Evaluation of G2350A Polymorphism of the Angiotensin-Converting Enzyme (*ACE*) Gene in Chronic Kidney Disease

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Chronic kidney disease (CKD) becomes a major problem for world health. Numerous studies have documented that the polymorphisms in angiotensin-converting enzyme (*ACE*) gene may contribute to an individual risk for the loss of kidney function. The present study was undertaken to evaluate the possible relationship between *ACE* G2350A gene polymorphism and the risk of CKD in Uttar Pradesh population. A total of 379 (159 CKD patients and 220 healthy controls) subjects were recruited for this study. All subjects were genotyped for G2350A polymorphism by PCR-RFLP method. The significant differences were reported between CKD patients and control groups in height, BMI, WC, WH ratio, SBP, DBP, FBS, serum creatinine, eGFR, triglyceride, total cholesterol, HDL and LDL ($p \leq 0.05$); while there was no difference in weight, WC, HC and VLDL. The frequency of AA genotype and A-allele were significantly higher in healthy controls than to patients. Conclusively, this study showed that the G2350A polymorphism may not contribute to CKD risk. Further investigations are warranted in larger sample size to confirm our results.

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

Chronic kidney disease (CKD) is a major health concern affecting the individuals of both developed and developing worlds. It is estimated that the global CKD prevalence ranges from 11 to 13%[1]. Generally, patients with early stage CKD (stages 1 to 3) are asymptomatic, this means that diagnosis is delayed and kidney dysfunction progresses further before it noticed[2]. The progressive and irreversible loss of kidney function that leads to end-stage renal disease (ESRD) (stage-5) and in this stage patients requires renal replacement therapies (dialysis or transplant)[3]. Many studies have indicated that CKD patients are not only high risk for ESRD, but also related strongly with cardiovascular disorder and premature mortality[4].

It is extensively reported that genetic factors may contribute to the pathogenesis of CKD. Several studies have explored the association of renin-angiotensin aldosterone system (RAAS) components with the susceptibility to CKD[5–7]. Angiotensin-converting enzyme (ACE, dipeptidyl carboxypeptidase) is a key component of the RAAS and a membrane-bound enzyme which converts angiotensin I to angiotensin II. The human *ACE* gene is located on chromosome 17q23 and contain 26 exons and 25 introns[8, 9]. Many studies were performed on the association of single nucleotide

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polymorphisms (I/D, G2350A and A–240T) in *ACE* gene with the risk of CKD, but the results were inconsistent and contradictory. In this regard, the association based studies have indicated that *ACE* I/D polymorphism may play a significant role in the development of CKD among Brazilian, Egyptian, Han-Chinese, Indian and Korean population[7, 10–14]. Similarly, the G2350A polymorphism also related significantly with CKD in the Han-Chinese subjects [15, 16]. While other studies in Hungarian, Kurdish of Western Iran, Taiwanese and Caucasians of Polish origin populations have failed to support the association between I/D polymorphism and CKD risk[17-20]. Moreover, there was no significant relationship between A-240T polymorphism and patients of ESRD in Han-Chinese[12].

On the basis of literature survey, we found that no study has yet been undertaken to explore the association between the *ACE* G2350A polymorphism and CKD risk in Uttar Pradesh population. Therefore, we designed this case-control study in order to clarify the association of *ACE* G2350 polymorphism with the risk of CKD.

MATERIAL AND METHODS

Study population

This case-control study recruited 159 cases and 220 controls from the Clara Swain Mission Hospital, Bareilly, India. All the participants included in this study were belongs to Uttar Pradesh ethnicity. This study was performed in accordance with the principles of the Declaration of Helsinki. The research protocol was approved by the Institutional Ethics Committee of Invertis University, Bareilly. All the selected subjects gave their informed written consent to participate in the study.

A *questionnaire* including demographic, clinical and biochemical data was *filled* out by interviewing the patient or their representative and reviewing the medical records. Two millilitre (mL) venous blood were collected from each subject in an ethylenediaminetetraacetic acid coated vacutainer.

DNA isolation and genotyping

Genomic DNA was isolated from whole blood samples using a commercially available isolation kit (Nucelospin from Macherey-Nagel, Germany) according to the manufacturer's protocol and stored at -20 R"C till further use.

Genotyping of G2350A variant was performed with polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP) method according to the previously reported protocol[21]. A set of primer: F 5'-CTGACGAATGTGATGGCCGC-3' and R 5'-TTGATGAGTTCCACGTATTTCG-3' was used to amplify segments of DNA through pre-programmed thermal cycler (Applied Biosystems, California, USA) with the following PCR cycling conditions: initial denaturation at 95 ÚC for 5 min, followed by 35 cycles of denaturation at 94 ÚC for 30 sec, annealing at 58 ÚC for 30 sec, extension at 72 ÚC for 30 sec, and final extension at 72 ÚC for 10 min. The resulting PCR products were digested with *Bst*UI (Thermo-Scientific) restriction enzyme. The digested products were resolved on 3% agarose gel stained with ethidium bromide. The G-allele was visualized as 122-bp fragment and A-allele as 100-bp and 22-bp fragments. For quality control purpose, 10% of the samples were randomly selected for re-genotyped to validate the results attained previously and they were 100% concordance rate.

Statistical analysis

Statistical analysis was performed with MedCalc software version 17.9 on Windows version 8.1 compatible computer. All values were expressed as mean and standard deviation (mean ± S.D.) for continuous data and as percentages for categorical data. The genotype and allele frequencies were calculated in Hardy-Weinberg equilibrium. The odd's ratio (OR) for different genetic models were calculated with 95% confidence interval (CI) limit from 2x2 contingency table. A *p*-value ≤ 0.05 was considered as statistically significant.

RESULTS

Clinical descriptions

The demographic, clinical and biochemical data of 159 cases and 220 controls from Uttar Pradesh descent are summarized in Table 1. All the variables were compared between the two groups. In the mean age, CKD patients were 1.76 year older than the controls. There were statistical significant differences between CKD cases and healthy

controls for height, body mass index (BMI), waist-hip ratio (WHR), systolic blood pressure (SBP), diastolic blood pressure (DBP), fasting blood sugar (FBS), Serum creatinine, eGFR, triglyceride (TG), serum total cholesterol (TCH), high-density lipoprotein (HDL) and low-density lipoprotein (LDL) ($p < 0.05$); but not for weight, waist circumference (WC), hip circumference (HC) and very low-density lipoprotein (VLDL).

Genotype and allele frequencies of G2350A polymorphism

The genotype and allele frequencies of G2350A polymorphism in *ACE* gene for both the case and control groups were calculated in accordance with the Hardy-Weinberg equilibrium, which are given in Table 2.

The allele frequency and genotype distribution of G2350A polymorphism were significantly different between the two groups ($p \leq 0.05$). The frequency of homozygous genotype (AA) was found to be lesser in patients (16.35%) as compared to controls (29.09%), that results a significant reduction in cases (OR: 2.39; 95% CI= 1.40 - 4.07). Likewise, the frequency of heterozygous genotypes (GA) was found to be lesser in cases (20.75%) as compared to controls (24.09%) (OR: 1.56; 95% CI= 0.93 - 2.61), but the frequency of this genotype was not found

Table 1. Distribution of Clinical and Biochemical parameters in cases and controls

Parameters	Cases (N= 159)	Controls (N = 220)	*p*-value
Age (years)	49.38 ± 10.05	47.62 ± 6.45	0.039*
Gender (male/female)	62.26 / 37.74	76.82 / 23.18	0.025*
Weight (kg)	58.02 ± 15.56	60.25 ± 15.25	Ns
Height (cm)	163.09 ± 10.13	160.52 ± 11.31	0.023*
BMI (kg/m^2)	21.77 ± 5.74	23.57 ± 4.2	0.0005*
WC (cm)	83.79 ± 11.32	81.27 ± 13.39	Ns
HC (cm)	86.37 ± 10.72	84.91 ± 12.71	Ns
WH ratio	0.98 ± 0.09	0.94 ± 0.08	< 0.0001*
SBP (mmHg)	155.09 ± 24.08	119.56 ± 12.71	< 0.0001*
DBP (mmHg)	94.91 ± 15.86	81.75 ± 7.45	< 0.0001*
FBS (mg/dl)	94.79 ± 23.97	101.13 ± 18.31	0.004*
Serum Creatinine (mg/dl)	8.01 ± 3.06	0.86 ± 0.23	< 0.0001*
eGFR (mL/min/1.73 m^2)	9.18 ± 7.02	124 ± 27.63	< 0.0001*
TG (mg/dl)	157.45 ± 18.79	149.5 ± 29.22	0.003*
TCH (mg/dl)	160.64 ± 61.12	137.52 ± 34.81	< 0.0001*
HDL (mg/dl)	41.79 ± 5.75	23.7 ± 6.82	< 0.0001*
LDL (mg/dl)	86.59 ± 16.79	81.35 ± 27.39	0.033*
VLDL (mg/dl)	31.76 ± 5.62	30.33 ± 13.52	Ns

Ns = Not significant; *significant value ($p \leq 0.05$)

Table 2. Genetic model analysis of *ACE* G2350A polymorphism in cases and controls

Genetic Model	Genotypes	Cases N=159 (%)	Controls N=220 (%)	OR (95% CI)	*p-value*
Genotype	GG	100 (62.89)	103 (46.82)	1.00 (reference)	—
frequency	GA	33 (20.75)	53 (24.09)	1.56 (0.93 - 2.61)	0.091
	AA	26 (16.35)	64 (29.09)	2.39 (1.40 - 4.07)	0.001*
Allele	G	233 (73.27)	259 (58.86)	1.00 (reference)	—
frequency	A	85 (26.73)	181 (41.14)	1.92 (1.40 - 2.62)	< 0.0001*
Dominant	GA + AA versus GG	59 / 100	117 / 103	0.52 (0.34 - 0.79)	0.002*
Recessive	AA versus GA + GG	26 / 133	64 / 156	0.48 (0.29 - 0.79)	0.005*
Codominant	GA versus GG + AA	33 / 126	53 / 167	0.83 (0.50 - 1.35)	0.445

significant in decrease of cases. The A-allele frequency also showed significantly lower risk for cases than the G-allele (OR: 1.92; CI= 1.40 - 2.62). Moreover, the genetic models were significantly different between cases and controls. For the dominant model (GA + AA versus GG) the OR were 0.52 (95% CI 0.34–0.79; p = 0.002) and recessive model (AA versus GA + GG) the OR were 0.48 (95% CI 0.29 - 0.79; p = 0.005). No significant difference in codominant model (GA versus GG + AA) between the two groups.

DISCUSSION

According to available literature data, this is the first study to evaluate the association of *ACE* G2350A polymorphism with the risk of CKD in Uttar Pradesh population.

The results of the present study indicates that the genotypic and allelic distributions of the G2350A polymorphism were found to be significantly different between CKD cases and healthy controls. The AA genotype showed a significantly lower risk for CKD than to GG genotype. The A-allele also showed a significantly lower frequency in cases. Our findings are similar to those of some previous studies. In this view, Han-Chinese population studies showed that the AA genotype and the A-allele were less frequent in patients of CKD. These findings suggest that the individuals with the AA genotype had protective effect from CKD[15, 16]. On the contrary, Yang et al. reported that the A-allele was significantly more frequent in patients with CKD than in healthy controls[12]. Our study showed the dominant and recessive models were statistically significantly different between CKD patients and control groups, while there was no significant difference in codominant model. However, no genetic model report is available on the association of G2350A polymorphism with CKD to compare our data.

In conclusion, the findings of this research are partially consistent with previous studies. Thus, the present study indicates that the G2350A polymorphism might not contribute to an individual's risk for CKD in the study population. Additionally, further validation of these results with large sample size are warranted to confirm the robustness.

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