Study on serum zinc and selenium levels in epileptic patients

Heidar N. Farahani, PhD, Alireza R. Ashthiani, PhD, Mher S. Masihi, MD.

ABSTRACT

Objective: To estimate serum zinc and selenium levels in a group of healthy subjects and correlate these with epileptic patients.

Methods: This case-control study was conducted in the Department of Neurology, Valiasr Hospital, Iran, between November 2011 and May 2012 on 40 patients aged 26.63±5.78 years who were diagnosed with generalized and a single epileptic form of epilepsy by a neurologist for the first time. The control group was selected from healthy individuals, and matched to the case group. Serum zinc was measured by the calorimetric method using a Randox kit. Selenium was measured by atomic absorption spectroscopy.

Results: The mean zinc level was 151.2±29.75 µg/dl in the cases, and 181.63±60.19 µg/dl in the controls (p=0.006). The mean selenium level was 73.37±13.31 µg/l in cases compared with 85.55±19.39 µg/dl in controls (p=0.002). We also found a significant difference between the selenium levels by gender in the 2 groups (p=0.03).

Conclusion: We observed statistically significant changes in serum zinc and selenium levels of epileptic adult patients in comparison with the control group. As zinc and selenium are used as potent antioxidants in cellular interactions, changes in their serum values may result in enzymatic changes, which in turn can cause neurological disorders.

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Behavioral disorders and epilepsy are one of many health problems in humans. After different types of cardiac and brain infarctions, epilepsy, which is a common neurological problem, has the highest prevalence. Various reports show that changes in levels of some electrolytes and trace elements in the body play a vital role in predisposing to conditions of epilepsy. The term “epilepsy” comes from the Greek word meaning to hold or to seize, available in Greek culture from fifth century BC. Therefore, human basic knowledge of the disease dates from at least 2400 years ago. Epilepsy is one of the most common neurological disorders, with a prevalence of 0.5%, and incidence of 45 in 100,000 in the general population. The prevalence of epilepsy in Iran is not available, though its prevalence in Tehran, the capital of Iran, is believed to be 0.83% by some reports, and after mild mental illnesses, and mental retardation has been the most common mental health problem. Attitudes and awareness among people towards epilepsy differs significantly in developing countries and developed countries. Recent studies show a positive attitude change in this area in developed countries, while there is a negative attitude towards epilepsy in developing countries due to lack of proper understanding.

Zinc (Zn) is an essential trace element for all organisms. In human subjects, body growth and development are strictly dependent on zinc. The nervous, reproductive, and immune systems are particularly influenced by zinc deficiency, as well as by increased levels of zinc. It is also a cofactor of more than 100 enzymes in the human body. Human zinc deficiency was first hypothesized with considerable supportive evidence in the early 1960s, which was related to adolescent nutritional dwarfism in Egypt and Iran and was also noted at younger ages. Also, various reports show common zinc deficiency in developing countries such as Iran, whose people have diets rich in rice. Symptoms resulting from zinc deficiency are as diverse as the enzymes with which it is associated and if chronic, severe, and untreated, this deficiency can be fatal. Symptoms include infections, hypogonadism, weight loss, emotional disturbance, dermatitis, alopecia, impaired taste acuity, night blindness, poor appetite, delayed wound healing, and elevated blood ammonia levels. The divalent cation zinc is abundant in the brain, particularly in the hippocampus. Recent evidence suggests that zinc is packaged into synaptic vesicles in this region and can be co-released with neurotransmitters. Zinc inhibits the activity of γ-aminobutyric acid type A (GABA(A) receptors and the sensitivity of the receptor to zinc is influenced by its alpha subunit type composition. The alpha 4, alpha 5, and alpha 6 subunits confer greater sensitivity to zinc than receptors containing other alpha subunits. The alpha 4 and alpha 5 subunits are highly expressed in hippocampal neurons, and likely mediate any effects of zinc on GABAergic neurotransmission in this area. Histidine 195 of the alpha 5 subunit plays an important role in determining the sensitivity of recombinant GABA(A) receptors to zinc and different gene mutations can increase or decrease this sensitivity.

Selenium (Se) is a nutritional trace element essential for various aspects of human health that exerts its effects mainly through its incorporation into selenoproteins as the amino acid, selenocysteine. Twenty-five selenoprotein genes have been identified in humans and several selenoproteins are broadly classified as antioxidant enzymes. As progress is made on characterizing the individual members of this protein family, it is becoming clear that their properties and functions are quite diverse. Some reports also show improvements in the antioxidant status of epileptic patients after treatment with antiepileptic drugs.

Since various studies have shown different results regarding the possible roles of zinc and selenium in epilepsy, it seems that zinc, selenium, and epilepsy have an unknown relationship with each other and there is no definite opinion on the effect of these elements on epilepsy. Thus, this study was carried out with the purpose of evaluating zinc and selenium levels in the serum of epileptic patients and comparing this with healthy individuals. We hope that with a better understanding of the etiology and pathogenesis of epilepsy, it will be possible to find more effective and modern treatments for this disease.

Methods. This case-control study was conducted in the Department of Biochemistry, Faculty of Medicine, Arak University of Medical Sciences, Arak, Iran between November 2011 and May 2012, and was approved by the Institutional Ethical Committee of Arak University of Medical Sciences. All patients involved in the study provided signed informed consent of the experimental protocol as recommended by the ethics committee and in accordance with the Helsinki declaration. Forty patients aged 18-50 years, admitted to Valiasr hospital in Arak, Iran, diagnosed with generalized and a single epileptic form of epilepsy by a neurologist for the first time were recruited as cases. Upon questioning, individuals who used supplements containing zinc, selenium, vitamin A and D, had a history of recent head trauma, weight loss, diarrhea, cigarette smoking and addiction, alcohol consumption, or use of certain medications were excluded from the study.
The control group (n=40) was selected from healthy male and female individuals, in whom no disease was diagnosed, and matched with the case group for age, gender, and economical status. After history taking, physical examination, and completing the questionnaire, with consideration of ethical issues and consent of subjects before the administration of any antiepileptic drug, 5ml of venous blood was taken and centrifuged at 3000 rpm for 10 minutes and the serum was isolated. Then, each sample of serum was divided into 2 equal parts, to measure zinc and selenium separately, and was frozen and stored at -30ºC until the time of analysis. The samples were collected during winter and spring. All the glassware and bottles used for the isolation and analysis of serum were previously soaked in diluted nitric acid (10%) and rinsed thoroughly with double distilled water.

Duplicated serum zinc was measured by the calorimetric method using a Randox kit (Randox Laboratories Ltd, Ardmore, Crumlin Co. Antrim, UK) in the Research Laboratory of Biochemistry of Arak University of Medical Sciences, and selenium was measured by atomic absorption spectroscopy in Noor Pathobiology Laboratory, Tehran, Iran. After Reducing Se (VI) to Se (IV) and cooling to room temperature (25ºC), the selenium concentration was measured using the hydride generation technique. Sodium borohydride solution was used as a reducing agent.

**Statistical analysis.** At first, Kolmogorov-Smirnov test (KS-test) was used to confirm the normal distribution of obtained data. Then, independent sample t-test was used to compare the data between the 2 groups. \( P \)-values of less than 0.05 were considered to be significant. For the comparison of zinc and selenium between the 2 groups by gender, a general linear model (GLM) was used. Results were calculated from logistic regression analyses with 95% confidence intervals. All data were analyzed using the Statistical Package for Social Sciences version 20 (SPSS Inc., Chicago, IL, USA).

### Results.
From 40 patients, 62.5% were single and 37.5% were married, while in the control group (n=40) 60% were single and 40% married. Regarding the residential status of subjects, 72.5% of patients were from urban areas and 27.5% from rural areas. While 85% of the control group was from urban areas and only 15% from rural areas. The ratio of males to females was the same between the 2 groups. Women comprised 57.5%, while men 42.5%. The average age ± standard deviation for members of the patient group was 26.6±5.7 years, while for the control group it was 27±5.4 years. Results comparing the average zinc and selenium levels in epileptic patients with the healthy controls by gender are shown in Table 1. There was no statistically significant difference observed between average zinc levels by gender in the 2 groups. However, a statistically significant difference was observed between serum selenium levels in epileptic patients, and there was a significant interaction between selenium levels by gender in the 2 groups (\( p=0.015 \)).

Table 1 summarizes the mean serum zinc and selenium levels in epileptic patients compared with healthy controls, both of which were found statistically significant (\( p=0.006 \) for zinc, and \( p=0.002 \) for selenium) (Table 2).

### Discussion.
The current study in patients who were diagnosed with generalized and a single epileptic form of epilepsy by a neurologist for the first time shows that there are changes in zinc and selenium levels of epileptic adult patients in comparison with healthy adults, with both parameters statistically significant. However, based
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on gender, only selenium levels showed a statistically significant difference. Our study limitation was that we did not correlate the present results with serum copper, lead, and other trace elements, which may have anti-

selenium or anti-zinc actions, and prevent their transfer into blood.

Previous studies, which were mainly focused on children with febrile seizures showed that serum levels of zinc in these children were lower than the children in the control group.15-18 Another study showed that variations in zinc levels in rats exacerbated their epilepsy and associated neurological disorders in which the GABAergic system played an important role.19 Studies conducted on convulsive children indicated a decrease in serum selenium levels is also associated with seizures.18,20 Decreased levels of selenium and the activity of selenium-dependent enzymes has also been studied in epileptic children, which showed a strong connection between their reduction and pathogenesis of epilepsy.21 A few studies have been carried out on adults, which also show that serum levels of zinc and copper in epileptic patients were lower than the control group, but these results were not statistically significant.2 While the results of our study are consistent with results of some previous studies,14-19,21 they also showed slight discrepancies with other studies.2,22,23 Mousali and coworkers reported that high cholesterol, hypertension, and diabetes mellitus may act as risk factors for epilepsy in elderly patients.3 Some other investigators showed management of epilepsy and a decrease of serum zinc levels have been reported.24,25

Selenium deficiency in humans is rare, but is the cause of some serious diseases such as Keshan disease (an endemic cardiomyopathy) and Kashin-Beck disease (an endemic osteoarthritis), which are treated with oral selenium supplements.26 Selenium deficiency and mutations or polymorphisms in selenoprotein genes and synthesis cofactors are also implicated in a variety of other diseases, including neurological disorders, muscle and cardiovascular disorders, immune dysfunction, cancer, and endocrine function.27 These selenoproteins include a number of glutathione peroxidases, iodothyronine 5-deiodinases, and thioredoxin reductase. Thus, selenium functions in the body as an antioxidant, in redox reactions, thyroid hormone metabolism, the reproductive system, and immune function.26

Mitochondrial dysfunction has also been implicated as a contributing factor in diverse acute and chronic neurological disorders. However, its role in the epilepsies has been only recently emphasized. Some animal studies show that epileptic seizures result in free radical production and oxidative damage to cellular proteins, lipids, and DNA. Mitochondria contribute to the majority of seizure-induced free radical production.28 According to more recent studies, oxidative stress resulting from excessive free radical release is likely involved in the initiation and progression of epilepsy.29 A complex defense system has evolved in living tissues to protect against free radicals and the consequent damage they might cause. Neurons are especially vulnerable to free radical attacks and impaired defenses and exposure to excessive free radicals can lead to neuronal death. It has been shown that free radicals contribute to neuronal loss in cerebral ischemia and hemorrhage, and also may be involved in the degeneration of neurons in epilepsy, schizophrenia, tardive dyskinesia, normal aging, Parkinson’s Disease, and Alzheimer’s Disease.12,30 Also, a study conducted to evaluate the antioxidant status in epileptic patients concluded that the antioxidant status in blood of epileptic patients was lower compared with healthy controls, and improved after the treatment.31 Considering the fact that selenium is a cofactor of some enzymes with antioxidant activity; one can assume that deficiency of this element makes the individual prone to various damages mediated by oxidative stress.29 Molecular biology recently played a special role in the recognition of properties of selenium and selenium-dependent enzymes as the modulators of brain activity.12 Given that in evaluation of zinc and selenium levels various studies have shown some discrepancies, differences and changes in climate and minerals in soil, as well as type of race and other conditions included in the studies (for example, time of the year when samples were collected, and so forth) may lead to changes in these parameters in the serum, and eventually result in harmful states. In addition, epilepsy has different etiologies, some of which are still not understood completely. As we know, Kashin-Beck disease and Keshan disease are found more commonly in Eastern Asia and China than in other regions of the world. However, the main sources of these diseases are unknown, and has been only confined to lack of selenium in the soil of these areas.32,33 Since zinc and selenium are both used as very potent antioxidants in cellular reactions, changes in the values of these 2 parameters in serum can lead to enzymatic changes and increased free radicals, which in turn can cause neurological disorders. As selenium measurement using atomic absorption spectroscopy is costly, it would be better in the future, after demonstration of the exact relationship between selenium and seizure, that attempts are made to find methods (for example, measurement of selenium-dependent enzymes) that are less expensive.
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We conclude that adults with epilepsy had lower levels of serum zinc and selenium, and zinc and selenium supplementation to reduce the incidence of epilepsy should be further investigated.

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References