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IL-1β, IL-6 and IL1Ra levels in temporal lobe epilepsy

Irem Fatma Uludag [a,*], Tarik Duksal [a], Bedile Irem Tiftikcioglu [a], Yasar Zorlu [a], Feriha Ozkaya [b], Guldal Kirkali [b]

[a] Izmir Tepecik Educational and Research Hospital, Neurology Clinic, Turkey
[b] Dokuz Eylul University Faculty of Medicine, Department of Medical Biochemistry, Turkey

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ABSTRACT

Purpose: There is now extensive evidence to support the involvement of inflammation in the course of epileptic seizures. Seizure-induced changes in serum IL-1β, IL-6 and IL-1Ra levels are reported in several studies. Serum cytokine levels may also be disturbed in inter-ictal period due to seizure activity.
Methods: Twenty-one patients (12 women; mean age 35 ± 12.3) with temporal lobe epilepsy (TLE), 17 patients (8 women; mean age 31.8 ± 10.4) with extra-temporal lobe epilepsy (XLE) and 20 normal controls (10 women; mean age 35.6 ± 8.8) were included in the study. Serum levels of IL-1β, IL-6 and IL-1Ra of the TLE, XLE groups in inter-ictal period and of the normal control group were compared.
Results: All three cytokine levels are found to be significantly elevated in epilepsy patients when compared to controls ($p < 0.05$). In TLE group, IL-1β serum levels were significantly higher than in the XLE group ($p < 0001$).
Conclusion: The major findings in our study were increased levels of IL-1β, IL-6 and IL-1Ra in epileptic patients and high levels of IL-1β in TLE group. Our results support the existence of a chronic inflammatory state in epileptic patients.

1. Introduction

Recent studies suggested involvement of inflammation and inflammatory cytokines in the pathogenesis and the course of epilepsy. Among different inflammatory cytokines studied such as TNF-α and IL-17A, it has been IL-6, IL-1β and IL-1Ra which have attracted most attention in clinical studies [1–4].

In 1998, Peltola et al. reported for the first time that IL-6 plasma and cerebrospinal fluid levels of patients with recent seizures (<72 h) were higher than seizure-free patients and controls [5]. Following studies investigated plasma levels of IL-6, IL-1β and IL-1Ra after epileptic seizures. Although seizure induced IL-6 increase in plasma is almost invariable, changes in IL-1Ra levels after epileptic seizures are less prominent and IL-1β levels are found to be increased but also to be unchanged in some studies probably due to local and transient production of the cytokine in low quantities [6–9]. Some publications on cytokine and epilepsy concentrated on localization related epilepsy and indicated that temporal lobe epilepsy (TLE) is more likely to affect serum cytokine levels, specially IL-6 serum levels, than extra-temporal lobe epilepsy (XLE) [8,10]. These studies demonstrated that seizures are associated with activation of a cytokine cascade caused by neuronal excitation. Several reports indicate that IL-1β, IL-6 and IL-1Ra may also have patho-physiological relevance to epilepsy. In experimental models the blockade of the IL-1β biosynthesis prevented the occurrence of epileptic EEG activity and the injection of exogenous IL-1β resulted in hippocampal neuro-degeneration. The effects of IL-1β are inhibited by IL-1Ra [11,12]. Chronic overexpression of IL-6 is associated with the development of spontaneous seizures but in contrast the lack of IL-6 increases seizure susceptibility [13,14].

In the present study we investigated elevated serum IL-1β, IL-1Ra and IL-6 levels in epileptic patients which cannot be explained by post-ictal cytokine increases and which may reflect pathologically high basal cytokine levels contributing to the pathogenesis in epilepsy.

We aimed to study the IL-1β, IL-1Ra and IL-6 levels in epileptic patients during interictal state and normal controls. Because of the selective temporal lobe degeneration caused by IL-1β and the higher post-ictal cytokine release reported in TLE, we focused on differences in cytokine profiles of the epileptic syndromes of temporal and extra-temporal origin.

* Corresponding author at: Tepecik Educational and Research Hospital Neurology Clinic, Izmir, Turkey. Tel.: +90 5304690368; fax: +90 2324570055.
E-mail address: fatmairem@yahoo.com (I.F. Uludag).

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2. Materials and methods

Thirty-eight patients with refractory localization-related epilepsy admitted to the video-electroencephalography (EEG) laboratory of the Izmir Tepecik Educational and Research Hospital and 20 normal controls (10 women; mean age 35.6 ± 8.8 [22–53]) were included in the study. The Ethics Committee of the hospital approved the study, and all patients gave their written informed consent.

All patients underwent five days of video-EEG monitoring. Throughout the five days inpatient monitoring period, blood samples were collected from those patients who did not have an epileptic seizure within the last 72 h. Patients with concomitant neoplasm, infectious or inflammatory diseases, hepatic or renal insufficiency, recent trauma, surgery or immune-modulatory treatment and with any central nervous system lesion other than hippocampal sclerosis in cranial magnetic resonance imaging (MRI) (obtained just before the inpatient period) were excluded. The control group consisted of age and sex matched healthy volunteers without any chronic diseases including obstructive sleep apnea and acute cerebrovascular or cardiovascular event, trauma or surgery.

Patients were divided into two groups: those with TLE and those with XLE. The diagnosis of TLE and XLE was based on seizure semiology, interictal and ictal EEG and/or video-EEG recordings, and high resolution 1.5 T MRI of the brain [15].

Serum blood samples were immediately centrifuged (3000 rpm, 4 °C) and frozen at −80 °C until further processing. Cytokine levels were measured with sandwich ELISA (Enzyme Linked Immunosorbent Assay) method by commercially available assays (Human IL-1β Platinum ELISA and Human IL-16 High Sensitivity ELISA, Bender MedSystems GmbH, Austria; Human IL-1Ra Cytoscreen ELISA, Biosource, Belgium) according to the manufacturer's instructions. Serum levels of IL-1β, IL-6 and IL-1Ra of the TLE, XLE and normal control groups were compared.

Statistical analysis was performed using the non-parametric (less than 30 subjects in each study group) Kruskal–Wallis test followed by Mann–Whitney *U* test. The correlations were analyzed by using the Spearman's test. A *p*-value of 0.05 was considered significant. SPSS version 22.0 was used.

3. Results

Demographical and clinical features of the patients are shown in Table 1. TLE, XLE and normal control groups were similar regarding age and sex. In patients with TLE, epileptic seizures were started earlier and the seizures were more frequent but the differences were not statistically significant.

Serum cytokine levels are given in Table 2. In epileptic patients, significantly higher levels of IL-6, IL-1β and IL-1Ra than controls were observed.

In TLE patients, we found significantly higher serum IL-1β levels than in XLE patients (1.36 ± 0.93 vs 0.54 ± 0.31, $p = 0.001$).

When TLE and XLE groups are compared separately to normal controls, in TLE group both three cytokine levels were higher than normal controls but in XLE group, only IL-1β and IL-1Ra levels were significantly higher.

IL1Ra/IL1β ratio was higher in normal controls and lower in TLE patients but the difference was not significant.

We did not find a correlation between IL-6 levels and IL-1Ra as well as IL-1Ra/IL-1β ratio in any of the study groups but IL-6 levels were correlated with IL-1β ($p = 0.003$, $R = 0.61$) in TLE group.

There were not any differences between TLE patients with and without mesial temporal sclerosis.

Serum cytokine levels were not correlated with disease years, seizure frequencies and number of antiepileptic drugs used.

Table 1
Demographic and clinical features of the patients.

	TLE $n = 21$	XLE $n = 17$
Age (years)[a]	35 ± 12.3 [19–59]	31.8 ± 10.4 [20–54]
Female/male	12/9	8/9
Disease years[a]	19.8 ± 12.9 [2–47]	12.8 ± 8.9 [3–30]
Age at disease onset[a]	15.2 ± 9.9 [1–40]	19.5 ± 16.8 [1–59]
Seizure/month[a]	3.1 ± 5.1 [0.1–20]	1.8 ± 2.6 [0.1–8]
Cranial MRI		
Normal	11	17
Unilateral HS (right/left)	3/5	–
Bilateral HS	2	–
Laterality		
Right/left/could not be determined	4/12/5	7/6/4
EEG		
Normal	2	–
Unilateral temporal EA (right/left)	4/12	–
Bilateral temporal EA	3	–
Unilateral frontal EA (right/left)	–	5/5
Bilateral frontal EA	–	4
Other focal EEG abnormalities	–	3
Generalized EA	–	–
Treatment		
Monotherapy	7	9
2 AED	7	5
≥3 AED	7	3
AED		
Valproic acid	15	7
Carbamazepine	9	9
Lamotrigine	11	8
Levetirasetam	11	2
Oxcarbazepine	3	1
Topiramate	2	1
Phenytoin	1	1
Pregabaline	2	–
Clonazepam	1	–
Zonisamide	1	–

TLE: temporal lobe epilepsy, XLE: extra-temporal lobe epilepsy, MRI: magnetic resonance imaging, HS: hippocampal sclerosis, EEG: electroencephalography, EA: epileptiform abnormality, AED: anti-epileptic drug.
[a] Values in mean ± SD.

4. Discussion

We found high IL-1β, IL-1Ra and IL-6 levels in epileptic patients. There are several reports which are convergent with our study results.

Hulkkonen et al. reported a trend toward elevated plasma levels of IL-6 in patients with treatment resistant epilepsy [16]. Lehtimäki et al. also showed a trend to increased basal IL-6 levels in epileptic patients when compared to controls [9]. In the study of Sinha et al, IL-1β and IL-6 levels of the epileptic patients in inter-ictal period ($n = 16$) were higher than normal controls ($n = 100$) [17]. Lehtimäki et al. found higher serum IL-6 levels in epileptic patients with intellectual disability than the controls [18]. Inter-ictal serum IL-1β levels were elevated in patients with epilepsy compared to controls [4]. A recent study found interictal elevation of IL-6 but not of IL-1β [1]. One of the causes of the high cytokine levels in epileptic patients may be frequent seizures. Seizure-dependent release of cytokines is related to the severity of the seizure and lasts up to 24 h [10,19,25,26]. Central nervous system lesions such as focal cortical dysplasia and glioneural tumors seen in epileptic patients may be associated with the over-expression of IL-1β in lesional or peri-lesional areas of chronic inflammation [20].

Table 2
IL-6, IL-1β, IL-1Ra levels of the patients and controls.

	IL-6	IL-1β	IL-1Ra	IL-1Ra/IL-1β
TLE				
Mean ± SD	0.76 ± 0.18*	1.36 ± 0.93*,‡	130.05 ± 50.56*	196.42 ± 371.21
Median	0.76	1.04	124.5	127.08
Range	0.47–1.11	0.04–3.34	61.43–234.63	32.8–1801.81
XLE				
Mean ± SD	0.68 ± 0.36	0.54 ± 0.31**	111.88 ± 78.58**	240.39 ± 125.86
Median	0.6	0.44	85.65	189.47
Range	0.47–2.02	0.11–1.05	47.31–351.21	81.66–460.48
TLE and XLE				
Mean ± SD	0.73 ± 0.27†	0.99 ± 0.83†	121.92 ± 64.31†	216.09 ± 265.34
Median	0.67	0.84	101.84	143.2
Range	0.47–2.02	0.04–3.34	47.31–351.21	32.80–1801.81
Normal control				
Mean ± SD	0.53 ± 0.12	0.24 ± 0.11	50.33 ± 14.06	292.48 ± 265.34
Median	0.49	0.25	44.87	209.48
Range	0.4–0.88	0.04–0.45	33.52–88.72	75.98–1291.05

Values given in pg/mL.
TLE: temporal lobe epilepsy, XLE: extra-temporal lobe epilepsy.
* TLE > normal controls: $p < 0.001$ for IL-6, IL-1β and IL-1Ra.
** XLE > normal controls: $p = 0.001$ for IL-1β and $p = 0.005$ for IL-1Ra.
† TLE and XLE > normal controls: $p = 0.04$ for IL-6, $p < 0.001$ for IL-1β and IL-1Ra.
‡ TLE > XLE: $p = 0.001$ for IL-1β.

Another explanation is the increased cytokine production from peripheral blood mononuclear cells to stimulation in epileptic patients [1,16,21]. Such alteration in reactions of peripheral blood mononuclear cells may cause a pro-inflammatory state leading to the facilitation of epileptic seizures.

In our study, high IL-1β, IL-1Ra and IL-6 levels in epileptic patients may not be explained by the seizure-induced cytokine changes since patients were seizure free for at least 72 h, a sufficiently long period for tested cytokines to return to baseline levels [5,6]. We excluded patients with central nervous system lesions other than mesial temporal sclerosis to rule out the effect of the cytokine increase related to the neuro-degeneration. Although an effect resulting from recurrent seizures cannot be totally excluded, we think that high cytokine levels in epileptic patients are the reflection of a trend toward chronic inflammatory state in epilepsy. This observation is supported by experimental models, showing that acute inflammation is associated with brain excitability which may lead to long-lasting increased sensitivity to seizures [22].

In addition to elevated levels of all three cytokines in epileptic patients, IL-1β was significantly higher in TLE patients than XLE patients and normal controls and we founded the lowest IL-1Ra/IL-1β ratio in TLE group. Because the difference in seizure frequency was not significant between two groups, we do not believe that the high IL-1 β level observed in TLE may be solely explained by the seizure frequency. Homozygosity for IL-1β gene polymorphism is found to be more frequent in TLE patients with hippocampal sclerosis than in TLE patients without hippocampal sclerosis or normal controls [23]. This genotypic variation leads to over-expression of the protein which may contribute to hippocampal damage in TLE. There have been other studies from different ethnic populations supporting or contradicting the high frequency of IL-1β polymorphism in TLE since then [24,25]. The significantly higher levels of IL-1β in our TLE patients may be related to a genetic predisposition contributing to the pathogenesis of TLE. Unfortunately we were not able to compare TLE patients with and without hippocampal sclerosis because of the small number of patients. The low number of patients used could also explain why we did not find any correlation between serum cytokine levels and disease years and seizure frequency.

Because the three groups were similar in age and sex, we do not believe that cytokine levels are affected by these factors. Likewise, there is not a statistically significant difference in disease years and seizure frequency between TLE and XLE groups which may be the cause of the high IL-1β levels in TLE group. Because of the limited numbers of patients and the diversity in their medications, the effect of each anti-epileptic drug in serum cytokine levels could not be analyzed. Also some other factors which may contribute to the changes in serum cytokine levels like the obesity and the stress due to hospitalization cannot be excluded [26].

In conclusion, we suggest that the elevated inter-ictal serum cytokine levels in epileptic patients point out the role of inflammatory processes in triggering or sustaining the seizures. This finding may have implications for future approaches targeting systemic inflammation in the treatment of epilepsy.

Conflict of interest

None.

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