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Cytokine Polymorphism and HLA Genotyping in Patients with Temporal Lobe Epilepsy Related to Hippocampal Sclerosis

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ABSTRACT

Objective: Hippocampal sclerosis (HS) is the most common pathological substrate associated with mesial temporal lobe epilepsy (MTLE), where inflammatory processes are known to play an increasingly important role in the pathogenesis. To further investigate the role of the immune system, both cytokine gene polymorphisms and human leukocyte antigen (HLA) genotyping in patients with MTLE–HS were investigated.

Methods: The DNA samples of 100 patients with MTLE–HS and 201 healthy individuals were genotyped for cytokines (IL-6,IL-10, TNF-α, TGF-β1 and IFN-γ) and HLA using polymerase chain reaction (PCR)-SSP and SSO methods. The results were statistically analyzed in patient and healthy control groups and then according to the presence of febrile seizures (FS) in the patient group.

Results: Analysis of cytokine genotyping did not reveal any significant difference between patients with MTLE–HS and controls and patients with or without FS. However, the HLA DRB1*13 allele was found to be more frequent in the patient population after Bonferroni correction.

Conclusion: This study suggests the possible role of HLA in the pathogenesis of MTLE–HS, although it failed to show any relationship with the cytokine system. However, data regarding the role of HLA are still lacking, and further studies are necessary to verify our results.

Keywords: Temporal lobe epilepsy, cytokine expression, HLA genotyping, hippocampal sclerosis

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INTRODUCTION

Mesial temporal lobe epilepsy (MTLE) is a form of intractable epilepsy that may highly benefit from surgery in which hippocampal sclerosis (HS) is the most frequent pathological substrate. Histopathology of human epileptic hippocampi shows a specific neuronal loss in the areas of CA and gliosis (1). Formerly, the relationship between prolonged febrile seizures (FS) and the development of MTLE has been shown with regard to cytokine gene expression (2), although other studies have failed to replicate it (3,4,5). Because altered immunity due to the etiopathogenesis of HS has been a point of interest, the role of proinflammatory cytokines and growth factors in the signaling process leading to a glial reaction have been investigated in animal models and human epilepsy (2,6,7,8). Despite recent studies, the pathogenesis of HS remains obscure, probably because of multifactorial reasons, including both environmental and genetic factors (9,10).

Previously, we performed HLA typing in patients with MTLE and found a significant difference in DR2, DR4, and DQ2 antigen distributions between the MTLE group and healthy population (11). Moreover, IL-1β/α gene polymorphism has been investigated in another group of patients with MTLE, but no significant difference has been observed (12).

To study the role of the immune system, we aimed to investigate other cytokine gene polymorphisms, including both pro- and anti-inflammatory cytokines (IL-6, IL-10, TNF-α, TGF-β1, IFN-γ), and HLA-DRB1 genotyping in patients with MTLE–HS.

METHODS

In total, 100 consecutive patients with MTLE–HS who were admitted to the epilepsy outpatient clinic of Cerrahpasa School of Medicine were studied. All these patients were surgical candidates because of medically intractable seizures and evaluated according to a standard protocol that included interictal and ictal video EEG recordings, cranial magnetic resonance imaging (MRI), and neuropsychological and psychiatric

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investigations. There were 68 female and 32 male patients, with a mean age of 25.8 years (range: 10–43) (Table 1). A history of FS was evident in 68% of the patients. HS was histopathologically confirmed in 70 patients who underwent anterior temporal lobectomy (the remaining were either under evaluation or in the waiting list for surgery). Informed consent was obtained from all the patients. The control group consisted of 201 healthy volunteers. This research was approved by the ethics committee of Istanbul University, Cerrahpaşa Faculty of Medicine (2006:18105).

DNA was extracted from the peripheral blood by using a standard method (13). Genotyping analysis of DRB1 loci and cytokine genes was performed at the Department of Medical Biology, Istanbul University. Cytokine genotypes were determined by the polymerase chain reaction (PCR)-SSP method using a commercial kit (One Lambda, Inc., Canoga Park, CA, USA) that contains sequence-specific primers for determining the following polymorphisms: TNF-a (−308 G/A), TGF-β1 (C/T codon 10, C/G codon 25), IL10 (−1082 G/A, −819 T/C, −592 A/C), IL-6 (−174 G/C), and IFN-δ (+874 A/T). DNA fragments corresponding to each cytokine were amplified. The allele frequencies of specified cytokines were analyzed. In patients with MTLE, the correlations between the genotype of cytokines and the presence of FS were also investigated.

Table 1. Demographics and clinical characteristics of patients

Number of patients	**100**
Age (years)	25.8 (range:10–43)
Gender	68 females (68%) 32 male (32%)
Presence of febrile seizures	68 (68%)
Age at onset (years)	8.9±5.8 (range: 1–28)
Disease duration (years)	18.4±8.1 (range: 1–41)
Operation	70 patients (70%)
Age at operation (years)	25.4 (range:12–44)
Seizure characteristics	
Presence of GTCS	47 patients (47%)
Status epilepticus	13 patients (13%)
Presence of aura	65 patients (65%)
GTCS: generalized tonic–clonic seizures	

Table 2. HLA-DRB genotyping in patients and controls

	Patient n=200	**Control n=402**	**χ2 test**	**p**
DRB1*01	9	21	0.189	0.664
DRB1*03	26	30	3.770	0.052
DRB1*04	37	63	1.387	0.239
DRB1*07	21	35	0.376	0.540
DRB1*08	3	13	1.995	0.158
DRB1*09	0	7	fisher	0.100
DRB1*10	3	9	fisher	0.757
DRB1*11	40	87	0.307	0.579
DRB1*12	3	16	2.747	0.097
DRB1*13	22	27	5.199	0.037*
DRB1*14	17	29	0.169	0.681
DRB1*15	11	41	3.986	0.046
DRB1*16	8	24	1.091	0.296
n refers to the number of HLA alleles. *p value was obtained from chi-square test and Bonferroni correction.				

For analysis of HLA-DRB1, all the samples were typed at the DRB1 loci by PCR-based methods (PCR-SSO) at low resolution (14,15,16).

Statistical Analysis

Statistical analysis was performed using Statistical Packages for the Social Sciences (SPSS Inc., Chicago, IL, USA) 12.0 version. Chi-square (χ2) test with Bonferroni correction and binary logistic regression models were also utilized for analysis.

RESULTS

Our results did not reveal any significant difference in the genotyping of pro- and anti-inflammatory cytokines between patients and controls. Further, allele distribution was not different in patients with or without FS. However, analyses of HLA-DRB1 showed a significant difference, where the DRB1*13 allele was found to be more frequent in patients ($p<0.05$), and it remained significant after Bonferroni correction.

DISCUSSION

Although previous studies have suggested a role of genetic, immunological, and environmental factors (9,10,17), the etiopathogenesis of MTLE remains obscure. In this study, we aimed to enhance the understanding of the etiology and identify potential immune markers in the susceptibility of disease.

Evaluation of Cytokines

Recent studies on epilepsy have suggested that the immune and inflammatory mechanisms play roles in some subgroups (18). In our study, the expressions of different cytokine genes (IL-6, IL-10, TNF-a, TGF-β1, and IFN- γ) and HLA-DR genotypes in Turkish patients with MTLE–HS were investigated to reveal the role of the immune system.

Cells in the central nervous system can produce cytokines upon activation (19), which may influence the transport systems by opening the blood–brain barrier (BBB). The administration of IL-1, IL-6, TNF-α, and IFN-γ may also increase the endothelial permeability (20,21,22,23). Impaired BBB and inflammatory states are common features of neurological diseases associated with late-onset epilepsy (24). The involvement of inflammation in the pathogenesis of epilepsy and seizure-induced brain damage have been emphasized in many studies (17,18,25). Vezzani et al. (25) showed that the manipulation of pro- and anti-inflammatory cytokines can modify the outcome as well as the neuropathological consequences of experimentally induced seizures. Moreover, in patients with treatment-resistant epilepsy, increased levels of IL-6 and decreased levels of anti-inflammatory cytokine IL-1RA have been demonstrated (26). Recently, it has been shown that IL-1β, among others, is upregulated in pediatric patients with MTLE during the developmental phase of refractory seizures related to HS (27). The role of IL-1β in the pathogenesis of MTLE has been supported by the finding that the inflammatory effects of IL-1β lead to the upregulation of the PI3K/Akt/mTor signaling pathway and could be suppressed by the administration of specific pathway inhibitors (28). The genetic susceptibility to inflammation has been found to be correlated with an increased risk of epilepsy (29). Weinberg et al. (30) explored the role of TNF-α and its two signaling receptors. They described a pro-ictogenic role of TNF-α mediated by TNFR1 and an anti-ictogenic role of this cytokine, probably mediated by TNFR2. The authors demonstrated that chronic induction of TNF-α, although inducing glial activation and BBB damage, did not provoke cell loss or seizures but enhanced the pathological consequences of seizures.

In many epilepsy models, acute seizures cause glial activation and increased expression of transcription factors and cytokines (31,32). The activated glia and elevated cytokines contribute to seizure-related hippocampal pathology such as neuronal death, reactive gliosis, and mossy fiber sprouting (33,34,35,36).

Differences in cytokine production are related to sequence variants in cytokine genes. Many of these polymorphisms occur in the regulatory regions, resulting in high or low cytokine production (37,38). We investigated different allelic variants in the promoter regions in pro-inflammatory (TNF-α, IFN- γ, and IL6) and anti-inflammatory (IL10 and TGFβ1) cytokine genes that have been identified previously (39). However, we did not find significant differences between patients with MTLE–HS and controls with regard to the genotype frequencies of IFN-γ, TNF-α, TGF-β1, IL-6, and IL-10.

Evaluation of HLA Genotype

HLA antigens and haplotypes have been described in connection with different epilepsies since the early 1970s; however, the results of such studies have been contradictory (40). We have reported a significantly high frequency of the HLA class II antigens DR4, DR7, and DQ2 in Turkish patients with MTLE–HS (11). Both our previous studies and several other studies have shown that HLA may play a role in different types of epilepsies (11,12,41). Moreover, the HLA-B*1502 allele has been found to be strongly associated with carbamazepine-induced Stevens–Johnson syndrome and its related disease, toxic epidermal necrolysis, in Southeast Asian countries (42).

In this study, we investigated the polymorphic exons of the HLA-DR locus (exon 2 of the class II HLA locus) in patients with MTLE–HS based on our previous results, which suggested that DR4, DR7, and DQ2 are independent risk factors for MTLE–HS (11). In correlation with previous results, the present results revealed that the DRB1*13 allele is more frequent in patients ($p<0.05$) than in healthy subjects (Table 2). Although the sample size is limited, our findings may suggest that a high frequency of HLA-DRB1*13 allele plays a role in the induction of hyperexcitability of the epileptic tissue. Recently, the HLA-DRB1*13:02 allele was found to be elevated in patients with MTLE–HS in comparison with controls, although the statistical significance was lost after Bonferroni correction (43). These findings may be taken into consideration as further evidence for immune-dependent genetic factors in the etiology of HS.

This study did not reveal any relationship between cytokine polymorphisms and human MTLE. However, the findings on HLA may suggest its role in the pathogenesis of HS, although this needs to be verified and requires further investigations, including a larger number of patients.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of İstanbul University Cerrahpaşa School of Medicine.

Informed Consent: Written informed consent was obtained from patients who participated in this study.

Peer-review: Externally peer-reviewed.

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