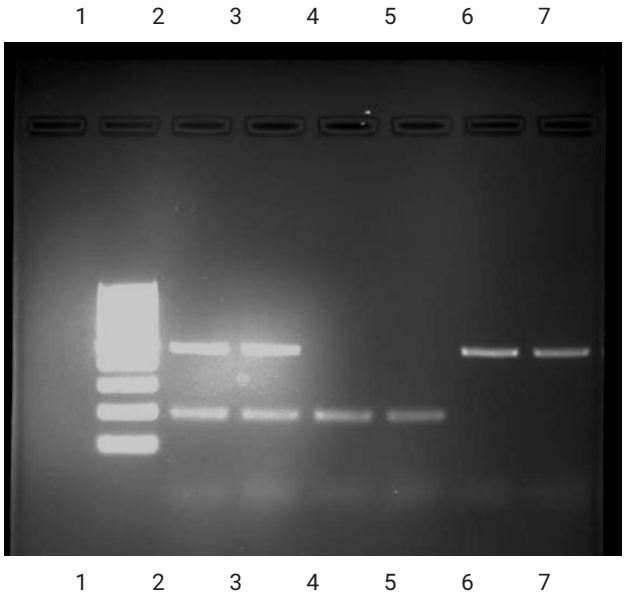

Figure 1: Shows 2% agarose gel electrophoresis image of alleles of ACE gene after standardization of PCR amplification D allele as lower bane of 191bp & I allele as upper band of 478 bp (1-100-100 BP DNA ladder, 2 &3 D/I ACE gene allele, 4 & 5 ACE gene DD allele, 6 & 7 ACE gene II allele)

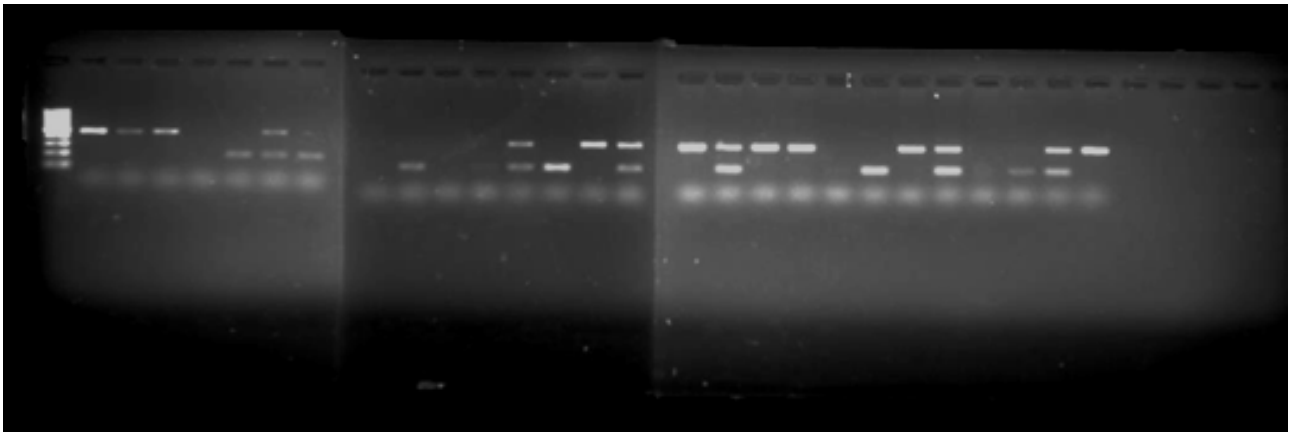

Figure 2: Shows 2% agarose gel electrophoresis representative image of ACE gene from samples after PCR amplification with 100-1000 BP DNA ladder

Table 1: Shows Distribution of cases as per the alleles of ACE gene

Total cases	DD allele	II allele	I/D allele	No I, D allele
58	17	12	18	11

The total no of cases studied were 58. On PCR amplification and gel electrophoresis 17 cases showed presence of ACE gene DD allele, 12 showed II allele,18 showed DI allele and 11 showed no band on gel electrophoresis indicating no deletion or insertion in ACE gene as shown in table 1.

Table 2: Showing the distribution of ACE genotype and allele frequency

ACE Genotype	CKD patients (N,%) n=58
DD	(17) 29.30%
II	(12) 20.68%
I/D	(18) 31.03%
No I,D	(11) 18.96%
Allele frequency	
I	(42) 44.68%
D	(52) 55.31%

As shown in table 2 the distribution of ACE genotype alleles was II 12 (20.68%), DD 17 (29.31%), ID 18 (31.03%) and no I/D 11(18.96%). Frequency of D allele observed in CKD patients was 55.31% which was higher than I allele 44.68%. By one way analysis of variance (ANOVA) the p value was 0.06, considered not quite significant.

Discussion:

Out of 58 cases studied on PCR amplification and gel electrophoresis 17 cases showed presence of ACE gene DD allele,12 showed II allele,18 showed DI allele and 11 showed no band on gel electrophoresis indicating no deletion or insertion in ACE gene as shown in table 1. In the present study we found the distribution of ACE genotype alleles was II (20.68%), DD (29.31%), ID (31.03%) and no I/D (18.96%). Frequency of D allele observed in CKD patients was 55.31% which was higher than I allele 44.68%. Similar frequency trend was also observed by other researchers as shown in the table no 3[3-5] except study by Bhagat M et al[5] The Bhagat M et al[5] showed the gender specific nature of ACE I/D polymorphism with CKD individuals and also the in the same study presence of DD-genotype association with CKD disease is reported.

Table 3: showing the distribution of ACE genotype and allele frequency between previous researchers and present study

ACE Genotype	Present Study 2022-Patients (N,%) n=58	Huda Rafaa Sabbar et al.,[3] 2018-Patients (N,%) n=100	Abuaisha et al.,[4] 2018-Patients (N,%) n=86	Meenakshi Bhagat M et al.,[5] 2017-Patients (N,%) n=200
II	12 (20.68%)	16 (16%)	4 (4.7%)	62 (31%)
DD	17 (29.31)	56 (56%)	53 (61.6%)	50 (25%)
ID	18 (31.03%)	28 (28%)	29 (33.7%)	88 (44%)
No I/D	11 (18.96%)			
I	42 (44.68%)	88 (44.0%)	37 (21.26%)	212 (53.0%)
D	52 (55.31%)	112 (56.0%)	135 (77.58)	188 (47.0%)

Huda Rafaa Sabbar et al.,[3] found DD allele mainly related with progression to ESRD among CKD patients. Abuaisha et al.,[4] reported high prevalence of DD genotype in their study was noted but association was not statistically significant between ACE genotypes. Finding of no I/D allele in ACE gene was reported by previous researchers[6].

On comparison of ACE genotype and its alleles (DD, II and I/D) with CKD patients our study showed results same that of to study done by Noel Pabalan et al.,[7]. They studied the ACE genotype and its alleles with CKD polycystic patients where 6-fold susceptibility was measured in DD homozygote carriers with high magnitude which were highly significant, indicating homogeneous and robust strong confirmation of relationship. Association of ACE genotype and dominance of DD allele was observed in studies done by Al-Awadi et al[8], Tripathi et al[9] and Mclaughin et al[10]. Few studies Abuaisha et al[6], Choudhary et al[11] and Noritika Kawada et al[12] were not in agreement with the role of ACE genotype in CKD patients where they found no association of ACE genotype with CKD patients. The difference would be due to changes in genetic and environmental heterogeneity in between cultural groups or it may be because of change in methodology and sample size in studied population[3].

ACE gene plays role in hypertension and secondarily affects kidney. This has been studied by many researchers. The ACE I/D polymorphisms may invite the utmost risk for growing CKD in hypertensive especially Asian males. Chin Lin et al[13] studied the ACE genotype and its allele (DD, II, and D/I) and found that CKD threat was elevated with the D allele as compare to I allele as Asian society and hypertension had affirmative moderate effects. The males were at higher risk in view of ACE I/D polymorphisms on CKD were the D allele measured showed 3.75 fold greater risks for CKD than I allele in hypertensive cases. Similar study done by Taposh Sarkar et al[14] found that the development of CKD is linked with DD genotype of ACE gene with hypertensive patients. Likewise Balaji Ramanathan[15] studied the role of ACE gene in Hypertensive and Diabetic neuropathy patients. They also observed that DD genotype and the D allele of the ACE I/D gene polymorphism can be a causative factor for type 2 diabetes mellitus, hypertension, and CKD in South Indian regional inhabitants. Likewise the effect of Ace gene and dominance of DD genotype in HTN was proved with the studies done by Patnaik M[16] and Srivastava K[17]. But some authors like Beige et al[18], Pei. Y et al[19] and Lind painter[20] disagreed the role of ACE gene for the development of HTN. However the study done by Suganya V et al[21] found the association of ACE 1 gene patients with CKD and HTN and evidence of an addictive role of ID genotype in the development of HTN and CKD in the population studied. Similarly ACE gene relation with diabetes

mellitus has also been studied. An inevitable gene of Renin-angiotensin-aldosterone system (RAAS) is ACE and its I/D polymorphism has been often accounted with T2DM[15]. Balaji Ramanathan[15] and Hyeong Cheon Park et al[22] study mentions that there is significant surplus role of DD homozygotes and possible gender growth relations among ACE gene in type 2 diabetes ESRF patients. Similar studies were done by Ha. Sk et al[23], Wong T.Y[24], Bjorck S[25] and Yoshida[26] however some studies do not found the association of ACE gene and DD allele among DM patients Parving HH[27], and Susanne Schmidt[28].

Various autoimmune diseases are studied to find out the prevalence of pathophysiological changes in blood vessels and the inflammatory process which are in concern with Insertion and deletion (I/D) of ACE gene polymorphism. Study done by Rashid L et al[29] observed that D allele appears to have a major role towards the development of vitiligo. CKD is a worldwide health burden that affects 8-16% of the universal inhabitants. ACE DD genotype is identified risk factor of cardiovascular diseases including left ventricular hypertrophy and coronary heart diseases that is considered as strong predictor of the mortality in dialysis patients[6]. This situation could result in noteworthy end-stage renal disease (ESRD) can also amplify the risk of cardiovascular disease. Hendri Susilo et al[30]. Correspondingly studies done by Badescu M.C[31] and Samani NJ[32] found the association of ACE gene DD allele in the development of CVD. However the study done by Suganya V[21] and Chin-Lin et al[13] showed the prevalence of II allele in the development of CVD. Notable studies done to understand the impact of ACE gene and the allele in case of Major depressive disorder and Sarcoidosis patients showed dominance of II allele. Sema Inanir et al[33] and Tomita[34].

Conclusion

In summary the study shows no association between ACE gene and the insertion or deletion of allele in CKD patients. On comparison between the alleles of ACE gene with CKD patients showed strong linkage with DD allele was observed. Limitations of the study were the control group was not taken and non-communicable diseases such as diabetes, hypertension and cardiomyopathies in accordance with CKD were excluded. Future study with longer follow up and comparison with associate diseases will add on to know the potential negative effects and intensity of genetic damage.

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