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Angiotensin Converting Enzyme (ACE) I/D Gene Polymorphism in susceptibility of Migraine.

KEYWORDS: Migraine, ACE, genotype, Jammu

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ABSTRACT *Angiotensin I-converting enzyme (ACE) gene has been shown to be involved in the pathophysiology of migraine. It is suggested that the ACE-DD genotype may play an imperative role in the determinism and in the frequency of migraine attacks. Therefore, the aim of the present study is to determine the frequency of ACE I/D genotypes and its association with susceptibility of migraine in the population of Jammu region of the Jammu & Kashmir (J&K) State. A total of 100 individuals including 50 migraine patients and 50 non-migrainous healthy controls were enrolled for the present study. Genomic DNA was extracted from whole blood using standard isolation methods. Genotyping of ACE I/D polymorphism was performed by polymerase chain reaction (PCR) technique. The frequency of DD-genotype was higher in patients in comparison to controls (32% vs 14%). The present study confirms a significant association of DD-genotype and D-allele of ACE gene with the susceptibility of migraine.*

Introduction

Migraine is a neurovascular disorder clinically characterized as episodic attacks of severe headache with certain associated symptoms such as nausea, hypersensitivity to light (photophobia) as well as sound (phonophobia) and/or head movement (1, 2). Broadly it is classified into two main subtypes: migraine without aura (MO) and migraine with aura (MA). Auras are usually visual alterations, such as hemianopsic field defects and scotomas that enlarge and spread peripherally or may be sensory alterations such as paraesthesias of the arm and face (3). Migraine is a genetic disease having polygenic and multifactorial mode of inheritance (4, 5). The changes in the vascular endothelial functioning have been observed in migraineurs (6). Genes involved in vascular functioning in particular Angiotensin I-converting enzyme (ACE), can modulate vascular tension and blood pressure and may be involved in migraine pathogenesis. The ACE gene is located chromosome 17 (17q23.3) in humans and has two polymorphic alleles, insertion (I), and the deletion (D) of 287 bp Alu sequence within the intron 16 (7). It is one of the key enzymes in the rennin-angiotensin-aldosterone system (RAAS) and performs two essential enzymatic cascades. Firstly, it converts angiotensin I into angiotensin II, which is a potent vasopressor and secondly, it degrades bradykinin, which is a vasodilator and helps in relaxation of vessels (8, 9, 10). However, the role of the ACE gene polymorphism in migraine pathophysiology is not fully understood yet. It is suggested that an increment in circulating levels of ACE can bring fluctuations in the levels of neurotransmitters thus, disturbing normal neurovascular activity (11). The ACE I/D gene polymorphism is associated with circulating ACE concentrations and has been implicated in migraine susceptibility (11, 12). The aims of the present study includes: 1. To assess the distribution of ACE gene genotypes and alleles in migraine patients and healthy control individuals in the population of Jammu region of the J&K State; and 2. To investigate the association of ACE I/D polymorphism in susceptibility of migraine in the population.

Material and Method

Subjects: A total of 100 individuals, 50 patients with clinically confirmed migraine and 50 healthy unrelated, age & sex matched controls were enrolled from Jammu region of the J&K state for the present study. Patients were recruited from the outpatient department of Neurology, Superspeciality Hospital, Government Medical College, Jammu (J&K). An informed written consent was obtained from each study participant before enrolment in the study.

DNA Isolation and Genotyping of ACE polymorphism: 2-5ml of venous blood was collected in EDTA coated vials and stored at -20°C. The DNA was isolated by organic method as given by Sambrook and Russel (13).The ACE I/D polymorphism was identified by polymerase chain reaction (PCR) technique. The PCR was performed by using forward primer: 5'- CTG GAG ACC ACT CCC ATC CTT TCC -3' and reverse primer: GAT GTG GCC ATC ACA TTC GTC AGA T -3'.

PCR reaction was performed in thermal cycler of Applied Biosystems thermal cycler (Make Veriti by life technology, Singapore), using 25µl of reaction mixture containing 2 µl DNA, 5 µl flexi buffer, 0.5 µl dNTPs (10Mm), 0.3 µl Taq (1U/µl), 2.5ul $MgCl_2$ (25Mm), 0.5 µl each primers (100 µM/µl), and 13.7 µl PCR water to make up the final volume of 25 µl. The conditions used for the amplification were as follows: an initial denaturation step at 95°C for 5 minutes; followed by

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35 cycles of 94°C for 1 minute, 55°C for 1 minute, and 72°C for 1 minute; and finally elongation at 72°C for 10 minutes. The amplified PCR products were checked using 1.5% agarose gel electrophoresis. The homozygous individuals for insertion allele (II) were identified by the presence of a single band of 490 bp, the homozygous for the deletion allele (DD) were identified by the presence of single 190 bp product fragment and the heterozygous individuals (ID) were identified by the presence of both bands of 490 bp and 190 Fig.1 Showing PCR products of 490 bp and 190 bp for ACE I/D Polymorphism bp.

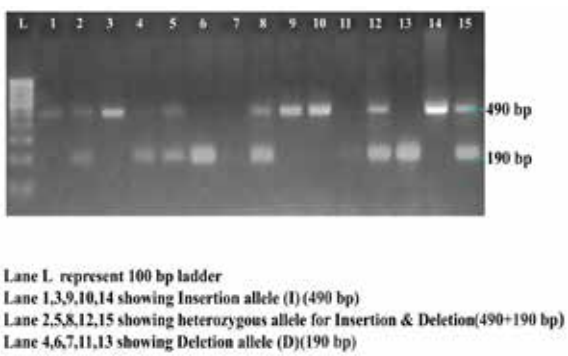

Fig.1 Showing PCR products of 490 bp and 190 bp for ACE I/D Polymorphism

Statistical analysis: Allelic frequencies were calculated by gene-counting method. Hardy-Weinberg equilibrium (HWE) and the genotypic as well as allelic distribution of polymorphism of ACE gene was analyzed using Pearson's goodness of fit chi-square test. The significant association of ACE I/D polymorphism with migraine susceptibility was analyzed by calculating the odds ratio (OR) with 95% confidence interval (CI). A p-value <0.05 is considered as statistical significant.

Results

Out of 50 patients, 34 cases had migraine without aura and 16 patients had migraine with aura. The genotype distribution and the allele frequencies of the ACE gene polymorphism for migraine patients and the control group are shown in figure.

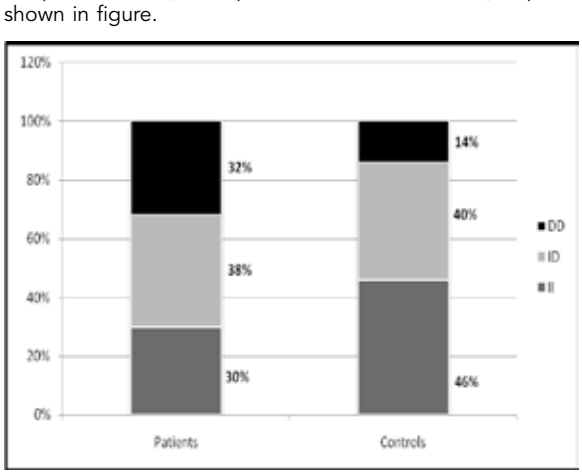

Figure 2: Genotypic distribution of ACE I/D polymorphism in study groups.

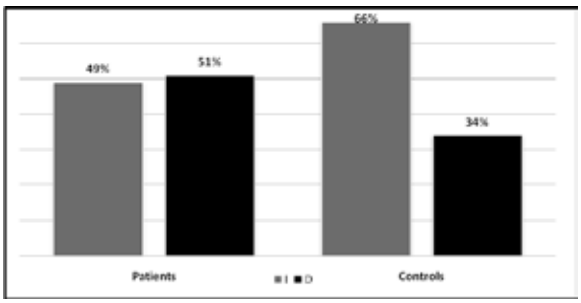

Figure 3: Allele distribution of ACE I/D polymorphism in study groups.

The genotypic frequencies were in accordance with HWE in both groups (patients: χ^2= 2.87, p=0.09 and controls: χ^2 =0.59, p>0.05). The association of ACE I/D polymorphism with migraine susceptibility can be obtained by calculating odds ratio (OR).

Genotypes/ Alleles	Migraine patients (n=50)	Controls (n=50)	OR (95% C.I)	p-value
II	15	23	1(Reference)	
ID	19	20	1.46 [0.59-3.60]	0.4
DD	16	7	3.50 [1.16-10.54]	0.02*
ID+DD	31	30	1.10 [0.87-4.52]	0.1
I	49	66	1(Reference)	
D	51	34	2.02 [1.14-3.57]	0.01*

Table 1: Association of ACE I/D Polymorphism with Migraine risk.

There was a significant difference in frequencies of DD vs II genotypes between the patients and controls (OR=3.5, p=0.02). OR for D vs I allele showed that the 'D' allele of ACE gene was adding 2- folds risk which was significant for the susceptibility of migraine in the study population of Jammu region.

Discussion

The aetiology of migraine is complex and usually involves alteration in vascular functioning. Angiotensin converting enzyme (ACE) gene which is one of the prime gene of vascular physiology has been implicated as a genetic factor associated with migraine. Previously, a higher incidence of the ACE gene DD-genotype was found in migraine patients without aura and a determining role of the ACE gene DD-genotype in the frequency of migraine attacks was suggested (12, 14). On the contrary, another study pointed out a slight protective effect of ACE-DD against migraine in male patients (15). In the present study, the frequency of DD-genotype was higher in patients in judgement to controls (32% vs 14%). Similarly, higher frequency of DD-genotype (39%) was reported by Pizza et al (3) in migraineurs. In the present study, we found that DD-genotype and D- allele of ACE gene was strongly associated with migraine [DD vs II, OR=3.50; 95% CI (1.16-10.54); p=0.02 and D vs I allele, OR=2.02; 95% CI (1.14-3.57); p=0.01] in the population of Jammu region of the J&K State. The results of the present study are consistent with some earlier studies done in other population by Paterna et al (12); Kowa et al (11); Horasinl et al (16). In a previous study on North-Indian population disclosed higher frequency of DD-genotype (12%) in migraine patients as compared to controls (8%), but the study failed to generate a significant association between ACE DD-genotype and migraine (17). Contradictory results were shown by Alasehirli et al (18); Özbey et al (19); Sezer et al (20) and Sipahi et al (21) and these studies were not in support of

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association of ACE I/D polymorphism with susceptibility of migraine.

Conclusion

The results of the present study support relationship between DD-genotype and D-allele of ACE gene I/D polymorphism with the susceptibility of migraine.

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