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Serum Copper Level among Children with Simple Febrile Seizures

Elham E. Elsakka[1*], Bayoumi A. Ghareib[1], Akram A. Deghady[2], Hebatallah F. Elsarraf[1]

[1]*Department of Pediatrics, Faculty of Medicine, University of Alexandria, Alexandria, Egypt*
[2]*Department of Clinical and Chemical Pathology, Faculty of Medicine, University of Alexandria, Alexandria, Egypt*

Abstract

***Background*: Febrile seizures (FS) are the most common seizure in children. Excess serum copper (Cu) can cause convulsions. *Aim*: to study serum Cu levels in children with simple febrile seizures (SFS) and whether elevated serum Cu could be a risk factor for SFS. *Subjects and Methods:* Sixty children were divided into 2 groups. Group I: 30 patients with SFS. Group II: 30 patients with febrile illness without seizures. All were subjected to history taking, weight, temperature, complete blood picture, C-reactive protein, and electrolytes measurement. *Results*: A significant difference was found between the two studied groups regarding past history of FS, family history of FS, hemoglobin level, and WBCs count. Serum Cu level was significantly higher in group I than in group II. A significant positive correlation was found between serum Cu level in group I patients and: their age at the first attack of seizure and their weight. Comparison within group I between patients having normal and high serum Cu levels regarding gender, family history of FS and past history of FS showed no significant difference. *Conclusions*: Children with SFS had a significantly high serum Cu level than children with febrile illness without seizures. Increased serum Cu level is positively correlated with age at first attack of seizure and weight of patients with SFS. Increased serum Cu levels could be a risk factor for SFSs.**

Keywords: family history, temperature, weight, family history, attack

Introduction

Febrile seizures (FS) are the most common type of seizure in childhood[(1,2)]. A FS is "a seizure accompanied by fever (38°C or greater) in an infant or a child 6 to 60 months of age, without central nervous system infection". FSs are classified as simple or complex[(1,3-5)]. A simple FS (SFS) is a FS characterized by: generalized seizure, lasting less than 15 minutes that does not recur within 24 hours from onset[(3,5)]. Pathophysiology of FS remains unknown. Genetic factors or electrolyte disturbances may play a role in seizures occurrence or recurrence[(6)]. The cause of FSs is multifactorial. There are some causes associated with higher risk, as: viral illnesses (the commonest) and some vaccinations together with genetic predisposition that affect the vulnerable, developing nervous system of infants and young children under fever

[*]*Corresponding Author: dr.elham.elsakka@alexmed.edu.eg*

stress[5,7,8]. FSs are common, occurring in up to 4% of infants and children in this age group. They may be single or multiple occurring several times in the specific age group[4,8]. Children with a SFS who are generally healthy and well-appearing do not require diagnostic tests, except as needed to diagnose the cause of the fever[5, 7]. SFSs are not associated with long-term mortality and do not affect the academic progress, the intellectual ability or behavior of the affected infant or child. Due to the benign nature of SFSs, the use of antiepileptics is not indicated. The use of antipyretics is not recommended to be used for prevention of SFS as they do not decrease their risk[5,7]. Copper (Cu) is an essential microelement that is found in all living organisms. It acts as an important catalytic cofactor for the proteins carrying important biological functions. It is important for development and growth[9]. The deficit of Cu can lead to many disorders, as; impaired energy production, increased oxidative damage and abnormal glucose and cholesterol metabolism. It has permanent effects on neurologic and immune system. Increased serum Cu level is found in many diseases like e.g.: Menke's disease or Wilson's disease[9]. Deficiency of Cu occurs when the requirements exceed the intakes. In about 20–40% of cases Cu deficiency is of unknown origin[10]. Cu is an important element for the function of the nervous system (NS) as Cu is important for normal brain development. Many researchers found that deficiency and excess may cause NS complications and convulsions[11-14]. There is few published research on the level of cupper in SFS and few compare it to febrile illness with no seizures. The aim of the present work was to study serum Cu levels in children with SFS and to assess whether elevated serum Cu could be a risk factor for SFS.

Subjects and Methods

This case control study was conducted on 60 infants and children, with inclusion criteria (age between 6-60 months, temperature 38° C or more) and exclusion criteria (including other causes of seizures than fever, such as: history of non-febrile seizures, electrolyte disturbances, neurological abnormalities, developmental delay, or intracranial infection), attending the emergency room at Alexandria University Children's Hospital (AUCH), Alexandria, Egypt. Concluded children were divided into 2 groups: Group I: included 30 infants and children with SFS (generalized seizure, lasting less than 15 minutes, and does not recur within 24 hours from onset), [3, 5] while group II: included 30 infants and children with febrile illness without seizures.

Methods

All cases were subjected to assessment by full history taking that included: socio-demographic data (age and gender), perinatal history, history of previous FSs, nutritional history, developmental history, and family history (consanguinity, history of FSs or epilepsy). Detailed history of the presenting symptom for group I: full seizures details (duration, number of seizure attacks/24 hours from onset, post-ictal period), symptoms of the present illness, other potential causes of seizure (e.g., trauma). For group II: onset and duration of fever and symptoms of the present illness. Full thorough examination of cases was done including core body temperature measurement in Celsius, weight measurement, systemic examination with emphasis on the source of infection, assessment of developmental milestones and neurological examination: consciousness, the signs of meningeal irritation (Kernig's sign, neck stiffness and Brudzinski's signs), muscle tone, power, and reflexes. Laboratory

investigations were done that included: Complete blood picture (CBC); using CBC analyzer machine. C-reactive protein (CRP); using Eurolyser. Serum electrolytes: serum sodium, calcium, and potassium (Na, Ca & K); using electrolyte analyzer machine, and Serum Copper (Cu)

Assessment of Serum Copper (Cu)
Serum Cu was measured by Colorimetric with Dibrom-PAESA[15]. Cu forms a chelate complex with 4- (3,5-dibromo-2-pyridylazo)-N-ethylsulfopropylaniline. The increase in the absorbance of this complex can be measured (which is proportional to the concentration of the total Cu in the sample). This assay requires 50 µL of serum for single determination. Blood was collected by venipuncture from the antecubital fossa. Samples were allowed to clot adequately before centrifugation, then sera were separated. Samples were firmly capped and were refrigerated at 2-8 °C if the assay is not completed in 8 hours. The samples are stored to freeze at -20 °C if not assayed within 48 hours. Reference Cu Values in serum are: Children (7-12 years): 73-154 µg/dl, Children (3-6 years):76-167 µg/dl and Infants: 72-178 µg/dl[16]

Statistical analysis

After collection of data, they were coded, entered, and processed using Statistical Package for Social Sciences system files (SPSS: package version 23). Interpretation and Analysis of the data were done. Descriptive statistics (distribution, mean, frequency, and standard deviation) were used to describe the different data characteristics. Bivariate analysis: Chi-Square, Mann-Whitney and t-tests were used to test the significance of the results. *P≤0.05* levels were used as cut off value for the statistical significance.

Ethical Considerations

The study was approved by the Ethics Committee of the Faculty of Medicine, Alexandria University (No 0105601, 19th July 2018. IRB No: 00012098, FWA No: 00018699). A written consents were taken from the parents of all infants and children included in the study.

Table 1: Demographic data of the two studied groups.

Demographic data	**Group I** (n=30)	**Group II** (n=30)	Significance	P-value
Age (months) mean ± SD	29.7±13.4	35.0±16.5	*Z = 1.442[a]*	*P=0.149*
Gender n (%)				
Male	12 (40%)	14 (46.7%)	*X^2= 0.271[b]*	*P=0.605*
Female	18 (60%)	16 (53.3%)		

**Significant at (P ≤ 0.05); a= Mann-Whitney test; b= Chi Square test*

Results

A total of 60 cases were included in the study, 30 has SFS and 30 has febrile illness without seizures. The mean age was 29.7±13.4 months for group I and 35.0±16.5 months for group II. In group I: 12 (40%) were males and 18 (60%) were females while in group II: 14 (46.7%) were males and 16 (53.3%) were females. There was no statistically significant difference between the two studied groups (*p=0.149, p=0.605 respectively*) (Table 1). Seventeen patients (56.7%) of group I had past history of FS,

while in Group II all patients had no past history of febrile seizures (*p=<0.001*). 10 (33.3%) patients of group I had family history of febrile seizures, while in group II; all patients did not have family history of febrile seizures. This was also statistically significant (*p=0.001*). No statistically significance difference was found between the two groups regarding their temperature at the ER and the duration of fever (Table 2).

Table 2: Past and family history of febrile seizures, temperature at ER and the duration of fever among the two studied groups

	Group I (n=30)		Group II (n=30)		Significance test	P-value
	N	%	n	%		
Past history of febrile seizures						
Yes	17	56.7	0	0.0	X^2= 23.721	P=<0.001*
No	13	43.3	30	100.0		
Family history of febrile seizures						
Yes	10	33.3	0	0.0	X^2= 12.00	P=0.001*
No	20	66.7	30	100.0		
Temperature at the ER						
Mean± standard deviation	38.8±0.5		38.7±0.4			
Median	38.9		38.7±0.4		t = 0.463	P=0.181
Minimum-maximum	37.8-39.7		38.7±0.4			
Duration of fever (in days)						
Mean± standard deviation	1.5±0.5		1.7±0.7			
Median	1.0		2.0		Z = 1.210	P=0.226
Minimum-maximum	1.0-2.0		1.0-3.0			

** Significant at (p ≤ 0.05)*

The distribution of cases of group I regarding revealed that their age at the 1st attack was 18.2± 7.3 months, their temperature at seizure attack was 39.4± 0.5°C and the number of previous attacks was 1.16 ± 1.2. The hemoglobin level was significantly higher (*P=0.042*) and the WBCs count was significant lower (*P=0.046*) in group I than group II. While the differential leucocytic count, the platelets count and the CRP values showed no statistically significant difference between the two studied groups (*P=0.302, P=0.701, P=0.585* respectively). The serum Sodium, Potassium and Calcium levels showed no statistically significant difference between the two studied groups (*P=0.726, P=0.716, P=0.336* respectively) (Table 3). The serum Cu level in group I was high in 33.3% of cases, while in group II it was high in 6.7% of cases. This proved to be statistically significant. (*P=0.010*) (Table 4). Comparison of group I patients regarding gender, family history of FS and past history of FS between cases with normal and high serum Cu level showed no statistically significant difference (Table 5). A positive correlation was found between serum Cu level in group I cases and their age at the first attack of seizure (*r=0.368, P=0.045*) and their weight (*r=0.413, P=0.001*) (Figures 1 and 2).

Discussion

Various studies have shown a correlation between serum Cu and cerebrospinal fluid

and the occurrence of FS[17]. The present study gives evidence of the high Cu level in infants and children with SFS. It highlighted the possibility that high serum Cu level could be a risk factor for the occurrence of SFS in infants and children.

Table 3: Laboratory parameters among the two studied populations

	Group I (n=30)		Group II (n=30)		*Test of significance*	P-value
	N	%	n	%		
Hemoglobin level (gm/dl)	11.9 ± 0.6		12.3 ± 0.7		*t = 2.080* [c]	*P=0.042**
WBCs (×1000) (mcL):	12.2 ± 2.9		10.8 ± 2.1		*t = 2.036* [c]	*P=0.046**
leucocytic count Lymphocytosis	13	43.3	17	56.7	X^2= 1.067[b]	P=0.302
Neutrophilia	17	56.7	13	43.3		
Platelets (×1000) (mcL)	262 ± 77		264 ± 67		*Z = 0.384*[a]	*P=0.701*
CRP (mg/L):						
Median	12.0		8.5		*Z = 0.546*[a]	*P=0.585*
Min-max	2.0 - 96.0		2.0 - 96.0			
Normal	12	40.0	12	40.0	X^2= <0.001 [b]	*P=1.00*
High	18	60.0	18	60.0		
Serum Sodium (mEq/L)	139.4±4.3		140.4±4.6		*t = 0.810* [c]	*P=0.726*
Serum Potassium (mEq/L)	4.3±0.4		4.2±0.4		*t = 0.923* [c]	*P=0.716*
Serum Calcium (mEq/L):	10.3±0.6		10.3±0.5		*t = 0.381*[c]	*P=0.336*

*Data are presented as mean ± Standard deviation (SD), WBCs: White blood cells, CRP: C-reactive protein, Min: minimum, Max: Maximum, a: Mann-Whitney test, b: Chi-Square test, c: Student t-test, *=Significant at (p ≤ 0.05)*

It also highlighted the significance of measuring serum Cu level in this group of patients. The current cross section study was conducted to assess the level of serum Cu level in infants and children with FS. The serum Cu level in FS group (I) cases was significantly higher (in 33.3% in comparison to group (II) cases (in 6.7%). The increased serum Cu level may be due to the effect of increased in the hepatic synthesis of Cu, decreased in the breakdown of copper binding proteins, alteration in intestinal absorption and in excretion patterns, changes in Cu distribution in the body tissues or it may be due to a combination of those factors[11]. Shokrzadeh et al[18] reported a significant higher level of serum Cu in children with FS than in the control children.

Table 4: Comparison between the two studied groups according to serum copper level.

Copper (µg/ml)	Group I (n=30)		Group II (n=30)		Test of significance	P-value
	N	%	n	%		
Normal	20	66.7	28	93.3	X^2= 6.667	P=0.010*
High	10	33.3	2	6.7		
Mean± SD	146.3 ± 49.2		129.7 ± 49.2		*t* = 1.307	P=0.196

** Significant at (P ≤ 0.05)*

Also, Prasad et al[11] reported that the level of serum Cu in children with seizures was significantly increased. In Gheini et al[19] study; the mean serum Cu level was significantly lower in the control children than the level in the simple febrile convulsions group. Kannachamkandy et al[20] concluded that there is a significant increase in serum Cu in children with FS. On the contrary; no significant change in the serum Cu level was found in patients with FS in the other studies[12, 21].

Table 5: High Copper level in relation to gender, and history and febrile seizures in group I

	Copper level				Chi Square test	P-value
	Normal (n=20)		High (n=10)			
	n	%	n	%		
• **Gender**						
Male	6	30.0	6	60.0	X^2= 2.500	P=0.114
Female	14	70.0	4	40.0		
• **Family history of febrile seizures**						
Yes	7	35.0	3	30.0	X^2= 0.075	P=0.784
No	13	65.0	7	70.0		
• **Past history of febrile seizures**						
Yes	12	60.0	5	50.0	X^2= 0.271	P=0.602
No	8	40.0	5	50.0		

** Significant at (P ≤ 0.05)*

Balci and Yılmaz[13] showed no significant difference in the serum Cu levels between the SFS group and the control group. Also, Aly et al.[22] found that the median serum Cu level in cases with febrile convulsions was non significantly higher than in controls. The difference between results of different studies may be attributed to the difference in age of participants or to the difference in sample size between studies. FSs may have familial tendency in some cases or may be sporadic in other cases, indicating that both genetic and environmental factors may contribute to the generation of seizures. It was listed also that around one-third of cases with a first FS have another one or more of FSs[12]. In the present study there was a statistically significant difference between the two studied groups regarding past history of FS and family history of FS, as no subjects in group II had past history nor family history of FS. The same was found by Aly et al.,2014[22] as their results showed a significant difference between cases and controls regarding positive family history of FS. The present study showed no statistically significant difference between the two studied groups regarding temperature at presentation at ER and the duration of fever. Our results are in line with the studies by Aly et al[22] and Bharathi & Chiranjeevi[23] as they found no significant difference regarding temperature at presentation between the patients with simple FS and the control infants and children. Many theories were postulated for the possible metabolic changes that occur during the rising phase of the body temperature in patients with FS have been suggested, as electrolyte disturbances, vitamin B6 deficiency, low gamma-amino-butyric acid, but they do not explain majority of cases[24]. In the present study there was no statistically significant

difference between the two studied groups in serum Na, K and Ca. Sadeghzadeh et al.[25] and Hamed et al.[26] did not find any clear abnormality in serum Cu levels in children with FS. On the contrary; Nadkarni et al[27] and Maksikharin[28] had noted that hyponatremia is common in cases of FS. Also, Hugen et al[29] and Nadkarni et al.[27] reported that hyponatremia can predict further seizures[30]. On the contrary; Akbayram et al[31] found that K concentrations in FS group were lower than in control group. This difference in electrolytes levels in patients with FS in different studies may be due to the difference in sample size, the age of the studied infants and children or may be due to the difference in timing of sample taking.

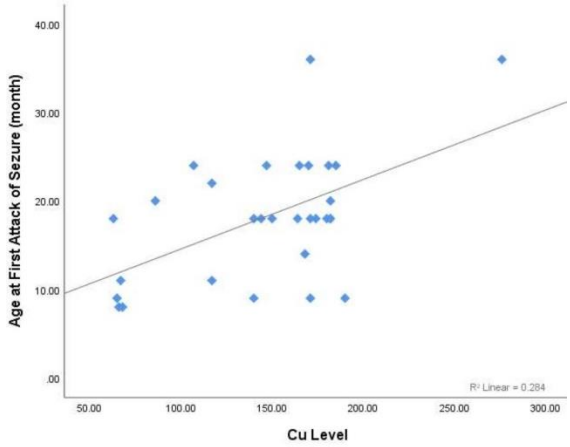

Figure 1: Correlation between Cu level and age at 1st attack.

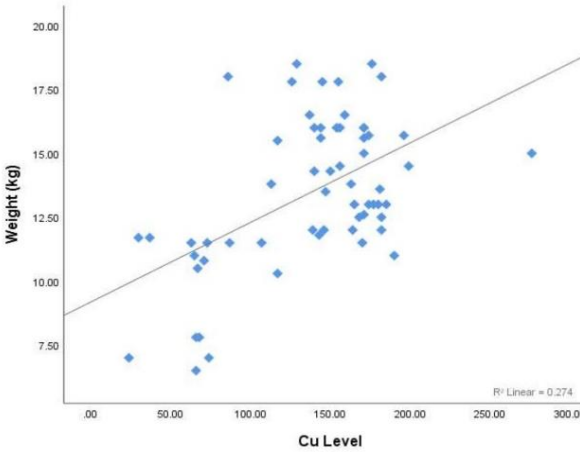

Figure 2: Correlation between Cu level and weight of studied sample.

There was no significant correlations in the present study between serum Cu level in group I cases and their gender, family history of FS and past history of FS. On the contrary; there was a significant positive relation between serum Cu level and each of age at first attack and weight of the patient. Serum Cu is increased with increase age and increase in weight. On the contrary; Aly et al.[22] found that children with body weight <10th percentile was in 32 in FS group versus 5 in control group. Also, Daoud et al.[32] reported that malnutrition increases the possibility of SFS. This difference may be attributed to the difference in ethnic groups and nutritional status between different studies subjects. In the current study a statistically significant difference was found between the two studied groups regarding HB level and the WBCs count; cases of group I has lower HB while higher WBCs count than cases of group I. Anemia may raises the threshold for FS[33]. Aly et al.[22] found that HB was statistically lower in the group of children with fever without FS levels than in the FS group. Also, Bidabadi et al.[33] and Donaldson et al. [34] found that RDW in the group with FS was significantly higher than in control group.

Conclusion

The present study gives evidence of the high serum Cu level in children with SFS. Children with SFS had a significant high serum Cu level than children with febrile illness without seizures. Increased serum Cu level is positively correlated with the age at the first attack of seizure and the weight of the patients with SFS. Increased serum Cu level could be considered a predisposing

risk factor for SFSs. A follow up study of children with SFS who have high serum Cu level would be of great interest to determine the incidence of subsequent FS after treatment.

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