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ORIGINAL ARTICLE

Cerebrospinal fluid and serum zinc, copper, magnesium and calcium levels in children with Idiopathic seizure

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ABSTRACT

Objectives: The present study was conducted to observe the alteration and their relations in cerebrospinal fluid (CSF) and serum Zinc (Zn), Copper (Cu), Magnesium (Mg) and calcium (Ca) levels in patients with different types of idiopathic seizure and to determine the ratios of serum and CSF Ca/Mg and Cu/Zn.

Methods: The children aged 1 to 14 years, having two or more unprovoked seizures, which were detected by normal MRI scan and abnormal EEG were included in the study group. The control group consisted of 40 healthy children without seizure. Zn, Mg and Cu levels in CSF and serum were analyzed by an atomic absorption spectrophotometer.

Results: The study subjects included 34 generalized seizures (GS), 5 cases of simple partial seizure (SPS) and 5 Complex partial seizures (CPS). Serum copper (Cu) was significantly elevated (P-0.01) in children with seizures. Within the seizure group, serum Mg was significantly increased in GS and serum copper (Cu) levels were significantly increased in CPS and GS as compared to controls (p-0.001). However, calcium and zinc levels did not show any significant change in all groups. CSF Calcium was significantly increased in CPS patients. The ratio obtained for the levels of these parameters revealed a significant increase in serum Cu/Zn ratio (P-0.002) and CSF Ca/Mg (P-0.04) in patients with idiopathic seizure as compared to controls. This ratio was also significant between SPS versus CPS and CPS versus GS.

Conclusion: The findings of the present study suggest that high serum Cu and the increased ratio of serum Cu/Zn and CSF Ca/ Mg may be responsible for enhanced neuronal excitability in children with idiopathic seizures.

Key Words: Idiopathic seizure, zinc, copper, magnesium, calcium

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Introduction

The exact pathogenesis of seizure is not fully understood but involves several factors like genetic predisposition, changes in the levels of neurotransmitters and some trace elements. Several reports suggested that the level of some trace elements play a vital role in causation of seizures [1], [2]. Among the trace elements, Zinc (Zn) acts as a co-factor of glutamic acid decarboxylase, an enzyme which maintains the production of GABA in the central nervous system. Decreased levels of Zn in CSF has also been observed in febrile seizures [2], [3]. Magnesium (Mg) is

also involved in neuronal function and inhibits the facilitatory effects of calcium on synaptic transmission and exerts a voltage dependent blockage of the N-methyl-D-aspartate (NMDA) receptor channel. Copper (Cu) inhibits Mg++-adenosine triphosphatase(ATPase) and Na+-K+-ATPase enzymes and disturbs sodium and potassium homeostasis, which results in the genesis of epileptiform discharges [4]. In some cases, the altered levels of trace elements in epileptic patients were attributed to anti-convulsant drug therapy or due to other unknown reasons.

A careful literature review reveals that a comprehensive record of zinc, copper, magnesium and calcium in serum and CSF and their ratios in children with idiopathic seizures is missing. Hence, to observe their significance in children, the current study was conducted to estimate their levels in children suffering from idiopathic seizures. Furthermore, an attempt has been made to find out the ratios of Ca/Mg and Cu/Zn levels and to correlate them with different types of seizures.

Materials and Methods

The present study was carried out in the Department of Pediatrics, at a tertiary care hospital in India from June 2006 to July 2008. A total of 44 children suffering from seizures, aged 1 year to 14 years were enrolled for the study.

Inclusion Criteria

Cases: Children of the age group of 1 year to 14 years, having two or more unprovoked seizures (first presentations in hospitals) which were detected by normal CT / MRI Scan and abnormal encephalogram (EEG) were included in the study group. Children with GS were treated with sodium and valproic acid, whereas cases with SPS and CPS received carbamazepine and valproic acid respectively.

Control: 40 healthy children (22 males and 18 females) without seizures.

Exclusion criteria

Children with malnutrition, acute bacterial meningitis and those taking Cu, Zn, Mg and Ca containing preparations were excluded from the study.

The protocol of the study was approved by the institute's Postgraduate Medical Board. Parents of each patient were explained about the illness of their child and informed consent was taken from the parent or legal guardian after explaining the procedure, which is an integral part of the study.

Collection and Storage Of Samples

Blood samples were collected through vein puncture using aseptic precautions. The serum was separated and transferred into plastic tubes. The patients underwent lumbar puncture and the CSF was collected in acid washed plastic tubes. One millimeter of CSF was used for the estimation of trace elements. Both CSF and serum samples were stored at -20°C until further analysis was done. The glass and polypropylene equipments used for the analysis of trace elements were soaked in 10% (v/v) nitric acid for 12 hours and were then rinsed with double distilled deionized water.

Estimation of Cu, Zn, Ca and Mg

Cu, Zn, Ca and Mg levels in CSF and serum were assayed by an atomic absorption spectrophotometer in the Department of Botany, Faculty of Science, Banaras Hindu University, Varanasi. Both CSF and serum samples were diluted with double deionized distilled water and the dilution factors were 1:3 and 1:6 for zinc and copper respectively and 1:10 for Ca and Mg estimation. The standard solution contained 100 μg/ml of each element and was used for calculation in the analysis of Cu, Zn, Ca and Mg levels in CSF and serum. Sample readings were taken thrice and the arithmetic mean was calculated.

Statistical Analysis

The data was analyzed using SPSS software

version 10. Student't' test and Man Whitney U-test were used to compare the significant difference of the means between the control and the patients. Data which did not follow normal Gaussian distribution were compared by the Wilcoxan Rank sum or the Kruskal-Wallis test. The post-hock test was used to find out the pair wise significant difference if one way ANOVA was significant. The ratio and correlation coefficients were also calculated. Categorical data were compared by calculating the chi-square value or by the Fischer exact test.

Results

In our study, there were 44 cases and 20 controls. The age of the subjects (34 males and 10 females) ranged from 1 – 14 yrs (mean 6.18 ± 3.12 yrs.) and the 40 age matched healthy controls ranged from 1 – 14 yrs (mean 5.96 ± 2.28 yrs.). The male to female ratio was 3:2. Out of 44 cases, 34 patients had generalized seizures and there were 5 patients each of simple partial and complex partial seizures.

In this study, Serum Cu levels in children with seizures (112.91 ± 41.88 µg /dl) was significantly increased as compared to controls [66.46 ± 9.76] (p-0.001). Although Serum Zn levels (64.82 ± 18.44) were found to be decreased, it was statistically insignificant. The present study demonstrated that serum Mg levels were low in cases (0.87 ± 0.34) as compared to controls (0.93 ± 0.18) but this was statistically insignificant (p-0.564). Serum calcium levels in the seizure group (10.47 ± 1.16) remained comparable to the control group. There was no significant difference in the levels of Ca, Mg, Cu and Zn in CSF in cases and controls [Table/Fig 1].

(Table/Fig 1) Serum and CSF Cu, Zn, Ca and Mg in study subjects

	Serum				CSF			
Parameter	Control (n=40)	Patients (n=44)	t	p	Control (n=40)	Patients (n=44)	t/u*	p
Calcium (mg/dl)	10.47 ± 1.16	10.76 ± 3.29	0.267	0.791	6.47 ± 0.87	5.85 ± 2.49	0.773	0.443
Magnesium (mg/dl)	0.93 ± 0.18	0.87 ± 0.34	0.580	0.564	1.31 ± 0.18	1.03 ± 0.58	1.481	0.145
Copper (µg/dl)	66.46 ± 9.76	112.91 ± 41.88	3.462	0.001	43.34 ± 19.32	36.69 ± 24.89	180*	0.373*
Zinc (µg/dl)	72.54 ± 23.56	64.82 ± 18.44	1.134	0.262	5.61 ± 2.02	6.64 ± 4.40	215*	0.911*

*CSF Man Whitney U- test for Cu and Zinc

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Within seizure group, serum Mg was significantly increased in GS as compared to SPS (p-0.03) and serum Cu levels were significantly increased in CPS and GS as compared to Control (p-0.001). CSF Ca was significantly decreased in children with SPS and GS [Table/Fig 2]. No significant change was observed in level of Mg, Zn and Cu in CSF between partial and generalized seizure group.

(Table/Fig 2) Cu, Zn, Ca and Mg in serum and CSF in sub-groups of study subjects

Parameter	Control[1] (n=40)	SPS[2] (n=5)	CPS[3] (n=5)	GS[4] (n=34)	F / H^	p
Serum Calcium (mg/dl)	10.47 ± 1.16	7.92 ± 1.96	10.76 ± 0.88	11.17 ± 3.48	1.807	0.158
Serum Magnesium# (mg/dl)	0.93 ± 0.18	0.51 ± 0.23	0.83 ± 0.25	0.92 ± 0.33	3.061	0.037
Serum Copper (µg/dl)†	66.46 ± 9.76	78.57 ± 21.97	131.33 ± 18.57	115.25 ± 44.15	6.171	0.001
Serum Zinc (µg/dl)	72.54 ± 23.56	71.5 ± 11.33	47.85 ± 18.02	66.34 ± 18.32	2.074	0.115
CSF Calcium (mg/dl)§	6.47 ± 0.87	5.17±1.29	8.31±1.75	5.59±2.54	2.657*	0.014*
CSF Magnesium (mg/dl)	1.31 ± 0.18	0.68±0.21	1.28±0.37	1.05±0.62	10.692*	0.682*
CSF Copper (µg/dl)	43.34 ± 19.32	39.79±13.30	32.95±31.29	36.78±25.76	1.500*	0.789*
CSF Zinc (µg/dl)	5.61 ± 2.02	5.13±3.67	6.83±5.01	6.83±4.49	1.051*	0.741*

*kruskal-wallis test, # SNK test showed significant difference in serum magnesium between group 2 and 4 at 0.05 level, † SNK test showed significant difference in serum magnesium between group 1 & 3 and 1 & 4 at level 0.05 and 0.01 respectively, §Man Whitney U- test showed significant difference in CSF calcium between groups 2 & 3 and 3 & 4 at 0.05 level.

Ratios between the levels of Ca/Mg and Cu/Zn were also calculated in serum and CSF [Table/Fig 3]. The serum Cu/Zn ratio in cases was significantly elevated as compared to controls (P-0.002) but no significant difference was observed in CSF. The Ca/Mg ratio in CSF was significantly increased (P-0.04) in patients with seizure as compared to controls but no changes were observed in serum [Table/Fig 3].

(Table/Fig) 3-Serum and CSF ratio of Ca-Mg and Cu-Zn in sub-groups of study subjects

Ratio	Parameter	Control[1] (n=40)	SPS[2] (n=5)	CPS[3] (n=5)	GS[4] (n=34)	H	p
Serum	Ca : Mg	11.66 ± 2.64	18.48 ± 10.18	14.26 ± 5.53	13.40 ± 5.72	2.13	0.546
	Cu : Zn	1.01 ± 0.38	1.14 ± 0.44	3.13 ± 1.35	1.98 ± 1.26	15.10	0.002
CSF	Ca : Mg	5.00 ± 0.73	7.97 ± 2.76	6.68 ± 1.55	6.55 ± 4.50	7.89	0.048
	Cu : Zn	9.24 ± 7.44	9.94 ± 4.85	5.47 ± 3.78	8.19 ± 7.69	2.62	0.454

The pairs of metal concentrations for correlations that were statistically significant ($p<0.05$) are shown in [Table/Fig 4]. Only 9 of all the possible correlations were significant in the control and study groups. The strongest correlations observed were between CSF calcium and CSF magnesium (r=0.39). Serum Cu had significant negative correlations with serum zinc(r = -0.324). CSF Ca also showed positive correlations with CSF Mg, serum Zn and Serum Ca.

(Table/Fig 4): Correlations of CSF and Serum Cu, Zn, Ca and Mg in study subjects.

Parameters	Control (n=40)		Study subjects (n=44)		Partial (n=10)		Generalized (n=34)	
	r	p	r	p	r	p	r	p
Mg CSF/ Mg Serum	0.460	0.181	0.354	**0.018**	0.598	0.068	0.321	0.064
Mg Serum / Cu Serum	-0.696	**0.025**	0.188	0.222	0.460	0.180	0.111	0.532
Ca CSF/ Mg CSF	0.432	0.213	0.390	**0.009**	0.840	**0.002**	0.340	**0.049**
Cu Serum / Mg CSF	-0.494	0.147	0.383	**0.008**	0.632	**0.050**	0.356	**0.039**
Zn CSF/ Ca Serum	-0.640	**0.047**	-0.126	0.415	0.071	0.846	-0.186	-0.292
Zn Serum / Ca CSF	-0.68	**0.03**	-0.222	0.147	-0.579	0.079	-0.105	0.553
Zn Serum / Ca Serum	0.548	0.101	0.341	**0.024**	-0.182	0.615	0.407	**0.017**
Zn Serum / Cu Serum	-0.010	0.978	-0.324	**0.032**	-0.466	0.175	-0.323	0.062
Zn Serum / Mg Serum	0.270	0.450	-0.192	0.211	-0.699	**0.024**	-0.153	0.386

Discussion

Serum copper levels in children with seizures and their subgroups were significantly increased, as observed by other workers [5], [6], [7], [8], [9] but Smith *et al*. [10] and Kurekci *et al*.[11] have reported thet there is no significant change. The increased copper levels in serum may be due to the effect of anti-epileptic drugs, increased hepatic synthesis or due to the decreased breakdown or both copper binding proteins, altered intestinal absorption and altered excretion patterns, changes in the distribution among body tissues or a combination of the above factors [8], [9]. CSF Cu was found to be comparable in both cases and controls, as observed by Goody et al.[12] This variation in serum and CSF values might be due to some unknown factors which need to be further evaluated.

Zinc is essential for the normal development of the brain. Zn levels in serum and CSF in patients with seizures were found to be decreased, but were statistically insignificant as observed by other workers [9], [10], [12]. Low Zn concentrations have been reported to be present in the serum and CSF of patients with epilepsy [13]. The mechanism by which the depletion of zinc facilitates seizure activity is hypothesized to be its inhibitory effect on GABA, an inhibitory neuro-transmitter. Zinc also plays an important role in both the synthesis and the function of GABA [14]. Goody et al [12] and Kapaki et al [15] had reported the presence of increased CSF concentrations in patients with neurological disorders.

The present study demonstrated low serum and CSF Mg levels in cases but this finding was insignificant. Our results are similar to those of other workers [16], [17], [18], [19]. However, Alwarez-Dominiguez et al. [20] reported the presence of higher serum magnesium levels in epilepsy. Rude [21] hypothesized that magnesium deficiency is responsible for the hyper- excitability of neurons. It is apparent from this study that in normal subjects, serum magnesium levels are lower than the levels in CSF, suggesting that some mechanism other than mere diffusion is responsible for maintaining these relatively higher concentrations in CSF.

It is well documented that low levels of calcium are responsible for the initiation of seizures. However, in the present study, serum calcium in seizures remained comparable to the control as reported by Rutter *et al* [22], but other workers [18] [19] reported the presence of higher levels of calcium in epileptic children. Calcium facilitates the release of acetylcholine by nerve impulses, which may be responsible for neuromuscular irritability. Hypomagnesaemia and hypercalcaemia combine to produce a membrane state, which becomes responsive to an otherwise sub-threshold stimulus.

The generalized seizure group had significantly higher levels of serum Mg as compared to the partial seizure group in the present study. Unlike our results, Shah et al

[16] had found nearly comparable levels of Mg between the two groups. The present study also showed a significant decrease in CSF Ca in children with SPS and CPS. We can hypothesize that the type of seizure had an influence on the concentrations of Mg and Cu in serum.

It was observed that serum Cu/Zn and CSF Ca/Mg ratios were significantly elevated in the study group. The elevated Cu/Zn ratio may be closely associated with the initiation and continuance of seizures. Leaver et al. [23] also observed a decline, both in calcium and magnesium concentrations and an increase in the serum Ca/Mg ratio, as was seen in our study. Our observation on the possible use of the Cu/Zn ratio is a new concept and may help to judge the enhancement of neuronal excitability.
The strongest correlations observed in our study were between CSF Ca and CSF Mg (r=0.39) as observed by Woodbury et al.[24] and Bogden JD et al. [25] The correlation between plasma and CSF Mg (r=0.35) although significant, is not strong enough to allow a useful estimate of the Mg concentration in CSF from its concentration in plasma.

The estimation of serum and CSF levels of Ca, Mg, Cu and Zn and their ratios are essential for the rational understanding of pathogenesis and for the management of childhood idiopathic seizures and their sub-groups. Changes in trace metals might be associated to the type of seizures, rather than to anti-convulsant therapy. However, it is suggested to conduct a study with greater sample size to evaluate the role of trace elements and their ratios in childhood seizures.

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