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Full Length Research Paper

Angiotensin converting enzyme insertion /deletion polymorphism in angiographically proven coronary artery disease subjects of South India

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The prevalence of Coronary artery disease (CAD) varies epidemiologically among different population. Environmental and genetic factors play an important role in the pathogenesis of CAD. Angiotensin converting enzyme (ACE) insertion/deletion polymorphism has an effect on the pathogenesis of CAD, through angiotensin II mediated mechanism. In the present study, we have evaluated the association of insertion/deletion polymorphism in angiographically proven CAD patients (n= 50) by comparing with controls (n=54). We found that DD polymorphism is highly significant in CAD subjects (46%) D allele frequency is high in CAD subjects (63%) vs Controls 33%. The odds ratio for developing CAD is high in DD genotype than in ID/II genotypes with p value of <0.001. Thus by this study we conclude that DD polymorphism is associated with CAD in south Indian subjects.

Key words: Angiotensin converting enzyme, gene polymorphism, coronary artery disease (CAD).

INTRODUCTION

Coronary Artery Disease (CAD) is a multifactorial disease involving a number of both environmental factors and genetic factors. India will be host to more than 45% of heart diseases in the world in next two decades (Gupta et al., 2008). Renin Angiotensin System (RAS) plays central role in cardiovascular homeostasis. Angiotensin Converting Enzyme (ACE), one of the enzymes of RAS responsible for conversion of Angiotensin I (Ang I) to Angiotensin II (Ang II), is a key peptide implicated in pathogenesis of atherosclerosis (Anna et al., 2008). Pharmacological inhibition of ACE, improves the out-comes in patients with cardiovascular diseases (Ferrari et al., 2010). Recent data suggests that ACE gene polymorphism appears to have a significant impact on prognosis of CAD (Kaiser et al., 2009).

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ACE gene is located on chr $17q^{23}$ and consists of 26 exons and 25 introns. Intron 16 contains polymorphism characterized by the presence (insert (I)) or absence (deletion (D)) of a 287 bp Alu repeat sequences (Domnita and Jean, 2000) resulting in 3 genotypes DD, II and ID. Individuals with DD genotype have high tissue and plasma ACE concentration. Different studies had proven the association of ACE gene polymorphism with CAD (Esmeray et al., 2005; Agarwal et al., 2004). Thus the purpose of this study is to analyze the role of ACE gene polymorphism in South Indian population with CAD.

MATERIALS AND METHODS

The study population consisted of 104 subjects of mean age 45 years, both males and females were included in the study. 50 subjects who were proven cases of CAD by diagnostic coronary angiography were included as cases. 54 normal healthy individuals without any CAD risk factors, or previous history of CAD were included as controls. The standard questionnaire, which takes the history on smoking, alcohol consumption, hypertension, diabetes,

Table 1. Demographic and biochemical parameters in study subjects.

Parameter		CAD (n = 50 (Mean ± SD))	Controls (n = 54)	p-value
Age (years)		51.72 ± 11.43	45.18 ± 5.5	0.0003*
Sex	Male	43	37	
	female	7	15	---
Smoking %		64	42	---
Hypertension%		16	-	---
Diabetes%		20		---
FBS mg/dl		103.47 ± 36.48	83.33 ± 7.48	0.000137*
TC mg/dl		162.5 ± 41.99	158.12 ± 34.32	0.5603
TGL mg/dl		100.3 ± 24.24	117.29 ± 49.75	0.0311*
HDL mg/dl		43.08 ± 12.45	43.12 ± 8.87	0.9849

n, Number of subjects, $p < 0.05$ significant.

family history of CAD was taken.

Blood sample collection

After 12 h of overnight fasting, 10 ml of venous sample was collected from anterior cubital vein of both cases and controls. 2 ml of it was transferred to EDTA tubes for genomic DNA extraction; 8 ml of the remaining blood in plain tubes was allowed to clot. Serum was separated by centrifugation at 2500 rpm and stored at -20℃ until analysis. Fasting blood glucose, lipid profile (TG, TGL, HDL) were measured by using enzymatic kit methods.

Angiotensin converting enzyme insertion/deletion polymorphism (genotypes) analysis

Genomic DNA was extracted from peripheral blood using spin column genomic DNA extraction kit (Axygen Biosciences USA) and ACE intron 16 gene was amplified by Polymerase chain reaction (MG series Thermo cycler USA). For amplification, a flanking primer pair (Zhu et al., 2001) 5'-CTGGAGACCACT CCCATCCTTTCT-3' and 5'-GATGTGGCCA TCACATTCGTCACGAT-3' (SYNTHESIZED BY Bioserve Biotechnology) were used. PCR amplification was performed with a 50µl reaction mixture containing 40 pmol of each primer, 200 µmol/L each dNTP, 1.5 mmol/L $MgCl_2$, 1U of thermo stable DNA polymerase (DYNAZYME II Espoo, Finland) and 20 mMol of TRIS-HCl (pH 8.8 at 25℃) PCR cycling conditi ons were carried out with an initial denaturation step of 5 min at 95℃, followed by 30 cycles of denaturation at 95℃ for 30 s, annealing at 58℃ for 1 min and extension at 72℃ before the stor age of sample at 4℃. PCR products were separated by agarose gel electrophoresis (GENI Bangalore India). DNA fragments were stained with ethidium bromide and visualized under UV light (Gel documentation system Biorad USA). The PCR fragments consist of three genotypes, a 490 bp bad (II), a 190bp band (DD), and both 490 and 190 bp band (ID), To increase the DD genotyping, PCR amplifications were also performed with insertion specific primer pair 5'-TGGGACCACAGCGCCCGCCACTAC-3' and 5'-TCGCCAGCCCTC CCATGCCCATAA-3' for each sample, which had the DD genotype to avoid mistyping of ID heterozygote's as D homozygote's. PCR cycling conditions were carried out with an initial denaturation step of 1 min at 95℃, followed by 30 cycles of denaturation at 95℃ for 30 s, annealing at 67℃ for 45 s and extension at 72℃ for 2 min. The PCR product shows a 335 bp band for I allele and no band for DD genotype.

Coronary angiography

Cornary angiogram was done using Phillips flat panel Cathlab system. Standard Judkin's technique was used to cannulate right and left coronary arteries. Iohexol was used in coronary angiography in standard projections. CAD was defined as more as >50% diameter stenosis of one or more coronary arteries or major branches.

Statistical analysis

Data was analyzed by using SPSS software system. The biochemical parameters were expressed as mean ± SD. Students t test was used to analyze the significance, p value < 0.05 considered as significant. We determined whether the distribution of the ACE insertion/deletion genotype was in Hardy–Weinberg equilibrium using Chi square test. For the ACE genotype, Odds ratio was calculated as a measure of association with the presence/absence of coronary artery disease. For each odds ratio, the 95% CI was calculated.

RESULTS

Table 1 shows the comparison of biochemical parameters between cases and controls. Table 2 represents genotype and allele frequencies of ACE I/D genepolymorphism in CAD subjects and controls. The results clearly showed that DD genotype frequency is high (46%) in CAD group compared to controls. Allele frequencies for I and D alleles in patients and controls explains that the D allele frequency was 63% in CAD vs 36% in controls. Table 3 shows the odds ratio of developing CAD for DD, ID and II genotypes .The odds ratio was high for DD vs II (3.6) with significant p value (<0.001). Figure 1 was the gel showing D and I alleles of

Table 2. Distribution of ACE genotypes and allelic frequencies of the study population.

Study group	ACE genotypes			Total	Allelic frequencies		Total
Genotype	II	ID	DD		I	D	
Controls(n)	29	14	11	54	72(0.66)	36(0.33)	108
Patient's(n)	10	17	23	50	30(0.37)	63(0.63)	100

Table 3. Distribution of ACE genotypes between CAD and controls.

Patients vs. Controls	Chi-square (x^2)	Odds ratio	CI 95%		p-value
			Limit	Unlimited	
DD vs. II	28.07	3.6	2.1	5.9	<0.001
DD vs. ID	16.14	2.7	0.9	2.6	<0.001
DD vs. II + ID	19.5	2.4	1.6	3.9	<0.001

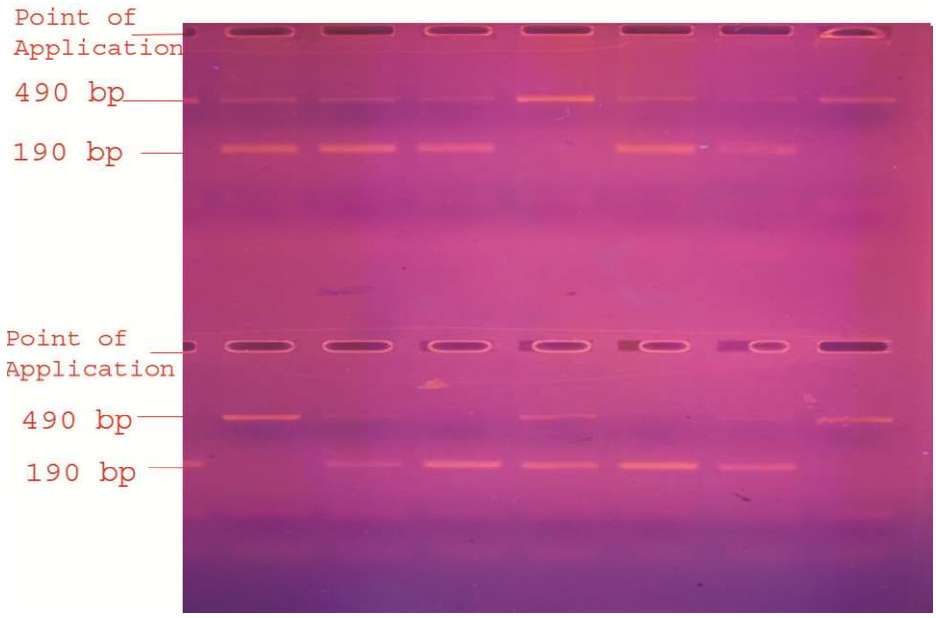

Figure 1. Gel showing I and D alleles in CAD patients.

ACE I/D genotype in CAD subjects.

DISCUSSION

Cardiovascular disease is the main cause of increasing morbidity and mortality in the world. This disease is (CVD) interplay of environmental risk factors and multiple genetic risk factors. RAAS functions as an endocrine system maintaining the cardiovascular haemodynamics. Conversion of Angiotensin I to Angiotensin II is the key reaction in the RAS pathway, which is mediated by ACE. Hence ACE activity results in the activation of a vasoconstrictor agent (Ang II) and inactivation of vasodilator bradykinin therefore, the beneficial effects of ACE inhibitors are due to antagonizing the above mentioned effects, as useful drugs in cardiovascular diseases (Ferrari and Fox, 2009), Since few decades researcher have defined various genes that play a role in the vascular disease. There are studies from India, proving the association ACE gene polymorphism with CAD (Pulla et al., 2010).

The ACE DD genotype is associated with increased circulating ACE levels (Rigat et al., 1990). D allele was found to be associated with increased risk of coronary artery disease. Cambien et al. (1992) found that DD

genotype frequency was high in subjects with MI compared to controls. Another study emphasizing the DD polymorphism for CAD was by Alvarez et al. (1998). According to Esbir et al. (2008) study, ID polymorphism is significantly associated with CAD in Turkish patients (Turgay et al., 2008). A Meta analysis of the effect of ACE gene polymorphism on CAD stated that the gene polymorphism predicts risk of IHD in small studies but not in large studies (Birgit et al., 2000). According to Copenhagen city heart study, US physicians' health study, there is no influence of ACE gene polymorphism on CAD (Lindpainter et al., 1995; Agerholm-Larsen et al., 1997). But this may be due to lack of large population based studies for different ethnic groups. Indian studies also gave different opinions regarding ACE I/D polymorphism on CAD. But all the Indian studies agreed upon increased D allele frequency in CAD (Kaiser et al., 2009; Agarwal et al., 2004).

The limitations of the different studies include lack of the data on ACE inhibitors usage. Effect of ACE inhibitors deferring according to genotype has to be evaluated in future. In the present study, we evaluated the association of ACE gene polymorphism (I/D) with CAD, and found that there is significant association with DD polymorphism. Allele frequencies were studied in both the patients and controls, the frequency of ACE deletion (D) allele has been found to be higher in CAD subjects than in the control group (63 vs 36%), the odds ratio for developing CAD was high for DD vs II genotype with P value of <0.001.

The association of DD polymorphism with CAD can be explained by increasing levels of ACE in the circulation. The role of ACE gene in the genetic control of plasma ACE levels was first reported by Rigat et al. (1990). There are various deleterious effects of Ang II that include hypertophy in non infracted areas, direct toxic effects on myocardial cells, remodeling of ventricles, proliferation of fibroblasts, promotion of smooth muscle hyperplasia, endothelial dysfunction, increase of left ventricular after load in addition to the main effect of vasoconstriction, coronary artery constriction and activation of sympathetic nervous system. This could be one of the explanations for DD genotype association with CAD. The increase in ACE may lead to increase in Ang II production thus causing CAD via Ang II medicated mechanisms (Emmanuel et al., 2005)

ACE activity in the blood vessel specimens from acute coronary syndrome patients was significantly increased (Shiro et al., 2001). DD polymorphism may be one of the genetic factors leading to increased ACE activity and hence to ACS/CAD. ACE is a local mediator of inflammation. There is an up-regulation of ACE activity at the shoulder region of unstable plaques. Ang II induced cytokine and oxidative stress may play a role in plaque instability (Bernhard et al., 2000). The higher level of circulating ACE will produce corresponding higher level of angiotensin II. It stimulate Ca^{++}, aldosterone pathways and vascular endothelial growth factors and the infusion of more cholesterol in the coronary vessel to cause pathophysiological condition (Griendling et al., 1994; Rajagopalan et al., 1996)

Conclusion

Thus the knowledge of genetic factors of coronary artery disease may help in explaining the molecular bases of the disorders and in designing prevention and treatment methods, as more than 50% of patients suffering from MI have no identifiable conventional risk factors. Polymorphism studies not only help in gene targeting but also in developing therapeutic interventions.

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