

# Possible Role of Heat Shock Protein 70 in Childhood Seizures

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## Abstract

**Background** There has been a long interest in investigating the relationship between heat shock protein (HSP) expression and the evidence of neuronal damage in the most susceptible brain areas after seizures. So, the present study aimed to assess heat shock protein (HSP70) in children with seizures (febrile seizures and epilepsy), and to find out the cutoff point of this marker that may help in confirming epilepsy diagnosis. The present study has been conducted to evaluate serum levels of HSP70 in children with epileptic and febrile seizures and to compare these results to that of healthy children.

**Materials and Methods** A prospective study included 85 children (32 females and 53 males) in Children and Maternity Unit, Minia University Hospital, Minia, Egypt. Children were subdivided into three groups, group (I) included 30 children with epilepsy, group (II) included 30 children with febrile seizures, and group (III) included 25 healthy children that served as a control group. HSP70 assay was performed for all included children using the enzyme-linked immunosorbent assay technique.

**Results** The overall results revealed significant high serum HSP70 levels in epilepsy and febrile seizures groups when compared with control group ( $p < 0.001$ ). Also, HSP70 serum levels were significantly higher in epilepsy group than in febrile seizures group ( $p < 0.001$ ). Serum HSP70 level at a cutoff point  $> 170$  ng/L showed 60% sensitivity and specificity equal to 83.3% in prediction of epilepsy.

**Conclusion** HSP70 level was significantly higher in epileptic and febrile seizures children than normal healthy children, and HSP70 may be beneficial in confirming the diagnosis of epilepsy.

## Keywords

- ▶ children
- ▶ epilepsy
- ▶ febrile seizures
- ▶ HSP70

## Introduction

Epilepsy is one of the most common neurological disorders in the world, affecting approximately 69 million people worldwide, with 90% living in low- and middle-income countries and it contributes nearly 1% to the global burden of diseases.<sup>1</sup>

Epilepsy exerts a significant physical, psychological, social, and economic toll on children and their caregivers. In the United States of America, between 25,000 and 40,000 children will have a first nonfebrile seizure each year.

The problem is further complicated in developing countries as they add approximately 75 to 80% of new cases of epilepsy.<sup>2</sup>

Febrile seizures (FS) are seizures that occur in the age from 6 to 60 months with a temperature of 38°C (100.4°F) or higher, that are not attributed to central nervous system infection or any metabolic disorder, and that occur in the absence of a history of previous FS. A simple FS is a primary generalized, usually tonic-clonic, attack accompanying with fever, lasting for a maximum of 15 minutes, and not reoccurred within a 24-hour period. A complex FS is more prolonged ( $> 15$  minutes),

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is focal, and/or reoccurs within 24 hours. Febrile status epilepticus is a FS long-lasting longer than 30 minutes.<sup>3</sup>

Heat shock protein 70 (HSP70) constitutes a very conserved family of protein chaperones which regulate protein homeostasis and promote cell survival; some HSPs are constitutively expressed, whereas others are strictly stress inducible.<sup>4</sup> The main function of these chaperones is to bind to denatured proteins and to support in their refolding, to inhibit their aggregation, and to guide them to their native conformations, in a manner requiring adenosine triphosphate, thus preventing cellular damage and apoptosis induced by unfolded aggregated proteins.<sup>5</sup> So, the aim of our study was to evaluate the serum levels of HSP70 in children presented with FS and epileptic fits and then compare these results to that of healthy volunteers.

## Materials and Methods

### Study Design and Participants

The current cross-sectional, hospital-based study included 85 children (32 females and 53 males), recruited from both the Children and Maternity Centre and the Pediatric Neurology Unit, Minia University Hospital, Minia, Egypt, in accordance with the ethical guidelines set by the Ethical Committee of the Faculty of Medicine, Minia University, Minia, Egypt. The study period was from January 2016 to April 2016. The involved children were classified into three groups: group (I) included 30 children with epileptic seizures, group (II) included 30 children with typical FS, and group III included 25 healthy children, not experience seizures previously which serve as a control group.

Children with electrolyte disturbances, with well-defined inborn metabolic error, abnormal computed topography suggestive of acute brain diseases or trauma and children with central nervous system infections, all were excluded from the study.

### Data Collections, Sampling, and Biochemical Assays

The included patients were subjected to full personal and family history, clinical and neurological examination; lumbar puncture if suspected central nervous system infection; computed topography; and blood electrolytes (sodium, potassium, magnesium, and calcium) were determined. Electroencephalograph (EEG) records were done using an EEG machine, flash lamp assembly (Model LS-703A/AA; Nihon-Kohden Corporation, Japan).

A total of 5 mL peripheral venous blood samples was drawn from every included child, evacuated into serum separator gel tube, where the sample was allowed to be clotted for 30 minutes at 37°C before centrifugation, and separated sera after centrifugation were aliquoted into 1 mL cryotubes and stored at -20°C till time of biochemical analysis of HSP 70 using microplate enzyme-linked immunosorbent assay reader (EMR-500; Labomed, Inc., United States), using commercially available kit (Wkea Med, Changchun, China), according to the manufacturer's instructions.

### Statistical Analysis

Data analysis was done using Statistical Package for Social Science version 20 (SPSS Inc., Chicago, Illinois, United States). Quantitative variables were described in the form of mean  $\pm$  standard deviation and range. Qualitative variables were described as number and percent. Student *t*-test and analysis of variance with post hoc Tukey test were used for comparisons of qualitative variables. Qualitative variables were compared using chi-square ( $\chi^2$ ) test or Fisher's exact test when frequencies were below five. Correlation studies were done using Spearman's rank correlation coefficient. Multiregression linear analysis was employed to determine the relationship between clinical and laboratory variables. Probability value < 0.05 was considered significant.

## Results

Epileptic seizures group (30 patients) included 21 males and 9 females in the age group of 2 to 11 years (mean age: 6.3 years). FS group (30 patients) included 20 males and 10 females in the age group of 6 months to 5 years (mean age: 2.0 years). The control group (25 children) included 12 males and 13 females in the age group of 1 to 10 years (mean age: 4.8 years). The results showed that children with FS were significantly younger in age than both epileptic and healthy control children.

As regarding family history, there was a significant difference between epilepsy group and both FS and control groups, and there was a significant difference between FS and control groups. No differences were observed regarding residence and socioeconomic status.

The onset of first attack of epileptic convulsion was at mean age of 2.5 years and ranged from 1 to 6 years. About 17 cases (56.7%) were treated with monotherapy, 9 cases (30%) were treated with double therapy, and only 4 patients (13.3%) were treated with triple therapy. For febrile group, most children, 24 cases (80%) with typical FS needed only antipyretics and only 6 cases (20%) needed diazepam.

Regarding the laboratory data (**Table 1**), there was a significant decrease in serum calcium levels in epileptic children than FS and healthy controls; however, this decrease was not significant in serum sodium concentration.

As regards serum HSP70 level, it was significantly higher in epilepsy and febrile groups (238.3 and 150.6 ng/L) than the control group (80.3 ng/L) with significant difference between epilepsy and febrile groups.

Serum HSP70 level showed significantly negative correlation with serum sodium level ( $r = -0.450$ ,  $p = 0.013$ ), and a significant positive correlation with the type of therapy (monotherapy, double therapy, and triple therapy), ( $r = 0.557$ ,  $p = 0.001$ ), among children with epilepsy. No relation was observed between age of children with FS and HSP70 levels. As well as, there was no relationship between serum calcium, potassium, and sodium levels with HSP70 levels in children with FS. There were no correlations between HSP70 levels and all these parameters in the control group (**Table 2**).

**Table 1** Comparison between groups in laboratory data

Variable	Group (I) epilepsy (n = 30)	Group (II) febrile seizures (n = 30)	Group (III) control (n = 25)	p-Value
Serum calcium Ca <sup>2+</sup> (mg/dL)	9.17 <sup>b</sup> ± 0.24	9.36 <sup>ab</sup> ± 0.33	9.46 <sup>a</sup> ± 0.36	0.004*
Serum potassium K <sup>+</sup> (mEq/L)	4.68 ± 0.41	4.68 ± 0.42	4.46 ± 0.36	0.077 <sup>NS</sup>
Serum sodium Na <sup>+</sup> (mEq/L)	134.5 ± 3.4	135.9 ± 2.2	136.2 ± 3.5	0.083 <sup>NS</sup>
Heat shock protein 70 (HSP70)	238.3 <sup>a</sup> ± 115	150.6 <sup>b</sup> ± 27.1	80.3 <sup>c</sup> ± 36.2	< 0.001*

Abbreviation: NS, not significant.

\*Significant ( $p < 0.05$ ).

<sup>a,b</sup>Means with the different superscripts are significantly different.

**Table 2** Correlation between HSP70 with different variables in all groups

Group		HSP70	
		R	p-Value
Group (I) epilepsy	Age	0.075	0.695
	Ca <sup>2+</sup>	0.174	0.357
	K <sup>+</sup>	0.174	0.359
	Na <sup>+</sup>	-0.450	0.013*
	Duration	-0.019	0.919
	Therapy	0.557	0.001*
Group (II) febrile seizures	Age	0.280	0.135
	Ca <sup>2+</sup>	-0.177	0.349
	K <sup>+</sup>	0.021	0.913
	Na <sup>+</sup>	0.356	0.053
Group (III) control	Age	0.051	0.809
	Ca <sup>2+</sup>	-0.047	0.822
	K <sup>+</sup>	-0.162	0.440
	Na <sup>+</sup>	-0.346	0.090

Abbreviation: HSP70, heat shock protein 70.

\* $p < 0.05$  (significant).

Results of receiver operating characteristic curve analysis of HSP70 for prediction of epilepsy in patients presented with convulsions indicated that HSP70 sensitivity was 60.0% and specificity was 83.3%, and accuracy was 71.7%, at a cutoff point > 170 ng/L (–Table 3, –Fig. 1).

## Discussion

Epileptic seizures affect 1 to 2% of the population and 4% of children. Developing countries may have a higher prevalence due to the lack of perinatal care, nutrition standards, public hygiene, and the greater risk of brain injury, cerebral infection, or other symptomatic cerebral conditions,<sup>6</sup> which encouraged us to conduct our research on epilepsy in children. The present results of family history were in accordance with van Esch et al<sup>7</sup> and Sfaihi et al,<sup>8</sup> both reported that family records are equally considered as a risk factor for recurrence of more attacks.

Serum calcium, sodium, and potassium levels were investigated in the current study, for exclusion of electrolyte imbalance, which may be the cause of seizures. Although every

child enrolled in our study had normal ranges regarding serum calcium, sodium, and potassium levels, a significant difference was found between epilepsy group versus both FS and control groups, and significant decrease in calcium levels in epilepsy group in relation to control group. This was in accordance with Chung and Ahn,<sup>9</sup> who found a significant increased frequency of hypocalcemia, radiological evidence of rickets, or decreased bone density, in children with epilepsy receiving long-term antiepileptic drug (AED) therapy. Hamed et al<sup>10</sup> and Gaitatzis and Sander<sup>11</sup> both reported the role of calcium in the pathophysiology of epilepsy, as hypocalcemia causes hyperexcitability of neurons and has been highly associated with seizures in adults and children and they also found that serum calcium was significantly lower in epileptics compared with the controls.

Heat shock protein 70 family constitutes one of the most conserved protein families in evolution. HSP70s are monomeric proteins that exist in any adenosine triphosphate-containing eukaryotic intracellular compartment and can also be found in cell membranes,<sup>12</sup> and the extracellular milieu,<sup>13</sup> as well as in bacteria and some Archaea. The expression of HSPs may be induced by insults other than thermal stress, including ischemia, heavy metals, nutrient deprivation, irradiation, infections, inflammation, and exposure to organics and oxidants.<sup>14</sup> There has been a long interest in investigating the relationship between HSP expression and the evidence of neuronal damage in the most susceptible brain areas after seizures.

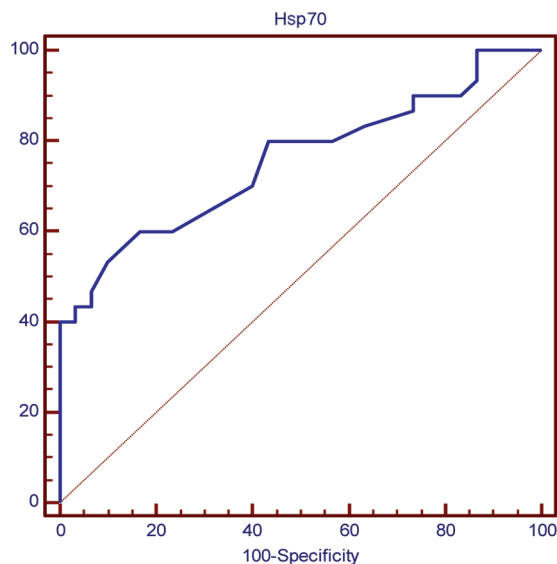
Regarding serum levels of HSP70, it was significantly higher in epilepsy and children with FS than control children and it was significantly higher in epileptic children than children with FS and our results were in accordance with Ekimova et al<sup>15</sup> and Chang et al.<sup>16</sup> They found that epilepsy, for which the underlying neuronal defects are different from those in conformational/misfolding diseases, has also been used to reveal an HSP70-mediated neuroprotective effect. In two different models of epilepsy, they demonstrated that exogenous heat shock cognate (HSC) and HSP70 can infiltrate into brain areas (e.g., cortex, thalamus, hypothalamus, hippocampus, and pontine reticular formation) involved in the introduction and propagation of generalized tonic-clonic seizures, where it acts to temper the severity of chemically induced seizures. Their study demonstrated for the first time that exogenous HSC and HSP70 have anticonvulsant properties and are able to permit through the cerebrospinal fluid-brain barrier and cross the plasma membrane of neurons.

**Table 3** Receiver operating characteristic curve analysis of HSP70 for prediction of epilepsy in patients presented with convulsions

Variable	Cutoff point	AUC	p-Value	Sensitivity	Specificity	PPV	NPV	Accuracy
HSP70 (ng/L)	>170	<b>0.756</b>	<b>&lt;0.001*</b>	60	83.3	78.3	67.6	71.7

Abbreviations: AUC, area under the curve; HSP70, heat shock protein 70; NPV, negative predictive value; PPV, positive predictive value.

\* $p < 0.05$  (significant).



**Fig. 1** Receiver operating characteristic curve of (HSP70) for prediction of epilepsy in patients presented with convulsions. HSP70, heat shock protein 70.

Our findings were also in accordance with Kilic et al<sup>17</sup> who reported that serum levels of HSP70 increased in both epileptic children and children with FS, but significantly increased more in children with epilepsy. This may be due to the fact that children with epilepsy may have recurrent attacks of convulsions that lead to more increase in levels of HSP70, but most children with FS may be presented with simple attacks or less recurrent attacks. Similarly, Oraby et al<sup>18</sup> reported that there was higher serum HSP70 levels in patients with temporal lobe epilepsy (TLE) compared with controls, and this finding may indicate that HSP70 is a stress marker in children with epilepsy.

In contrast, our study disagrees with Turturici et al,<sup>19</sup> who found an uncertain role for HSP70 in an epilepsy model. A model used commonly for studying the pathological changes of human TLE is the kainic acid (KA)-induced seizure model in rodents, which reproduces a lot of clinical features of TLE. KA induces HSP70 expression in hippocampal neurons or more generally throughout the brain, depending on the dose of KA.

In the present study, regarding the correlation between HSP70 and serum sodium levels in epilepsy group, there was a negative significant correlation in which epileptic children with high level of HSP70 had low serum level of sodium, but in FS and healthy children, there was no relationship between serum HSP70 and serum sodium levels. These results agree

with that reported by Lu and Wang,<sup>20</sup> who found that children with high levels of HSP70 had low sodium levels, which could be attributed to the fact that uncontrolled convulsions in epileptic children may lead to increase in serum levels of HSP70, and patient with uncontrolled convulsions need poly-therapy of AEDs that may lead to AEDs-induced hyponatremia.

A positive significant correlation was found between HSP70 and type of therapy (monotherapy, double therapy, and triple therapy) in epilepsy group in which children with high levels of HSP70 were treated with more than one drug. This was in agreement with Rejdak et al<sup>21</sup> and Chang et al,<sup>16</sup> who reported that there was a positive correlation between HSP70 and type of therapy in epileptic children as children with uncontrolled convulsions due to epilepsy have high levels of HSP70 and also need more than one therapy of antiepileptic drugs.

## Conclusions

Serum HSP70 level was significantly higher in epileptic children than febrile and healthy control children. HSP70 level was significantly higher in FS children than healthy controls. Also, there was a positive correlation between HSP70 level and type of therapy in epileptic children. HSP70 may be beneficial in diagnosis of epilepsy. Further studies are recommended for the assessment of other types of HSPs in epileptic and FS children for comparing our results of HSP70 in epileptic and FS children and also to assess HSP70 at specific period after seizures.

## Limitations of the Study

One of the limitations of this study is the small sample size owing to which these results may not be generalized to other patients with epilepsy or children with FS. Also, assessment of the relationship between EEG findings and HSP70 levels is lacking, which could be considered in future studies.

## Conflict of Interest

None declared.

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