



## Association Between A561C Polymorphism of E-Selectin Gene and Coronary Arterial Disease in Southeastern Iranian Population

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### ABSTRACT

**Background:** Numerous factors including genetic factors play a role in pathogenesis of coronary atherosclerosis. It has been reported that polymorphisms of genes encoding adhesion molecules are associated with atherosclerosis.

**Objectives:** The present research aimed to evaluate A561C polymorphism of the E-selectin gene in patients with coronary arterial diseases (CAD).

**Materials and Methods:** Eighty seven CAD patients and 93 age- and sex-matched control subjects were enrolled in this research. The polymorphism of A561C in the E-selectin gene was defined by polymerase chain reaction followed by restriction fragment length polymorphism (PCR-RFLP).

**Results:** The prevalence's of AA, AC and CC genotypes were 55.2%, 24.1% and 20.7% in CAD patients and 51.6%, 40.9% and 7.5% in the control subjects, respectively. The frequencies of the C allele were significantly higher in CAD patients compared with control groups ( $P < 0.05$ ). Logistic regression analysis revealed a significant association between the C allele and the risk of CAD (OR=1.61, 95%CI=1.03-2.51).

**Conclusions:** Our results displayed that presence of C allele at position 561 E-selection gene is associated with increased risk of atherosclerosis disease in the southeastern Iranian population. This polymorphism may be able to affect leukocyte-endothelial interactions, which may account for the pathogenesis of atherosclerosis.

**Keywords:** Coronary Artery Disease; E-Selectin; Polymorphism, Genetic

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This article explained association between polymorphism of E-selectin gene and coronary arterial disease. Polymorphism studies are useful in identifying genetic risk factors of diseases. Based on present study, in who have C nucleotide in position 561 of E-selectin gen, risk of CAD diseases is high and, therefore these individuals with avoid from environmental risk factors may be can reduce the risk of disease.

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## 1. Background

Coronary artery disease (CAD), also entitled coronary heart disease is a multi-factorial disease that results from the interaction between genetic and environmental risk factors such as smoking, physical inactivity, obesity, high stress, hypertension, elevated level of LDL cholesterol and diabetes mellitus (1). Atherosclerosis is the major cause of CAD, in which atherosclerotic changes are present within the walls of the coronary arteries (2). Atherosclerosis seems to be a chronic inflammatory process that is converted to an acute clinical event by the induction of plaque rupture, which in turn leads to thrombosis (3, 4). The recruitment of leukocyte into the arterial wall intima is a principal stage in the formation of atherosclerotic plaques (5, 6). The process of leukocyte recruitment is a multistep cascade that involves leukocyte adhering followed by rolling, firm adhesion, and emigration from the circulatory system to the intima (7).

The numerous adhesion molecules including members of the selectins family (P, E, and L) play a role in early stages of leukocyte recruitment during the development of atherosclerosis (8, 9). P and E-selectin are expressed in cytokine-activated endothelium and directly attach leukocytes to endothelium (10). By contrast, L-selectin and one of its ligands, P-selectin glycoprotein ligand-1 (PSGL-1), are found solely on leukocyte surface and can mediate leukocyte-leukocyte interaction (11).

The selectins are transmembrane proteins that have three domains in their extracellular segment which include: N-terminal  $\text{Ca}^{2+}$ -dependent lectin domain that is analogous to domain of C-type lectin, a single epidermal growth factor-like (EGF-like) domain and 2-9 numbers of short consensus repeats homologous to the domains found in complement binding proteins (12). In humans, E-selectin is encoded by the 13 Kbp gene containing 14 exons. The several polymorphisms have been described within E-selectin gene which affects function of encoded protein. A Single nucleotide polymorphism (SNP) in the coding region of the gene (A561C) causes replacement of serine (S) with arginine (R) at codon 128 (13).

## 2. Objectives

Since E-selectin has a major role in inflammation and atherosclerosis; we aimed to explore the association between this polymorphism with CAD in a sample from southeastern Iranian population.

## 3. Material and Methods

### 3.1. Subjects

The study population was composed of 87 patients suffering from CAD and 93 age and sex-matched control

subjects. The CAD patients were selected from subjects admitted at cardiology service of hospitals of Zahedan University of Medical Sciences. The CVD patients were defined by the presence of recognized myocardial infarction and coronary insufficiency (unstable angina with demonstrated ischemic electrocardiographic changes). The control group was selected from the Zahedan population who participated in a metabolic syndrome project and had normal blood pressure, normal triglycerides, normal blood glucose, normal body mass index, and no history of CAD or negative family history for CAD. The study was approved by local ethical committee of Zahedan University of Medical Sciences.

### 3.2. Sampling and Methods

Following 12 hours of fasting, blood samples with/without EDTA were taken from subjects. Total cholesterol, triglycerides (TG) and HDL cholesterol (HDL-C) concentrations were measured by standard enzymatic methods using commercially available kits (Pars Azmoon Co, Iran). Low-density lipoprotein cholesterol (LDL-C) was calculated with the Friedewald formula.

DNA was extracted from EDTA blood using the standard salting out method (13). Primers were designed with Fast-PCR software and are identified as ESF: 5'-GCTGATGTCTCTGTTGCACACTG3' and ESR: 5'-CCATATGACACCATCTGCACCAG3'. The region of E-selectin gene containing A561C site was amplified from genomic DNA by polymerase chain reaction (PCR). PCR was performed using commercially available PCR premix (AccuPower PCR PreMix, BIONEER, Daejeon, Korea) based on manufacturer recommended protocol. PCR mixture contains lyophilised AccuPower PCR PreMix, 2  $\mu\text{L}$  template DNA (~80 ng/ $\mu\text{L}$ ), 1  $\mu\text{L}$  of each primer (10  $\mu\text{M}$ ) in total volume 20  $\mu\text{L}$  with DNase-free water. The reaction mixture was subjected to denaturation at 95°C for 5 minutes, followed by 30 cycles at 95°C for 45 seconds, 59°C for 45 seconds, 72°C for 45 seconds, then by a final extension at 72°C for 5 minutes in a My Cycler system (Bio Rad Co, USA). PCR product was confirmed by electrophoresis in 1.5% agarose gel prestain with ethidium bromide. Subsequently, 10  $\mu\text{L}$  PCR product (323bp) was digested by 10 units Pst I and resulting fragments were separated by gel electrophoresis in a 2.5% agarose gel.

### 3.3. Statistical Analysis

Student's t-test was used for analysis of the serum lipid levels and demographic characteristics. Genotype frequencies in patient and control groups were compared by Chi-Square test. Binary logistic regression was used to compute ORs and 95% CIs. Data were analyzed using SPSS 15 software and p value less than 0.05 was considered statistically significant.

## 4. Results

The demographic characteristics of the patients and age and gender-matched controls are summarized in Table 1. There was no significant difference in smoking between two groups ( $P = 0.18$ ). Serum triglyceride, total cholesterol and LDL cholesterol were higher ( $P < 0.001$ ), while HDL cholesterol concentration was lower ( $P < 0.001$ ) in patients when compared with controls. The PCR product (323bp) following digestion yielded bands of 204 and 119bp in AA homozygote, and 323, 204, and

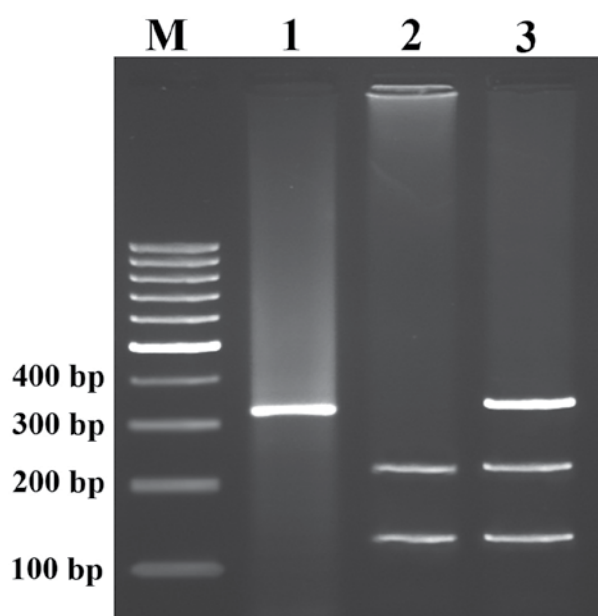
119bp in AC heterozygote. The PCR product in CC homozygote remained intact (Figure 1).

The frequency of CC genotype was statistically different between CAD patients and controls: 20.7% versus 7.5%, OR = 2.96 [95% CI = 1.36–6.44,  $P < 0.01$ ]. The C allele frequency was also significantly different between patients with CAD and controls: 32.7% versus 27.9%, OR = 1.61 (95% CI = 1.03–2.51,  $P < 0.05$ ) (Table 2). The genotypes in controls ( $\chi^2 = 0.052$ ,  $P = 0.819$ ) but not in cases were in HEW ( $\chi^2 = 17.78$ ,  $P < 0.001$ ).

**Table 1.** Characteristics of Patients and Control Subjects (Mean  $\pm$  SD)

|                                 | CAD (n = 87)     | Control (n = 93) | P-Value |
|---------------------------------|------------------|------------------|---------|
| <b>Gender</b>                   |                  |                  |         |
| Male/Female                     | 38/49            | 51/42            | 0.053   |
| <b>Smoking</b>                  |                  |                  |         |
| Yes/No                          | 14/64            | 6/54             | 0.180   |
| <b>Age, y</b>                   | 59.3 $\pm$ 12.1  | 59.4 $\pm$ 10.6  | 0.900   |
| <b>Total cholesterol, mg/dl</b> | 208.6 $\pm$ 39.0 | 171.0 $\pm$ 27.0 | < 0.001 |
| <b>LDL cholesterol, mg/dl</b>   | 130.1 $\pm$ 30.9 | 105.9 $\pm$ 31.4 | < 0.001 |
| <b>HDL cholesterol, mg/dl</b>   | 37.5 $\pm$ 5.7   | 50.9 $\pm$ 7.5   | < 0.001 |
| <b>Triglyceride, mg/dl</b>      | 214.2 $\pm$ 40.2 | 109.0 $\pm$ 37.4 | < 0.001 |

**Figure 1.** Electrophoresis Pattern of A561C Polymorphism of E-selectin Gene Using PCR-RFLP



Abbreviations: M: DNA Marker; Lane 1: CC; Lane 2: AA; Lane 3: AC

## 5. Conclusions

The various genetic factors including genetic variants

(single nucleotide polymorphisms) may play a role in pathogenesis of atherosclerosis. The SNPs replace a nucleotide with another nucleotide in gene structure. When SNPs arise within a gene or in a regulatory region near a gene, they may play a direct role in disease by affecting the gene's function (14, 15). If a SNP take place in coding regions of genes, it causes replacement of an amino acid in a protein structure with another amino acid, which results in the alteration of protein activity (16).

In the present study, we found that the frequency of AA genotype in the A561C polymorphism of the E-selectin gene was greater in CAD patients compared with control subjects. Whereas, the prevalence of and AC genotype was less in CAD patients than controls. However, these differences were not statistically significant ( $P > 0.05$ ). In contrast, the prevalence of CC genotype in control groups was significantly less than CAD patients. The C allele was significantly less common in control subjects than in CAD patients, indicating that C allele in A561C polymorphism site of E-selectin gene may be a risk factor or coronary artery disease in south-eastern Iran.

The relationship of between A561C polymorphism of E-selectin gene and CAD was evaluated in numerous studies (17-21). In contradiction with our results, Tripathi et al. reported that S128R polymorphism in E-selectin gene in Indian peoples has no relationship with CAD (17).

Moreover, in another study, it was observed that in subjects suffering from diabetes type 2 the E-selectin S128R polymorphism is not associated with CAD (18). Contrarily, Li et al. reported that the presence of allele C in A561C polymorphism was associated with CAD in a Chinese population (19). A significant association between C allele and CAD was observed in Arab patients (20). Furthermore, it was shown in a Japanese population that substitution of Ser with Arg in EGF domain of E-selectin may be a risk factor for severe atherosclerosis (21). Our results support conclusions of these two research studies.

The possible molecular mechanism that link A561C polymorphism with CAD may be the effect of substitution of Ser with Arg on selectin properties. The initial step in atherogenesis is attachment of leukocytes to endothelium that mediates by selectin molecules (22). The EGF-like domain of P- and E-selectin may play a direct role in ligand recognition and leukocyte adhesion (8, 9). The substitution of Arg instead of Ser at positions of 128 within either the E-selectin EGF domain can increase binding activity of these selectin to its ligand in leukocyte surface (23).

**Table 2.** E-Selectin Genotypes and Alleles Frequencies in CAD Patients and Control Group

| Genotypes      | CAD, No. (%) | Control, No. (%) | OR (95%CI)         | P-value |
|----------------|--------------|------------------|--------------------|---------|
| AA             | 48 (55.20)   | 48 (51.60)       | Ref.               | -       |
| AC             | 21 (24.10)   | 38 (40.90)       | 0.586 (0.30-1.147) | 0.120   |
| CC             | 18 (20.70)   | 7 (7.50)         | 2.96 (1.36-6.44)   | 0.006   |
| <b>Alleles</b> |              |                  |                    |         |
| A              | 117 (67.30)  | 134 (72.05)      | Ref.               | -       |
| C              | 57 (32.70)   | 52 (27.95)       | 1.61 (1.03-2.51)   | 0.040   |

The consequence of S128R polymorphism on binding properties of E-selectin to its ligand was also evaluated by in vitro studies. Wenzel et al. showed that S128R polymorphism decreased attachment of E-selectin expressing COS cells to HL-60 cells (24), whereas their study on German population identified S128R polymorphism as risk factor for CAD (25). An in vitro study by Rao et al. showed that substitution of Ser with Arg in S128R polymorphism significantly increased affinity and specificity of lymphocyte binding to CHO cell line (26).

In summary, in spite of small sample size, we found out a relationship between A561C polymorphism of E-selectin gene and CAD in southeastern Iranian population. Our results revealed that presence of C nucleotide in position 561 of E-selectin gene may be a genetic risk factor for CAD.

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## Authors' Contribution

Nakhaee A and Hashemi M: Designed and conducted the project, Afzali M: Participated in all parts of study, Tabatabaei SP and Tirgar Fakheri K: Participated in design of study and patients selection.

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