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The insertion I/deletion D polymorphism of angiotensin converting enzyme (ACE) gene in type II diabetic

R Sapkota, R Dasgupta, Banashree Das and Luna Rai

Department of Microbiology/ Biotechnology Baba farid Institute of Technology, Sudhowala, Dehradun, Uttarakhand 248001, India.

Abstract

Purpose: The causes of T2DM are mainly unknown, but they arise from interplay between several genetic and environmental factors. The I/D allele of the ACE gene has been studied in Indian population in relation to T2DM with contradictory results. Hence the present study was aimed to investigate whether the I/D polymorphism of ACE gene increase the susceptibility to T2DM.

Material and methods: ACE gene was genotyped in 119 patients, 59 (aged matched and sex no matched) patients and 50 (age matched and no sex matched) controls.

Results: The Hardy Weinberg equilibrium was tested for the entire group. The χ^2 (chi-square) test with Fisher's correction was used to compare the genotype and allele frequency in the cases, on the one hand, and the controls, on the other . The II genotype occurred more frequently in North Indian population with type II diabetes mellitus patient (30%) than in the control (12%). The genotypic and allele frequencies differed significantly in case from control subjects ($p<0.05$).

Conclusion: From the present data it was observed that in T2DM patient genotypic and allele frequencies were significantly deviated from Hardy-Weinberg equilibrium ($p<0.05$).The involvement of II genotype occurred more frequently among the case subjects.

Keywords: Polymorphism, type II diabetes, Angiotensin converting enzyme.

INTRODUCTION

Patients with diabetes have a high risk of developing severe complications, such as diabetic nephropathy, cardiovascular disease and retinopathy. Type 2 diabetes is an important complication of hypertension and is observed in more than 30% of patients with hypertension[1]. The report of insertion/deletion(I/D) polymorphism of ACE gene was first characterized by the presence (insertion) or absence (deletion) of a 287 bp *AluYa5* element inside introns 16 producing three genotype (II homozygous, ID heterozygous, DD homozygous), the ACE I/D polymorphism is also associated with overall plasma ACE level[2].

Renin angiotensin system may play an important role in blood pressure regulation and acts as a key regulator of sodium homeostasis. The gene coding for Angiotensin converting Enzyme (ACE) regulates vascular tone through the activation of angiotensin II, apotent vasoconstriction[3]. Patients homozygous for the D allele are characterized by elevated plasma levels of ACE compared with patients homozygous for the I allele, which might explain a diversity in the response to ACE inhibition[4].

The distribution of the ACE polymorphism varies across different geographical regions and ethnic groups in India and respectively the frequency of the I allele was found to be significantly higher in Dogras, Assamese and Kumaonese, while that of the D allele was higher in Punjabis[5]. The study conducted on south Indian population had found the association between the D allele (ID and DD genotype) of the ACE polymorphism and diabetic nephropathy in south Indian type 2 diabetic patients[6]. The ACE insertion (I)/ deletion (D) polymorphism is not only effective in playing a role in diabetes, but also participate in cardiac complications[7]. However, these studies yielded conflicting results: some claiming positive association with ACE genotype, and others contradicting. Several case- control studies of diabetes have demonstrated a positive association of the ACE I/D polymorphism[8]. Hence the present study was carried out to investigate the association of I/D polymorphism and its role in increasing susceptibility to T2DM.

MATERIAL AND METHODS

Subject selection

The patients were recruited from those visiting the diabetic clinic in Department of Medicine, ERA'S Lucknow Medical College & Hospital, Lucknow. The study covered 109 subjects, all of whom were from Lucknow and the adjoining areas and belong to the Indo-European ethnic group. Data has been collected from each patient on clinical variables including age, height, weight, body mass index, cigarette smoking, alcohol consumption and family history. Of the 109 subjects, 59 (no age and sex matched samples) had type 2 diabetes mellitus .Diagnosis of Diabetes was based on the physical and clinical examination of patients by the doctors followed by appropriate laboratory and other investigations. Type 2 diabetes mellitus is defined as a fasting plasma glucose level of more than 126mg/Dl. The control groups consists of 50 individuals (no age matched individuals) having fasting glucose level below 110mg/Dl

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*Corresponding Author
R Sapkota
Department of Microbiology,Baba Farid Institute of Technology,
Suddhowala, Uttrakhand, India.

Email: azzusapkota72@gmail.com

without family history of diabetes, none of them were receiving any medications at the time of participation.

Sample collection and DNA extraction

Five milliliters of peripheral blood was collected from all the subjects and anti-coagulated by collecting in EDTA blood tubes. The DNA was extracted by using standard Phenol-Chloroform method [9]

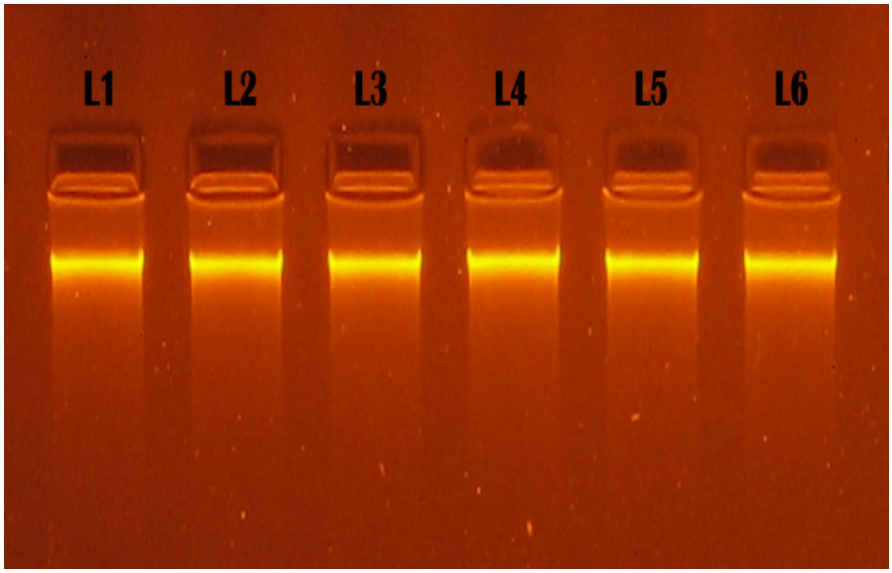

Fig 1. Lane 1, 2 & 3 showing DNA of sample and lane 4,5 & 6 showing DNA of control patients.

Genotyping method for ACE I/D polymorphism

The ACE I/D polymorphism was determine by Polymerase chain reaction (PCR-Thermo cycler, Biometra, U.SA). The primer sequences used for amplification were 5'-CTG GAG ACC ACT CCC ATC CTT TCT-3' (forward primer), and 5'-GAT GTC GCC ATC ACA TTC GTA CGA T-3' (reverse primer)[2]. The final concentration of the PCR mixture was 1.5mM $MgCL_2$, 50mM KCl, 10mM Tris-HCL (pH 8.8), 0.1% gelatin, 1% Triton X-100, 0.3mM each of dNTPs, and 2U Taq DNA polymerase(Bangalore Genei, India) in each reaction tube. The PCR amplification will be carried out under the following condition: initial denaturation at 94°C for 5 minutes, followed by 35 cycles of denaturation at 94°C for 45 seconds, annealing at 60.4°C for 1.15 min , extension at 72°C for 2:30 minutes and final extension at 72°C for 5 minutes. The PCR products must be of 490bp for I allele and 190bp for D allele.

Thus there are three genotypes:

1. 490bp band (genotype II)
2. 190bp band (genotype DD)
3. Both band (I/D genotype)

The product was checked on 1.5% agarose gel by ethidium bromide staining[2].

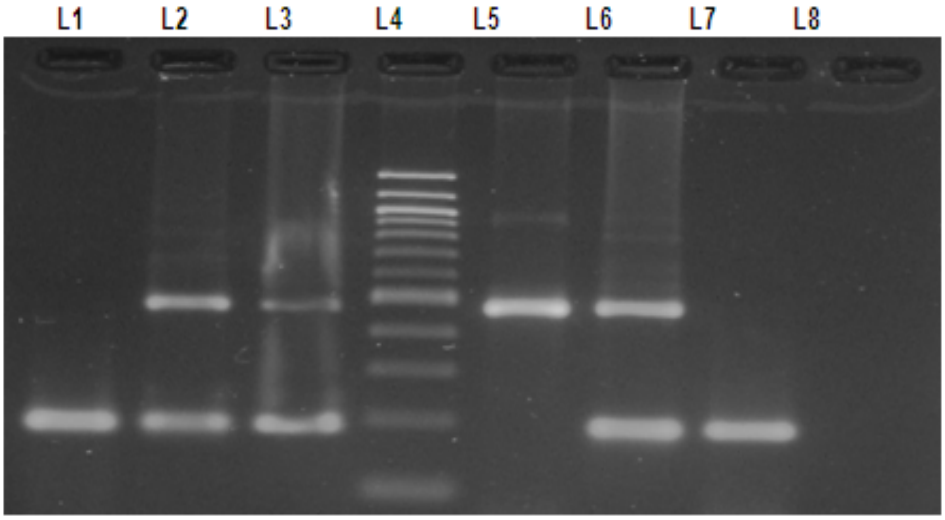

Fig 2. Agarose gel picture showing PCR product for ACE polymorphism. Lane 1 & 7 is D/D genotype (490bp), lane 2, 3 & 6 is I/D genotype, lane 5 is I/I genotype (190bp) and lane 4 is 100bp ladder

Statistical Analysis

The clinical characteristics of all the subjects were expressed as mean and standard deviation. All the statistical analysis was carried out using XLSTAT 2011.2.06. The allele frequencies were deduced from the genotype distribution. The Hardy Weinberg equilibrium was tested for the entire group. The χ^2 (chi-square) test with Fisher's correction was used to compare the genotype and allele frequency in the cases, on the one hand, and the controls, on the other. The P value, t value and 95% CI have been calculated for all available clinical parameters. Two tailed alpha P value less than 0.05 were considered significant. The odd's ratios (OR) together with the 95% confidence interval (CI), comparing the allelic distribution in the all case and control subjects.

RESULT

The general characteristics of the patients and controls, together with their biochemical parameters, are shown in[Table1] . Two groups were matched for age , but not for sex. The angiographic features were shown in[Table2] .. The allele frequencies and genotype distribution for I/D polymorphism in the ACE gene differed significantly between the case and the controls. The study population could also be divided into three groups according to genotype: II(n=23), ID(n=42) and DD(n=12) in patients and II(n=6), ID(n=34) and DD(n=10) in controls. The frequency of the I allele was significantly higher among the case (88%) than the controls (46%).Similarly, the II genotype occurred more frequently in North Indian population with type II diabetes mellitus patient (30%) than in the control (12%). The genotypic and allele frequencies differed significantly in case from control subjects ($p<0.05$). The blood glucose levels gave significant difference in case subjects analysed.

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.

Table 1. Clinical data of 76 North Indian type 2 diabetic patients

	Observation	Minimum	Maximum	Mean	S.D.	t-value	P-value	95% CI
BMI	76	17.03	37.66	25.63	4.44	2.08	<0.0001	85.02,143.27
HBA1C	76	0.045	0.149	0.063	0.013	2.59	<0.0001	0.054,0.073
S.Chol	76	90	283	199.5	39.4	2.11	<0.0001	204.08,216.69
TG	76	95	764	221	137.81	2.11	<0.0001	158.88,283.29
HDL	76	24.9	80.9	50.87	9.6	2.11	<0.0001	46.7,55.05
LDL	76	74.4	146.3	114.14	91.49	2.11	<0.0001	85.02,143.27
VLDL	76	19	152.8	44.22	27.55	2.11	<0.0001	31.77,56.65

BMI**:** Body mass index ;TG: Triglyceride; HDL: high density lipid; LDL: low density lipid; VLDL: very low density lipid.

Table 2. Genotype distributions and allele frequency for polymorphisms in study group

		Genotype				Allele	
ACE gene		II	I/D	DD	I/D+DD	I	D
Case	Number	23	42	12	54	88	66
	Frequency	0.3	0.55	0.15	0.7	0.57	0.42
Controls	Number	6	34	10	49	46	54
	Frequency	0.12	0.68	0.2	0.98	0.46	0.54

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 ho-mo-zygotes and I/D hetero-zygotes. Alu insertion polymorphisms, like ACE I /D polymorphism, are also suit-able 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. The distribution of the ACE genotypes differs between races and it is used as a marker in population structure analyses.

The ACE gene I/D polymorphism were identified in 1990 by Rigat and co-workers. Their study showed that the activity of circulating ACE depended on the insertion/deletion (I/D) polymorphism. Subjects with the genotype II displayed the highest mean activity of serum ACE, those with the genotype DD displayed

the lowest, and those with the genotype ID displayed the intermediate. Since then, it has been speculated that these differences in serum ACE activity associated with the ACE genotype might affect therapeutic response of ACE inhibitors, explaining inter individual variability in Cardiovascular or renal response to equivalent doses of ACE inhibitor.

The aim of our study was to look for an association between ACE and type 2 diabetes mellitus. We found difference between patients and controls in the case of allele frequency and genotype distribution for the I/D polymorphism of the ACE gene. The I allele frequency, combined frequency of I allele carriers and II genotype were significantly higher in the type 2 diabetes mellitus than in the controls.

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