

The Correlation Between Cerebrospinal Fluid and Levels of Serum Zinc and Calcium in Children With Febrile Seizure

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Background: Febrile seizure (FS) is one of the most common neurological conditions in childhood. It seems that zinc and calcium deficiency are related to seizure activity.

Objectives: The aim of the present study was to measure zinc and Calcium levels in the serum and cerebrospinal fluids of febrile convulsive children referred to Zanjan University, Valie Asr hospital.

Patients and Methods: Three hundred and ten consecutive children with febrile seizure, who were admitted from January 2009 to December 2010, were studied. Forty five children required lumbar puncture based on the discretion of treating physician. Levels of zinc and calcium were measured in the serum and cerebrospinal fluid specimens of patients using atomic absorption spectrometry method.

Results: The mean levels of serum calcium, serum zinc, cerebrospinal fluid calcium and cerebrospinal fluid zinc were 14.51 ± 3.69 mg/dL; 184.04 ± 65.49 µg/dL; 5.23 ± 2.21 mg/dL and 87.06 ± 28.25 µg/dL respectively.

Conclusions: We did not find any clear abnormality in plasma or cerebrospinal fluid calcium and zinc levels in children with febrile seizures in our patient population.

Keywords: Calcium; Cerebrospinal Fluid; Seizures, Febrile; Serum; Zinc

1. Background

Febrile seizure (FS) is one of the most common neurological conditions in childhood (1-4). The reported prevalence rate for most developed countries varies between 2% and 5% (5-8). Febrile seizure has been defined by the International League Against Epilepsy (ILAE) as "a seizure occurring in childhood after one month of age, associated with a febrile illness not caused by an infection of the central nervous system, without previous neonatal seizures or a previous unprovoked seizure, and not meeting the criteria for other acute symptomatic seizures" (1, 3, 7, 9, 10). The pathogenesis of this condition is still unknown. Several potential hypotheses such as genetic basis, immunologic disorders, low serum iron as well as reduction of serum and cerebrospinal fluid (CSF) zinc, calcium, and magnesium levels and low gamma-aminobutyric acid have all been proposed to explain the pathophysiology of this condition (11-16).

Zinc plays an important role in children's growth and development and as one of the most prevalent trace elements in brain, it contributes to normal brain function (12, 14, 17). It is believed that as a co-factor of glutamic

acid decarboxylase, zinc modulates the production of Gamma-aminobutyric acid in central nervous system (11, 14). A low Gamma-aminobutyric acid level in cerebrospinal fluid, seems to be related to seizure disorders (13). The role of low ionized calcium for initiation and manifestations of seizures is well known (14, 18). The release of acetylcholine and thereby neuromuscular irritability are facilitated by calcium (14). Therefore, study of these trace elements could clarify the pathogenesis and influence the treatment of febrile seizures.

In some studies, CSF zinc levels in FS patients were significantly lower than control group (19-22); whereas other studies revealed no significant difference in CSF zinc levels (14). Although, many investigations showed significantly lower level of serum zinc comparing to control group (12, 19, 22, 23). In some studies, serum zinc levels were within normal limit (24). Similar controversies exist about CSF calcium levels. Regarding these inconsistent data, and the fact that although our city Zanjan is located in a region with large number of zinc mines and major sources of zinc in Iran, we still have a high prevalence of febrile seizures, and also because similar studies have not yet been done in this region, we conducted the cur-

Implication for health policy/practice/research/medical education:

Study of these trace elements could clarify the pathogenesis and influence the treatment of febrile seizures.

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rent study to estimate serum and CSF levels of zinc and calcium in children suffering from febrile seizures in our geographic area.

2. Objectives

The aim of the present study was to measure the cerebrospinal fluid level of zinc and calcium in children with febrile convulsion.

3. Materials and Methods

This cross-sectional study was carried out in the Department of Pediatrics of Zanjan Vali-e-Asr hospital, a teaching hospital of Zanjan University of Medical Sciences, Zanjan, Iran. Three hundred and ten consecutive children with febrile seizure, who were admitted from January 2009 to December 2010, were studied. Forty five children who met our inclusion criteria and required lumbar puncture based on the discretion of the treating physician were enrolled in the study. All children of the age group of nine months to five years, with axillary temperature ≥ 37.4 who were admitted with diagnosis of febrile seizure in the paediatric ward and underwent lumbar puncture in the first twelve hours of admission, entered this study.

Children with seizures longer than 15 minutes, recurrences in 24 hours, focal neurologic signs, acute bacterial meningitis and abnormal EEG were excluded from the study. The project was approved by scientific advisory and ethical committees of Zanjan University of Medical Sciences. Parents of the patients were informed about the illness of their child and written informed consent was

obtained from them before inclusion of the children in the study.

3.1. Collection and Storage of Samples

All children, admitted to the hospital with the diagnosis of febrile seizure who needed lumbar puncture, entered the study until the completion of sample size. The sample size was estimated to be 42, but we enrolled 45 patients in the study. Within 12 hours of admission to the hospital, blood samples were collected and 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 milliliter of CSF was used for trace elements estimation. Both CSF and serum samples were stored at -70°C until the analysis time. Serum and CSF zinc and calcium levels were assayed by Atomic Absorption Spectrophotometer in the Faculty of Chemical Sciences in Institute for Advanced Studies in Basic Sciences of Zanjan (IASBS). Some regional and environmental factors which could affect serum zinc levels such as using zinc supplements, antibiotics and antipyretics and medicines could interfere with the results, but unfortunately these factors could not be omitted.

3.2. Statistical Analysis

Statistical analysis was performed using SPSS for windows (version 16) software; employing Student's t-test for comparing continues variables in two groups and Pearson correlations for association between variables. All of the statistical tests were two-sided. P values of less than 0.05 were considered significant.

Table 1. Gender and Age Differences in Serum and CSF Levels of Zinc and Calcium in Patients With Febrile Seizure

Levels of Element	Groups	Levels, Mean \pm SD	P Value
Serum levels of calcium, mg/dL	sex	Girls, n = 24	15.1 \pm 3.3
		Boys, n = 21	13.8 \pm 4.1
	age	Less than 18 mo, n = 30	14.41 \pm 3.55
		More than 18 mo, n = 15	14.7 \pm 4.07
CSF levels of Calcium, mg/dL	sex	Girls, n = 24	5.05 \pm 2.3
		Boys, n = 21	5.64 \pm 2.4
	age	Less than 18 mo, n = 30	5.56 \pm 2.34
		More than 18 mo, n = 15	4.58 \pm 1.81
Serum levels of zinc, $\mu\text{g/dL}$	sex	Girls, n = 24	194.12 \pm 61.0
		Boys, n = 21	172.52 \pm 69.9
	age	Less than 18 mo, n = 30	183.3 \pm 60.26
		More than 18 mo, n = 15	185.53 \pm 77.15
CSF levels of zinc, $\mu\text{g/dL}$	sex	Girls, n = 24	85.08 \pm 31.5
		Boys, n = 21	89.33 \pm 24.6
	age	Less than 18 mo, n = 30	85.40 \pm 29.5
		More than 18 mo, n = 15	90.4 \pm 26.09

4. Results

Of a total of 45 children, 24 (53.3%) patients were females, and 21 (46.7%) patients were males. Patients' ages were between nine and 59 months with the mean age of 18.45 ± 11.67 months. The body temperature at the time of admission differed between 37.5°C and 40.5°C and the mean admission temperature was $38.47 \pm 0.8^\circ\text{C}$. The mean age of girls and boys were 17.34 ± 9.68 months and 19.73 ± 13.74 months respectively. The mean body temperature in girls was $38.38 \pm 0.97^\circ\text{C}$ and in boys was $38.58 \pm 0.61^\circ\text{C}$. There was no significant difference in the age and body temperature range between boys and girls ($P = 0.5$, $P = 0.42$ respectively). Mean serum calcium was 14.51 ± 3.69 mg/dL and mean serum zinc level was 184.04 ± 65.5 $\mu\text{g/dL}$ with the range of 90 - 380 $\mu\text{g/dL}$. The mean calcium and zinc levels of CSF were 5.33 ± 2.2 mg/dL and 87.06 ± 28.2 $\mu\text{g/dL}$ respectively. The range of calcium in CSF was between 0.7 $\mu\text{g/dL}$ and 9.90 mg/dL and zinc level of CSF

varied between 20 $\mu\text{g/dL}$ to 150 $\mu\text{g/dL}$. Serum and CSF levels of zinc and calcium in both sexes and in different age groups are shown in Table 1.

In addition, no significant differences in cerebrospinal fluid zinc and calcium levels were found when measures such as age, gender and maximal temperature were compared (Table 1). Although our results showed a significant correlation between serum and CSF calcium levels ($r = 42\%$, $P < 0.003$), no significant correlation between serum and CSF zinc levels was detected ($r = 8\%$, $P = 0.57$). There was statistically significant correlation between the levels of serum zinc and patients presenting body temperature. ($r = 0.33$, $P = 0.02$), no significant correlation was found between the body temperature and serum calcium, CSF calcium and zinc levels. A reverse statistically significant correlation between serum zinc level and serum and CSF calcium levels was observed ($r = -0.35$, $P = 0.02$ and $r = -0.43$, $P = 0.003$ respectively) (Table 2).

Table 2. Correlations of Serum and CSF Zinc and Calcium Levels With Body Temperature

Levels of Element	Calcium Level of Serum, No. (%)	Calcium Level of CSF, No. (%)	Zinc Level of Serum, No. (%)	Zinc Level of CSF, No. (%)
Calcium level of serum	1	-	-	-
Calcium level of CSF	0.42 ^a (0.003)	1	-	-
Zinc level of serum	-0.35 (0.01)	0.43 (0.003)	1	-
Zinc level of CSF	-0.08 (0.58)	0.04 (0.77)	0.08 (0.57)	1
Temperature	-0.20 (0.17)	-0.06 (0.69)	0.33 (0.02)	0.19 (0.21)

^a pearson correlation

5. Discussion

Several hypotheses have been proposed to explain the possible relationship between zinc, calcium, GABA, and convulsions (9, 11, 14). In our study, a total of 45 children with febrile seizure who underwent a lumbar puncture were studied. The mean serum calcium and zinc level were 14.51 ± 3.69 mg/dL and 184.04 ± 65.5 $\mu\text{g/dL}$ respectively, also the mean calcium and zinc level of CSF were 5.33 ± 2.2 mg/dL and 87.06 ± 28.2 $\mu\text{g/dL}$ respectively.

In this study, no patient had hypocalcemia which is similar to findings of Sakha's study (15) in Tabriz. The same results were shown in the study of Seyedzadeh (25) and Nikavar (26) in which no significant differences in serum calcium levels between patients with febrile seizure and control groups were seen. In the current study, CSF to serum calcium ratio was low which may be explained by higher serum calcium level. The mean serum zinc level in our study was higher than other studies (11, 13, 19, 23). The normal value for serum zinc level in our laboratory was considered 70 - 200 $\mu\text{g/dL}$ and no hypozincemia was detected. Although our results are different from many studies (11, 13, 19, 20, 23), a recent study (24) supports our findings. In the study of kafadar et al. at Turkey in which

febrile convulsive patients were compared with febrile but non convulsive and also healthy children, serum zinc concentration did not show any significant differences (24).

The mean CSF zinc level in this study was higher than others, (19, 20) and was similar to the study of Papierkowski (27). We did not find any low zinc level in CSF, which was similar to the study of Tutuncuoglu et al. who didn't show a significant difference between CSF zinc level of patients and febrile seizure and control groups (22). No significant differences in cerebrospinal fluid and serum zinc levels were found when parameters such as age and gender were compared, which is similar to results of other studies (13, 21, 24). We found statistically significant correlation between the body temperature and serum zinc level. Mollah in Bangladesh found no significant relation between both serum and CSF zinc levels and duration of fever (19). A positive and significant correlation between body temperature and serum zinc levels in our study may suggest that a higher body temperature is needed to cause seizure in FS patients with already higher serum zinc levels.

Our results do not support the assumption that febrile seizures are related to reduced zinc or calcium

levels in the serum or central nervous system. However these findings should be interpreted cautiously due to multiple limitations present in our study. In the present study, only 14.5% of patients presenting with febrile seizure underwent lumbar puncture. Because of ethical considerations, we did not perform LP in all FS patients and lumbar puncture was performed only when it was considered clinically necessary based on the discretion of the treating physician. Also we could not recruit a matched control group due to exposure of the control group to the risks of unnecessary lumbar puncture. All children received some medications such as antibiotics and antipyretic agents before and or after admission in the hospital which can affect measurements of serum or possible CSF levels of calcium and zinc. A common practice of zinc supplements, to augment the growth and development, and control diarrhea in our medical community, may also affect our data. Geographic, regional and environmental factors affecting serum zinc levels further limit the generalization of our findings. High level of zinc may be attributed to the presence of zinc mines in our geographic area.

Although we didn't have a control group, the fact that our patients still had febrile seizure despite high levels of zinc in their serum or CSF is noteworthy and makes doubts about the role of zinc in febrile seizure and its prophylactic use in all geographical areas. Further studies in different geographic areas, with large sample sizes and different control groups are recommended to support the existing hypothesis that low serum and CSF zinc have important roles in febrile seizures.

We have not found any clear abnormality in serum or cerebrospinal fluid calcium and zinc levels in children with febrile seizures in our patient population. Our findings do not support the role of reduced serum zinc concentration in febrile seizure. More studies in different geographical areas are of great consideration.

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Authors' Contribution

Study concept and design: Mansour Sadeghzadeh, Parisa Khoshnevisasl; acquisition of data: Samane Nabi, Mansour Sadeghzadeh; analysis and interpretation of data: Mansour Sadeghzadeh, Parisa Khoshnevisasl, Nooreddin Mousavinasab; drafting of the manuscript: Parisa Khoshnevisasl, Mansour Sadeghzadeh, Nooreddin Mousavinasab; critical revision of the manuscript for important intellectual content: Parisa Khoshnevisasl, Mansour Sadeghzadeh; statistical analysis: Nooreddin Mousavinasab; administrative, technical, and material support: Parisa Khoshnevisasl, Mansour Sadeghzadeh,

Samane Nabi; study supervision: Mansour Sadeghzadeh.

Financial Disclosure

The authors declare that they have no competing interests.

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