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Serum Electrolyte Values and Blood Oxidative Stress Parameters in Patients with Focal and Generalized Epilepsy

Gamze Sarı[1], Aysu Kılıç[2], Ferda Ilgen Uslu[3], Şahabettin Selek[4], Savas Ustunova[2], Ismail Meral[2*]

[1]Bezmialem Vakif University, Faculty of Medicine, Istanbul, Turkey
[2]Bezmialem Vakif University, Faculty of Medicine, Department of Physiology, Istanbul, Turkey
[3]Bezmialem Vakif University, Faculty of Medicine, Department of Neurology, Istanbul, Turkey
[4]Bezmialem Vakif University, Faculty of Medicine,Department of Biochemistry, Istanbul, Turkey

ABSTRACT

Because epilepsy pathogenesis is affected by serum minerals and antioxidant enzymes, this study was designed to evaluate whether there was a relationship among electrolytes (Na, Ca, K, Cl, P, Mg), trace elements (Fe, Zn, Cu) and oxidative stress in patients with generalized or focal epilepsy.

Twenty healthy individuals (control group), and 20 focal and 20 generalized epilepsy patients (patient groups) were included in the study. Control and patient groups were matched with age (30-40 years old), body mass index (25 -28) and sex (10 males and 10 females). Serum Na, Ca, K, Cl, P, Mg and Fe levels were analyzed in an autoanalyzer, serum Zn and Cu were analyzed in a mass spectrophotometer, total antioxidant status, total oxidant status and myeloperoxidase levels were measured spectrophotometrically.

The oxidative stress index increased ($p<0.001$) and the serum Na levels decreased ($p<0.001$) in focal epilepsy patients compared to the controls.

It has been concluded these changes may make focal epilepsy patients more susceptible to seizures than generalized epilepsy patients.

Keywords: Focal epilepsy, generalized epilepsy, oxidative stress, trace elements, minerals

Introduction

Epilepsy is a chronic disorder that causes periodic abnormal movements, abnormal sensation and behavior, and sometimes loss of consciousness. The formation of seizures is caused by the release of spontaneous, fast and local electrical impulses from gray matter (1). Epilepsy may affect all age groups regardless of ethnicity (2). In epilepsy, symptoms vary depending on the type of seizure. However, a person has usually a type of seizure with similar symptoms. Seizures are simply divided into focal and generalized seizures depending on whether the origin of the abnormal impulses in the brain is regional or generalized (3). Although the etiology of epilepsy is not fully defined, infections in the brain, tumors, prenatal injuries, head injuries, changes in body mineral level and genetic factors can affect epilepsy formation. Epilepsy has idiopathic, symptomatic and cryptogenic etiologies (4).

Oxidative stress is one of the major factors of the pathogenesis of many acute and chronic diseases. Some studies have indicated that oxidative stress plays a critical role in the development of epilepsy (5). There are some studies showing that cholinergic markers and muscarinic receptors such as acetylcholine decrease and epilepsy develops due to oxidative stress and lipid peroxidation (6). Although the reactive oxygen and nitrogen products are involved in the pathology of many neurological diseases such as stroke, spinal cord injury, Parkinson, Alzheimer, Huntigton and amyotrophic lateral sclerosis (7), the role of these products in epilepsy is still not fully understood.

Minerals and trace elements have a crucial role in sustaining immunity by providing antioxidant defense (8). Some studies suggest that antioxidant trace elements are abnormal in epileptic patients and may have role in the pathogenesis of epilepsy (9, 10). It is speculated that the reduction of

***Corresponding Author:** Ismail Meral, Department of Physiology, School of Medicine, Bezmialem Vakif University, 34093 Istanbul, Turkey
E- mail: imeral@bezmialaem.edu.tr, Phone: +90 212 5232288/3193, Fax: +90 212 6217578
ORCID ID: Gamze Sarı: 0000-0002-8089-3683, **Aysu Kılıç:** 0000-0002-8593-1415, Ferda Ilgen Uslu: 0000-0002-2124-5037, **Şahabettin Selek:** 0000-0003-1235-3957, **Savas Ustunova:** 0000-0003-1870-229X, **Ismail Meral:** 0000-0002-0973-4305
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antioxidant protective mechanisms and the increase of active oxygen metabolites may increase the risk of seizures and may play a role in neuronal death due to convulsion-induced oxidative damage. It has been suggested that there might be a direct relationship between trace elements and epilepsy (11). However, the number of studies carried out to investigate the serum mineral levels in epilepsy patients are limited. Studies with epilepsy and serum mineral concentrations have mostly concentrated on Zn and Cu, and different results were obtained.

In this study, the serum levels of many minerals (Na, Ca, K, Cl, P, Mg) and trace elements (Fe, Zn, Cu), and blood oxidative stress parameters were evaluated in patients with focal and generalized epilepsy.

Materials and Methods

The study included 20 healthy individuals, 20 focal epilepsy patients, and 20 generalized epilepsy patients who applied to Neurology Services and Polyclinics, Bezmialem Vakif University Hospital, Istanbul, Turkey. All ethical procedures were approved by the non-interventional research ethics committee, Bezmialem Vakif University. An informed consent form was read and signed to obtain the participants' consent that they voluntarily participate. Compliance of the patients who will participate in the study with the inclusion and exclusion criteria has been checked. Inclusion criteria include similar age (between 30 - 40 years old) and body mass index (25 -28).

Considering the distribution of the patients, 20 patients were idiopathic generalized epilepsy, and 20 patients were focal epilepsy. Focal epilepsy etiologies of patients were 5 symptomatic and 15 cryptogenic-focal epilepsy of unknown cause. All patients were diagnosed for the first time and 12 were receiving levetiracetam low dose (starting dose), 2 were receiving low dose valproic acid therapy, 26-patient was yet drug-free. Exclusion criteria includes chronic additional diseases and related drug use, psychiatric diseases, pregnancy, smoking, alcohol, or drug use.

Biochemical Analysis: Analyzes was made on routine blood taken from the participants and new vascular access was not opened. The blood of the control group was made up of those who applied to the hospital but could not be diagnosed with any disease. The serum was separated by centrifugation at 3.500 x g for 5 min and were stored at −80°C until the day of analysis.

Serum Trace Elements and Minerals Analysis: Serum Na, Ca, K, Cl, P, Mg and Fe levels were analyzed in an auto-analyzer (Abbott C16000, Abbott Diagnostics, Abbott Park, IL), and serum Zn and Cu levels were analyzed in a mass spectrophotometer using appropriate kits according to manufacturers' instructions (Rel Assay Diagnostics, inductively coupled plasma - mass spectrometer - ICP / MS - Agilent 7700x; Agilent Technologies Japan, Tokyo, Japan).

Total Antioxidant Status, Total Oxidant Status and Myeloperoxidase Analysis: Erel's method was used for total antioxidant status (TAS) and total oxidant status (TOS) and myeloperoxidase (MPO) measurements. (12, 13). TAS, TOS and MPO were measured in serum samples at 240nm and 520nm wavelengths, respectively, with a plate reader (Thermo Scientific Multiskan FC, 2011-06, USA). Trolox, a water-soluble vitamin E compound, was used as the calibrator for TAS measurements, and the results were expressed as mmol.Trolox equivalent/L. H_2O_2 was used as the standard for TOS measurements and the results were expressed as μmol·H_2O_2 equivalent/L. After TAS and TOS levels were determined, the results were calculated according to the following formula. Results were expressed as Arbitrary Unit to calculate the oxidative stress index (OSI) value.

Statistical Analysis: All data are shown as mean ± standard error of mean (mean ± SEM). The Shapiro–Wilk test was used to determine the distribution of the data. Data were analyzed by One-way ANOVA and Kruskal-Wallis test followed by Bonferroni and Dunn post-hoc tests using GraphPad Prism 6.0 (GraphPad Prism Software, San Diego, CA, USA). $P < 0.05$ indicated a statistically significant result.

Results

Serum Trace Elements and Minerals: Serum trace element and mineral values of groups are presented in table 1. The Zn, Cu, Mg, Ca, and K levels were not different ($p>0.05$) in both patient groups comparing to controls. The P level was higher ($p<0.05$), and both Cl and Na levels were lower ($p<0.05$, $p<0.001$, respectively) in focal epilepsy patients comparing to controls. The Fe level was higher ($p<0.05$) in generalized epilepsy patients comparing to controls. However, P, Cl and Fe levels were still within the normal ranges despite the changes obtained when compared to controls.

Oxidative Stress: TAS level was found to be the lowest ($p<0.001$) in the focal epilepsy patients

Table 1. Levels of serum minerals and trace elements in control (C), focal epilepsy patients (F) and generalized epilepsy patients (G)

PARAMETER	C	F	G
	n=20	n=20	n=20
Cu (μg/dl)	101,7±4,63	99,12±4,35	95,35±2,67
Zn (μg/dl)	81,67±1,98	79,42±1,5	84,42±1,52
Fe (mg/dl)	71,17±3,24	69,04±3,67	97,08±6,8*
Mg (mg/dl)	2,025±0,02	2,023±0,03	2,015±0,03
P (mg/dl)	3,071±0,09	3,388±0,11*	3,325±0,12
Ca (mg/dl)	9,642±0,07	9,488±0,1	9,608±0,07
Cl (mEq/L)	102,3±0,37	100,2±0,99*	101,3±0,51
K (mEq/L)	4,38±0,06	4,323±0,06	4,351±0,08
Na (mEq/L)	140±0,46	135,5±1,12***	138±0,53

Data are mean ± SEM
*; p<0.05, **; p<0.01, ***; p<0.001 statistical significance comparing to controls
P, Cl and Fe levels are still within the normal ranges

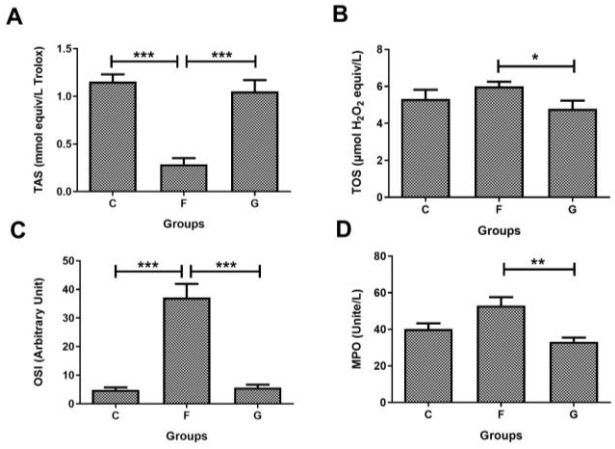

Fig. 1. The serum TAS (A), TOS (B), OSI (C) and MPO (D) levels of the study groups (n=20). Data is presented as mean ± SEM. *; p<0.05, **; p<0.01, ***; p<0.001 statistical significance

comparing to the control and generalized epilepsy patients (Fig. 1A). TOS levels were found to be higher (p<0.05) in focal epilepsy patients comparing to generalized epilepsy patients (Fig. 1B). The OSI level was significantly (p<0.001) higher in focal epilepsy patients comparing to controls and generalized epilepsy patients (Fig. 1C). The MPO level was also found to be higher (p<0.05) in focal epilepsy patients comparing to generalized epilepsy patients (Fig. 1D).

Discussion

Oxidative stress contributes to the pathogenesis of many neurological diseases, such as Alzheimer's disease, Huntington's disease, etc. according to previous studies (7, 14). It has been also shown that the oxidative stress due to excessive free radical production may be related to epilepsy formation and progression (15, 16). Low antioxidant levels were found in patients with epilepsy than healthy individuals, in a study conducted to evaluate the antioxidant status in epileptic patients (17). In our study, serum TOS levels were higher and serum TAS levels were lower in focal epilepsy patients compared to control and generalized epilepsy patients. The group with the highest oxidative stress index was focal epilepsy group. To support this finding, MPO values were increased in patients with focal epilepsy. Similarly, Wu, Liu (18) showed that the level of oxidative damage marker malondialdehyde increased and the antioxidant marker glutathione (GSH) decreased in patients with focal epilepsy. Increased oxidative stress leads to alteration in neurotransmitter release and expression of ion channels, resulting in a neuronal hyperexcitability by changing the neuron's physiological functions (19). The changes that occur due to oxidative stress may induce neuronal death by causing necrosis and apoptosis, that can cause the development of epilepsy (20). In addition, oxidative stress can also cause mitochondrial dysfunction, thus leading to an increase in neuronal excitability by causing a decrement in ATP production and a decrement in Na^+/K^+ ATPase activity, which is responsible for the maintenance of resting membrane potential (21, 22).

Electrolyte imbalance such as Na, K, Ca and Mg aberrations are factors that facilitate seizures in epileptic susceptibility and disease (23-25). There is no significant difference was detected in the serum Ca, K and Mg levels of patients in both epilepsy groups in our study. However, serum Na level decreased in patients with focal epilepsy. The

Na ion is essential for neuronal firing. Some changes in Na currents can lead to abnormal neuronal activity, such as in epilepsy (26). The main excitatory neurotransmitter of the central nervous system is glutamate, and the main inhibitory neurotransmitter is gamma-aminobutyric acid (GABA). The ionotropic receptors of glutamate are N-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) and kainate receptors, which are highly permeable to Na flow. It is also known that increased NMDA, AMPA and kainate receptor activity can cause epileptic seizures in animal and human studies (27-29). A decrease in Na level also increases susceptibility to epileptic seizures (23). Under hypoosmotic conditions, the extracellular concentration of glutamate increases in the brain. Increased Na influx into the cell is initially due to Na/K ATPase dysfunction and Na influx through over activated ionotropic glutamate receptors (30). Accordingly, hyperosmolarity increases the amplitude of excitatory postsynaptic potentials via stimulating hyperexcitability and epileptiform activity (31). Nigro, Winzeler (32) suggested in their prospective study that, 5% of patients with severe hyponatremia had acute epileptic seizures and focal neurological deficits.

It has been concluded that focal epilepsy patients have higher blood oxidative stress and lower serum Na comparing to healthy individuals, which may make focal epilepsy patients more susceptible to seizures than generalized epilepsy patients. However, more studies are needed.

Conflict of Interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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