RESEARCH ARTICLE

CYPRUS
JOURNAL OF MEDICAL SCIENCES

DOI: 10.4274/cjms.2022.2022-11
Cyprus J Med Sci 2022;7(5):609-613

Investigation of Total Antioxidant Status and Total Oxidant Status with Seizure Types in Patients with Epilepsy

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Abstract

BACKGROUND/AIMS: As epilepsy is a complex disease group, it is difficult to diagnose and classify. Oxidative stress plays a vital role in the pathogenesis of epilepsy. This study investigated the total oxidant/antioxidant status levels in patients with focal onset and generalized onset seizures. The results we obtained may help find the etiological cause in patients with seizure complaints and may guide their treatment. In addition, knowing the seizure type of the patient can give an idea about the prognosis of their disease.

MATERIALS AND METHODS: The total number of patients included in this prospective study was 58. There were also 57 people in a control group. The patients were classified according to their type of seizure: focal or generalized onset. The serum oxidative stress index (OSI) and total oxidant/antioxidant status values of all patients and control group members were measured. The patients (focal/generalized groups) and control group members were compared.

RESULTS: This prospective study was completed with 58 eligible patients who met the inclusion criteria. There were 57 people in the control group. Total oxidant status (TOS) and OSI levels were higher in the seizure groups compared to the control group ($p<0.05$). The difference between the serum TOS and OSI levels of patients with generalized or focal onset seizures was statistically significant.

CONCLUSION: Classifying patients according to their seizure types by looking at their oxidative stress levels can guide treatment (in terms of investigating antioxidant activity) and give an idea about prognosis. This study showed the importance of TOS and OSI levels in patients presenting with seizures. This was particularly evident in generalized onset seizures. An evaluation of serum TOS and OSI levels in patients presenting with seizures may help us in clinical diagnosis, treatment, and classification.

Keywords: Epilepsy, total oxidant status, seizure types, total antioxidant status

INTRODUCTION

Epilepsy is a common disorder characterized by seizures. It affects people of all ages. Anamnesis, neurological examinations, neuroimaging, and electroencephalography (EEG) examinations are essential in diagnosis. Since epilepsy is a complex disease group, it is difficult to diagnose and classify. Classification is necessary for the prognosis and treatment of this disease. According to the seizure types, epilepsies were determined by the International League Against Epilepsy (ILAE) in 2017; seizures were classified as focal onset, generalized onset, and seizures of unknown origin.[1,2]

The presence of oxidative stress has been shown in the pathogenesis of this disease.[3,4] Studies have shown that oxidative stress levels are higher in epilepsy patients than in healthy individuals.[5,6] Structural changes resulting from oxidative stress appear as an increased risk of seizures or

To cite this article: Taşlıdere B, Uslu F, Sönmez E, Selek Ş. Investigation of Total Antioxidant Status and Total Oxidant Status with Seizure Types in Patients with Epilepsy. Cyprus J Med Sci 2022;7(5):609-613

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Received: 25.03.2022
Accepted: 03.06.2022

recurrent seizures.[7] Total antioxidant status (TAS), a marker of oxidative stress, and total oxidant status (TOS) have been defined in recent years. The presence of high TOS and oxidative stress index (OSI) and low TAS levels in epilepsy patients have been shown in studies.[8,9]

Studies on epileptogenesis are noteworthy. This study investigated the levels of oxidative stress markers (TOS, TAS, OSI) in patients with focal and generalized onset seizures. We hope that the results we obtained will contribute to the classification of patients with seizure complaints. This may be particularly beneficial for patients in the group whose seizure type cannot be classified. In addition, knowing the seizure type of the patients can give an idea about the prognosis of the disease and can guide antioxidant treatment strategies.

MATERIALS AND METHODS

This study was conducted in the Emergency Medicine and Clinical Biochemistry departments. Bezmialem Vakıf University Ethics Committee approval (approval number: 2021/177, date 29.04.2021) was obtained for this study. The research was completed in accordance with the criteria specified in the Declaration of Helsinki. The number of patients included in this study was 58. The study was completed by forming a control group of 57 healthy individuals. Informed consent was obtained from the patients with epilepsy (or their primary relatives) and those in the control group. All patients were diagnosed with epilepsy according to the ILAE diagnostic criteria by evaluating their clinical symptomology, EEGs, and imaging findings. The age, gender, seizure type, body mass index, and clinical characteristics of the people included in this study were recorded. Patients were classified according to their type of seizure: either focal or generalized onset.

According to the results of the EEG, they were grouped into those with or without epileptogenic findings. Those whose seizure type was not determined (seizures that could not be classified according to ILAE 2017 seizure classification), without EEG, those with chronic systemic diseases, those using immunosuppressive drugs, pregnant women, substance abusers, chronic drug users (excluding anti-epileptics), those under the age of 18 years, and those who did not give consent were excluded from this study (Figure 1). The physical and neurological examinations of the control group were normal. They had similar age and gender characteristics to the patient group. The blood TOS, TAS, and OSI values of all patients and control group individuals were measured. Serum samples were obtained from those patients with epilepsy who visited the emergency department with seizures, during or immediately after a seizure, within the first hour for patients who had seizures at home and at any time in the healthy controls. Venous blood samples of 10 cc were taken from the seizure and control groups, centrifuged at 5,000 rpm for 10 minutes, and stored at -80 ºC. Biochemical measurements were made at the Clinical Biochemistry Laboratory of our hospital.

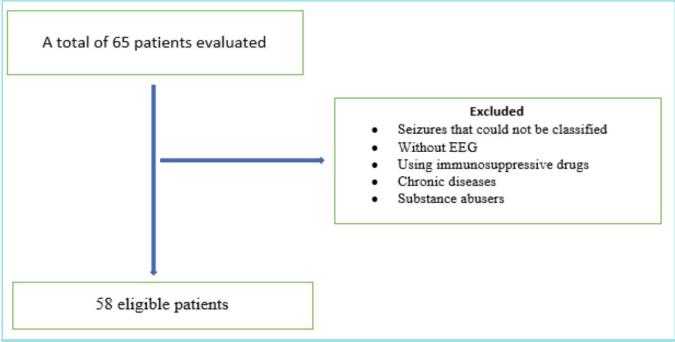

Figure 1. Patient flow diagram for inclusion in this study.
EEG: electroencephalography.

The OSI, TAS and TOS values of the patient (focal and generalized) and control groups were compared. The laboratory parameters and EEG findings of the epileptogenic and non-epileptogenic patient groups were statistically compared.

Measurement of the TOS: A method developed by Erel[10] was used to measure the TAS level of the serum. In this method, hydroxyl radical is formed by using the iron solution and hydrogen peroxide. Thus, the anti-oxidative effect against free radical reactions is measured.[10]

Measurement of TAS: The TAS level was measured via the colorimetric method developed by Erel[10]. He used the hydroxyl radical in this method. Oxidative reactions initiated by the addition of a plasma sample provide an effective measure of the plasma TAS level.[11]

Determination of OSI: The formula used for the OSI value is OSI = TOS (mmol)/TAS (mmol).[12]

Statistical Analysis

Quantitative variables were determined using centralization and measures of variance (mean ± standard deviation). The Kruskal-Wallis and the Mann-Whitney U tests were used in cases where the assumptions of normality and homogeneity were not met to show behavioral differences of the group averages. A value of $p=0.05$ or below was determined to be statistically significant. Statistical analyses were carried out with the IBM Statistics Package for Social Sciences for Windows program. According to an ANOVA test, the required sample size was 58 for the effect size - Cohen's $(f)=0.4$ and power $(1-\beta)=0.80$ at ($\alpha=0.05$ statistical significance).

RESULTS

This study was completed with 58 eligible patients who met the inclusion criteria. It was a prospective study. There were thirty males (51.7%) and 28 females (48.3%) in the patient group with a mean age was 43.5±19.3 years. The control group had 57 people. There was no significant demographic difference between the seizure and control groups. The clinical features of the control and epilepsy groups are shown in Table 1. An EEG examination of all patients was performed. Epileptogenic activity was found in 16 (27.6%) patients. Computed tomography was performed in 21 (36.2%) patients. Thirty-seven (63.8%) patients were using a single anti-epileptic, thirteen (22.4%) used multiple anti-epileptics, and eight (13.8%) were not using any anti-epileptics. There were 42 patients with generalized onset seizures (36.5%), 16 with focal onset seizures (13.9%), and a control group which consisted of 57 subjects (49.6%). The routine laboratory results obtained from the patients are given in Table 1.

The TOS and OSI levels were significantly higher in the seizure group compared with the control group (Table 1) ($p<0.05$). In the serum taken from the patients, the TOS level was 32.44±23.78, and the OSI level was 4.67±3.81. When the TAS levels were examined, no statistically significant difference was found between the seizure and control groups. (TAS=0.71±0.19). There was a statistically significant difference between the serum TOS and OSI levels in the generalized

Table 1. The clinical and laboratory features of the control and seizure groups

		Seizure (n=58) (mean ± SD)	Control (n=57) (mean ± SD)	p-value
Age	Years	43.59±19.33	43.15±9.24	0.617
Gender	Female	28 (48.3%)	28 (49.1%)	0.908
	Male	30 (51.7%)	29 (50.8%)	
BMI	Kg/m^2	25.76±2.65	26.89±2.44	0.377
TAS	mmolTrolox Eq/L	0.71±0.19	0.74±0.09	0.574
TOS	µmol H_2O_2 Eq/L	32.44±23.78	3.78±4.13	<0.05
OSI	Arbitrary unit	4.67±3.81	0.54±0.58	<0.05
Antiepileptic	Single	37 (63.8%)	-	-
	Two or more	13 (22.4%)	-	-
	Not using	8 (13.8%)	-	-
Seizure types	Generalized	42 (36.5%)	-	-
	Focal	16 (13.9%)	-	-
WBC	$10^{\wedge}3/\mu L$	11.05±4.65	8.35±2.05	<0.05
Hemoglobin	g/dl	13.43±2.35	13.62±1.95	0.690
Platelet	$10^{\wedge}3/\mu L$	267.64±88.09	275.67±89.62	0.554
Sodium	mmol/L	137.71±3.93	135.65±4.15	0.060
Potassium	mmol/L	4.09±0.47	3.6±0.62	0.529
Calcium	mg/dL	9.38±0.53	8.76±0.75	0.316
BUN	mg/dL	14.48±6.64	12.8±1.6	0.334
Creatine	mg/dL	0.89±0.48	0.95±0.24	0.790
AST	U/L	27.76±23.45	31.77±17.46	0.433
ALT	U/L	24.55±14.25	29.98±16.51	0.352
Albumin	g/L	3.8±0.53	3.76±0.75	0.252

TAS: total antioxidant status, TOS: total oxidant status, OSI: oxidative stress index, WBC: white blood cell, AST: aspartate aminotransferase, ALT: alanine aminotransferase, BUN: blood urea nitrogen, BMI: body mass index.

onset and focal onset patient groups (p^1<0.05) (Table 2). Likewise, there was a statistically significant difference between the serum TOS and OSI values of patients with generalized onset seizures and the control group (p^3<0.05) (Table 2). There was no significant difference between the focal onset seizure patients and the control group in terms of TAS, TOS, and OSI levels (p^2>0.05) (Table 2). No statistically significant difference was found between the serum TOS, TAS, and OSI levels obtained from those patients with normal and epileptiform activity in the EEG (Table 3).

DISCUSSION

The presence of high oxidative stress levels and low antioxidant status in patients with epilepsy has been shown in many studies.[13-15] This study evaluated the relationship between oxidative stress biomarkers (TAS, TOS, and OSI) and seizure types in patients with epilepsy. TOS and OSI levels were higher in the group with epileptic seizures. The difference between them was statistically significant when compared with the control group. At the same time, TAS levels were lower in the epilepsy groups compared with the control group (Table 1). This result suggests that low antioxidant levels contribute to the epileptogenesis process. The brain has a high oxidative metabolism, and when antioxidant capacity decreases, seizures can be seen through neuronal hyper-excitability.[16,17] In fact, the deterioration of the oxidant/antioxidant balance, which occurs with a decrease in antioxidant enzyme activities, may pave the way for generalized seizures.[18]

In this study, serum TOS and OSI levels were significantly higher in the generalized onset seizure group compared with both the focal and control groups (Table 2: p^1, p^3). The difference between serum TAS, TOS and OSI levels measured in the focal-onset group and the control group was not statistically significant (Table 2: p^2). Focal seizures are caused by abnormal electrical activity originating in an area of our brain.[19,20] Can it be said that focal seizures do not affect the oxidative balance

Table 2. The TOS, TAS and OSI levels in focal, generalized and control group

	Seizure types (mean ± SD)		Control (57) (mean ± SD)	p-value*		
	Focal (n=16)	Generalized (n=42)		p^1	p^2	p^3
OSI	3.43±3.07	5.14±3.99	0.54±0.58	**<0.05**	0.277	**<0.05**
TAS	0.68±0.22	0.72±0.18	0.74±0.09	0.898	0.574	1
TOS	22.91±17.03	36.07±25.13	3.78±4.13	**<0.05**	0.358	**<0.05**

p^1: focal/generalized, p^2: focal/control, p^3: generalized/control, *Kruskal-Wallis test, SD: standard deviation, TOS: total oxidant status, TAS: total antioxidant status, OSI: oxidative stress index.

Table 3. TOS, TAS, and OSI levels obtained from patients with activity in the EEG

	Electroencephalography		p-value*
	Epileptogenic (n=16) (mean ± SD)	Normal (n=42) (mean ± SD)	
OSI	5.32±4.68	4.42±3.46	0.645
TAS	0.69±0.22	0.71±0.18	0.709
TOS	34.0±25.79	31.85±23.28	0.830

*Mann-Whitney U test, TOS: total oxidant status, TAS: total antioxidant status, OSI: oxidative stress index, EEG: electroencephalography, SD: standard deviation.

significantly? In our study, it was revealed that the use of biomarkers, such as TAS, TOS, and OSI, might not be useful in focal-onset seizures.

Whether high TOS is a cause, or a consequence of seizures should be further investigated. If it is the cause of seizures, we can conclude that high TOS and OSI levels may trigger a generalized seizure. If it is the result of seizures, we can say that focal seizures (because they remain more localized) do not increase TOS and OSI levels as much as generalized seizures. In the classification of seizure types made by the ILAE in 2017, patients whose seizure type could not be determined were included in the "unknown seizures" group.[21,22] However, the classification of epilepsy patients according to seizure type is clinically valuable because such classifications guide treatment and can give us an idea about the prognosis of the disease.[23,24] In epilepsy, the distinction between focal and general seizures should be made whenever possible. This information is important in deciding which treatment plan is more effective. Some drugs of choice for treatment (e.g., carbamazepine and lamotrigine) are often used to treat focal seizures, while others (e.g., sodium valproate) are usually used for generalized tonic-clonic seizures. TAS, TOS, and OSI levels can help determine the type of seizure.

It should be investigated whether it is possible to classify seizures of unknown onsets by looking at the serum TAS and TOS levels of the patients. Knowing and recognizing EEG findings is also important in the early diagnosis of diseases, in selecting appropriate antiepileptic drugs, and in evaluating the response to treatment.[25] Therefore, our study evaluated the TAS, TOS, and OSI levels of patients with typical EEG findings and patients whose EEG was considered normal, but we observed no statistically significant difference. About half of all EEGs for patients who have already had seizures are interpreted as normal.[26] EEG detects interictal epileptiform discharges in about 50% of patients with epilepsy.[27] In our study, no relationship was shown between EEG results and oxidative stress markers, but using video EEG in future studies may be illuminating in this regard.

The seizure group had higher white blood cell levels than the control group, which may be a result of muscle activity during seizures. Many studies support this result.[28] Renal and hepatic function tests (urea, creatinine, aspartate aminotransferase, alanine aminotransferase, an albumin) were within normal limits in all patients (Table 1). Even if laboratory parameters do not have a place in the diagnosis of epileptic seizures, they can be a guide for etiology and treatment (e.g. electrolyte imbalances can manifest with seizures). It is reasonable to check the hemogram, blood urea nitrogen, creatinine, electrolyte, and liver panel.

Study Limitations

Our study was limited by its small sample size. This study's limitations were the absence of video EEG and the fact that some seizures were experienced at home and could not be monitored by clinicians.

CONCLUSION

Classifying patients according to seizure types by looking at their oxidative stress levels can guide treatment (in terms of investigating antioxidant activity) and give an idea about prognosis. This study showed the importance of TOS and OSI levels in patients presenting with seizures. This was particularly evident in generalized onset seizures. An evaluation of serum TOS and OSI levels in patients presenting with seizures may help in the clinical diagnosis, treatment, and classification of epilepsy.

MAIN POINTS

- Since epilepsy is a complex disease group, it is difficult to diagnose and classify.
- This study evaluated the relationship between oxidative stress biomarkers and seizure types in patients with epilepsy.
- Serum TOS and OSI levels were significantly higher in the generalized onset seizure group compared with both the focal group and the control group.
- Evaluating serum TOS and OSI levels in patients presenting with seizures may help in the clinical diagnosis, treatment, and classification of epilepsy.

ETHICS

Ethics Committee Approval: Bezmialem Vakıf University Ethics Committee approval (approval number: 2021/177, date 29.04.2021) was obtained for this study.

Informed Consent: Informed consent was obtained from the patients with epilepsy (or their primary relatives) and those in the control group.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Concept: B.T., F.U., E.S., Ş.S., Design: B.T., F.U., E.S., Ş.S., Data Collection and/or Processing: B.T., F.U., E.S., Ş.S., Analysis and/or Interpretation: B.T., F.U., E.S., Ş.S., Literature Search: B.T., F.U., E.S., Ş.S., Writing: B.T., F.U., E.S., Ş.S., Critical Review: B.T., F.U., E.S., Ş.S.

DISCLOSURES

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The author declared that this study had received no financial support.

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