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MULTİPLE SKLEROZ HASTALARINDA APOE GEN EKSPRESYONUNUN BELİRLENMESİ

DETERMINATION OF APOE GENE EXPRESSION IN MULTIPLE SCLEROSIS PATIENTS

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ÖZ

AMAÇ: Apolipoprotein E (ApoE), lipoprotein parçacıklarının reseptör aracılı endositozunda ligand olarak işlev gören bir glikoproteindir. ApoE'nin Alzheimer ve multipl skleroz gibi nörojeneratif hastalıklar ile ilişkisini gösteren çalışmalar vardır. Bu çalışmanın amacı ApoE gen ekspresyonu ile MS arasında bir ilişki olup olmadığını araştırmaktır.

GEREÇ VE YÖNTEM: ApoE gen ekspresyonunun multiple skleroz hastlığı üzerinde etkisini saptamak amacıyla yaptığımız çalışmamıza 35 hasta ve 20 sağlıklı birey dahil edilmiştir. ApoE mRNA ekspresyonunun seviyesi, Revers-Transkriptaz Polimeraz Zincir Reaksiyonu (qRT-PCR) ile belirlendi. İstatiksel analiz için SPSS paket programı kullanılarak,t testi, ki-kare testi, Kruskal-Wallis testi ve Mann-Whitney U testi yapıldı ve p <0,05 istatistiksel olarak anlamlı kabul edildi.

BULGULAR: Sonuç olarak, ApoE gen ekspresyonunun çoklu skleroz hastalarında ve sağlıklı kişilerin gerçek zamanlı PCR teknikleri kullanılarak karşılaştırılması, gruplar arasında istatistiksel olarak anlamlılık göstermedi (p =0.95).

SONUÇ: Çalışmamız ApoE gen ekspresyonunun MS hastalığı ile ilişkili olabileceğini desteklememektedir. ApoE'nin Multipl Skleroz hastalığında rolünü belirlemek için ileri çalışmalara ihtiyaç vardır.

ANAHTAR KELİMELER: ApoE, Multiple sklerozis, Gen ekspresyonu

ABSTRACT

OBJECTIVE: Apolipoprotein E (ApoE) is a glycoprotein that functions as a ligand in receptor-mediated endocytosis of lipoprotein particles. There are studies showing the association of APOE with neurogenerative diseases such as Alzheimer's disease and multiple sclerosis (MS). The aim of this study was to investigate whether there is a relationship between APOE gene expression and MS.

MATERIAL AND METHODS: To determine the effect of APOE gene expression on multiple sclerosis patients, we have included 35 MS patients and 20 healthy subjects into the study. The level of APOE mRNA expression was determined by real-time quantitative reverse transcription polymerase chain reaction (qRT-PCR). For statistical analysis, t-test, chi-square test, Kruskal-Wallis test and Mann-Whitney U test were performed using SPSS package program and p <0.05 was considered as statistically significant.

RESULTS: As a result, the comparison of ApoE gene expression in multiple sclerosis patients and healthy people using real-time PCR technique did not show any statistical significance between the groups (p=0.95).

CONCLUSIONS: Our study does not support that ApoE gene expression may be related to MS. Further studies are needed to determine the role of ApoE in Multiple Sclerosis disease.

KEYWORDS: ApoE, Multiple sclerosis, Gene expression

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INTRODUCTION

Multiple sclerosis (MS) is a chronic, inflammatory, central nervous system demyelinating disease. Previous studies have reported a significant increase in the prevalence rates of MS worldwide. Although the etiology and pathogenesis of MS are still obscure, it is demonstrated that the causes of MS are multifactorial and include genetic predisposition together with environmental factors (1, 2).

Apolipoprotein E (APOE) plays a functional role in lipid transport, immunoregulation, neuroplasticity and repair mechanisms. APOE is the highest expression in the brain and liver and is the lipoprotein that regulates the metabolism of lipids in the body (3). The apolipoprotein E (ApoE) gene localized on the chromosome 19q13 region is found in three common allelic forms: the APOE gene (Apo e2, e3, e4). The frequency of the ε4 allele is approximately 10-23 % in the general population (4-6). Research on APOE polymorphisms has found Apo E genotype being involved in other neurodegenerative diseases and ApoE-ε4 allele carriers seem to have a worse prognosis (7). When mRNA expression level of ApoE decreases, it reduces the protective properties of ApoE in both inflammatory and immune responses. Studies have reported that APOE is a genetic risk factor for Alzheimer's disease pathology. In addition, APOE-2 has been reported to have protective properties. mRNA expression level of APOE may contribute to the risk of developing MS, but this is controversial (6, 8-10).

The aim of this study was to investigate whether there is a relationship between APOE gene expression and MS.

MATERIAL AND METHODS

Study Subject

Case-control study consisted of 55 people. The study groups included 35 MS patients (7 males and 22 females; mean age 37.2 ± 11.9) and 20 age- and sex-matched healthy volunteers (4 males and 16 females; mean age 38.5 ± 14.7).

All patients and controls were sex and age-matched (no difference was calculated, $p > 0.05$).

Disease type was defined as relapsing-remitting (RRMS), primary progressive (PPMS) or secondary progressive (SPMS). All of patients were diagnosed according to the revised McDonald criteria.

Blood Sample Collection and Gene Expression Analysis

Venous blood samples (5 ml) were collected from both control and patient groups. Total RNA was extracted from whole blood using AccuZol RNA isolation kit according to the manufacturer's instructions (Bioneer, South Korea).

Spectrophotometry showed optical density ratio from 260 nm to 280 nm (1.8–2.1) for extracted RNA confirming purity and quantity of extracted RNA in optimal range. Complementary DNA (cDNA) was synthesized with oligo (dT) primer using a reverse transcription kit (Bioneer, South Korea). APOE mRNA expression was quantified by real-time PCR using GreenStar qPCR Master Mix kit) (Bioneer, South Korea). Real-time PCR amplification for APOE was performed using a total volume of 20 µL that contained 10 µl qPCR mix, 5 µl of each primer, 2µ cDNA and distillate water. The PCR conditions were as follows: 94 °C for 3 min, and then 40 cycles of 95 °C for 30 s, 60 °C for 30 s, and 72 °C for 30 s, and finally 72 °C for 5 min. The primers set were as follow: APOE, F: 5'-TGGACAAGTCTGGGATCCTT-3' (forward) and R: 5'-CATCTTCCTGCCTGTGATTG-3' (reverse); GAPDH, 5,-TGCACCACCAACTGCTTAGC-3, (forward) and 5,-GGCATGGACTGTGGTCATGAG-3,. The threshold cycle (Ct) is the intersection point. The relative gene expression of the APOE was normalized to GAPDH as the reference gene. The Delta Ct is the difference in threshold cycles between the target and reference. Delta Ct values were used for statistical analysis.

STATISTICAL ANALYSIS

Results are presented as mean ± SD. The normal distribution of clinical variables and gene expression data were checked using Kolmogorov–Smirnov test. Two-tailed t-test, chi-square test, Kruskal-Wallis test, and Mann–Whitney U test were used for comparison of the patients and controls by SPSS19 (SPSS, IBM Company). p-values <0.05 are taken as statistically significant.

RESULTS

35 MS patients, including 31 RRMS, 2 SPMS and 2 PPMS subjects, were analysed for the presence of APOE and compared with 20 age- and gender-matched control group. The characteristics of all patients are presented in Table 1.

Multiple sclerosis patients showed no significant correlation between age and APOE mRNA level (rs=0.31, p=0.19). Two-tailed t-test revealed no significant difference in the mRNA expression level of APOE between the male and female (p=0.27). No relationship exists between the Apo gene expression and MS subgroups (p=0.07). There was a decrease of APOE relative expression level in MS group when compared to the control group but there was no significant statistical difference (p =0.95) **(Table 1)**.

Table 1: Demographic and clinical details of study subjects

	MS (n=35)	Control (n=20)	p Value
Age (mean years ± SD)	37.2 ± 11.9	38.5 ± 14.7	0.17
Sex (n (%))			0.64
Male	7 (20.0)	4 (20.0)	
Female	28 (80.0)	16 (80.0)	
MS-Type (n (%))			
RRMS	31 (88.6)		
PPMS	2 (5.7)		
SSMS	2 (5.7)		
APOE relative expression level	0.62±0.22	0.68±0.25	0.95

SD: Standard Deviation; RRMS: Relapsing-remitting MS; PPMS: Primary progressive MS; SPMS: Secondary progressive MS

ETHICS COMMITTEE

Ethical Committee of Çanakkale Onsekiz Mart University Faculty of Medicine has approved the experiments and informed consents were obtained from each participant before enrollment (2011-KAEK-27/2019-E.1900037101).

Patients with infection diseases or other autoimmune diseases, cardiovascular or renal disease, cancer and blood systemic disorders were excluded from study.

DISCUSSION

In the present study, we found lower APOE gene expression in peripheral blood of MS patients compared to control subjects. Our results are in agreement with the findings reported by several studies indicating decreased plasma and APOE gene expression in MS patients (11, 12).

It is reported that some studies indicated a positive association between ApoE gene polymorphism and the risk of MS progression whereas other studies generated negative results (5, 13-17). However, the results remain controversial. To generate robust data, a much larger sample size in each subgroup might be required.

Previous studies have reported an increased APOE expression associated with Alzheimer's disease. Regarding the role of APOE genotype in AD risk, differences in these expressions may be significant (13, 18, 19). Bekris et. al. reported no significant difference in APOE mRNA levels between brain regions of AD patients and control subjects. It has been reported to correlate with low APOE plasma levels when cognitive impairment occurs in AD (3). There are conflicting reports that APOE mRNA or APOE protein levels are increased or decreased in autopsy specimens from AD patients. (20-24).

Previous studies on serum and CSF ApoE levels in MS patients reported variable results (16, 17, 25, 26). Pirtilla et al. showed that serum or CSF ApoE levels or ApoE-index did not differ between controls and MS patients (12). In contrast, Gaillard et al. reported decreased CSF ApoE levels in patients with definite MS (11).

Our results showed that ApoE expression levels were not statistically different between the patient and control groups. The reasons for these discrepant results remain unknown. The methods and reagents employed for the measurement of ApoE were different. Other explanations for the discrepant results may be related to differences in patient populations.

CONCLUSION

In conclusion, our data indicate that ApoE gene expression is not associated with MS. Although ApoE levels are not useful as an activity marker of MS, the role of some ApoE isoforms in the pathogenesis of MS cannot be excluded. The association between ApoE and multiple sclerosis should be examined in a larger population to clarify the role of ApoE in the degenerative form of MS.

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