Int J Diabetes & Metab (2010) 18:114-118

ACE Insertion/Deletion gene polymorphism and genomic sequence in Diabetic nephropathy

SF Haque[1], M Ahmad[1], AU Khan[2], V Gupta[1], AS Khan[2]
Department of Medicine[1], Interdisciplinary Biotechnology,[2] Aligarh Muslim University, Aligarh, India

Abstract
Insertion/deletion polymorphism of the angiotensin I converting enzyme (ACE) genetically determines most of the plasma ACE activity. Modulation of ACE gene activity might have an important bearing on the rate of progression of renal disease, though its exact role in the nephropathy of type 2 diabetes (T2DM) is far from clear. This prospective, cross-sectional, observational study was designed to study the correlation between insertion/deletion polymorphism of ACE gene in diabetic nephropathy. T2DM cases (n=30) were evaluated, regarding duration, onset and degree of albuminuria, renal insufficiency and hypertension. All patients underwent detailed clinical and biochemical evaluation. Genomic DNA intron 16 of the ACE gene was amplified by polymerase chain reaction (PCR) followed by sequencing. The mean age of this study group was 45.21±2.34 yrs. PCR amplification of ACE gene fragments revealed insertion/insertion (I/I, n =8); insertion/deletion (I/D, n = 18) and deletion/deletion (D/D, n = 4) alleles. All three groups were matched age-wise. Micro- and macro-vascular complications were more prevalent in DD type (p=0.012). The majority (75%) of patients with II allele took a longer time to develop overt albumiuria, having lesser hypertension, renal dysfunction, and dyslipidemia than ID and DD allele (p<0.005). On the other hand, urinary albumin excretion, systemic and diastolic blood pressures, triglycerides, serum creatinine and low density lipoprotein-C were significantly higher (p<0.005), in patients of DD type than II and ID groups. This finding suggests that patients with DD allele of the ACE gene are more likely to have progressive diabetic nephropathy with micro- and macro-vascular complications.

Key words: *ACE Gene, ID polymorphism, diabetic nephropathy*

Introduction

Genetic susceptibility to the microvascular complication of diabetic nephropathy in patients with type 2 diabetes mellitus (T2DM) is not clearly understood. Genes that seem to be of importance is angiotensin 1 converting enzyme (ACE) which is involved in the pathogenesis of diabetic kidney disease.[1] The ACE gene is an excellent candidate for determining the prognosis for cardiovascular and renal risks in patients with diabetes mellitus. Interest in gene polymorphism research has led to DNA genotyping of diabetic patients with retinal, renal and cardiovascular complications. The angiotensin I converting enzyme is encoded by the human ACE gene, which has been cloned and sequenced. The ACE gene is located on chromosome 17p23 and spans approximately 21kb DNA.[2,3] The ACE gene polymorphism, first described by Rigat and co-workers,[4,5]conventionally refers to the insertion (I) or deletion (D) of a 287 bp sequence in intron 16 of the gene. The development of a single-step method for detection of the ACE gene (I/D) polymorphism by use of polymerase chain reaction (PCR),[6] which amplifies DNA, facilitated large-scale research in the field of genomics of diabetes mellitus in relation to complications. Insertion/deletion (I/D) polymorphism of the angiotensin I converting enzyme determines most of the plasma ACE activity genetically and it has also been shown to modify response to ACE inhibition. Subjects with DD genotypes have the highest level of plasma ACE, while those with II phenotypes have lowest levels and those with ID phenotypes exhibiting intermediate levels of plasma ACE. It has been reported that response of drugs was less in patients with ACE D/D genotype than in patients with I/I allele of the ACE gene. It seems likely that the risk for diabetes-associated kidney disease is magnified by inheriting risk alleles at several susceptibility loci. Genome-wide linkage studies have recently identified several chromosomal regions that likely contain diabetic nephropathy susceptibility genes. Pharmacological inhibition of ACE protects against nephropathy, and retards progression to end-stage renal failure (ESRF)[8] mainly in type 1 diabetes, while it reduces cardiovascular risk, mainly in type 2diabetes.[9] The aim of the present study is to evaluate the reliability of ACE I/D polymorphism in identifying patients at risk and those who may benefit the most from renoprotective therapy with ACE inhibitors or angiotensin receptor blockers (ARBs). This may provide a guide in pharmacotherapy in individual patients and help design clinical trials in progressive nephropathies.[10] Moreover, it might help optimize

Received on: 18/12/2009
Accepted on 3/7/2010
Correspondence to: Dr. SF Haque, Department of Medicine, AMU, Aligarh-202002, India E-mail: sfhalig@yahoo.co.in

prevention and intervention strategies at population levels, in particular, in developing countries including India, where resources are extremely limited and 1 million patients continue to die every year of cardiovascular or renal disease.

Materials and Methods

Study design: Prospective, cross-sectional, observational study.

Patient selection: Type 2 diabetic patients with albuminuria attending the Medicine Outpatient and Renal Clinic of our Hospital between June 2006 and May 2007. All patients had established diabetic nephropathy, defined as persistent albuminuria (>300 mg/24 h or >200 µg/min or >200 mg/L) in two of three consecutive measurements on sterile urine samples with or without renal failure (serum creatinine >150 µmol/L). Excluded from the study were patients having renovascular and/or uncontrolled hypertension, congestive heart failure, chronic kidney disease, urinary tract infection, heamaturia, acute febrile illness, and patients taking ACE Inhibitors or ARB's in last one month. All patients underwent detailed clinical and biochemical evaluation including duration of diabetes, onset of microalbuminuria, overt proteinuria, renal insufficiency and hypertension. Blood urea, serum creatinine, blood glucose (fasting and postprandial), fasting lipid profile, 24-h urinary albumin excretion (enzyme immunoassay) were measured. Serum creatinine concentration was assessed by a kinetic Jaffe method. Lipid profile was measured by a conventional laboratory technique. The patients had diabetic diet (45 to 55% carbohydrates, 30 to 35% fat, and 15 to 20% protein) without restriction in sodium or protein intake.

ACE Genotyping

All patients gave written informed consent to study only the polymorphism of the ACE gene, thus excluding exploratory studies of other candidate genes. Oligos were designed amplify insertion/deletion of Alu repetitive element in human ACE gene. Lymphocytes were isolated from blood and DNA was prepared by standard techniques. PCR was used to detect the two alleles of the ACE-ID polymorphism. DNA was amplified using primers and PCR-cycling conditions followed by sequencing. The study protocol was approved by the Local Ethical Committee.

Statistical analysis

Data are expressed as the means ± SD. Differences among mean values were evaluated by analysis of variance (ANOVA) or ANCOVA, as appropriate. A p value (two sided) of < 0.05 was considered to be significant. All calculations were performed using SPSS-11.0 (Chicago, IL, USA).

Results

The mean age of this study group was 45.21±2.34 years. The mean age of thirteen females was 44.36 ±4.21years and that of seventeen males was 46.08±3.74 years.

Mean systolic blood pressure was 170.0 ± 8.0 and 92.0 ± 6.0 mm of Hg diastolic blood pressure. Mean fasting blood glucose, postprandial glucose, total cholesterol, triglyceride level were 148.0 ± 8.6 mg/dl, 242.0 ± 9.8 mg/dl, 219.09 ± 47.07mg/dl and 222.06 ± 59.58 mg/dl, respectively. Mean blood urea and serum creatinine (n=30) were 94.0 ± 12.8 mg/dl and 2.5 ± 0.3 mg/dl, respectively. In general, those patients who had no nephropathy were older, had diabetes for a longer period of time (>5 years) and their time to retinopathy onset was longer than the others.

ACE Genotyping

PCR amplification of ACE gene fragment followed by sequence analysis revealed insertion/deletion polymorphism. Subjects were classified according to the presence (I) or absence (D) of a 287 base pair insertion in intron 16 of the ACE gene into II, ID or DD genotypes. Two sequences have already been submitted to gene bank *vide* accession number: GQ-449380 and GQ-449383.

PCR primers

Forward sequence: 5′CTGGAGACCACTCCCATCCTTTCT-3′, Reverse sequence: 5′-GATGTGGCCATCACATTCGTCA GAT-3′

Patient characteristics (n =30) according to ACE I/D genotypes are shown in Table 1. The distribution of patients as per genotyping, ID (n = 18), II (n = 8) and DD (n = 4) are also presented. The genotype frequencies for ACE I/D polymorphism were in Hardy–Weinberg equilibrium.

All patients were compared. The age of all three groups matched (p=0.012). Micro- and macrovascular complications were more prevalent in DD type, whereas retinopathy and coronary artery disease (CAD) was present in 100% and 75% cases, respectively. In patients having genotype II (n = 8), majority (75%) of patients took longer time >5 year, to develop overt albumiuria, having lesser degree of hypertension, renal dysfunction, and dyslipidemia than ID and DD genotypes ($p<0.005$). On the other hand, urinary albumin excretion, systemic and diastolic blood pressures, triglycerides, serum creatinine and low density lipoprotein-C rose significantly in patients with DD type compared to II and ID groups ($p<0.005$).

Discussion

Angiotensin I converting enzyme (ACE) gene is one of the most intensely studied genes because of the key role it plays in the rennin-angiotensin system (RAS). ACE gene is located on chromosome 17q23 and consists of 26 exons and 25 introns. The insertion deletion (I/D) polymorphism in this gene refers to an Alu repetitive sequence 287 bp long, in intron 16, found in three forms: D/D and I/I homozygotes and I/D heterozygotes. Alu insertion polymorphisms, like ACE I /D polymorphism are also suitable markers for studying genetic variation in human populations. They can be easily detected by PCR amplification and gel electrophoresis and they are stable markers that represent a unique evolutionary event.

Table 1: Patient Characteristics and Distribution of ACE ID genotypes

Characteristic		ID Genotype			
		DD	ID	II	P value
		n = 4	n=18	n= 8	95% C.I
Age, (yr.)		43.82±1.34	47.61±2.56	45.21±2.64	0.012
Sex:	Male (17) /Female (13)	2/2	10/8	5/3	
T2DM	<5 yr, (%)	3 (75.0)	4 (22.22)	2 (25.00)	
	>5 yr, (%)	1 (25.0)	14 (77.77)	6 (75.00)	
Retinopathy		4 (100%)	8 (44.44%)	2 (25%)	
CAD/MI		3 (75%)	3 (75%)	0	
SBP mm Hg		178.0 ± 8.0	162.0 ±8.0	146.0 ±4.0	<0.05
DBP mmHg		92.0 ± 6.0	82.0±4.0	76.0±6.0	<0.05
UAE mg/24 hr.		1364±76.0	1193±28.0	566±12.0	<0.05
S.Creat.mg/dl		3.85 ±0.68	2.87 ±0.24	1.85 ±0.34	<0.05
T.Chol.mg/dl		230.0±32.0	225.8±46.0	176.5±26.0	0.020
TG mg/dl		280.9±48.0	232.4±28.0	190.8±30.2	<0.05
LDL mg/dl		168.4±28.3	150.3±24.2	104.1±18.6	<0.05
HDL mg/dl		41.4 ±4.8	45.5±5.1	52.2±4.2	<0.2

The distribution of the ACE genotypes differs between races and it is used as a marker in population structure analyses.[11] In our study, ID genotype was the most frequent allele present in 60.0%, followed by II in 26.66% and DD found in only 13.33% of total cases of diabetic nephropathy. In an Iranian cohort of patients, Golmohamadi and co-workers, found the frequency of DD, ID and II genotypes in patients with nephropathy to be 30.6%, 55.3%, 14.1%, respectively.[12] While in a North Indian diabetic nephropathy population the frequency was 17.0%, 54.2%, 17 28.8% for DD, ID and II genotypes, respectively.[13]

In the present study 73.33% patients belonged to ID and DD genotype. Clinical correlation revealed that most patients in this group have macro as well as microvascular complications: coronary artery disease, retinopathy, greater degree of proteinuria, severe renal insufficiency ($p<0.05$). We conclude from the study that haplotypes, including the deletion allele (D) of the ACE gene are associated with greater risk of diabetic nephropathy compared with haplotype insertion (I) allele. Furthermore, the prevalence, onset and progression of diabetic nephropathy was greater in patients with DD genotype. Jeffers and co-workers have also shown an association between ACE DD genotype and diabetic nephropathy.[14]

The association of DD allele with diabetic nephropathy (13.33%) observed in the present study differs from that of the 17.0% measured in North India,[13] 22.75% in South India,[15] 28.8% in Brazil,[16] and 30.6% in Iran.[12] This difference is attributed mainly to difference in frequency of ACE genotypes in patients with diabetic nephropathy, diabetes without nephropathy and non-diabetics in different ethnic groups.[17] The DD genotype is known as an independent risk factor and has a high prognostic value for the onset and progression of diabetic nephropathy in T2DM. Furthermore, many studies have established that the DD genotype leads to a higher ACE expression and activity and may predispose individuals to T2DM and its complications.[4] All previous studies in non-diabetic and diabetic nephropathies have demonstrated that the deletion polymorphism of the ACE gene, particularly the homozygote DD, is a risk factor for an accelerated loss of kidney function.[18] Besides diabetes mellitus, it was found that ACE insertion/deletion polymorphism is associated with essential hypertension.[19,20] In addition, individuals who are homozygous for the D allele of the ACE gene are more likely to have essential hypertension.[21]

Polymorphisms in the RAS system are associated with clinically significant renal and cardiovascular disease morbidity and altered serum ACE activity through a pro-inflammatory mechanism. Individuals with DD genotype have twice as much as tissue and plasma ACE concentrations as I/I subjects, with ID subjects having intermediate levels. The explanations for the deleterious effect of the deletion polymorphism on albuminuria may be elevated intrarenal angiotensin II formation and/or insufficient angiotensin converting enzyme in DD type individuals.[22] A meta-analysis showed that the risk of nephropathy increased in the presence of DD or ID genotypes in Asian patients with types 2 diabetes.[23]

Conclusion

Consequently, genotypic abnormalities in the renin-angiotensin system have emerged as potential risk factors for the development of diabetic nephropathy. Evaluation of the ACE I/D polymorphism is a reliable tool to identify patients at risk of diabetic nephropathy and those who

may benefit the most from ACE inhibitors or ARB's in retarding the progression of chronic kidney disease and end stage renal disease. This may help optimize prevention and intervention strategies at population levels, especially in developing countries including India, where renal replacement therapies are neither accessible nor affordable to millions of diabetics who continue to die every year of cardiovascular and/or renal disease.

Acknowledgment
We are indebted to faculty and staff, especially of Interdisciplinary Biotechnology Unit, AMU, where most of the investigations were carried out.

References

1. Barnett A. Prevention of Loss of Renal Function over Time in Patients with Diabetic Nephropathy. Am J Med 2006; 119:S40-47.
2. Erdös EG. Angiotensin I converting enzyme and the changes in our concepts through the years. Hypertension 1990; 16:363-370.
3. Hubert C, Houot AM, Corvol P, Soubrier F. Structure of the angiotensin I-converting enzyme gene: two alternate promoters correspond to evolutionary steps of a duplicated gene. J Biol Chem 1991; 266: 15377-15383.
4. RigatB, Hubert C, Alhenc-Gelas F, Cambien F, Corvol P, Soubrier F. An insertion/deletion polymorphism in the angiotensin 1-converting enzyme gene accounting for half the variance of serum enzyme levels. J Clin Invest 1990;86:1343-1346
5. Rigat B, Hubert C, Corrol P, Sourbier F. PCR detection of the insertion/ deletion polymorphism of the human angiotensin converting enzyme gene (DCP1) (dipeptidyl-carboxy peptidase 1). Nucleic Acids. Res, 1992; 20: 1433-5
6. Ruggenenti P, Perna A, Gherardi G, Garini G, Zoccali C, Salvadori M, Scolari F, Schena FP, Remuzzi G. Renoprotective properties of ACE-inhibition in non-diabetic nephropathies with non-nephrotic proteinuria. Lancet 1999; 354: 359-364
7. Cambien F, Poirier O, Lecerf L, Evans A, Cambou JP, Arveiler D, Luc G, Bard JM, Bara L, Ricard S, Tiret L, Amouyel P, Alhenc-Gelas F, Soubrier F. Deletion Insertion in the gene for angiotensin converting enzyme is a potent risk factor for myocardial infarction. Nature 1992; 359:641-644.
8. Keavney B, McKenzie C, Parish S, Palmer A, Clark S, Youngman L, Delépine M, Lathrop M, Peto R, Collins R, International Studies of Infarct Survival (ISIS) Collaborators: Large-scale test of hypothesized associations between the angiotensin-converting-enzyme insertion/deletion polymorphism and myocardial infarction in about 5000 cases and 6000 controls. Lancet 2000 355: 434–442
9. Marre M, Jeunemaitre X, Gallois Y, Rodier M, Chatellier G, Sert C, Dusselier L, Kahal Z, Chaillous L, Halimi S, Muller A, Sackmann H, Bauduceau B, Bled F, Passa P, Alhenc-Gelas F. Contribution of genetic polymorphism in the renin-angiotensin system to the development of renal complications in insulin-dependent diabetes: Genetique de la Nephropathie Diabetique (GENEDIAB) study group. J Clin Invest 1997; 99:1585-1595
10. Scharplatz M, Puhan M A, Steurer J. Does the angiotensin converting enzyme (ACE) gene insertion/deletion polymorphism modify the response to ACE inhibitor therapy? A systematic review Cardiovasc Med 2005; 6: 16
11. Barbalić M, Skarić-Jurić T, Cambien F, Barbaux S, Poirier O, Turek S, Vrhovski-Hebrang D, Cubrilo-Turek M, Rudan I, Rudan P, Narancić NS. Gene polymorphisms of the rennin-angiotensin system and early development of hypertension. Am J Hypertens 2006; 19: 837-842.
12. Golmohamadi T, Nikzamir A, Nakhjavani M, Zahrai M, Amirzargar A, Saffari R. Association of angiotensin converting enzyme (ACE) gene polymorphism and diabetic nephropathy. Iran J Public Health 2006; 35:14-21.
13. Kumar A, Mohindru K, Sehajpal PK. Angiotensin-I Converting Enzyme Polymorphism and Diabetic Nephropathy in North India. Int J Hum Genet 2005; 5: 279-283.
14. Jeffers BW, Estacio RO, Raynolds MV, Schrier RW. ACE gene polymorphism in NIDDM and its relationship with diabetic nephropathy. Kidney Int 1997; 52:473-477.
15. Movva S, Alluri RV, Komandur S, Vattam K, Eppa K, Mukkavali KK, Mubigonda S, Saharia S, Shastry JC, Hasan Q. Relationship of angiotensin-converting enzyme gene polymorphism with nephropathy associated with Type 2 diabetes mellitus in Asian Indians. J Diabetes Compl 2007; 21: 237-241
16. Canani LH, Costa LA, Crispim D, Gonçalves Dos Santos K, Roisenberg I, Lisbôa HR, Sarturi Tres G, Maia AL, Gross JL. The presence of allele D of angiotensin-converting enzyme polymorphism is associated with diabetic nephropathy in patients with less than 10 years duration of Type 2 diabetes. Diabet Med 2005; 22:1167-1172.
17. Costerousse O, Allegrini J, Lopez M, Alhenc-Gelas F. Angiotensin I converting enzyme in human circulating mononuclear cells: genetic polymorphism of expression in thymphocytes. Biochem J 1993; 290: 33-40.
18. Samuelsson O, Attman PO, Larsson R, Mulec H, Rymo L, Weiss L, Ricksten A. Angiotensin I-converting enzyme gene polymorphism in non-diabetic renal disease. Nephrol Dial Transplant 2000; 15:481-486.
19. Harrap SB, Davidson HR, Connor JM, Soubrier F, Corvol P, Fraser R, Foy CJ, Watt GC. The angiotensinconverting enzyme gene and predisposition to high blood pressure in man. Hypertension 1993; 21:455-60.
20. Das M, Pal S, Ghosh A. Angiotensin converting enzyme gene polymorphism (insertion/deletion) and hypertension in adult Asian Indians: A population-based study from Calcutta, India. Human Biol 2008; 80: 303-312.

21. Yoshida H, Kuriyama S, Atsumi Y, Tomonari H, Mitarai T, Hamaguchi A, Kubo H, Kawaguchi Y, Kon V, Matsuoka K, Ichikawa I, Sakai O. Angiotensin I converting enzyme gene polymorphism in non-insulin dependent diabetes mellitus. Kidney Int 1996; 50: 657-664.

22. Fujisawa T, Ikegami H, Kawaguchi Y, Hamada Y, Ueda H, Shintani M, Fukuda M, Ogihara T. Meta-analysis of association of I/D polymorphism of ACE gene with diabetic nephropathy and retinopathy. Diabetologia1998; 41: 47-53.