ORIGINAL ARTICLE

Brain-Derived Neurotrophic Factor as a Biomarker in Children with Attention Deficit-Hyperactivity Disorder

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Abstract :

Background: Evidence suggests that Brain-Derived Neurotrophic Factor (BDNF) is involved in the pathogenesis of Attention-Deficit Hyperactivity Disorder (ADHD), although experimental data regarding the contribution of BDNF concentration to this psychiatric disorder are controversial. *Aim:* To evaluate the plasma levels of BDNF in patients with ADHD. *Material and Methods*: In this cross sectional study, ADHD and controls were recruited from the outpatient clinic of the Shafa Hospital, Rasht; between March 2012 and April 2013. Clinical data concerning ADHD diagnosis and blood samples for patients were collected before treatment. Medical, neurological and psychiatric co-morbidities were excluded. The mean of BDNF concentration measured and compared with healthy controls. BDNF assay was determined using ELISA kits according to manufacturer's instructions. Descriptive statistical analysis was used with analysis of variance to find the significance of data. *Results*: Statistical analyses showed that the mean BDNF levels were significantly lower in ADHD patients and its subgroups as compared with normal control subjects ($p<0.001$). *Conclusion*: This study showed a dramatically lower BDNF plasma levels in untreated patients with ADHD, which might be useful adjunct method for diagnosis of ADHD in society.

Keywords: Brain-Derived Neurotrophic Factor (BDNF), Attention-Deficit Hyperactivity Disorder (ADHD), BDNF Blood Level

Introduction:

Attention deficit-hyperactivity disorder (ADHD) is a mental and neurobehavioral disorder characterized by inattention, impulsivity and hyperactivity. Diagnosing ADHD is based on its symptoms to inattention (ADHD-I), hyperactivity-impulsiveness (ADHD-H) or a combination of inattention and hyperactivity (ADHD-C) [1]. ADHD affects children globally and is diagnosed about twelve percent of Iranian kindergartens and school-aged children [2]. Moreover, its symptoms can be difficult to differentiate from other disorders, increasing the likelihood that the diagnosis of ADHD would be missed.

Although, the definite causes of ADHD are ambiguous, some factors such as genetics, dietary and the social environmental factors might be important to contributors in this disorder [3, 4]. Recently, there is evidence, which suggests that brain-derived neurotrophic factor, is involved in the pathogenesis of ADHD [5].

Brain-derived neurotrophic factor (BDNF) is a 25-kDa member of the neurotrophin family and highly expressed in cortical and hippocampal structures. It enhances the growth and maintenance of several neuronal systems as well

as participates in mechanisms of neuronal plasticity. Accumulating evidence suggests BDNF as a candidate molecule involved in the pathophysiology of mental disorders [6]. In the absence of normal levels of BDNF, mice exhibit enhanced aggressiveness, hyperactivity [7]. In addition, Tsai et al suggest that reduction of BDNF activity, especially in the midbrain region, might play a role in the pathogenesis of ADHD [8]. The experimental data regarding the contribution of BDNF levels to this psychiatric disorder are controversial. The purpose of this study was to evaluate the plasma levels of BDNF in patients with ADHD.

Material and Methods:

Study Population:

In this cross sectional study, participants were recruited from the outpatient clinic of the Shafa Hospital, Rasht; between March 2012 and April 2013. Sample size was calculated on n = 29 per group based on previous measurements of BDNF, assuming a minimum difference of 264.6 pg/mL with a SD of 53.5 pg/mL and 32.3 pg/mL, and the detection of this difference at a significance level of 5%. The total studied sample consisted of 58 participants divided into ADHD and controls matched by age and gender. Twenty-nine healthy children, living in the same area with similar demographic characteristics and without any acute or chronic medical problem, were included in the study as a control group. They were chosen from children that met no criteria for a psychiatric diagnosis after a careful evaluation by a specialized child and adolescent psychiatrist. The Raven's progressive matrices were applied to the control group and children with IQ below 70 were excluded. The ethical guidelines for study protocol were followed in accordance with Guilan University of Medical Sciences ethical committee acts. All participants and their parents provided their informed consents.

Exclusion Criteria :

Participants with previous history of major Axis I disorders, conduct disorder, pervasive developmental disorders, tic disorders, impulse control disorders, elimination disorders, eating disorders as well as individuals with an IQ<70 were excluded. In addition, subjects with history of neurological, metabolic, cardiac, liver, kidney and respiratory disease as well as Axis III disorders were not included in the present study.

Instruments:

The diagnosis of ADHD was made by DSM-IV-TR structured clinical interview. Kiddie-Schedule for Affective Disorders and Schizophrenia for School-Age Children-Present and the Lifetime-Persian Version (K-SADS-PL-P) were conducted for participants. The IQ was measured by the Raven's progressive matrices. The severity of ADHD symptoms were assessed using Conner's Parents Rating Scale (CPRS). The CPRS is a well-designed instrument that promises to be instrumental in the evaluation of children with ADHD behavior problems such as hyperactivity, impulsivity, and anxiety. All the (K-SADS-PL-P) interviews and IQ tests were made by one person.

Clinical Assessments:

Clinically two experienced psychiatrists performed complete medical history, physical examination and the K-SADS-PL-P interviews with all ADHD and control children and their parents. Their agreement on ADHD and its subtypes was acceptable for all diagnoses. Disagreements that could not be resolved by discussion would lead to exclusion, but in this study agreement could be achieved and all subject could retained. In addition, the confirmatory evaluation was made by one board-certified child psychiatrist (M. Kosha) with a combination of review of the completed questionnaire and a short interview.

Blood Samples and BDNF Measurement:

Blood samples were drawn from antecubital vein between 8 a.m. and 12 a.m. The samples were collected into tubes containing heparin. After sampling, tubes were centrifuged at 1000×g for 10 min immediately and aliquots of plasma were stored at ?20 ?C. After blood sampling, routine hematology tests were done within a few hours.

BDNF levels were determined using the human BDNF Quantikine® immuno assay Kit (R&D Systems, Minneapolis, USA), according to the manufacturer's instructions. Briefly, the BDNF in the samples was bound by the immobilized antibody in each well. An enzyme linked monoclonal antibody specific for BDNF was added to the wells. Following a washing, a substrate was added and color changed in proportion to the amount of BDNF. The reaction was stopped with sulfuric acid and the absorbance was measured at 450 nm.

Statistical Analyses:

The differences in plasma levels of BDNF between two mentioned groups were compared using the Student's t test. BDNF levels were tested for normality of distribution by means of the Kolmogorov-Smirnov test. Comparison between ADHD subgroups for parametric data was performed using Analysis of Variance (ANOVA). The analyses were performed using the SPSS statistical package, version 16.0 and P <0.001 were considered significant.

Results:

In this study, 29 ADHD patients and controls matched by age and gender were enrolled. Some demographic and psychometric data of ADHD patients and control subjects are shown in Table 1.

Table 1: Results of ADHD Patients and Control Subjects is Stratified by Demographic and Psychometric Data

Parameters	ADHD Subjects (N=29)	Control subjects (N=29)	P
Sex (female/male)	5/24	6/23	0.80
Age (years)	7.59 ± 2.01	7.43 ± 1.95	0.77
CPRS	68.26 ± 8.78	42.14 ± 6.84	<0.001

Age and CRPS are shown as mean ± SD.

The age of patient varied from 3.5-10 years (Mean 7.59 ± 2.01). Control subjects and ADHD subjects showed no differences between age ranges and sex, but they showed statistical differences between symptoms (*p<0.001). There were no significant difference in the Mean body weight and IQ scores of the two groups.

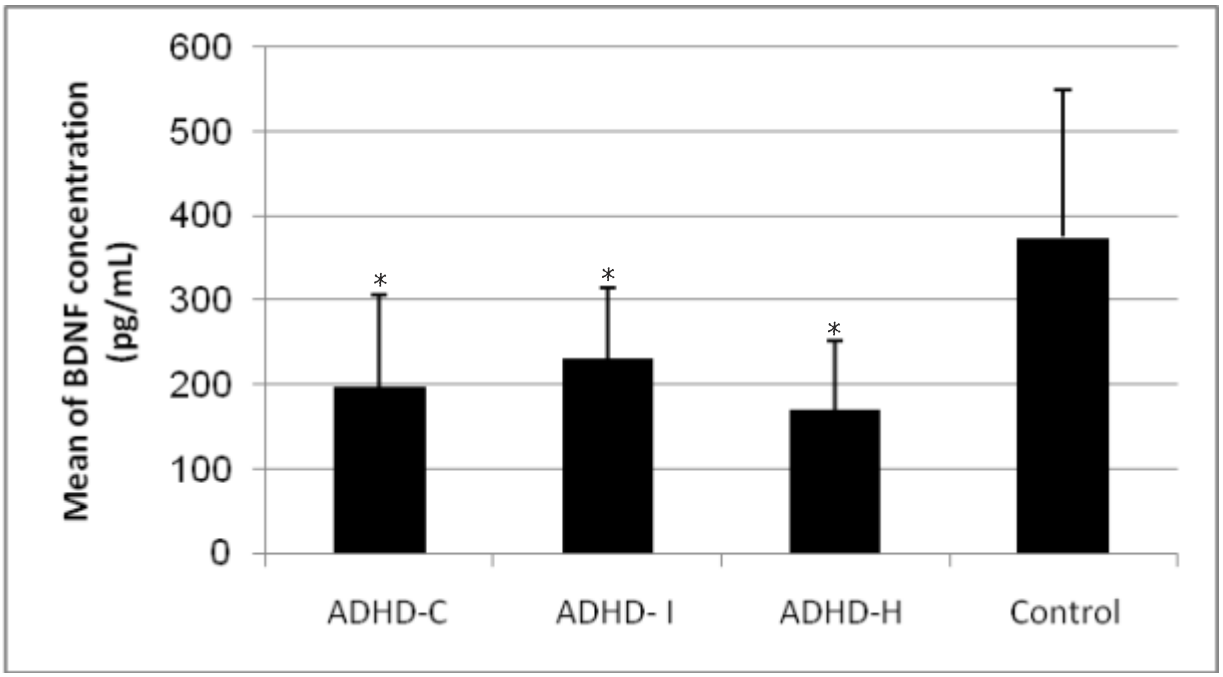
Based on DSM-IV-TR structured clinical interview and K-SADS-PL-P, all participants with attention deficit hyperactivity disorder were categorized inattentive ADHD (13.8%), hyperactivity-impulsiveness ADHD (34.5%) and combined ADHD-c (51.7%) subtypes.

According to data obtained by Conner's' Parent Rating Scales, there were significant statistical differences between different diagnostic subtypes of ADHD patients regarding, conduct problems (F= 11.743, P< 0.001), impulsive (F= 42.023, P< 0.001), anxiety (F= 7.405, P= 0.001) and hyperactivity (F= 26.303, P< 0.001). Although there is no significant differences in ADHD subtypes, severity of learning problems being the highest in the hyperactive type (Table 2).

Table 2: Psychometric Variables of Different Subtypes of ADHD Assessed Using Analysis of Variance (N=29)

Psychometric variables	ADHD-C Mean BDNF ± S.D (N=15)	ADHD-I Mean BDNF ± S.D (N=5)	ADHD-H Mean BDNF ± S.D (N=9)	F	P value
Conduct problems	64.79 ± 12.825	62.75 ± 12.738	77.20 ± 11.243	11.743	<0.001*
Learning problems	69.07 ± 18.223	71.00 ± 10.033	76.90 ±16.954	2.010	0.1403
Psychosomatic	65.14 ± 18.288	77.50 ±15.264	67.40 ± 22.80	3.466	0.0358*
Impulsivity	64.64 ± 7.143	60.00 ± 0.000	74.20 ± 7.584	42.023	<0.001*
Anxiety	62.21 ± 15.146	70.25 ± 21.156	54.30 ± 8.381	7.405	0.0011*
Hyperactivity	69.21 ± 9.325	60.00 ± 15.535	83.20 ± 11.094	26.303	<0.001*
Total	65.84 ± 7.840	67.33 ± 4.680	72.20 ± 10.304	1.659	0.211

Plasma levels of BDNF in ADHD group and healthy group were 190.70 ± 99.06 pg/mL and 374.91 ± 175.60 pg/mL, respectively. This difference was statistically significant (P <0.001). The concentration of BDNF in patients with various ADHD groups is depicted in Fig. 1.



ADHD = attention deficit hyperactivity disorder; BDNF = Brain-derived neurotrophic factor
**= No significant difference among subtypes*
The difference of BDNF concentration between ADHD subtypes and control was significant (P < 0.001)*

Fig 1: The Means Plasma Levels of BDNF in Different ADHD Subtypes and Control Obtained from Immunoassay Test

According to our results the differences in plasma BDNF levels between ADHD subtypes were not statistically significant (P=0.550). However, the mean plasma BDNF levels in different subtypes of ADHD groups (ADHD-I, ADHD-H, ADHD-C) were statistically significant as compared with normal (P<0.001). Moreover, according to Pearson's correlation test, there was no correlation between the BDNF titer and conduct problems, learning problems, psychosomatic, impulsive-hyperactive and anxiety of ADHD symptoms assessed using CPRS.

Discussion:

The finding of this study reveals a significant decrease in plasma levels of BDNF in children with all subtypes of ADHD compared with normal controls. Additionally, we could not demonstrate any association between plasma BDNF levels and psychometric symptoms according to Conner's Adult Rating Subscales.

As we excluded all co-morbidities and attendance of drug naive ADHD children in the survey, lower level of BDNF cannot be attributed to co-morbidities or previous medication. Nowadays, the diagnosis of ADHD has been based on history taking and diagnostic interview. It means that no simple objective test such as blood test, can aid in making diagnosis. Thus, according to the powerful P value between plasma levels of BDNF of the two groups, it seems to be a sensitive maker for this disorder.

Decreased in BDNF levels in ADHD children

may be primary, as an underlying neuro-developmental deficit in ADHD or secondary to dysregulation of neural system in this disorder. Since, plasma levels of BDNF are a marker of central levels of this protein, our finding support the hypothesis of Tsai about the role of BDNF in the pathogenesis of ADHD [8]. The hypothesis of BDNF as primary underlying deficit in ADHD is supported by increasing evidence about the role of BDNF in the development of dopamine system [5, 8]. However, more studies with molecular and biological methodology either are necessary to clarify the exact role of BDNF in neurobiological pathology of ADHD or may provide a new direction for treatment of this disorder.

Lower levels of BDNF in untreated ADHD children is consistent with animal studies [9-11] and Roso's study in adult ADHD [5]. Roso et al have reported a significant decrease in serum BDNF in adult ADHD compared to control. They also have found no significant correlations between serum BDNF and different subtypes of ADHD or scores on CPRS [5]. These findings are congruent with our results. As, adult ADHD is in the continuum of childhood disorder and the etiologic factors are similar, it seems the role of BDNF may also persist for the disorder at all ages [12]. Our ADHD children were drug naive, so previous treatment could not affect on the results. As like as ADHD children here, adult patients with ADHD also have shown decreased levels of BDNF. It may be explained by the fact that BDNF is acting as an imperative mediator in the complex neural system dysfunction.

On the other hand, our result was not consistent with the results of Shim et al, who have reported higher BDNF levels in ADHD children compared to controls. They have suggested a compensatory mechanism by upregulation of BDNF in response to dopaminergic and serotonergic dysregulaition for their results [13]. Though we believe the compensation mechanism, the age ranges of our sample were lower than Shim's study; it may be acceptable that the smaller ADHD children do not fulfill this mechanism. Moreover, decrease in serum levels of BDNF have also been reported in adults with ADHD compared to healthy controls [5]. These results suggest a role for BDNF in ADHD, at least in those patients whose disorder persists throughout life. Therefore, the compensation mechanism might not contribute in this process.

Although higher BDNF levels in ADHD children have been reported by Shim et al they have not considered the influence of physical activity on BDNF levels in their samples. Some researchers studied the role of exercise on serum brain-derived neurotrophic factor concentrations in human subjects. BDNF levels in humans are significantly elevated in response to exercise, and the magnitude of increase is exercise intensity dependent [14, 15]. This phenomenon can be expanded to ADHD children, as they are naturally hyperactive.

There are some clues on the association of BDNF polymorphism with different subtypes of ADHD [16]. A genetic study revealed that there is an association between the met allele of BDNF val66met SNP and ADHD symptoms of hyperactivity and impulsivity in European populations [17]. Based on these finding, the role of specific genetic subtype in our region should

considered.

Recently, some researchers report no alteration of serum BDNF levels in untreated patients with ADHD [18, 19]. These contradictory results may attribute to heterogenicity of ADHD syndrome, multi factorial pathophysiology of this disorder, different blood sampling methods, genetic patterns and laboratories differences.

Although, we made an effort to check all the known reasons affecting on BDNF levels, some limitations could be noted in our study. These limitations are the small sample size and few females included in the study. Cho et al, 2010 provided a preliminary evidence for a gender-specific association between BDNF and ADHD [20]. According to our findings, there is no obvious difference between male and female in both healthy and ADHD groups. Due to a limited age range and sex, we cannot expand our results to all ADHD patients. Another limitation of this study was that it did not measure BDNF levels of platelets. As Lommatzsch et al [21] have found, BDNF levels in plasma to some extent correlated with BDNF levels in platelets. Since, no significant differences between platelet counts of our samples are seen; platelet count does not seem to confound our results.

Conclusion:

Data obtained from this study showed a dramatically detrimental plasma level of BDNF in untreated patients with attention deficit-hyperactivity disorder. It may consider as a valuable adjunct method for diagnosis of ADHD in society. Further studies are required to elucidate the role of this neurotrophin in ADHD.

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