

Serum levels of zinc and copper in epileptic children during long-term therapy with anticonvulsants

Mohamed A. Talat, MD, Anwar Ahmed, MD, Lamia Mohammed, MD.

ABSTRACT

الأهداف: لتقدير مستويات الخارصين و النحاس في الأطفال الذين يعانون من الصرع ويتناولون العلاج على المدى الطويل مع مجموعة التحكم .

الطريقة: أجريت هذه الدراسة الاستيعادية في مجموعة مستشفيات في قسم طب الأطفال، كلية الطب، جامعة الزقازيق، الزقازيق، مصر خلال الفترة من نوفمبر 2013م حتى أكتوبر 2014م على عدد 90 طفل من الذين يعانون من الصرع متوسط أعمارهم 7.1 ± 3.6 عاماً والذين شخضوا بالصرع من طبيب الأعصاب. اختير المجموعة الضابطة من الأفراد الأصحاء المطابقة للحالة. تم قياس الزنك في الدم والنحاس من خلال طريقة المسعر باستخدام QCA .

النتائج: قد أظهرت الدراسة أن نسبة الخارصين في مرضى الصرع كانت 60.1 ± 22.6 أقل من نسبة الزنك في الأطفال الطبيعيين 102.1 ± 18 بقيمة ذات دلالة احصائية ($p < 0.001$). ولوحظ أيضاً أن نسبة النحاس في مرضى الصرع 180.1 ± 32.4 كانت أكثر من نسبة النحاس في الاطفال الطبيعيين 114.5 ± 18.5 بقيمة ذات دلالة احصائية ($p < 0.001$).

الخاتمة: تناول أدوية الصرع على المدى الطويل يؤثر على نسبة النحاس والزنك في الدم. ولذلك من المهم قياس مستوى الزنك والنحاس في هؤلاء الاطفال والحاجة إلى إضافة عنصر الزنك ضمن برنامج العلاج إلى الأطفال الذين يتناولون أدوية الصرع وعلاج زيادة النحاس في هؤلاء الاطفال .

Objective: To evaluate the serum levels of zinc and copper in epileptic children during the long-term treatment of anticonvulsant drugs and correlate this with healthy subjects.

Methods: A hospital-based group matched case-control study was conducted in the Department of Pediatrics, Faculty of Medicine, Zagazig University, Zagazig, Egypt between November 2013 and October 2014. Ninety patients aged 7.1 ± 3.6 years were diagnosed with epilepsy by a neurologist. The control group was selected from healthy individuals and matched to the case group.

Serum zinc and copper were measured by the calorimetric method using a colorimetric method kit.

Results: The mean zinc level was 60.1 ± 22.6 ug/dl in the cases, and 102.1 ± 18 ug/dl in the controls ($p < 0.001$). The mean copper level was 180.1 ± 32.4 ug/dl in cases compared with 114.5 ± 18.5 ug/dl in controls ($p < 0.001$).

Conclusion: Serum zinc levels in epileptic children under drug treatment are lower compared with healthy children. Also, serum copper levels in these patients are significantly higher than in healthy people. No significant difference in the levels of serum copper and zinc was observed in using one drug or multiple drugs in the treatment of epileptic patients.

*Neurosciences 2015; Vol. 20 (4): 341-345
doi: 10.17712/nsj.2015.4.20150336*

From the Department of Pediatrics (Talat, Ahmed), and the Department of Clinical Pathology (Mohammed), Faculty of Medicine, Zagazig University, Zagazig, Egypt.

Received 14th May 2015. Accepted 8th July 2015.

Address correspondence and reprint request to: Dr. Mohamed A. Talat, Department of Pediatrics, Faculty of Medicine, Zagazig University, Zagazig, Egypt. E-mail: Abo.talat@yahoo.com

Epilepsy is a common neurological disorder with heterogeneous nature affecting 50 million people world wide, with more than 85% living in the developing world, and it begins in childhood in more than half of the cases. An estimated 4.7 million people with epilepsy live in the Eastern Mediterranean Region.¹ The prevalence of epilepsy in Egypt is not accurately determined, although some studies reported that the lifetime prevalence rate of epilepsy is 12.67/1000.² Epilepsy is a disorder of brain electrical activity that may

Disclosure. The authors declare no conflicting interests, support or funding from any drug company.

lead to recurrent seizures. The type of seizure depends on the part of the brain involved, and various causes can lead to seizures. The absence of a specific cause of the seizures is called primary or idiopathic epilepsy.³ Some of the main causes of epilepsy include: low oxygen during birth; head injuries that occur during birth or from accidents during youth or adulthood; brain tumors; genetic conditions that result in brain injury, such as tuberous sclerosis; infections such as, meningitis or encephalitis; stroke; or any other type of damage to the brain and abnormal levels of substances such as, sodium or blood sugar.⁴ Different mineral elements are critical for normal functioning of the central nervous system, and several studies have demonstrated that changes in different electrolytes of the body, such as sodium, potassium, magnesium, and the trace elements such as copper (Cu) and zinc (Zn) subsequently are effective on the incidence of convulsions and epilepsy.⁵ The routine treatment of the epilepsy is using anticonvulsant agents. The use of such drugs mainly controls the disease, or can reduce the times of the seizure. After initial recognition, approximately 70% of patients have controlled seizures with antiepileptic drugs (AEDs). Approximately 25% of patients with epilepsy do not have any observed improvement in the reduction of the amount of seizures, even when 2 or 3 AEDs are used. Some studies have shown the importance of a specific diet, hormones, and micronutrients in the management of patients with epilepsy.⁶ The aim of the present study was to evaluate serum levels of Cu and Zn in patients with epilepsy in long-term treatment with anticonvulsants and comparing this with healthy individuals.

Methods. Study Design. A hospital-based group matched case-control study was conducted in the Department of Pediatrics, Faculty of Medicine, Zagazig University, Zagazig, Egypt between November 2013 and October 2014. The Institutional Ethical Committee of Zagazig University approved the study. All parents of the 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.

Study population and sampling. We evaluated the serum levels of Cu and Zn in 90 children with epilepsy during long-term treatment with the commonly used anticonvulsant, such as sodium valproate, carbamazepine, phenytoin, clonazepam, and levetiracetam, and compared with 90 healthy subjects matched with the case group for age and gender. The sample size was determined by using EPI-Info version (EPI-Info, Atlanta, USA) 6 statistical packages at 80% power, and 95% confidence interval then estimated

sample will be 90 in each. The inclusion criteria included age less than 15 years, 1-10 years duration of epilepsy, absence of seizures in at least 24 hours before sampling, and normal liver tests and renal tests. The exclusion criteria included using compounds containing Cu and Zn in the past 6 months, patient with obvious intracranial pathology, child has a disease that affects the level of Zn or Cu, such as Wilson disease-acrodermatitis enteropathica-malnutrition, and MRI or CT evidence of brain abnormality. The control group was 90 healthy subjects matched with the case group for age, gender, height, and weight, and had the following criteria: 1) No history of simple or complex febrile seizures; 2) No history of seizures or seizure-like episodes; 3) Not diagnosed with neurological disease (cerebral palsy, stroke, meningitis, and neurodegenerative disorders); 4) No family history of an immediate relative (parent, sibling) with epilepsy, or febrile convulsions; and 5) Not used Cu and Zn containing compound in the past 6 months (drugs, tonic and anabolic).

Data collection procedure. All children in this study passed through:

1. Full history taking with special emphasis on: age; convulsion; onset of epilepsy type of seizure, seizure count per month (frequency), onset of treatment, type of treatment, number of drugs, duration of treatment and response to treatment; motor development; speech disturbance; and gait disturbance.

2. Clinical examination. Height for age; weight for age; general examination including: cardiac examination (to exclude arrhythmia or any side effect from the drug); abdominal examination (especially for the liver); neurological examination (to exclude ataxia, Wilson disease or permanent sequelae of recurrent convulsion).

3. Laboratory investigation. Cell blood count (CBC) on Sysmex KX-21, (Sysmex, Kobe, Japan) and SGOT, SGPT, total protein and albumin (liver function tests on Cobas Integra 400 plus [Roche, Penzberg, Germany] and urea and creatinine (kidney function tests on Cobas Integra 400 plus [Roche, Penzberg, Germany], and serum Cu and Zn levels by direct colorimetric method.

Sample collection. After overnight fasting, 5 ml venous blood samples were taken from the patients then the serum was divided into 2 parts. The first part was immediately analyzed for liver function and kidney function tests and CBC. The second part was preserved in an Ependorff tubes at -20° for measurement of serum levels of Cu and Zn with colorimetric method. The normal range of serum Cu was 70-150 $\mu\text{g}/\text{dl}$ or 10.7-22 $\mu\text{mol}/\text{L}$ and the normal range serum Zn was 60-90 $\mu\text{g}/\text{dl}$ between the ages of 1-12 months, 80-110 $\mu\text{g}/\text{dl}$ between the ages of 1-10 years, 90-120 $\mu\text{g}/\text{dl}$ between the ages of 10-15 years. Concentrations were determined by direct colorimetric method. In order

to eliminate confounding variables, tests of liver and kidney were noted.

4. Electroencephalogram for diagnosis of epilepsy and CT or MRI for exclusion of intracranial pathology.

Statistical analysis. The data was checked, entered and analyzed statistically by Statistical Package for Social Sciences version 15 (SPSS Inc., Chicago, IL, USA). For quantitative variables, the data was expressed as mean±standard deviation. The relation between qualitative data was evaluated using Chi-square test. Also, the relation between quality and quantity data was evaluated using T-test, ANOVA tests, and the relation between the variables were evaluated using Pearson and Spearman correlation coefficient. $P<0.05$ was considered statistically significant.

Results. Our study included 90 epileptic patients, 63 males (70%) and 27 females (30%) presenting to Zagazig University Pediatric Hospital, Zagazig, Egypt, who were suffering from epileptic seizures. The mean age of the cases was 7.1 ± 3.6 years with a range of 1.3-14 years. They were 90 gender- and age-matched healthy children that served as a control group, 66 males (73.3%) and 24 females (26.7%), with a mean age of 6.1 ± 2.2 years with a range of 3-10 years. The 2 groups were matched for age, gender, weight, and height. The study showed that the mean of beginning of convulsion was 3 ± 2.2 years (range first day of life-9.5 years), the mean of onset of treatment was 3.1 ± 2.4 years with a range of 18 days-10 years, the mean frequency /month was 3 ± 2.6 with a range of 1-12/months, and the treatment duration mean was 2.8 ± 1.2 years with a range of 1.1-5 years. The most common type of convulsion was generalized on 69 (76.6%) followed by partial on 21 (23.4%). The Zn level mean in children with epilepsy was 60.1 ± 22.6 µg/dl, and in the control group was 102.1 ± 18 µg/dl, which was significantly lower in epileptic patients ($p<0.001$). The Cu level mean in patients with epilepsy was 180.1 ± 32.4 µ/dl, and in the control group was 114.5 ± 18.5 µ/dl, which was significantly higher in the case group ($p<0.001$). Laboratory parameters of both groups are shown in Table 1.

Table 2 shows no significant differences between the groups regarding type of seizure (generalized or partial), and type of treatment (single or multiple) and response to treatment (non-responder, moderate responder, complete responder) based on gender. Table 3 shows no significant differences between the groups regarding Zn and Cu levels based on response to treatment. Also, our study shows no significant differences between the studied group regarding laboratory findings based on type of seizure. The Cu was 185.1 ± 39.4 with generalized

epilepsy and 176.6 ± 21.1 with partial epilepsy, and Zn was 61.7 ± 26.9 with generalized epilepsy and 57.7 ± 17.8 with partial epilepsy. In this study, 39 patients used single drug for epilepsy control (27 used sodium valproate and 12 used carbamazepine), and 51 patients used multiple drugs. No significant differences between the studied group regarding Zn and Cu levels based on regimen of treatment (mono or poly therapy) as serum Cu was 155.1 ± 60 µg/dl with single drug, and 191.5 ± 70 µg/dl with multiple drugs, and serum Zn was 75.8 ± 30 µg/dl with single drug, and 54.7 ± 28 µg/dl with multiple drugs.

Table 4 shows that the Zn levels were 52 ± 8.1 µg/dl and Cu levels were 195 ± 11 µg/dl in patients treated with carbamazepine and the Zn levels were 65 ± 0.05 µg/dl, and Cu levels were 144.7 ± 8 µg/dl in patients treated with sodium valproate. These results revealed that carbamazepine was more effective than sodium valproate in reducing serum Zn and increasing serum Cu in epileptic patients.

Table 1 - Significant differences between the groups regarding laboratory findings of serum zinc and copper only (N=90).

Laboratory findings	Cases (mean± standard deviation)	Controls	t-test	P-value
Copper	180.1±32.4	114.5±18.5	7.245	<0.001**
Zinc	60.1±22.6	102.1±18	6.262	<0.001**
Total bilirubin	0.5±0.2	0.4±0.1	1.212	0.232
SGOT	28.3±12	29.1±11	0.07	0.789
SGPT	38.2±10	37.4±12	0.08	0.78
Albumin	3.9±0.5	4±0.5	0.431	0.668
Total protein	6.8±2	7.9±3	2.79	0.099
Urea	30.8±6.8	27.3±4	1.82	0.076
Creatinine	0.6±0.1	0.5±0.1	1.348	0.06

SGOT - serum glutamic-oxaloacetic transaminase, SGPT - serum glutamic-pyruvic transaminase, ** $p>0.05$ were statistically significant

Table 2 - Type of seizure, type of treatment, and response to treatment based on gender (N=90).

Convulsion	Gender		X ²	P-value
	Male n=63	Female n=27		
	n (%)			
<i>Type of seizure</i>				
Generalized	45 (71.4)	24 (88.9)	1.07	0.3
Partial	18 (28.6)	3 (11.1)		
<i>Type of response to treatment</i>				
Non responder	12 (19.1)	6 (22.2)	0.04	0.979
Moderate responder	36 (57.1)	15 (55.6)		
Complete responder	15 (23.8)	6 (22.2)		
<i>Type of treatment</i>				
Single drug	18 (28.6)	15 (55.6)	1.98	0.159
Multiple drugs	45 (71.4)	12 (44.4)		

Table 3 - Laboratory findings between patients groups based on response to treatment.

Laboratory findings	Response to treatment			F	P-value
	Non responder	Moderate responder	Complete responder		
	(mean± standard deviation)				
Copper	167.2±31	175±26.7	184.7±24.4	0.603	0.561
Zinc	73.8±33.4	57±24.3	58.2±8.2	0.863	0.443
Total bilirubin	0.4±0.2	0.5±0.1	0.4±0.2	0.505	0.614
SGOT	28±7.2	29±6.9	28.2±8.9	0.025	0.975
SGPT	35.5±5.6	38.6±5.4	39.8±5.6	0.968	0.404
Albumin	3.8±0.4	3.9±0.5	4.2±0.6	0.699	0.514
Total protein	6.7±0.2	6.9±0.4	6.7±0.2	0.825	0.601
Urea	30.3±5.5	32.8±5.8	7.7±3.1	0.198	0.822
Creatinine	0.6±0.2	0.6±0.1	0.5±0.1	0.345	0.714

SGOT - serum glutamic-oxaloacetic transaminase,
SGPT - serum glutamic-pyruvic transaminase

Table 4 - Serum levels of zinc and copper based on single drug usage.

Serum level	Sodium valproate	Carbamazepine	T-test	P-value
	n=27	n=12		
	Mean±SD			
Serum Zinc	65±0.05	52±8.1	5.58	0.024
Serum Copper	144.7±8	195±11	9.47	0.004

Discussion. Seizure disorder is one of the most common neurological diseases in children and occurs at least one time in 4-10% of children in the first 16 years of life. The prevalence of epilepsy is 0.5-1% per year with a lifetime cumulative incidence of 3%. Recurrent unprovoked seizures called epilepsy, and its diagnosis are carried out when 2 or more unprovoked seizures have occurred at intervals longer than 24 hours intervals.⁷ Zinc is a regulator of the glutamic acid decarboxylase enzyme that has a major role in the production of gamma amino butyric acid (inhibitory neurotransmitter), and the deficiency of this enzyme can lead to epileptic disorders. Many ionic channels, such as sodium and T-Type channels and gamma-aminobutyric acid (GABA) receptors are activated by Zn and Cu affect specific forms of epilepsy.⁸ But, the role of Zn in seizure is controversial as at one hand, it plays a role in the synthesis and function of inhibitory neurotransmitter GABA, and on the other hand, it also has an inhibitory effect on GABA and thus facilitating seizure activity.⁹ Serum Zn in cases was significantly lower than the control. This result is in agreement with previous studies.¹⁰⁻¹³ Also, in Seven et al's¹⁴ study, patients with idiopathic intractable epilepsy had significantly decreased levels of serum Zn in comparison with healthy children. However, other studies found no changes in serum Zn concentration

in epileptic patients compared with the control group as described in some studies.^{9,15,16,17} On the other hand, Hamed et al¹⁸ demonstrated that the untreated epileptics exhibited unaltered Zn levels and sodium valproate treated epileptics showed significantly higher levels of Zn ($p<0.05$). The real mechanism of the possible effect of AEDs on Zn concentrations is not completely understood, valproate binds Zn protects GABA from the inhibitory effect of Zn; hence, increased level of GABA. Another study carried out by Sarangi et al¹⁹ on epileptic patient were on monotherapy anti epileptic drugs (valproic acid- carbamazepine- phenytoin- levetiracetam) in whom was found statistically significant increase serum Zn, which can be due to increased antioxidant enzymes, and Zn being an integral part of these.

In our study, serum Cu levels in epileptic patients undergoing treatment with anticonvulsants drugs were significantly higher than the control group that is consistent with other studies^{11,12,20} and this was attributed to the effect of AEDs, increased hepatic synthesis, or due to the decreased breakdown or both Cu binding proteins, altered intestinal absorption, and altered excretion patterns, changes in the distribution among body tissues, or a combination of these factors.^{13,20} Also, these results are in agreement with a study carried out by Sarangi et al¹⁹ who found statistically significant increase serum Cu as enzyme - inducing potential of anti epileptic drugs leading to increase in hepatic synthesis of ceruloplasmin has been suggested as a possible mechanism. Hamed et al¹⁸ reported higher levels of serum Cu in untreated epileptics ($p<0.05$), which was attributed to inverse relationship between Zn and Cu concentration. However, the results in the treated epileptics are contradictory. But, other authors found no changes in serum Cu concentration in epileptic patients as compared with the control group.^{5,10,15} There was no significant differences between the groups regarding the type of seizure (generalized or partial), and type of treatment (single or multiple), and response to treatment (non-responder, moderate responder, complete responder) based on gender. Also, our study shows no significant differences between the studied group regarding laboratory findings based on type of seizure, these results were in accordance with the results reported by Saboktakin et al.¹² In this study, we classify our patient into non-responder, moderate responder, and complete responder according to the recurrent of seizures /month.²¹ If more seizures recurrent within a month (non-responder) and if more seizures recurred within 6 months (moderate responder) and if no seizures within 6 months (complete responder). There was no significant differences between the studied group regarding Zn and Cu levels based on response to treatment, However a study carried out

by Kheradmand et al²² revealed that patients with the intractable epilepsy had significantly decreased levels of serum Zn in comparison with the control group ($p < 0.001$), and there was no statistically significant difference between serum Cu levels of intractable and controlled epilepsy group. Table 1 shows no statistically significant differences between the 2 groups regarding liver function and kidney function test, these results were in accordance with the results reported by Sarangi et al.¹⁹ On the other hand, Saboktakin et al¹² found that creatinine was higher in patients using multiple drugs than single drug with a statistically significant difference.

The limitation of this study was inability to reveal the impact of Zn and Cu effect on epileptogenesis, and effect of treatment of high Cu or low Zn in the management of epilepsy, and we believe that this will contribute to the development of future research in the area of pathogenesis and management of epilepsy in order to improve the quality of life of patients with epilepsy.

In conclusion, long-term therapy with anticonvulsants drug affects serum level of Zn and Cu. Serum Cu levels in epileptic children under drug treatment are higher than in healthy children. Also, serum Zn levels in these patients are strongly lower than in healthy people. No significant difference in the levels of serum Cu and Zn on using one drug or multiple drugs in the treatment of epileptic patients. We should measure Zn and Cu levels in patients on long-term anticonvulsant therapy. We need to supply the decreased elements (Zn in our study) with anticonvulsant therapy.

Acknowledgments. *The authors would like to extend gratitude to their assistance team and their students.*

References

- World Health Organization. Epilepsy in the WHO Eastern Mediterranean Region: Bridging the gap. Cairo (EG): WHO Regional Office for Mediterranean Region; 2010.
- Khedr EM, Shawky OA, Ahmed MA, Elfetoh NA, Al Attar G, Ali AM, et al. A community based epidemiological study of epilepsy in Assiut Governorate/Egypt. *Epilepsy Res* 2013; 103: 294-302.
- Volpe SL, Schall JI, Gallagher PR, Stallings VA, Bergqvist AG. Nutrient intake of children with intractable epilepsy compared with healthy children. *J Am Diet Assoc* 2007; 107: 1014-1018.
- Guerrini R. Epilepsy in children. *Lancet* 2006; 367: 499-524.
- Tutor-Crespo MJ, Hermida J, Tutor JC. Assessment of copper status in epileptic patients treated with anticonvulsant drugs by measuring the specific oxidase activity of ceruloplasmin. *Epilepsy Res* 2003; 56: 147-153.
- Sołowiej E, Sobaniec W. [The effect of antiepileptic drug therapy on antioxidant enzyme activity and serum lipid peroxidation in young patients with epilepsy]. *Neurol Neurochir Pol* 2003; 37: 991-1003. Polish
- Mikati MA. Seizures in childhood. In: Kliegman RM, Stanton BF, Schor NF, Geme JWS, Behrman R, editors. *Nelson Textbook of Pediatrics*. 19th ed. Philadelphia (PA): Saunders Elsevier; 2011. p. 2013-2033.
- Mathie A, Sutton GL, Clarke CE, Veale EL. Zinc and copper: pharmacological probes and endogenous modulators of neuronal excitability. *Pharmacol Ther* 2006; 111: 567-583.
- Kumar L, Chaurasiya OS, Gupta AH. Prospective study of level of serum zinc in patients of febrile seizures, idiopathic epilepsy and CNS infections. *People's J Scient Res* 2011; 4: 1-4.
- Saad K, Hammad E, Hassan AF, Badry R. Trace element, oxidant, and antioxidant enzyme values in blood of children with refractory epilepsy. *Int J Neurosci* 2014; 124: 181-186.
- Wojciak RW, Mojs E, Stanislawska-Kubiak M, Samborski W. The serum zinc, copper, iron, and chromium concentrations in epileptic children. *Epilepsy Res* 2013; 104: 40-44.
- Saboktakin L, Barzegar M, Hagh Jo AG, Emamalizadeh M. Study on serum Copper and Zinc level of children with epilepsy during long term therapy with anticonvulsants. *Life Sci J* 2012; 9: 1250-1254.
- Armutcu F, Ozerol E, Gurel A, Kanter M, Vural H, Yakinci C, et al. Effect of long-term therapy with sodium valproate on nail and serum trace element status in epileptic children. *Biol Trace Elem Res* 2004; 102: 1-10.
- Seven M, Basaran SY, Cengiz M, Unal S, Yuksel A. Deficiency of selenium and zinc as a causative factor for idiopathic intractable epilepsy. *Epilepsy Res* 2013; 104: 35-39.
- Verrotti A, Basciani F, Trotta D, Pomilio MP, Morgese G, Chiarelli F. Serum copper, zinc, selenium, glutathione peroxidase and superoxide dismutase levels in epileptic children before and after 1 year of sodium valproate and carbamazepine therapy. *Epilepsy Res* 2002; 48: 71-75.
- Alshafei MM, Kassem SS, Abdel kader MM. Effect of long term treatment with antiepileptic drugs on oxidant status, zinc and magnesium in epileptic patients. *World Applied Sciences Journal* 2013; 28: 316-323.
- Tutor-Crespo MJ, Hermida J, Tutor JC. Possible induction of cholinesterase in epileptic patients treated with anticonvulsant drugs: relationship with lipoprotein levels. *J Clin Pharmacol* 2004; 44: 974-980.
- Hamed SA, Abdellah MM, El-Meley N. Blood levels of trace elements, electrolytes, and oxidative stress/antioxidant systems in epileptic patients. *J Pharmacol Sci* 2004; 96: 465-473.
- Sarangi SC, Tripathi M, Kakkar AK, Gupta YK. Effect of antiepileptic therapy on trace elements status in Indian population in a tertiary care hospital from northern India: a cross sectional study. *Epilepsy Res* 2014; 108: 917-927.
- Prasad R, Singh A, Das BK, Upadhyay RS, Singh TB, Mishra OP. Cerebrospinal fluid and serum Zinc, Copper, Magnesium and Calcium levels in children with idiopathic seizure. *Journal of Clinical and Diagnostic Research* 2009; 3: 1841-1846.
- Park KM, Hur Y, Kim HY, Ji KH, Hwang TG, Shin KJ, et al. Initial response to antiepileptic drugs in patients with newly diagnosed epilepsy. *J Clin Neurosci* 2014; 21: 923-926.
- Kheradmand Z, Yarali B, Zare A, Pourpak Z, Shams S, Ashrafi MR. Comparison of serum Zinc and Copper levels in children and adolescents with intractable and controlled epilepsy. *Iran J Child Neurol* 2014; 8: 49-54.