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Original Article

The relationship of the apolipoprotein E gene polymorphism in Turkish Type 2 Diabetic Patients with and without diabetic foot ulcers

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ABSTRACT

Aims: The aim of this study was to investigate the association between Apolipoprotein E (ApoE) gene polymorphism in the development of diabetic foot ulcers in Type 2 diabetes Turkish patients.
Materials and methods: The ApoE genotypes were determined retrospectively in 50 patients with diabetic foot and 50 without diabetic foot and a control group of 50 healthy individuals.
Results: The genotype ApoE distribution did differ between the control group (E2E3 44%, E3E3 38%, E3E4 18%) and Type 2 Diabetic Patients (E2E3 6%, E3E3 81%, E3E4 16%) ($p < 0.001$). The genotype ApoE distribution did not differ between Type 2 Diabetic Patients group (E2E3 4%, E3E3 86%, E3E4 4%) and diabetic foot ulcers (E2E3 8%, E3E3 76%, E3E4 16%) ($p > 0.05$). The frequency of the E2,E3,E4 allele in between the control group and Type 2 Diabetic Patients were no similar for the groups (E2 22%, E3 69%, E4 9% and E2 3%, E3 90.5%, E4 6.5%, respectively) ($p < 0.001$). The frequency of the E2–E4 allele in between the Type 2 Diabetic Patients and diabetic foot ulcers were similar for the groups (E2 2%, E3 93%, E4 5% and E2 4%, E3 88%, E4 8%, respectively) ($p > 0.05$).
Conclusions: The gene polymorphism of ApoE and E3 allele are a risk factor for diabetes, but gene polymorphism of ApoE is not an independent risk factor for diabetic foot. Lack of association between ApoE gene polymorphism and Type 2 diabetic foot ulcers might be due to ethnic differences.

1. Introduction

Diabetic foot disorder, the major source of disability and morbidity, is a significant burden for the community and a true public health problem [1]. The most important factor related to the development of foot ulcer is peripheral neuropathy, associated with loss of pain sensation. Neuropathy can be associated with peripheral vascular disease and foot deformities [2]. Diabetic neuropathy is the most common complication of diabetes, affecting 50% of diabetic patients [3]. Diabetic foot ulcerations result from different pathophysiological mechanisms; a clear understanding of them is crucial to reduce their incidence, provide early care, and finally delay the amputation risk. The three main diabetes complications involved in foot ulcerations are neuropathy, peripheral artery disease, and infection [4]. Peripheral vascular disease is one of the components of the diabetic foot. Diabetic patients should be assessed for lower limb arterial disease. Medical management includes minimization of vascular risk factors, antithrombotic therapy, and walking rehabilitation. Vascular testing is required in the presence of a foot wound [5]. Infection is always the consequence of a preexisting foot wound whose chronicity is facilitated by the diabetic peripheral neuropathy, whereas peripheral vascular disease is a factor of poor outcome, especially regarding the risk for leg amputation [6]. Foot infections are common in patients with diabetes and are associated with high morbidity [7].

Genetic influences play an important role in the development of diabetic foot ulcerations. ApoE gene plays a key role in lipid metabolism. The apolipoprotein E gene (APOE), which is well known to have a polymorphism (ε2, ε3, and ε4) in exon 4, has been considered a candidate gene susceptible to this complication. ApoE polymorphism affects plasma lipoprotein concentrations. Beyond

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the known influence of ApoE polymorphisms on serum lipid profile, the pathogenesis of atherosclerosis, and the development of neurodegenerative disorders, ApoE also has a major role in the pathogenesis and progression of a variety of renal diseases, as well as in the atherosclerotic complications associated with them [8,9]. ApoE2 allele induces high triglyceride levels and is associated with lower cholesterol compared with apoE3 allele. ApoE4 allele often has increased plasma cholesterol levels and correlation with cardiovascular disease and especially Alzheimer's disease [10–12]. ApoE polymorphisms have been proposed as the risk factor for the development of diabetic complication. A number of studies have investigated the association between the ApoE isoforms and diabetic complication. However the findings remain inconclusive. The association between APOE gene polymorphisms and diabetic foot ulcers is still unclear.

We aims investigate Turkish Type 2 Diabetic Patients with/without diabetic foot ulcers and healthy group and examined the contribution of ApoE gene polymorphism to the development of diabetic foot ulcers.

2. Subjects and methods

After getting approval for the Ethics Committee, 50 Type 2 Diabetic Patients with diabetic foot ulcers, 50 Type 2 Diabetic Patients without diabetic foot ulcers and a control group of 50 healthy individuals enrolled in the study. A detailed medical history of each patient was obtained. The phenotypic characteristics were determined. Informed consent was obtained from each participant. Age, sex, body mass index (BMI), blood pressure, duration of diabetes were recorded. Exclusion criteria were having thyroid disorders, diseases with acute inflammation, Type 1 Diabetes Mellitus,, serious hepatic, cardiac, renal failure, malignancy and psychiatric disorders.

Blood samples were taken in the morning after overnight fasting. Serum total-cholesterol, LDL-cholesterol, HDL-cholesterol, triglycerides, glycolizated hemoglobin (4.5–6%), high sensitive C-reactive protein (hs-CRP), erithrocyte sedimentation rate (ESR) creatinine, uric acid, microalbuminuria levels and thyroid stimulating hormone were measured. Biochemical parameters were studied with Roche/Hitachi Moduler autoanalyzer, complete blood count was studied with Roche Symex autoanalyzer.

Macrovascular disease is defined as; presence of ischemic heart disease, stroke, transient ischemic attack or peripheral artery disease [13,14]. Peripheral artery disease is diagnosed by absence of both feet pulses and/or ankle-brachial pressure index [ABPI]) lower than 0.90 [15–17]. ABPI measurement is made by a hand doppler (Hadeco ES-101EX, 8 Mhz).

Peripheral sensory neuropathy was defined as insensitivity to the 5.07 (10-g) Semmes–Weinstein monofilament at any one of ten sites on either foot (dorsal midfoot, plantar aspect of foot including pulp of the first, third, and fifth digits, the first, third and fifth metatarsal heads, the medial and lateral midfoot and the calcaneus).

Wagner classification was used for diabetic foot ulcers. Foot ulcer is defined as full-thickness skin defect that did not heal within 14 days [17].

Microalbuminuria was diagnosed when albumin excretion rate (AER), measured by radioimmunoassay (RIA), was 30–300 mg/24-h in at least two out of three 24-h urine collections over a 3-month period. Cretinine clearance, is calculated by Cockroft–Gault formula [GFR = (140 − age) × weight (kg)/Plasma creatinine × 72 (in women × 0.85)] [17,18].

Infection was defined as at least two of the following; local heat increment, purulent discharge, local erythema, edema, lymphangitis, fever and bad odor [14,19]. Retinopathy is diagnosed by ophthalmologic evaluation [13].

2.1. Genetic analysis

2.1.1. ApoE genotyping

Genomic DNA was extracted from peripheral leukocytes of the subjects using the High Pure PCR Template Preparation Kit (Roche Applied Science).

For the detection of the presence of the three ApoE E alleles ε2, ε3 and ε4 (codon 112 and 158) were analyzed by the commercial LightCycler ApoE Mutation Detection Kit (Roche Diagnostics, Mannheim, Germany). All experiments were carried out on the LightCycler™ Instrument (Roche Applied Science) according to the protocols provided by the manufacturer. Polymorphic alleles were identified by the specific melting temperature (Tm) of the resulting amplicons.

In the case of ApoE codon 112 and 158 were analyzed simultaneously and therefore two Tm values were obtained for each allele: for ε2 these were 56 °C and 57.5 °C, for ε3 56 °C and 66 °C, and for ε4 62.5 °C and 66 °C, respectively.

2.2. Statistical analysis

SPSS 18.0 for windows was used for statistical analysis of results. Distributions of continuous variables in groups were expressed as mean ± S.D. The distribution of alleles and genotypes between groups was compared using χ^2 analysis or Fisher's exact test. Differences between the mean of biochemical parameters were examined by means of ANOVA. A value of $p < 0.05$ was considered to be significant.

3. Results

3.1. Clinical investigation

Neuropathy, retinopathy and infection were statistically different between diabetic patient groups. Systolic and diastolic blood pressures were significantly different between patients and controls groups (Table 1). CRP, ESR levels and HDL-cholesterol

Table 1
Anthropometric and clinical features of diabetic patients and healty controls.

	Healty controls (*n*:50)	Diabetic patients without foot ulcers (*n*:50)	Diabetic Patients with foot ulcers (*n*:50)	*p*-Value
Numbers (M/F)	50 (25/25)	50 (24/26)	50 (26/24)	
Age (years)	57.95 ± 4.68	58.92 ± 8,48	59.32 ± 10.59	
BMI (kg/m^2)	27.86 ± 4.38	29.82 ± 4.48	28.32 ± 5.87	
Diabetes duration (years)	–	11.95 ± 7.78	12,25 ± 6.82	
Smoking (%)	16	18	20	
Systolic blood pressure (mm/Hg)	110.40 ± 11.16	128.19 ± 12.94	127.40 ± 18.26	<0.001***
Diastolic blood pressure (mm/Hg)	70,10 ± 7,54	77,10 ± 8,62	76,50 ± 9,36	<0.001***
Retinopathy (%)	–	20	42	<0.05*
Nepropathy (%)	–	80	86	
Macrovascular disease (%)	–	54	60	
Neuropathy (%)	–	40	98	<0.001***
Infection (%)	–	0	56	<0.001***

Retinopathy, neuropathy and infection were statistically different between diabetic patient groups. Systolic and diastolic blood pressures were significantly different between patients and controls.
n, number of individuals.
* Statistically significant difference was determined between two groups ($p < 0.05$).
*** Statistically significant difference was determined between two groups ($p < 0.001$).