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Evaluation of Serum Brain-Derived Neurotrophic Factor Levels in Children with Attention Deficit Hyperactivity Disorder: Preliminary Data

Dikkat Eksikliği ve Hiperaktivite Bozukluğu (DEHB) Tanısı Olan Çocuklarda Serum Beyinden Köken Alan Nörotrofik Faktör (BDNF) Seviyelerinin Değerlendirilmesi: Öncül Bulgular

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ABSTRACT

Objective: Brain-derived neurotrophic factor (BDNF) has an important role in the survival, differentiation and synaptic plasticity of a series of neuronal systems including dopaminergic neurons. For this reason, it is proposed that BDNF plays a role in the attention deficit hyperactivity disorder (ADHD) pathophysiology. In this study, serum BDNF levels in children diagnosed with ADHD were compared with those in healthy subjects.

Methods: 30 children diagnosed with ADHD and 31 healthy control subjects aged 6-12 years were recruited to the study. The psychiatric diagnoses were determined by applying a semi-structured interview using the Kiddies Schedule for Affective Disorders and Schizophrenia for School-Age Children-Present and Lifetime Version (K-SADS-PL) and serum BDNF levels of the subjects were measured. Both parents were asked to fill out the Child Behavior Checklist (CBCL) for children 4-18 years of age.

Results: When ADHD cases and the controls were compared, no statistically significant difference was found in mean levels of serum BDNF (p=0.885). In ADHD cases, no statistically significant correlation was found between serum BDNF levels and ADHD subtypes (p=0.093). However, although there was no statistically significant difference, serum BDNF levels were found to be low in children diagnosed with attention deficit predominant type ADHD compared to children diagnosed with combined type ADHD.

Conclusion: This study does not support the hypothesis that BDNF plays a role in the etiology of ADHD. However, to interpret accurately the levels of BDNF, the factors that affect the levels, the reliable measurement source of the peripheral blood BDNF and its method must be enlightened. *(Archives of Neuropsychiatry 2012; 49: 96-101)*

Key words: Brain-derived neurotrophic factor, attention deficit hyperactivity disorder, children, neurotrophin

Conflict of interest:
The authors reported no conflict of interest related to this article.

ÖZET

Amaç: Beyinden köken alan nörotrofik faktör (BDNF) nöronların gelişim ve sağ kalımını destekler ve dopaminerjik nöronları da içeren pek çok nöronda sinaptik plastisite mekanizmalarında çok önemli rol oynar. BDNF'nin nöronal gelişimde önemli bir role sahip olması nedeniyle çocukluk başlangıçlı nörogelişimsel bir hastalık olan DEHB'nin de patogenezinde rolü olabileceği hipotezi öne sürülmüştür. Bu çalışmada, DEHB tanısı alan hastalarda serum BDNF düzeylerinin sağlıklı kontrollerle karşılaştırılması amaçlanmıştır.

Yöntem: Bu çalışmaya 6-12 yaş arasında 30 DEHB'li ve 31 sağlıklı kontrol çocuk alınmıştır. Çalışmaya katılan tüm çocuklarda psikiyatrik tanıları belirlemek için DSM IV'e göre uyarlanmış Okul Çağı Çocukları İçin Duygulanım Bozuklukları ve Şizofreni Görüşme Çizelgesi - Şimdi ve Yaşam Boyu Versiyonu (Kiddie Schedule for Affective Disorders and Schizophrenia for Shool Aged Children- Present and Lifetime Version) K-SADS-PL uygulanmıştır ve serum BDNF düzeyleri ölçülmüştür. Ayrıca, çocukların anne ve babalarından, 4-18 yaş Çocuk ve Gençler için Davranış Değerlendirme Ölçeğini (CBCL) doldurmaları istenmiştir.

Bulgular: Bu çalışmada DEHB tanısı alan olgularda serum BDNF düzeylerinin araştırılmış ancak DEHB'li olgular ile sağlıklı kontroller arasında istatistiksel olarak anlamlı bir fark saptanamamıştır (p=0,885). DEHB olgularında, serum BDNF düzeyleri DEHB alt tipleri açısından karşılaştırıldığında istatiksel olarak anlamlı bir fark bulunmamıştır (p<0,093). Ancak istatistiksel olarak anlamlı farklılık olmasa da DE baskın tip DEHB olgularında bileşik tip DEHB olgularına göre serum BDNF düzeyleri düşük bulunmuştur.

Sonuç: Bu çalışma BDNF'nin DEHB etyolojisinde rol oynadığı hipotezini desteklememektedir. Ancak, BDNF düzeylerini anlamlı bir şekilde yorumlayabilmek için, düzeyleri etkileyen faktörleri ve aynı zamanda periferik kan BDNF'sinin kaynaklarının aydınlatılması gerekmektedir. *(Nöropsikiyatri Arşivi 2012;49: 96-101)*

Anahtar kelimeler: Beyinden köken alan nörotrofik faktör (BDNF), dikkat eksikliği hiperaktivite bozukluğu (DEHB), çocuk

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Introduction

Attention deficit hyperactivity disorder (ADHD) is an important psychiatric illness affecting 5% of children globally. It is characterized by core symptoms like hyperactivity, attention deficit and impulsivity far beyond the developmental levels and has negative impact on quality of life of both patients and their family members (1).

Even though the pathogenesis of ADHD is still greatly unknown, evidences from different research studies point out primarily the dopaminergic system (2). Molecular genetic studies show that the genetic abnormalities in D4, D5 receptors and the dopamine transporter gene are related to ADHD (3).

As a member of the neurotrophin family, brain-derived neurotrophic factor (BDNF) is the most widely expressed neurotrophin in the brain (4). Preclinical preliminary studies have shown that BDNF plays an important role in the survival and differentiation of the dopaminergic neurons in the midbrain (5,6). Besides, it has been shown that BDNF prevents the spontaneous deaths of dopaminergic neurons in rat's mesancephalic culture (7), prevents neurotoxic effects (5) and protects nigrostriatal dopaminergic neurons from neurotoxicity (8). Since the midbrain dopaminergic system is very important in the ADHD pathogenesis (9), it was proposed that reduced midbrain BDNF activity may cause midbrain dopaminergic dysfunction and that this may lead to ADHD (10).

Psychostimulants can increase the release of dopamine and norepinephrine in the midbrain by various mechanisms. It has been shown that BDNF modulates dopamine release. It has been seen that the dopamine release induced by the psychostimulants was repressed by BDNF and due to this, BDNF was thought to be related to psychostimulant-induced dopamine release (11). Also, it has been demonstrated that the psychostimulants and antidepressants commonly used in the treatment of ADHD increase central BDNF (12,13). These findings showed that central BDNF activity has an important place in the treatment of ADHD (10).

The dopamine transporter (DAT) has a key role in terminating dopaminergic neurotransmission and genetic, pharmacological and neuroradiological evidences point out that DAT is related to the pathogenesis of ADHD (2). Since the DAT knockout mice show characteristics of ADHD, including hyperactivity and cognitive impairments, a preclinical model for ADHD was proposed (14). Fumagali et al. (2003) found that the DAT knockout mice had reduced expression of BDNF gene in their frontal cortices (15).

Mice with BDNF ablation die soon after birth and show abnormalities both in peripheral and central tissues. In mice with only one functional BDNF allele (heterozygous null mutant's mice, BNDF +/-) reduced BDNF gene expression, and locomotor hyperactivity has been detected (16). In rats which BDNF was only eliminated in the brain after birth using the cre-loxP recombination system technology, it was shown that hyperactivity, locomotor activity and aggression were increased in "conditional knockout mice" exposed to stress when compared to controls (17).

Currently, there is only one study that investigates ADHD and peripheral BDNF levels. In that study, ADHD patients with no former history of medication were compared to healthy controls and the plasma BDNF levels were found to be significantly higher in the children with ADHD. Indeed, the plasma BDNF levels showed a positive correlation with the severity of attention deficiency symptoms. The authors interpreted the increase in the BDNF levels was as a compensatory response to dopaminergic and serotonergic dysfunction proposed in the pathogenesis of ADHD (18).

BDNF is found in both brain and peripheral blood. Serum levels of BDNF have been found to be higher than plasma levels (19). In addition, human platelets contain BDNF (20). Pan et al. investigated the ability of BDNF to cross the blood-brain barrier and they concluded that intact BDNF in the peripheral circulation crosses the blood-brain barrier by a high-capacity (21). Although, Shim et al. suggested that there is an increase of plasma BDNF levels in untreated children with ADHD (18), the reliable measurement source of the peripheral blood BDNF is still unknown. Since BDNF is known to cross the blood-brain barrier in both directions, circulating BDNF might originate from neurons and glial cells of the brain (21). Accordingly, serum levels of BDNF have been found to be 200-times higher than plasma levels (19). However, so far there has been no study on serum BDNF levels in children diagnosed with ADHD. In the present study, therefore, we examined the serum levels of BDNF in both children with drug-naive ADHD and normal controls. We then explored whether there are any correlations of serum BDNF levels with clinical subtypes and comorbid conditions of ADHD.

In this study, we investigated the differences in serum BDNF levels of untreated children with ADHD diagnosis and healthy controls as well as whether there was a correlation between the serum BDNF levels and severity of ADHD symptoms.

Methods

Participants

30 children with ADHD and 31 healthy controls were recruited. The group of children diagnosed with ADHD was chosen from the children referred to the Child and Adolescent Psychiatry Department at Dokuz Eylul University, School of Medicine. The cases consisted of children between 6 and 12 years with no former history of psychiatric medication and medical illnesses. Children with history of seizures, epilepsy, progressive neurological illnesses, pervasive developmental disorders, substance abuse disorders, psychotic disorders, mood disorders, mental retardation (IQ below 70) and a history of severe head trauma (within the last 1 year) were excluded. Thirty-one healthy children, living in the same area with similar demographic characteristics (age, gender, mother's age, mother's education, and number of siblings) 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 Wechsler Intelligence Scale for Children-Revised (WISC-R) was applied to the control group and children with IQ below 70 were excluded. In addition, children with chronic medical and neurological illnesses were excluded. The study protocol was approved by the institutional ethics committee of Dokuz Eylul University and all parents gave their informed consents for their children to participate in the study and children gave assent.

Measurements

For all children diagnosed with ADHD, psychiatric diagnoses were made by an experienced child and adolescent psychiatrists using the Kiddies Schedule for Affective Disorders and Schizophrenia for School-Age Children-Present and Lifetime Version (K-SADS- PL) (22) that was prepared according to the DSM-IV TR

criteria (23). Besides, parents were asked to fill out the Child Behavior Checklist (CBCL) for 4-18 yrs (24) and the ADHD Rating Scale-IV (25). Teachers were asked to complete the Conners' Teacher Rating Scale (26). The symptom severity of ADHD was measured by the Clinical Global Impression (CGI)-ADHD-severity scale.

Collecting Blood Samples and BDNF Measurement Method

For the measurement of BDNF, blood samples (10 mL) were collected from the children at morning hours between 09.00 and 10.00 a.m. after at least 12 hours of fasting. The samples were collected in tubes without anticoagulants and were immediately sent to the laboratory where they stayed at room temperature for 30 minutes. Then, the samples were centrifuged at 3000 g for 10 minutes and the serums collected were pipetted to the Eppendorf tubes with 2-3 layers. The samples were stored in the deep freezer at -85 °C until the day of analysis.

For the measurement of BDNF, commercial sandwich ELISA kit was used (Millipore, Chemikine, and CYT306). The study was conducted after preliminary studies to determine appropriate dilution percentages and according to the manufacturer guidelines. After the serum samples were diluted by 1/7-1/10, the diluted sample (100mL) was pipetted to 96- spaced microplates covered with monoclonal antibodies against human BDNF protein produced in rats. The plates were incubated at 4°C for 17 hours. After incubation, all factors in the serum that were not bound were removed by four subsequent wash-outs. The wash-outs were carried out by automatic equipment. Then, biotin-bound BDNF antibodies (100 mL) were added to the spaces, incubated at room temperature for 2 hours and washed four times. Then, 100 mL horseradish-peroxidase (HRP) enzyme conjugated with streptavidine was added to the spaces. After an hour of incubation, the washing procedure was repeated as defined above. After this process, 100 mL peroxidase substrate tetramethyl benzidin (TMB) was added to each space, was kept at room temperature for 15 minutes and then, 100 mL HCL solution was added to each space to stop the reaction, and the absorbance of the reaction was read at 450 nm. By using recombinant BDNF standards for the samples in the same way, a calibration graphic was constructed and the BDNF values in the samples were defined quantitatively.

Table 1. Clinical features of children diagnosed with ADHD

	CASE GROUP (n=31)	
	n	(%)
ADHD SUBTYPE		
Attention deficit predominant type	4	12.9
Hyperactivity predominant type	5	16.1
Combined type	22	71.0
COMORBIDITY		
Yes	21	67.7
No	10	33.3
CLINICAL GLOBAL IMPRESSION(CGI)ADHD-SEVERITY SCALE		
Medium severity	5	16.1
Definite severity	14	45.2
Severe	12	38.7
Much severe	0	0
	mean±SD	range
DU PAUL'S ADHD RATING SCALE		
Mothers	27.29±7.11	(9-39)
Fathers	23.10±8.93	(1-41)
Teachers	26.32±8.18	(9-42)
CONNERS' TEACHER RATING SCALE		
Conduct	30.87±8.81	(13-46)
Inattentive and Passive	33.48±8.51	(16-51)
Hyperactivity	32.42±8.78	(15-48)
10-Item Hyperactivity	27.13±8.75	(12-48)

Table 2. The distribution of CBCL scores of case and control groups

	CASE GROUP (n=31) mean±SD	CONTROL GROUP (n=30) mean±SD	p
CBCL subtests			
Withdrawn	63.68±11.76	53.17±4.19	0.000
Somatic complaints	64.32±13.13	52.80±4.44	0.000
Anxious/depressed	68.84±8.93	56.67±5.62	0.000
Social problems	66.23±11.40	56.20±6.36	0.000
Thought problems	69.29±13.44	62.00±8.46	0.014
Attention problems	71.90±10.18	53.03±3.88	0.000
Delinquent behavior	67.90±12.69	59.67±7.24	0.003
Aggressive behavior	68.23±10.86	51.17±2.48	0.000
Internalizing problems	68.48±10.97	52.60±7.87	0.000
Externalizing problems	68.39±10.67	49.70±6.99	0.000
Total problems	72.52±11.00	54.07±8.00	0.000

Statistics

Statistical evaluations were performed using SPSS version 11.0. For the comparison of categorical variables, chi-square test and the Fisher's exact chi-square test, when needed, were used. Continuous variables were compared by using independent sample t-test as well as the Mann-Whitney U test. Continuous variables were defined as mean±standard deviation, while categorical variables were identified as percentages. To test whether there was a difference in BDNF levels between the ADHD subtypes (a triple variable), the Kruskal-Wallis analysis was performed. The WISC-R scores, CBCL scores, the class children were attending, age, number of children in the family in the ADHD and control groups and their correlations with BDNF levels were determined by the Pearson's correlation test. With covariance analysis, the difference between sexes was prevented from being a confounding factor. Statistical significance was determined at $p < 0.05$.

Results

The study group consisted of 31 children diagnosed with ADHD and 30 healthy controls between ages 6 and12 yrs. The mean age of the ADHD cases recruited was 8.45±1.57 and of healthy controls - 8.87±1.92 years. No statistically significant difference was found between the ADHD and control groups regarding age ($p>0.05$). 26 of the ADHD cases (83.9%) were male and 5 (16.1%) were female. 11 of the healthy controls (36.7%) were male and 19 (63.3%) were female.

The statistically significant difference was found between children diagnosed with ADHD and control groups regarding gender ($p=0.000$). The average education of mothers ($p=0.632$), average

education of fathers (p=0.505), marital status of the mothers (p=0.354), family income (p=0.418), and the number of siblings (p=0.178) were similar in both groups.

The mean WISC-R verbal scores of the ADHD cases and healthy controls were 93.10±12.59 and 95.00±9.87, respectively. The mean WISC-R performance scores of the ADHD cases and healthy controls were 100.97±18.09 and 96.43±11.54, respectively. The mean WISC-R total scores of the ADHD cases and healthy controls were 96.45±14.40 and 95.00±10.03, respectively. No statistically significant differences were found between the ADHD and control groups regarding the mean WISC-R verbal, performance and total scores (p>0.05).

The distributions of ADHD subtypes, comorbidity and the CGI-ADHD-severity scale, Du Paul's ADHD Rating Scales and the Conners' Teacher Rating Scale are shown in Table 1.

The average subscale scores of the CBCL applied to the case and control groups are presented in Table 2. Overall, average subscale scores were found to be statistically higher in the patient group compared to the control group (p<0.05).

In the present study, the types of ADHD were found to be 12.9% cases with attention deficit predominant type, 16.1% - hyperactivity predominant type and 71.0% with combined type. 67.7% of the cases had one or more comorbidities. Since mood disorders, psychotic disorders and mental retardation were exclusion criteria in our ADHD cases, common comorbid diagnoses were oppositional defiant disorder (ODD), anxiety disorders (separation anxiety disorder, specific phobia, social anxiety disorder), enuresis nocturna and tic disorders, in order of frequency.

Serum BDNF Levels

No statistically significant difference was found between the BDNF levels of ADHD cases and the control group (t-test, p =0.885), (Table 3).

The ADHD cases and control group were statistically similar regarding age but different by gender. With covariance analysis, the difference between sexes was prevented from being a confounding factor. When matched for gender, ANCOVA showed no significant difference the between groups.

Regarding gender analysis in control and case groups, the Mann-Whitney U test showed no significant difference between males and females for BDNF levels (Table 3). Besides, within both groups, no significant difference was found between sexes (Table 4).

The correlation between clinical features of ADHD cases and BDNF levels is shown in Table 5.

In ADHD cases, no statistically significant association was found between serum BDNF levels and ADHD subtypes (p<0.093). However, although there was no statistically significant difference, serum BDNF levels were found to be lower in children with attention deficit predominant ADHD compared to children with combined type ADHD. No statistically significant difference was found within the ADHD group when serum BDNF levels were compared regarding comorbid diagnoses (p<0.300).

Correlations

As for the correlation of age and WISC-R scores in ADHD and control groups with serum BDNF levels, no statistically significant difference was found between the two groups (p>0.05).

No significant relation was found between serum BDNF levels in the case and control groups and CBCL subscore points (p<0.05). The CBCL scale scores in case and control groups and its correlation with serum BDNF levels are presented in Table 6.

Table 3. The comparison of levels of serum BDNF in children diagnosed with ADHD and control group

	CASE GROUP		CONTROL GROUP		p
	n	mean±SD	n	mean±SD	
BDNF	31	2124.45±1044.31	30	2157.63±694.94	0.885*
MALE	26	2233.40±1060.23	11	2196.40±632.76	0.855**
FEMALE	5	1557.90±821.91	19	2135.19±744.44	0.145**

*T-test was used
** The Mann-Whitney U test was used

Table 4. Comparison of serum BDNF levels in ADHD cases and control groups according to gender

	BDNF levels mean±SD	p
CASE GROUP		
Male (n=26)	2233.40±1060.23	0.179
Female (n=5)	1557.90±821.91	
CONTROL GROUP		
Male(n=11)	2196.40±632.76	0.983
Female(n=19)	2135.19±744.44	

The Mann-Whitney U test was used

Table 5. The comparison of serum BDNF levels among children diagnosed with ADHD; ADHD subtypes and the presence of comorbidity

	BDNF levels mean±SD	p
ADHD SUBTYPE		
Attention deficit predominant type (n=4)	1222.45±955.39	0.093*
Hyperactivity predominant type (n=5)	1768.22±633.44	
Combined type (n=22)	2369.41±1050.29	
COMORBIDITY		
Yes (n=21)	2271.62±1153.50	0.300**
No (n=10)	1815.37±722.35	

*The Kruskal-Wallis test was used.
** The Mann-Whitney U test was used

Table 6. The correlation between the CBCL scale scores and serum BDNF levels in case and control groups

	Correlation with BDNF			
	Case(n=31)		Control (n=30)	
	r	p	r	p
CBCL subtests				
Withdrawn	-0.094	0.614	-0.038	0.842
Somatic complaints	-0.064	0.733	-0.051	0.787
Anxious/depressed	0.012	0.948	0.236	0.210
Social problems	0.078	0.675	0.125	0.510
Thought problems	0.045	0.810	0.191	0.311
Attention problems	0.053	0.779	0.105	0.581
Delinquent behavior	0.063	0.735	0.251	0.180
Aggressive behavior	-0.009	0.961	0.028	0.883
Internalizing problems	-0.080	0.668	0.146	0.443
Externalizing problems	0.027	0.886	0.197	0.296
Total problems	0.035	0.852	0.217	0.249

No statistically significant relationship was found between impairment severity (CGI-ADHD-severity scale), Du Paul's and Conners' scale scores in ADHD cases and serum BDNF levels ($p<0.05$). The impairment severity in ADHD cases (CGI-ADHD-severity scale), Du Paul's and Conners' scale scores and their correlations with BDNF levels are presented in Table 7.

Discussion

In the present study, no statistically significant difference was found between children diagnosed with ADHD and control group for BDNF levels. Regarding the analysis of gender and BDNF levels in both groups, no statistically significant difference was found when males and females were compared. Also, no significant difference was found within the two groups regarding gender.

As far as we know, the present study is the first study to investigate serum BDNF levels in peripheral blood in children diagnosed with ADHD and having no history of former psychiatric medication. The only study that compared ADHD cases and healthy controls regarding BDNF levels was conducted by Shim et al. The authors found that plasma BDNF levels were significantly higher in the ADHD cases compared to controls (18). In the study conducted by Shim et al., the groups consisted of 41 drug naive ADHD patients and 107 normal controls within the age range of 6 and 14 years (18). The groups were age-matched, similar to the present study, but it was stated that there was a statistically significant difference regarding gender distribution, as observed in the present study. When the groups were age- and gender-standardized , the covariance analysis showed that the mean plasma BDNF levels were significantly higher in the children diagnosed with ADHD (840.5±53.5 pg/mL) when compared to healthy controls (575.9±32.2 pg/mL) (18). Additionally, similar to the present study, no significant difference in plasma BDNF levels was found between the genders within both ADHD and control groups (18).

In the present study, no significant relation was observed between serum BDNF levels in the patient and control groups and CBCL subscore points, and no statistically significant relationship was found between impairment severity (CGI-ADHD-severity scale), Du Paul's and Conners' scale scores in ADHD cases and serum BDNF levels. However, Shim et al. found that there was a significant positive correlation between attention deficit symptom severity and plasma BDNF levels. The results of the present study do not support these results.

BDNF is the most common neurotrophic factors and is found in the central and peripheral nervous systems in high quantities. It has been shown that BDNF may pass the blood-brain barrier bilaterally (21,27) and that there is a significant correlation between serum and cortical BDNF levels in mice (28). There are studies proposing that serum BDNF levels are 100 times (29) or 200 (19) times higher than the plasma ones. The majority of serum BDNF originates from thrombocytes. It has been shown that BDNF is not produced in human pre-megacaryocyte cells and that the thrombocytes store BDNF from external resources (20). The potential resources of BDNF are vascular endothelial and smooth muscle cells (30,31,32). Other potential resources are activated macrophages or lymphocytes (33,34,35). Since BDNF passes the blood-brain barrier bilaterally, it has been hypothesized that an important portion of its serum levels originates from neurons and glial cells (21,28). Therefore, the BDNF stored in thrombocytes possibly originates from brain cells. When taking into consideration the grand differences between serum, thrombocyte and plasma BDNF levels and that the serum BDNF levels are affected, while the plasma ones are less affected by the BDNF stored in the thrombocytes, it may be concluded that plasma BDNF reflects current BDNF levels from central BDNF levels and that serum BDNF levels do not. From this point of view, the presence of no difference between ADHD and control groups in the present study assessing serum levels and the significant difference between case and control groups in the study of Shim et al. that measured plasma levels may indicate that serum BDNF is a non-specific peripheral marker, while plasma BDNF level is a marker in the pathogenesis of the illness (18). On the other hand, bearing in mind that BDNF passes the blood-brain barrier bilaterally (21,27), there was a significant correlation between serum and cortical BDNF levels in mice (28) and that thrombocyte cells have a half life of 11 days, so that the effect size would be small, it may be concluded that serum BDNF might reflect the central BDNF. From this point of view, our study does not support the hypothesis that BDNF plays a role in the etiology of ADHD. When taking into consideration that it has not been yet enlightened by which means the measurement of BDNF (complete blood, serum, plasma, and thrombocytes) is the accurate and most reliable procedure, one of the main limitations of this study is that the BDNF levels were studied only in the serum.

In the present study, we observed no statistically significant correlation between serum BDNF levels and ADHD subtypes. However, although there was no statistically significant difference, serum BDNF levels were found to be lower in children diagnosed with attention deficit predominant type ADHD when compared to children diagnosed with combined type ADHD. Tsai proposed a speculative hypothesis that decreased BDNF activity in the midbrain region may play a role in the therapeutic action and pathogenesis of ADHD (10). The result of the present study could support the hypothesis.

BDNF also seems to be involved in the pathophysiology of depression (36) and has been recognized as a mediator of the antidepressant drug response (37). Because of these data, we excluded the affective disorders in the present study. In addition, no statistically significant difference was found within the ADHD group when serum BDNF levels were compared regarding comorbid diagnoses.

In a study evaluating the age-related changes in serum BDNF levels in healthy individuals, it was found that serum BDNF levels within first 10 years were same as in 30-39 yrs which is accepted as the adult level. However, the BDNF levels significantly started to decrease within 10-19 yrs. No significant difference was found in serum BDNF levels regarding gender within any age range (38). In an adult study, effects of age, gender and weight on serum, thrombocyte and plasma BDNF levels were evaluated in 140 healthy adults within age range 20-60 yrs. The authors observed that plasma BDNF decreased as age or weight increased. In Lommatzsch et al. (1999) study, the female subjects had lower thrombocyte BDNF levels compared to male subjects. However, no difference was detected in plasma BDNF levels between the genders (31). Within this context, to reduce the confounding

Table 7. The impairment severity in ADHD cases (CGI-ADHD-severity scale), Du Paul's and Conners' Scale Scores and their correlation with BDNF levels

	Correlation with BDNF	
	r	p
CLINIC GLOBAL IMPRESSION (CGI) -ADHD severity	0.250	0.174
Du Paul mother score (n=31)	0.236	0.200
Du Paul father score (n=31)	0.227	0.228
Du Paul teacher score (n=31)	0.072	0.700
Conners' behavior score (n=31)	0.303	0.098
Conners' hyperactivity score (n=31)	0.163	0.381
Conners' attention deficiency score(n=31)	0.254	0.168
Conners' hyperactivity index(n=31)	0.243	0.187

effect of different age and developmental phases on neurotrophins, all children recruited in our study were chosen from preadolescence period forming a narrow age range. A prior study has shown that BDNF levels decrease with body weight (31). As for this reason, another limitation of the study is that the body weights of the children recruited were not measured.

Because higher serum BDNF levels were shown to exist in patients with mental retardation (39), WISC-R was applied to ADHD and control groups and no significant difference regarding intelligence quotations was found when the groups were compared.

In preclinical studies, it was shown that exercise stimulates neurogenesis and increases brain BDNF levels. In another study conducted on healthy individuals, it was found that physical activity increased BDNF levels (40). When these findings are taken into account, another limitation of this study is that the exercises were not standardized.

In conclusion, our results indicate no change of serum BDNF levels in drug-naive children with ADHD. Further large studies are required to investigate the serum BDNF levels in children with ADHD. The accurate interpretation of BDNF levels in the blood, the factors affecting them and the reliable measurement source of the peripheral blood BDNF and its method need to be enlightened.

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