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CLINICAL STUDY

Major and minor bio-element status in children with febrile seizure

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Abstract: Febrile seizures (FS) are the most common cause of seizures in children. The exact etiopathogenesis is unknown but involves factors like genetic predisposition and alterations in the levels of neurotransmitters and some trace elements. The study includes 48 consecutive children with FS, and 55 healthy age matched control subjects. Calcium, magnesium and potassium concentrations in the febrile study group were lower than in the control group ($p<0.05$). Iron and Gallium levels in the study group were lower than in the control group ($p<0.01$). Serum Selenium ($p<0.001$), Zinc ($p<0.001$) and Strontium ($p<0.05$) levels were significantly decreased in the study group when compared to the control group. There was no significant difference between the control and study groups in Serum Barium, Beryllium and Copper levels ($p>0.05$). The aim of the present prospective analytical case-control study was to determine whether there was any change in element levels in children with FS *(Ref. 33)*. Full Text in PDF *www.elis.sk*.
Key words: febrile seizures, children, trace elements.

Febrile seizures (FS) are defined as an event during infancy or childhood, usually between 3 months and 5 years of age, associated with fever and without any evidence of intracranial infection or a definite cause for the seizure (1, 2). As the mechanisms underlying FS have multifactorial etiology, and so far remain unclear, its genetic causes are being studied (3–5). However, FS represents the point between a low seizure threshold and genetic components, recognized to be susceptible for FS and caused by mutations in several gene maps, such as FEB-1 to FEB-4 (5–7). Moreover, some mutations causing neural hyperexcitability could be responsible for FS especially with polygenic multifactorial genetic trait (5, 6, 8).

Bio-elements as trace and major elements refer to chemical minerals required in different quantities by an organism to maintain its normal physiological function (9). Function of bio-elements in cells is generally complex and these functions in different ways. Cells require metal ions as co-factors of metalloproteins. Most metal ions are directly incorporated into their cognate sites in proteins involved in various cellular pathways. However, some metal ions become a part of the prosthetic groups, co-factors, or complexes prior to insertion of these moieties into the target proteins (10, 11). Several essential elements play important roles in redox reactions, in connective tissue or cell membranes, in stabilization of biological molecules and in control of biological processes by facilitating the binding of molecules to receptor sites on cell membranes (12).

In this study, serum major and trace elements were studied in children with FS. The aim of the present prospective analytical case-control study was to determine whether there was any change in element levels in children with FS.

Patients and methods

The study included 48 consecutive children with FS, and 55 healthy age matched control subjects, followed in Yuzuncu Yil University, Faculty of Medicine, Department of Pediatrics. All these children had normal neurological examination. The local ethical committee of our hospital approved the study.

Blood samples were collected from the patients with FS and healthy control subjects, which were included in the study. It was obtained from children with FS within a few hours after seizure attack. Venous blood samples were obtained from each subject and transferred to normal tubes. Serum was obtained by centrifugation at 2000 *g* for 10 min of blood samples taken without anticoagulant and stored at –20 ºC until analysis date.

Hydrogen peroxide, nitric acid, perchloric acid, and suprapur ICP multielement standard solutions were purchased from Merck. All other chemicals and reagents used in this study were of analytical grade. Ultra-distilled water was used as the solvent.

All the plastic containers were previously washed in 10 % nitric acid (ultrapure grade) and then repeatedly rinsed with ultra water. Decomposition of the organic matrix in the tissue samples were performed using a microwave oven. A Milestone Start D (Italy) microwave oven equipped with a Pro 24 High Throughput Rotor

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and a temperature control program was used to digest simultaneously 24 samples of tissue in one cycle. The rest of the elements were determined after mineralization of the samples. The serum samples of exactly 0.1 g were weighed (wet weight) and put in high-pressure Teflon vessels and added with 3 ml of concentrated nitric acid, 1 ml of hydrogen peroxide and 0.5 ml perchloric acid (ultrapure, Merck, Germany). The Teflon vessels were then sealed with a Teflon lid and put into the steel bombs, which were sealed with exactly the same momentum. The mixture in the bombs was heated in a microwave oven according to the following sequence (temperature/time): 90 °C/15 min, 120 °C/15 min, 140 °C/ 60 min, 150 °C/60 min. After cooling to room temperature, this solution was quantitatively transferred and adjusted in a flask to 10 mL with 18.2 MΩ.cm ultra water (Millipore DirectQ UV, Japan). Trace and major element concentrations in the digest were determined by inductively coupled plasma-optical emission spectroscopy (ICP-OES; Spectro Genesis, Germany). The operating conditions of the ICP-OES are given in Table 1. Accuracy of the analysis was verified by the determination of the mineral content of the ICP multielement standard obtained from Merck (Germany).

Statistical analysis

Statistical analysis was performed with the Statistical Package for the Social Sciences for Windows (SPSS version 10.0, Chicago, IL, USA). Data were expressed as the mean ± standard deviation (SD). Student's *t*-test was used to compare the mean values of different bio-element parameters between the study and control groups. In all data analysis, a value of $p<0.05$ was considered statistically significant.

Results

A total of 48 children 17 girls (35.5 %) and 31 boys (64.5 %), aged 0.5 years to 5 years (mean 22.2±13.7 months) with FS and 55 healthy children 28 girls (51 %) and 27 boys (49 %), aged 0.5 years to 5 years (mean 28.8±17.0 months) were included in the study.

Table 2 shows serum major element levels in the control and study groups. Calcium (Ca) and potassium (K) concentrations in the febrile study group were lower than the control group ($p<0.05$). Magnesium (Mg) concentrations was also statistically decreased in the study groupwhen compared to the control group ($p<0.01$).

Serum trace element levels in the control and study groups are shown in the Table 3. Iron (Fe) and Gallium (Ga) levels in the study group were lower than in the control group ($p<0.01$). Serum Selenium (Se) ($p<0.001$), Zinc (Zn) ($p<0.001$) and Strontium (Sr) ($p<0.05$) levels were significantly decreased in the study group when compared to the control group. There was no significant difference between the control and study groups in Serum Barium (Ba), Beryllium (Be) and Copper (Cu) levels ($p>0.05$).

Discussion

FS are the most common cause of seizures in children. It has been known since ancient times that seizures frequently accompany fever in young children. The exact pathogenesis is unknown but involves factors, such as genetic predisposition and alterations in the levels of neurotransmitters and some trace elements (8, 13, 14). There is a growing evidence that the balance between the bio-elements, such as Mg, Ca, Fe, Cu, Zn, and Se in the nervous tissue is of crucial importance for maintaining human health (15). In biological systems, these mainly metallic elements are usually bound to proteins. Metals in metalloproteins are components of enzymatic systems, and fulfill structural and storage functions. Hence, these elements are responsible for various metabolic processes, including those occurring in the brain (16). Existing evidence suggests that epileptic seizures significantly change the metabolism and distribution of trace elements in the nervous tissue (17–19). On the other hand, metal contents determine susceptibility to convulsions (20).

In the present study, we investigated the levels of trace and major element concentrations in children with FS. Our results of this study showed that bio-element levels were affected in children with FS. The changes in bio-elements in FS explained the respond of the metabolism. Amiri et al found significantly low levels of Se and Zn in patients with FS (21).

Zinc is a component of more than 300 different enzymes that function in many aspects of cellular metabolism, involving metabolism of proteins, lipids, and carbohydrates (22). Moreover, according to different reports, Zn is considered either as a convulsion promoting factor or as an anticonvulsant (23, 24). Decreased Zn levels have been reported in the serum of epileptic patients and in the cerebrospinal fluid of infants with benign postnatal seizures (25–27). In our study, we found that serum Zn ($p<0.001$) levels were significantly decreased in children with FS.

It was noted that there was a significant reduction in serum Mg values in FS in comparison to encephalitis and fever with meningismus. Papierkowski et al (28) also reported similar findings in their series. In our study, we found that Mg ($p<0.01$) levels were significantly decreased in the FS group.

Fe is involved in the metabolism of several neurotransmitters, and monoamine and aldehyde oxidases are reduced in iron-deficiency (29). Kobrinsky et al (30) reported that iron deficiency raises the threshold for seizures. In our study, Fe ($p<0.01$) levels were significantly decreased in the study groupwhen compared to the control group.

Cu is an essential trace element in living organisms (31). Decreased Cu levels in given regions of the animal brain after an acute phase of epileptic seizures may suggest the activity of Cu. The observed decrease could also be the result of postepileptic changes in the permeability of brain blood barrier leading to a massive outflow of the elements from the tissue affected by seizures (32). Donaldson et al (33) showed that Cu exhibits an inhibitory activity against Mg^{2+}-ATPase, as well as Na^{+}–K^{+}-ATPase enzymes found in abundance in hippocampus and hypothalamus and disrupting the mechanisms, which maintain the correct intraneuronal sodium and potassium ratio. This disruption is associated with a membrane instability and paroxysmal discharges. In our study, no significant difference was found between the control and study groups for serum Cu levels.

In conclusion, our findings showed that some trace elements including, Ca, K, Mg, Fe, Ga, Se, Zn and Sr were decreased in

children with FS. Therefore, we suggest that deficiencies of these elements may be responsible for the etiopathogenesis in some children with FS.

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