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Research Article

SERUM BRAIN DERIVED NEUROTROPHIC FACTOR AS BIOMARKER FOR EPILEPSY DIAGNOSIS IN EGYPTIAN CHILDREN WITH EPILEPSY; RELATIONSHIP TO DISEASE SEVERITY AND COGNITIVE FUNCTION

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ABSTRACT

Objectives: This study was designed to estimate serum brain-derived neurotrophic factor (BDNF) concentration in a group of Egyptian children with epilepsy to clarify its utility as a biomarker for epilepsy diagnosis and to evaluate its relationship with their cognitive function and disease severity.

Methods: Intelligence was assessed using Arabic version of the WISC-R test. Serum concentration of BDNF was assessed using enzyme-linked immunosorbent assay in 40 children with epilepsy and 40 apparently healthy children of matched age and sex controls.

Results: Of total 40 epileptic patients aged 6–12 years, the mean age was 8.32 ± 0.7 years and male to female ratio was 1.5:1. The mean serum BDNF concentration and cognitive IQ scores were statistically significantly reduced in the studied patient's group versus to controls ($p<0.001$). There were highly significant differences in serum BDNF concentration as regards epileptic severity. Serum concentration of BDNF showed significantly positive correlation with cognitive, verbal, performance, and totals IQ scores and negative correlation with the age at the onset of seizures, and duration of therapy. The linear regression analysis showed a statistically significant association between age at the onset of seizures, duration of therapy, cognitive function, and serum BDNF concentrations among the studied patients.

Conclusions: Concentration of BDNF in serum is involved in the mechanism of epileptogenesis in children with epilepsy. It should be used as a helpful marker for epilepsy diagnosis and detection of severity.

Keywords: Epilepsy, Brain-derived neurotrophic factor, Egyptian children, Cognitive function, Disease severity.

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INTRODUCTION

Epilepsy is one of the most common chronic neurological diseases in children, which is manifested by a sudden transient disturbance of brain function [1]. It is often associated with changes or loss of consciousness that have a burden insult on the cognitive function of epileptic children [2]. At school age, the prevalence of epilepsy is between 4 and 7/1000 children [3].

Brain-derived neurotrophic factor (BDNF) is one of the neurotrophin family of growth factors promoting neuronal differentiation, neurogenesis, and neural plasticity [4,5]. It is found throughout the brain, particularly in the cerebral cortex and hippocampi. The dysregulation of BDNF leads to a number of neurologic and psychiatric disorders [6].

BDNF concentration was found to be elevated in brain tissues of children with resistant temporal lobe epilepsy [7]. Kanemoto *et al*. [8] also demonstrated a relation between BDNF gene polymorphism and partial epilepsy. Otherwise, BDNF serum concentration in epileptic children has not been previously well described. Therefore, the aim of our present study was to compare serum BDNF concentration in a group of Egyptian children with epileptic seizures between epileptic attacks and healthy controls to assess its utility as a diagnostic tool and evaluate its relationship with their cognitive function, and disease severity.

METHODS

Design and setting of the study

This descriptive comparative case–control study was conducted on 40 patients with epilepsy over a period of 2 years (from March 2013 to February 2015). They referred to the Center of Excellence, National Research Centre (NRC) in Egypt from the outpatient epilepsy, Clinic Abu Elrich Hospital, for assessment of their cognitive function.

Patients

Epilepsy was defined as the separate occurrence of two or more unprovoked seizures, manifested by involuntary motor, sensory, or autonomic, alone or in combination, and not diagnosed as neonatal or febrile seizures [9,10]. Epileptic children were examined by a pediatric neurologist. The diagnosis of epilepsy in the children was made on the basis of history and confirmed by video-electroencephalogram (EEG) monitoring. They were diagnosed according to the international classification of epileptic seizures, epilepsies, and epileptic syndromes of league against epilepsy for the determination of seizure types, epilepsy disorders, and age-specific epileptic syndromes [11]. Children with epilepsy were also assigned to subgroups with regard to epilepsy type (generalized/partial), drug therapy (poly/monotherapy). Our study was conducted on 80 children; 40 children with epilepsy and 40 apparently healthy children with no chronic illness of matched age, sex, and social class as controls. They were selected from the outpatients Child Health Clinic at the NRC. They were attending the Clinic for follow-up.

Cases included in this study were randomly selected among children who recently had seizures over a period of 1 year. Exclusion criteria were (i) a history of epilepsy surgery, (ii) a history of psychogenic seizures or uncertain diagnosis, (iii) presence of an active neurological disorder, and (iv) active comorbid psychiatric illness.

Controls

Age, sex, and social class matched 40 apparently healthy children without CHD were included as the control group. They were selected

from the outpatients' Clinic at NRC while they were coming for follow up.

Standard protocol approvals and consents

This study was approved by the Research Ethics Committee of the NRC with Ethics Committee Number 17082. All studied cases provided signed written consent form of the Medical Ethical Committee of NRC to participate in the assessments from the parents of the children before study enrollment.

Methods

Assessments

- Epilepsy severity assessment

All patients were subjected to a detailed assessment of history including a detailed description of the epilepsy severity variables including age at the onset of seizures, type of epilepsy, duration of seizures, its frequency, duration of therapy, and the degree of control upon treatment.

- Intellectual assessment

Cognitive abilities were assessed by the administration of the Arabic version of the WISC-R [12]. It is the most widely used scale to assess the intelligence of children aged between 6 and 15 years, and it takes around 1.5 h. It provides scores for verbal, performance, and full-scale IQ score. Its subtests include different types of tasks, allowing the observation of the child's strengths, and weaknesses. Results from IQ tests help guide diagnosis, treatment, and educational planning. The average or normal IQ for children ranged from 90 to 110.

Laboratory diagnosis

After an overnight fast, venous blood samples were collected from all participants and the separated sera were stored at −20°C. Quantitative determination of BDNF concentration in serum samples was performed using enzyme-linked immunosorbent assay kit purchased from Glory Bioscience, USA, according to manufacturer's instructions.

Statistical analysis

All analyses were performed using the statistical package for the social sciences (SPSS), version 22 computer program (SSPS Inc, Chicago, USA), and the results were presented as Tables and Figures. Quantitative variables are expressed as the mean ± SD. The sample size was calculated to assess the risk factors probably affecting serum BDNF concentration of children with epilepsy between attacks. To compare cognitive score and serum BDNF concentration between groups, Student's t-test was used. Categorical data were expressed as frequencies and percentages and analyzed with the two-tailed Chi-square test. Pearson's correlation coefficient was used to assess for association between serum BDNF concentration, cognitive scores, and seizures' variables. As the concentration of BDNF has a normal distribution, linear regression analysis was used to estimate the effects of different risk factors as duration of therapy with antiepileptic medications (years), age of the onset of seizures, and IQ scores in potentially changing the BDNF concentrations. $p<0.05$ was considered significant and $p<0.001$ was considered highly significant.

RESULTS

Demographic data and characteristics of the participants

A total number of 40 epileptic patients (60% males and 40% females) were enrolled in this study, giving a male to female ratio of 1.5:1 and a significant sex distribution ($p<0.05$). The patients' age ranged from 6 to 12 years with a mean of 8.32 ± 0.7 years. Generalized tonic-clonic seizures were seen in 50% epileptic children being treated with polytherapy while partial seizures were diagnosed in 50% children being treated with monotherapy. 16 patients (40%) had severe epilepsy, as indicated by a seizure frequency of more than one attack per month, and 24 (60%) of patients were controlled. The therapeutic period ranged from 2 to 6.5 years with a mean of 3.63 ± 1.53 years (Table 1).

Serum BDNF concentration in controls and children with epilepsy

The mean serum concentration of BDNF was significantly lower in the studied patient's group (6.93 ± 0.34 ng/ml) compared to controls (8.08 ± 0.56 ng/ml) ($p<0.001$), as shown in Fig. 1 and Table 2. The mean cognitive, verbal, performance, and total IQ scores showed highly significantly lower in patients relative to control group ($p<0.001$) as shown in Table 2.

Relation of serum BDNF concentration to types of seizures and seizures frequencies

The mean concentration of serum BDNF showed insignificant differences between generalized epilepsy and partial epilepsy ($p>0.05$).

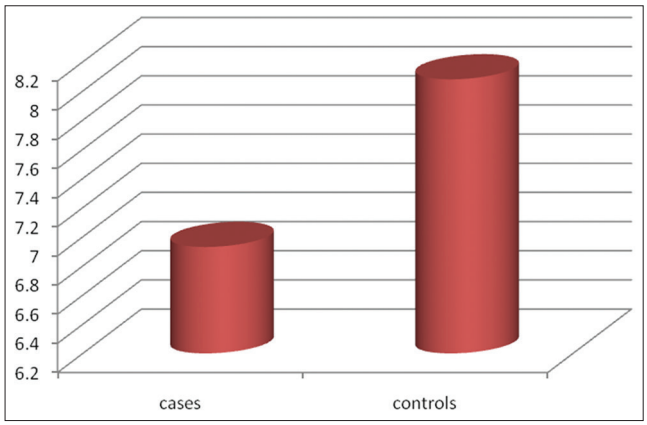

Fig. 1: Comparison of serum brain-derived neurotrophic factor concentration (ng/ml) between the studied cases and control group

Table 1: Demographic data and clinical characteristics of the studied patients

Variables	Mean±SD	Range
Gender (%)	Male	60%
	Female	40%
Age (years)	8.32±0.7	6-12
Age at the onset of symptoms	1.18±0.73	0.3-2.5
Duration of therapy	3.63±1.53	2-6.5
Seizure frequency	n	Percent
Controlled	24	60
Uncontrolled	16	40
Types of seizures		
Generalized	20	50
Partial	20	50

Table 2: Comparison of serum BDNF concentration and cognitive variables between the studied patients and control group

Variables	Cases	Controls	t	p value
Serum BDNF (ng/ml)				
Mean±SD	6.93±0.34	8.08±0.56	−7.41	0.00**
Range	6.3–7.2	7.4–8.9		
Verbal IQ score				
Mean±SD	83.5±3.2	95.8±6.4	−9.46	0.00**
Range	79–88	90–108		
Performance IQ score				
Mean±SD	79±11.32	91.3±2.54	−3.3	0.009**
Range	57–89	88–96		
Total IQ score				
Mean±SD	81±6.96	93.5±3.1	−7.26	0.00**
Range	68–88	90–99		

**Highly significant difference at $p<0.001$, BDNF: Brain-derived neurotrophic factor

However, there was highly significant association between serum BDNF concentration and disease severity ($p<0.05$) as shown in Table 3.

Relation of serum BDNF concentration to patients' gender

Insignificant differences in serum BDNF concentration were found between female and males patients ($p>0.05$), as shown in Table 3.

Serum BDNF concentration related to epilepsy risk factors

Serum concentration of BDNF showed significant positive correlation with cognitive, verbal, performance, and total IQ scores and negative correlation with duration of therapy with antiepileptic drugs, and the age at the onset of seizures (Table 4).

The linear regression analysis showed a statistically significant association between serum BDNF concentration and early disease onset, long duration of therapy with antiepileptic medications, as well as low cognitive function (total, verbal, and performance IQ scores) among the studied patients ($p<0.01$) as shown in Table 5.

DISCUSSION

The importance of BDNF stems from its functions as a regulator of neuronal survival, growth and differentiation, connectivity during development in addition to its role in synaptic plasticity [13]. Recently, it has been found that decreased plasma BDNF level in adults is reduced with epileptic seizures [14], while serum BDNF concentration in children with epilepsy has not been previously well described. Connolly *et al*. [15] recorded no differences in serum BDNF concentrations between epileptic children and controls. Therefore, the purpose of our study was to elucidate the potential use of BDNF in early diagnosis of epilepsy by determining serum BDNF concentrations in children with epilepsy and compare with the healthy controls between epileptic attacks to clarify its utility as a biomarker for epilepsy diagnosis and severity.

Forty epileptic patients, 60% males and 40% females, were recruited into this study giving a male to female ratio of 1.5:1 and a significant sex distribution ($p<0.05$). This is in respect with the findings of Shinnar and Pellock [16], who mentioned that the frequency rate is higher in males (63.6%) than females (36.4%), with a male to female ratio of 1.9:1.

The age of our patients ranged from 6 to 12 years with a mean of 8.32 ± 0.7 years. 16 patients (40%) of the children were controlled meaning that they had severe epilepsy, as defined by a seizure frequency of more than one attack per month, and 24 (60%) of patients were controlled. The duration of therapy with antiepileptic drugs ranged from 2 to 6.5 years with a mean of 3.63 ± 1.53 years.

Epileptic children are more likely to have cognitive dysfunction than normal children [17-19]. In our present study, the mean cognitive, verbal, performance, and total IQ scores were statistically highly significantly lower in patients versus control group ($p<0.001$). Learning disabilities in our study as in previous reports [19-21] may be due to multifactor acting individually or synergistically. These include the detrimental effects of antiepileptic drugs and repeated seizures on the developing brain.

Our results showed that the mean serum concentration of BDNF was statistically significantly lower in the studied patient's group (6.93 ± 0.34 ng/ml) compared to controls (8.08 ± 0.56 ng/ml) ($p<0.001$). This is in agreement with Waragai *et al*. [22] who reported that BDNF concentration in plasma is significantly reduced in patients with epilepsy relative to controls. There are interesting documents for the implication of the neurotrophin BDNF in the pathogenesis of epilepsy. However, our results are not in agreement with Connolly *et al*., [15] who observed no difference in BDNF concentration between children with epilepsy and controls.

In our present study, the effect of different risk factors (i.e., gender, seizures frequency, severity, age at the onset of epileptic seizures, and duration of therapy with antiepileptic drugs) on the potential changes in serum BDNF concentration was detected. There are no significant differences in the mean of the serum BDNF concentration between female and male patients ($p>0.05$). Our results are in the agreement with Erickson *et al*. [23], Ziegenhorn *et al*. [24], and Yasutake *et al*. [25], who mentioned that BDNF concentration does not display any significant gender difference.

Table 3: Comparison of serum BDNF concentration as regards gender, type of seizures, and disease severity

Variables	Serum BDNF concentration (ng/ml)	Chi-square	p value
	Mean±SD		
Gender			
Female	6.97±0.28	0.822	0.43
Male	6.75±0.63		
Type of seizures			
Generalized	6.73±0.28	0.78	0.35
Partial	6.97±0.63		
Disease severity			
Controlled	7.12±0.07	7.8	0.00**
Uncontrolled	6.46±0.2		

**Highly significant difference at $p<0.001$, BDNF: Brain-derived neurotrophic factor

Table 4: Correlation between age at the onset of seizures, duration of therapy, and IQ scores with serum BDNF concentration

Variables	Age at the onset of seizures	Duration of therapy	Verbal IQ scores	Performance IQ scores	Total IQ scores
Serum BDNF (ng/ml)					
r	−0.67*	−0.76	0.485**	0.528**	0.547**
p	0.034	0.11	0.001	0.000	0.000

*Significant difference at $p<0.05$, **highly significant difference at $p<0.01$, BDNF: Brain-derived neurotrophic factor

Table 5: Linear regression analysis of risk factors associated with serum BDNF concentrations among the studied patients

Variables	Unstandardized coefficients		Standardized coefficients	p value	95% confidence interval	
	B	Standard error	Beta		Lower bound	upper bound
Constant	5.762	0.835		0.00	4.064	7.46
Early onset of seizures	0.088	0.074	0.192	0.045*	0.002	0.178
Long duration of therapy	0.126	0.024	0.568	0.00**	0.173	0.078
Verbal IQ score	0.089	0.044	0.868	0.049*	0.001	0.178
Performance IQ score	0.120	0.043	3.912	0.009**	0.032	0.209
Total IQ score	0.207	0.086	4.155	0.022*	0.381	0.032

Dependent factor: BDNF concentration. *Significant difference at $p<0.05$, **highly significant difference at $p<0.01$, BDNF: Brain-derived neurotrophic factor

In the current study, the mean concentration of serum BDNF showed insignificant differences between generalized epilepsy and partial epilepsy ($p>0.05$). These results come in line with those previously published by LaFrance *et al*. [17].

According to data obtained in our study, serum concentration of BDNF showed significant positive correlation with cognitive, verbal, performance, and total IQ scores ($p<0.001$). This is in agreement with Angelucci *et al*. [26] and Forlenza *et al*. [27] who reported that serum BDNF concentration is considered as a key player in the activity-dependent synaptic plasticity that is involved in the learning and memory processes.

The other observation in the current study was the significant association between low serum BDNF concentration and low cognitive function among the studied patients with epilepsy by linear regression analysis ($p<0.001$). This is in agreement with Unalpa *et al*. [28].

The association between epilepsy severity and serum BDNF concentration was investigated. The present findings suggested that serum BDNF might have a benefit as a biomarker of epilepsy severity, as there were highly significant differences in serum BDNF concentration as regards epilepsy severity (determined by seizure frequency) ($p<0.001$). Furthermore, serum BDNF concentration was negatively correlated with epilepsy severity (determined by age at onset of seizures and duration of antiepileptic therapy). These findings are in agreement with Unalpa *et al*. [28] who reported that a decreased serum BDNF is correlated with epilepsy severity.

The other observation in the current study was the significant association between serum BDNF concentration and early disease onset, long duration of antiepileptic therapy among the studied patients with epilepsy by linear regression analysis ($p<0.001$) which is in concert with the report from Unalpa *et al*. [28].

In conclusion, our data proved the significant decline in serum BDNF concentration and cognitive IQ scores in the epileptic children compared to control counterparts. Therefore, along with video-EEG, serum BDNF concentration should be used as a helpful biomarker for epilepsy diagnosis and severity.

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AUTHORS CONTRIBUTION:

Inas R. EL-Alameey: responsible for concept, collection of samples, analysis and interpretation of data; drafting and revising of the manuscript. Hanaa H. Ahmed: responsible for biochemical analysis, interpretation of data and revising of the manuscript.

CONFLICTS OF INTERESTS

The authors declare that they have no conflict of interest.

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