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Research Article

ASSOCIATION ANALYSIS OF ACE (DD) GENOTYPE WITH GYNOID CHRONIC KIDNEY DISEASE PATIENTS OF JAMMU REGION (J&K)

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ABSTRACT

Background: Angiotensin converting enzyme (ACE) is one the important candidate marker for susceptibility of chronic kidney diseases (CKD), as it is a key regulator of blood pressure and renl physiology.
Aim: The present study is aimed to investigate the role of ACE (I/D) polymorphism in Kidney Disease in Jammu Population.
Methodology: In the present study a total of 400 individuals (200 cases & 200 controls) were enrolled from Jammu population. The ACE I/D polymorphism was amplified with the flanking primers by polymerase chain reaction (PCR) and was further visualized by 1.5% agarose gel electrophoresis.
***Results*:** The overall frequency of variant genotype (DD) was higher in CKD patients in comparison of controls (25% vs 19. 5%). The observed frequency of DD- genotype was lower in male CKD patients than male controls (23.8% vs 26.7%) whereas vice versa was reported for female patients when compared to female controls (26.3% vs 12.1%). The applied genetic models did not reveal any association of ACE I/D polymorphism with risk of CKD. However, [DD vs II (OR=?)] , [DD vs ID + II (OR =?)] and [D vs I (OR =?)] models were significantly associated with riskm of CKD in gynoid group.
***Conclusion*:** A significant role of ACE (I/D) polymorphism was reported in gynoid CKD patients of Jammu region (J&K).

INTRODUCTION

Chronic Kidney Disease (CKD) is an international public health problem reaching high prevalence and demanding elevated health costs. It is characterized by a slow, progressive and irreversible decline of renal function; it is usually asymptomatic and thus untreated (Nagamani *et al*., 2015 & Levey *et al*., 2003). It includes a variety of phenotypes and each phenotype is a combination of an underlying kidney disease and superimposing risk factors both at environmental and genetic and non-genetic level (Nagamani *et al*., 2015). The elaborate RAAS pathway is a key regulator of blood pressure, renal hemodynamic and volume homeostasis regulator (Weirt *et al.,* 1999). Thus, the genes encoding the RAAS pathway are the potent genes for the development and progression of CKD. ACE is an important component of RAAS, which is involved in the conversion of angiotensin I to angiotensin II. It is a vasoactive peptide which has different effects on kidney like vasoconstriction, release of aldosterone, control of glomerular filtration rate and release of nitric oxide (Wolf., 1999). The gene for ACE is located on chromosome number 17 having 25 introns and 26 exons . The polymorphism consist of presence (I allele) or absence (D allele) of a 287 bp Alu repeat sequence within intron 16, therefore three genotypes are described DD, II and ID. The D allele is associated with high level serum activity and is concerned with CKD suseptibility (Choudhry *et al*., 2012). The aim of the present study was to genotype the loci of ACE of RAAS for I/D polymorphism and to evaluate its role in CKD risk in population of jammu region.

METHODOLOGY

Subjects Recruitment: The study included 400 unrelated participants (200 CKD cases and 200controls) belonging to

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Jammu region. CKD patients were collected from the out patient Deptt. Of Nephrology, Superspeciality hospital, Government Medical College, Jammu. Control group consisted of sex and age matched healthy participants with neither clinical signs of renal disease nor past or present family history of renal disease. An informed written consent was also dully taken from each study participant. The study was approval by the ethical committee of University of Jammu.

Blood Collection: 3ml of venous blood was collected in EDTA coated vials from each study participant and was transported in ice bags to the Major Research Lab of Human Genetics Research cum Counseling Centre, University of Jammu and was further stored in -20°c till further analysis.

DNA Isolation & Genotyping: The ACE I/D mentioned polymorphism was screened using Polymerase Chain Reaction (PCR). The PCR amplification products were obtained using 25 µL reaction system containing; 50 ng µL-1 genomic DNA, ddH20 15.7 µL, 5X buffer, dNTPs, 0.5 µL of each primer, Mg CL2 and Taq Polymerase. After an initial denaturation at 95°C for 5 minutes, DNA was amplified by 35 cycles for denaturation at 94°C for 1 minute, different annealing temperatures for 1 minute followed by final elongation at 72°C for 10 minutes. PCR products were separated and sized by electrophoresis on 1.5% agarose gel stained with ethidium bromide, at 100 V for 1 hour. After electrophoresis the products were visualized under UV transilluminator.

Statistical Analysis

Continuous and categorical variables were compared using Student's T-test and Chi-square test respectively. Genotype distributions were tested at each polymorphic locus (among controls) for departure from Hardy-Weinberg Equilibrium (all p>0.05). Differences between the two groups were analyzed using t test. Data are presented as mean ±SD. Statistical analysis between patients and controls were performed using SPSS software version 21. Multiple logistic regression models were used to calculate odds ratio (OR) with 95% confidentiality intervals. Models were adjusted for gender.

RESULTS

Distribution of Ace Genotype

Case Control association studies between genetic polymorphisms are hallmark for the analysis of the complex diseases. However, studying the relationship between allele and genotype frequencies of candidate genes in the patients and controls subjects to understand the genetic etiology of complex human traits is an efficient method for molecular dissection of the disease pathogenesis. Based on these, we determined the possible association between ACE I/D polymorphism and CKD patients among the North Indian population.

The distribution of II, ID and DD genotypes in patients (n= 200) was 31%, 44% and 25%, however the frequency in healthy controls (n=200) was 34%, 46.5% and 19.5%. (Table No 1, 2 and 3). After performing logistic regression analysis we did not find any significant association of above said polymorphism with CKD in the studied population. However, when we segregated our data into android (male) and gynoid (female) CKD , it was observed that the distribution of variant DD genotype of ACE (InDel) polymorphism was higher in gynoid patients (26.3%) as compared to android patients (23.8%). None of the applied genetic models have shown a positive association of ACE I/D polymorphism with either overall CKD patients nor with android CKD patients.

Table No 1 Showing allelic and genotypic distribution of ACE ID polymorphism along with corresponding Odd ratio and p-values for total no of cases and controls.

Genotypes	Cases (n=200)	Control (n=200)	OR(CI)	p-value
II	62 (31%)	68 (34%)	Ref.	
ID	88 (44%)	93 (46.5%)	1.04 [0.66-1.63]	0.9
DD	50 (25%)	39 (19.5%)	1.41 [o.81-2.42]	0.2
ID + DD vs II (dominant)	138/62	132/68	1.15[0.75-1.74]	0.5
DD vs ID + II (recessive)	50/150	39/161	1.38 [0.86-2.21]	0.2
D vs I (allele)	188/212	171/229	1.19 [0.89-1.57]	0.2

Table No 2 Showing allelic and genotypic distribution of ACE ID polymorphism along with corresponding Odd ratio and p-values for male cases and controls.

Genotypes	Male Patients (n=101)	Male Controls (n=101)	OR (CI)	p-Value
II	32 (31.7%)	29(28.7%)	Ref.	
ID	45 (45.5%)	45 (44.5%)	0,91 [0.47-1.34]	0.8
DD	24 (23.8%)	27 (26.7%)	0.81 [0.38-1.69]	0.6
ID + DD vs II	69/32	72/29	0.87[0.48-1.58]	0.6
DD vs ID + II	24/77	27/74	0.85 [0.45-1.61]	0.6
D vs I	93/109	99/103	0.88 [0.60-1.31]	0.5

Table No 3 Showing allelic and genotypic distribution of ACE ID polymorphism along with corresponding Odd ratio and p-values for female cases and controls.

Genotypes	Female Patients (n=99)	Female Controls (n=99)	OR (CI)	p-Value
II	30 (30.3%)	39 (39.4%)	Ref	
ID	43 (43.4%)	48 (48.5%)	1.16 [0.62-2.18]	0.6
DD	26 (26.3%)	12 (12.1%)	2.82 [1.22-6.48]	0.01
ID vs DD +II	69/30	60/39	1.49 [0.83-2.69]	0.2
DD vs ID +II	26/73	12/87	2.58 [1.22-5.47]	0.01
D vs I	95/103	72/126	1.61 [1.08-2.41]	0.02

None the less, a significant association of DD vs II {OR = 2.82, 95% CI [1.22-5.47],p-0.01} and D vs I model {OR= 1.61, 95% ,CI [1.08-2.41],p-0.02} was observed with CKD risk in the gynoid patients.

Clinical Characteristics

The distribution of clinical characteristics of the study participants is given in table. The mean age of patients was higher than controls [65.75±16.10 and 63.68±10.48 respectively]. The mean SBP and DBP were higher in patients and were significantly associated with CKD. The average BMI in patients was high [26.62±3.70] in judgment to controls [22.98±3.80]. Among biochemical parameters, S. Urea was significantly associated with CKD risk (p<0.05) but not S. Creatine (p>0.05).

DISCUSSION

Chronic Kidney Disease (CKD) being a complex polygenic disorder is often termed as state affected by "Genetic

Predisposition". RAAS is known for its regulation and maintenance of salt balance, systemic and glomerular blood pressure. The hyperactivation of this system leads to an increase in systemic and glomerular blood pressure followed by progressive loss of renal functions (Anbazhagan *et al*., 2009). ACE gene is considered to be a main player of RAAS pathway and is the major determinant for CKD. In the present study the role of ACE gene I/D polymorphism in CKD was investigated in population of Jammu region. In our study we focused on both the genetic and non genetic factors as they altogether give a clear representation of reasons behind disease causation in our studied phenotypes. Our study reported that the ACE InDel polymorphism was not associated with CKD occurrence in our population. However, when we segregated our data gender wise we reported that the polymorphism was significantly involved in CKD pathogenesis in gynoid population. Mansoor *et al* in 2010, also reported in their study that the ACE I/D polymorphism was responsible for CKD in female population [OR: 0.81 (CI 0.52-1.26) p =0.35] rather than the male population. Similar findings were reported by other workers and Nagamani *et al*., (2015). Nagamani and coworkers in their study reported the association of DD genotype with the female population of Tamilnadu [OR 2.40 (1.05-5.51) p= 0.03]. In another study by Pinon and associates, they reported that the DD genotype of ACE I/D polymorphism was risk factor for the development of renal disease. According to a meta analysis it has been suggested that the gender wise effect of ACE polymorphism was common in different populations (Lin et al 2014) and the DD genotype was a major risk determinant for the development of CKD. Azmy *et al* (2013) also reported the association of ACE I/D polymorphism with CKD.

Null association of ACE I/D polymorphism with CKD observed in our study. Choudhry *et al* (2012) in their study also reported the non association of ACE InDel polymorphism with nephropathy in Pakistani population of Punjab. Another study conducted by Arfa *et al* (2008) analysed the null association of ACE gene with nephropathy and reported no association.

Apart, from these genetic data evaluation, several non genetic factors were also taken into account. CKD mainly affects populace in elderly age. Therefore, the mean age of patients was higher (65.75 ± 16.10) and lower (63.68 ± 16.48) for controls. This result was in concordance with the other studies done in different parts of world viz. UK (Chowdhury *et al*., 1996), Denmark (Tarnow *et al*., 1995), Germany (Schmitd *et al*., 1995). Elevated levels of blood pressure were observed in our patient group as compared to controls, as HTN is the major reason for CKD and our results were in consistent with the study done by (Shaikh *et al*., 2012).

CONCLUSION

A gender specific nature of ACE I/D polymorphism with development of CKD was observed in the present study. The presence of DD-genotype or D-allele was associated towards risk of CKD in gynoid population of Jammu region. The possible limitation of the present study includes small sample size and also only one candidate gene of RAAS was taken into account. There is a need to investigate other candidate genes of RAAS which we will investigate in future.

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