ICAM-1 E469K and E-Selectin S128R Polymorphisms with Non-Diabetic Metabolic Syndrome

Non-Diabetik Metabolik Sendromda İCAM-1 E469K ve E-Selektin S128R Polimorfizmleri

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Abstract

Backgrounds: Metabolic syndrome (MetS) is a cluster of abdominal obesity linked to an excess of visceral fat, insülin resistance, dyslipidemia and hypertension. Inflammation biomarkers and endothelial dysfunction is associated with metabolic syndrome (MetS) and inflammatory condition. Recent studies suggest that genetic variation in inflammatory genes plays a pivotal role in MetS. We aimed to investigate the polymorphisms of two inflammatory genes, ICAM-1 and E-selectin with non-diabetic MetS in Turkish population.

Methods: The study included 132 patients with non-diabetic MetS. and 118 control subjects. Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method were used to determine the E selectin S128R (561A>C;rs5361) and ICAM-1 E469K (1462A>G;rs5498) polymorphisms.

Results: There was no change in genotype and allele frequencies in both ICAM-1 E469K and E selectin S128R polymorphisms compared to control subjects.

Conclusions: The realtionship between ICAM-1 and E-selectin polymorphisms and non-diabetic MetS was investigated firstly examined in this study. The data of this study suggest that ICAM-1 E469K and E-selectın S128R polymorphisms are not in susceptibility to non-diabetic MetS in the Turkish population.

Key words: Metabolic syndrome, ICAM-1, E-Selectin, gene polymorphism, Polymerase Chain Reaction

Öz

Amaç: Metabolik sendrom (MetS), abdominal obezite ile ilişkili viseral yağ, insulin direnci, dislipidemi ve hipertansiyon gibi sistemik bozuklukların bir grubudur. İnflamasyon biyomarkırları ile endotelyal disfonksiyon, metabolik sendrom ve inflamatuar şartlarla ilişkilendirilmiştir. Son çalışmalar inflamatuar genlerdeki genetik varyasyonların metabolik sendromda kilit rol oynadığını ileri sürmektedirler. Bu çalışmada non-diabetik MetS li hastalarda, inflamatuar genlerden İCAM-1 ve E-selektin gen polimorfizmlerinin araştırılması amaçlanmıştır.

Metod: Çalışmaya non-diabetik MetS'li 132 hasta ve 118 kontrol örneği dahil edilmiştir. İCAM-1 E469K (1462A>G ;rs5498) ve E-selektin S128R (561A>C;rs5361) gen polimorfizmlerini belirlemek için polimeraz zincir reaksiyonu- restriksiyon parça uzunluk polimorfizmi (PCR-RFLP) tekniği uygulanmıştır.

Bulgular: Kontrol grubuna kıyasla, non-diabetik MetS'li hastalarda Hem İCAM-1 E469K hemde E selektin S128R polimorfizmlerinin genotip dağılımı ve allel frekanslarında herhangi bir değişiklik saptanmamıştır.
Sonuç: Bu çalışma, non-diabetik MetS'lu hastalarda İCAM-1 E469K ve E selektin S128R polimorfizmlerinin araştırıldığı ilk çalışmadır. Bu çalışmadan elde edilen veriler, İCAM-1 E469K ve E selektin S128R gen polimorfizmlerinin Türk populasyonundaki non-diabetik MetS ile ilişkili olmadığını göstermiştir.

Anahtar Kelimeler: Metabolik sendrom, İCAM-1, E-Selektin, gen polimorfizmi, Polimeraz zincir reaksiyonu.

Introduction

Metabolic syndrome (MetS) is a complex syndrome with clustering of multiple cardiovascular risk factors including central obesity, atherogenic dyslipidemia, hyperglycemia (1). Recently, important increase have been reported in the prevalence of MetS(2) especially in all western society and in Asia, where obesity is epidemic (3,4). In general, it has been estimated that approximately 10%-30% of the world's adult population has the MetS (5). At the molecular level, MetS is accompanied by dysregulation in the expression of adipokines (cytokinesa nd chemokines) and is associated with activation of pro-inflammatory cytokines making metabolic syndrome an inflammatory condition (6,7). Patients with MetS and acute ischemic stroke are with a higher degree of immune-inflammatory markers compared to stroke controls without metabolic syndrome (8,9) reported that serum cellular adhesion molecule (sCAM)s are one of the reasonable markers for early metabolic abnormalities and endothelial activation leading to the MetS and atherosclerosis. Genetic susceptibility and environmental factors are involved in the progression of MetS (10). Genome-wide association studies (GWAS) have been applied in the search of gene variants for the MetS and several loci having pleiotropic effects on multiple MetS-related traits have been reported for the individual components of the MetS. Several single nucleotid polymorphisms (SNPs) have been shown to be associated with body mass index (BMI), other measures of obesity or fat distribution and metabolic syndrome (11,12).

Recent studies suggest that genetic variation in inflammatory genes plays a pivotal role in MetS. It was shown that ICAM-1 gene rs5491 (13) nd TNF-α gene rs1800629 (14) associated with MetS. Hovewer, the association of ICAM-1 E469K (rs5498) and E- selectin S128R (rs5361) polymorphisms with non-diabetic MetS has not been studied yet. In this study, we aimed to investigate the role of these two polymorphisms in non-diabetic MetS in Turkish population.

Materials And Methods

One hundred and thirty two non-diabetic MetS patients and 118 age matched control subject without MetS enrolled in the study. A standard 75 g oral glucose tolerance test (OGTT) was administered to all participants, and non -diabetic subjects included to the study according to their affected glucose metabolism.

The diagnosis of the MetS was done by clinicians according to the the NationalCholesterol Education Program (NCEP) Adult Treatment Panel (ATP) III criteria which is an acceptable and well-recognized criterion for MetS diagnosis (15, 16)

MetS is defined according to the criteria accepted in the Third Report of the National Cholesterol Education Program (NCEP) (15,16)

A metabolic syndrome diagnosis was made when a subject fulfilled three of the following five criteria: WC ≥102 cm in men and ≥88 cm in women,

triacylglycerol ≥150 mg/dL or treatment of dyslipidemia, HDL cholesterol <40 mg/dL in men and <50 mg/dL in women or treatment of dyslipidemia, systolic/diastolic BP ≥130/85 mm Hg or antihypertensive treatment, and fasting blood glucose ≥100 mg/dL.

Control subjects were sex and age-matched, healthy and had no symptoms of both MetS and diabetes. Presence of coronary artery disease, peripheral occlusive arterial disease, coagulopathy, vasculitis, autoimmune disease, severe kidneyand hepatic diseases, cancer, pregnancy and diabetes were exclusion criteria for control subjects.Age, weight, height, body mass index (BMI:body weight (kg)/height (cm)2), and systolic (SBP) and diastolic blood pressures (DBP) of all subjects were recorded.

The study was approved by the local ethics committee, and all participants gave signed informed consent.

Biochemical analysis

The venous blood samples of each subject after ≥8 h or overnight fasting samples were stored at -80°C until biochemical assay byblinded investigators. All routine chemistry was conducted by the standard laboratory techniques in the Clinical Biochemistry laboratory.

Genetic Analysis

Heparinised peripheral venous blood (2ml) was collected from each subject and stored at -20°C until the extraction of the DNA. Genomic DNA extraction was performed using GeneJET™ whool blood genomic DNA purification kit (Thermo Scientific, St. Leon-Rot, Germany) according to our previous studies (17).

Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method were used to determine the E selectin gene 561A>C (S128R;rs5361) and ICAM-1 gene 1462A>G (E469K;rs5498) polymorphisms with appropriate primer sets and restriction enzyme as previously described (18)The primer sets and enzymes used in this study is shown in table 2.

The PCR reaction was carried out in a 20 µl reaction volume containing 1×PCR buffer, 2 mMMgCl2, 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°Cannealing for 30 second ,(for annealing of E selectin S128R and ICAM-1 E469K respectively) and extension at 72°C for 30 second, with a final extensionstep of incubation at 72°C for 5min.

Genotyping of E-selectin gene S128R polymorphism

For Genotyping of E-selectin gene 561A>C (**S128R)** polymorphism, RFLP analysis was carried out by PCR-amplified products followed by *PstI* restriction enzyme digestion at 37 °Covernight (18). The digested products were separated by 2% agarose gel along with a 100 to 1,500 bp DNA ladder (BioBasic Inc.) and stained with ethidium bromide. Ethidium bromide-stained gels analyzed using the AlphaImager Imaging System (AlphaInnotech, San Leandro, California, USA). The homozygous polymorphic RR genotype yielded one fragment of 357 bp, the homozygous wild SS genotype yielded digested two fragments of 219, and 138 bp, and the heterozygous SR genotype yielded 357, 219, and 138 bp (Fig. 1).

Genotyping of ICAM-1 gene E469K polymorphism

For Genotyping of ICAM-1 gene 1462A>G (E469K) polymorphism, RFLP analysis was carried out by PCR-amplified products followed by BstUI restriction enzyme digestion at 37°C overnight (18).

The digested products were separated by 3% agarose gel along with a 100 to 1,500 bp DNA ladder (BioBasic Inc.) and stained with ethidium bromide. Ethidium bromide-stained gels analyzed using the AlphaImager Imaging System (AlphaInnotech, San Leandro, California, USA). The homozygous EE genotype yielded one fragment of 223 bp, the polymorphic KK genotype yielded digested two fragments of 136 and 87 bp, and the heterozygous EK genotype yielded three fragment of 357, 219, and 138 bp (Fig. 2).

Statistical analysis

The differences in frequency of genotype and allels of the E-selectin and ICAM, were analyzed by the chi-squared test. Deviation from Hardy-Weinberg equilibrium (HWE) for genotypes was analysed. Statistical analyses were performed using SPSS software (version 11.5 for Windows, SPSS Inc., Chicago, IL, USA). Fisher's exact test was used to analysis genotype and allele frequencies of the polymorphisms. Statistical significance was defined as $p \leq 0.05$ and all statistical tests were two-sided. The results were expressed as mean SD if the variables were continuous and as percentage if the variables were categorical.

Results

The genotype frequencies of both ICAM-1 E469K and E-selectin S128R polymorphisms in control group were consistent with Hardy-Weinberg equilibriumin. The genotype frequencies were not significantly different ($P > 0.05$) in ICAM-1 E469K polymorphism between the groups of patients with non-diabetic Mets and controls. Heterozigous EK genotype was 62,1% in non-diabetic Mets and 44,9% in control group. Polymorphic KK genptype was 9.1 % in non-diabetic MetS and 20.3 % in control group. There was also no significant difference in allele frequencies of this polymorphism ($P > 0.05$). K allele was 40% in patients and 42.4 % in healthy controls. The distrubition of the genotypes and allele frequencies of ICAM-1 E469K are listed in Table 3.

There was no statistically significant difference in both genotype and allele frequency for E-selectin S128R polymorphism between patients with non-diabetic MetS and control subjects ($P>0.05$). The polymorphic homozygous RR genotype was 1.5% in patients and 0.84% in control group. The heterozigous SR genotypes were 27.3 % in patients and 23.9% in control group. The R allele was 15.2 % in patients and 12.7 % in control subjects. The distributions of genotype and allele frequencies of ICAM-1 E469K are presented in Table 4.

Discussion

The non-synonymous SNPs ICAM-1E469K (rs5498) and E-selectin S128R (rs5361) have been identified in patients with non-diabetic MetS and control subjects in a Turkish population in this study. This is the first study to investigate the association of two inflammatory genes, ICAM-1 and E-selectin with the risk of developing non-diabetic MetS to the best of our knowledge. We did not find any association between these polimorphisms and the disease.

In the present study polymorphic RR genotype frequency was very less, as seen only two patients of 132 and one control subject of 118 had RR genotype of E-selectin S128R similar to other studies (19,20) and in our previous study (unpublished data). Additionally, the heterozygous genotype was high for ICAM-1 E469K similar to that reported in earlier studies (21,18) and also in our previous study (unpublished data).

ICAM-1 E469K, a non-synonymous SNP of ICAM, resides in the fifth immunoglobulin-like domain, is essential for the structure and function of ICAM-1 (22). This polymorphism is common in all populations and is involved in several inflammatory diseases (23). It has been shown that ICAM-1 E469K influence the binding of ICAM-1 on endothelial cells and leukocyte function associated antigen-1 (LFA-1)

and macrophage adhesion ligand-1(Mac-1) on leukocytes (22).

In this study, we found that ICAM-1 E469K (rs5361) is not associated with the increased risk of development of non-diabetic MetS in Turkish population. Recently, Hsu et al. found that ICAM-1 rs5491 which another functional variant of ICAM-1 is associated with MetS in Taiwan population differently from our data. But their study group is different since we included the patiens with non-diabetic MetS and also they studied a different SNP of ICAM from us (13). The results of the studies concerning ICAM-1 E469K in cardiovascular conditions that component of MetS are in fact controversial. Homozygous KK of ICAM-1 E469K had a higher risk of restenosis after coronary stenting, especially in the case of obese or hyperlipemia patients (24) and this polymorphism was related to ACS recurrence and cardiovascular mortality (25). It was reported that ICAM-1 E469K might increase the risk for coronary artery disease in males of Uygur patients (26). Hovewer Barresi et al (2014) found that ICAM-1 E469K is not associated with symptomatic peripheral artery disease, supporting our data (27).

E-selectin is a cytokine-inducible endothelial cell adhesion molecule that participates in the initial tethering and rolling of leukocytes before their extravasation at sites of inflammation. E- selectin S128R is of particular interest since it is functional that modifies ligand affinity and also causes increased adhesiveness of leukocytes to the endothelium. This polymorphism results into decreased binding specificity and increased affinity for additional ligands and provides a mechanistic link for the development of diseases such as atherosclerosis and stroke (28,29). We investigated firstly the association of E-selectin S128R in non-diabetic MetS and we found that it is not involved in the susceptibility of the disease. Several previous studies have examined the relations of this polymorphism in hypertension and coroner arter disease, the components of the metabolic syndrome. Tripathy et al (19) supports our study, showing that E-selectin S128R is not a predictor of coronery artery disease in Indian population. Hovewer, it was reported that E-selectin S128R is strongly associated with essential hypertension in Han individuals but weakly associated in Uygur individuals (30) and might affect blood pressure in Chines (31).

There is a growing body of evidence suggesting inflammation is a key feature in MetS . Abdominal obesity, the important component of MetS is also associated with inflammation (32). Obesity itself can precipitate an inflammatory response and lead to free radical generation (33). The increase in oxidative stress is associated with visceral fat accumulation and MetS (34, 35). Infiltration of monocyte-derived macrophages into adipose tissue has been associated with tissue and systemic inflammation. Adipocytes might also contribute to systemic chronic low-grade inflammation associated with human obesity (36). Obesity induces inflammation in adipose tissue (AT) by expressing many cytokines and chemokines resulting with insulin resistance, type 2 diabetes and cardiovascular disease (37). Hovewer, we didn't find an association between non-diabetic MetS and polymorphisms of ICAM-1 and E-selectin, the inflammatory genes, in this study. A more comprehensive study involving multiple ethnicity and larger study sample are required to validate these results. We also suggest to investigate polymorphisms of the other inflammatory genes associated with MetS to reveal new insights for pathophysiology of MetS.

Conclusion

In conclusion, our results did not show a predisposition to non-diabetic MetS in patients with the E- selectin S128R or ICAM-1 E469K polymorphisms in Turkish population. This is the

first study to investigate the involvement of E-selectin S128R or ICAM-1 E469K polymorphisms in susceptibility to non-diabetic MetS. Further studies in larger populations and other ethnic groups are needed to explain the role of these polymorphisms in MetS.

Conflicts of interest

Author(s) disclose no funding sources and no potential conflicts of interest.

Table 1.Baseline demographic and clinical characteristics of Metobolic Sendrom patients and controls

	Patients (n=132 , 29 M and 103 F)	Control (n=134, 34M and 100F)	P
Age (years)	41.15±12.16	4.93±9.49	0.05
Smoking(n, %)	25 (18.93)	28 (20.89)	0.05
Alcohol intake(n, %)	4 (2.8)	3	0.05
BMI (kg/m^2)	41.09±5.94	23.06±1.02	<0.0001
Waist circumference (cm)	118.58±11.98	82.54±6.29	<0.0001
Systolic BP (mm Hg)	132.05±14.80	116.19±9.09	<0.0001
Diastolic BP (mm Hg)	85.98±13.99	73.99±5.48	<0.0001
Fasting plasma glucose (mg/dl)	97.83±18.81	86.08±6.47	<0.0001
HbA1c (%)	5.8±0.57	-	
Total cholesterol (mg/dl)	198.88±44.98	149.98±16.08	<0.0001
LDL (mg/dl)	129.30±30.57	98.54±12.88	<0.0001
HDL (mg/dl)	48.53±12.83	44.10±5.84	<0.0001
TG (mg/dl)	167.99±74.49	123.15±26.6	<0.0001
Insulin (pmol/l)	20.60±11.34	-	

M, male; F, female; BMI, body mass index; HDL/LDL, high-density lipoprotein/low-density lipoprotein; MetS, metabolic syndrome; SBP/DBP,systolic blood pressure/diastolic blood pressure;TG, triglyceride; Waist-C, waist circumference.

*Data in which non-parametric tests were used and expressed as median (range).

P0.001: MetS versus control;

Table 2.gene polymorphisms, primer sequences, annealing temperatures, restriction enzymes and allele sizes used in this study.

Polymorphisms	Primer sequences	Annealing temp (°C)	Restriction enzyme	Allele size	NCBI SNP
E selectin S128R	5' ATGGCACTCTGTAGGACTGCT-3' 5'GTCTCAGCTCACGATCACCAT 3'	56	Pst I	A:357 C:219+138	rs5361
ICAM-1 E469K	5' GGAACCCATTGCCCGAGC -3' 5'GGTGAGGATTGCATTAGGTC-3'	58	BstUI	A:223 G:136+87	rs5498

Table 3. Genotype and allele frequencies of ICAM-1 E/K polymorphism in patient and control groups.

ICAM-1 (E/K)	Patients n=132	Healthy controls* n=118	P	OR (CI 95%)
Genotypes				
EE	38	41	1	
EK	82	53	0.07	1.66(0.95-2.92)
KK	12	24	0.13	0.53 (0.23-1.22)
Alleles				
E	158	135	1	
K	106	101	0.54	0.89 (0.62-1.28)

*Control group is consistent with HWE

Table 4. Genotype and allele frequencies of E-Selectin S/R polymorphism in patient and control groups.

E-Selectin (S/R)	Patients n=132	Healthy controls* n=118	P	OR (CI 95%)
Genotypes				
SS	94	89		1
SR	36	28	0.5	1.2(0.68-2.15)
RR	2	1	0.59	1.8(0.16-21.25)
Alleles				
S	224	234		1
R	40	34	0.43	1.2(0.73-2.04)

*Control group is consistent with HWE

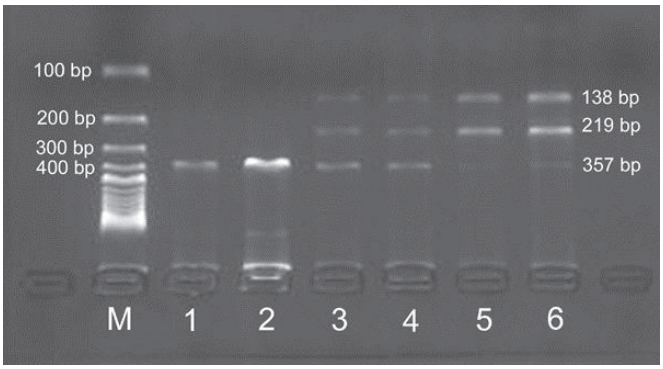

Figure 1. PCR-RFLP products of E-Selectin gene S128R polymorphism obtained by 2% agarose gel electrophoresis. Lane M shows 100bp DNA marker. Lanes 1,2 shows homozygous polymorphic RR alleles, lane 3,4 shows heterozygous SR alleles and lane 5,6 shows homozygous SS alleles.

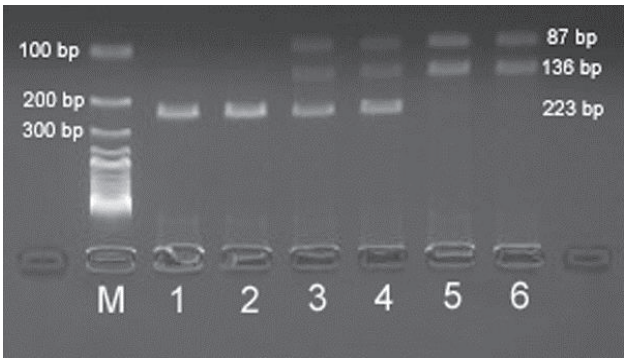

Figure 2. PCR-RFLP products of ICAM-1 gene E469K polymorphism obtained by 3% agarose gel electrophoresis. Lane M shows 100bp DNA marker. Lanes 1,2 shows homozygous EE alleles, lane 3,4 shows heterozygous EK alleles and lane 5,6 shows homozygous KK alleles.

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