ASSOCIATION BETWEEN INSERTION/DELETION POLYMORPHISM OF THE ANGIOTENSIN-CONVERTING ENZYME GENE AND CORONARY ARTERY DISEASE IN BOSNIAN POPULATION

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Abstract

One of the genes considered as a risk factor for coronary artery disease (CAD) is the angiotensin-converting enzyme (ACE) gene. Many studies have been published regarding the relation between the ACE gene insertion/deletion (I/D) polymorphism and CAD. However, studies have provided controversial results. To explore this further in the population of Bosnia and Herzegovina, we compared the ACE I/D genotypes and alleles distribution between two groups: 100 CAD patients and 100 healthy control subjects. The higher distribution of DD genotype (47.0%) and D allele (65.5%) were found in CAD patients compared to controls (DD 34.0%; D allele 51.0%). Genotype odds ratio, (DD + ID) on the II, was 2.471 (1.252 – 4.876; 95% CI; $p < 0.05$). This leads to the conclusion that the DD genotype of the ACE I/D polymorphism affects the risk for development of coronary artery disease in Bosnian population.

Key words: *Coronary artery disease, angiotensin-converting enzyme gene, insertion/deletion polymorphism*

Introduction

Coronary artery disease is a complex disorder resulting from the interaction between genetic background and various environmental factors. One of the genes implicated in the pathogenesis of CAD is the angiotensin-converting enzyme (ACE) gene. Angiotensin-converting enzyme is the primary component of the renin-angiotensin system (RAS). It converts inactive form of angiotensin I to the potent vasoconstrictor angiotensin II. It also inhibits the vasodilator bradykinin (Griendling et al., 1993). Thus, products of this enzyme have important roles in modulation of vascular tone, aldosterone secretion, water intake, renal sodium absorption, sympathetic activity and vasopressin release (Balakumar & Jagadeesh, 2014). Typical plasma levels of ACE are associated with the insertion/deletion (I/D) polymorphism of ACE gene, located on chromosome 17q23, identified by Rigat et al. (1990). The I/D polymorphism is based on presence (allele I) or absence (allele D) of the 287-bp Alu repeat sequence in intron 16. ACE activity levels in DD homozygote are almost doubled compared to II homozygote.

In 1992, Cambien et al. first reported positive association between D allele and myocardial infarction (MI). Since then, many scientists have investigated the ACE I/D polymorphism in relation to coronary artery disease and demonstrated positive association between D allele and CAD (Badenhop et al., 1995; Peterlin et al., 2000; Terzić et al., 2003; Acarturk et al., 2005; Istrati et al., 2006; Seckin et

al., 2006; Freitas et al., 2008; Guney et al., 2013; Sahin et al., 2015). However, some other studies, conducted in different populations, did not establish strong correlation between the I/D polymorphism and CAD (Friedl et al., 1995; Agerholm-Larsen et al., 1997; Hernández et al., 2002; Oei et al., 2005; Kähönen et al., 2007).

The aim of this study was to investigate the genotypes frequency of the ACE I/D polymorphism and their association with coronary artery disease in Bosnian population.

Materials and methods

The study population included 200 unrelated subjects: 100 healthy individuals without apparent signs and symptoms of CAD and 100 patients with angiographically confirmed CAD. The diagnosis of CAD was confirmed at the Cardiovascular Clinic Tuzla from 2012 to 2015. All patients were from the northeast Bosnia and Herzegovina and they all received oral and written information about the study prior to signing a written informed consent.

DNA was isolated from peripheral blood leukocytes using the FlexiGene DNA extraction kit (Qiagen GmbH. Hilden, Germany). DNA fragment of the ACE gene (intron 16) was amplified by polymerase chain reaction (PCR) (Labnet Multigene Thermal Cycler, Labnet International, Inc., North America) using primers (Sigma-Aldrich, St. Louis, USA) described by Rigat et al. (1992). PCR conditions were shown in Table 1. The presence (allele I) or absence (allele D) of the 287-bp were determined by evaluating the size of amplicons: II genotype 1 amplicon of 490 bp, DD genotype 1 amplicon of 190 bp and ID genotype 2 amplicons of 490 bp and 190 bp. DNA ladder 100bp (New England BioLab, The UK) was used for fragment size evaluation. The fragments were separated using 2% agarose gel electrophoresis (agarose Sigma-Aldrich, St. Louis, USA) with ethidium bromide staining and genotypes were scored by visualizing the fragments under UV light (VWR GenoMini, VWR International, BVBA Leuven, Belgium).

Differences in allele and genotypes distribution between the patients and healthy subjects were assessed using the chi-square test (χ^2 test). A value of p lower than 0.05 or less was considered as statistically significant. The odds ratio (OR) and their 95% confidence interval (CI) were also calculated. The statistical analysis was performed with the SPSS Statistics, version 20.0 (IBM Corporation, 2011).

Table 1. PCR cycling and running parameters

Cycle step	Temp (°C)	Time (min)	Number of cycles
Initial denaturation	94	5	1x
Denaturation	94	1	
Annealing	58	1	30x
Extension	72	1	
Final extension	72	5	1x
Storage	4	hold	

Results and Discussion

After electrophoresis, each DNA sample revealed one of three possible genotypes of the ACE I/D polymorphism: II, ID or DD (Figure 1).

The frequency of the deletion allele (D) was higher (65.5%) in patients with CAD than in healthy subjects (51.0%). This difference was significant ($p < 0.01$). Table 2 shows the distribution of the ACE I/D genotypes among the CAD group and controls. Also, the incidence of DD genotype was significantly higher in CAD patients than in controls ($p < 0.05$). Both DD and ID genotypes were more strongly associated with coronary artery disease compared to II genotype. Genotype odds ratio, (DD + ID) on the II, was 2.471 (1.252 – 4.876; 95% CI; $p < 0.05$).

In this study, CAD patients and controls were genotyped for the ACE I/D polymorphism regarding to define a role of this polymorphism in occurrence of coronary artery disease. It was found that the DD genotype associates with a 2.471-fold increased risk for CAD. This is in agreement with some previous reports that described a positive association between CAD and the DD genotype (Badenhop et al., 1995; Peterlin et al., 2000; Terzić et al., 2003; Istrati et al.,

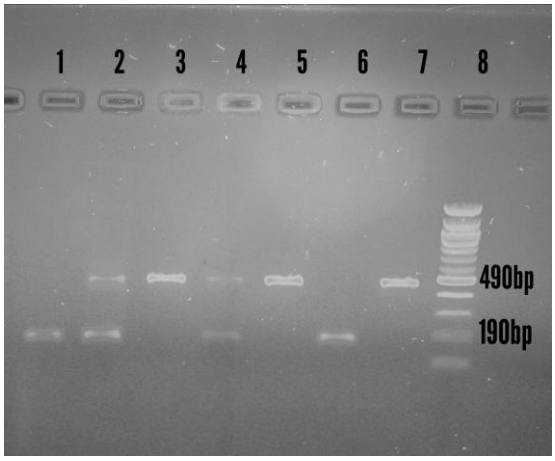

Figure 1. Agarose gel electrophoresis. Detection of the ACE I/D genotypes: DD (190bp, lane 1 and 6), ID (490/190bp, lane 2 and 4), II (490bp, lane 3, 5 and 7), DNA ladder (lane 8)

2006; Freitas et al., 2008). Several studies in Turkish population (Acarturk et al., 2005; Seckin et al., 2006; Guney et al., 2013; Sahin et al., 2015) have also described the ACE I/D polymorphism as a risk factor for CAD. Research in Poland, reported by Niemiec et al. (2007) suggested that the DD genotype/D allele increases the risk of CAD associated with the presence of traditional risk factors (smoking, hypercholesterolemia and obesity). The genotype combinations of the ACE I/D polymorphism and the 4G/5G polymorphism of the plasminogen activator inhibitor type 1 increases the risk for early onset of coronary heart disease (CHD) in German population (Leow et al., 2006). Study in Lebanon (Abchee et al., 2010) has described a protective role of the ACE I allele in individuals who may be at risk of developing CAD. The meta-analysis reported by Zintzaras et al. (2008) has demonstrated evidence of a modest positive association between ACE I/D polymorphism and coronary artery disease. However, in populations of Austria (Friedl et al., 1995), Denmark (Agerholm-Larsen et al., 1997), Spain (Hernández et al., 2002), Netherlands (Oei et al., 2005), Finland (Kähönen et al., 2007) and some other populations, authors failed to find significant correlation between the DD genotype and CAD. The studies on Iranian (Shafiee et al., 2010; Vaisi-Raygani et al., 2010; Poorgholi et al., 2013), Indian (Joseph et al., 1998; Dhar et al., 2012), Chinese (Ko et al., 1997; Deng et al., 2002; Jiang et al., 2006; Zhou et al., 2012) and Japanese population (Fujimura et al., 1997; Kondo et al., 2015) have shown contradictory results.

Table 2. Distribution of the ACE I/D genotypes among the CAD patients and controls

Genotype	CAD patients N (%)	Controls N (%)
DD genotype	47 (47.0%)	34 (34.0%)
ID genotype	37 (37.0%)	34 (34.0%)
II genotype	16 (16.0%)	32 (32.0%)

Barley et al. (1994) reported that the ACE I/D genotypes and alleles frequencies were associated with ethnic groups and suggested that ethnic origin should be considered in the studies on the association between the ACE I/D polymorphism and disease etiology. The discrepancies among studies results could also be explained in terms of different number of study subjects (Sayed-Tabatabaei et al., 2006) and different selection criteria (age, sex) (Marković et al., 2007; Hamelin et al., 2011) .

Conclusions

The results of the present study show that the DD genotype of the ACE I/D polymorphism is associated with an increased risk for coronary artery disease in Bosnian population. Since the mechanisms in the pathogenesis of coronary artery disease are very complex, we cannot exclude a possible interaction of the ACE gene polymorphism with other genes, as well as an interaction between the ACE I/D polymorphism and environmental risk factors.

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