Angiotensin-Converting Enzyme Gene Polymorphism in Patients with Coronary Artery Disease

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Abstract: Several genetic investigations have been attempted to elucidate the association of gene polymorphism of angiotensin-converting enzyme (ACE) in coronary artery disease. This study was conducted to investigate the role of gene polymorphism of ACE in patients with coronary artery disease. The study included fifty-six numbers of patients with atherosclerotic coronary artery disease where proven angiographically and fifty-six numbers of healthy individuals of sex matched as a control group. The patients and control group were subjected to routine investigations, assays like, serum cholesterol, triglycerides, high-density Lipoprotein-cholesterol (HDL-C), low-density lipoprotein-cholesterol (LDL-C). Genomic DNA was extracted and analyzed for angiotensin-converting enzyme insertion/deletion polymorphism using polymerase chain reaction (PCR). When we compared the genotypes of patients with coronary artery disease and controls, it was observed that all three genotypes were not statistically different also no significant difference of alleles in ACE gene genotypes was found. Inpatient serum cholesterol, triglyceride and HDL-C (P <0.001, P <0.001 and P <0.001: respectively) showed a significant increase than the control group. In patients, LDL-C level was not more significant than controls. In the evaluated population, we conclude that the gene I/D polymorphism for ACE are not risk associated and may not be a useful marker for coronary artery disease.

Keywords: Angiotensin-Converting enzyme; Genotypes; Polymorphism; Coronary artery disease.

1. Introduction

Coronary artery disease (CAD) is the underlying cause for most of the ischemic cardiac events and can result in myocardial infarction, congestive heart failure, cardiac arrhythmias, and sudden cardiac death. Clinically significant CAD in men under 40 and premenopausal women is uncommon, but the risk is more with increased age as well as in the presence of risk factors such as smoking, hypertension, diabetes, high cholesterol, and family history of heart disease [1]. The prevalence of CAD varies epidemiologically and among different populations. Familial studies have shown that genetic and environmental factors co-operate in the pathogenesis of cardiovascular disease [2].

Angiotensin-Converting Enzyme (ACE) has an important impact on the structure and function of cardiovascular biology. ACE has a key role in the production of angiotensin-II and catabolism of bradykinin. Two peptides of ACE involved in the modulation of vascular tone and proliferation of smooth muscle cells [3]. Several studies have suggested that the genes encoding components of the Renin-Angiotensin System (RAS) is the candidate genes for cardiovascular disease, and recent reports suggest that genetic polymorphisms of the RAS have been associated with cardiovascular diseases [4, 5].

The human gene for ACE is located on chromosome 17 and contains a length polymorphism consisting the presence (insertion, I) or absence (deletion, D) of a 287 base pair DNA of intron 16,

which results in three genotypes: homozygotes DD and II and heterozygotes, ID [6]. ACE is a zinc metallopeptidase that converts angiotensin I to the potent vasoconstrictor angiotensin II which degrades bradykinin, a powerful vasodilator, both angiotensin I and II are for the regulation of vascular tone and cardiac functions [7]. The polymorphism is shown to be associated with the interpersonal variability of ACE levels in circulating blood [8]. The deletion allele at the gene site of ACE is associated with increased activity of plasma ACE [9]. Conflicting results have been reported regarding the association of ACE polymorphism with coronary artery disease [10, 11]. The purpose of this study was to investigate the correlation of the ACE/ID polymorphism with CAD in the evaluated population.

2.   Materials and Methods

2.1 Materials

The present study included 56 patients with CAD (42 males and 14 females, ages 35 to 55 years old) and 56 apparently healthy individuals (41 males and 15 females, ages 35 to 56 years old) as a control group. We collected information about the presence of categoric cardiac risk factors in every patient. Risk factors data were derived from patient interviews, contacting the referring physicians, and existing medical records. Systemic arterial hypertension was defined from documented history of high blood pressure or treatment with medication, diet, and/or exercise. A history of current smoking or cessation of smoking within 3 months before testing was defined as the status of positive smoking. Hypercholesterolemia was determined on the basis of the answers to the following questions: "Has your physician ever told you that you need medications for high cholesterol?" "Are you currently taking cholesterol medications?" Answers to these questions often identified patients who were currently receiving cholesterol-lowering medications. Individuals were classified as having diabetes mellitus if they were previously diagnosed and determined by blood glucose levels and were in treatment with insulin or oral hypoglycemic agents.

2.2 Collection of blood samples

The 5ml of venous peripheral blood samples were collected in 15 ml polypropylene centrifugation tubes with screw cap that contained 20% EDTA with 3ml of nuclei lysis buffer (10 mM Tris-HCl, 400 mM NaCl and 2mM Na_2EDTA, pH 8.2). The cell lysates were digested overnight at 37°C with 0.2ml of 10% SDS and 0.5ml of a protease K solution (1mg protease K in 1% SDS and 2mM Na_2EDTA). After digestion, 1 ml of saturated NaCl (approximately 6M) was added to each tube and shaken vigorously for 15 seconds, followed by centrifugation at 2500 rpm for 15 minutes. The supernatant containing the DNA was transferred to

another 15ml polypropylene tube. Exactly 2 volumes absolute ethanol was added and mixed until the DNA was precipitated. The precipitated DNA was removed and transferred to Microcentrifuge tube containing 100-200μl TE buffer (10mM Tris-HCl, 0.2mM Na_2EDTA, pH 7.5). The DNA was allowed to dissolve for 2 hours at 37°C for quantification.

2.3 Determination of ACE genotype

According to the procedures of Miller *et al.*, (1998) [12], the ACE genotypes of genomic DNA isolated from whole blood samples were determined by polymerase chain reaction. PCR amplification was carried for 35 cycles in a thermal cycler the mixture was denatured at 95°C for 5 minutes, then denaturing at 95°C for 45 minutes, annealing at 65°C for 45 seconds and extension at 72°C for 2 minutes, the final extension cycle running for 10 minutes under the standard set of reaction conditions. The flanking primer pair 5'CTGGAGACCACTCCCATCCTTTCT3'   and 5'GATGTGGCCATCACATTCGTCACGAT3' for the DNA amplification was used and subsequently to increase the specificity of DD genotyping, PCR amplifications were also performed with I-specific primer   pair 5'TGGGACCACAGCGCCCGCCACTAC3'   and 5'TCGCCAG CCCTCCCATGCCCATAA3' in all the samples that were found to be DD after amplification with the flanking primers. The amplicon of the specific fragments was identified on 2% agarose gel containing ethidium bromide.

2.4 Statistical analysis

The statistical significance of all data was analyzed using the SPSS Version 12.0. Data were double entered, and the resulting sets of data were compared and checked for completeness and accuracy of the entry. The significant differences between the mean of the two groups was assessed with Student's t-test, and the proportions were tested with the chi-square statistic. Differences in Vessel diseases were tested using ANOVA. A p-value <0.05 was considered to be statistically significant. Descriptive statistics were applied for calculating the distribution of various characteristics estimates.

3.   Results and Discussion

The Angiotensin-I converting enzyme a key component of the renin-angiotensin system was responsible for the development of cardiovascular disease. An ACE inhibitor is known to significantly reduce mortality or the incidence of myocardial infarction in patients who have hypertension or ischemic heart disease [13-15]. In our study, we selected the individuals free from coronary risk factors as a control group. The age and gender distributions of patients with CAD in the study group and controls were

not similar. The Demographic and clinical characteristics of the patients and control subjects are presented in Table 1. The result of the table 1 is comparable with the previous study group of earlier reports which suggest that the prevalence of the ACE genotype specifically the insertion/deletion polymorphism in association with dilated cardiomyopathy, coronary-artery restenosis, hypertrophic cardiomyopathy, parental history of

myocardial infarction [16] and cardiac hypertrophy with variable results was reported in a number of small case-control studies [17].

Total cholesterol, triglycerides and HDL-C levels showed significant differences between the study groups with CAD and were higher than the control group. LDL-C level was not significant in patients when compared to control (Table 2).

Table 1. Demographic and clinical characteristics of the patients and controls

Variables	Coronary artery Disease (n = 112)	Healthy control Subjects (n = 52)
Age (years)	57.87 ± 8.91	50.23 ± 4.97
Gender (n)		
Men	42 (80.4)	18 (72.0)
Women	14 (19.6)	7 (28.0)
Smoking habit (n)		
Smokers	22 (40)	6 (24)
Ex- smokers	14 (25.5)	6 (24)
No smokers	19 (34.5)	13 (52)
Hypertension (n)	26 (47.3)	0
Diabetes (n)	16 (29.1)	0
Dislipidemia (n)	14 (25)	0
Drug therapy		
Nitrates	44 (78.5)	0
Aspirin	45 (80.3)	0
ACE inhibitors	26 (46.4)	0
Calcium- channel blockers	33 (58.9)	0

Values in patients and controls are percentages

Table 2. Serum lipid profile in both control and coronary artery disease patient groups.

	CAD group (N = 56)	Control group (N = 56)	t- Value	P- Value
T. Cholesterol (mg/dl)	172.3 ± 39.42	148 ± 15.85	4.28	P < 0.001
Triglyceride (mg/dl)	155.7 ± 76.53	110.71 ± 11.25	4.35	P < 0.001
LDL-C (mg/dl)	114.28 ± 54.15	112 ± 13.07	-0.30	ns
HDL-C (mg/dl)	45.39 ± 14.97	49.85 ± 3.85	-2.16	P < 0.05

Values are expressed as mean ± SD, n = 56 (control) n = 56 (coronary artery diseases) P values difference between the control and the coronary artery disease statistical significant difference in two groups of (TC, TG and HDL-C) P < 0.001. Comparison of LDL-C statistical difference between from control and patients' values are not significant. > 0.05. CAD: coronary artery disease, HDL-C: high-density lipoprotein cholesterol, LDL-C: low-density lipoprotein cholesterol, ns: nonsignificant TC: total cholesterol.

The present result agrees with the previous study which instigates that the lack of association between ACE gene polymorphism and serum cholesterol levels (HDL-C, LDL-C and triglycerides) in a group of patients with hypertension was observed [18]. On the other hand, Kawamoto (2002) has found a significant association between DD genotype and total cholesterol in a group of patients with hypertension and carotid atherosclerosis [19], while Chowdhury (1998) has found no association between DD genotype and total cholesterol in a group of Bangladesh patients with hypertension [20].

Comparison of the distribution of ACE gene genotypes among single, double and triple vessel CAD group and control group have shown no significant difference in gene polymorphism [2, 21]. However, these reports have been challenged by a series of negative studies [22, 23]. Our result on descriptive statistics of the population study with coronary angiography is presented in Table 3a.

Table 3a. Distribution of ACE gene genotypes in CAD and the control group (overall).

Genotype	CAD n (%)	Control n (%)	t - value	P value
DD	14 (25)	12 (21.4)	0.45	ns
ID	26 (46.6)	19 (35.7)	0.19	ns
II	16 (28.6)	25 (42.9)	0.605	ns

Values are expressed as mean ± SD, n = 14 (DD) n = 26 (ID) n = 16 (II) P values difference between the three groups control and the coronary artery disease statistical difference is not significant P >0.05 P test values (0.45, 0.19 and 0.60). n = no of samples; CAD: coronary artery disease; DD: deletion; ID: insertion deletion II: insertion.

Angiotensin-converting enzyme is responsible for the conversion of angiotensin I to the angiotensin II. Angiotensin II has been implicated in the pathogenesis of atherosclerosis CAD through the induction of hyperplasia and hypertrophy of smooth muscle cells, which increased the expression of platelet-derived growth factor and proto-oncogenes [24, 25]. In the present study, we have evaluated the association of the

ACE gene with CAD, and found that between CAD (single, double and triple vessel disease) and control groups (Table 3b, c, d) there was no significant difference in D and I allele frequency because of no association with the DD, ID, or II genotypes. The genotype analysis was observed that the DD genotype was slightly higher in CAD patients than controls (25% *v.* 21.4%). The ID genotype is 46.4% in patients while it is 44.6% in controls. The present data show contrary results to the previous reports of two studies performed in Turkish population which instigates that there was a significant association between DD polymorphism of ACE gene and CAD [26, 27]. In our study, there was no significant increase in the frequency of the DD genotype in CAD patients which were shown in Gel bands (Fig. 1 & 2a, b, c). The II genotype was little higher in controls (33.9%) than the patients (28.6%). However, the differences were not significant in genotype/phenotype interactions.

Table 3b. Comparison of the distribution of ACE gene genotypes in single vessel CAD and the control group.

Genotype	CAD n (%)	Control n (%)	t - value	P value
DD	4 (26.7)	12 (21.4)	0.4364	ns
ID	7 (46.6)	19 (35.7)	0.7724	ns
II	4 (26.7)	25 (42.9)	1.14	ns

Values are expressed as mean ± SD, n = 14 (DD) n = 26 (ID) n = 16 (II) P values difference between the three groups control and the coronary artery disease statistical difference is not significant P >0.05 P test values (0.45, 0.19 and 0.60). n = no of samples; CAD: coronary artery disease; DD: deletion; ID: insertion deletion II: insertion.

Table 3c. Comparison of the distribution of ACE gene genotypes in double vessel CAD and the control group.

Genotype	CAD n (%)	Control n (%)	t - value	P value
DD	6 (22.2)	12 (21.4)	0.0829	ns
ID	13 (48.1)	19 (35.7)	1.0815	ns
II	8 (29.6)	25 (42.9)	1.1662	ns

Values are expressed as mean ± SD, n = 6 (DD) n = 13 (ID) n = 8 (II) P values difference between the three groups control and the coronary artery disease statistical difference is not significant P > 0.05 P test values (0.08, 1.08 and 1.16). n = no of samples; CAD: coronary artery disease; DD: deletion; ID: insertion deletion II: insertion.

Table 3d. Comparison of the distribution of ACE gene genotypes in triple vessel CAD and the control group.

Genotype	CAD n (%)	Control n (%)	t - value	P value
DD	4 (28.6)	12 (21.4)	0.5740	ns
ID	6 (42.8)	19 (35.7)	0.4918	ns
II	4 (28.6)	25 (42.9)	0.9767	ns

Values are expressed as mean ± SD, n = 4 (DD) n = 6 (ID) n = 4 (II) P values difference between the three groups control and the coronary artery disease statistical difference is not significant P > 0.05 P test values (0.57, 0.49 and 0.97). n = no of samples; CAD: coronary artery disease; DD: deletion; ID: insertion deletion II: insertion.
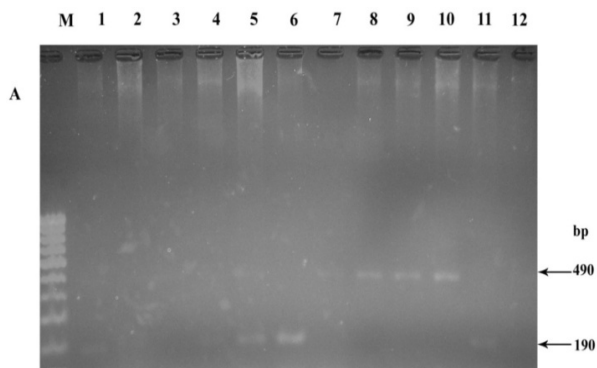

Fig. 1. Analysis of Polymerase chain reaction for angiotensin-converting enzyme gene polymorphism in CAD and control. The gel is showing the I and D alleles in control.
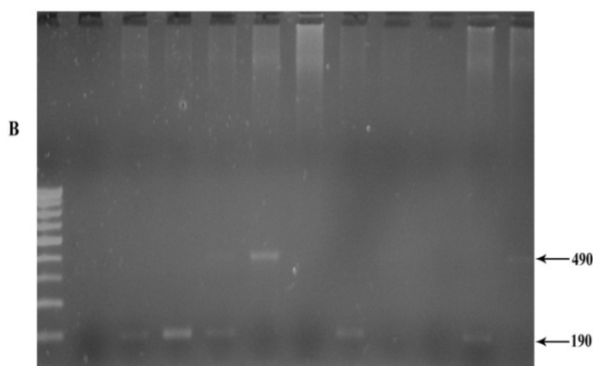

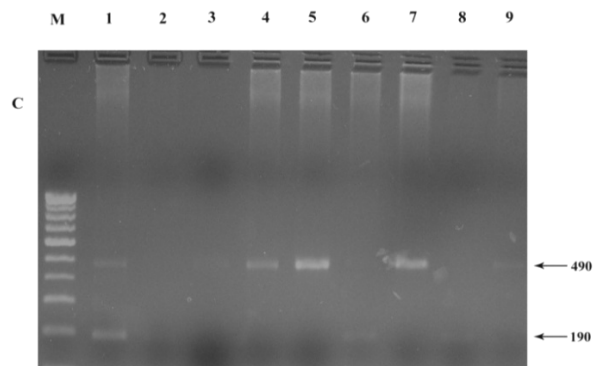**Fig. 2a. Gel shows the I and D alleles in CAD patients.**

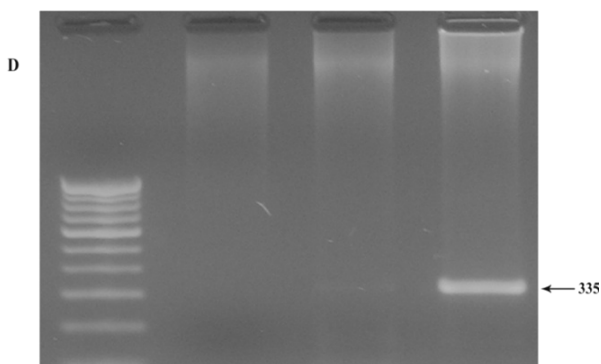**Fig. 2b. Gel shows the I and D alleles in CAD patients.**

Fig. 2c. Gel shows the I specific alleles in CAD patients.

Although the precursors of ACE gene polymorphism on the prediction of clinical manifestation of CAD may be genetically determined, the CAD event rates and their occurrences with differences in the population appear to be largely determined by environment and lifestyle [28]. Some studies have suggested that ACE polymorphism may be relevant to the factors that predispose individuals to the development of atherosclerosis than being the direct cause of CAD itself. The mechanism by which the ACE I/D genotype may predispose an individual to the development of CAD remains unclear. Further efforts should be made to elucidate the specific nature of epidemiology.

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