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Assessment of *ACE* A-240T Polymorphism with Chronic Kidney Disease in North Indian Population

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Chronic kidney disease (CKD) is a major public health problem with high risk of morbidity and mortality. Angiotensin converting enzyme (*ACE*) gene plays a significant role in the pathogenesis of chronic kidney disease (CKD) in different ethnic groups. This study aimed to investigate an association between *ACE* (A-240T) gene polymorphism and CKD in North Indian population. This case-control study was conducted in 385 subjects- 165 patients with CKD and 220 healthy controls. Genotyping of *ACE* A-240T polymorphism was performed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. The AA genotype and the A allele distributions were higher in both groups than the TT genotype and the T-allele. But, the genotypic and allelic distributions were not statistically significant difference between CKD patients and healthy controls. Also, no significant difference was found between the two groups in dominant, recessive and codominant genetic models. Our study suggested that the *ACE* A-240T variant not seems to be a risk factor for CKD in North Indian population. Further studies with a larger sample size are needed to confirm these results.

Keywords: *ACE*, allele, CKD, genotype, polymorphism.

Chronic kidney disease (CKD) has become a major public health problem with high risk of morbidity and mortality. Several epidemiological studies were carried out to estimate the prevalence of CKD across the world, it ranges between 7% to more than 18% in the population of many countries (Bartmañska and Wiêcek 2016). In 2002, the National Kidney Foundation's Kidney Disease Outcomes Quality Initiative published clinical practice guidelines for CKD. According to these guidelines- CKD is slow, progressive and irreversible decline in kidney function with estimated glomerular filtration rate (eGFR) of less than 60 mL/min/1.73 m^2 and / or kidney damage over a period of 3 or more months. On the basis of eGFR the CKD have been classified into five stages (Inker *et al.* 2014). Advance stage (stage-5) CKD also known as end-stage renal disease (ESRD) or renal failure and in this stage patients require renal replacement therapy (peritoneal / hemodialysis or receive a renal transplant) for the survival (Smyth 2012). In addition, CKD patients are at greater risk for multiple co-morbidities including both acute or chronic conditions such as hypertension, acute kidney injury, cardiovascular disease, mineral and bone disorders, pruritus, restless legs syndrome,

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anxiety and depression, and cognitive impairment or sleep disturbance (Murtagh *et al.* 2007; Yang *et al.* 2011; Stengel *et al.* 2014).

Many studies have revealed that a combination of multiple genetic and environmental factors may involve for the onset of CKD and its progression (McClellan and Flanders 2003; Luttropp *et al.* 2009). In humans, the renin-angiotensin-aldosterone system (RAAS) act as an endocrine system which regulates blood pressure and fluid-electrolyte balance. But, the alter activity of the RAAS leads to the development of kidney diseases (Ferrario and Strawn 2006). Angiotensin-converting enzyme (ACE, dipeptidyl carboxypeptidase) is one of the key component of the RAAS system. It is a membrane-bound enzyme which converts inactive angiotensin I to active angiotensin II. The *ACE* gene is located on chromosome 17q23 and contain 26 exons and 25 introns (Mattei *et al.* 1989; Hubert *et al.* 1991). Many clinical studies have investigated the association between polymorphisms on the *ACE* gene and CKD risk, but the results of these studies were inconsistent and contradictory. A significant association of the *ACE* (I/D and G2350A) polymorphisms with CKD were reported in the Chinese, Egyptian, Lebanese Indian, Malaysian populations (Elshamaa *et al.* 2011; Ali *et al.* 2015; Lu *et al.* 2016; Sarkar *et al.* 2016; Fawwaz *et al.* 2017), while other studies failed to find such genetic association (I/D and A-240T) in Hungarian and Han-Chinese of Taiwan populations (Yang *et al.* 2015; Kiss *et al.* 2017).

To the best of our knowledge, the association between A-240T polymorphism in the *ACE* gene and CKD has not been studied in North Indian population. Therefore, the present study aimed to investigate the association of *ACE* A-240T polymorphism with CKD risk.

MATERIALS AND METHODS

Study population

One hundred and sixty-five CD patients and 220 healthy controls from the Clara Swain Mission Hospital, Bareilly, India were included in the study. All the study participants were belong to North India ethnicity. The study was conducted in accordance with the principle outlined in the Helsinki declaration for the investigation of human subjects. This study was reviewed and approved by the Institutional Ethics Committee of Invertis University, Bareilly, India. Written informed consent was obtained from each participant before participating in the study.

Blood Collection

Two millilitre of venous blood was collected from each participant in an ethylenediaminetetraacetic acid coated tubes and stored at -20°C till further use.

Genomic DNA isolation

Total genomic DNA was extracted from whole blood samples using a commercially available genomic DNA isolation kit (Nucelospin® from Macherey-Nagel, Germany) according to the manufacturer's protocol. The quality of DNA was analysed by 0.8% agarose gel electrophoresis and the quantity was measured by absorption at 260/280 nm in a standard spectrophotometer. Extracted DNA samples were stored at -20°C till further use.

Genotyping

Genotyping of *ACE* A-240T variant was carried out by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method as previously described by Firouzabadi *et al.* 2005. The oligonucleotide sequences of the primers were as follows: forward primer 5' TCGGGCTGGGAAGATCGAGC 3' and reverse primer 5' GAGAAAGGGCCTCCTCTCTCT 3'. The DNA was amplified for initial denaturation at 94°C for 5 min followed by 35 cycles of 95°C for 30 sec, 58°C for 30 sec and 72°C for 45 sec with a final extension of 72°C for 5 min on a 9700 thermal cycler (Applied Biosystems, California, USA). The PCR products were digested with *Xba*I restriction endonuclease and the resulting fragments were separated by electrophoresis on 2% agarose gel and visualized by ethidium bromide staining. The digested products were of the size 137 bp and 114 + 23 bp for A and T-allele, respectively. In addition, 10% samples were randomly selected for re-genotyped to confirm the authenticity of the results obtained earlier and they were found to be in 100% concordance.

Statical Analysis

All statistical analysis were performed with the MedCalc software version 17.9 on Windows version 8.1 compatible computer. All values were expressed as percentages for

Table 1. Genotype distribution and allele frequencies of *ACE* A-240T gene polymorphism in the study population

ACE A-240T variant		Cases N= 165 (%)	Controls N=220 (%)	OR (95% CI)	*p*-value
Genotype frequency	AA	56 (33.94)	81 (36.82)	1	—
	AT	81 (49.09)	104 (47.27)	0.89 (0.57–1.39)	0.60
	TT	28 (16.97)	35 (15.91)	0.86 (0.47-1.58)	0.64
Allele frequency	A	193	266	1	0.58
	T	137	174	0.92 (0.69–1.23)	

Table 2. Genetic model studies of *ACE* A-240T gene polymorphism in the study population

Genetic Model	Genotype	Cases N=165	Controls N=220	OR (95% CI)	*p*-value
Dominant	AT + TT versus AA	109/56	139/81	1.13 (0.74 – 1.73)	0.56
Recessive	TT versus AT + AA	28/137	35/185	1.08 (0.63 – 1.86)	0.78
Codominant	AT versus AA + TT	81/84	104/116	1.08 (0.72 – 1.61)	0.72

categorical data. The observed frequencies were compared with expected frequencies and tested for Hardy-Weinberg equilibrium. The odds ratio (OR) were calculated with 95% confidence interval (CI) limit from 2 x 2 contingency table. A value of $p \leq 0.05$ was regarded as statistically significant.

RESULTS

Patients with CKD (N=165) and healthy controls (N=220) were recruited in our study. The genotypic and allelic frequencies of the A-240T polymorphism in the *ACE* gene and their association with CKD risk are shown in Table 1.

The frequency of TT genotype of A-240T polymorphism was found slightly higher in patients (16.97%) as compared to controls (15.91%) (p = 0.64; OR: 0.86; 95% CI= 0.47 – 1.58). Likewise, the frequency of heterozygous genotype (AT) was slightly higher in patients (49.09%) than controls (47.27%) (p = 0.60; OR: 89; 95% CI= 0.57 – 1.39). The genotypic and allelic frequencies were not statistically significant differences between CKD patients and healthy controls ($p \geq 0.05$). The A-allele frequency was higher in both the groups (p = 0.58; OR: 0.92; CI= 0.69 – 1.23) than the T-allele.

Also, there were no significant differences in genetic models between CKD patients and healthy controls ($p \geq 0.05$), which are shown in Table 2. In dominant model (AT + TT versus AA) the OR was 1.13 (95% CI 0.74 – 1.73; p = 0.56), in recessive model (TT versus AT + AA) the OR was 1.08 (95% CI 0.63 – 1.86; p = 0.78) and in codominant model (AT versus AA + TT) the OR was 1.08 (95% CI 0.72 – 1.61; p = 0.72).

DISCUSSION

To our knowledge, this is the first study to investigate the relationship between *ACE* A-240T polymorphism and CKD risk in North Indian population.

Limited studies were reported on the association of *ACE* A-240T polymorphism with CKD risk. In the present study, no significant association was reported between A-240T polymorphism and CKD. Similar results were also shown in previous studies. In this regard, no association reported between A-240T polymorphism and ESRD in a Han-Chinese population of Taiwan (Yang *et al.* 2015). In addition, the genotypes of the A-240T polymorphism was not associated with immunoglobulin A nephropathy in Japanese patients (Narita *et al.* 2003).

In conclusion, the findings of this study are consistent with previous studies. Hence, the present study suggested that the *ACE* A-240T variant cannot be risk factor for CKD in North

Indian population. Additionally, further validation of these results in larger sample size are needed to confirm the robustness.

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