

The association of ICAM E469K with cardiovascular characteristics of acromegaly

E. OGUZ¹, S. TABUR², H. AKBAS³, I.V. DUZEN⁴, H. KARABAG⁵,
F. AKKAFA³, M. OZKAYA², B. ALASEHIRLI⁶

¹Department of Medical Pharmacology, Faculty of Medicine, Harran University, Sanliurfa, Turkey

²Division of Endocrinology, Department of Internal Medicine, Faculty of Medicine, Gaziantep University, Gaziantep, Turkey

³Department of Medical Biology, Faculty of Medicine, Harran University, Sanliurfa, Turkey

⁴Department of Cardiology, 25 Aralik State Hospital, Gaziantep, Turkey

⁵Department of Neurosurgery, Faculty of Medicine, Harran University, Sanliurfa, Turkey

⁶Department of Medical Pharmacology, Faculty of Medicine, Gaziantep University, Gaziantep, Turkey

Abstract. – OBJECTIVE: It has been shown that genetic factors have a role in the development of acromegaly. We aimed to investigate the association between intercellular adhesion molecule (ICAM)-1 E469K polymorphism and some cardiovascular clinical parameters of acromegalic patients.

PATIENTS AND METHODS: We included 41 patients with acromegaly and 65 healthy subjects with similar age and sex to the study. Fasting plasma glucose (FPG), high-density lipoprotein-cholesterol (HDL-C), low-density lipoprotein-cholesterol (LDL-C), triglyceride (TG) were analyzed. Genotyping was made by polymerase chain reaction-restriction fragment length polymorphism.

RESULTS: The frequency of genotype and allele ICAM-1 E469K was not significantly different between control and patients ($p > 0.05$). Systolic blood pressure (SBP), diastolic blood pressure (DBP) and FPG levels were significantly higher, and HDL-C was significantly lower in patients with KK genotypes compared to patients with EE genotype in acromegaly group ($p < 0.05$).

CONCLUSIONS: This is the first study to investigate the role of ICAM gene polymorphism in acromegaly and its cardiovascular characteristics. ICAM E469K may not be a risk factor for the acromegaly in Turkish population but may be associated with hypertension, higher FPG and lower HDL-C in acromegalic patients.

Key Words:

Acromegaly, Cardiovascular, Intercellular adhesion molecule, Polymorphism.

growth factor-1 (IGF-1), which causes most of the clinical manifestations of acromegaly¹. Genetic factors are involved in the development and comorbidities of acromegaly^{2,3}.

The prevalence of acromegaly was estimated 30 to 70 individuals per million in Europe, demonstrating that it is a rare disease^{4,6}. Cardiovascular complications are the most relevant causes of the increased risk of mortality in acromegaly. It is unclear whether this is related to increased conventional risk factors or a result of the direct effect of GH excess on cardiovascular function⁷. Hypertension (HT) is one the most cardiovascular risk factors in acromegaly, occurring in more than 40% of patients. There is also an increase likelihood of developing insulin resistance, diabetes mellitus (DM) and hyperlipidemia (HL) in this disease^{7,8}.

The increased levels of serum tumor necrosis factor (TNF) and interleukin (IL)-8 levels reveal the role of inflammation in the pathogenesis of acromegaly⁹. Also, a recent study¹⁰ concerning the evaluation of endothelial adhesion molecules in these patients indicated that intercellular adhesion molecule (ICAM)-1 and vascular cell adhesion molecule (VCAM)-1 are increased in both active and remission acromegaly groups as compared to the control group.

ICAM-1 is a member of the immunoglobulin superfamily. ICAM-1 has an important role in both innate and adaptive immune responses since it is involved both in the migration of leukocytes to sites of inflammation and interactions between antigen-presenting cells and T cells¹¹.

ICAM-1 gene is located in 19p13.3–p13.2, and includes 7 exons and 6 introns. ICAM-1

Introduction

Acromegaly results from persistent hypersecretion of growth hormone (GH). Excess GH stimulates hepatic secretion of insulin-like

E469K gene polymorphism is located in the ICAM-1 gene coding region. This gene polymorphism might influence the serum level and activity of ICAM-1¹². ICAM-1 E469K polymorphism is known to be common in all populations, and the association of this polymorphism has been shown with several inflammatory diseases¹³⁻¹⁵.

To the best of our knowledge, there is not any study investigating the association between polymorphisms of ICAM-1 and acromegaly. The aim of this study was to investigate the frequency of ICAM-1 E469K polymorphism in acromegaly and the relation of this polymorphism with cardiovascular clinical parameters of acromegaly.

Patients and Methods

Forty-one acromegalic patients who were in the follow-up of the Endocrinology Clinic enrolled in the study. Patients were evaluated according to the clinical parameters, comorbidity status and the treatments received before and currently. The surgical intervention, histopathological examination, pituitary imaging and radiotherapy applied to the patients previously were recorded. The activity of the disease determined according to Cortina criteria¹⁶.

The control group included 65 (32 female, 33 male) healthy age and gender matched subject. Both control and patients groups were chosen from the same geographical area, the South East Region of Turkey. The study was approved by the Ethics Committee of Gaziantep University and written informed consent was taken from patients and healthy volunteers after the procedures were explained.

Blood samples were collected in the morning after an 8-hour fasting period. Fasting plasma glucose (FPG), high-density lipoprotein-cholesterol (HDL-C), low-density lipoprotein-cholesterol (LDL-C), triglycerides (TG) of all subjects were analysed.

IGF-1 levels were measured using chemiluminescent immunometric assay (Immulite 2000, Siemens, Munich, Germany).

Genetic Analysis

Heparinized peripheral venous blood (2 ml), collected from both controls and patients, were stored at -20°C until the extraction of the DNA. Genomic DNA extraction was performed using

GeneJETTM whole blood genomic DNA purification kit (Thermo Fisher Scientific, St. Leon-Rot, Germany) according to manufacturer's instructions.

For determining the ICAM-1 gene E469K (1462A > G; rs5498) polymorphism, polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method was used with appropriate primer sets and restriction enzyme as previously described¹⁷. The PCR reaction was carried out in a 20 μl reaction volume containing 1 \times PCR buffer, 2 mM MgCl_2 , 0.2 mM each deoxynucleotide triphosphate (dNTPs; Fermentas, St. Leon-Rot, Germany), 80 ng of DNA, 0.2 μM of each primer (Bio Basic Inc., Markham, ON, Canada), and 1 unit of Taq DNA Polymerase (Fermentas). The PCR conditions were: 3 minutes of initial denaturation at 94°C , followed by 30 amplification cycles. Each cycle consisting of denaturation at 94°C for 30 seconds, 56°C or 58°C annealing for 30 seconds (for annealing of ICAM-1 E469K) and extension at 72°C for 30 second, with a final extension step of incubation at 72°C for 5 min.

Genotyping of ICAM-1 Gene E469K Polymorphism

RFLP analysis was carried out by PCR-amplified products followed by BstUI restriction enzyme digestion at 37°C overnight for genotyping of ICAM-1 E469K (1462A > G; rs5498). The digested products were separated by 3% agarose gel along with a 100 to 1,500 bp DNA ladder (BioBasic Inc., Markham, ON, Canada) and stained with ethidium bromide. AlphaImager Imaging System (AlphaInnotech[®], San Leandro, CA, USA) was used to analyze ethidium bromide-stained gels. The fragment length of the KK, EE and KE genotypes were 223 bp; 136 and 87 bp respectively (Figure 1).

Statistical Analysis

Continues variables were expressed as means \pm standard deviation (SD). Independent sample *t*-test were used to analyse differences in continuous variables between control and acromegalic patients. Kruskal-Wallis test was used to analyze differences in continues variables among three genotypes. Mann-Withney U test was used to analyze differences in continues variables between two genotypes. The Chi-squared test was used to analyze the differences in frequency of genotype and alleles of the E-selectin and ICAM. *p* values

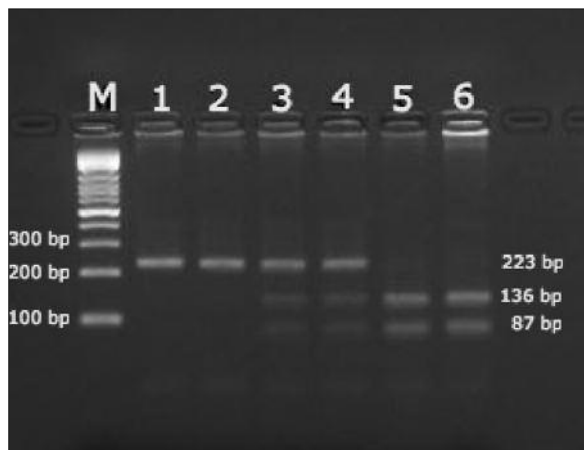


Figure 1. 2% Agarose gel electrophoresis stained with ethidium bromide showing the PCR-RFLP analysis of the ICAM-1. Lanes M: DNA marker. Lanes 1, 2: AA allelic polymorphism; Lane 3, 4: AG allelic polymorphism; Lane 5, 6: GG allelic polymorphism.

< 0.05 were accepted as statistically significant. Deviation from Hardy-Weinberg equilibrium (HWE) for genotypes was analyzed. Statistical analyses were performed using SPSS software, version 11.5 for Windows (SPSS Inc., Chicago, IL, USA). The results were expressed as mean SD if the variables were continuous and as percentage if the variables were categorical.

Results

The mean age and gender distribution of patients and controls were similar ($p > 0.05$). Instead of 6 patients the tumor size was over 1 cm (macroadenoma) in the acromegalic group.

Transsphenoidal pituitary surgery was performed to 33 patients while 8 of them received primary medical treatment.

The clinical parameters of both acromegaly and control group are shown in Table I. Blood pressure and biochemical measurements of patients were significantly different in acromegalic patients compared to controls (Table I).

The genotype frequencies of ICAM-1 K469E polymorphism was assessed in Hardy-Weinberg equilibrium in both for the patients and healthy controls. Genotypes for ICAM E469K were expressed as EE for homozygote normal, EK for heterozygote and KK for homozygote polymorphic. The distribution of the ICAM E469K genotype was as follows: 11 (26.8%) patients had EE, 19 (46.4%) had EK and 11 (26.8) had KK genotype in acromegalic patients and 22 (33.9%) had EE, 32 (49.2%) had EK and 11 (16.9%) had KK genotype in control group (Table II). The homozygote KK was two folds higher in patients with acromegaly compared to control. But no statistically significant difference was found among three genotypes between acromegaly patients and healthy control subjects. Allel frequency was also not different regarding E and K allel between control subjects and acromegalic patients.

The comparisons of cardiovascular clinical characteristics among the genotypes in acromegalic patients were shown in Table III.

Systolic blood pressure (SBP), diastolic blood pressure (DBP), FPG and HDL-C showed significant difference among the three ICAM E469K E/K genotypes, EE, EK and KK in acromegalic patients ($p < 0.05$). SBP, DBP and FPG levels were significantly higher and HDL-C was significantly lower in patients with KK genotypes compared to patients with EE genotype in acromegaly group (Table III).

Table I. Demographic and laboratory parameters of acromegalic patients and controls.

	Acromegaly	Healthy control	<i>p</i>
Age	46.56 ± 13.05	43.84 ± 102.54	0.127
Gender (F/M)	22/19	32/33	0.776
FPG (mg/dl)	131.41 ± 62.59	93.50 ± 9.00	< 0.001
SBP (mmHg)	131.95 ± 11.22	118.75 ± 6.65	< 0.01
DBP (mmHg)	82.07 ± 7.49	71.62 ± 9.17	< 0.01
HDL-C (mg/dl)	34.19.00 ± 7.79	47.50 ± 17.00	< 0.001
LDL-C (mg/dl)	133.00 ± 34.66	117 ± 40.00	< 0.001
TG (mg/dl)	148.00 ± 44.55	101.00 ± 48.00	< 0.001
IGF-1	461.95 ± 313.82	209 ± 59.62	< 0.001

DBP: diastolic blood pressure; FPG: fasting blood glucose; HDL-C: high density lipoprotein cholesterol; LDL-C: low density lipoprotein cholesterol, TG: triglycerides; GH: growth hormone; IGF-1: insulin-like growth factor 1.

Table II. Genotype and allele frequencies of ICAM-1 E/K polymorphism in patient and control groups.

ICAM-1 (E/K)	Patients n = 41 (%)	Healthy controls* n = 65	OR (CI 95%)	p
Genotypes				
EE	11 (26.8)	22 (33.9)	1	
EK	19 (46.4)	32 (49.2)	1.18 (0.47-2.97)	0.71
KK	11 (26.8)	11 (16.9)	1.57 (0.66-6.04)	0.21
Alleles				
E	41 (50.0)	76 (58.5)		
K	41 (50.0)	54 (41.5)	1.4 (0.80-2.45)	0.22

Discussion

The increase in proinflammatory cytokines such as TNF, IL-8 and soluble ICAM-1 (sICAM-1) shows the role of inflammation in acromegaly^{9,10}. In this study, we aimed to investigate the distribution of ICAM-1 E469K gene polymorphism in acromegaly patients and the association with cardiovascular characteristics of the disease.

We identified a non-synonymous SNP ICAM-1 E469K (rs5498) in patients with acromegaly and control subjects in a Turkish population. The heterozygous index was high for rs5498 both in patients and controls similar to the other studies^{18,19}. To the best of our knowledge, this is the first study investigating the association of the ICAM-1 gene polymorphism in acromegalic patients. We found that KK genotype was two times higher in patients with acromegaly compared to controls whereas it was not significant ($p > 0.05$). We think that this situation is due to the limited number of acromegalic patients.

ICAM-1 belongs to the superfamily of immunoglobulins, composed of 507 amino acids.

Its coding gene is located in p13.3-p13.2 of human chromosome 19 with the total length of 15.5kb, including 7 exons and 6 introns. The sixth exon has a common polymorphism (E/K), influencing 469th codon and changes glutamic acid to lysine. This substitution could influence ligand binding and may affect the plasma concentration and activity of ICAM-1²⁰⁻²³. Genome-wide association analyses indicate that ICAM-1 K469E polymorphism had a role in the genetic regulation of sICAM-1 levels²².

Acromegalic patients carry increased risks of insulin resistance, DM, HT and HL due to the effects of increased GH⁷.

In the present study, the cardiovascular risk factors such as SBP, DBP, HDL-C, LDL-C, triglycerides and FPG were found significantly higher and HDL-C levels were significantly lower in acromegalic patients compared to controls. We investigated the association of ICAM-1 E469K polymorphism with these cardiovascular risk factors in acromegalic patients. We found that that SBP, DBP and FPG levels were significantly higher and HDL-C levels were significantly lower in acromegalic patients with

Table III. The comparisons of cardiovascular risk characteristics among the genotypes of ICAM E469K in acromegalic patients.

Genotypes	EE	EK	KK
SBP (mmHg)	127.27 ± 10.57* ^a	129.44 ± 7.64	141.81 ± 11.67
DBP (mmHg)	77.72 ± 6.84* ^a	80.55 ± 4.16	89 ± 7.56
FPG (mg/dl)	102.63 ± 41.55* ^a	118.94 ± 35.04	181.72 ± 88.00
Triglyceride (mg/dl)	126.45 ± 39.40	152.83 ± 49.48	163.54 ± 37.27
LDL-C (mg/dl)	126.45 ± 30.12	136.83 ± 35.18	132.63 ± 41.44
HDL-C (mg/dl)	49.18 ± 10.02* ^a	42.05 ± 9.40	34.72 ± 6.01

*Shows significance among three ICAM E469K A/G genotypes (Kruskal-Wallis test). ^aShows significance between EE and KK genotypes (Mann-Whitney U test). Data are mean ± SD. DBP: diastolic blood pressure; FPG: fasting blood glucose; HDL-C: high density lipoprotein cholesterol; LDL-C: low density lipoprotein cholesterol; TG: triglycerides.

KK genotype ($p < 0.05$). These data suggest that ICAM E469K polymorphism may be a risk factor for the development of HT, FPG and low level of HDL-C in acromegalic patients. The studies concerning ICAM E469K polymorphism in cardiovascular diseases also supports our data. Liu et al²⁶ indicated that K469E polymorphism of ICAM-1 might contribute to the recurrence of acute coronary syndrome (ACS) and cardiovascular death by affecting HT and HDL-C similar to our data. Jiang et al¹³ have found that ICAM E469K polymorphism is associated with coronary heart disease and myocardial infarction. It was suggested that K allele of the ICAM-1 E469K gene might predispose coronary artery disease (CAD) susceptibility²³ and it was reported that individuals who carry the KK or KE genotype might have an 80% increased CAD risk compared with EE genotype carriers²⁴. ICAM E469K polymorphism was indicated as a risk factor for peripheral arterial occlusive disease²⁵.

Inflammation plays a key role in the initiation, progression, and development of HT, atherosclerosis since endothelium dysfunction is related with inflammatory process^{26,27}. ICAM-1 and other inflammatory factors such as VCAM-1, TNF, IL-6, and C-reactive protein have key roles in mediating vascular inflammation²⁸. ICAM-1 directly contributes to inflammatory responses within the blood vessel wall by increasing endothelial cell activation and augmenting atherosclerotic plaque formation¹¹. ICAM-1 expression can be upregulated by several cytokines involved in HT, such as C-reactive protein, angiotensin II and oxygen free radicals²⁹. Some clinical studies^{30,31} have found that sICAM-1 is increased in the plasma of hypertensive patients. sICAM-1 levels are correlated with HT³²⁻³⁵, low HDL-cholesterol³⁶ and hypercholesterolemia and/or hypertriglyceridemia³⁷. A recent study³⁸ showed that ICAM-1 level was correlated with systolic and mean pulmonary artery pressures and it was suggested that ICAM-1 might have potential use as a biomarker for the diagnosis and follow-up of pulmonary artery HT. Hyperglycaemia may play a major role in the onset and development of atherosclerotic disease, which causes arterial wall dysfunction, haematological disturbances and lipid abnormalities. Hyperglycaemia may induce damage to the endothelium through increased levels of pro-adhesion proteins such as ICAM-1³⁹.

Conclusions

This study reveals that ICAM-1 E469K polymorphism is not involved in the susceptibility of acromegaly but may be associated with FPG, SBP, DBP and HDL-C, the cardiovascular risk factors of acromegaly. We think that more comprehensive studies in larger study sample are required to explain the role of ICAM E469K polymorphism in acromegaly. Additionally, the studies in larger patient groups, preferably involving multiple ethnicity and multicenter studies are needed to reveal the association of ICAM E469K polymorphism and the cardiovascular risk factors of acromegaly.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

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