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RESEARCH

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Serum protein S-100B as a novel biomarker of diagnosis and prognosis of childhood epilepsy

Mohamed Khamis*, Nahed Salah El Din, Maha Ali Nada and Hossam El Din Mahmoud Afifi

Abstract

Background Elevated levels of S-100B in serum are increasingly considered a potential biochemical marker of nervous system damage. To our knowledge, limited number of research studies have tested the serum S-100B protein levels in children with epilepsy. The objective of our study is to measure the serum levels of S-100B protein in pediatric cases with epilepsy.

Results The mean serum concentration of S-100B protein was 0.135 ± 0.014 mg/L in the patient group and 0.082 ± 0.018 mg/L in the control group. The patients showed significantly high S-100B protein levels compared with healthy controls ($P < 0.001$).

Conclusion Our data suggest that increased S-100B protein levels in the serum potentially indicate neuronal damage in the brains of children with epilepsy.

Keywords Childhood epilepsy, S-100B level, Biological marker, Children

Background

Epilepsy is a common disease that affects the central nervous system. it has a prevalence of around 1% worldwide, and in spite of the high prevalence, it can be frequently misdiagnosed because there is no way to reach for a certain diagnosis of epilepsy until seizures appear, in addition to that, epileptic disorders do not have a trustable and reliable biomarker for either epileptogenesis, epileptogenicity, presence, severity or progression of an epileptic state. Lately, many researches have been conducted to show the significance of astrocytosis in epilepsy [1, 2]. Astrocytosis has an important relation to the etiology and pathogenesis of epileptic process, Reactive astrocytosis can lead to local synaptic dysfunction causing some deficits in mechanisms of neuronal inhibition. Thus a disproportion between excitatory and inhibitory neuronal pools firing may incite epileptiform discharges in these patients [3, 4].

A number of proteins have been suggested as peripheral biochemical markers of neuronal injury and astrocyte activation, such as neuron-specific enolase (NSE) and S-100B protein. S-100B protein is a calcium-binding protein that exists essentially in astroglial and Schwann cells. [5–7]. Some research reports have tested serum S-100B protein levels in adults with epileptic conditions, but the results were non-conclusive [8, 9] and very few studies were made on children with epilepsy [10].

In our study, to evaluate the probable neuronal and/or glial pathology at the cellular and molecular levels in children with epilepsy, we have measured the levels of S-100B protein which is considered a potential biomarker of neuronal damage in children with different types of epilepsy.

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Methods

This case-controlled cross-sectional study was performed at Epilepsy outpatient clinic and pediatric outpatient clinic in Ain Shams University, Cairo.

The study consisted of 90 patients in 2 groups. Patient group of 45 subjects, (14 females, 31 males) diagnosed with epilepsy and a control group of 45 healthy individuals (18 females, 27 males). All of the 45 subjects in the patient group were diagnosed with epilepsy on the basis of their clinical Presentation of seizures, magnetic resonance imaging (MRI) and EEG results. We classified the seizures types according to ILAE 2017 expanded version to focal onset; generalized onset and unknown onset [11] and seizures severity is assessed according to Liverpool seizure severity scale 2 [12]. Positive EEG results showed epileptogenic discharges in the form of focal or generalized spikes and spike-slow wave discharges, patients with symptomatic epileptic disorders due to other medical problems, such as electrolyte disturbances, metabolic disorders, parenchymal brain insults or traumatic lesions, and pseudo-epileptic paroxysmal incidents were all excluded from the study.

Ethical research approval was gained from the Ethics committee of College of medicine Ain shams university at, 22nd of December 2019, Informed written consent was obtained from all children or their legal guardians, S-100B samples were withdrawn within 1 h of seizures onset and were put to centrifuge at rate of 5000 rpm for 10 min time then were kept stored at − 20 °C until analysis, Serum S-100B protein levels were measured with DRG® S-100B (Human) ELISA (EIA-4555) DRG International, Inc., USA and hemolyzed samples were all excluded.

The data underwent statistical analysis using SPSS1 for Windows version 11.5 (Chicago, USA). Statistical values for each group were expressed as the mean/SD. Parametric tests were used due to the homogenous distribution of the study groups. The differences between groups were measured with the help of *T*-Test. Analysis of variance [ANOVA], linear correlation coefficient and Chi-square tests were also used. The results are illustrated as the mean ± S.D.

$P < 0.05$ was considered as statistically significant and other statistical methods were also used such as receiver operator curve (ROC) curve—receiver operating characteristic curve analysis.

Results

The age and gender of the study subjects are given in Tables 1 and 2. Nonsignificant statistical differences were found between the study groups in relation to age or gender distribution ($P = 0.378$, ($P = 0.285$, respectively). In the study group, patients were classified into generalized or focal epilepsy, and seizure severity were shown according to Liverpool Seizure Severity Scale 2. EEG and MRI brain were done for all patients and the results are given in Table 1.

The observed mean serum concentration of S-100B protein was 0.13 ± 0.02 mg/L in the study group and 0.08 ± 0.01 mg/L in the control group. We found that the serum levels of S-100B protein were significantly higher in children with epilepsy compared to controls ($P < 0.001$) (Table 2).

S-100B protein levels were also found to be significantly increased in patients with severe epilepsy conditions, evident structural changes in their MRIs and in generalized rather than focal epilepsy (Table 3)

The receiver operating curve (ROC) analysis of S-100B levels in epileptic individuals showed that the area under the ROC curve was 0.980 and this curve analysis indicated the presence of S-100B as a marker of epilepsy with high sensitivity (area under the curve of 0.980; 95% confidence interval of (0.00–1.00) (Table 4, Fig. 1).

Discussion

S-100B is an integral structural protein of the central nervous system present essentially in astrocytes. It is considered primarily a potential peripheral biochemical marker of astroglial activation [6, 13]. Studies made on animal surrogates of epilepsy and brain biopsy specimens from epileptic cases have obviously illustrated an increased S-100B protein levels in brain tissue [14–16].

Also in many studies previously done, elevated S-100B protein levels were related to different neurological conditions, including subarachnoid hemorrhage, cerebrovascular ischemic strokes, traumatic brain lesions, CNS infections and other various neurological illnesses [17–19]. However, only few research studies have examined serum S-100B protein levels in epilepsy and only exclusively in adults population [20].

In 1999, Steinhoff and his colleagues reported that serum S-100B protein was in much higher levels in cerebrospinal fluid (CSF) of cases with temporal lobe epilepsy (TLE) compared to control group levels [21], while a study by Portela and colleagues reported that S-100B protein has no relation to pathogenesis of epilepsy and its levels are in normal ranges in adults with focal epilepsy [9].

Generally, S-100B protein has a short serum half-life (ranges around 25–113 min), and this short half-life put ahead challenges for its testing [22]. In this context, another study by Leutmezer and his colleagues investigated the serum S-100B levels in 10 temporal lobe

Table 1 Demographic and descriptive data of the study subjects

Age	Group		*T*-Test	
	Patient	Control	*t*	*P*-value
Range	1–16	1–15	1.075	0.285
Mean ± SD	7.133 ± 3.539	6.400 ± 2.903		

Gender	Group				Chi-square	
	Patient		Control			
	n	%	*n*	%	X^2	*P*-value
Male	31	68.89	27	60.00	0.776	0.378
Female	14	31.11	18	40.00		
Total	45	100.00	45	100.00		

	n	%
Classification of epilepsy		
Generalized	32	71.11
Focal	13	28.89
Total	45	100.00
Severity		
Mild	14	31.11
Moderate	15	33.33
Severe	16	35.56
Total	45	100.00
EEG localization		
Generalized	31	68.89
Temporal	9	20.00
Frontal	3	6.67
Central	2	4.44
Total	45	100.00
MRI		
Normal MRI	34	75.56
Abnormal MRI	11	24.44
Total	45	100.00

T T test, *SD* standard deviation

Table 2 S-100B levels in study groups

S-100B	Group		*T*-Test	
	Patient	Control	*t*	*P*-value
Range	0.09–0.16	0.06–0.12	15.732	< 0.001*
Mean ± SD	0.135 ± 0.018	0.082 ± 0.014		

T T test, *SD* standard deviation

epilepsy(TLE) patients at baseline and at 30 min, 3 h, 12 h, and 24 h after an epileptic seizure and did not notice any differences in S-100B levels at these different time beings [23].

On the other side, Palmio and his colleagues also compared S-100B levels in 2 patient groups, a group with temporal lobe epilepsy and another group with other types of epilepsy outside temporal region and he illustrated—in his research article—that the plasma levels of S-100B in the temporal group showed a significantly higher level after an epileptic seizure, While in the other group, insignificant changes in levels of S-100B protein were noticed after an epileptic seizure [24].

In a study performed on children with epilepsy, Mustafa Calik and colleagues investigated the serum S-100B levels in 19 children with (TLE) at time interval of 30 min after an epileptic attack and found impressive differences in the serum S-100B levels in comparison to healthy subjects [10].

In our study, we found also a significant increase in serum S-100B protein levels in children with epilepsy

Table 3 Correlation statistics of S-100B protein levels in patient group

	S-100B		*T*-Test or ANOVA	
	n	Mean ± SD	*T*	*P*-value
Gender				
Male	31	0.136 ± 0.018	0.824	0.415
Female	14	0.131 ± 0.018		
MRI				
Normal MRI	34	0.131 ± 0.016	− 2.229	0.031
Abnormal MRI	11	0.145 ± 0.020		
Classification of epilepsy				
Generalized	32	0.140 ± 0.018	3.315	0.002
Focal	13	0.122 ± 0.008		
Severity				
Mild	14	0.119 ± 0.006	12.270	< 0.001
Moderate	15	0.139 ± 0.008		
Severe	16	0.144 ± 0.022		

T T test, *SD* standard deviation, *ANOVA* analysis of variance

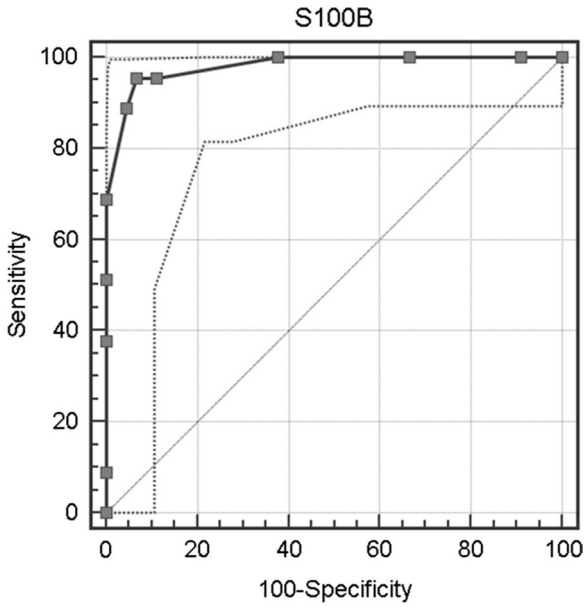

Fig. 1 The receiver operating curve (ROC) analysis of S-100B levels

that were higher than its peers' levels of the control group.

Our clinical samples for S-100B were obtained from plasma. These samples were taken 1 h after an epileptic seizure. Our results in children with epilepsy conditions were remarkably consistent with the previous studies that illustrated a higher S-100B protein levels after seizures in adult patients. In addition, we found significantly increased S-100B levels in correlation with the more severe epilepsy states and much higher levels in children with structural changes in their MRI and in generalized rather than focal epilepsy. Thus, the observation of elevated S-100B levels in children with epilepsy made us to suggest that brain damage is in relation to the existence and severity of epileptic seizures.

Unfortunately, we were not able to test baseline levels of S-100B in the epilepsy cases in our study. However, a significant difference have been shown between the patient and control groups with respect to S-100B levels. This study might be considered the first to exclusively report that serum S-100B protein levels were elevated in children with different epilepsy types, and its findings may contribute to the literature regarding this topic.

Through ROC analysis, our study has also demonstrated that serum S-100B levels may have predictive value that could foster the beneficial results of radiological and non-radiological tests, such as MR imaging, or EEG monitoring in diagnosis and prognosis of epileptic conditions and that S-100B protein may be a novel biological marker with high sensitivity that might aid in the diagnosis of epilepsy and exclusion of other paroxysmal epileptic-like incidents.

A disadvantage of this study is that it was conducted with no relation to genetic or familial epilepsy syndromes. This limitation may have restricted the predefined cut-off value of the ROC analysis.

Conclusion

The serum S-100B protein level may be a sensitive biochemical marker for neuronal damage in childhood epilepsy. It is clear that there is a need for further studies with a larger patient population and with comparison to different other causes of central nervous system damage.

Table 4 The receiver operating curve (ROC) analysis of S-100B levels

ROC curve between patient and control						
	Cutoff	Sensitivity	Specificity	PPV	NPV	Accuracy
S-100B	> 0.1	95.56	93.33	93.5	95.5	98.1%

ROC curve the receiver operating curve, *PPV* positive predictive value, *NPV* negative predictive value

Abbreviations

EEG	Electroencephalography
GTCs	Generalized onset tonic–clonic seizures
ILAE	International League Against Epilepsy
MRI	Magnetic resonance imaging
SD	Standard deviation
SPSS	Statistical Package for Social Science
ANOVA	Analysis of variance
ROC-curve	Receiver operating characteristic curve
PPV	Positive predictive value
NPV	Negative predictive value
CNS	Central nervous system
TLE	Temporal lobe epilepsy
CSF	Cerebrospinal fluid
XTLE	Extra-temporal lobe epilepsy
NSE	Neuron specific enolase
BBB	Blood–brain barrier

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Author contributions
MK, MN, and NS conceived of the study, participated in its design and coordination, helped in analysis, interpretation draft the manuscript. MK and HA drafted and critically revised the manuscript and performed the statistical analysis. All authors have agreed to conditions noted on the Authorship Agreement Form and have read and approved the final version submitted. All authors read and approved the final manuscript.

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Availability of data and materials
Dataset is available as master sheet in Excel format and publicly available in Neurology Department, Ain Shams University, through communicating with the corresponding author.

Declarations

Ethical approval and consent to participate
Research ethics approval was obtained from the Ethics Committee of the Medical School of Ain shams University at 22-12-2019. Written informed consent was obtained from all children/legal guardians, after informing them about the study rationale and their right to withdraw from the study at any time without any consequences.

Consent for publication
Not applicable.

Competing interests
All authors declare no competing interests.

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