Study of Trace Elements and Role of Zinc Supplementation in Children with Idiopathic Intractable Epilepsy

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Abstract

Background Trace elements have physiological effects on neuronal excitability that may play a role in the etiology of intractable epilepsy. The aim was to evaluate the possible associations between some trace elements and idiopathic intractable epilepsy in children, and also the role of zinc supplementation in reduction of seizures in such patients.

Materials and Methods Our study was designed as a case-control study with 80 idiopathic epileptic patients between the ages of 10 months and 14 years enrolled in the study, 45 intractable to treatment (Group I) and 35 controlled by treatment (Group II). Serum levels of selenium, zinc, and copper were measured with atomic absorption spectrophotometer. Group I patients were subdivided according to zinc supplementation into two subgroups. Group IA included 31 epileptic patients with refractory response and oral zinc supplementation for 4 months and Group IB included 14 epileptic patients with refractory response and without zinc supplementation with continuous follow-up of patients for further 6 months for evaluation of seizure recurrence.

Results We found that Group I patients had significantly lower levels of serum Se and Zn compared with those of Group II patients ($p < 0.05$). Serum copper levels were not significantly lower in Group I than Group II. Zinc supplementation resulted in a significant reduction of seizure in Group I A compared with Group IB. Recurrence of seizure activity after discontinuation of zinc supplementation was significant in Group IA ($p < 0.01$).

Conclusions We found significantly lower serum levels of zinc and selenium in patients with intractable epilepsy as compared to the controlled epilepsy group. Zinc supplementation had a significant role in reduction of seizures in such patients.

Keywords
- trace elements
- intractable epilepsy
- controlled epilepsy
- zinc supplementation

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Introduction

Epilepsy can be defined as a condition in which seizures are recurrent. Seizures may develop as a result of the spreading of excitatory postsynaptic potentials, which discharge synchronously from either abnormal neurons or metabolic derangements that lower seizures potential. A seizure is the manifestation of an abnormal, hypersynchronous discharge of a population of cortical neurons.[1] The annual prevalence of epilepsy is 0.5 to 1% and its lifetime cumulative incidence is 3%.[2] Approximately 50 million people in the world have epilepsy and up to one-third continue to have seizures despite appropriate drug treatment.[3] It is important to identify those with intractable epilepsy, due to its comorbidities and sometimes mortalities. Patients are categorized as intractable when they developed at least one seizure in a 6-month period despite being treated with at least two antiepileptic drugs (AEDs).[4] By definition, trace elements are found in small quantities in the body but have important structural functional roles in a variety of biological processes.[5] The equilibrium of trace elements is essential for a healthy nervous system due to their key roles in activation of specific enzymes in many pathways of the central nervous system function and metabolism. Antioxidative defense mechanisms are important pathways involving trace elements. The accumulation of free radicals may lead to seizures and increases the risk of their recurrence, because oxidative stress produces peroxidated membrane lipids and damages the cells. Glutathione peroxidase (GPx) and superoxide dismutase (SOD) are two major enzymes that are involved in antioxidative defense mechanisms. Selenium (Se), zinc (Zn), and copper (Cu) are important trace elements that participate in the structure of these enzymes.[6–8] Imbalance in trace elements may be involved in epileptogenesis.[7] Zn and Cu are endogenous transition metals that can be synaptically released during neuronal activity. Synaptically released Zn and Cu likely function to modulate neuronal excitability under normal conditions. However, Zn and Cu can be neurotoxic, and it has been proposed that they may contribute to the neuropathology associated with a variety of conditions, such as Alzheimer disease, stroke, and seizures.[9] The purpose of this study was to evaluate the possible associations between these trace elements and idiopathic intractable epilepsy in children by comparing the levels of Se, Zn, and Cu between patients with idiopathic intractable epilepsy and idiopathic controlled epilepsy. We also sought to evaluate role of zinc supplementation in reduction of seizures in intractable patients.

Materials and Methods

The study design was case-control study. The study included 80 children with idiopathic epilepsy enrolled from pediatric neurology outpatient clinic, Al Hada and Taif military hospitals, Saudi Arabia. Their ages ranged from 10 months to 14 years. The study was conducted in the period from June 2012 to October 2014 after informed consent. Ethical clearance was obtained for the research study. The study protocol conforms to the ethical guidelines of the 1964 Declaration of Helsinki and its later amendments. All patients were treated for at least 1 year with an AED. All patients were idiopathic with normal weight, height, physical, and neuroradiological examination. Patients with symptomatic epilepsy due to underlying brain lesion were excluded from the study. Other exclusion criteria were clinical symptoms of Zn, Cu, and Se deficiency, administration of Zn or Zn-containing compounds, poor compliance to AED or Zn supplementation, uncompleted periods of follow-up, and malnutrition. According to their response to AED, they were divided into two groups:

- Group I: Included 45 idiopathic epilepsy patients with refractory response to AED (intractable epilepsy group). Patients were considered refractory when at least one seizure occurred in a 6-month period despite being treated by at least two appropriately selected AEDs (with proper doses, good compliance, and within the therapeutic range of AED).
  Group I patients were further subdivided according to Zn supplementation into two subgroups:
  - Group IA: Included 31 epilepsy patients with refractory response and Zn supplementation.
  - Group IB: Included 14 epilepsy patients with refractory response and without Zn supplementation. Zn supplementation was determined based on the presence of decreased serum Zn levels compared with normal reference values according to age and sex.
- Group II: Included 35 idiopathic epileptic children with good response to AEDs (controlled epilepsy group).

Oral Zn supplementation was added to AEDs in the form of oral Zn sulfate according to recommended daily allowance for 4-month duration. Dosing was as followed: 2 mg per day for children less than 1 year of age, 4 mg per day for children between 1 and 3 years, 5 mg per day for children between 4 and 8 years, and 8 mg per day for children between 9 and 14 years.[10] Zn supplementation was discontinued after 4-month duration with subsequent follow-up of patients for a further 6-month period for evaluation of seizure frequency. Response to Zn therapy was assessed during frequent follow-up visits in an outpatient clinic according to seizure reduction ratio (below) with therapy in comparison to intractable epileptic patients without Zn supplementation.

- Complete response: complete cessation of seizures
- Good response: more than 50% reduction in seizure frequency
- Poor response: less than 50% reduction in seizure frequency
- No response: no change in seizure frequency

All patients were further evaluated by:

- Complete history including age at time of evaluation, sex, age at onset of seizure, duration of illness, type of AED, frequency of seizure, response to therapy, and review of previous medical documentation
- Comprehensive neurological examination

- Routine electroencephalogram for characterization of the epilepsy type
- Laboratory investigations including:
  - Routine laboratory tests (complete blood count and liver and kidney function)
  - Determination of serum trace element levels of Se, Zn, and Cu

It was completed for all patients at the beginning of the study. Serum zinc levels were repeated again in patients receiving Zn supplementation at the end of the treatment.

A sample of 10 mL of venous blood was drawn aseptically into plastic disposable syringes for each individual in the study. All blood samples were digested by the microwave-induced acid digestion method and transferred to acid-washed centrifuge tubes provided with plastic cups which were immersed in HNO_3 5% v/v for 24 hours, then allowed to stand 2 hours at room temperature and centrifuge at 3,000 rpm for 30 minutes to separate the serum. The samples were preserved at −20°C until the time of analysis.

Apparatus

A Shimadzu model AA-670 (GBC Scientific Equipment Pty Ltd, SENSAA, Australia) flame atomic absorption spectrophotometer (FAAS) was used for the determination of Cu and Zn levels, and a Shimadzu model AA-670 (E-Chrom Tech, Taiwan) graphite flameless atomic absorption spectrophotometer (GFAAS) was used for the determination of Se levels.

Chemicals

All chemical substances used were of the highest purity (analytical-reagent grade), obtained from Fluka (Buchs, Switzerland) and BDH (Poole Dorset, UK) companies.

Standard Stock Solutions and Their Commercial Sources

We used 1,000 µg mL^{-1} selenium in 1% HNO_3 from BDH, 1,000 µg mL^{-1} copper in 1% HNO_3 from Riedel-de Haen, and 1,000 µg mL^{-1} zinc in 1% HCl from Riedel-de Haen.

Analytical Methods of GFAAS

A nitrate mixture of 0.1% Ni^{2+}, 0.1% Mg^{2+}, and 0.1 Cu^{2+} was added as a modifier to both standard solution and serum samples (which was diluted twofold with deionized water) for determination of Se at wavelength 196.0 nm.

Analytical Method for FAAS

Serum samples were diluted fivefold with deionized water and introduced into nebulizer burner system by the injection method. In view of viscosity of diluted serum, 3% (v/v) glycerin was added to the standard solutions for matching the surface tension between samples and calibrators for Zn and Cu to determine their levels at wavelength 213.9 and 324.8 nm, respectively. A blank was used for setting of zero absorbance of spectrophotometer. Calibration curves have been prepared for each element, separately. Finally, serum content of elements being determined was estimated.[11,12]

The outcome variables of our study included serum levels of trace elements and response to zinc supplementation.

Statistical Methods

All data were analyzed with SPSS and variables were analyzed with *t*-test and chi-square test. All *p*-values below 0.05 were considered statistically significant.

Results

Our study included 80 children with idiopathic epilepsy. They consisted of 46 males and 34 females. Their ages ranged from 10 months to 14 years with a mean age of 87.3 ± 59.7 months. Seventy percent of the patients were treated with conventional AEDs and 30% of them were treated simultaneously with both conventional and new AEDs. Seizure- and epilepsy-type classifications of patients were as follows: generalized tonic–clonic (72%), complex partial seizure (20.8%), infantile spasm (5.8%), and simple partial seizure (1.4%). All routine laboratory studies were within normal reference ranges. Normal selenium levels show a decrease from the age <1 month (0.64 µmol/L) to 4 months (0.44 µmol/L), an increase to 0.62 µmol/L in the 4 to 12 months' age group, constant values in children between 1 and 5 years of age (0.90 µmol/L), and an additional slight increase to reach a plateau between 5 and 18 years (0.99 µmol/L). The normal range of zinc was considered as 60 to 90 µg/dL between the ages of 1 and 12 months, 80 to 110 µg/dL between the ages of 1 and 10 years, and 90 to 120 µg/dL between the ages of 10 and 15 years, and the normal range of copper was considered as 40 to 80 µg/dL in all age groups.[13,14]

Our results are shown in ►**Table 1**.

►**Table 1** shows clinical characteristics of studied groups.

The mean ages of Group I and Group II were 79.1 ± 48.8 and 84.4 ± 46.5 months, respectively. Group I included 57.8% males and 42.2% females and Group II included 54.3% males and 45.7% females. For the anthropometric measurements, all patients had appropriate weight and height for age; the average weights were 24.3 ± 10.5 and 26.1 ± 8.9 kg and the average heights were 117 ± 24.8 and 119 ± 14.4 cm in Group I and Group II, respectively. Ages at first seizure were 4.8 ± 2.7 and 5.3 ± 2.3 years in Group I and Group II, respectively. Regarding frequency of seizures in Group I, 17.8% of patients had more than five epileptic seizures per day, 22.2% had seizure frequency of one to five times per day, 42.2% had seizure frequency of one to five times per week, and 17.8% had one to five epileptic seizures per month. There were no significant differences in the age, gender, anthropometric measurements, and age at onset of seizure between Group I and Group II.

The serum selenium levels in patients with intractable and controlled epilepsy were determined (►**Fig. 1**).

Twenty eight patients (62.2%) in Group I had Se deficiency and 17 patients (37.8%) had normal Se levels. Eleven patients (31.4%) in Group II had Se deficiency and 24 patients (68.6%) had normal Se levels.

The mean serum level of Se was 4.22 ± 1.58 and 5.34 ± 1.74 µmol/L in Group I and Group II, respectively.

Table 1 Patient clinical characteristics

Values	Intractable epilepsy (Group I) ($n = 35$)	Controlled epilepsy (Group II) ($n = 45$)	*p*-Value
Age (mo) (mean ± SD)	79.1 ± 48.8	84.4 ± 46.5	ns
Sex (no. %)			
Male	26 (57.8%)	19 (54.3%)	ns
Female	19 (42.2%)	16 (45.7%)	
Weight (kg) (mean ± SD)	24.3 ± 10.5	26.1 ± 8.9	ns
Height (cm) (mean ± SD)	117 ± 24.84	119 ± 14.36	ns
Age at first seizure (y) (mean ± SD)	4.8 ± 2.7	5.3 ± 2.3	
Frequency of seizure (no. %)			
>Five per day	8 (17.8%)		
<Five per day	10 (22.2%)		
One to five per week	19 (42.2%)		
One to five per month	8 (17.8%)		

Abbreviation: ns, not significant.
P value > 0.05.

Patients with the intractable epilepsy had significantly decreased levels of serum Se in comparison with the controlled epilepsy group (*p*-value < 0.05).

Serum zinc levels were measured at the initiation of the study (►**Fig. 2**).

Thirty-one patients (68.9%) in Group I had Zn deficiency and 14 patients (31.1%) had normal Zn levels. Ten patients (28.6%) in Group II had Zn deficiency and 25 patients (71.4%) had normal Zn levels. The mean serum level of Zn was 1.67 ± 0.33 and 2.01 ± 0.48 mg/L in Groups I and II, respectively. Patients with the intractable epilepsy had significantly decreased levels of serum Zn in comparison with the controlled epilepsy group ($p < 0.01$).

Serum copper levels in patients at study onset were also quantified (►**Fig. 3**).

Twenty-three patients (51.1%) in Group I had normal serum Cu level and 22 patients (48.9%) were Cu deficient. Twenty patients (54.5%) in Group II had normal serum Cu level and 15 patients (45.5%) were Cu deficient. The mean serum level of Cu was 0.96 ± 0.28 and 1.02 ± 0.42 mg/L in Group I and Group II, respectively. There was no statistically significant difference between serum Cu levels of intractable and controlled epilepsy groups (*p*-value > 0.05).

Those given zinc supplementation in the intractable epilepsy group had their seizure frequency characterized (►**Fig. 4**). Complete response was found in 12 patients (38.8%) in Group IA and none in Group IB. Good response was found in nine patients (29%) and in one patient (7.4%) in Group IA and Group IB, respectively. Poor response was found in seven patients (22.6%) and three patients (21.2%) in Group

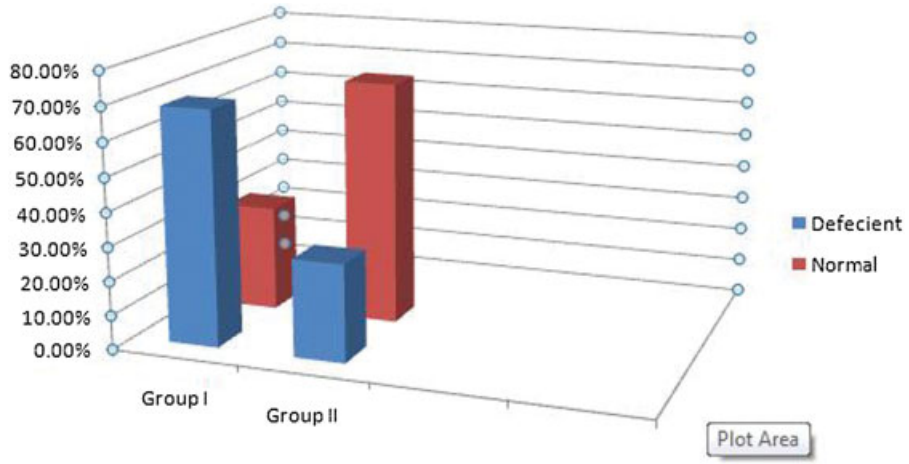

Fig. 1 Serum selenium levels in patients with intractable epilepsy and controlled epilepsy groups.

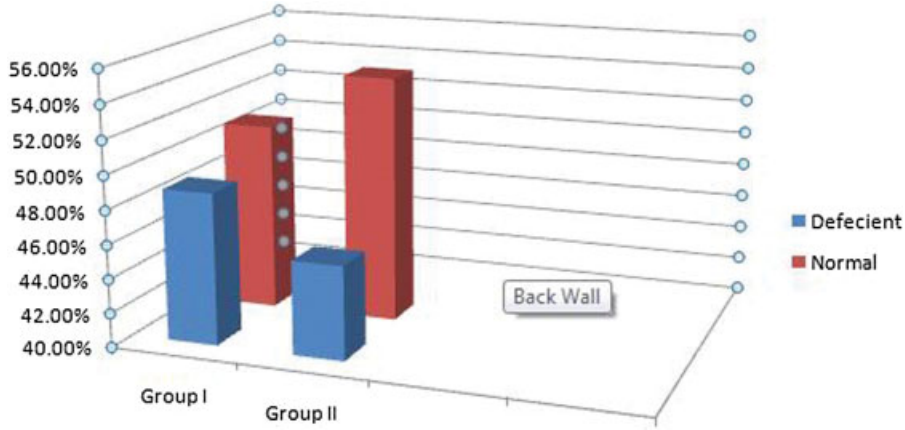

Fig. 2 Serum zinc levels in patients with intractable epilepsy and controlled epilepsy groups.

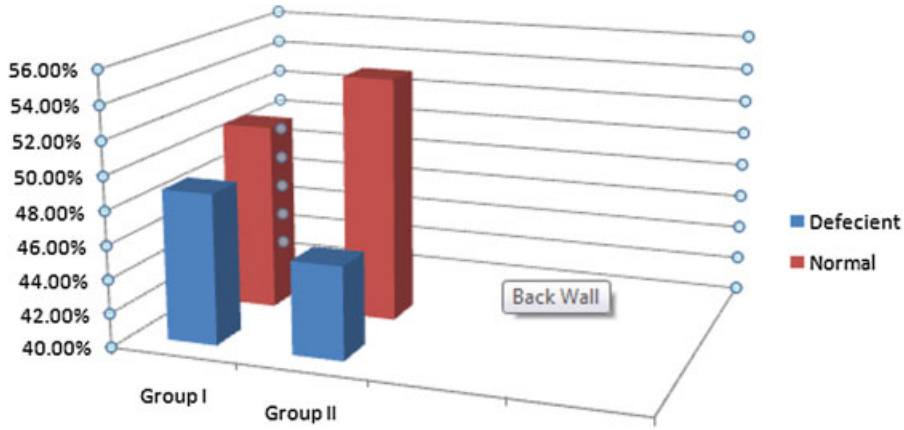

Fig. 3 Serum copper levels in patients with intractable epilepsy and controlled epilepsy groups.

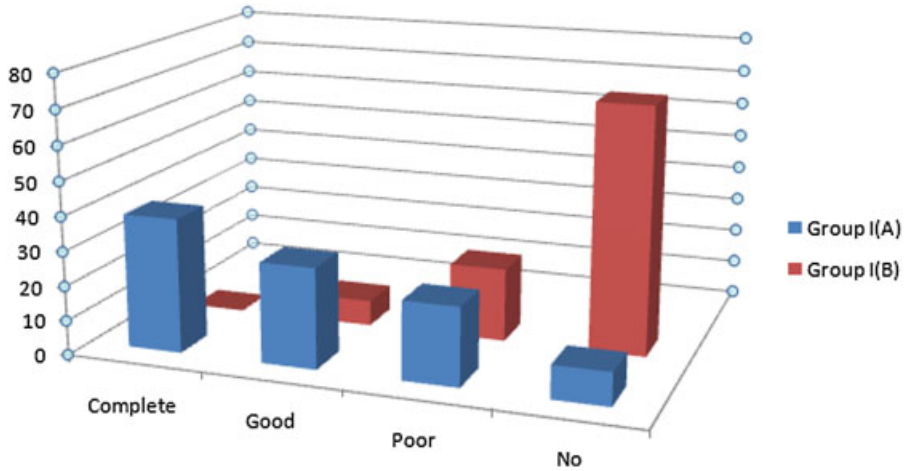

Fig. 4 Response of seizure to zinc supplementation in intractable epilepsy groups.

IA and Group IB, respectively. No response was found in 3 patients (9.6%) and in 10 patients (71.4%) in Group IA and Group IB, respectively.

Patients with the intractable epilepsy and Zn supplementation had significant response in comparison with the intractable epilepsy group without Zn supplementation ($p < 0.05$). It was observed that the seizure recurrences after discontinuation of Zn supplementation were significant ($p < 0.01$).

Serum zinc levels repeated in patients on Zn supplementation at the end of the treatment showed normal levels.

Few adverse effects of Zn supplementation were reported: nausea (two patients) and bad taste (three patients).

Discussion

Epilepsy affects a significant ratio of the population (2–3%) and is a major health problem. Medical therapy with AEDs is effective in most patients, but about 30% of the patients remain medically refractory with intractable epilepsy.[15] The relationship between epilepsy and trace element levels on path physiological basis is a matter of debate; however, there is a growing concern as abnormal trace element levels have been correlated with epilepsy treatment response. Most of the epilepsy-related research that has been done on trace elements has been conducted in patients with epilepsy that were not classified.[9] In the current study, we included patients with idiopathic epilepsy who did not respond to medical treatment that included at least two AEDs and who developed at least one seizure in the previous 6-month period.

The cascade of neurotoxic events that lead to epileptic seizures is highly complex, but the main event involves the accumulation of free oxygen radicals. Adding more complexity to the situation, oxygen radical formation has been found to be both the cause and the result of epileptic seizures.[7,16] We found significant changes in trace elements levels in our patients (Zn and Se). The study results suggest a vital role of certain trace elements in epilepsy pathogenesis and warrant larger studies for the associations between intractable epilepsy and trace element levels.

In our study, patients with intractable epilepsy had significantly decreased levels of serum Se and Zn in comparison to the controlled epilepsy group.

Se, Zn, and Cu are the three trace elements that are involved in the metabolism of oxygen radicals. For example, Se is involved in the reduction of peroxide by participating in the structure of GPx, which is a very important antioxidant enzyme.[7] Low levels of Se and GPx have been found in patients with epilepsy.[17,18] Se-deficient rats have been found to be more susceptible to excitotoxicity.[19] In another study that was conducted on two patients with intractable epilepsy, the seizures ceased with Se supplementation in addition to antiepileptic therapy, which suggested the benefit of this supplementation and which was confirmed by the return of the seizures after cessation of the Se supplementation.[20]

In a recent study by Seven et al, it was concluded that decreased levels of Zn and Cu could have etiopathophysiological correlation with drug-resistant epilepsy.[21] In another recent study, it was found that children with epilepsy had increased levels of Cu and decreased levels of iron, Zn, and chromium concentration.[22] Similar findings to our results were reported by Prasad et al.[23] Glutaric acid decarboxylase enzyme activity is regulated by zinc. This enzyme plays a critical role in the synthesis of gamma-amino butyric acid (GABA), which is the major inhibitory neurotransmitter in the brain, and it has been shown that levels of GABA are reduced in the cerebrospinal fluid of children with seizure disorders.[24] Many ionic channels such as sodium and T-type channels and GABA receptors which are activated by zinc and copper are implicated in specific forms of epilepsy. However, specifying the impact of these ions as excitatory or inhibitory is not easily possible without great effort.[24,25] SOD is another antioxidant enzyme that acts by removing free oxygen radicals from the intracellular environment and SOD requires Cu and Zn for its action.[26] In animal studies, epileptic seizure activity in mice has been reduced by supplementing with copper and increased by Zn deprivation.[24] Similarly, in humans, Wojciak et al found significantly lower serum Zn levels in patients with epilepsy, in comparison with a controlled healthy group. They also found serum Cu level of epileptic patients were higher than healthy control group.[22] In the Seven et al study, patients with idiopathic intractable epilepsy had significantly decreased levels of serum Zn in comparison with healthy children.[21] Our findings are in line with those of Wojciak et al and Seven et al, but our study performed a comparison of serum Zn levels between intractable and controlled epilepsy groups. This may confirm the role of Zn in the pathogenesis of medically refractory epilepsy.[21,22] In our study, there was no statistically significant difference between serum Cu levels of intractable and controlled epilepsy group ($p > 0.05$), although 48.9% of the intractable epilepsy group and 45.5% of the controlled epilepsy group were Cu deficient. This was observed by others as well.[27] Other authors have observed higher levels of serum Cu in epilepsy patients than in the control group[5,7,22,28–30]. This was attributed to the effect of AEDs, increased hepatic synthesis, decreased breakdown of Cu binding proteins, altered intestinal absorption, altered excretion patterns, changes in the distribution among body tissues, or a combination of these factors.[28,29,31] Wojciak et al. demonstrated serum Cu levels of epileptic patients were higher than in the control group and they postulated that epilepsy may increase the Cu level, but our study failed to confirm this hypothesis.[22] Our results are similar to those of Kheradmand et al, who showed significantly low serum Zn levels of patients with intractable epilepsy in comparison with controlled epilepsy group. Also, they reported insignificant changes in serum Cu between study groups.[32] Saad et al studied trace elements, oxidant, and antioxidant enzyme values in blood of children with refractory epilepsy in comparison to healthy control children and found significantly lower serum Zn, Se, and erythrocyte GPx but no statistical difference in Cu and SOD values among patient group than those in the control group.[27] Zinc deficiency by itself is known to be a cause of seizures and that seizures tend to improve with zinc supplementation.[33]

Children with epilepsy have been found to have significantly lower levels of serum zinc as compared to controls. More important, it appears that children with epilepsy may have elevated copper-to-zinc ratio. Seizures may be triggered when zinc levels fall, as is seen in the absence of adequate taurine. Although the exact role of zinc, or the copper-to-zinc ratio, is not clearly understood, it appears that anticonvulsants may cause a deficiency in zinc, either by reducing zinc absorption in the intestines or by causing diarrhea. Therefore, zinc supplementation may be warranted.[24]

The reason for the pharmacoresistance of some types of epilepsy is not clear. Several studies have been published regarding the role of antioxidants and some trace elements in the pathogenesis of seizure disorders. Experimental observations showed that zinc have both stimulatory and inhibitory effect on seizure activities.[7,17,21,24,25,34] Our study considered the first therapeutic trial of zinc supplementation in children with idiopathic intractable epilepsy. In our study, complete cessation of seizure was found in 38.8% and greater than 50% reduction in seizure activity was found in an additional 29% with Zn supplementation along with antiepileptic therapy in children with idiopathic intractable epilepsy. No one showed complete cessation of seizure in the group without Zn supplementation and only 7.4% showed >50% reduction in seizure activity. Zinc ions limit the excitatory responses in the dentate granule cells of those with temporal lobe epilepsy presumably by blocking the *N*-methyl-D-aspartate receptors. Studies involving three different animal models of epilepsy showed that zinc supplementation protected against the development of seizures, which suggest that zinc may be an essential component of a natural anticonvulsant tissue response to abnormal excitation.[33] It was found that mice which were zinc-deficient had increased seizure susceptibility and those with adequate dietary zinc had no change, but those who took supplemental zinc had decreased seizure susceptibility. Based on other reports, some conventional AEDs could make oxidative pressure and subsequently alter the metabolism of trace elements; alterations in Zn metabolism could be effective on causing seizure.[35,36] Our study showed high seizure susceptibility in the group without Zn supplementation with 71.4% showing no response to AEDs and <50% reduction in seizure activity was found in an additional 21.2%. Seizure susceptibility was low in the group with Zn supplementation as no response to AEDs was found in only 9.6% and greater than 50% reduction in seizure activity was found in 22.6%. Approximately 10% of the total Zn in the brain, probably ionic Zn, exists in the synaptic vesicles, and may serve as an endogenous neuromodulator in synaptic neurotransmission. However, dietary Zn deprivation affects Zn homeostasis in the brain. Vesicular zinc-enriched regions (e.g., the hippocampus) are responsive to dietary zinc deprivation, which causes brain dysfunction. On the other hand, the susceptibility to epileptic seizures, which may decrease vesicular Zn, is also enhanced by Zn deficiency.[37] Our results demonstrated a significant role of Zn supplementation in reduction of seizures in children with idiopathic intractable epilepsy. These results were further supported by significant recurrence rate of seizure following discontinuation of Zn supplementation.

Contradictory to our results, previous studies report that variations in serum Zn concentrations are a normal physiological process and are unlikely to be related to anticonvulsant drugs or epilepsy.[38] Also, Karimooy et al found that serum Zn concentration is irrelevant with the incidence of epilepsy and seizure.[39] These findings may be attributed to the fact that their reported cases were not on any AED. Their findings may have been different if they investigated after administrating proper antiepileptic medication treatment. Based on our findings, we can conclude that Zn and Se values were lower in children with intractable epilepsy than in controlled epilepsy and they may play a prominent role in the pathogenesis of intractable epilepsy. Zn administration in patients with intractable epilepsy has a significant role in reduction of seizures. These study results further warrant larger study of the associations between idiopathic intractable epilepsy and trace element levels to delineate the etiological basis of intractable epilepsy and its possible therapeutic options. Additionally, a future investigation comparing the pre- and posttreatment levels of the trace elements in patients with idiopathic intractable epilepsy should be performed given the relationships between AEDs and the levels of trace elements.

Conflict of Interest
None.

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