INVESTIGATION OF THE ROLE OF ICAM-1 E469K AND E-SELECTIN S128R POLYMORPHISMS IN DIABETIC NEUROPATHY

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Abstract

Introduction: Diabetic peripheral neuropathy is one of the commonest complications of diabetes. The soluble adhesion molecules, E-selectin and intracellular adhesion molecule (ICAM)-are indicators for endothelial activation. Recent studies demonstrated that these molecules are increased in patients with diabetes and diabetes-related complications. The present study was designed to investigate the possible association of two mutations, S128R in E-selectin and K469E in ICAM-1 with diabetic peripheral neuropathy (DPN) in Turkish population.

Materials and methods: One hundred thirty eight patients with DPN and 138 non-diabetic control subjects by similar age were enrolled in the study. Genotyping was done by polymerase chain reaction-restriction fragment length polymorphism.

Results: No significant differences were found in the genotype and allele frequencies of ICAM E469K polymorphism between the groups of DPN patients and non-diabetic control subjects. There was also no statistically significant difference for E-selectin S128R polymorphism between DPN patients and non-diabetic control subjects.

Conclusions: We firstly investigated the involvement ICAM-1 and E-selectin gene variants in development of DPN. These results suggested that the ICAM-1 E469K and S128R polymorphisms are not associated with susceptibility to DPN in Turkish population. Further studies in larger series and in different ethnic populations should be carried out to validate our findings.

Key words: Diabetic peripheral neuropathy, E-selectin, intercellular adhesion molecule-1, polymorphism.

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Introduction

Diabetes is a common disorder with various systemic complications including diabetic peripheral neuropathy (DPN), which may decrease the quality of life by causing severe painful clinical symptoms1,2.

Recent studies emphasize the role of inflammation in DPN as well as diabetic retinopathy and diabetic nephropathy, the other micro-vascular complications of Type 1 and Type 2 diabetes3-6. E-selectin and serum intercellular adhesion molecule-1 (sICAM-1), circulating markers of systemic inflammation, are members of cell adhesion molecules and responsible for the rolling and adhesion of the leukocytes to endothelial cells. It is suggested that E-selectin and ICAM-1 may play an important role in the development and progression of peripheral neuropathy in diabetes7. ICAM-1 was found to be increased in DPN7,8 and it was reported that elevated levels of E-selectin have important role and may be useful for estimating deterioration of nerve function in diabetic patients.

ICAM-1 is a 90-kDa cell surface glycoprotein belonging to the immunoglobulin superfamily and it is involved in the firm attachment of leukocytes to endothelium9. Genome-wide scans have predicted
that Type I diabetes susceptibility genes may reside at chromosome 19p13(10,11). ICAM-1 gene is located in 19p13.3-p13.2, which is linked to Type 1 diabetes(12). ICAM-1 E469K, a non-synonymous SNP of ICAM, resides in the fifth immunoglobulin-like domain and is identified as Lys (K) or Glu (E) at codon 469 (exon 6)(13). This polymorphism is known to be common in all populations and several associations have been reported between this polymorphism and inflammatory diseases(14). The A allele corresponds to Lys at codon 469 (E469), and the G allele to Glu. It was demonstrated that the allele A of SNP rs5498 E469K is increased in Type 1 diabetic patients compared to nondiabetic subject(15,16).

E-selectin is specifically synthesized by endothelial cells and plays an important role in mediating leukocyte-endothelial adhesion. Plasma concentration of sE-selectin correlates with its expression on the surface of endothelial cells(17), and therefore, is a marker of endothelial dysfunction(18). It was reported that serum E-selectin levels have been increased in type 2 diabetes and may be a sensitive marker of endothelial activation and damage seen in diabetes(19,20,21). Baseline plasma E-selectin was found to be significant predictors of incident diabetic retinopathy in Type 1 diabetes and E-selectin was associated with progression of diabetic retinopathy and incidence of proliferative diabetic retinopathy(22). E-selectin was considered as a valid marker predicting onset and progression of nephropathy(23). The human E-selectin gene is located on chromosome number 1q24.2, spans approximately 13 kb long and consists of 14 exons and 13 introns. The S128R polymorphism (A to C mutation), a functional polymorphism of E-selectin gene was shown by Wenzel et al.(24).

Recent studies have revealed that rs5361 S128R polymorphism is associated with atherosclerosis and myocardial infarction, the diseases related to elevated adverseness of leukocytes to the endothelium(25,26).

In this study we aimed to investigate a possible association between ICAM-1 E469K and E-selectin S128R gene polymorphisms and DPN.

Material and methods

This study was conducted on 276 Turkish subjects: a test group of 138 patients (46 males and 92 females) with DPN and 138 age matched non-diabetic control subjects (49 males and 89 females).

Patients with diabetic peripheral neuropathy had symptomatic symetrical distal neuropathy (i.e., hypoactive deep tendon reflexes, reduced tactile, pinprick, and/or position sensation) with at least moderate severity of one or more of the typical symptoms (pain, burning, paresthesia, numbness or cramps) in the lower extremities.

The diagnosis of diabetic peripheral neuropathy was made according to clinical symptoms, neurologic examination and electrophysiologic investigation. Nerve conduction studies were performed with a standart electromyography equipment.

Subjects had infectious diseases, inflammatory diseases, liver failure, malignancies, neurodegenerative diseases, renal failure, cerebrovascular diseases, medical history of serious trauma to the limbs, use of neurotoxic medication, B12 vitamin deficiency, excessive alcohol comsumption and smokers were excluded from the study both in study and control groups.

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. Blood samples were collected in the morning after an 8-hour fasting period. Fasting plasma glucose (FPG), high-density lipoprotein-cholesterol (HDLC), low-density lipoprotein-cholesterol (LDLC), total cholesterol, triglyceride, Hemoglobin A1c (HbA1C) and high-sensitivity C-reactive protein (hs-CRP) levels were measured in diabetic and healthy control groups.

The local ethics committee of Harran University Medical Faculty approved the study and informed consent was obtained from all subjects.

Genomic DNA was extracted from peripheral blood leucocytes using GenJet whole blood genomic DNA purification kit (Thermo Scientific, St. Leon-Rot, Germany) according to manufacturer’s instructions. To determine the ICAM-1 E469K (A/G) and E selectin S128R (A/C), single nucleotide polymorphisms of ICAM-1 and E-selectin genes polymerase chain reaction restriction fragment length polymorphism (PCR -RFLP) assay was used as previously described(27). The genomic region flanking the K469E (rs5498; accession no. NT-011295) polymorphism in exon 6 of ICAM-1 was amplified with forward (5’ GGAACCCATTGCGCCGAGC -3’) and reverse (5’GGTGAGGATTGCATTAGTGC 3’), and flanking the Ser128Arg (rs5361) polymorphism in exon 4 of E-selectin was amplified with forward (5’ATGGCAGCTCTGTAGGACTG -3’) and reverse
(5'GTCTCAGCTCACGATCACCAT 3') using the following protocol: the PCR reaction was carried out in a 10 μl reaction volume containing 1xPCR buffer, 2 mM MgCl2, 0.2 mM each deoxynucleotide triphosphate (dNTPs; Fermentas, St. Leon-Rot, Germany), 40 ng of DNA, 0.2 μM of each primer (Bio Basic Inc., Markham, ON, Canada), and 0.5 unit of Taq DNA Polymerase (Fermentas). The PCR conditions were: 3 minutes of initial denaturation at 94 oC, followed by 30 cycles at 95 oC for 30 seconds, 30 seconds at 58 oC for annealing, and 30 seconds at 72oC for extension, followed by 5 minutes at 72oC for final extension.

**Genotyping of ICAM-1**

The K469E polymorphism was amplified using two specific primers. The PCR products were identified by enzyme digestion with BstUI that cuts theE469 allele but not K469. The digested products were separated on a 2% agarose gel along with a 100 to 1,500 bp DNA ladder (BioBasic Inc.). Ethidium bromide-stained gels were visualized under ultraviolet light using the Alpha Imager System (Alphalnnotech, San Leandro, CA, USA). The fragment length of the KK, EE and KE genotypes were 223 bp; 136 and 87 bp; 223, 136, and 87 bp, respectively (Figure 1).

**Genotyping of E-selectin**

Detection of the S128R polymorphism was carried out by PCR amplification followed by Pst I restriction enzyme digestion 13. The digested products were separated on a 2% agarose gel along with a 100 to 1,500 bp DNA ladder (BioBasic Inc.). Ethidium bromide-stained gels were visualized under ultraviolet light using the Alpha Imager System (Alphalnnotech, San Leandro, CA, USA). The fragment length of the CC genotype was 357 bp; the fragment lengths of the heterozygous AC genotype were 357, 219, and 138 bp (Figure 2).

**Results**

The mean age and gender distribution of patients and controls were similar (p>0.05). BMI, FPG, and HbA1c levels of diabetic patients were significantly higher than the controls (p<0.01) (Table 1).

The genotype frequencies of both ICAM-1 K469E and E-selectin S128R polymorphisms were assessed in Hardy-Weinberg equilibrium for both the patients and healthy controls. No significant differences were found in the genotype and allele frequencies of ICAM E469K polymorphism between the groups of DPN patients and non-diabetic control subjects (p > 0.05). The distribution of AG and GG genotypes were 47.8% and 54.3 in patient group and 24.0% and 16.0% in control subjects respectively. There was no statistically significant difference between the groups regarding allele G (p>0.05). The distrubition of polymorphic G allele was 47.8% and 43.1% in patient group and healthy controls respectively.
Genotypes and single allele polymorphism of ICAM-1 are illustrated in Table 2. There was also no statistically significant difference for E-selectin S128R polymorphism between DPN patients and non-diabetic control subjects according to the frequency of AC genotype, the heterozygote one (p > 0.05). The homozygote genotype (CC) was not seen in both the patients and non-diabetic control subjects. No significant differences in the allele frequencies were found between the groups of DPN patients and non-diabetic control subjects (p > 0.05). C allele was 10.9% and 9.1% in patients and control subjects respectively. The distribution of genotype and single allele polymorphism of E-selectin are showed in Table 3.

**Discussion**

In the present study, we have genotyped non-synonymous SNPs rs5498 E469K in ICAM-1 gene and rs5361 S128A in E-selectin in DPN patients and non-diabetic control subjects in a Turkish population. In the present study we did not find the CC genotype of rs5361 S128A in both patients and the control group as described in other studies (28,29). Secondly, SNP rs5498 E469K presented a high heterozygous index in the genotype distribution of Turkish population similar to the previous data (30,31). This is the first study to investigate the involvement of these two polymorphisms in the susceptibility of DPN. The data of this study demonstrated that these polymorphisms are not associated with the increased risk of development of DPN in the Turkish population.

ICAM-1 K469E polymorphism results in a non-conservative amino acid substitution in the fifth immunoglobulin-like domain of ICAM-1. Although this domain is not known to be involved in ligand binding of ICAM-1, it may play a role in an immunodominant epitope of B lymphocytes and dendritic cells (9). We found that there was no statistically significant difference (p > 0.05) between the control group and the patients with DPN regarding both in terms of the ICAM-1 genotypes and ICAM-1 alleles. These results are in agreement with those reported by Kristiansen et al (32) and Nejentsev et al. (33) who found the association of this SNP with type 1 diabetes was not detectable in Danish, Finnish and British Caucasians. However, some other studies demonstrated this polymorphism was associated with adult-onset type 1 diabetes (34) and diabetic nephropathy conversely to our findings (16,30).

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<th>Table 1: Clinical and metabolic parameters of healthy controls and patients with diabetic neuropathy.</th>
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<td><strong>Body Mass Index, BMI; Dyastolic Blood Pressure, DBP; Fasting Blood Glucose, FBG; High density lipoprotein cholesterol, HDL-C; Hemoglobin A1C, HbA1C; Low density lipoprotein cholesterol, LDL-C; Systolic Blood Pressure, SBP; Triglyceride, TG.</strong></td>
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<td><strong>ICAM-1 (A/G) Patients n=138 (%) Healthy controls n=138 (%) P OR (CI 95%)</strong></td>
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**CI, Confidence Intervals; Odds Ratio, OR.**

Genotypes and single allele polymorphism of ICAM-1 are illustrated in Table 2.

There was also no statistically significant difference for E-selectin S128R polymorphism between DPN patients and non-diabetic control subjects according to the frequency of AC genotype, the heterozygote one (p > 0.05). The homozygote genotype (CC) was not seen in both the patients and non-diabetic control subjects. No significant differences in the allele frequencies were found between the groups of DPN patients and non-diabetic control subjects (p > 0.05). C allele was 10.9% and 9.1% in patients and control subjects respectively. The distribution of genotype and single allele polymorphism of E-selectin are shown in Table 3.
E-selectin gene S128R affects the process of binding of E-selectin to the endothelium and may increase leukocyte adherence to endothelium. In the present study it was determined that no statistically significant (p > 0.05) difference existed between the control group and the patients with DPN groups regarding both the E-selectin genotypes and E-selectin alleles. The studies concerned with the association between E-selectin S128R polymorphism and diabetes are contradictory. In agreement with our findings Meigs et al. showed that E-selectin S128R polymorphism is not an important genetic risk factor for type 2 diabetes in women. However, a study pointed a possible association of E-selectin S128R polymorphism in type 2 diabetic patients who had risk for coronary heart disease.

In conclusion, our results showed that patients with the E469K and S128R polymorphism have not a predisposition to DPN in Turkish population. This is the first study to investigate the involvement of ICAM-1 and E-selectin polymorphisms in susceptibility to DPN. We suggest further studies in larger populations and expression analysis to reveal the role of E-selectin S128R and ICAM-1 E469K polymorphisms in DPN. Further studies are required to test the association of these two polymorphisms with DPN in other ethnic populations.

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