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Expression analysis of cytokine coding genes in epileptic patients

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ABSTRACT

Epilepsy is a chronic disorder in which immune dysregulation is shown to be involved. Imbalances in the cytokine levels both in serum and brain tissue have been demonstrated in epileptic patients. In the present study, we assessed mRNA expression of TNF-α, TGF-β, IFN-γ, CXCL8, IL-1β, IL-2, 1L-4, IL-6, IL-17 and CXCL8 in blood samples of 40 epileptic patients compared with age- and sex-matched healthy controls by means of quantitative real time PCR. The relative levels of CXCL8 transcripts were significantly higher in total epileptic patients compared with healthy subjects (P = .023). Relative mRNA expression of IFN-γ was significantly higher in female patients compared with female healthy subject (P = .048). In addition, significant correlations have been found between the mRNA levels of mentioned cytokines. Such imbalance between expression of pro-inflammatory and anti-inflammatory cytokines might be implicated in the pathogenesis of epilepsy.

1. Introduction

Epilepsy is a chronic neurological disorder distinguished by intermittent seizures due to uncontrollable neural firing in the brain [1]. Studies aimed at identification of the pathogenesis of epilepsy have noted a complex model for epileptogenesis consisted of both neuronal and nonneuronal elements including glial cells, brain vasculature and immune system components [1]. Moreover, impairment of the blood-brain barrier (BBB) may lead to efflux of serum proteins and penetration of immune cells into brain tissues resulting in abnormal neuronal excitation [1]. Supporting evidences for the role of inflammatory responses in epileptogenesis come from the effects of direct anti-inflammatory modalities in suppression of certain kinds of epileptic seizures that are refractory to routine anti-epileptic drugs (AEDs) [2]. Furthermore, intravenous immunoglobulin (IVIG) administration has decreased seizure frequency in some other types of refractory epilepsy [3]. Cytokines have been recognized as facilitators of spontaneous seizures in animal models [4,5]. Moreover, IL-1, IL-6, tumor necrosis factor (TNF)-α, and interferon (IFN)-γ have been demonstrated to cause BBB failure [6]. The disparity between proinflammatory and anti-inflammatory cytokines has been suggested to exacerbate organ damage and be involved in the pathogenesis of epilepsy [7]. Certain proinflammatory cytokines such as IL-6 have been shown to be increased in blood and brain tissues of epileptic patients [7]. The experimental data also advocate stimulation of cytokine networks during postictal period [7]. However, data regarding the role of cytokines in the pathogenesis of epilepsy are limited.

In the present study, we compared transcript levels of proinflammatory cytokines including IL-1β, IL-2, IL-6, IL-17, IFN-γ, and TNF-α, anti-inflammatory cytokines including IL-4 and TGF-β as well as CXCL8 chemokine in whole blood cells of epileptic patients and healthy subjects.

2. Material and methods

In the present case-control study we enrolled 40 patients affected with generalized epilepsy (19 females and 21 males, age mean: 36.66 ± 2.8 years) and 40 age/gender matched healthy controls. All patients were on treatment with Depakene (valproic acid) for a period of 3–18 months and did not have any seizure attack during the 6 months prior to sampling. Patients were not on any other medications unrelated to epilepsy. The diagnosis was based on patients' report of seizure occurrence as well as findings of electroencephalogram (EEG) and brain magnetic resonance imaging (MRI). The local ethical committee approved the study. Informed consent forms were provided by all participants. Healthy subjects were chosen from volunteers in whom the

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existence of any neurological, psychiatric or systemic disorder was ruled out. None of study participants has a history of systemic or autoimmune disorder.

2.1. Sampling and RNA extraction

Whole venous blood (3 mL in ethylene-diamine-tetra-acetic acid [EDTA] prepared tube) was obtained through venepuncture from all study participants. Hybrid-RTM blood RNA extraction Kit (Geneall Biotechnology Co Ltd, South Korea) was used for extraction of RNA from whole blood samples. Nanodrop equipment (Thermo Scientific, MA, USA) was used to evaluate the suitability of extracted RNA in the terms of both quantity and quality.

2.2. cDNA synthesis and quantitative RT-PCR

cDNA was synthesized from total RNA samples using High-Capacity cDNA Reverse Transcription kit (Applied Biosystems). The mRNA expression levels of cytokines were quantified by using SYBR® Premix Ex TaqTM (TaKaRa, Japan) according to the manufacturer protocol on the rotor gene 6000 corbett Real-Time PCR System using *HPRT1* gene was as normalizer. Specific primers and probes for assessment of transcript levels of mentioned cytokines genes were extracted from Allele ID 7 for x64 windows software (Premier Biosoft, Palo Alto, USA). The nucleotides sequences of primers and probes as well as PCR product length are demonstrated in Table 1.

2.3. Statistical analysis

The differences in mean values were compared between two groups using Bayesian estimation. Parameters with 200,000 iterations were supposed to have normal distribution. Spearman rank order correlation test was used for the calculation of the correlation between cytokines transcript levels. The effects of possible confounding variables were measured through application of Quantile regression. P values less than .05 were considered as significant.

3. Results

3.1. General demographic data

Demographic and clinical data of patients and healthy subjects are summarized in Table 2.

3.2. Relative mRNA expression of CXCL8 in patients compared with healthy subjects

The relative levels of CXCL8 transcripts were significantly higher in total epileptic patients compared with healthy subjects (P = .023) (Table 3). When comparing mRNA expression of this gene in distinct age- and sex-based subgroups, such difference was only significant in female patients compared with the corresponding control subgroup (P = .002). After using Quantile regression to examine the effect of age and sex on relative mRNA expression of CXCL8, there was a statistically significant difference in CXCL8 expression between patients and healthy subjects (P = .005).

3.3. Relative mRNA expression of IFN-γ in patients compared with healthy subjects

Relative mRNA expression of IFN-γ was significantly higher in female patients compared with female healthy subject (P = .048) (Table 4). After using Quantile regression to examine the effect of age and sex on relative mRNA expression of IFN-γ, there was not a statistically significant difference in IFN-γ expression between patients and healthy subjects (P = .495).

Table 1
Nucleotide sequences of primers and probes used in the study.

Gene name	Primer and probe sequence	Primer and probe length	Product length
HPRT1	F: AGCCTAAGATGAGAGTTC R: CACAGAACTAGAACATTGATA FAM-CATCTGGAGTCCTATTGACATCGC-TAMRA	18 21 24	88
IL-1β	F: ATAGCCTGGACTTTCCTGTTGTC R: GTGAGTAGGAGAGGTGAGAGAGG FAM-ACACCAATGCCCAACTGCCTGCCT-TAMRA	23 23 24	163
IL-2	F: GGGATCTGAAACAACATTCATGTG R: AGTCAGTGTTGAGATGATGCTTTG FAM-TGATGAGACAGCAACCA-TAMRA	24 24 17	109
IL-4	F: TGCTGCCTCCAAGAACACAAC R:GTCCTTCTCATGGTGGCTGTAG FAM-CCGGAGCACAGTCGCAGCCCT-TAMRA	21 22 21	88
IL-6	F: ATGCAATAACCACCCCTGACC R: CCATGCTACATTTGCCGAAGAG FAM-ACCACAAATGCCAGCCTGCTGACG-TAMRA	23 21 22	160
CXCL8	F:CGGAAGGAACCATCTCACTGTG R:AGAAATCAGGAAGGCTGCCAAG FAM-TGACTTCCAAGCTGGCCGTGGCTC-TAMRA	22 22 24	77
IL-17	F: CAGCAAGAGATCCTGGTCCTG R: GGTCGGCTCTCCATAGTCTAAC FAM-AGCCTCCACACTGCCCCAACTCCT-TAMRA	21 22 24	176
IFN-γ	F: GGCAAGGCTATGTGATTACAAGG R: CATCAAGTGAAATAAACACACAACCC FAM-GGGGCCAACTAGGCAGCCAACCT-TAMRA	23 26 24	96
TGF-β	F: GCTCCACGGAGAAGAACTGC R: GTTGGCATGGTAGCCCTTGG FAM-CCACTTCCAGCCGAGGTCCTTGCG-TAMRA	20 20 24	101
TNF-α	F: TCCACCCATGTGCTCCTCAC R: TCTGGCAGGGGCTCTTGATG FAM-CTACCGAGTCCGTGTCTACCA-TAMRA	20 20 21	97

Table 2
Demographic and clinical data of patients and controls.

Variables	Patients	Controls
Female/Male [No. (%)]	19 (47.5%)/ 21(52.5%)	19 (47.5%)/ 21(52.5%)
Age (mean ± SD, Y)	36.66 ± 2.8	34.06 ± 1.9
Age range (Y)	21–58	23–62
Age at onset (mean ± SD, Y)	28 ± 8.6	–
Generalized Epilepsy (No. %)	100 (100%)	–
Disease Duration (mean ± SD, Y)	8.18 ± 4.1	–

3.4. Relative mRNA expression of IL-1β in patients compared with healthy subjects

The transcript levels of IL-1β were not significantly different either between patients and healthy subjects or between age- and sex-based subgroups (Table 1 supplementary material (Table 1S)). After using Quantile regression to examine the effect of age and sex on relative mRNA expression of IL-1β, there was not a statistically significant difference in IL-1β expression between patients and healthy subjects (P = .338).