Assessment of the Level of GABA and Some Trace Elements in Blood in Children who Suffer from Familial Febrile Convulsions

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Abstract

Febrile seizure is one of the most common neurological problems during childhood. The etiology and pathogenesis of febrile seizure remain unknown. However, several factors such as vitamin B6 deficiency, electrolyte disturbances, and reduction in serum zinc, selenium, magnesium levels, and low gamma-aminobutyric acid (GABA) levels are thought to play a role in the pathogenesis of febrile seizure. The present study included twenty children from 10 families, 11 were male and 9 were female. Each family has at least 2 members with a history of febrile convulsion. All cases were subjected to the following: Determination of serum levels of copper, zinc, magnesium, selenium level in serum, and plasma level of γ-aminobutyric acid (GABA). Serum levels of selenium and GABA were statistically significantly low in comparison with controls. Serum copper was statistically significantly higher in cases than controls, while serum zinc showed no significant changes in the cases of febrile convulsion compared with the control group. The mean Zn level in the serum of febrile convulsion was found to be at lower level than in the control group. The serum magnesium was significantly low in cases than controls. The cumulative incidence of febrile convulsions among children ranges from about 1% in China to more than 8% in Japan and 14% in Guam. The peak incidence of a first febrile convulsion occurs in the second year of life [2].

Febrile seizure is one of the most common neurological problems during childhood [10]. Approximately 2-5% of children are estimated to experience at least one epileptic seizure during a febrile illness before they are 5 year old. The incidence of febrile seizures varies between 2% and 4% in Western countries, whereas the incidence is 7% in Japan and higher in developing countries [10-13]. Febrile seizures occur during infancy or childhood, typically between 3 months to 5 year of age. Seizures occurring with fever in children who have suffered a previous non-febrile seizure are excluded from the

Introduction

Seizure or convulsion is a paroxysmal, time limited change in motor activity and/or behaviour that results from abnormal electrical activity in the brain [1]. Between 2% and 4% of all children in Europe and the United States experience at least one convulsion associated with a febrile illness before the age of 5 years [2].

Febrile seizures (FS), generally defined as seizures taking place during fever, and without an obvious central nervous system (CNS)-invasive infection, are the most common type of convulsive events in infants and young children [3]. FS lasting less than 10 [4, 5] or 15 min [6] have not been associated with subsequent epilepsy or cognitive deficits in prospective or retrospective studies [7-9].

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definition of febrile seizures. Febrile seizures should also be distinguished from epilepsy, which is characterized by recurrent non-febrile seizures [14].

Febrile seizures occur in a strongly age-specific manner, supporting the strong contribution of factors that selectively characterize specific stages of brain development [15, 16]. The seizures are familial in some cases and sporadic in others, suggesting that both genetic and environmental elements contribute to their generation [17].

The pathophysiology of febrile convulsion is still unknown; however, there are different hypotheses about neurotransmitters and trace elements changes in the biological fluids which can have a role in the pathogenesis of febrile convulsion [18]. Several factors such as vitamin B6 deficiency, electrolyte disturbances, reduction in serum zinc, selenium, and magnesium levels, and low gamma-aminobutyric acid (GABA) levels are thought to play a role in the pathogenesis of febrile seizure [19, 20]. Several genes have been implicated in the susceptibility to febrile seizures, including those coding sodium channels [21, 22], GABAA receptors [23-25], and interleukins. In addition, interactions among several genes might contribute to the occurrence of these seizures in a more complex manner [26, 27].

Selenium is an essential trace element in humans that has antioxidant effects in cells, especially in brain cells. Brain contains a high quantity of selenium, especially in gray matter [28]. Zinc is one of the most important of the trace elements in human body and is essential for the normal development of the central nervous system. Zinc modulates the activity of glutamic acid decarboxylase which is a rate-limiting enzyme in the synthesis of gamma-aminobutyric acid (GABA), also it increase the affinity of neurotransmitters such as glutamate to their receptors and facilitates the inhibitory effect of calcium on N-methyl-d-aspartate receptors [29]. Magnesium (Mg) is involved in neuronal function and it inhibits the facilitatory effects of calcium on synaptic transmission and also exerts a voltage dependent blockage of N-methyl-D-aspartate (NMDA) receptor channel. It is suggested to use supplementary zinc and magnesium as preventive measure for febrile convulsion in children [30]. Copper inhibits Mg++-adenosine triphosphatase (ATPase) and Na+-K+-ATPase enzymes and disturbs the sodium and potassium homeostasis which results in the genesis of epileptiform discharges [31].

Low cerebrospinal fluid GABA values were reported in adults with seizure disorders and low GABA in cerebrospinal fluid was found in children with febrile convulsions compared with seizure free children [31].

The aim of this work is to determine the relationship between serum levels of zinc, magnesium, copper, selenium and gamma-aminobutyric acid and familial febrile convulsions.

Subjects and Methods

Subjects

The present study included twenty children from 10 families, with a mean age of 3.9 ± 1.5 years; 11 were male and 9 were female. Each family has at least 2 members with a history of febrile convulsion; aged from 6 months to 6 years. All children were referred to Outpatient Clinic of Children with Special Needs Department, National Research Center. The study protocol was approved by the ethical committee of the National Research Center and informed written consents were obtained from the parents of the cases and controls. A comparable twenty children with matched age and sex were included in the study as a control group. All these children had a normal neurological examination.

Inclusion criteria

Children presenting with history of fever with generalized tonic clonic convulsion persisting less than 15 minutes in the absence of symptoms or signs suggestive of CNS infection and without postictal neurological deficit were included.

Exclusion criteria

Children who had a systemic infection within 2 weeks before the study and those who had chronic disease, malnutrition, known to be epileptic, or on zinc supplement for therapeutic purposes were excluded.

Blood Sampling

Venous blood sample were obtained from the patients and controls and separated into two parts. 3 ml in vacutainer tubes containing potassium EDTA and mixed by gentle inversion and 3 ml in vacutainer tubes it was left to coagulate spontaneously. The samples were then centrifuged at 3000 x g for 10 min to obtain serum and plasma, which was then frozen at -25°C for transportation and storage in the laboratory.

All cases were subjected to the following analysis: determination of copper level in serum; determination of zinc level in serum; determination of magnesium level in serum; determination of selenium level in serum; and determination of γ-aminobutyric acid (GABA) in plasma.

Methods

Measurement of serum Cu, Zn, Mg and selenium was done by an atomic flame spectrophotometer method. The flame spectrophotometer machine was an Australian-made Varian Spectra AA220 model (ULvi et al., 2002) [32].
**Plasma GABA Analysis**

Plasma GABA levels were measured by a modification of the high-performance liquid chromatography-electrochemical method previously reported by Donzanti and Yamamoto 1988 [33] using the ESA Coulometric Electrode Array System (ESA Inc., Chelmsford, MI).

**Results**

The current study included 20 patients with a history of febrile convulsion with a mean age of 3.9 ± 1.5 years; 11 were male and 9 were female. There was a positive family history in all selected cases. In addition, there was consanguineous marriage between parents of 10 cases. As regards the control group, their mean age was 4.1 ± 1.5; 10 were male and 10 were female. Furthermore, there was consanguineous marriage between parents of 9 controls. Both groups were matched as regards age, sex, state of parent consanguinity, and anthropometric measures (Table 1).

### Table 1: Epidemiological data.

<table>
<thead>
<tr>
<th></th>
<th>Cases (20)</th>
<th>Controls (20)</th>
<th>t-test</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years ± SD)</td>
<td>3.9 ± 1.5</td>
<td>4.1 ± 1.5</td>
<td>0.637 (NS)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>11</td>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>9</td>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Consanguinity</td>
<td>10</td>
<td>9</td>
<td>0.752 (NS)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Age of onset</td>
<td>Median 1</td>
<td>None</td>
<td>0.8 (NS)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td></td>
<td>0.5-2 years</td>
<td></td>
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</table>

As regards serum selenium, in the current study, it was found that the serum selenium level was statistically significantly lower in cases than controls (<0.001*), with mean selenium level in cases (51.3 ± 15.9) and in controls (91.4 ± 24.8) (Table 2).

### Table 2: Serum selenium (ng/ml) in cases and control.

<table>
<thead>
<tr>
<th></th>
<th>Mean ± SD</th>
<th>Range</th>
<th>t</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Case</td>
<td>51.3 ± 15.9</td>
<td>28.0–80.0</td>
<td>-6.088</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Control</td>
<td>91.4 ± 24.8</td>
<td>48.0–127.0</td>
<td></td>
<td></td>
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</tbody>
</table>

As regards GABA, in the current study, it was found that serum GABA is statistically significantly lower in cases than in controls (0.003*), with mean GABA level in cases and controls are 76.4 ± 25.0 and 104.7 ± 30.6 respectively (Table 3).

### Table 3: Serum GABA (ng/ml) in cases and control.

<table>
<thead>
<tr>
<th></th>
<th>Mean ± SD</th>
<th>Range</th>
<th>t</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Case</td>
<td>76.4±25.0</td>
<td>34.0–113.0</td>
<td>-3.202</td>
<td>&lt;0.003*</td>
</tr>
<tr>
<td>Control</td>
<td>104.7±30.6</td>
<td>48.0–157.0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

As regards serum copper, in the current study, it was found that serum copper is statistically significantly higher in cases than controls (P<0.001), with mean copper level of 154.0 ± 22.9 in cases and 122.5 ± 23.6 in controls (Table 4).

### Table 4: Serum Copper (µg/dl) in cases and control.

<table>
<thead>
<tr>
<th></th>
<th>Mean ± SD</th>
<th>Range</th>
<th>t</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Case</td>
<td>154.0 ± 22.9</td>
<td>117.0–191.0</td>
<td>4.276</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Control</td>
<td>122.5 ± 23.6</td>
<td>83.0–163.0</td>
<td></td>
<td></td>
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</tbody>
</table>

As illustrated in Table 5, serum zinc showed no significant changes in the cases of febrile convulsion compared with the control group. The mean Zn level in the serum of febrile convulsion group (52.8 µg/dl ± 24.4) was found to be at lower level than in the control group (56.1 µg/dl ± 23.5).

### Table 5: Serum Zinc (µg/dl) in cases and control.

<table>
<thead>
<tr>
<th></th>
<th>Mean±SD</th>
<th>Range</th>
<th>t</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Case</td>
<td>52.8±24.4</td>
<td>13.0–104.0</td>
<td>-0.436</td>
<td>0.665</td>
</tr>
<tr>
<td>Control</td>
<td>56.1±23.4</td>
<td>13.0–104.0</td>
<td></td>
<td></td>
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</tbody>
</table>

The serum magnesium (Mg) in the study was significantly lower in cases than controls (P<0.011), with a mean serum Mg level in cases (1.38 ± 0.37) and in controls (1.74 ± 0.46) (Table 6).

### Table 6: Serum Magnesium (mEq/l) in cases and control.

<table>
<thead>
<tr>
<th></th>
<th>Mean±SD</th>
<th>Range</th>
<th>t</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Case</td>
<td>1.38±0.37</td>
<td>0.76–1.99</td>
<td>-2.688</td>
<td>0.011*</td>
</tr>
<tr>
<td>Control</td>
<td>1.74±0.46</td>
<td>1.20–2.50</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The logistic regression model in our study shows that Selenium and Magnesium have protective effects, while Copper has causative effect. The impact of Magnesium was the most prominent factor (Table 7).

### Table 7: Logistic regression model for independent factors Causing febrile convulsions (cases).

<table>
<thead>
<tr>
<th>Element</th>
<th>B</th>
<th>SE</th>
<th>OR</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mg</td>
<td>-6.43</td>
<td>2.79</td>
<td>0.002</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Selenium</td>
<td>-0.12</td>
<td>0.05</td>
<td>0.889</td>
<td>0.028*</td>
</tr>
<tr>
<td>Copper</td>
<td>0.10</td>
<td>0.05</td>
<td>1.109</td>
<td>0.033*</td>
</tr>
<tr>
<td>Constant</td>
<td>4.04</td>
<td>6.40</td>
<td>56.935</td>
<td>0.526</td>
</tr>
</tbody>
</table>

The ROC curve in the current study shows that only copper (the largest AUC) has a suggestive value to predict febrile convulsions (Table 8, Figure 1).

### Table 8: ROC curve to evaluate the value of Selenium, Copper, Magnesium, Zinc and GABA to differentiate cases (febrile convulsion condition) from controls.

<table>
<thead>
<tr>
<th>Element</th>
<th>AUC</th>
<th>SE</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Selenium</td>
<td>0.094</td>
<td>0.045</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>GABA</td>
<td>0.249</td>
<td>0.077</td>
<td>0.007*</td>
</tr>
<tr>
<td>Copper</td>
<td>0.281</td>
<td>0.067</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Zinc</td>
<td>0.439</td>
<td>0.092</td>
<td>0.508</td>
</tr>
<tr>
<td>Mg</td>
<td>0.311</td>
<td>0.083</td>
<td>0.241*</td>
</tr>
</tbody>
</table>

**Discussion**

In the current study, serum selenium in our cases was significantly lower, as compared with controls (<0.001*). And these results was agreed by Similar studies which done by Mahyar et al., 2010 and Amiri et al., 2010 [34, 35]. In both studies the serum selenium level in the children who had febrile convulsion was significantly lower than controls (P<0.0001). In a study done by Wirth et al., 2010, [36]...
they found that cerebral selenium deficiency is associated with the increased incidence of seizure in mice; they concluded that cerebral selenium deficiency negatively affect glutathione peroxidase concentration.

In our study serum copper in cases was significantly higher than controls (P<0.001), and this is agreed with study done by Prasad et al., 2009 [41], they found that serum copper levels in children with seizures were significantly increased and this may be due to the effect of increased hepatic synthesis, decreased breakdown, altered intestinal absorption, and altered excretion patterns, changes in the distribution among body tissues or a combination of the above factors. Copper (Cu) is known to enhance genesis of epileptiform discharges through its inhibitory effects on Mg$^{2+}$ adenosine triphosphate (ATPase) and Na$^{+}$-K$^{+}$ ATPase enzymes and disturbance of sodium and potassium homeostasis [42]. On the other hand Mishra et al., 2009 [43], found that there was no significant change in the serum copper level in cases of febrile convulsion. However, they found a significant reduction in the CSF copper level in the group of febrile convulsion compared to the other two groups with encephalitis and fever with meningismus. Strangely enough, they could not elicit a correlation between the CSF and the serum copper levels.

Serum zinc of cases in our study was lower than controls; however the reduction in its level were not statistically significant in the cases of febrile convulsion compared with the control group. Low Zn concentrations have been reported to be present in the serum and CSF of patients with febrile convulsion. The mechanism by which the depletion of zinc facilitates seizure activity is hypothesized to be due to its inhibitory effect on GABA, an inhibitory neurotransmitter. Zinc also plays an important role in both the synthesis and the function of GABA [41]. The reason for reduction of serum zinc levels in patients affected with febrile seizure is not known. However, fever and acute infections may have some role in developing such conditions. It is believed that the release of tumor necrosis factor (TNF) and interleukin during fever or tissue injury may result in reduction of serum zinc level, mostly due to shifting from blood to the liver [44]. Hypozincemia trigger the NMDA receptor which is one of the members of glutamate family receptor, so hypozincemia may play an important role in initiation of epileptic discharge [45]. Many studies have reported that the mean serum zinc level is significantly lower in children with febrile seizure [46]. However, these studies have some limitations as they were done in small number of patients, in addition these studies had enrolled many patients with infection as respiratory tract infection that can itself act as a confounding factor by reducing the serum zinc concentrations [47]. Also, the dietary habits of the Egyptian children that were enrolled in the current study can give a clue for this controversy, as the daily diet usually consumed by the Egyptian are very rich in zinc, e.g.: pulses (especially beans), wheat, rice and green leafy vegetables used for salad especially arugula (gargeer) and lettuce [48].

The serum magnesium (Mg) of cases in the current work was significantly lower than controls (P<0.011). The result comes in accordance with recent studies done by Talebian et al., 2009 [30] their
study showed that the mean serum level of magnesium in the febrile convulsion group was significantly lower (P<0.05) than controls. It is known that normally Mg^{2+} binds to NMDA receptor channel producing a voltage dependent block, thereby decreasing synaptic transmission. Therefore, a decrease in Mg concentration in children of FS may also act as precipitating events for seizures with the onset of fever [31].

No significant correlation was observed between age and Selenium, Copper, Magnesium, Zinc and GABA in cases; however selenium was the only one which had a significant positive correlation with age in control only. A similar result was obtained in a study that found an increase in the serum selenium concentration with age in the children younger than 10 years old, however in the older subjects (10-19 years) there was no correlation between serum selenium level and age [49].

In the current work the serum GABA levels was significantly lower in cases as compared with controls (0.003*). These results are compatible with a study done by Mishra et al., 2007 [31] they enrolled 20 cases of febrile seizure, 26 patients with encephalitis and 22 children with fever and meningismus. The authors found no significant difference in the mean values of CSF and serum GABA between cases of febrile convulsion and encephalitis. However, the values were found to be significantly decreased when comparing the group of febrile seizure and the group suffering from fever with meningismus (P<0.05). It appears that at higher body temperature, serum GABA levels decrease, which in turn can change the brain GABA levels and may lead to precipitation of seizures during febrile episode [31]. Gamma-aminobutyric acid (GABA), is a major inhibitory neurotransmitter, which produced by decarboxylation of L-glutamate. Zinc modulates the activity of glutamic acid decarboxylase, the rate Limiting enzyme in the synthesis of gamma amino butyric acid (GABA), which is a major inhibitory neurotransmitter. Low cerebrospinal fluid GABA values have been reported in association with several seizure disorders, including febrile convulsions. Fever and/or infections cause a reduction in serum zinc concentrations so affects the level of GABA [50].

We concluded that there is no significant correlation between Selenium, Copper, Magnesium, Zinc and GABA in case group, however Zinc in control had a significant negative correlation with Copper (P<0.001) and Magnesium (P<0.001), while Copper had a significant positive correlation with Magnesium (P<0.001). Using logistic regression model, selenium and magnesium have protective effects (OR<1), and magnesium has the most prominent effect. On contrary, copper has a causative effect (OR>1). By drawing the ROC curve to evaluate the value of different trace elements to differentiate febrile convulsion cases from controls; only copper was found to have a suggestive value to predict febrile convulsion and serum copper level ≥1.38 (µg/dl) has a moderate characteristics to differentiate cases from controls.

References


27. Tan NC, Mulley JC, Berkovic SF. Genetic association studies in epilepsy: “the truth is out there” Epilepsia. 2004;45:1429–1442.


