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Original article

ACE I/D gene polymorphism in diabetic nephropathy: Clinical implications

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Abstract

Diabetic nephropathy (DN) is a major microvascular complication accounting for about 30% of End-Stage Renal Disease (ESRD) cases. An insertion/deletion (I/D) polymorphism of the gene encoding angiotensin-I converting enzyme (ACE) is reported to be a candidate gene predisposing to diabetic nephropathy. Accordingly, we investigated the ACE I/D gene polymorphism in 52 Type 2 diabetes mellitus (T2DM) cases suffering from nephropathy as assessed by 24 hrs urinary protein levels. 50 age and sex matched healthy subjects served as controls. ACE I/D genotyping was carried out by polymerase chain reaction (PCR) amplification using allele specific primers. The frequencies of ACE DD, ID and II genotypes in the diabetic nephropathy patients were 38.5% , 50% and 11.5% and in the control subjects, 22%, 38% and 40% respectively. There was an increase of 16.5% in the frequency of DD genotype in the patients compared to controls. The frequency of D allele in the patients was 63% which was found to be statistically significant ($p< 0.05$, Odds ratio=2.6) compared to 41% in the controls. These results indicate that Type 2 diabetic patients with D allele (those with DD & ID genotypes) have more than two fold risk of developing nephropathy. Clinical implications of ACE genotyping in planning for patient's management have been discussed.

Key words: ACE gene polymorphism, diabetic nephropathy, genetic risk, clinical implications

Type 2 diabetes mellitus (T2DM) is the most common endocrine disorder which results from insulin resistance and inadequate secretion of insulin[1]. In the last two to three decades there has been a dramatic increase in the incidence of this disorder. T2DM is associated with macrovascular and microvascular complications like diabetic retinopathy, diabetic nephropathy and peripheral neuropathy which are responsible for considerable morbidity and mortality[1].

Diabetic nephropathy (DN), an important complication of T2DM, is observed in about 30% of these cases[2]. DN is now considered to be commonest

cause of End-Stage Renal Disease (ESRD) requiring dialysis. There is evidence that blood sugar control and ACE inhibition therapy might prevent or at least delay the onset of DN and progressive renal failure in T2DM cases[3]. DN occurs in stages; the first stage is characterized by increased glomerular filtration rate (GFR) which is followed by micro albuminunia (30-300 mg urinary albumin/24 hrs). This can progress to macroalbuminuria or overt nephropathy (more than 300 mg urinary albumin/24 hrs)[4,5]. Further progressive decline in renal function is characterized by decreased GFR and results in renal insufficiency and ESRD[1].

Important risk factors for predisposition to DN include hyperglycemia, hypertension and genetic susceptibility. The significance of genetic factors in predisposition to this microvascular complication can be realized from the fact that various genes have been implicated in susceptibility to DN[5,6] including ACE gene of the RAS. This gene codes for angiotensin-I converting enzyme (ACE). ACE is a potent vasoconstrictor which catalyzes the conversion of angiotensin-I to angiotensin-II. It also inactivates bradykinin, a vasodilator, by bringing about its proteolysis[7]. The ACE gene has 26 exons; of which exons 1-12 code for the amino domain and remaining 13-26 code for the carboxyl domain[6]. An insertion and deletion polymorphism of a 287 bp Alu repetitive sequence in the intron 16 of this gene has been reported[8] which results in three genotypes (DD & II homozygotes and ID heterozygotes). Quantitative variations in the serum activity of ACE has been reported in individuals with different I/D genotypes, with highest activity in DD homozygotes, intermediate in ID heterozygotes and lowest in II homozygotes. There are a sizeable number of reports available on ACE I/D gene polymorphism in DN[8-10] claiming high frequency of D allele in DN patients. The number of reports on ACE I/D genotyping in DN from India are limited.

In a study carried out on South Indian diabetic nephropathy patients, Vijay Vishwanathan et al[11] reported significantly high frequency (80.2%) of the D allele compared to T2DM cases with normal urine albumin. However Kumar et al[12] from Punjab (North India) restricted their study to T2DM patients with ESRD and claimed that the frequency of D allele was similar in patients and healthy subjects studied. In view of this we felt that there was a need for further studies on ACE I/D gene polymorphism in Indian DN patients. The present study was carried-out with this objective.

Materials and methods

Selection of cases

Patients visiting the Diabetic Clinic of Deccan College of Medical Sciences and allied hospitals, Hyderabad, were selected for the present study after obtaining informed consent. The duration of the study was from June 2003 to June 2005. The patients selected had a history of T2DM for 5-10 years and were on oral hypoglycemic agents and / or insulin. All the patients had undergone cataract surgery and had diabetic retinopathy. The diagnosis of DN was confirmed by 24 hr urine albumin excretion. All the patients had persistent albuminuria on three consecutive urine examinations. Adult T2DM cases belonging to both the sexes with micro albuminuria or overt nephropathy or ESRD were included in the present study. A total of 52 DN cases were selected with a mean age of 56.07 ± 8.34 years for the study. Of these 25 were males and the remaining 27 were females. Details of the patients like age at sampling, age at onset, sex, history of T2DM in the family and laboratory results like 24 hrs urinary protein, serum creatinine and serum triglycerides were recorded in a proforma. Fifty, age and sex matched healthy subjects were selected as controls for the above tests as well as for ACE I/D genotyping.

Isolation of genomic DNA

Blood samples (4-5 ml) were collected from patients as well as controls in tubes containing EDTA and DNA was extracted from the pelleted leukocytes according to the method described by Miller et al[13].

Genotyping method for ACE I/D polymorphism

Template DNA (1 µg) was used in a PCR under stringent conditions to avoid the possibility of false positives for ACE genotyping. Reactions were performed with 10 pmol of each primer: forward primer 5'-CTGGAGACCACTCCCATCCTTTCT-3', reverse primer 5'-GATGTGGCCATCTTCGTCAGAT -3', in a final volume of 50 µl containing 3 mM $MgCl_2$, 50 mN KCl, 10 mM Tris-HCl (pH 8.4), 0.5 mM of each dNT and 1 unit Taq polymerase (Bangalore Genei, India). Amplification was carried out in a DNA Thermal Cycler for 30 cycles with denaturation steps at 94^{o}C for 1 minute, annealing at 58^{o}C for 1 minute and extension at 72^{o}C for 2 minutes. PCR products were separated on 3% agarose gel and DNA was visualized by ethidium bromide staining. The DNA product is a 190 bp fragment in the presence of deletion (D) allele, and a 490 bp fragment in the presence of insertion (I) allele. Thus there are three genotypes: a 490 bp band (genotype II),

Table I. Mean levels of biochemical parameters in DN patients and controls

Group	Number	Urinary protein (mg/24 hrs)	Serum creatinine (mg/dl)	Serum triglycerides (mg/dl)
DN patients	52	550.38 ± 28.50*	4.87±1.82*	228.46± 35.80*
Controls	50	28.50± 2.80	1.23± 0.30	155.00± 32.70

*p< 0.05

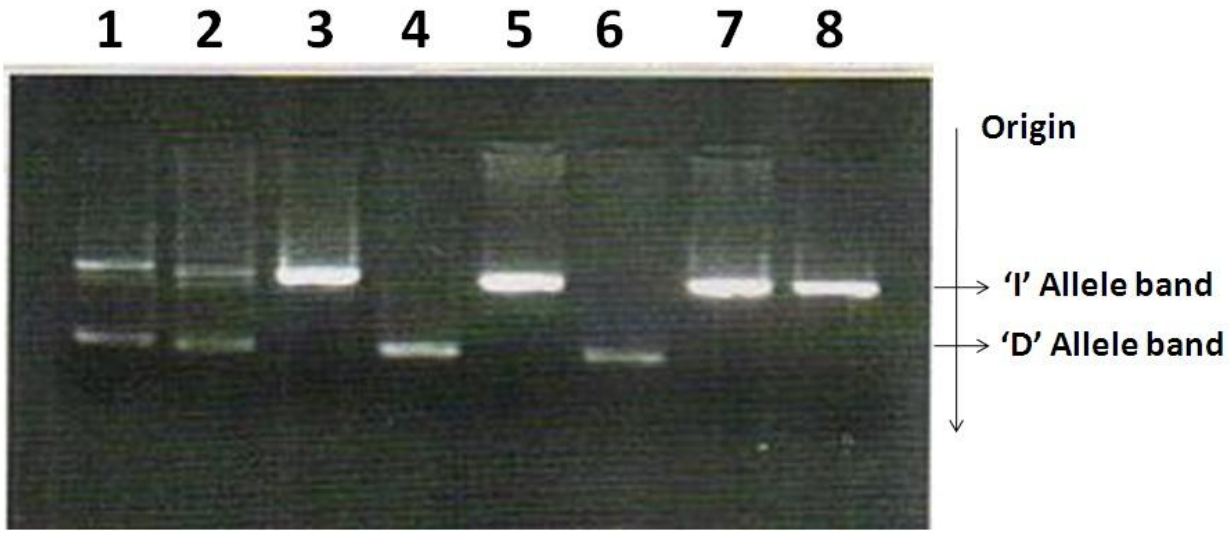

Fig 1. Agarose gel electrophoresis patterns of ACE I/D gene polymorphism. Lane number 1 and 2 represent ID genotype; 3,5,7,8 represent II genotype and; 4 and 6 DD homozygotes

a 190 bp band (genotype DD) and both (ID heterozygote). Template (genomic) DNA was used in excess to avoid mistyping of heterozygotes. Hence the need to retest each sample with DD genotype employing insertion specific primers could be avoided[14].

Results

Clinical laboratory investigations

The patients as well as controls were compared for mean levels of biochemical parameters employing a student's 't' test (Table I). The mean level of 24 hrs urinary protein was significantly higher in DN group compared to controls (p< 0.05). Similarly mean serum creatinine levels were also significantly higher in patients compared to controls (p< 0.05). Apart from the above markers of nephropathy, serum triglycerides (TG) were also estimated in view of reported derangement of TG levels in DN. The mean serum TG levels were also significantly higher in the patients compared to controls (p<0.05).

Distribution of ACE genotypes

The frequencies of the three ACE I/D genotypes in the diabetic nephropathy patients and controls are shown in Table II. Fig 1 depicts the band pattern observed in each genotype. The frequencies of the genotypes DD, ID and II in the DN group were 38.5%, 50% and 11.5% respectively. In the control group these frequencies were DD - 22%, ID - 38% and II - 40 %. A comparative analysis revealed that there was an increase of 16.5% in the frequency of DD genotype in diabetic nephropathy

Table II. Distribution of ACE I/D genotypes and allele frequencies in study groups

	Type 2 diabetics with nephropathy (n=52)		Controls (n=50)	
Genotypes	**n**	**%**	**N**	**%**
DD	20	38.5	11	22
ID	26	50	19	38
II	6	11.5	20	40
Alleles				
D	33	63.5*	21	41.0
I	19	36.5	29	59.0

n = number of individuals, % = percentage of individuals, *p <0.05

patients compared to that in controls. With regard to the frequency of ID genotype, there was an increase of 12% in the patients. The II genotype percentage was significantly reduced in patients (40 % in the controls versus 11.5% in the patients). The frequencies of D and I alleles among the patients and controls are also provided in Table II. The frequency of D allele in the patients group was 63.5% compared to 41% in the controls. An increase of 22.5% was observed in the frequency of D allele in the patients group which was statistically significant (p<0.05; Odds ratio=2.6) (using Z-test for comparing proportions). These results indicate that type 2 diabetic patients with D allele (those having genotype DD or ID) have increased risk of developing diabetic nephropathy as assessed by proteinuria.

Discussion

Familial clustering of patients with diabetic nephropathy and beneficial effects of ACE inhibition has led most researchers to investigate genetics of renin-angiotensin system (RAS)[5]. It is known that ACE gene is one of the important genes of RAS. The first study on ACE I/D gene polymorphism in diabetic nephropathy was that of Marre et al[8] who proposed a protective role of II genotype against the development of diabetic nephropathy. Subsequently a considerable number of studies have investigated the possible role of ACE I/D polymorphism in the pathophysiology of DN and most of them have reported association of D allele as a risk factor[10]. However, some studies have claimed lack of D allele association[6,12] with diabetic nephropathy and suggested a possible role of ACE-D allele in the progression of nephropathy rather than susceptibility to nephropathy[15]. An important factor contributing to discrepancies in the results reported by various studies is considerable ethnic variation in the distribution of ACE I/D genotypes[6].

In the present study, we observed a significant association of ACE-D allele (DD and ID genotypes) with susceptibility to diabetic nephropathy. Similar results were reported in another study carried out in DN patients from South India[11]. In view of reported increased activity of serum angiotensin-I converting enzyme in individuals with DD and ID genotypes, determination of ACE genotypes of DN patients may be beneficial in assessing prognosis of DN response to ACE inhibition therapy. Patients with II genotype are likely to have a favorable progress and good response to ACE inhibitors, whereas DN patients with DD and ID genotypes may progress to ESRD at a relatively faster rate and the response to ACE inhibitors may not be as favorable as in those with II genotype.

Conflict of interest: None

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