Int J Hum Genet, 14(3,4): 183-187 (2014)
PRINT: ISSN 0972-3757 ONLINE: 2456-6360
DOI: 10.31901/24566330.2014/14.03-4.10

Endothelin-1 Gene Polymorphism in Preoperative Myocardial Infarction with /or without Coronary Artery Bypass Graft

Sefa Senol[1], Ilker Akar[2], Kürsat Kargün[3], Ali Bayram[3], Murat Kara[4] and Özlem Secen[5]

[1]Department of Cardiovascular Surgery, Education and Resarch Hospital, Elazig, Turkey
[2]Gaziosmanpasa University, School of Medicine, Department of Cardiovascular Surgery, Tokat, Turkey
[3]Firat University, Elazig High School of Health Sciences, Elazig, Turkey
[4]Mugla Sitki Kocman University, School of Medicine, Department of Genetic Medicine, Mugla, Turkey
[5]Department of Cardiology, Education and Research Hospital, Elazig, Turkey
E-mail: [1]<ssenol2015@gmail.com>,[2]<ilker.akar@gop.edu.tr>[3], <kargun_@hotmail.com>, [3]<abayram@firat.edu.tr>, [4]<muratkara@mu.edu.tr>, [5]<ozlemsecen@hotmail.com>

KEYWORDS Endothelin-1. Gene Polymorphism. Myocardial Infarction. Coronary Artery Bypass Graft

ABSTRACT Atherosclerosis is a complex multifactorial and polygenic disorder resulting from endothelial dysfunction and excessive inflammatory response to various forms of injurious stimuli to the arterial wall. In this study, researchers aimed to investigate endothelin-1 (Lys198Asn and rs10478694) polymorphisms which are supposed to play roles in atherothrombotic process. Study group included 100 patients who had coronary artery bypass operation (CABG) without any history of a myocardial infarction with ST elevation (Group 1), and 80 patients who had a CABG operation after a myocardial infarction with ST elevation (Group 2). The control group consisted of 80 healthy people without coronary artery disease. Endothelin-1 gene polymorphisms of the subjects were determined by real time polymerase chain reaction method. Comparison of study groups, Groups 1 and 2, and control group (Group 3) did not show any statistically significant differences for endothelin-1 gene polymorphisms ($p>0.05$).The polymorphisms endothelin-1 gene which is thought to play a role in atherothrombotic process, were not supposed as risk factors in CABG patients. Studies on different polymorphisms of endothelin-1 gene may be beneficial.

INTRODUCTON

Current standards for pre-operative cardiac evaluation support the use of non-invasive cardiac tests to improve perioperative risk stratification , but predictive value remains > 2 percent (Senol et al. 2014). Due to lack of precision in individual classification, the search for novel risk factors predicting perioperative cardiovascular adverse events is important. The need for improved perioperative risk profiling is further justified by a worrisome growing surgical burden, owing to accelerated population aging and increased reliance on surgery for disease treatment. Over 40 million patients undergo surgery annually in the U.S., resulting in costs of $450 billion per year (Senol et al. 2014). With approximately one-third of surgical patients ≥65 years of age and 40% having atherosclerosis risk factors, approximately 1.25 million perioperative cardiovascular complications occur annually, resulting in an additional $25 billion in health care expenditures (Senol et al. 2014). It is projected that by 2020 the number of surgeries will increase by 25 percent, associated costs by 50 percent, and likelihood of atherosclerotic-related cardiac, cerebral, and renal complications by 100 percent (Senol et al. 2014). Evidence is accumulating that genetic variations, or polymorphisms, can significantly affect an individual's susceptibility to adverse postoperative events (Senol et al. 2014).

Atherosclerosis is a complex multifactorial and polygenic disorder resulting from endothelial dysfunction and excessive inflammatory response to various forms of injurious stimuli to the arterial wall (Garcia et al. 2005). Disruption of endothelial structure and inflammation play important roles in the onset and progression of atherosclerotic process. A complicated inflammatory and fibroproliferative response also develops against arterial intimal deposition of

Address for correspondence:
Dr. Sefa Senol
Department of Cardiovascular Surgery,
Educational and Resarch Hospital,
Elazig,Turkey
E-mail: ssenol2015@gmail.com

atherogenic lipoproteins derived from plasma (Hansson et al. 2004).

Several molecules have been associated with the vascular physiology and severity of coronary artery diseases (Topol et al. 2006). Endothelin-1 is a polypeptide composed of 21 amino acids, produced by endothelium and smooth muscle cells of vessels, and it is the most potent vasoconstrictor ever known exerting an autocrine effect (Nova et al. 1991).Endothelin 1(ET-1) is encoded by the EDN1 gene located in chromosome 6p21–24 and is a potent vasoconstrictor that acts as a modulator of vasomotor tone and vascular remodeling (Rankinen et al. 2007).

Endothelial injury causes chronic activation of intravascular coagulation system (Kobayashi et al. 1987).When characteristics of endothelin-1 such as strong and long-lasting vasoconstrictor effect, release from the region with the endothelial injury, and proliferative effects on vascular and smooth muscle cells are taken into account, it is not surprising for this compound to play an important role in vascular function disorders in the case of atherosclerosis, hypertension, and postangioplastic restenosis (Gandhi et al. 1994).

Objectives

In this paper, the patients in the study groups who did not have pre-operative myocardial infarction and had a myocardial infarction with ST elevation preoperatively, and the control group were compared for endothelin-1 polymorphisms.

METHODOLOGY

The study group included 100 patients (71 males, 29 females; mean age: 58.2 ± 10.9 years) who had a coronary artery bypass graft (CABG) operation without any history of myocardial infarction with ST elevation (Group 1), and 80 patients (61 males, 19 females; mean age: 59.6 ± 9.3 years) who had a CABG operation after a myocardial infarction with ST elevation (Group 2). The control group consisted of 80 healthy people (60 males, 20 females; mean age: 55.6 ± 10.6 years) without coronary artery disease.

All patients had complete physical examinations, and their demographic characteristics and cardiovascular risk factors were noted. The ones with a fasting glucose level ≥ 126 mg/dl, and the ones on antidiabetics or insulin treatment were regarded as diabetics. The patients with a systolic blood pressure ≥ 140 mmHg and a diastolic blood pressure ≥ 90 mmHg, and the ones on an antihypertensive treatment were considered as hypertensive patients. The blood samples were obtained from the patients for routine hematological and biochemical tests, following a 12-hour fasting period. Myocardial infarction was diagnosed in accordance with Word Health Organisation (WHO) criteria, taking symptoms, high levels of cardiac enzymes and electrocardiographic changes into account. Because of high mortality, the patients who had myocardial infarction underwent coronary bypass surgery when the cardiac enzymes became normal and the blood samples were taken before surgery. So these enzymes in all groups were normal.The control group is the composed of subjects without coronary artery disease. The patients on drugs affecting the coagulation cascade such as aspirin, clopidrogel and warfarin, the ones with a hematological disease causing hypercoagulability or bleeding diathesis, the patients with any malignancy, and the patients with a thrombocyte count below 150,000/ ml or above 400,000/ml, or the ones with an INR above 1.5 were excluded. In addition, patients with cardiomyopathy, valvular disease, renal or liver dysfunction or with any known systemic disorders were excluded. The patients were informed about the study, their informed consents were obtained, and the study protocol was approved by the university ethics committee.

2-3 ml of venous blood sample of the patients was put into tubes with EDTA, and the DNA isolations were done in Medical Genetics Laboratory using the DNA isolation kit (PureLink™ genomic DNA kits). Isolated DNAs were stored at -20 0 C until the analyses were performed. Genotyping was performed with ABI StepOne Plus Real Time Polymerase Chain Reaction (PCR) equipment.

Statistical Analysis

The statistical analyses were performed using SPSS 11.5 package program (SPSS Inc, Chicago, IL, USA). Differences in genotype distribution and consistency with Hardy–Weinberg equilibrium were tested by χ^2 test. Chi-square test was used for the categorical variables. Intergroup comparisons were done with one way variance analysis (One-Way ANOVA) test. In-

tragroup comparisons of the parameters were done using t-test and Mann-Whitney U test. The results were analyzed with a confidence interval of 95%, and $p<0.05$ was regarded as statistically significant.

RESULTS

The baseline demographic and clinical characteristics of the groups are presented in Table 1. The study group, composing of Groups 1 and 2, and the control group (Group 3) were similar for gender, age, levels of total cholesterol and LDL- cholesterol, as well as body mass index (BMI), smoking and family history ($p>0.05$). On the other hand, fasting blood glucose levels, HDL-cholesterol , triglyceride, rates of diabetes and hypertension were statistically significantly higher in the study groups (Groups 1 and 2) when compared to Group 3 ($p<0.05$). However, comparison of all genotypes with categorical variables did not show any relation between the control and the study groups ($p>0.05$). The comparison of Groups 1 and 2 for demographic and clinical characteristics did not yield statistically significant differences ($p>0.05$).

Table 2 shows genotype distributions of endothelin-1 gene polymorphisms in the study and the control groups. Table 3 shows p values for genotype distributions of endothelin-1. The allele frequency for endothelin-1 is given in Table 4. The p values for allele frequencies for endothelin-1 is given in Table 5.

Thirty-five percent of the patients in Group 1, 30 percent of the patients in Group 2, and 29 percent of the subjects in the control group were homozygous (Lys198Lys) for endothelin-1. Forty five percent of the patients in Group 1, 48 percentof the patients in Group 2, and 49 percent of the subjects in the control group were heterozygous (Lys198Asn). Mutant region (Asn198Asn) was seen in 20 percent of the patients in Group 1, 22 percent of the patients in Group 2, and 22 percent of the subjects in the control group. The comparison of the study groups and the control group for endothelin-1 (Lys198Asn) results did not show statistically significant results ($p>0.05$).

Table 1: The demographic and baseline clinical characteristics of the groups

	Group 1	*Group 2*	*Group 3*
Age (years)	58.2 ± 10.9	59.6 ± 9.3	55.6 ± 10.6
Gender (F/M)	29/71	19/61	20/60
Hypertension (%)	39	37.5	10
Fastin Glucose (mg/dl)	102.6 ± 24.6	103.8 ± 27.4	91 ± 18.6
Total cholesterol (mg/dl)	219 ± 44	198 ± 53	189 ± 42
Triglyceride (mg/dl)	238 ± 196	223 ± 162	148 ± 96
HDL cholestero (mg/dl)	37 ± 13	40 ± 11	48 ± 11
LDL cholestero (mg/dl)	134 ± 59	128 ± 54	124 ± 48
Diabetes Mellitus (%)	35	30	12.5
Body mass index (kg/m2)	28.9 ± 4.3	28.4 ± 3.2	26.9 ± 2.9
Cigarette (%)	34	32.5	25
Family history (%)	29	22.5	20

F: Female, M: Male, FBG: Fasting blood glucose, T-Cholesterol: Total cholesterol DM: Diabetes mellitus, BMI: Body Mass Index.
Group 1: MI (st elevation) Group 2: CABG Group 3: Control

Table 2: Genotype distributions of endothelin-1 gene polymorphisms

Polymorphisms	*Genotypes*	*Group 1 (n=100)*	*Group 2 (n=80)*	*Group 3 (n=80)*
Endotelin 1	Lys198Lys *n (%)*	35 (35)	24 (30)	23 (29)
Lys198Asn	Lys198Asn *n (%)*	45 (45)	38 (48)	39 (49)
	Asn198Asn *n (%)*	20 (20)	18 (22)	18 (22)
Endotelin 1*	3A/3A *n (%)*	49 (49)	37 (46)	36 (45)
rs10478694	3A/4A *n (%)*	42 (42)	33 (42)	36 (45)
	4A/4A *n (%)*	9 (9)	10 (12)	8 (10)

*A: insertion (I) /deletion (D) , 3A/3A (wild type/deletion), 3A/4A, 4A/4A (mutation/ insertion).

Table 3: P values for genotype distributions of endothelin-1

	**p1*	**p2*	**p3*
Lys198Asn	0.69	0.66	0.98
rs10478694	0.74	0.84	0.83

*p1 = Group 1/Group 2 *p2 = Group1/Group 3 *p3 = Group 2/Group3

For endothelin-1 (rs10478694), 49 percent of the patients in Group 1, 46 percent of the patients in Group 2, and 45 percent of the patients in Group 3 were 3A/3A (wild type/deletion). On the other hand, 42 percent of the patients in Group 1, 42 percent of the patients in Group 2, and 45 percent of the patients in the control group were 3A / 4A. 4A/4A (mutation/insertion) was seen in 9 percent of Group 1, 12 percent of Group 2, and 10 percent of Group 3. The differences among the study and the control groups were not statistically significant (p>0.05).

Table 5: The p values for allele frequencies

	**p1*	**p2*	**p3*
Lys/Asn Lys 198 Asn	0.41	0.40	0.91
Allel	OR:0.84	OR:1.19	OR:1.02
3A/4A rs10478694	0.52	0.61	0.90
Allel	OR:0.86	OR:1.12	OR:0.97

*p1 = Group1/Group2 *p2 = Group1/Group3 *p3 = Group2/Group3
OR= Odss Ratio

DISCUSSION

Coronary artery disease is a complex disease appearing due to environmental and genetic factors in the developed countries. World Health Organisation has estimated that annually 7 million patients, most of them living the developing countries, die due to coronary artery disease, and atherosclerosis will be the leading cause of death all over the world (Garcia et al. 2005). The candidate genes and their loci have been shown to be related with a tendency to myocardial infarction (Um et al. 2005).

Endothelin-1 dysfunction has been shown to have different effects on a number of diseases such as atherosclerosis, coronary artery disease and acute coronary syndrome (Böhm et 2007). Endothelin-1 causing smooth muscle cell proliferation, and platelet-derived growth factor were found high in hypercholesterolemic patients, with a positive correlation in between (Battistini et al. 1993). In this study, there was no correlation between endothelin-1 polymorphism and all cholesterol types studied. Experimental studies showed that ET-1 played an important role in the development of atherosclerotic plaques (Duerrschmidt et al. 2000; Böhm et al. 2007). Some authors emphasized the need for studies investigating endothelial gene modifications on atherosclerosis and coronary artery disease / myocardial infarction due to considerations on proatherogenic activity of endothelin-1 (Winkles et al. 1993; Kanaya et al. 2007)

Some studies investigated the relation of the genes coding endothelin-1 with risk of hyper-tension, atherosclerosis, coronary artery disease and myocardial infarction (Iwey et al. 2008; Palacin et al. 2009, Dzholdasbekova et al. 2010, Vargas et al. 2010). No studies in the literature have investigated endothelin-1 in patients who had preoperative myocardial infarction with ST elevation, and in the patients who had CABG without any history of preoperative myocardial infarction.

Palacin et al. (2009) did not find any significant correlation between endothelin-1 polymorphism and acute myocardial infarction in 316 patient with early onset acute myocardial infarction (<55 years of age). In this study, comparison of Group 1 and Group 2, or the comparison of Groups 1 and 2 with Group 3 did not yield significant results for endothelin-1 polymorphisms.

Dzholdasbekova et al. (2010) performed a study on 120 Kazakh patients with grade 2-3 hypertension, and found that 198Asn and As-

Table 4: Allele frequencies

	Allel	*Group 1*	*Group 2*	*Group 3*
Endotelin 1	Lys, n (%)	115 (57.5)	86 (53.8)	85 (53.1)
Lys198Asn	Asn, n (%)	85 (42.5)	74 (46.2)	75 (46.9)
Endotelin 1	3A, n (%)	140 (70)	107 (66.9)	108 (67.5)
*rs10478694	4A, n (%)	60 (30)	53 (33.1)	52 (32.5)

nAsn allele genotypes of endothelin-1 were risk identifiers for development of arterial hypertension. In this study, the researchers did not find any significant relation of hypertensive patients in both study groups and the control group with endothelin-1 polymorphisms.

Vargas et al. (2010) reported that rs1412444 and rs246833 gene polymorphisms of endothelin-1 had correlations with coronary artery disease. The researchers did not find any correlation of Lys198Asn and rs10478694 polymorphisms of endothelin-1 with Group 1 and Group 2 patients.

CONCLUSION

The polymorphisms endothelin-1 gene, a gene which is thought to play a role in atherothrombotic process, were not supposed as risk factors in CABG patients. Studies on different polymorphisms of endothelin-1 gene may be beneficial. One limitation of this paper is the small number of patients in the study groups, and a larger patient group could have increased the statistical power of this study.

REFERENCES

Battistini B, Chailler P, D'Orleans-Juste P, Briere N, Sirosis P 1993. Growth regulatory properties of endothelins. *Peptides*, 14: 385-399.

Böhm F, Pernow J 2007. The importance of endothelin-1 for vascular dysfunction in cardiovascular disease. *Cardiovasc Res:* 76: 8–18.

Duerrschmidt N, Wippich N, Goettsch W, Broemme H.J, Morawietz H 2000. Endothelin-1 induces NAD(P)H oxidase in human endothelial cells. *Biochem Biophys Res Commun*, 269: 713–717.

Dzholdasbekova AU, Gaipov AE 2010. The association between polymorphism of Lys198Asn of Endothelin-1 gene and arterial hypertension risk in kazakh people. *Eu J Gen Med*, 7: 187-191.

Gandhi CR, Berkowitz DE, Watkins WD 1994. Endothelins. *Anesthesiology*, 80: 892-905.

Garcia-Moll 2005. Inflammatory and anti-inflammatory markers in acute coronary. syndromes. Ready for use in the clinical setting? *Rev Esp Cardiol*, 58: 615–617.

Hansson GK, Nilsson J 2003. Pathogenesis of atherosclerosis. MH Crawford, JP DiMarco (Eds.): *Cardiology.* 1st Edition. England: Mosby International Ltd.

Ivey M.E, Osman N, Little PJ 2008.Endothelin 1 signalling in vascular smooth muscle: Pathways controlling cellular functions associatedwith atherosclerosis. *Atherosclerosis*, 199: 237-247.

Kanaya AM, Barrett-Connor E, Wassel Fyr CL 2007. Endothelin-1 and prevalent coronary heart disease in older men and women (the Rancho Bernardo Study). *Am J Cardiol*, 99(4): 486-490.

Kobayashi T, Terao T 1987. Preeclampsia as chronic disseminated intravascular coagulation. *Gynecol Obstet Invest*, 24: 170-178.

Nova A, Sibai BM, Barton JR, Mercer BM, Mitchell MD. 1991. Maternal plasma levels of endothelin. *Am J Obstet Gynecol*, 165: 724-727.

Palacín M, Rodriguez-Pascual F, Reguero JR, Rodríguez I, Avanzas P et al. 2009. Lack of association between endothelin-1 gene variants myocardial infarction. *J Atheroscler Thromb*,16: 388–395.

Rankinen, T. Rankinen T, Church T, Rice T, Markward N, Leon AS et al. 2007. Effect of endothelin 1genotype on blood pressure is dependent on physical activity or fitness levels. *Hypertension*, 50: 1120–1125.

Senol S, Es MU, Gokmen G, Ercin O, Tuylu BA, Kargun K 2014. Genetic polymorphisms in preoperative myocardial infarction. *Cardiovasc Thorac Ann*, Aug 13. pii: 0218492314547724 (In Press).

Topol, EJ, Smith J, Plow EF, Wang QK 2006. Genetic susceptibility to myocardial infarction and coronary artery diseases. *Hum Mol Genet*,15: R117–R123.

Um JY, Kim HM 2004.Tumor necrosis factor alpha gene polymorphism is associated with cerebral infarction. *Brain Res Mol Brain Res*, 122: 99–102.

Vargas-Alarcon G, Vallejo M, Posadas-Romero C, Juarez-Rojas JG, Martinez-Rios MA et al. 2013. The-974C>A (rs3087459) gene polymorphism in the endothelin gene (EDN1) is associated with risk of developing acute coronary syndrome in Mexican patients. *Gene*, 542(2): 258-262.

Winkles JA, Alberts GF, Brogi E, Libby P 1993. Endothelin-1 and endothelin receptor mRNA expression in normal and atherosclerotic human arteries. *Biochem Biophys Res Commun*, 191(3): 1081-1088.