CrossMark

ORIGINAL CONTRIBUTION

Serum brain-derived neurotrophic factor levels in treatment-naïve boys with attention-deficit/hyperactivity disorder treated with methylphenidate: an 8-week, observational pretest–posttest study

Aynur Pekcanlar Akay[1] · Halil Resmi[2] · Sevay Alsen Güney[1] ·
Handan Özek Erkuran[3] · Gonca Özyurt[4] · Enis Sargin[5] · Ahmet Topuzoglu[6] ·
Ali Evren Tufan[7]

Abstract Brain-derived neurotrophic factor (BDNF) is an important neurotrophin in the brain that modulates dopaminergic neurons. In this study, we aimed to investigate the changes in serum BDNF levels of children with attention-deficit/hyperactivity disorder (ADHD) in response to OROS methylphenidate treatment. We also aimed to determine whether there were any pre–post-differences between ADHD subtypes and comorbid psychiatric disorders in serum BDNF levels. Fifty male children with ADHD and 50 male healthy controls within the age range of 6–12 years were recruited to the study. The psychiatric diagnoses were determined by applying a structured interview with Kiddie schedule for affective disorders and schizophrenia for school-age children—present and lifetime version. The symptom severity of ADHD was measured using the Clinical Global Impression ADHD Severity Scale (CGI-S).

Physicians completed Du Paul ADHD questionnaires. The levels of serum BDNF were assessed before and after 8 weeks of treatment with effective dosages of OROS methylphenidate. In the present study, the mean serum BDNF levels of boys with ADHD and of the healthy controls were 2626.33 ± 1528.05 and 2989.11 ± 1420.08 pg/mL, respectively. Although there were no statistically significant difference between the ADHD group and healthy controls at baseline ($p = 0.22$), the increase of serum BDNF was statistically significant from baseline to endpoint in the ADHD group ($p = 0.04$). The mean serum BDNF levels at baseline and endpoint of the ADHD group were 2626.33 ± 1528.05 and 3255.80 ± 1908.79 pg/mL, respectively. The serum BDNF levels of ADHD-inattentive subtype were significantly lower at baseline ($p = 0.02$), whereas BDNF levels post-treatment showed no significant difference. The increase of serum BDNF levels with methylphenidate treatment after 8 weeks was significantly higher in the inattentive group ($p = 0.005$). The increase of serum BDNF levels with methylphenidate treatment after 8 weeks in boys with ADHD may support the potential role of BDNF in the pathophysiology of ADHD. The role of BDNF in ADHD subtypes in particular should be evaluated with further, larger studies.

✉ Gonca Özyurt
  goncaenginozyurt@gmail.com

[1] Department of Child and Adolescent Psychiatry, Dokuz Eylul University Medical Faculty, Izmir, Turkey

[2] Department of Biochemistry, Dokuz Eylul University Medical Faculty, Izmir, Turkey

[3] İzmir Dr. BehcetUz Training and Research Hospital for Pediatric Disorders and Surgery, Izmir, Turkey

[4] Department of Child and Adolescent Psychiatry, Katip Çelebi University Medical Faculty, Izmir, Turkey

[5] İzmir Tepecik Training and Research Hospital, Izmir, Turkey

[6] Department of Public Health, Kurtalan State Hospital, Siirt, Turkey

[7] Department of Child and Adolescent Psychiatry, Abant Izzet Baysal University Medical Faculty, Bolu, Turkey

⚫ Springer

Introduction

Attention-deficit/hyperactivity disorder (ADHD) is one of the most important neuro-developmental disorders, characterized by age-inappropriate and impairing symptoms of inattention, hyperactivity and impulsivity, and having a prevalence of 5% worldwide [1]. Although catecholaminergic neuro-transmission, especially involving dopamine and noradrenaline, was previously thought mostly responsible for its pathogenesis, the current consensus is that of a more complex disorder arising from an interaction of bio-psycho-social factors [2–4]. The clinical heterogeneity of patients with ADHD, both in terms of symptoms and in treatment responsiveness, the changes observed in neuro-anatomic structures across development, and the protracted time course for treatment response, especially with noradrenergic agents, argues for a more complex pathophysiology [5, 6].

Neurotrophins are a family of proteins involved in neuronal growth, differentiation, maturation, and survival, and include brain-derived neurotrophic factor (BDNF), nerve growth factor (NGF), and Neurotrophin-3 and 4/5 (NT3 and NT4/5) among others. They are increasingly being linked to the etiology of various psychopathologies including ADHD [4, 7, 8]. BDNF is widely expressed in the brain. It helps in survival and differentiation of the dopaminergic neurons in the midbrain, protects neurons against neurotoxicity, modulates dopamine release, and prevents spontaneous death of rat mesencephalic dopaminergic neurons in culture [9, 10]. As a consequence of its relationships with dopaminergic innervation, reduced BDNF activity was suggested among the etiologies for ADHD [11].

It was previously shown that psycho-stimulant-induced dopamine release correlated with that of BDNF, and treatment-naïve children with ADHD had elevated levels of BDNF, whereas adults with ADHD had reduced levels. Polymorphisms in BDNF interacted with socio-economic status to change inattentive symptoms in ADHD families; the valine allele of the BDNF polymorphism was reported to transmit preferentially from fathers in families with ADHD and the same allele correlated with clinical response to treatment with OROS methylphenidate (MPH) in Korean children with ADHD [4, 12–17]. However, other studies failed to find significant differences in BDNF levels of children with ADHD and matched controls [18–20].

The effects of treatment on BDNF levels of patients with ADHD are also not clear. Amiri et al. [21] reported that 6 weeks of MPH treatment increased BDNF levels significantly, yet Sahin et al. reported that 8 weeks of treatment significantly lowered BDNF levels in children with ADHD

[22]. It was shown recently that 3 months of atomoxetine treatment had no significant effect on BDNF levels in adult patients with ADHD [23]. As a result, it can be said that further studies focusing on BDNF levels in ADHD and their response to treatment are needed.

Consequently, this study aimed to evaluate the pre–post-difference of BDNF levels after 8 weeks of treatment with OROS-MPH in ADHD. We also aimed to determine whether there were any pre–post-differences between ADHD subtypes in serum BDNF levels. In addition, the differences in serum BDNF levels of untreated boys diagnosed with ADHD and healthy controls were investigated at the beginning of study.

Materials and methods

Participants

Boys aged 6–12 years who attended the Department of Child and Adolescent Psychiatry at DokuzEylul University in Izmir (Turkey) between 2009 and 2012 and were diagnosed as having ADHD in accordance with the Diagnostic and Statistical Manual of the American Psychiatric Association (DSM-IV-R) criteria using a Turkish version of the Kiddie Schedule semi-structured clinical interview were enrolled in the present study. Twenty-five of the patients and 12 of the healthy controls' first data were derived from a preliminary BDNF case–control study conducted by Sargın et al. from our team, published in Archives of Neuropsychiatry in 2012 [18]. The patient and healthy control groups comprising 50 each were assigned to the groups, all of whom were male. The controls were healthy boys matched by age. Controls had BDNF levels obtained once at baseline and parents of controls completed a clinical interview and the K-SADS-PL and CBCL. Potential controls with evidence of ADHD or comorbid psychiatric conditions were excluded (Table 1).

To check for mental retardation, each boy's performance on the Wechsler Intelligence Scale for Children-Revised (WISC-R) was tested [24, 25].

Patients with past psychiatric treatment, chronic medical conditions (e.g., history of seizures, progressive/static neurologic illnesses), history of severe head trauma within the last year (resulting in loss of consciousness for any duration), pervasive developmental disorders, substance use disorders, psychotic, anxiety and mood disorders, or intelligence quotient (IQ) <70 were excluded. We only included oppositional defiant disorder (ODD) as comorbidity with ADHD. Four participants dropped out because their drug treatment adherence was inappropriate (Table 2).

All parents had to give consent before any study procedure was applied to the patient. The final sample's (boys with ADHD and healthy controls) mean age was 8.8 years.

Table 1 Age, IQ and CBCL Rating scales at baseline in ADHD group and healthy controls

	ADHD group	Healthy controls	p*
Age (Mean ± SD)	8.8 ± 1.5 years	8.8 ± 1.1 years	0.999
IQ (Mean ± SD)			
Verbal	91.6 ± 11.97	96.26 ± 13.22	0.68
Performance	99.48 ± 16.99	99.22 ± 12.42	0.93
Total	95.10 ± 13.82	97.62 ± 12.50	0.34
CBCL (Mean ± SD)			
CBCL withdrawal	63.52 ± 12.40	53.58 ± 5.16	**<0.001**
CBCL social problem	67.94 ± 8.11	55.30 ± 5.47	**<0.001**
CBCL anxiety/ depression	64.90 ± 10.93	55.10 ± 4.84	**<0.001**
CBCL thoughts problem	66.44 ± 12.26	60.660 ± 7.14	**0.005**
CBCL attention problem	71.22 ± 9.19	52.64 ± 3.45	**<0.001**
CBCL delinquent behavior	64.84 ± 12.10	57.28 ± 5.61	**<0.001**
CBCL aggression	66.08 ± 10.61	50.30 ± 0.91	**<0.001**
CBCL total internalizing score	66.00 ± 10.47	47.40 ± 4.28	**<0.001**
CBCL total externalizing score	67.54 ± 10.10	51.22 ± 8.59	**<0.001**
CBCL total problem	70.60 ± 10.42	52.58 ± 8.52	**<0.001**

*p values determined by independent samples t test was used. Bold values indicate statistically significant results. *ADHD* attention deficit hyperactivity disorder, *IQ* intelligence quotient, *CBCL* the child behavior checklist

Baseline neurologic/physical examinations on all cases were conducted in Departments of Pediatrics to achieve consensus. International criteria of the World Health Organization (WHO) were used to calculate body mass index (BMI) percentiles. BMI of all subjects were within normal range for their age (http://www.who.int/growthref/who2007_bmi_for_age/en/, Accessed on 10/25/2014). The research protocol was approved by the DokuzEylul University of Medical Sciences Research Ethics Committee. All of the study procedures were conducted in accordance with the principles listed in the Declaration of Helsinki and local laws and regulations. The study was supported by the DokuzEylul University Scientific Research Project Committee.

Treatment and assessments

Methylphenidate dosage

All patients were treatment naïve. The parents were advised to use OROS methylphenidate daily with no drug holidays at the weekends. Four participants dropped out because their drug treatment adherence was inappropriate.

At each visit, the parents were asked about medication adherence and patients who skipped their doses more than three times during the study period were deemed to be non-adherent (n = 4) and were removed from the analyses. Drug dosages were calculated from prescription refills and

Table 2 Baseline and endpoint variables in boys (aged 6–12 years) with ADHD

	ADHD group
OROS-MPH dosage at the baseline	36 mg/day (46.0%), 27 mg/day (28.0%) 18 mg/day (18.0%) and 54 mg/day (8.0%)
ADHD IA-subtype % (n)	8.0% (4)
ADHD C-subtype % (n)	72.0% (36)
ADHD HI-subtype % (n)	20.0% (10)
ARS baseline (Mean ± SD)	
ARS total score	41.64 ± 6.4
ARS- inattention score	22.3 ± 3.7
ARS-hyperactivity impulsivity score	19.1 ± 4.7
ARS endpoint (Mean ± SD)	
ARS total score	27.3 ± 7.9
ARS- inattention score	14.7 ± 4.3
ARS-hyperactivity impulsivity score	12.5 ± 4.3
CGI-S baseline (Mean ± SD)	5.34 ± 0.8
CGI-S endpoint (Mean ± SD)	3.5 ± 1.1
CGI-I, good responder, %.	3.5 (IQR = 1.3)
	Very much improved, 24.0 % Much improved, 24.0 %

ADHD attention deficit hyperactivity disorder, *OROS-MPH* osmotic controlled release oral delivery system- methylphenidate, *IA* inattentive, *C* combined, *HI* hyperactive/impulsive, *BDNF* brain-derived neurotrophic factor, *ARS* ADHD rating scale, *CGI-S* clinical global impressions-severity

pill count. OROS methylphenidate treatment was naturalistic with the daily dosage individually titrated in accordance with the clinical response on the CGI Scale [26]. The starting dose was 18 mg, titrated up to 54 mg in 4 weeks to yield an average dosage of 1 mg/kg/day. Follow-up visits were conducted at baseline, 1st, 2nd, 3rd, 4th, and at 8th week. Height, weight, blood pressure, adverse effects, and concomitant drug therapies were assessed at each visit and CGI-S [26] and ARS [27] were assessed at baseline and endpoint visits. First and third visits were made by phone calls.

Blood samples were collected at baseline and at week 8 to detect BDNF levels. ADHD symptom severities at these points were assessed by physicians by applying the Du Paul ADHD Rating Scale IV (ARS) (25) and Clinical Global Impression-severity (CGI-S) [26].

Kiddie schedule for affective disorders and schizophrenia for school-age children—present and lifetime version (K-SADS-PL)

K-SADS-PL is a widely used semi-structured diagnostic interview tool [24]. It inquiries about current and past episodes of child and adolescent psychiatric disorders and allows a diagnosis to be made. The Turkish version of the K-SADS-PL was reported to have good test–retest and inter-rater reliability [25]. In the current study, K-SADS-PL was used to make the diagnosis of ADHD along with allowed comorbidities (i.e., enuresis, tic disorders).

Wechsler Intelligence Scale for Children-Revised (WISC-R)

The WISC-R was designed to measure the IQ of children aged between 6 and 16 years [28, 29]. The standardization of the WISC-R for Turkish children was conducted by Savasir and Sahin [28]. The WISC-R along with impaired functioning was used to rule out mental retardation in the present study.

Clinical Global Impression (CGI) Scale

The CGI is a 7-item Likert-type scale that allows physicians to evaluate the severity of the disorder at the time of assessment, relative to the physician's past experience with patients who have the same diagnosis. The scores range from 1 (normal, not at all ill) to 7 (extremely ill). CGI-Improvement (CGI-I) evaluates the effect of treatment from 1 (very much improved) to 7 (very much worse, 28). Treatment response is also evaluated in accordance with CGI in terms of therapeutic efficacy and treatment-related adverse effects [0 (marked improvement and no adverse effects) to 4 (unchanged/worse and adverse effects outweigh the therapeutic effects, 28)]. Global improvement of the patients were evaluated with the physician-rated CGI-I Scale in this study and those with CGI-I scores of 1 (very much improved) or 2 (much improved) were judged as "good responders," and those with scores of 3–7 were judged as "poor responders" as per previous studies [26].

The child behavior checklist (CBCL)

The CBCL is a standardized form that parents fill out to describe their children's behavioral and emotional problems. The version for ages 4–18 years (CBCL/4–18) includes competence items and problems. The problems section consisted of 113 items on a three-point scale. Eight psychopathology scales (withdrawn/depressed, somatic complaints, anxious/depressed, social problems, thought problems, attention problems, rulebreaking behavior, and aggressive behavior) can be evaluated with CBCL [30].

Du Paul ADHD Rating Scale IV (ARS)

This scale is composed of 18 items that tap into symptoms of ADHD listed in the DSM-IV criteria. Each item has a 4-point scale of 0–3. Nine of the items are related to inattention and the rest are related to hyperactivity and impulsivity [24]. This scale was previously used in Turkish studies of ADHD [31].

In the present study, a senior physician (APA) who was blinded to the evaluations and treatments of children with ADHD conducted in the outpatient department completed the questionnaires according to parental reports at baseline and after 2 months of methylphenidate treatment.

Laboratory procedures

For the measurement of BDNF, 10 mL of blood was collected from children between 09:00 and 10:00 a.m. after at least 12-h fasting to avoid circadian variation [32]. Samples were collected in tubes without anticoagulants and were sent to the laboratory immediately to wait in room temperature for 30 min. The blood tubes were then centrifuged at $3000 \times g$ for 10 min and the sera collected were pipetted into Eppendorf tubes. The resulting sera were stored in deep freezer at $-85\ ^\circ C$ until the day of analysis.

A commercial sandwich enzyme-linked immunosorbent assay (ELISA) kit was used to measure BDNF (ChemiKine BDNF Sandwich ELISA CYT306, Merck Millipore, Data Sheet, http://www.emdmillipore.com/, Accessed on 08/06/2014). The study was conducted after preliminary studies to determine appropriate dilution factor according to the manufacturer's guidelines. After the serum samples were diluted by 1/7–1/10, the diluted sample (100 μL) was pipetted into 96-well plates pre-coated with monoclonal antibodies against human BDNF protein produced in mice.

Plates were incubated at 4 °C for 17 h. After incubation, all factors in the serum that were not bound were removed by four respective wash-outs. The wash-outs were performed by automatic equipment. Biotin-bound BDNF antibody (100 μL) was then added to the wells, which was incubated at room temperature for 2 h and then washed four times. Then, 100 μL of horseradish-peroxidase (HRP) enzyme conjugated with streptavidin was added to the wells. After an hour of incubation, the washing procedure was repeated as defined above. After this process, 100 μL peroxidase substrate, 3, 3′, 5, 5′-tetramethylbenzidine (TMB) was added to all wells, the mixture was left at room temperature for 15 min and then 100 μL of HCL solution was added to each well to stop the reaction and the absorbance of the reaction was read at 450 nm. Using recombinant BDNF standards with the samples in the same way, a calibration graphic was constructed and the BDNF values in the samples were defined quantitatively.

Analysis and statistics

Statistical evaluations were performed using SPSS 15.0 program (SPSS Inc., Chicago, IL., USA). When the 50 male subjects with ADHD and 50 male healthy controls' results were compared, power analysis was found as 100% in our study. The Chi-square test and when needed, Fisher's exact Chi-square test were used for the comparison of categorical variables. Continuous variables were compared using the t test. Continuous variables are reported as means (with standard deviations) and categorical variables are identified as percentages. Ordinal variables are reported as median and inter-quartile ranges (IQR). Pairwise comparisons were used to determine the source of differences. Bonferroni adjustment was used for multiple comparisons. Statistical significance was determined at $p < 0.05$. The range of data was investigated and the range was normalized with logarithmic transformation, thus the independent t test was used for determining differences between the case and control groups' serum BDNF levels. Difference of serum BDNF (week 8)—serum BDNF (baseline) was calculated using the paired samples t test. Descriptive statistics were calculated and Box plot was used for graphical presentations. Differences in mean BDNF change between ADHD subtypes were analyzed.

Results

The primary outcome of the present study was the change of plasma BDNF from pretreatment to post-treatment in boys with ADHD. The mean plasma BDNF levels at baseline and endpoints were 2626.33 ± 1528.05 pg/mL and 3255.80 ± 1908.79 pg/mL, respectively, and the increase

of BDNF was statistically significant from baseline to endpoint ($p = 0.04$).

However, there were no statistically significant differences between boys with ADHD and the healthy controls ($p = 0.22$). The mean baseline serum BDNF levels of boys with ADHD and healthy controls were 2626.33 ± 1528.05 and 2989.11 ± 1420.08 pg/mL, respectively.

Considering baseline and endpoint differentiation in serum BDNF levels between ADHD subtypes, there was no statistically significant difference. Even though inattentive subtype had much more increased serum BDNF levels from baseline to endpoint compared with combined subtype ($p = 0.68$), combined and hyperactive/impulsive subtypes did not differ significantly ($p = 0.5$; $p = 0.29$, respectively). ADHD subtypes differed for baseline BDNF levels. Pairwise comparisons showed that baseline BDNF was significantly lower in ADHD-inattentive subtype ($p = 0.02$), whereas combined and hyperactive/impulsive subtypes did not differ significantly. At baseline, the serum BDNF level was 1224.45 ± 955.40 pg/mL in the inattentive group, 2774.29 ± 1281.12 pg/mL in combined subtype ADHD, and 2655.21 ± 2006.39 pg/mL in the hyperactive/impulsive group; after treatment, serum BDNF levels increased to 5053.91 ± 2594.60, 2992.94 ± 1820.41, and 3482.87 ± 1713.14 pg/mL, respectively. The increase of serum BDNF levels with methylphenidate treatment after 8 weeks was significantly higher in the inattentive group ($p = 0.005$) (Tables 3, 4).

The median CGI-S score of the study group at the 8th week was 3.5 (IQR = 1.3). Patients with CGI-I scores of 1 (very much improved, 24.0%) and 2 (much improved, 24%) at the end of the study formed almost one half of the total sample. Overall, 96.0% of the sample responded to treatment with 4.0% remaining unchanged or worsened after 8 weeks of treatment. The reported adverse effects were moderate loss of appetite (7 patients), moderate headache (4 patients), moderate agitation (3 patients), moderate nausea (2 patients), moderate sleep disturbance (2 patients), moderate fatigue (1 patient), and tachycardia (1 patient).

Most of the patients with ADHD-inattentive type ($n = 3$; 75.0%) were judged as "good responders," whereas the rates of "good responders" among combined and hyperactive/impulsive subtypes were 50.0% ($n = 18$) and 30.0% ($n = 3$), respectively. When patients judged to be "good responders" (i.e., CGI-I 1 or 2) were compared with poor responders for post hoc analyses, no significant differences in baseline and endpoint BDNF and total methylphenidate dose received could be found. Post hoc comparisons also revealed that comorbidity had no effect on BDNF levels (Fig. 1).

ARS and CGI assessments revealed no significant differences of mean symptom severity between ADHD diagnostic subtypes according to K-SADS-PL.

Table 3 Comparing baseline and endpoint serum BDNF levels of in boys with ADHD according to ADHD subtypes

	BDNF-Baseline (Mean ± SD)	BDNF-Endpoint (Mean ± SD)	p*
ADHD TOTAL (*n* = 50)	2626.33 ± 1528.05	3255 ± 1908	**0.04**
ADHD-IA (*n* = 4, 8%)	1224.45 ± 955.40	5053.91 ± 2594.60	0.68
ADHD-C (*n* = 36, 72%)	2774.29 ± 1381.12	2992.94 ± 1820.41	0.51
ADHD- HI (*n* = 10, 20%)	2655.21 ± 2006.39	3482.87 ± 1713.14	0.29

Bold values indicate statistically significant results

p values determined by paired samples *t* test

ADHD attention deficit hyperactivity disorder, *IA* inattentive, *C* combined, *HI* hyperactive/impulsive, *BDNF* brain-derived neurotrophic factor

Table 4 Comparing baseline and endpoint serum BDNF levels of in boys with ADHD within ADHD subtypes

	ADHD-IA (*n* = 4)	ADHD-C (*n* = 36)	ADHD-HI (*n* = 10)	p*
BDNF-Baseline	1224.45 ± 955.40	2774.29 ± 1381.12	2655.21 ± 2006.39	**0.02**
BDNF-Endpoint	5053.91 ± 2594.60	2992.94 ± 1820.41	3482.87 ± 1713.14	0.06

Bold values indicate statistically significant results

p values determined by Kruskal–Wallis test

ADHD attention deficit hyperactivity disorder, *IA* inattentive, *C* combined, *HI* hyperactive/impulsive, *BDNF* brain-derived neurotrophic factor
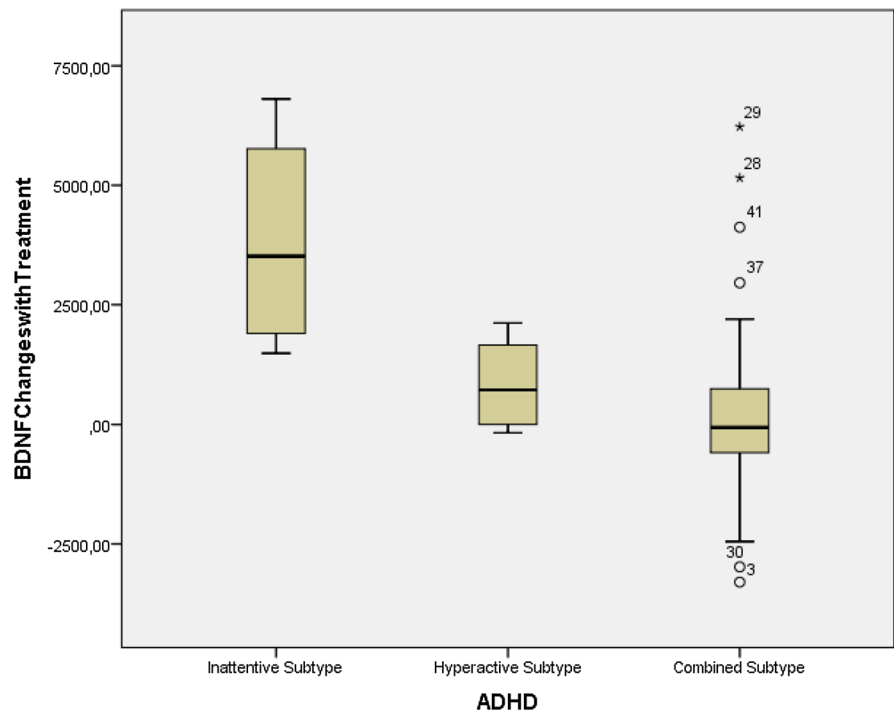
Fig. 1 BDNF changes after 8 weeks of treatment with OROS methylphenidate in boys (aged 6–12 years) with ADHD according to ADHD subtypes

The effect of ODD comorbidity on BDNF was also evaluated. A statistically significant finding could not be obtained.

Discussion

In this 8-week, single-center, observational prospective study, serum BDNF levels in boys ADHD and healthy controls were compared and the effects of OROS methylphenidate treatment on BDNF levels in boys aged 6–12 years were evaluated. Fifty patients and fifty healthy controls were enrolled in the study. Although there was no statistically significant difference between boys with ADHD and healthy controls in terms of baseline serum BDNF levels, after 8-week OROS methylphenidate treatment, we found that there was a statistically significant

increase of serum BDNF levels from pretreatment to post-treatment in boys with ADHD.

Different from results derived from a previous study by Shim et al. [14], there was no significant difference between baseline mean BDNF levels of boys with ADHD and the healthy controls. However, the findings in the present study were in concordance with the pretreatment BDNF results of Sahin et al. and our preliminary data [18, 22]. In the study by Shim et al., plasma BDNF levels were evaluated instead of serum BDNF levels different from our study. In addition to this difference, Shim et al. recruited both sexes in their study different from our study group, which contained only boys.

As mentioned above, the primary outcome of the present study was the increase of serum BDNF levels from pretreatment to post-treatment in boys with ADHD. Previous studies on the effects of treatment on BDNF levels of patients with ADHD gave contradictory results [21–23].

In a previous study, Amiri et al. assessed levels of plasma BDNF in 28 children with ADHD aged 3.5–10 years (24 male, 4 female) before and after 6 weeks of treatment with methylphenidate [21]. They reported that post-treatment BDNF levels were significantly higher than pretreatment levels in agreement with our findings. In another study, Sahin and colleagues reported that 8 weeks of treatment in a study group consisting of 30 children and adolescents aged 6–18 years (24 males, 6 females) significantly lowered BDNF levels in children with ADHD [21]. The contrast between those results may be explained by the evaluation methods of BDNF levels. In our study, we evaluated serum BDNF levels. However, in the previous studies BDNF levels were evaluated in plasma.

Tsai suggested that decreased BDNF activity in the midbrain region might play a role in the therapeutic action and pathogenesis of ADHD, especially by causing dopaminergic dysfunction [11]. The increase of serum BDNF levels with methylphenidate treatment after 8 weeks in boys with ADHD could support the hypothesis proposed by Tsai. However, this position should be viewed with caution due to our limited sample size and needs replication.

The study group was mostly formed of ADHD-combined subtype, and more than half of the patients had a comorbid diagnosis. Patients were mostly treated with OROS MPH 27 or 36 mg/day at baseline and received an estimated mean total dose of 887.2 mg of MPH during the study. Almost one half were judged as much or very much improved according to the physicians and 96.0% responded to treatment. ADHD subtypes tended to differ for baseline BDNF levels; in the comparison of combined, hyperactive/impulsive and inattentive subtypes, inattentive subtype differed significantly. Baseline serum BDNF was significantly lower in the inattentive group. The increase

of serum BDNF levels with methylphenidate treatment after 8 weeks was significantly higher in the inattentive group. The difference of serum BDNF levels between the ADHD-inattentive subtype and other subtypes at baseline may support the hypothesis that BDNF levels could affect the severity of inattention symptoms [33].

In genetic studies, some clues were revealed for the relationship of BDNF with different subtypes of ADHD [21]. The findings suggested that changes of plasma BDNF levels could affect the severity of inattention symptoms, which were called 'omission errors'. Shim et al. showed that there was a significant positive correlation between plasma BDNF levels and omission errors [14]. In another study designed by Corominas-Roso et al., BDNF levels in adults were found higher in inattentive subtype serum but there were no statistically significant differences between subgroups [17]. Similarly, in the study by Amiri et al., plasma BDNF levels in the inattentive subtype were higher than in the other subgroups, but not with statistical significance. These inconsistent findings may be the result of using different methodology and/or different age groups. We recommend that more studies with larger sample sizes in children and adolescents should be performed to clarify this issue.

In our findings, there was no significant difference between good and poor responders to treatment in the whole sample for BDNF levels. Comorbidity was found to have no effect on BDNF changes.

It is known that BDNF in serum is higher than plasma levels because circulating platelets also contain BDNF and this is released during clotting [34]. Platelets themselves cannot synthesize BDNF but take it from plasma [34]. The cellular sources of BDNF in plasma are not known but it is thought to be secreted by endothelial, epithelial and muscular cells, macrophages, and leucocytes [35–37]. A study on adult patients who were abstinent alcoholics and adult social drinkers reported that plasma BDNF showed higher within-individual and within-group variation compared with serum BDNF, and some subjects displayed greater daily variation in serum BDNF levels [38]. Incidentally, the same study reported low correlation between plasma and serum BDNF levels, which supports the view that they are separate pools [38].

The stress of fasting and blood drawing on children in our study, differing age ranges of study samples with variable levels of pubertal development [20, 21, 39], inclusion of male patients only [39], and probable clotting [33] may have contributed to our results. Repeated sampling of children during our study may also have helped to determine individual variation in our sample. Accordingly, further studies on changes of BDNF in children with ADHD who are being treated with stimulants would be warranted.

The main limitations of our study are its limited sample size, which precludes further analyses on ADHD subtypes,

lack of female patients, and lack of evaluation of post-pubertal male children. There is one additional limitation. Although comorbid psychiatric conditions were screened with the K-SADS-PL, the significant differences between patients and controls on the CBCL may mean that some patients with ADHD had sub-syndromal psychiatric conditions. Larger and more varied treatment arms with IR MPH and atomoxetine may also have enriched our results. It was also previously shown that BDNF levels could be affected by exercise, stress, anxiety, time of birth, and intra-uterine nicotine exposure [40–45]. The strength of our study would be increased if we had controlled for these variables. Finally, as can be seen in the reports including serum BDNF levels, there is a wide range of distribution. This may cause a limitation in the comparison of BDNF levels among different studies. A quality control system including standardized control material accompanying BDNF ELISA kits from different companies can be proposed. Regardless of those limitations, our results may support the potential role of BDNF in the pathophysiology of ADHD, which should be evaluated with further, larger studies.

Compliance with ethical standards

Conflict of interest Aynur Pekcanlar Akay is an Advisory Board Member of Janssen, Sanofi and Lilly Pharmaceutical Companies. Other authors have no conflicts of interest to declare.

References

1. Polanczyk G, de Lima MS, Horta BL et al (2007) The worldwide prevalence of ADHD: a systematic review and meta-regression analysis. Am J Psychiatry 164:942–948
2. Faraone SV, Perlis RH, Doyle AE et al (2005) Molecular genetics of attention-deficit/hyperactivity disorder. Biol Psychiatry 57:1313–1323
3. Biederman J, Milberger S, Faraone SV et al (1995) Family-environment risk factors for attention deficit hyperactivity disorder: a test of Rutter's indicators of adversity. Arch Gen Psychiatry 52:464–470
4. Lasky-Su J, Faraone SV, Lange C et al (2007) A study of how socioeconomic status moderates the relationship between SNPs encompassing BDNF and ADHD symptom counts in ADHD families. Behav Genet 37:487–497
5. Faraone SV, Biederman J (2004) Neurobiology of attention deficit hyperactivity disorder. In: Charney DS, Nestler EJ (eds) Neurobiology of mental illness, 2nd edn. Oxford University Press, Oxford, pp 979–999
6. Shaw P, Lerch J, Greenstein D et al (2006) Longitudinal mapping of cortical thickness and clinical outcome in children and adolescents with attention-deficit/hyperactivity disorder. Arch Gen Psychiatry 63:540–549
7. Li X, Zhang T, He S et al (2014) Elevated serum levels of FGF-2, NGF and IGF-1 in patients with manic episode of bipolar disorder. Psychiatry Res 218:54–60
8. Sen S, Duman R, Sanacora C (2008) Serum brain-derived neurotrophic factor, depression, and antidepressant medications: meta-analyses and implications. Biol Psychiatry 64:527–532
9. Hyman C, Juhasz M, Jackson C et al (1994) Overlapping and distinct actions of the neurotrophins BDNF, NT-3 and NT-4/5 on cultured dopaminergic and GABAergic neurons of the ventral mesencephalon. J Neurosci 14:335–347
10. Hung HC, Lee EH (1996) The mesolimbic dopaminergic pathway is more resistant than the nigrostriatal dopaminergic pathway to MPTP and MPP toxicity: role of BDNF gene expression. Mol Brain Res 41:14–26
11. Tsai SJ (2007) Attention-deficit hyperactivity disorder may be associated with decreased central brain-derived neurotrophic factor activity: clinical and therapeutic implications. Med Hypotheses 68:896–899
12. Meredith G, Callen S, Scheuer D (2002) Brain-derived neurotrophic factor expression is increased in the rat amygdala, piriform cortex and hypothalamus following repeated amphetamine administration. Brain Res 949:218–227
13. Narita M, Aoki K, Takagi M et al (2003) Implication of brain-derived neurotrophic factor in the release of dopamine and dopamine-related behaviors induced by methamphetamine. Neuroscience 119:767–775
14. Shim SH, Hwangbo Y, Kwon YJ et al (2008) Increased levels of plasma brain-derived neurotrophic factor (BDNF) in children with attention deficit hyperactivity disorder (ADHD). Prog Neuropsychopharmacol Biol Psychiatry 32:1824–1828
15. Kent L, Green E, Hawi Z et al (2005) Association of the paternally transmitted copy of common valine allele of the Val-66Met polymorphism of the brain-derived neurotrophic factor (BDNF) gene with susceptibility to ADHD. Mol Psychiatry 10:939–943
16. Bung- Nyun K, Cummins TDR, Kim J-W et al (2011) Val/Val genotype of BDNF val66Met polymorphism is associated with a better response to OROS-methylphenidate in Korean ADHD children. Int J Neuropsychopharmacol 14:1399–1410
17. Corominas-Roso M, Ramos-Quiroga JA, Ribases M et al (2013) Decreased serum levels of brain-derived neurotrophic factor in adults with attention-deficit hyperactivity disorder. Int J Neuropsychopharmacol 16:1267–1275
18. Sargın E, Akay AP, Resmi H et al (2012) Evaluation of serum brain-derived neurotrophic factor levels in children with attention deficit hyperactivity disorder: preliminary Data. Arch Neuropsychiatry 49:96–101
19. Sayyah H (2009) BDNF plasma level in ADHD children: correlation to different symptomatology. Curr Psychiatry [Egypt] 16:284–294
20. Scassellati C, Zanardini R, Tiberti A et al (2014) Serum brain-derived neurotrophic factor (BDNF) levels in attention deficit-hyperactivity disorder (ADHD). Eur Child Adolesc Psychiatry 23:173–177
21. Amiri A, TorabiParizi G, Kousha M et al (2013) Changes in plasma brain-derived neurotrophic factor (BDNF) levels induced by methylphenidate in children with attention-deficit/hyperactivity disorder (ADHD). Prog Neuropsychopharmacol Biol Psychiatry 47:20–24
22. Sahin S, Yuce M, Alacam H et al (2014) Effect of methlyphenidate treatment on appetite and levels of leptin, ghrelin, adiponectin, and brain-derived neurotrophic factor in children and adolescents with attention deficit and hyperactivity disorder. Int J Psychiatry Clin Pract 18:280–287
23. Ramos-Quiroga JA, Corominas-Roso M, Palomar G et al (2014) Changes in the serum levels of brain-derived neurotrophic factor in adults with attention deficit hyperactivity disorder after treatment with atomoxetine. Psychopharmacology 231:1389–1395

24. Kaufman J, Birmaher B, Brent D et al (1997) Schedule for affective disorders and schizophrenia for school-age children-present and lifetime version (K-SADS-PL): initial reliability and validity data. J Am Acad Child Adolesc Psychiatry 36:980–988

25. Gokler B, Unal F, Pehlivanturk B et al (2004) Reliability and validity of Schedule for affective disorders and schizophrenia for school age children-present and lifetime version-Turkish version (K-SADS-PL-T) (in Turkish). Turkish J Child Adolesc Psychiatry 11:109–116

26. Guy W (1976) ECDEU assessment manual for psychopharmacology-revised (DHEW Publ No ADM 76-338)

27. Du Paul GJ (1991) Parent and teacher rating of ADHD symptoms-psychometric properties in a community-based sample. J Clin Child Psychol 20:245–253

28. Savasir I, Sahin N (1995) Manual for the wechsler intelligence scale for children-revised (WISC-R). Turkish Psychology Association Publication, Ankara (in Turkish)

29. Wechsler D (1974) WISC-R manual for the Wechsler Intelligence Scale for Children-Revised. Psychological Corporation, New York

30. Achenbach TM (1991) Child behavior checklist/4-18. University of Vermont, Psychiatry

31. Pekcanlar A, Turgay A, Miral S, et al. (1999) Family functioning in attention- deficit/hyperactivity disorder. Turk J Child Adolesc Mental Health 6:99–107 (Turkish)

32. Bergliuomini S, Lenzi E, Ninni F et al (2008) Plasma brain derived neurotrophic factor daily variations in men: correlation with cortizol circadian rhythm. J Endocrinol 197:429–435

33. Dovis S, Van der Oord S, Huizenga HM et al (2015) Prevalence and diagnostic validity of motivational impairments and deficits in visuospatial short-term memory and working memory in ADHD subtypes. EurChild Adolesc Psychiatry 24:575–590

34. Fujimura H, Altar CA, Chen R et al (2002) Brain-derived neurotrophic factor is stored in human platelets and released by agonist stimulation. ThrombHaemost 87:728–734

35. Nakahashi T, Fujimura H, Altar CA et al (2000) Vascular endothelial cells synthesize and secrete brain-derived neurotrophic factor. FEBS Lett 470:113–117

36. Chaldakov GN, Tonchev AB, Aloe L (2009) NGF and BDNF: from nerves to adipose tissue, from neurokines to metabokines. RivPsichiatr 44:79–87

37. Linker RA, Gold R, Luhder F (2000) Function of neurotrophic factors beyond the nervous system. Crit Rev Immunol 29:43–68

38. D'Sa C, Dileaone RJ, Anderson GNM, Sinha R (2012) Serum and plasma brain-derived neurotrophic factor (BDNF) in abstinent alcoholics and social drinkers. Alcohol 46:253–259

39. Begliuomini S, Casarosa E, Pluchino N et al (2007) Influence of endogenous and exogenous sex hormones on plasma brain derived neurotrophic factor. Hum Reprod 22:995–1002

40. Yochum C, Doherty-Lyon S, Hoffman C et al (2014) Prenatal cigarette smoke exposure causes hyperactivity and aggressive behavior: role of altered catecholamines and BDNF. ExpNeurol. 254:145–152

41. Jeong HI, Ji ES, Kim SH et al (2014) Treadmill exercise improves spatial learning ability by enhancing brain-derived neurotrophic factor expression in the attention-deficit/hyperactivity disorder rats. J Exerc Rehabil 10:162–167

42. Nowacka MM, Paul-Samojedny M, Bielecka AM et al (2014) Chronic social instability stress enhances vulnerability of BDNF response to LPS in the limbic structures of female rats: a protective role of antidepressants. Neurosci Res 88:74–83

43. Dhobale MV, Pisal HR, Mehendale SS et al (2013) Differential expression of human placental neurotrophic factors in preterm and term deliveries. Int J Dev Neurosci 31:719–723

44. Zegers D, Hendrickx R, Verrijken A et al (2014) Screening for genetic variants in BDNF that contribute to childhood obesity. PediatrObes. 9:36–42

45. Mueller SC, Aouidad A, Gorodetsky E et al (2013) Gray matter volume in adolescent anxiety: an impact of the brain-derived neurotrophic factor Val (66) Met polymorphism? J Am Acad Child Adolesc Psychiatry 52:184–195