Study on serum Copper and Zinc level of children with epilepsy during long term therapy with anticonvulsants

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Abstract: Epilepsy is a disorder of brain electrical activity that may lead to recurrent seizures. Changes in serum levels of some trace elements such as Zinc and Copper can be observed in patients with Epilepsy. The aim of this study was evaluating the serum levels of copper and zinc in patients with epilepsy on long term treatment of anticonvulsant agents in Tabriz children's hospital. In a case control descriptive –analytical study in the children's diseases department of Tabriz children's hospital we evaluated the serum levels of copper and zinc in patients with epilepsy and compared them with healthy subjects. The two groups were matched for age, gender, weight and height. The mean zinc level in children with epilepsy is 0.30 ± 0.13 and 0.93 ± 0.25 µg/ml respectively which was lower meaningfully in epileptic patients. The copper level in patients with epilepsy was 1.06 ± 0.36 µg/ml and in control group was 0.39 ± 0.21 µg/ml respectively which was significantly higher in the case group. Serum copper levels in epileptic children under drug treatment are higher than in healthy children. Also, serum zinc levels in these patients are significantly lower than in healthy people. The use of one drug or multiple drugs in the treatment of epileptic patients have made a significant difference in the levels of serum copper and zinc and also the serum level of Zinc in patient under treatment with Phenobarbital, Phenytoin, Carbamazepine, Valproate sodium, Clonazepam, Topiramate and Primidone was significantly lower and the serum level of Copper in patients winder treatment by Phenobarbital, Phenytoin, Carbamazepine and Valproate sodium was significantly higher.
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1. Introduction

Epilepsy is a disorder of brain electrical activity that may lead to recurrent seizures. Type of seizure depends on the part of the brain involved and various causes can lead to the seizure. The absence of a specific cause for the seizures Epilepsy is called primary or idiopathic (Gaby, 2007).

The exact mechanism of epilepsy is largely unknown. The routine treatment of the epilepsy is the using of the anticonvulsants agents. The use of such drugs mainly controls the disease or can reduce the times of the seizure, but in some patients, treatment is not effective or patient suffers repeated seizures. Recent researches have shown that a specific diet, micronutrients, and hormones are useful in the treatment of patients with Epilepsy (Sołowiej and Sobaniec, 2003). With regard to the fact that the Zinc is a regulator of the glutamic acid decarboxylase enzyme that has a major role in the production of the gamma amino butyric acid-an inhibitory neurotransmitter- and the deficiency of this enzyme can lead to epileptic disorders. Changes in serum levels of some trace elements such as copper can be observed in patients with Epilepsy. Besides these micronutrients to prevent or overcome the effects of certain deficiencies in the use of anticonvulsant drugs are effective (Tutor-Crespo, 2003).

Barbeaus and colleagues examined the changes in serum levels of Zinc in patients with Epilepsy and stated that serum levels of zinc is low in these patients (Barbeau and Donaldson, 1974). Schott and colleagues showed that patients with epilepsy have a normal level of zinc and an elevated level of copper (Schott and Delves, 1978). Plum and colleagues in a study on patients with Epilepsy concluded that increased serum levels of copper in these patients (Plum and Hansen, 1960).

In a study by the Tutor and his colleagues on 49 patients with generalized and partial Epilepsy serum copper and zinc were compared to control subjects, it showed no difference in plasma levels of copper and zinc in these patients (Tutor-Crespo, 2004).

Sherifa and colleagues in a study on 70 patients with epilepsy and 40 healthy children as controls Epilepsy showed that serum levels of Zinc

in patients on treatment (particularly with sodium valproate) is high whereas Copper levels in these patients are low (Sherifa, 2004)

With considering the fact that epilepsy and its frequents attacks have psychological and financial burden in the family and with regard to the previous studies result which has reported a variable results in the serum copper and zinc levels in these patients we designed a study to evaluate the serum levels of copper and zinc in patients with epilepsy on long term treatment of anticonvulsant agents in Tabriz children's hospital.

2. Material and Methods

In a case control descriptive –analytical study in the children's diseases department of Tabriz children's hospital we evaluated the serum levels of copper and zinc in patients with epilepsy and compared them with healthy subjects.

In this study, all children and adolescents with Epilepsy in 2010 during a visit to Children's Hospital and neurology clinic were enrolled in the study.

Inclusion criteria included age under 18 years, over one year duration of epilepsy, the top 25 percentile in weight not using compounds containing copper and zinc in the past 6 months, not receiving other drugs but anticonvulsant drugs, no obvious intracranial pathology, absence of disease leading to recent surgery, Absence of seizures in at least 24 hours before sampling, normal liver tests and renal tests, and other diseases were not.

The control group has been selected from children attending in Children's hospital general clinics to periodically controlling of the growth who had no known diseases.

The control group was matched with the case group for age, gender, height and weight and had not used copper and zinc containing compounds in the past 6 months. For exact measurement of height and weight in children we used SECA weight and height meter.

After overnight fasting venous blood samples was taken from the patient .the sample was centrifuged and serum was isolated in the laboratory and in Eppendrof tubes transported to the biochemistry laboratory of applied drug research center in -70° to measurement of serum levels of copper and zinc with atomic absorption spectrophotometry method.

The serum Copper(sensitivity: 0.03 mg/l, detection Limit: 0.004 mg/l, Working Range: 0.018-40. mg/l) and Zinc(sensitivity: 0.01 mg/l, detection Limit: 0.003 mg/l, Working Range: 0.01-30. mg/l) concentrations was determined by Atomic absorption methods, using CTA 3000 Atomic Absorption Spectrometer(ChemTech Analytical Instruments Limited, UK) equipped with Air/acetylene flame.

In order to eliminate confounding variables, tests of liver and kidney were noted. The National Center for Health Statistics (NCHS) standard curve was used to convert weight and height to the standard Deviation Score (SDS).

Statistical analysis:

All data were analyzed using descriptive and deductive statistics methods by SPSS Ver. 15. The relation between qualitative data was evaluated using Chi-square test. And the relation between quality and quantity data were 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 meaningful.

3. Results

We studied the serum levels of copper and zinc in 50 epileptic patients with 50 healthy patients as control group. The two groups were matched for age, gender, weight and height. The demographic findings of both groups are shown in the table 1.

The mean zinc level in children with epilepsy is 0.30 ± 0.13 and 0.93 ± 0.25 µg/ml respectively which was lower meaningfully in epileptic patients (figure 1). The copper level in patients with epilepsy was 1.06 ± 0.36 µg/ml and in control group was 0.39 ± 0.21 µg/ml respectively which was significantly higher in the case group (figure 2). Laboratory parameters of both study groups are shown in table 2.

The family history of epilepsy and febrile convolution were positive in 9% and 2% of epileptic patients, respectively that positive family history of epilepsy was significantly more in case group.

Type of seizure and treatment and Response to treatment in patients with epilepsy upon gender is shown in table 3.

Laboratory findings between two groups based on Type of seizure and treatment and Response to treatment were shown in table 4.

The serum copper levels in patients with epilepsy had no linear correlation with laboratory parameters of study whereas the zinc levels of this group had a straight linear correlation with height percentile of patients($R=0.411, P=0.003$) and an inverse linear correlation with the age of onset of epilepsy($R=-0.279, P=0.045$).

The serum level of Zinc and Copper of patients with epilepsy according to the type of medication used are shown in table 5 indicating the serum level of Zinc in patient under treatment with Phenobarbital, Phenytoin, Carbamazepine, Valproate sodium, Clonazepam, Topiramate and Primidone was

significantly lower and the serum level of Copper in patients winder treatment by Phenobarbital, Phenytoin, Carbamazepine and Valproate sodium was significantly higher.

Onset of Epilepsy and treatment, Seizure count per month, Number of Drugs and Duration of treatment based on Type of seizure and treatment and Response to treatment were shown in table 6.

Table 1. Demographics parameter of patients between two groups

		Group		
		Case	Control	
Gender	Male	32	32	1
	Female	18	18	
Age		61.72 ± 39.99	61.52 ± 43.86	0.981
Height		107.55 ± 20.41	109.56 ± 21.88	0.636
Height Percentile-for-age		45.32 ± 21.82	53.90 ± 25.62	0.075
Weight		19.85 ± 9.16	21.48 ± 11.49	0.434
Weight Percentile-for-age		61.60 ± 20.51	63.15 ± 20.64	0.708

Table 2. Laboratory findings between two groups

	Group		P
	Case	Control	
Copper	1.06 ± 0.36	0.39 ± 0.21	<0.001
Zinc	0.30 ± 0.13	0.93 ± 0.25	<0.001
Ceruloplasmin	0.37 ± 0.09	0.37 ± 0.09	0.699
SGOT*	29.52 ± 6.43	26.96 ± 6.76	0.055
SGPT¥	11.44 ± 4.45	10.06 ± 3.34	0.083
Alkaline Phosphatase	446.38 ± 164.90	474.31 ± 162.28	0.395
GGT€	10.94 ± 4.63	9.48 ± 3.18	0.069
Total Protein	7.39 ± .66	7.57 ± .46	0.130
Albumin	4.31 ± .36	4.44 ± .41	0.123
Urea	23.22 ± 5.74	24.36 ± 5.59	0.321
Creatinine	0.55 ± 0.12	0.54 ± 0.14	0.770

*_ Serum Glutamine Oxaloacetice Transaminase
¥_Serum Glutamine Pyruvate Transaminase
€_Gamma-glutamyl Transpeptidase

Table 3. Type of seizure and treatment and Response to treatment based on gender

		Gender		P
		Male	Female	
Type of seizure	Generalized	28	16	0.631
	Partial	4	2	
Type of response to treatment	Non responder	10	8	0.638
	Moderate responder	17	8	
	Complete responder	5	2	
Type of treatment	Single drug	12	6	0.768
	Multiple drugs	20	12	

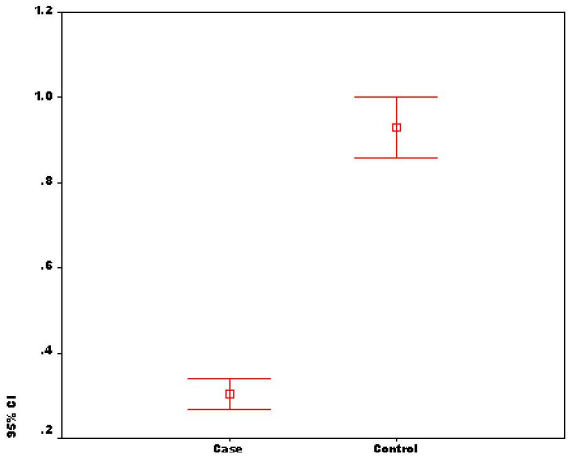
4. Discussions

Epilepsy is an important disease with an incidence rate of 0.5% rising up to 3% in case of a history of one time convulsion, including a wide range of symptoms and etiologies (Barbeau and Donaldson, 1974). Despite their low amount, micronutrients play an important role in metabolic processes and are effective in the development of central and peripheral nervous system. Deficiency of these micronutrients can lead to various health problems in central and peripheral nervous system (Motta, 1998).

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 and zinc are effective on incidence of convulsions and subsequently, epilepsy (Liu, 1998).

Free radicals and decreased antioxidant activity may be effective in increasing incidence risk of convulsions and their recurrence; and micronutrients (copper, zinc and magnesium) may prevent the incidence and progression of convulsions and subsequent epilepsy by increasing the antioxidant activity (Ilhan, 2004).

In our study, serum copper levels in epileptic patients undergoing treatment with anticonvulsant drugs were significantly higher than in control group; and in return, serum zinc levels in epileptic patients were significantly lower.



two groups

Motta et al demonstrated that prolonged use of anticonvulsant drugs decreases patients' serum copper levels, but has no effect on their serum zinc levels (Motta, 1998).

Evaluating copper and zinc levels in epileptic patients, Verrotti et al demonstrated that in the group treated with anticonvulsant drugs only the serum zinc level was significantly lower than in control group, but no significant difference in levels of other elements (copper and magnesium) (Verrotti, 2002).

In our study, the serum level of Zinc in patient under treatment with Phenobarbital,

Table 4. Laboratory findings between two groups based on Type of seizure and treatment and Response to treatment

	Type of seizure		P	Response to treatment			P	Type of treatment		P
	Generalized	Partial		Non responder	Moderate responder	Complete responder		Single drug	Multiple drugs	
Copper	1.08 ± 0.35	1.02 ± 0.46	0.720	1.15 ± 0.35	0.98 ± 0.38	1.08 ± 0.29	0.329	1.03 ± 0.31	1.09 ± 0.39	0.603
Zinc	0.29 ± 0.12	0.40 ± 0.09	0.038	0.32 ± 0.15	0.30 ± 0.12	0.26 ± 0.07	0.565	0.30 ± 0.12	0.31 ± 0.13	0.781
Ceruloplasmin	0.37 ± 0.09	0.43 ± 0.08	0.09	0.38 ± 0.09	0.38 ± 0.09	0.37 ± 0.06	0.90	0.35 ± 0.09	0.38 ± 0.08	0.24
SGOT*	29.52 ± 6.22	29.50 ± 8.55	0.99	28.94 ± 5.80	30.29 ± 7.48	28.29 ± 4.82	0.70	29.33 ± 6.75	29.63 ± 6.36	0.88
SGPT¥	12.80 ± 4.56	9.83 ± 2.32	0.12	12.28 ± 3.98	13.33 ± 4.95	10.29 ± 3.45	0.27	11.56 ± 3.94	12.94 ± 4.69	0.29
Alkaline Phosphatase	458.39 ± 164.18	358.33 ± 155.16	0.16	459.83 ± 131.81	417.96 ± 188.67	529.86 ± 144.94	0.27	434.00 ± 200.52	453.34 ± 144.19	0.69
GGT€	12.57 ± 4.37	15.67 ± 5.96	0.12	12.94 ± 4.18	12.79 ± 5.08	13.86 ± 4.91	0.87	14.06 ± 5.55	12.31 ± 3.99	0.20
Total Protein	7.27 ± 0.67	7.03 ± 0.57	0.40	7.53 ± 0.37	7.07 ± 0.82	7.20 ± 0.40	0.08	7.13 ± 0.47	7.31 ± 0.75	0.37
Albumin	4.12 ± 0.36	4.12 ± 0.43	0.99	4.26 ± 0.36	4.03 ± 0.33	4.16 ± 0.29	0.10	4.07 ± 0.30	4.15 ± 0.39	0.49
Urea	21.49 ± 5.71	19.33 ± 6.12	0.39	21.29 ± 6.11	20.46 ± 5.70	24.57 ± 4.20	0.25	22.83 ± 5.23	20.29 ± 5.90	0.13
Creatinine	0.45 ± 0.12	0.43 ± 0.13	0.66	0.42 ± 0.08	0.43 ± 0.12	0.55 ± 0.13	0.04*	0.42 ± 0.08	0.50 ± 0.15	0.04*

*_ Serum Glutamine Oxaloacetice Transaminase ¥_Serum Glutamine Pyruvate Transaminase €_Gamma-glutamyl Transpeptidase 0.42 ± 0.08

Table 5. Serum level of Copper and Zinc based on several drugs usages

		Copper	P	Zinc	P
Phenobarbital	No	0.48 ± 0.30	<0.001	0.85 ± 0.30	<0.001
	Yes	1.11 ± 0.35		0.27 ± 0.10	
Phenytoin	No	0.67 ± 0.42	<0.001	0.66 ± 0.37	<0.001
	Yes	1.13 ± 0.41		0.31 ± 0.11	
Carbamazepine	No	0.70 ± 0.45	0.021	0.64 ± 0.37	<0.001
	Yes	1.06 ± 0.34		0.34 ± 0.16	
Valproate sodium	No	0.67 ± 0.45	0.005	0.68 ± 0.38	<0.001
	Yes	0.99 ± 0.36		0.35 ± 0.15	
Clonazepam	No	0.71 ± 0.45	0.233	0.64 ± 0.37	<0.001
	Yes	0.91 ± 0.35		0.30 ± 0.06	
Nitrazepam	No	0.72 ± 0.44	0.101	0.63 ± 0.37	0.148
	Yes	1.15 ± 0.53		0.31 ± 0.18	
Topiramate	No	0.72 ± 0.45	0.201	0.62 ± 0.37	0.007
	Yes	1.13 ± 0.58		0.22 ± 0.06	
Epilim	No	0.73 ± 0.45	0.986	0.62 ± 0.37	0.348
	Yes	0.72		0.27	
Thioridazine	No	0.73 ± 0.45	0.986	0.62 ± 0.37	0.404
	Yes	0.72		0.31	
Lamotrigine	No	0.73 ± 0.45	0.674	0.62 ± 0.37	0.522
	Yes	0.54		0.38	
Primidone	No	0.71 ± 0.44	0.154	0.63 ± 0.37	0.001
	Yes	1.01 ± 0.40		0.29 ± 0.11	
Vigabatrin	No	0.73 ± 0.45	0.232	0.62 ± 0.37	0.513
	Yes	1.27		0.37	

Table 6. Onset of Epilepsy and treatment, Seizure count per month, Number of Drugs and Duration of treatment based on Type of seizure and treatment and Response to treatment

	Type of seizure		P	Response to treatment			P	Type of treatment		P
	Generalized	Partial		Non responder	Moderate responder	Complete responder		Single drug	Multiple drugs	
Onset of Epilepsy	26.36 ± 27.59	38.17 ± 43.65	0.365	26.44 ± 30.77	26.92 ± 30.22	38.00 ± 27.04	0.656	24.78 ± 23.84	29.47 ± 32.66	0.596
Seizure count per month	3.20 ± 2.74	-	-	2.50 1.29	3.40 ± 3.78	-	0.666	2.33 ± 2.31	3.57 ± 2.99	0.545
The Onset of treatment	26.77 ± 27.55	38.17 ± 43.65	0.381	27.56 ± 30.69	26.83 ± 30.20	38.00 ± 27.04	0.675	24.78 ± 23.84	30.03 ± 32.56	0.552
Number of Drugs	2.00 ± 1.01	2.50 ± 0.84	0.254	2.44 ± 0.92	2.00 ± 1.02	1.43 ± 0.79	0.059	1.00 ±0.00	2.66 ± 0.75	<0.001
Duration of treatment	32.16 ± 28.40	39.17 ± 24.89	0.569	26.78 ± 14.81	40.6 ± 35.96	24.14 ± 17.36	0.189	34.50 ± 31.43	32.16 ± 26.14	0.779

Phenytoin, Carbamazepine, Valproate sodium, Clonazepam, Topiramate and Primidone was significantly lower.

Barbeaus et al demonstrated that serum levels of copper and zinc in epileptic patients is lower than in normal people (Barbeau and Donaldson, 1974).Liu demonstrated that anticonvulsant treatment is associated with elevated serum copper level and ceruloplasmin (Liu, 1998).

In our study, the serum level of Copper in patient's winder treatment by Phenobarbital, Phenytoin, Carbamazepine and Valproate sodium was significantly higher.

Ilhan et al demonstrated that there is no significant difference between serum zinc level in epileptic patients and in normal people (Ilhan, 2004). Hamed et al demonstrated that levels of zinc and selenium in patients treated by anti-epileptic drugs, especially valproate, was higher than in the control group (Hamed, 2004).

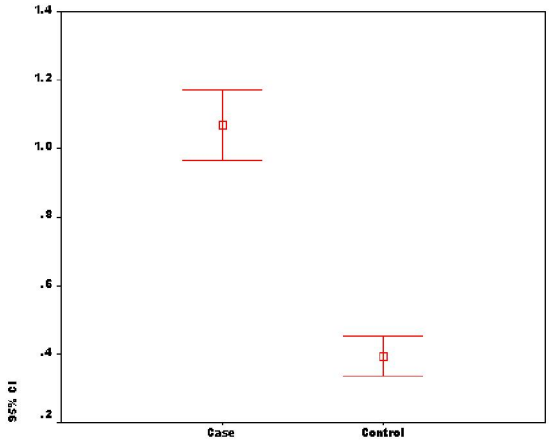


Figure 2. Distribution serum level of Copper between two groups

In our study, like the results of the above mentioned study, serum zinc levels in epileptic patients were lower than in normal people.

Verrotti's study demonstrated that the use of Phenobarbital was more effective than Sodium Valproate reducing serum zinc level of patients (Verrotti, 2002).

In this study, zinc and copper levels were 0.27 ± 0.10 and 1.11 ± 0.35 respectively in patients treated with Phenobarbital, and 0.35 ± 0.15 and 0.99 ± 0.36 respectively in patients treated with Sodium Valproate that Phenobarbital was more effective than Sodium Valproate reducing serum zinc level of patients.

Conclusion

Serum copper levels in epileptic children under drug treatment are higher than in healthy children. Also, serum zinc levels in these patients are significantly lower than in healthy people.The use of one drug or multiple drugs in the treatment of epileptic patients have made a significant difference in the levels of serum copper and zinc and also the serum level of Zinc in patient under treatment with Phenobarbital, Phenytoin, Carbamazepine, Valproate sodium, Clonazepam, Topiramate and Primidone was significantly lower and the serum level of Copper in patients winder treatment by Phenobarbital, Phenytoin, Carbamazepine and Valproate sodium was significantly higher.

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