Effects of endothelin (G8002A) gene polymorphism on serum endothelin levels in Turkish coronary artery disease patients

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Objectives: The purpose of this study, is to investigate whether endothelin-1 (END-1) polymorphisms are associated with serum endothelin levels and coronary artery disease (CAD) in Turkish population.

Methods: We investigated the effect of G8002A polymorphisms in intron 4 of the END-1 gene on serum END-1 levels in 57 coronary artery disease (CAD) individuals and 32 controls. PCR (Polymerase Chain Reaction), RFLP (Restriction Fragment Length Polymorphism), and agarose gel electrophoresis techniques were used to determine the END-1 G8002A genotypes. Serum END-1 levels were determined by enzyme-linked immunoassay.

Results: Distribution of END-1 genotypes (AA, GG, AG) in patients with CAD are: 9 (15.8%), 21 (36.8%), 27 (47.4%), and in control subjects: 1 (3.1%), 18 (56.3%), 13 (40.6%) respectively. Patients with END-1 A alleles (AA+AG) had higher serum END-1 levels than patients with GG genotypes (3.41±0.34 pg/ml vs 3.20±0.29 pg/ml, p<0.01).

Conclusion: The present study demonstrates that the END-1 A alleles may be responsible with high serum END-1 levels and predisposition of coronary artery disease in Turkish patients.

Key words: Endothelin, polymorphism, coronary artery disease


Introduction

Endothelin-1 (END-1) was implicated in the pathophysiology of various cardiovascular diseases,¹ including atherosclerosis.² Raised plasma levels of END-1 have been described in patients with coronary artery disease.³ Elevated plasma END-1 concentrations have also been demonstrated in patients with increased cardiovascular risk factors such as hypercholesterolaemia, who do not have symptomatic atherosclerosis.³ However, plasma levels in atherosclerosis are lower than those in patients with established coronary artery disease.³ Raised plasma END-1 levels have also been described in patients with hypertension, diabetes mellitus,⁶,⁷ and in cigarette smokers.⁸ In patients with established atherosclerosis, a significant correlation between plasma END-1 levels and the number of atherosclerotic lesions have been shown.³ Together with the fact that END-1 has a short half-life in the circulation,⁸ these raised plasma levels are likely to be due to increased tissue END-1 production.

Because of the role of END-1 in vascular pathophysiology, the gene coding for END-1 is an obvious

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candidate gene for coronary heart disease and hypertension. In the present study we examined whether molecular variation at the END-1 G8002A polymorphism might be involved in the predisposition to coronary heart disease and the determination of serum endothelin levels.

Materials and Methods

Subjects

The study consisted of a study group of 57 (45 male, 12 female; mean age: 61.7±8.5 year) patients with symptomatic CAD documented with coronary angiography. In all CAD patients, coronary angiography showed more than 50% stenosis in at least one major coronary vessel due to atherosclerosis. Patients were included irrespective of concomitant risk factors for atherosclerosis such as smoking, arterial hypertension, hyperlipidemia, increased body mass index, and diabetes mellitus.

Healthy persons (14 male, 18 female; mean age: 62.3±6.7) without any symptoms of cardiovascular disease were selected for inclusion in the control group. Coronary angiography was not performed on these individuals, and therefore the presence of atherosclerotic coronaries cannot be excluded. However, none of these individuals had any history of a vascular event.

Serum endothelin measurement

For the measurement of END-1 immunoreactivity blood was collected in ethylenediaminetetra-acetic acid (EDTA)-coated polystyrene tubes and centrifuged immediately. The EDTA plasma was stored at -80°C until plasma END-1 levels were determined by enzyme-linked immunoassay (Cat. No. BBE5; R&D Systems Inc. Minneapolis, America).

Isolation of DNA: Blood specimens were collected in tubes containing EDTA, and DNA was prepared from leucocyte pellet by SDS lysis ammonium acetate extraction and ethanol precipitation.

Identification of Polymorphisms of the END-1 Gene: Genotypes for END-1 isoforms were determined from leucocyte DNA by Restriction Fragment Length Polymorphism (RFLP) of amplified END-1 sequences. Genomic DNAs (0.5-1.0 mg) were amplified by using a forward primer END-1 G8002A 1: 5’-AGTAGCAGAGAGATCTATGCATCC-3’ and a reverse primer END-1 G8002A 2: 5’-CAGCATGTTCTAAATTCTACCAACCC-3’. Amplification was achieved by 30 cycles of denaturation (1 min at 94°C), annealing (1 min at 55°C), and extension (1 min at 72°C) followed by extension for 3 minute at 72°C. The amplified 748 bp PCR product was directly digested with the restriction enzyme Taq I.

Statistical analyses, using SPSS version 10.0, included the X2 test for genotype and allele frequency comparison. The significance between patient and control subjects was evaluated using the ampered Student’s t test. Statistical significance was accepted at P < 0.05.

Results

The distribution of endothelin genotypes and allele frequencies in the CAD and control groups was shown in Table 1. In patient group, END-1 AA genotype was higher than control group (15.8% vs 3.1%) (X2:3.29, P:0.069 Odds ratio: 0.172; %95 CI: 0.021-1.426 Fisher exact test p:0.088). Patients with END-1 A allele (AA+AG genotypes) was higher than control group (63.2% vs 43.8%) (x2:3.13, P: 0.077 Odds ratio: 2.20 %95 CI: 0.91-5.32).

As shown Figure 1, mean serum END-1 levels were significantly higher in CAD patients compared with the control subjects (p<0.001).

The END-1 polymorphism did have a major effect on serum END-1 levels (Figure 2). GG homozygotes had significantly lower serum END-1 levels than AG genotypes (p<0.05). Patients with END-1 A allele (A+) had significantly higher serum END-1 levels than without A allele (A-) group (p<0.01).

Discussion

We examined the effect of END-1 gene polymorphisms on serum END-1 levels in patients with CAD and healthy controls in Turkish subjects.

Table 1

<table>
<thead>
<tr>
<th>END-1 genotypes</th>
<th>Control</th>
<th>CAD</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA</td>
<td>1 (3.1%)</td>
<td>9 (15.8%)</td>
</tr>
<tr>
<td>GG</td>
<td>18 (56.3%)</td>
<td>21 (36.8%)</td>
</tr>
<tr>
<td>AG</td>
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Figure 1
END-1 levels in study groups

Figure 2
END-1 levels according to END-1 genotypes in study group
END-1 plays a role in increasing blood pressure, cell proliferation, and modulation of vasomotor tone; it also interacts with the pathophysiology of a variety of vascular diseases, such as hypertension, arteriosclerosis, and ischemic heart disease.1,2

In our study we showed that there is an increased plasma END-1 levels in patients with coronary artery disease as compared to healthy controls. Physiologically, END-1 is primarily secreted abnormally from the endothelial cell layer toward the medial layer of the vessel’s smooth muscle cells,11 indicating that endothelins predominantly exert paracrine and autocrine effects and that increased plasma END-1 levels may indicate early disturbances of endothelial function (leakage of END-1 into the circulation). These occur before complications (e.g., atherosclerotic processes) are clinically present. Due to low plasma levels of circulating END-1 and its short half-life, END-1 may be come undetectable within a few minutes after blood sample collection. Therefore, great care was taken in our study to process the blood immediately for END-1 measurement to prevent END-1 degradation in the plasma.

Plasma END-1 concentrations are known to be elevated under various pathologic conditions.12-15 We found that mean serum END-1 levels were significantly higher in CAD patients compared with the control subjects (p<0.001).

Brugada et al.6 showed that G8002A substitution located in the fourth intron of the END-1 gene may influence the median left ventricular hypertrophy score in patients with hypertrophic cardiomyopathy. Greater left ventricular mass was found in patients who carried the A allele as compared to those who did not. We found that an association between the END-1 AA genotypes and CAD in Turkish subjects. These results in agreement with the study of Brugada et al.6 However, we observe a significant correlation between plasma END-1 levels and and genotypes. We found that patients with END-1 A allele had significantly higher serum END-1 levels than without A allele group (p<0.01).

In conclusion, our results suggest that the serum END-1 levels affected by END-1 genetic variability in Turkish CAD patients and controls. END-1 AA polymorphisms and high END-1 levels were suggested as an independent risk factor for CAD.

References