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- Title: Increased serum brain-derived neurotrophic factor, glial-derived neurotrophic factor, nerve growth factor and galanin levels in children with attention deficit hyperactivity disorder, and the effect of 10 weeks methylphenidate treatment
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Increased serum brain-derived neurotrophic factor, glial-derived neurotrophic factor, nerve growth factor and galanin levels in children with attention deficit hyperactivity disorder, and the effect of 10 weeks methylphenidate treatment

Abstract

Objective: This study aimed to investigate the levels of serum brain-derived neurotrophic factor (BDNF), nerve growth factor (NGF), glial cell-derived neurotrophic factor (GDNF) and galanin in children with attention deficit hyperactivity disorder (ADHD).

Methods: The study included 58 cases with ADHD and 60 healthy controls. K-SADS-PL together with DSM-5 criteria were used for diagnostic evaluation. Sociodemographic data form and Conners' Parent/Teacher Rating Scale-Revised:Long Form were applied to all cases. The serum levels of BDNF, NGF, GDNF, and galanin were evaluated in all subjects. Afterwards, methylphenidate was started in the ADHD group. ADHD cases were reevaluated in terms of the serum levels of BDNF, NGF, GDNF, galanin at the 10th week of treatment.

Results: Before the treatment, the levels of BDNF, NGF, GDNF, galanin were significantly higher in the ADHD group compared to the control group. The levels of BDNF, NGF, GDNF, galanin were found to be significantly lower after treatment in ADHD group compared to pre-treatment. No correlation was between scale scores and the serum levels of BDNF, NGF, GDNF, galanin.

Conclusion: The levels of neurotrophic factors and galanin were thought to be parameters worth evaluating in ADHD. Further studies on the subject with longer-term treatments and larger sample groups are required.

Keywords: Attention deficit hyperactivity disorder, BDNF, NGF, GDNF, Galanin, Methylphenidate

Introduction

Attention deficit hyperactivity disorder (ADHD) is a neurodevelopmental disorder with multiple etiologies. In its etiology; genetic, neuroanatomical, neurochemical, environmental, and psychosocial factors are considered to play a role [1]. Recently, it has been suggested that neurotrophic factors (neurotrophins), which are thought to play an important role in the regulation of neuronal function, may be associated with the etiopathogenesis of ADHD [2]. Neurotrophins affect various neurotransmitter systems such as dopamine and glutamate. In addition, neurotrophins control the development, function, and survival of neurons in both the central and peripheral nervous systems, and play an important role in synaptic formation and plasticity [3].

Brain-derived neurotrophic factor is produced in the form of precursor pro-BDNF. A 14 kDa mature BDNF is obtained through processes that take place in the intra or extracellular area [4]. It has been reported that BDNF is required for homeostasis of the dopaminergic system [5]. In clinical studies, it has been pointed out that BDNF may be associated with the pathophysiology of mental disorders such as major depressive disorder (MDD) [6], obsessive-compulsive disorder (OCD) [7, 8]. BDNF is the most studied molecule among neurotrophic factors in ADHD in children and adolescents, but its impact is not yet clear. In two previous studies, blood BDNF levels were found significantly higher in ADHD patients than in controls [9, 10]. Other reports did not find any significant differences between the groups [11-18] and in some cases, lower BDNF levels were reported in ADHD subjects [19-21]. The discrepancy between study results may be due to the differences of methodology and the characteristics of the sample groups used in the studies.

NGF weighs approximately 26 kDa and is produced from a 33.8 kDa precursor called pre-pro-NGF. NGF plays an important role in basal forebrain cholinergic neurons involved in learning processes and attention systems. Changes in the levels of NGF can lead to various

neuropsychiatric disorders since it plays a role in maintaining neuronal homeostasis [22]. The studies shows that NGF may be associated with the pathophysiology of psychiatric diseases such as depression [23], schizophrenia [24], OCD [7]. On the other hand, four studies examining circulating NGF levels in children with ADHD were found, all with inconclusive results. Güney et al. found that NGF levels were significantly higher in of ADHD subjects compared to controls [25]. In three studies, it was reported that serum NGF levels did not differ significantly between ADHD and control groups [15, 18, 21].

GDNF is a 32-42 kDa disulfide-linked homodimer, and as a result of proteolysis of the precursor of 211 amino acids called pro-GDNF, mature GDNF of 134 amino acids is formed. GDNF is widely in the brain and it has a neuroprotective effect against neuroinflammatory and oxidative damage and have important roles for the survival and protection of both serotonergic and dopaminergic neurons [26]. It has also been reported to be associated with cognitive functions in animals, including learning and memory [27]. When the literature is searched, there were studies found that GDNF might be associated with psychiatric diseases such as anxiety disorders [28], depression [29], OCD [7]. Four studies were found in the literature that evaluated peripheral GDNF levels in children and adolescents with ADHD. In three of these studies, GDNF levels were found to be higher in children with ADHD compared to the healthy controls [15, 18, 30] and there was no significant difference in the other study [21].

Galanin is a neuropeptide, synthesized as a 123 amino acid pre-propeptide molecule encoded by the GAL gene, which is converted to a mature 30 amino acid peptide in humans. Galanin plays a role in many physiological tasks such as sleep regulation, nociception, awakening, nutrition, cognition, and regulation of blood pressure. In addition, galanin has neuroprotective activity [31]. Existing studies have shown that galanin and its receptors are widely present in the peripheral and central nervous systems. Therefore, galanin is involved in various

pathological and physiological processes in the central nervous system [31]. It was stated that galanin may be a biomarker for various neuropsychiatric disorders. Serum galanin levels are altered in some cases such as depression and autism spectrum disorders and have been correlated with symptoms severity [32, 33]. Murck et al. [34] showed that intravenous administration of galanin had a fast antidepressant effect and affected sleep electroencephalogram. According to results of that study, intravenously administered galanin rapidly crossed the blood-brain barrier and produced various effects on the central nervous system. In addition, there may an association between serum galanin and alcohol addiction [35]. Collectively, the evaluation of peripheral galanin levels may be important in neuropsychiatric disorders such as ADHD. However, no studies have been published evaluating serum galanin levels in children and adolescents with ADHD.

It is seen that the results of existing studies on neurotrophic factors in attention deficit hyperactivity disorder have not been clarified yet, and more studies are needed on this subject. In this study, inconsistencies that were not resolved in the previous studies were evaluated by considering some of limitations stated in those studies, such as comorbidity (except oppositional defiant disorder), clinical features of sample, well age-and sex-matched to a healty control group. In addition, no study in the literature evaluated galanin levels in ADHD cases. By considering all these findings, this study aimed to evaluate serum neurotrophic factors and galanin levels in ADHD and to compare the findings with healthy controls. In addition, the effect of OROS (osmotic release oral system) methylphenidate treatment applied to ADHD cases on BDNF, NGF, GDNF, and galanin levels was also evaluated in our study. It was thought that our study could help to create a more holistic perspective on the subject.

Methods

Study Design and Participants

This study was carried out between October 2018 and January 2020 at the Fırat University Faculty of Medicine Child and Adolescent Psychiatry Outpatient Clinic. The study was approved by Fırat University Ethics Committee (21.06.2018-11/14). All procedures followed the principles listed in the Declaration of Helsinki.

Sixty cases with ADHD and 60 healthy control subjects aged 6-12 years were initially included in the study. The ADHD group consisted of cases who were admitted to the polyclinic with ADHD symptoms and met the diagnostic criteria. Two patients in the ADHD group were excluded from the study, since cardiac side effects developed in these patients during methylphenidate treatment. The control group, on the other hand, was formed from the children of voluntary families, taking into account the age and gender distribution of the ADHD group.

Procedure

Psychiatric Evaluation

Diagnostic assessment was made according to the Schedule for Affective Disorders and Schizophrenia for School Age Children-Present and Lifetime Version-Turkish version (K-SADS-PL) and DSM-5 criteria. Written consent forms were obtained from the parents of all cases before the evaluation. Sociodemographic data of all cases were recorded in the sociodemographic data form. The Conners Parent/Teacher Rating Scale-Revised:Long Form was filled in by the families and teachers of the cases.

In the ADHD group; those who has a comorbid psychiatric diagnosis other than ODD as a result of the K-SADS-PL and DSM-5 diagnostic criteria, those who have used any psychotropic drug in the last 3 months (including stimulants/non-stimulants), those who have used any drugs other than psychotropics in the last month, and those with chronic systemic diseases were excluded from the study. In the control group; those who had a psychiatric

diagnosis as a result of the K-SADS-PL and DSM-5 diagnostic criteria, those who had used any medication in the last month, and those with chronic systemic diseases were excluded from the study. According to the detailed information obtained from the families/teachers and clinical evaluation for all cases, the cases who were thought to show age-appropriate development of neuromotor development and basic adaptive functions (for example, understanding/learning, academic achievement, reasoning, etc.) were included in the study. Afterwards, all cases were evaluated in terms of serum BDNF, NGF, GDNF, and galanin levels. Then, OROS methylphenidate treatment was planned for the cases with ADHD (initial dose 18 mg/day). It was increased to 54 mg/day in 4 weeks, with an average of 1 mg/kg/day. After the drug treatment was started, the cases were evaluated every two weeks. Interviews at week 0, 4, 8 and 10 were made face-to-face in the clinic, and the evaluations in the 2nd and 6th weeks were made via phone. The cases were controlled in terms of severity of disease symptoms, level of benefit from treatment and possible drug side effects. At the tenth week, the cases with ADHD were re-evaluated for serum BDNF, NGF, GDNF, and galanin levels.

Assessment Tools

Sociodemographic Data Form: It was developed by the researchers for the sociodemographic data of the participants. Information about the child's age, gender, developmental stages, school success, medical background information, parents' age/educational status, average monthly income level of the family, and psychiatric and medical disease history among first/second degree relatives was requested.

Schedule for Affective Disorders and Schizophrenia for School Age Children-Present and Lifetime Version (K-SADS-PL): It is a semi-structured interview form used to determine past and present psychopathologies of children and adolescents. It was developed by Kaufman et al. [36]. The validity and reliability study of the Turkish version was made by Gökler et al. [37].

Conners' Parent Rating Scale-Revised:Long Form (CPRS-R:L): It was developed by Conners et al. [38]. It allows parents to evaluate their children's behavior outside of the school environment. The scale, which contains 80 items in total, consists of 14 subscales. Turkish validity and reliability study was performed by Kaner et al. [39].

Conners' Teacher Rating Scale-Revised:Long Form (CTRS-R:L): It was developed by Conners et al. in order to evaluate the situation of the cases in the classroom [40]. It consists of a total of 59 items and has 14 subscales. It is rated by teachers. The validity and reliability study in Turkish population was made by Kaner et al. [41].

Biochemical Analysis

Biochemical analyzes were made in the Biochemistry Laboratory at Firat University Hospital. From all cases, 5 ml of venous blood was drawn after 12 hours of fasting. These samples were centrifuged for 10-20 minutes at 2500-3000 rpm and separated as serum/plasma and stored at -80 C° under appropriate conditions.

Serum neurotrophic factors and galanin levels were measured by ELISA method in ELx50 (washer) and ELx800 (reader) devices using commercial ELISA kits (SinoGeneClon Biotech Co., Ltd, China) in accordance with the manufacturer's instructions. All kits and samples were kept at room temperature before starting the measurements. Standards and chemicals of the kits were prepared and then standard and serum samples were placed in the wells of the plate. Afterwards, the samples were colored according to their concentration by following the steps specified in the manufacturer's prospectus. After the color formation, the absorbance values of the wells were determined with the ELx800 reader device at 450 nanometers (nm) and the concentrations were calculated. Concentrations found are in picograms/milliliter (pg/ml) for BDNF, GDNF and galanin, and nanograms/milliliter (ng/ml) for NGF.

Statistical analysis

The findings were analyzed in 22 package programs of SPSS (Statistical Package for Social Sciences; SPSS Inc., Chicago, IL). Kolmogorov-Smirnov test was used to evaluate whether the non-categorical data were in accordance with the normal distribution. Among the groups; variables with normal distribution were compared with the Independent Samples t-test, and variables not normal distribution were compared with the Mann-Whitney U test. Chi-square analysis (Pearson Chi-square, $\chi 2$ test) was used to compare categorical data between groups; Fisher's exact test was applied if more than 20% of the expected value was less than 5. Correlation analysis of normally distributed variables was performed with the Pearson correlation test, and the correlation analysis of non-normally distributed variables was performed with the Spearman correlation test. Paired Samples t test was used to compare normally distributed variables at two different times, and Wilcoxon test was used to compare non-normally distributed variables at two different times. Statistical significance level is $p<0.05$.

Results

Sociodemographic Data

Sixty cases with ADHD and 60 healthy control subjects were included in the study. Cardiac side effects developed in two patients during methylphenidate treatment, therefore two patients in the ADHD group were excluded from the study. ODD was observed to accompany 25.9% (n=15) of the cases in the ADHD group. The mean age of ADHD and control groups was 10.10±1.60 and 10.07±1.31, respectively (p=0.895). 67.2% (n=39) of the cases in the ADHD group were male, 32.8% (n=19) were female; 68.3% (n=41) of the participants in the control group were male and 31.7% (n=19) were female (p=0.899).

There was no significant difference between the groups in terms of body mass index, parental age, parental education, family type, and living place. Sociodemographic data of the groups are shown in Table 1.

- Table 1 -

According to the predominant appearance, the ADHD group was distributed as 51.7% (n=30) predominantly inattentive type and as 48.3% (n=28) combined type.

Biochemical Data

During the pre-treatment evaluation, serum BDNF, NGF, GDNF, and galanin levels were found to be significantly higher in the ADHD group compared to the control group ($p<0.001$ for all). Pre-treatment data is shown in Table 2.

- Table 2 -

After 10 weeks of OROS methylphenidate treatment in ADHD cases, serum BDNF, NGF, GDNF, and galanin levels were found to be significantly lower than pre-treatment levels ($p=0.019$, $p<0.001$, $p=0.002$, $p<0.001$, respectively). Post-treatment data are shown in Table 3.

- Table 3 -

In cases with ADHD, no significant correlation was found between the CPRS-R:L and CTRS-R:L scores and pre-treatment blood parameters. Correlation analyzes are shown in Table 4 and Table 5.

- Table 4 -

- Table 5 -

When the blood parameters in the ADHD group were evaluated according to the predominant appearance; BDNF, NGF, GDNF, and galanin levels did not differ significantly between the groups before or after treatment (Table 6-7).

- Table 6 -

- Table 7 -

Discussion

Pre-treatment Evaluation

Our study found the serum BDNF, NGF, and GDNF levels in the ADHD group before treatment to be significantly higher compared to the control group. Although BDNF is the most studied neurotrophin in ADHD, the probable role of neurotrophins in the etiopathogenesis of ADHD is still not clear. Studies reveal the important effects of BDNF on the survival and nutrition of the dopaminergic neurons during brain development [42]. The necessity of BDNF for the homeostasis of the dopaminergic system has been asserted [5]. Similarly, studies divulge GDNF as also being a protector for dopaminergic neurons and reversing the toxin-induced damage of midbrain dopaminergic neurons under in vivo conditions [43]. Studies put forth the necessity of NGF for the functional integrity of cholinergic neurons in the central nervous system [44]. Prefrontal cholinergic inputs are very important in attention processes. There may be reason to believe that the neurotrophin elevation we detected in ADHD cases in our study may be the result of a compensatory mechanism working to compensate the dopaminergic or cholinergic dysfunction.

Neurotrophins may also be associated with ADHD through the glutamatergic system. Studies reveal that BDNF synthesis in the cerebral cortex is regulated by neuronal activity and that glutamate stimulates BDNF expression [45]. With the activation of glial metabotropic glutamate receptors, the formation of various neurotrophic factors such as BDNF, NGF and GDNF are promoted [46]. Literature documents studies pointing to increased glutamate signals in various parts of the brain in cases with ADHD [47]. The probable glutamatergic changes reported in ADHD may be affecting the neurotrophin levels. Further studies are needed to determine whether or not a cause-effect relationship exists.

Another system pointing to the possible relationship of neurotrophins with ADHD is the hypothalamo-pituitary-adrenal (HPA) axis and the glucocorticoid system. Glucocorticoids

may be involved in the regulation of BDNF expression and signaling. A study revealed decreasing BDNF mRNA levels in all hippocampal regions after giving dexamethasone [48]. As far as can be discerned from the literature, although studies on the subject mostly focus on BDNF, there are also results indicating that there may be a relationship between NGF and GDNF and the HPA axis [49]. There are reports indicating changes related to the HPA axis in ADHD as well. In a meta-analysis, salivary cortisol levels were found to be lower in ADHD cases compared to those in the control groups [50]. The elevation of BDNF, NGF, and GDNF found in ADHD cases in our study may also be associated with possible HPA axis changes.

A possible relationship between ADHD and neurotrophins has also been shown in various animal studies. It is observed that studies on the subject mostly focus on BDNF. In some animal models associated with BDNF, impairments in hippocampus-dependent learning have been reported, similar to academic dysfunctions seen in ADHD patients [51]. A study determined that in rats with a damaged DAT gene, (which is thought to be important in the etiology of ADHD), there were changes in locomotor activity, deficit in working memory, and dysregulation in frontostriatal BDNF functions [52]. Through the literature, we came across various genetic studies investigating the possible relationship between ADHD and BDNF, NGF, and GDNF genes, however, we may surmise that the results of these studies remain to be clarified [53-55].

When the literature is examined, we found that the results of studies evaluating peripheral BDNF levels in children and adolescents were inconsistent. As far as can be discerned, in two studies, blood BDNF levels were found to be significantly higher in ADHD patients than in control subjects, which is consistent with our study [9, 10]. However, in some other studies, no significant difference was found between the groups [11-18], while lower BDNF levels were reported in ADHD cases in some studies [19-21]. The study results diversity may be based on the characteristics of the sample groups used in the studies and the differences in the

methodological characteristics of the studies. In a recently published meta-analysis reviewing ten studies, it was reported that no significant difference was found between ADHD and control groups in terms of peripheral BDNF levels; however, the researchers pointed out that the studies showed high heterogeneity in terms of various factors such as sample size and sample characteristics [56]. When existing studies are examined, it is seen that serum BDNF levels are studied in some and plasma BDNF levels in others. It has been reported that serum and plasma BDNF levels can vary considerably and serum BDNF levels give more consistent results than plasma [57]. In our study, the evaluation of serum levels of BDNF was thought to be important in this respect. In some studies, it is seen that psychopathologies such as depression, anxiety disorders, and OCD accompanying ADHD are not evaluated or excluded [14, 15]. There are studies in the literature indicating that BDNF levels may vary in depression, anxiety disorders, and OCD [5, 6, 8]. It would not be incorrect to say that the exclusion of all comorbid psychopathologies other than ODD, also empowered our study.

As far as can be discerned from the literature, four studies evaluating NGF levels in children and adolescents with ADHD could be found. In the study conducted by Güney et al., serum NGF levels of 44 ADHD and 36 healthy control subjects were compared; consistent with our study, NGF levels of ADHD cases were found to be significantly higher than control cases [25]. In the other two studies, it was reported that serum NGF levels did not differ significantly between ADHD and control groups [15, 18]. In another study published quite recently, it was determined that there was no significant difference between the ADHD group and the control group in terms of βeta-NGF levels [21]. Upon examination of existing studies, in one of those studies, we discern that psychopathology such as depression, anxiety disorders, and OCD, which are comorbid with ADHD, are not excluded [15]. In the examination of Yurteri et al. study, we see that the sample size is smaller than in our study

[18]. Our study investigated the serum NGF levels. On the other hand, the Chang et al. study evaluated plasma levels [21]. These factors may have caused the results of the study to differ.

As far as can be ascertained from the available literature, we came across four studies evaluating the GDNF levels of children and adolescents diagnosed with ADHD. In three of the four studies, in congruence with our study, the GDNF levels in the ADHD group were determined to be significantly higher compared with the control group [15, 18, 30]. In the Chang et al. study, the GDNF levels between the ADHD and control groups did not show significant difference [21]. In review of that study, we have to draw attention to the fact that the number of control subjects (n=12) whose GDNF levels were being evaluated compared with ADHD subjects (n=54) was remarkably lower. In our study, the groups are considerably proximate to each other (n=58, n=60). In addition, the Chang et al. study analyzed plasma sample [21] while our study measured serum GDNF levels. These conditions may have varied the results.

Our study found the serum galanin levels in the ADHD subjects to be significantly higher than that of the control group. As far as we could discern from review of the available literature, we did not come across any other study analyzing the galanin levels in children and adolescents with ADHD diagnosis. In this respect, our study is the first of its kind in literature.

There is evidence of an association between galanin and the dopaminergic system that plays an important role in the etiology of ADHD. In a study carried out with rats, galanin was found to inhibit the expression of tyrosine hydroxylase (the rate-inhibiting enzyme found in dopamine synthesis) in midbrain dopaminergic neurons [58]. Galanin regulates dopamine activity in the mezocorticolimbic region. A study found diminishing dopamine synthesis in the ventral tegmental region following administration of galanin [59]. Nevertheless, in another study, increased dopamine levels in the nucleus accumbens were observed following

galanin microinjection into the paraventricular nucleus [60]. The available data in literature led us to think that the modulator effect of galanin on dopamine levels may be complex and may act through more than one pathway. Further studies on the subject are necessary.

Literature discloses of an association between galanin and ADHD aside from the dopaminergic system. In an animal study with an epilepsy model, it is asserted that endogenous galanin inhibits serotonergic transmissions in the raphe nucleus and noradrenergic transmissions in the locus ceruleus, and may play a role in the pathophysiology of epilepsy-related ADHD [61].

Our study found no significant correlation between the CPRS-R:L and CTRS-R:L scores of ADHD cases and their serum neurotrophin and galanin levels. Upon analyses of the literature on the subject, it may be discerned that various scales are used in evaluating the severity of ADHD symptoms. As far as may be gleaned, only a single study reported that BDNF levels may affect the severity of inattention symptoms [9], while existing findings indicate no significant correlation between the BDNF levels and ADHD symptom severity in general [11, 14, 15, 19]. Upon review of studies evaluating the NGF levels in children and adolescents with ADHD, we find that two of the existing studies researched the relationship between the symptom severity of the disorder and the serum NGF levels. Both of these studies, in line with ours, found no correlation between the serum NGF levels and ADHD symptom severity [15, 18]. In only one of the four studies evaluating GDNF levels in children and adolescents with ADHD was a positive correlation found between the plasma GDNF levels and inattention, hyperactivity/impulsivity, and total scale scores [30]. The two studies put forth results consistent with ours; no significant correlation was found between the ADHD symptom severity and the serum GDNF levels [15, 18]. In general, the results of our study seem to be compatible with the literature, where the elevated neurotrophin levels detected in ADHD cases may be related to the presence of the disorder more so than its severity.

Post Treatment Evaluation

Our study found the BDNF, NGF, GDNF, and galanin levels in the ADHD group following treatment to be significantly lower compared with before the treatment. In literature, we came across four studies evaluating the BDNF levels of children and adolescents diagnosed with ADHD based on their treatment. The results are inconsistent with each other. In two of the four studies, while the BDNF levels are reported to have significantly increased following methylphenidate treatment compared with before; in one study there was no significant variation of the BDNF levels compared with before and after treatment [16, 20, 62]. Congruous with our study, another study found that the BDNF levels showed a significant decrease after the treatment [12]. As far as it may be ascertained from the literature, no studies were found evaluating peripheral NGF, GDNF, and galanin levels in ADHD diagnosed cases based on treatment status.

Preclinical studies affirm that dopaminergic stimulation may play a role to varying degrees in regulating neurotrophin levels, yet it is found that the studies gleaned from literature present conflicting findings on this subject. The Schmitz et al. study divulges there was a decrease of BDNF and NGF levels in the rat hippocampus following the administration of chronic methylphenidate [63]. Another study, carried out by Oakes et al., found that no significant change occurred in BDNF and GDNF levels following chronic methylphenidate exposure [64]. In a study carried out by Sadasivan et al., it was found that the methylphenidate exposure decreased the GDNF mRNA levels in the substantia nigra of mice [65]. For now, the effects of psychostimulants on neurotrophin levels seems to be complex. Our study brings to mind that the decrease in serum neurotrophin levels following treatment may be associated to the decrease in the need for a compensatory mechanism alongside the treatment. Additionally, literature presents studies reporting the neuroprotective effect of methylphenidate [66]. The probable neuroprotective effect of the methylphenidate used in our

study may have also caused the decrease in the compensatory increase of neurotrophin levels. More studies are needed on the subject.

This study should be evaluated together with its strengths and limitations. The strengths of our study are the use of semi-structured interview, the exclusion of confounding factors as possible, the use of a control group of equivalent size, keeping the case age range as narrow as possible. In review of the literature on the subject, this is the first study to examine change in the serum NGF and GDNF levels of ADHD diagnosed children undergoing methylphenidate treatment. Additionally, it is also the first study to examine the serum galanin levels and the change of this molecule in the serum level following the administration of methylphenidate in ADHD cases. While our study has its own merits, the small sample size is a limitation. We evaluated blood sample parameters in our study. Although it was shown that these molecules can generally cross the blood-brain barrier, we wanted to evaluate these molecule functions directly with their receptor activities in the central nervous system. In addition, the concentration in cerebral blood flow and other tissue will yield more comparable results. The level of intelligence was only evaluated based on clinical observation, and the subjects were not put through a standard intelligence test. Since ODD is frequently an associated comorbidity with ADHD, it could not be excluded.

As a result; serum BDNF, NGF, GDNF, and galanin levels were found to be significantly higher in the ADHD diagnosed subjects compared with the control subjects. Post-treatment serum BDNF, NGF, GDNF, and galanin levels were found to be significantly lower compared with pre-treatment levels. No correlation was found between the blood parameters and ADHD scale scores. Our findings lead us to believe further studies are necessary in this area.

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Table 1. Socio-demographic data of the ADHD and healthy control groups

	ADHD (n=58)	**Control (n=60)**			**p**
Age, year, mean, (SD)	10.10 (1.60)	10.07 (1.31)	MWU=1716.0	z=-0.132	0,895[a]
Gender, %, (n)			x^2=0.016	df=1	0,899[b]
Boy	%67.2 (39)	%68.3 (41)			
Girl	%32.8 (19)	%31.7 (19)			
Body mass index, kg/$m^{2,}$ mean, (SD)	18.17 (4.05)	18.54 (2.63)	MWU=1625.5	z=-0.616	0.538[a]
Mother age, year, mean, (SD)	36.41 (6.23)	37.88 (5.54)	MWU=1438.0	z=-1.629	0.103[a]
Father age, year, mean, (SD)	41.16 (7.11)	41.20 (5.68)	MWU=1502.5	z=-0.538	0.590[a]
Mother education, %, (n)			Fisher's Exact=5.056		0,284
No education	%8.6 (5)	%3.3 (2)			
Elementary school	%48.3 (28)	%38.3 (23)			
Secondary school	%20.7 (12)	%18.3 (11)			
High school	%17.2 (10)	%28.3 (17)			
University	%5.2 (3)	%11.7 (7)			
Father education, %, (n)			Fisher's Exact=2.769		0,626
No education	%3.4 (2)	%0,0 (0)			
Elementary school	%25.9 (15)	%23.3 (14)			
Secondary school	%24.1 (14)	%20,0 (12)			
High school	%31.0 (18)	%35.0 (21)			
University	%15.5 (9)	%21.7 (13)			
Family income monthly %, (n)			x^2=3.020	df=3	0,389[b]
0-1500 TL	%27.6 (16)	%21.7 (13)			
1501-2500 TL	%56.9 (33)	%50.0 (30)			
2501-3500 TL	%10.3 (6)	%16.7 (10)			
>3500 TL	%5.2 (3)	%11.7 (7)			
Living place, %, (n)			Fisher's Exact=0.415		1,000
City	%81.0 (47)	%80.0 (48)			
Town	%17.2 (10)	%16.7 (10)			
Village	%1.7 (1)	%3.3 (2)			

a: Mann-Whitney U test, b: Chi-Square, ADHD: Attention deficit hyperactivity disorder, SD: Standart deviation.

Table 2. Pre-treatment BDNF, NGF, GDNF, and galanin levels

	ADHD (n=58)	**Control (n=60)**			**p**
BDNF, pg/mL, mean, (SD)	2067.93 (580.24)	1712.68 (289.29)	t=4.187	df=83.051	<0.001[a]
NGF, ng/mL, mean, (SD)	157.11 (28.59)	123.07 (18.99)	MWU=535.0	z=-6.488	<0.001[b]
GDNF, pg/mL, mean, (SD)	1699.59 (484.82)	1318.33 (224.73)	MWU=877.0	z=-4.646	<0.001[b]
Galanin, pg/mL, mean, (SD)	271.57 (55,90)	187.50 (37,23)	t=9.582	df=98.804	<0.001[a]

a: Independent samples t test, b: Mann-Whitney U test, ADHD: Attention deficit hyperactivity disorder, BDNF: Brain-derived neurotrophic factor, GDNF: Glial-derived neurotrophic factor, NGF: Nerve growth factor, SD: Standart deviation.

Table 3. Comparison of blood parameters pre and post-treatment in ADHD group

	Pre-treatment (n=58)	**Post-treatment (n=58)**			**p**
BDNF, pg/mL, mean, (SD)	2067.93 (580.24)	1857.66 (316.07)	t=2.408	df=57	0.019[a]
NGF, ng/mL, mean, (SD)	157.11 (28.59)	94.26 (32.46)		z=-6.267	<0.001[b]
GDNF, pg/mL, mean, (SD)	1699.59 (484.82)	1436.71 (402.09)		z=-3.062	0.002[b]
Galanin, pg/mL, mean, (SD)	271.57 (55.90)	205.71 (51.33)	t=6.121	df=57	<0.001[a]

a: Paried sample t test, b: Wilcoxon test, ADHD: Attention deficit hyperactivity disorder, BDNF: Brain-derived neurotrophic factor, GDNF: Glial-derived neurotrophic factor, NGF: Nerve growth factor, SD: Standart deviation.

Table 4. Correlation of pre-treatment blood parameters with CPRS-R: L scores in ADHD group

		BDNF[a]	**NGF[b]**	**GDNF[b]**	**Galanin[a]**
Oppositional	r	-0.138	-0.089	-0.072	0.076
	p	0.302	0.506	0.594	0.569
Cognitive problems/inattention	r	-0.171	-0.226	-0.132	-0.102
	p	0.200	0.089	0.325	0.445
Hyperactivity	r	-0.125	0.177	-0.019	0.133
	p	0.351	0.184	0.886	0.320
Anxious/shy	r	0.049	-0.161	-0.098	-0.007
	p	0.715	0.228	0.464	0.960
Perfectionism	r	-0.033	-0.043	0.121	0.029
	p	0.808	0.751	0.366	0.831
Social problems	r	-0.067	-0.071	0.044	0.149
	p	0.620	0.597	0.740	0.264
Psychosomatic	r	0.210	0.013	0.015	-0.181
	p	0.113	0.921	0.913	0.173
ADHD index	r	-0.238	0.067	-0.156	-0.028
	p	0.072	0.616	0.241	0.832
Conners' global index-restless/impulsivity	r	-0.215	-0.025	-0.104	-0.052
	p	0.104	0.853	0.437	0.700
Conners' global index-emotional lability	r	0.139	0.065	0.250	-0.015
	p	0.298	0.627	0.058	0.910
Conners' global index-total	r	-0.099	0.051	0.089	-0.051
	p	0.462	0.706	0.508	0.703
DSM-IV-inattention	r	-0.095	-0.182	-0.063	-0.138
	p	0.477	0.172	0.638	0.302
DSM-IV-hyperactivity/impulsivity	r	-0.152	0.110	-0.142	0.030
	p	0.255	0.412	0.288	0.824
DSM-IV-total	r	-0.191	-0.063	-0.144	-0.115
	p	0.150	0.641	0.280	0.390

a: Pearson correlation test, b: Spearman correlation test, ADHD: Attention deficit hyperactivity disorder, BDNF: Brain-derived neurotrophic factor, GDNF: Glial-derived neurotrophic factor, NGF: Nerve growth factor, r: Correlation coefficient.

Table 5. Correlation of pre-treatment blood parameters with CTRS-R: L scores in ADHD group

		BDNF[a]	NGF[b]	GDNF[b]	Galanin[a]
Oppositional	r	-0.076	-0.099	-0.162	0.123
	p	0.571	0.458	0.223	0.359
Cognitive problems/inattention	r	-0.051	-0.129	0.098	0.001
	p	0.704	0.333	0.466	0.992
Hyperactivity	r	-0.152	0.095	-0.078	0.090
	p	0.254	0.476	0.561	0.501
Anxious/shy	r	0.221	-0.053	-0.094	-0.103
	p	0.095	0.690	0.482	0.443
Perfectionism	r	0.116	-0.017	0.258	-0.053
	p	0.387	0.897	0.050	0.695
Social problems	r	-0.118	0.003	0.015	0.131
	p	0.378	0.981	0.913	0.327
ADHD index- inattention	r	-0.062	0.179	-0.147	0.255
	p	0.642	0.178	0.272	0.053
ADHD index- hyperactivity	r	-0.058	-0.034	0.006	0.090
	p	0.667	0.800	0.963	0.502
Conners' global index-restless/impulsivity	r	-0.117	0.056	-0.029	-0.127
	p	0.380	0.677	0.826	0.344
Conners' global index-emotional lability	r	0.122	0.236	0.073	-0.064
	p	0.361	0.075	0.588	0.632
Conners' global index-total	r	0.000	0.178	0.065	-0.137
	p	0.998	0.181	0.630	0.307
DSM-IV-inattention	r	-0.051	-0.225	0.082	-0.128
	p	0.705	0.089	0.539	0.337
DSM-IV-hyperactivity/impulsivity	r	-0.165	0.081	-0.009	0.037
	p	0.216	0.545	0.944	0.782
DSM-IV-total	r	-0.151	-0.061	0.038	-0.046
	p	0.257	0.650	0.778	0.731

a: Pearson correlation test, b: Spearman correlation test, ADHD: Attention deficit hyperactivity disorder, BDNF: Brain-derived neurotrophic factor, GDNF: Glial-derived neurotrophic factor, NGF: Nerve growth factor, r: Correlation coefficient.

Table 6. Pre-treatment blood parameters according to ADHD predominantly appearance

	Predominantly inattentive (n=30)	**Combined (n=28)**			**p**
BDNF, pg/mL, mean, (SD)	2170.97 (679.18)	1957.52 (436.90)	t=1.433	df=49.884	0.158[a]
NGF, ng/mL, mean, (SD)	154.38 (31.26)	160.04 (25.66)	z=-0.716	MWU=374.0	0.474[b]
GDNF, pg/mL, mean, (SD)	1651.95 (498.12)	1750.63 (473.80)	z=-0.989	MWU=356.5	0.323[b]
Galanin, pg/mL, mean, (SD)	272.12 (57.73)	270.98 (54.92)	t=0.077	df=57	0.939[a]

a: Independent samples t test, b: Mann-Whitney U test, ADHD: Attention deficit hyperactivity disorder, BDNF: Brain-derived neurotrophic factor, GDNF: Glial-derived neurotrophic factor, NGF: Nerve growth factor, SD: Standart deviation.

Table 7. Post-treatment blood parameters according to ADHD predominantly appearance

	Predominantly inattentive (n=30)	**Combined (n=28)**			**p**
BDNF, pg/mL, mean, (SD)	1810.56 (324,99)	1908.13 (303.90)	t=-1.179	df=56	0.243[a]
NGF, ng/mL, mean, (SD)	94.35 (33.52)	94.16 (31.90)	z=-0,093	MWU=414.0	0.926[b]
GDNF, pg/mL, mean, (SD)	1350.74 (380.29)	1528.83 (411.07)	z=-1.658	MWU=313.5	0.097[b]
Galanin, pg/mL, mean, (SD)	206.31 (53.08)	205.08 (50.35)	t=0.091	df=56	0.928[a]

a: Independent samples t test, b: Mann-Whitney U test, ADHD: Attention deficit hyperactivity disorder, BDNF: Brain-derived neurotrophic factor, GDNF: Glial-derived neurotrophic factor, NGF: Nerve growth factor, SD: Standart deviation.