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Original Article

Clinical value of serum S100B in children with epilepsy

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Abstract: Objective: To explore the clinical value of serum S100B in children with epilepsy. Methods: Ninety eight children with clinical diagnosis of epilepsy (Case group) from Jan 2014 to May 2017 from Zhoushan Maternity Infant Hospital were recruited. For the included 98 cases, 41 patients were diagnosed of generalized seizure epilepsy (GSEP) and the left 57 subjects were diagnosed of focal seizures epilepsy (FSEP). 87 healthy children from our hospital for regular physical examination were included as the control group in the same period of time. The serum S100B protein concentration was measured by enzyme linked immunosorbent assay (ELISA) at the time of 6 hr and 24hr after epileptic seizure for case group. The serum level of S100B was compared between the two groups. Using the serum S100 as the biomarker for epilepsy diagnosis, the diagnostic sensitivity and specificity were calculated. The area under the receiver operating characteristic (ROC) curve (AUC) was drawn to investigate its clinical diagnostic value. Results: The serum S100B concentration of 87 healthy control showed a skewed distribution, which declines gradually with the increasement of children age. The level of serum S100B in children with epileptic attack for 6 h was significantly higher than that in normal controls ($P < 0.05$), and the serum S100 concentration in GSEP group was significantly higher than that of FSEP group ($P < 0.05$). However, 24 hours after the onset of epilepsy in case group, the serum S100B concentration was not statistical different to control group ($P > 0.05$). But the serum S100 in GSEP group is significantly higher than that of FSEP group after 24 h epileptic attack ($P < 0.05$); And the serum S100B level decreases significantly from 6 h to 24 h after epileptic attack ($P < 0.05$). Using 6 and 24 h serum S100B as the diagnosis reference, the diagnostic sensitivity and specificity of epileptic children were 62.07% (95% CI: 51.03%-72.26%), 60.20% (49.82%-69.96%) and 56.32% (39.73%-60.27%), 51.02% (39.73%-60.27%), respectively. The areas under the ROC curve (AUC) were 0.71 and 0.52. The sensitivity and specificity for differential diagnosis of GSEP and FSEP were 75.44% (62.24%-85.87%), 51.22% (35.13%-67.12%) and 66.67% (52.94%-78.60%), 53.66% (37.43%-69.34%), respectively. The differential diagnosis AUC were 0.69 and 0.69 respectively. Conclusion: Serum S100B level was a sensitivity index for the evaluation of the nerve damage in children with epilepsy which can be used as a serum biomarker for epilepsy diagnosis and assessment of severity of this disease.

Keywords: S100B protein, epilepsy, diagnosis, generalized seizure epilepsy, focal seizures epilepsy

Introduction

Epilepsy is a type of complicated and recurrent neurological syndrome that is commonly observed in childhood and is a type of convulsive attack caused by paroxysmal, temporary brain disorders [1]. Generally, etiology is divided into primary and secondary categories. Clinical manifestations are featured by repeated episodes of muscle twitching and short-term abnormality of consciousness, feeling, and emotion [2, 3]. The cerebral cortex is stimulated to cause excessive abnormal discharge because children's nervous system is not mature. At present, the biochemical markers generally used in brain injury are neuro-specific enolase, brain creatine kinase, myelin basic protein, and S100B protein. The sensitivity and specificity of S100B in prediction of brain injury is better than other marks, and the degree of serum elevation is positively correlated with the degree of injury. S100B protein is mainly distributed in the neuroepithelial cells of nervous system and mostly distributed in the brain tissue. After the brain tissue is injured, the elevated S100B in the cerebrospinal fluid enters the blood through the damaged blood-brain barrier. And the S100B protein in the peripheral blood is paral-

Table 1. The base line of the two groups

Parameter	Case (*n* = 98)	Control (*n* = 87)
Gender		
Male	54 (55.1%)	45 (51.7%)
Female	44 (44.9%)	42 (48.3%)
Age	7.6 ± 5.1	8.4 ± 5.3
0~	14 (14.3%)	11 (12.6%)
3~	30 (30.6%)	28 (32.2%)
6~	28 (28.6%)	25 (28.7%)
12~16	26 (26.5%)	23 (26.4%)

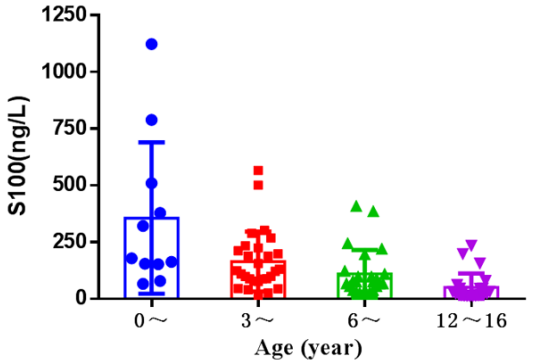

Figure 1. Box plot distribution of serum S100B protein levels in normal children of different age groups.

lel to the level of cerebrospinal fluid [4]. Therefore, the elevation of serum S100B can be used as an marker of specificity and sensitivity for central nervous system damage, and blood detection is more convenient, practical, and operational than cerebrospinal fluid measurement [5-7]. This study aims to investigate the expression of S100B protein in serum of children with epilepsy and its clinical value in judging generalized seizures (GSEP) and focal seizures (FSEP).

Materials and methods

Patients

Ninety-eight epileptic children in Zhoushan Maternity Infant Hospital from May 2013 to April 2017 were recruited in our present study (case group), including 41 cases of GSEP and 57 cases of FSEP. Of the included 98 cases, 54 were male and 44 are female with an average age of 7.6 ± 5.1 years old. In the same period of time, 87 healthy children for regular physical examination were included as the control group. Among them, there were 45 boys and 42 girls, of which 11 cases are in the 0 year-old group, 28 cases in 3 year-old group, 25 cases in 6 year-old group, 23 cases in 12-16 year-old group, with an average age of 8.4 ± 5.3 years. The general characteristics of the two groups are showed in **Table 1**.

The patients inclusion criteria were: Children (1) aged 0 to 16 years; (2) diagnosed according to the classification of epilepsy regulated by International League Against Epilepsy in 1981 and 1989 (cases that cannot be clearly classified are excluded); (3) who suffered from epilepsy for the first time.

Exclusion criteria: children with (1) abnormal birth or perinatal disorders; (2) severe mental illness; (3) congenital major hereditary disorders, chromosomal abnormalities; (4) cardiovascular and endocrine disorders, tumors and hematologic diseases; (5) skin disorders and obesity; (6) recent surgery, fractures or brain trauma (within 3 months); (7) strenuous exercise before sampling. This study has been approved by Medical Ethics Committee of Zhoushan Maternity and Infant Hospital and been consented by pediatric patients' guardians who voluntarily joined the clinical study.

Instrument and equipment

Microplate reader (Model: 550) was purchased from BIO-RAD, USA; Automatic biochemical analyzer (AC5800) was purchased from Beckman Coulter, USA; -80°C ultra-low temperature refrigerator was purchased from Thermo, USA; high-speed centrifuge was purchased from Beckman Coulter, USA.

Detection of serum S100B level

3 ml peripheral blood of epilepsy children was extracted at 6 h and 24 h after epileptic attack, placed in non-anticoagulation tube, and sent to the laboratory for centrifugal operation within 30 min (1500 rpm × 10 min). The supernatant was stored in -70°C for the rest examination (the hemolysis specimens were removed). The serum S100B assay kit was purchased from AB Sangtec Medica, Sweden. The experimental procedure was conducted strictly in accordance with the kit instructions.

Table 2. Distribution of serum S100B protein levels in normal children of different age groups

Age	*n*	Minimum	25%	Median	75%	Maximum
0~	11	66.02	152.8	179.0	508.8	1123.0
3~	28	25.11	87.27	122.90	221.00	566.00
6~	25	22.11	38.94	76.98	116.50	409.90
12-16	23	11.78	15.76	23.00	45.11	234.11

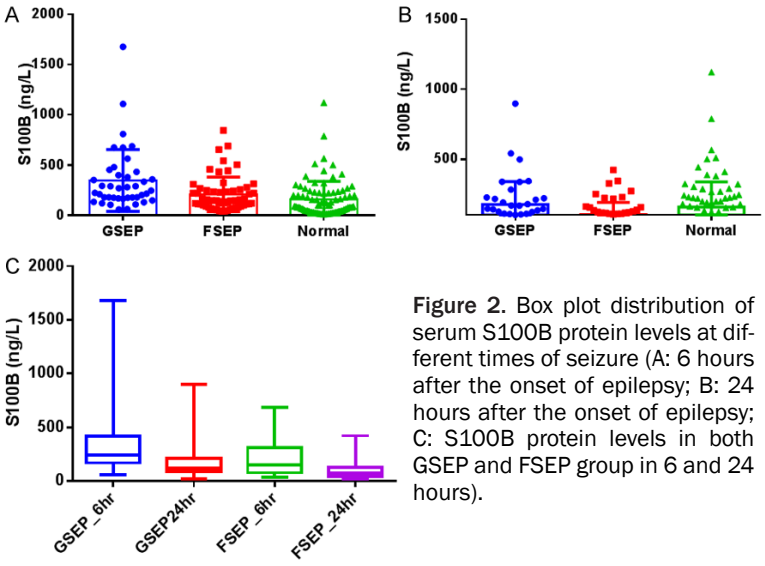

Figure 2. Box plot distribution of serum S100B protein levels at different times of seizure (A: 6 hours after the onset of epilepsy; B: 24 hours after the onset of epilepsy; C: S100B protein levels in both GSEP and FSEP group in 6 and 24 hours).

Statistical analysis

All data were analyzed by Stata11.0 and SPSS16.0 statistical software. The children's age and serum S100B level were taken as the measurement data. Firstly, normal distribution test was conducted. If the data was in accord with normal distribution, the data was expressed by $\bar{x} \pm s$ and compared by student-t test between groups. However, for non-normal distribution data, median and quartile interval was used for expression and rank-sum test for the group-to-group comparison was performed. Rate was used to express counting data, and X^2 test was used for the group-to-group comparison. $P < 0.05$ means significant difference.

Results

Serum S100 level declined with the age increasing

The level of serum S100B protein of 87 healthy control children shows a skewed distribution, which declines gradually with the increasing of children's age (**Figure 1**). The median and quartile interval of serum S100B in healthy control children of all ages are shown in **Table 2**.

Serum S100B protein levels differs for different groups at different time

Serum S100B level in children with epileptic attack at 6 h was significantly higher than that of normal controls ($P < 0.05$), and GSEP group was significantly higher than FSEP group ($P < 0.05$) (**Figure 2A**, Supplementary Data). However, 24 hours after the onset of epilepsy in case group, the serum S100B concentration was not statistical different to control group ($P > 0.05$) (**Figure 2B**). But the serum S100 level in GSEP group was significantly higher than that of FSEP group after 24 h epileptic attack ($P < 0.05$), (**Figure 2C**); And the serum S100B level decreases significantly from 6 h to 24 h after epileptic attack ($P < 0.05$), (**Figure 2C**). The median and maximum and minimum values of serum S100B protein level in the epilepsy group at 6 and 24 h are shown in **Table 3**.

Serum S100B as potential biomarker for epilepsy

Using 6 and 24 h serum S100B as the diagnosis reference, the diagnostic sensitivity and specificity of epileptic children were 62.07% (95% CI: 51.03%-72.26%), 60.20% (49.82%-69.96%) and 56.32% (39.73%-60.27%), 51.02% (39.73%-60.27%), respectively (**Table 4**). The areas under the ROC curve (AUC) were 0.71 and 0.52, **Figure 3A**, **3B**). These results indicated that early detection (6 h) serum S100B can be a potential clinical practical method for diagnoses of children epilepsy.

S100B as sensitive biomarker for differentiating GSEP and FSEP

The sensitivity and specificity for serum S100B in differential diagnosis of GSEP and FSEP were

Table 3. Serum S100B protein levels in children with epilepsy at different times (ng/L)

Groups	6 hr			24 hr		
	Minimum	Median	Maximum	Minimum	Median	Maximum
GSEP (n = 41)	56.98	245.20	1678.89	26.08	130.45	879.90
FSEP (n = 57)	38.88	145.90	845.70	20.21	69.67	422.80
Control (n = 87)	11.78	88.09	1123.00	8.90	96.40	1321.11

Table 4. Clinical value of serum S100B in diagnosis of epilepsy

Parameter	S100B_6 hr	S100B_24 hr
Sensitivity		
Point estimation	62.07%	56.32%
95% CI	51.03%-72.26%	39.73%-60.27%
Specificity		
Point estimation	60.20%	51.02%
95% CI	49.82%-69.96%	45.27%-66.94%
Cut off	154.80	100.06
AUC	0.70	0.52
Likelihood ratio	1.56	1.13

75.44% (62.24%-85.87%), 51.22% (35.13%-67.12%) and 66.67% (52.94%-78.60%), 53.66% (37.43%-69.34%), respectively (**Table 5**). The differential diagnostic AUC were 0.69 and 0.69 respectively, (**Figure 3C**, **3D**). This result indicated that serum *S100B* was a sensitive biomarker for differentiating GSEP and FSEP.

Discussion

In 1965, Moore et al. first discovered S100B protein in bovine brain tissue [4], which is named for its 100% dissolution in neutral saturated ammonium sulfate. S100B is a kind of small molecular EF hand-type acidic calcium binding protein [8]. At present, the specific high expression of the protein is found in a variety of neurological and nerve damage diseases [8-10]. Recent studies showed that S100B protein can play the role of cell's internal and external signaling by regulating cell proliferation, differentiation, or recruiting cellular inflammatory mediators [11-13].

In our present study, the results showed that serum S100B level in the epilepsy group at 6 h is significantly higher than in the normal group ($P < 0.05$), and GSEP group is significantly higher than FSEP group ($P < 0.05$); the brain damage in GSEP is more severe than in FSEP. The level of S100B protein in the epilepsy group at 24 h is significantly lower than at 6 h. S100B protein in the early seizures significantly increases and rapid decreases at 24 h, which is consistent with EP definition (paroxysmal, sudden, temporary brain dysfunction), suggesting that brain damage recovers rapidly after EP attack. These finds provides an important theoretical basis for our clinical practice in the rational application of brain function protection drug. Moreover, basing on Bayesian theorem, we take serum S100B as a reference to evaluate its sensitivity and specificity in diagnosing pediatric epilepsy. The findings showed that the early detection of S100B protein is acceptable for identifying GSEP and FSEP. However, the clinical significance and value of 24 h test were limited with low sensitivity and specificity. The dynamic observation can be used to determine the evolution and prognosis of disease because of the shortterm S100B biological half-life. In this study, no significant difference was observed between the serum S100B level in FSEP group at 24 h and in the normal group, which also indicates that S100B protein degrade fast in vivo especially in the early phase of epilepsy.

In this study, S100B concentrations gradually decrease with the increase in ages of the normal children. S100B concentration is at a high level among infants and young children, then declines faster, basically stable at the preschool age or school age, and rapidly declines at early puberty, which shows that serum S100B protein concentration is negatively correlated with age, but the correlation is not linear. Therefore, this age associated S100 serum level character should be considered when the serum S100B protein reference value of normal childhood is developed. The most profound study of the relationship between children's age and topical S100B protein level is carried out by Diego et al. [14]. They collected a total of 1004 cases of normal children in 3 years, with the age distribution from 1 month to 15 years old. They used ELISA to determine the plasma S100B protein value.

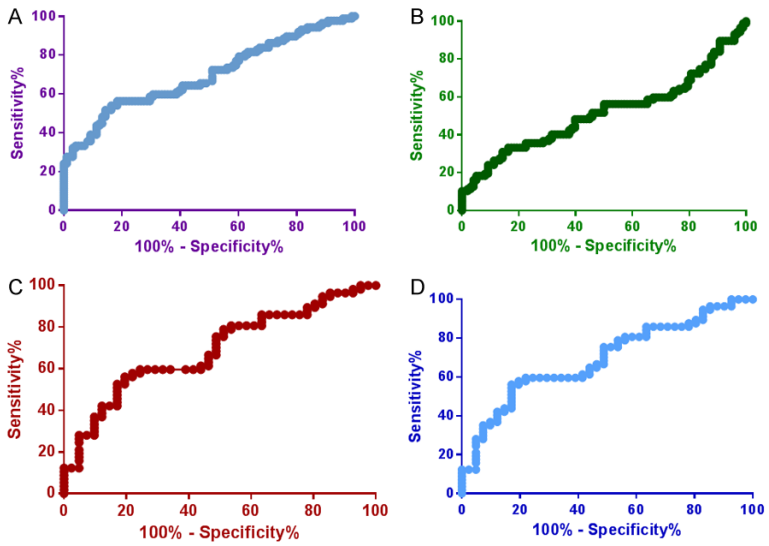

Figure 3. ROC curve of serum S100B in assessment and identification of GSEP and FSEP. (A: ROC curve of serum S100B in the diagnosis of epilepsy in the time point of 6 hours after the onset of epilepsy; B: ROC curve of serum S100B in the diagnosis of epilepsy in the time point of 24 hours after the onset of epilepsy; C: ROC curve of serum S100B in assessment and identification of GSEP and FSEP in the time point of 6 hours after the onset of epilepsy; D: ROC curve of serum S100B in assessment and identification of GSEP and FSEP in the time point of 24 hours after the onset of epilepsy).

Table 5. Serum S100B in assessment and identification of GSEP and FSEP

Parameter	S100B_6 hr	S100B_24 hr
Sensitivity		
Point estimation	75.44%	66.67%
95% CI	62.24%-85.87%	52.94%-78.60%
Specificity		
Point estimation	51.22%	53.66%
95% CI	35.13%-67.12%	37.43%-69.34%
Cut off	245.20	110.50
AUC	0.69	0.69
Likelihood ratio	1.55	1.44

The result showed that plasma S100B protein reduced gradually at 0-7 years old but increased at 7-13 years old and finally reduced at 14-15 years old. Compare to Diego' study, the statistical power of our present work is limited with 87 normal children in the control groups. Thus, well designed prospective diagnostic studies with large samples was needed to further clarify the exact distribution of Serum S100B protein of normal children in China, which provides more evidence for the clinical diagnosis of pediatric epilepsy.

Disclosure of conflict of interest

None.

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Supplementary Data. Supplement Data for GSEP and FSEP in **Figure 2A**.

GSEP	FSEP
293.1	168.8
132.11	119.89
178.98	217.98
398.9	228.7
146.89	139.9
106.9	143.91
178.98	89.9
289.98	89.9
478.87	145.88
278.97	154.87
200.98	165.12
351.27	245.12
378.98	654.22
1678.89	312.76
178.88	78.65
809.98	45.12
676.45	56.89
564.11	98.78
356.78	112.34
167.9	145.89
1109.78	219.11
118.47	234.14
245.23	456.87
56.98	845.67
334.78	219.88
432.09	122.89
164.89	67.98
104.98	79.941
129.78	114.23
214.89	134.61
269.65	145.66
178.09	154.13
687.43	56.26
674.98	42.98
209.87	51.67
452.11	78.93
60.98	109.98
174.67	241.78
289.84	118.97
222.22	267.88
173.98	432.11
	321.67
	276.89
	254.98
	309.23
	211.56
	109.69

116.89
234.56
98.89
67.98
41.22
543.11
443.24
502.1
689.9
38.88

Table Analyzed	6 h
Column B	FSEP
Vs.	Vs.
Column A	GSEP
Unpaired t test	
P value	0.0062
P value summary	**
Significantly different (P < 0.05)?	Yes
One- or tow-tailed *P* value?	Tow-tailed
t, df	t = 2.8, df = 96
How big is the difference?	
Mean ± SEM of column A	345.2 ± 48.12, n = 41
Mean ± SEM of column B	208.3 ± 22.93, n = 57
Difference between means	-136.9 ± 48.91
95% confidence interval	-234 to -39.86
R squared (eta squared)	0.0755
F test to compare variances	
F, DFn, Dfd	3.169, 40, 56
P value	< 0.0001
P value summary	****
Significantly different (P < 0.05)?	Yes