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The role of plasma cytokine levels in the differential diagnosis of epileptic and psychogenic non-epileptic seizures

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Abstract
Aim: In this study, we aimed to evaluate the use of plasma cytokine levels in the differential diagnosis of epileptic and psychogenic nonepileptic seizures.
Material and Methods: Thirty-three epilepsy patients with generalized seizures, 23 patients with psychogenic nonepileptic seizures and 31 control patients were included in the study. Blood was drawn from all patients at 1 and 24 hours after seizures, and IL-1β, IL-1Ra, IL-6 and TNF-α levels were measured. The results were evaluated in terms of differences by groups and hours.
Results: A significant increase was observed in IL-1β, IL-6 and IL-1Ra levels in the epileptic seizure group compared to controls in the first hour. This increase is significantly different only for IL-1β with the psychogenic non-epileptic seizure group. IL-1β appears to be the marker that best distinguishes the epileptic seizure group from both control and psychogenic non-epileptic seizure groups. IL-1β values were not significantly different between the 1st and 24th- hour measurements in the epileptic seizure group. TNF-α levels decrease significantly in psychogenic non-epileptic seizure patients compared to both epileptic seizure and control groups, but show significant increase over time between 1 and 24 hours.
Discussion: Checking blood cytokine levels can be used as an adjunct method in the differential diagnosis when distinguishing between epileptic and psychogenic non-epileptic seizures. In our study, the importance of blood cytokine levels in the differential diagnosis and the time at which they are checked were shown.

Keywords
Cytokines; Epileptic Seizure; PNES

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Introduction

According to the definition of the World Health Organization, epilepsy is a dysfunction of the whole or a part of the brain, which is seen as a result of repetitive, abnormal discharges of neurons that have become over-excitable in the brain, which manifests itself with clinical features of sudden and transient, motor, sensory, autonomic and psychic nature [1]. Psychogenic non-epileptic seizure (PNES) is mainly used to describe non-epileptic clinical conditions that are not associated with epileptic electro encephalogram (EEG) disorders, but have an epileptic seizure-like appearance and often seen secondary to psychiatric disorder [2].

Many clinical and experimental studies have shown an increase in inflammatory cytokine levels in epileptic seizures [3]. Pro-inflammatory cytokines are concentrated in low amounts within the brain, increasing after seizures. After the seizure, mRNA expression of interleukin-1 beta (IL-1β), IL-6 and TNF-α has been shown to be upregulated in the hippocampus [4-6].

In experimental studies, it has been shown that IL-1β is proconvulsant and neurotoxic, and interleukin-1 receptor antagonist (IL-1Ra) is anticonvulsant and neuroprotective [7]. IL-1β, a member of the interleukin receptor family, activates the GluN2B subunit of the N-methyl-D-aspartate (NMDA) receptor and induces seizures by causing upregulation of NMDA receptors on postsynaptic cells [8].

It was found that IL-1β in the cerebrospinal fluid (CSF) increased significantly in epileptic pediatric patients compared to the control group, indicating that IL-1β plays an important role in the onset and progression of epilepsy [9].

It has also been shown that IL-1β is proconvulsive in rabbit epilepsy models induced with kainic acid, with worsening of seizures and prolongation of seizure activity after intrahippocampal injection of IL-1β [10]. Indirect evidence of the proconvulsive effects of IL-1β is the reduction in induced convulsions after intracerebral injection of IL-1Ra in mice [11]. IL-1β not only induces nitric oxide production and increases seizure sensitivity, but also increases neuronal excitability by inhibiting direct Gamma-Aminobutyric acid (GABA) receptors, increasing NMDA receptor function, and reducing potassium efflux [7,12]. It is known that IL-1Ra cells bind to IL-1β receptors and prevent IL-1β from binding. In some studies, it has been found that intracerebral administration of IL-1Ra (this is a natural antagonist of endogenously produced IL-1β) has a very strong anticonvulsant effect. Similarly, mice that produce excess IL-1Ra have resistance against seizures [11].

In humans, it has been shown that IL-6 and IL-1Ra levels increase in the CSF and blood, and there is no significant change in the level of IL-1β after focal seizures and secondary generalized tonic clonic seizures [13-15].In animal studies, it has been shown that IL-6 mRNA increases rapidly in the hippocampus, amygdala, dentate gyrus, cortex, and meninges [16].

Neither CSF nor blood TNF-α levels were observed to change within 24 hours of tonic clonic seizure and partial secondary generalized seizure. Again, no changes were found in TNF-α concentrations in the blood and CSF of patients with febrile seizures [17].

Studies show that the effect of TNF-α is concentration dependent. TNF-α shows its proconvulsive effect at low concentration and anticonvulsive effect at high concentration in seizures mediated by Shigella. In addition, the p55 pathway is activated at low concentrations of TNF-α, while the p75 pathway is activated at higher concentrations. In conclusion, while TNF-α creates a proconvulsive effect through the p55 receptor at low concentrations, it has an anticonvulsive effect through the p75 receptor at high concentrations [18].

In our study, we looked at postictal blood cytokine levels at 1 and 24 hours in a group of patients with epileptic seizures and PNES and compared the groups with both themselves and with the control group, we searched the availability of the difference, if detected, as an auxiliary method in the differentiation of epileptic seizures and PNES.

Material and Methods

In this study, 33 epilepsy patients (17 females, 16 males) with primary generalized seizures and 23 patients with PNES (18 females, 5 males) and 31 healthy volunteers (18 females, 13 males) who applied to the neurology service, neurology outpatient clinic and emergency service of İzmir Tepecik Training and Research Hospital were included. Local ethics committee approval was obtained for the study.

In epileptic seizure and PNES groups, blood cytokine levels were measured by taking 2 tubes of blood at 1 and 24 hours after the seizure and 1 tube of 5 cc blood from the control patients.

Patients with acute cerebrovascular disease, alcohol or substance abuse, pregnancy and lactation, oral contraceptive, hormone or anti-inflammatory drug use, chronic renal failure or dialysis patients, and those with signs of infection were excluded from the study.

EEG, 1.5 Tesla Cranial MRI, liver function tests, kidney function tests, and hemogram examinations were performed in all patients and the control group.

TNF-α, IL-1β, IL-1Ra, IL-6 levels in serum samples were measured by the "sandwich" ELISA (Enzyme Linked Immunosorbent Assay) (Human TNF-α Ultrasensitive ELISA, Invitrogen, Camarillo, CA 93012) method. Serum samples were diluted with dilution buffer at a ratio of 1/2 and studied. According to the ELISA method, patient samples were sandwiched between immobilized polyclonal antibodies and biotin-labeled polyclonal specific antibodies conjugated with streptavidin-peroxidase. After the unbound material was removed by washing, the process was stopped after a while after the addition of peroxidase enzyme substrate (tetramethylbenzidine) and the resulting color was measured at 450 nm wavelength. Values were calculated as pg/mL using the standard graph drawn. The determined values were multiplied by the dilution coefficient and the real values were obtained.

The SPSS (version 26.0) program was used for the analyses. After all data were transferred to the digital environment and controlled, the frequency and percentage values for categorical variables, mean and standard deviation values for continuous variables were given. The normality of the distribution of continuous variables was evaluated using the Kolmogorov-Smirnov test. Since there was no normal distribution in variables in all groups, non-parametric tests were preferred. The Chi-square test for categorical variables, the Kruskal-Wallis test for continuous variables and Dunn's test for post hoc analysis

were used for intergroup comparisons. The Wilcoxon Signed Ranks test was used to compare TNF-α, IL-1β, IL-1Ra and IL-6 values at 1 and 24 hours. Test constants and absolute p-values were given for all analyzes. The general significance limit in the study was accepted as p<0.05.

Results

The age and gender characteristics of the epileptic seizure, PNES and control groups included in the study are given in Table 1. There is no statistically significant difference between the gender distribution and mean age of the cases according to the groups (p>0.05).

IL-1β, IL-6, IL-1Ra and TNF-α values of the seizure patients' 1st hour samples and control group are shown in Table 2. There was a significant difference between the groups for all four variables measured.

Postictal first-hour interleukin levels in the study groups are shown in Figure 1. In the post-hoc comparison of IL-1β levels between the groups, there was a significant difference between the epileptic seizure and the PNES group (Z=17.308; p=0.035) and between the epileptic seizure group and the control group (Z=28.533; p<0.001), while there was no significant difference between the PNES group and the control group (Z=11.225; p=0.317).

In the post-hoc comparison of IL-6 levels between the groups, there was no significant difference between the epileptic seizure group and the PNES group (Z=14.408; p=0.170). There was a significant difference between the control group and epileptic seizure group (Z=31.577; p<0.001) and control group and PNES group (Z=17.168; p=0.040).

In the post-hoc comparison of IL-1Ra levels between the groups, there was a significant difference between the epileptic seizure group and the control group (Z=21.660; p=0.002), while there was no significant difference between the epileptic seizure and PNES group (Z=8.689; p=0.616) and control group and the PNES group (Z=12.971; p=0.186).

In the post-hoc comparison of TNF-α levels between the groups, there was a significant difference between the epileptic seizure group and the PNES group (Z=17.397; p=0.034) and between the PNES group and the control group (Z=21.503; p=0.006), while there was no significant difference between the epileptic seizure group and the control group (Z=4.106; p=1,000).

Comparison of IL-1β, IL-6, IL-1Ra and TNF-α values at 1 and 24 hours, obtained according to the study groups of the participants is shown in Table 3. There was significant difference only in IL-6 values in the epileptic seizure group, and in IL-1β, IL-6, and TNF-α values in the PNES group.

Table 1. Demographic case characteristics by groups

	Epileptic seizure (n=33)	Psychogenic Nonepileptic Seizures (n=23)	Control (n=31)	Statistical analyze
Age	31,89±10,98	30,48±12,19	31,52±6,99	X^2=0,295* p=0,863
Gender				
Women	17 (%51,5)	18 (%78,3)	18 (%58,1)	X^2=4,237† p=0,120
Men	16(%48,5)	5 (%21,7)	13 (%41,9)	

*Kruskal-Wallis test †Chi-square test

Table 2. 1st hour cytokine values of the participants according to the groups

	Epileptic seizure (n=33)	Psychogenic Nonepileptic Seizures (n=23)	Control (n=31)	Statistical analyze
IL-1β (pg/mL)	0,37±0,19	0,25±0,14	0,19±0,08	X^2=20,790* p<0,001
IL-6 (pg/mL)	0,46±0,32	0,31±0,30	0,13±0,15	X^2=25,018* p<0,001
IL-1Ra (pg/mL)	202,17±144,95	138,38±60,97	105,61±54,24	X^2=11,843* p=0,003
TNF-α (pg/mL)	2,04±1,29	1,43±0,79	1,98±0,75	X^2=10,391* p=0,006

*Kruskal-Wallis test
IL-1β (interleukin-1 beta), IL-1Ra (interleukin-1 receptor antagonist), IL-6 (interleukin-6), TNF-α (tumor necrosing factor alpha)

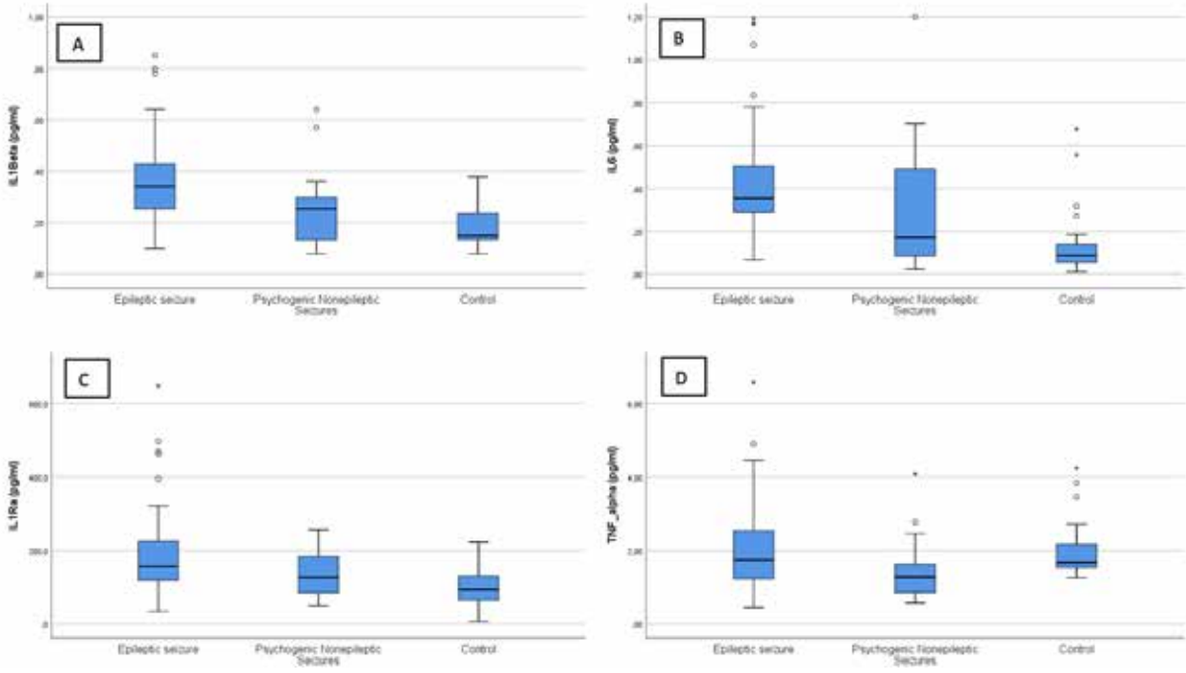

Figure 1. Postictal first hour interleukin levels in study groups. A) IL-1β levels. B) IL-6 levels C) IL-1Ra levels D) TNF-α levels

Table 3. Comparison of the 1st and 24th hour cytokine values of the participants according to the patient groups

	1st hour	24th hour	Statistical analyze*
Epileptic seizure			
IL-1β (pg/mL)	0,37±0,19	0,31±0,12	Z=1,534; p=0,125
IL-6 (pg/mL)	0,46±0,32	5,08±5,83	Z=3,824; p<0,001
IL-1Ra (pg/mL)	202,17±144,95	179,24±135,96	Z=1,510; p=0,131
TNF-α (pg/mL)	2,04±1,29	2,29±1,74	Z=1,081; p=0,280
Psychogenic Nonepileptic Seizures			
IL-1β (pg/mL)	0,25±0,14	0,31±0,18	Z=2,487; p=0,013
IL-6 (pg/mL)	0,31±0,30	2,27±4,46	Z=1,977; p=0,048
IL-1Ra (pg/mL)	138,38±60,97	128,32±84,48	Z=0,213; p=0,831
TNFα (pg/mL)	1,43±0,79	2,11±2,28	Z=2,160; p=0,031

* Wilcoxon Signed Ranks test
IL-1β (interleukin-1 beta), IL-1Ra (interleukin-1 receptor antagonist), IL-6 (interleukin-6), TNF-α (tumor necrosing factor alpha)

Discussion

For years, clinicians have been trying to define the nature of episodic neurological symptoms. Events associated with marked motor activity or altered consciousness are often predicted to be epileptic seizures. However, the event actually represents one of a broad spectrum such as syncope, parasomnias and movement disorders or conversive paroxysmal events. It is known that an important type of episodic behavior is the psychogenic conversion seizure.

In our study, we examined the possibility of using postictal blood cytokine levels to identify and differentiate seizures. When the first-hour measurements were compared with the control group, a significant increase was observed in the levels of IL-1β, IL-6 and IL-1Ra for the epileptic seizure group. This increase is significantly different for only IL-1β with the PNES group. IL-6 was also significantly higher than in the control group in the PNES group, while PNES group values for IL-1Ra levels did not differ from the epileptic seizure and control groups. On the other hand, TNF-α levels were significantly decreased in the PNES group compared to the control and epileptic seizure groups. IL-1β appears to be the marker that best distinguishes the epileptic seizure group from both the control and PNES groups. IL-1β values were not significantly different between the 1st and 24th hour measurements in the epileptic seizure group. This can be interpreted as the differential value of this marker continues for the following hours in the differentiation of epileptic seizures. However, it should be noted that there is a small but significant increase in IL-1β values between the 1st and 24th hours in the PNES group. Significant reduction of TNF-α levels in both epilepsy and control groups in PNES patients may support diagnostic power. However, it should be noted that TNF-α levels significantly increase over time between the 1st and 24th hours in PNES patients and lose this power. Significant increases in IL-1β, IL-6 and IL-1Ra in the epileptic seizure group can be evaluated as the diagnostic power will increase when all of these markers are used together, but mixed and contradictory situations may also be encountered in all patients due to the lack of a change in the same direction.

Increased IL-1 production was detected in temporal lobe epilepsy in the literature. The detection of IL-1β, IL-1Ra, and IL-1α gene polymorphism in drug-resistant epilepsy patients suggests a relationship between haploid types of cytokine genes and the development of focal seizures [19].

In a study conducted on rats, it was found that there was an increase in IL-1β and IL-1Ra immunoreactivity, mainly in microglial cells after kainic acid-induced seizures [20]. It was found that the changes in plasma IL-6 level seen in patients with complex partial epilepsy were very low compared to patients with secondary generalized tonic clonic epilepsy, which showed that seizure severity and IL-6 level were correlated [21]. There was no significant change in IL-1β levels in the postictal period when compared with preictal levels in the studies. A striking increase in IL-1Ra levels was detected at the second and twelfth hours after the seizure. These increases seen in IL-1Ra were higher, especially after generalized seizures, but this difference was not statistically significant [15]. In another study, an increase in IL-6 levels was found in postictal blood samples taken immediately after the seizure. The average increase was 51% after 1 hour and 87% after 24 hours. No difference was found in the postictal IL-1β and TNF-α levels. IL-6 levels were slightly higher in patients with secondary generalized tonic clonic epilepsy compared to those with focal seizures, but this difference was not statistically significant. Gender was not found to have an effect on serum cytokine levels. Basal IL-6, TNF-α, IL-1β levels did not differ in patients with hippocampal sclerosis (HS) or without HS, but patients with HS had significantly less elevated levels of postictal IL-6 compared to others and were taken to preictal measurements. Those with right-sided temporal seizure onset had higher serum IL-6 levels in all measurements than those with left-onset [23]. Our study showed that blood cytokine levels can be used to differentiate between epileptic seizures and PNES.

Conclusion

Results associated with epilepsy and blood cytokine levels are few and contradictory. Existing changes in the patient profiles taken in the studies and the seizure types of the patients affect the results of different studies. Epilepsy patients and healthy controls were compared in the studies conducted. Our study performed a separate comparison between patients with epileptic seizures and PNES patients and their comparison with healthy control groups. In our study, the increase in IL-1β, IL-6 and IL-1Ra levels after epileptic seizures was significant, the most distinctive difference was associated with IL-1β levels. TNF-α levels were significantly lower in the PNES group than in the control group and epileptic seizure patients. When evaluated together with the results of other studies, cytokine levels can be considered to be used diagnostically, but it should be kept in mind that there are many differences in interpersonal genetics, seizure type, drugs used, and the etiological factors of seizures. More studies should be conducted on the relationship between blood cytokine levels, which, in our opinion, may have an important place in the differential diagnosis of epilepsy patients. The place of blood cytokine levels in differential diagnosis with long-term and large-group studies will become clearer in the upcoming years.

Scientific Responsibility Statement
The authors declare that they are responsible for the article's scientific content including study design, data collection, analysis and interpretation, writing, some of the main line, or all of the preparation and scientific review of the contents and approval of the final version of the article.

Animal and human rights statement
All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. No animal or human studies were carried out by the authors for this article.

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Conflict of interest
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