

Altered Arginine/Nitric Oxide Pathway in Children Diagnosed Attention Deficit Hyperactivity Disorder, and the Effect of 10 Weeks Methylphenidate Treatment

Ebru Doneray¹, Kemal Utku Yazici², Ipek Percinel Yazici², Bilal Ustundag³

¹Department of Child and Adolescent Psychiatry, Sanliurfa Training and Research Hospital, Sanliurfa, ²Department of Child and Adolescent Psychiatry, Firat University Faculty of Medicine, ³Department of Biochemistry, Firat University Faculty of Medicine, Elazig, Turkey

Objective: In this study, we investigated the levels of arginine, nitric oxide (NO), asymmetric dimethylarginine (ADMA), and adrenomedullin that are presumed to play a role in attention deficit hyperactivity disorder (ADHD) etiology, and to compare the findings with healthy controls.

Methods: Thirty ADHD patients and thirty healthy control subjects aged 6–12 years were included in the study. Sociodemographic data form, Schedule for Affective Disorders and Schizophrenia for School Age Children-Present and Lifetime Version; Conners' Parent/Teacher Rating Scale-Revised: Long Form; Children's Depression Inventory; and The State-Trait Anxiety Inventory for Children were applied to all cases. All participants included in the study were evaluated in terms of their serum arginine, NO, ADMA, and adrenomedullin levels. Subsequently, methylphenidate treatment was started in ADHD patients and blood parameters were tested again in the tenth week of treatment.

Results: At the start of the study, arginine and ADMA levels were significantly higher and NO and adrenomedullin levels were significantly lower in the ADHD group compared to the control group. Post-treatment arginine and ADMA levels were found to be significantly lower than in the pre-treatment period. There were no significant differences in NO and adrenomedullin levels before and after treatment. There was no correlation between scale scores and blood parameters.

Conclusion: These variations in the blood parameters of the ADHD group seem to be worth further investigation. Studies to be conducted with larger sample groups after longer-term treatment may provide new information about the alterations in neurobiological processes related to ADHD etiology and treatment.

KEY WORDS: Attention deficit hyperactivity disorder; ADMA; Adrenomedullin; Arginine; Nitric oxide.

INTRODUCTION

Although attention deficit hyperactivity disorder (ADHD) is a very common disorder in childhood, its etiology is still unclear. Today ADHD is accepted as a neurodevelopmental disorder of multiple etiologies in which genetic, neuro-anatomical, neurochemical, neurophysiological, neuropsychological, environmental, and psychosocial factors play a role [1]. Nevertheless, studies investigating the etiology and pathophysiology of this disorder are continuing

at a faster pace.

Arginine is a positively charged amino acid that is mainly metabolized in three major pathways in the human body. In one of these pathways, arginine is transformed into urea and ornithine by the arginase enzyme. In another pathway, arginine is converted to agmatine via arginine decarboxylase. The resulting agmatine forms other polyamines such as putrescine, spermidine, and spermine by the enzyme agmatinase. The third pathway is the nitric oxide (NO) pathway. In this pathway, NO is formed from L-arginine via the enzyme nitric oxide synthase (NOS) (Fig. 1) [2,3].

The arginine/nitric oxide pathway has high importance in the central nervous system. High levels of NO synthesis take part in the cerebellum, cerebral cortex, hypothal-

Received: February 9, 2021 / **Revised:** June 26 2021

Accepted: July 2, 2021

Address for correspondence: Kemal Utku Yazici
Department of Child and Adolescent Psychiatry, Firat University
Faculty of Medicine, Elazig 230000, Turkey
E-mail: dr.kemal.utku@outlook.com
ORCID: <https://orcid.org/0000-0001-8659-6340>

© This is an Open-Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (<http://creativecommons.org/licenses/by-nc/4.0>) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

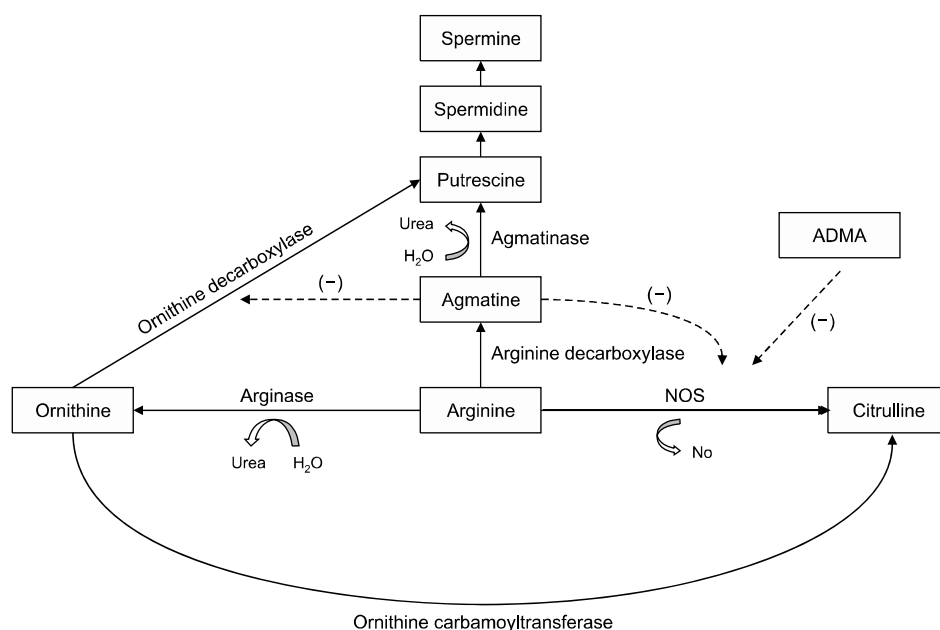


Fig. 1. Arginine metabolism. ADMA, asymmetric dimethylarginine; NO, nitric oxide; NOS, nitric oxide synthase; (-), inhibition.

amus, hippocampus, and amygdala [4]. In addition to being a vasodilator, nitric oxide is known to act as a neurotransmitter in both the central nervous system and the peripheral nervous system. NO in the central nervous system is associated with circadian rhythm, sleep control, and cognitive functions such as memory and learning. It is also closely related to the regulation of the release of neurotransmitters such as noradrenaline, dopamine, glutamate, and GABA [5,6]. Arginine is the only substrate of the NOS enzyme in the process of NO formation [7]. For these reasons, it has been frequently suggested in recent years that both arginine and NO may be associated with various psychiatric disorders [8-11].

As far as can be seen from the reviewed literature, two studies have been found evaluating the levels of arginine in children and adolescents with ADHD; however, the results of these studies are inconsistent [12,13]. In the study by Jansen *et al.* [12], arginine levels in children with ADHD did not differ significantly from the control group. On the other hand, Sari *et al.* [13] reported that the arginine levels in children with ADHD were significantly higher than in control subjects. There are a relatively high number of studies evaluating NO levels in children and adolescents with ADHD in the literature. However, the results of these existing studies are not consistent [12-16].

Asymmetric dimethylarginine (ADMA) is a methylated arginine derivative. After the arginine residues in proteins are methylated by the protein arginine methyl transferase

enzyme, ADMA is formed as a result of the proteolysis of these proteins. Most of the ADMA formed is degraded by the dimethylarginine dimethylaminohydrolase (DDAH) enzyme. ADMA is an inhibitor of the NOS enzyme [17,18]. Through this inhibition, ADMA is closely associated with the arginine/NO pathway and functions as an important regulator of this pathway (Fig. 1). Since ADMA is one of the important regulators of the arginine/NO pathway, ADMA may play an important role in the etiopathogenesis of psychiatric diseases. There have been limited studies in the literature investigating ADMA levels in psychiatric diseases, and the existing studies seem to focus on schizophrenia and major depressive disorder in general [19-23]. When the literature was reviewed, only one study was found evaluating ADMA levels in children and adolescents with ADHD. Jansen *et al.* [12], in their study, found higher levels of plasma ADMA in ADHD compared to controls.

Adrenomedullin is both a hormone found in the circulatory system and a local paracrine mediator involved in various biological activities [24]. Studies suggest that adrenomedullin may also act as a neurotransmitter, neuromodulator, neurohormone, and a cytoprotective factor in ischemic/hypoxic conditions [25]. It acts as a vasodilator by recruiting the NO/cGMP pathway [26]. Adrenomedullin has been shown to activate endothelial NOS and to stimulate hypothalamic NO production and hypothalamo-pituitary adrenal (HPA) axis activity by activating

central neuroendocrine and autonomic pathways [26-29]. It may be important to include adrenomedullin in psychiatric studies evaluating the arginine/NO pathway, as it is associated with NO synthesis. When the literature is reviewed, there has been only one study evaluating adrenomedullin levels in children and adolescents with ADHD. In that study, Gürbüz Özgür *et al.* [14] found no significant difference between the ADHD and the healthy control groups.

In light of all these findings, in this study, we aimed to evaluate arginine, NO, ADMA, and adrenomedullin levels. Furthermore, the relationship between serum arginine, NO, ADMA, and adrenomedullin levels with ADHD symptom severity was investigated. As far as can be seen in the literature, no study was designed that evaluates these molecules in children and adolescents with ADHD simultaneously.

In addition, in our study, the effect of OROS (osmotic release oral system) methylphenidate treatment on arginine, NO, ADMA, and adrenomedullin levels was also evaluated in patients with ADHD. As far as we can see in the literature, there are no studies that evaluate the relationship between serum arginine, NO, ADMA, and adrenomedullin levels in children and adolescents with ADHD treatment with a methodology similar to our study. In one study, children with ADHD who received methylphenidate and children with ADHD who did not receive methylphenidate were compared, and there was no significant difference between the two groups in terms of NO levels [16]. In another study, 23 ADHD cases who used methylphenidate and 19 ADHD cases who did not use methylphenidate were evaluated, and there was no significant difference in plasma ADMA and arginine levels between those who used methylphenidate and those who did not [12]. In that study, plasma nitrite and nitrate concentrations reflecting NO synthesis were also evaluated. Although plasma nitrite levels were found to be significantly higher in children with ADHD who received methylphenidate compared to children with ADHD who did not receive methylphenidate, plasma nitrate levels did not differ significantly between groups [12]. In those studies [12,16], the effect of methylphenidate on blood levels of molecules was evaluated in different cases. Therefore, it is thought that our study can help to create a more holistic perspective on the subject.

METHODS

Study Design and Participants

This study was conducted in Firat University Faculty of Medicine Child and Adolescent Mental Health and Diseases Outpatient Clinic between June 2018 and May 2020. All procedures were carried out in accordance with the principles listed in the Declaration of Helsinki. The study was approved by Firat University Ethics Committee (21.06.2018-11/15). It was supported by the decision of Firat University Scientific Research Projects Coordination Unit TF.18.43.

Thirty patients diagnosed with ADHD and thirty healthy control cases between the ages of 6 and 12 years were included in the study. The ADHD group consisted of patients who applied to the outpatient clinic with ADHD symptoms and met the diagnostic criteria. The control group was chosen from the children of volunteer families, considering the age and sex distribution of the ADHD group.

Procedure

Psychiatric evaluation

Written consent forms were obtained from the families of all participants before the evaluation. Diagnostic evaluation was performed according to the Schedule for Affective Disorders and Schizophrenia for School-Age Children (K-SADS-PL) and the Diagnostic and Statistical Manual of Mental Disorders 5th edition (DSM-5) criteria. Sociodemographic data of all participants were recorded in the sociodemographic data form. The Conners' Parent/Teacher Rating Scale-Revised: Long Form was filled in by the parents and teachers of the participants. Depressive symptoms of the participants were evaluated with Children's Depression Inventory, and anxiety symptoms were evaluated with The State-Trait Anxiety Inventory for Children.

For the ADHD group, those who had additional psychiatric diagnoses other than oppositional defiant disorder (ODD), those who had used any psychotropic medication in the previous three months (including stimulants/non-stimulants), those who used a drug other than psychotropic medications in the previous one month, and those with chronic systemic disease were excluded from the study. For the control group, those who were diagnosed with a psychiatric diagnosis, who used any medication

within the previous one month, and those with chronic systemic diseases were excluded from the study. According to the detailed information obtained from the families and teachers of the participants, the participants that did not have any problems in basic adaptation functions such as academic achievement, comprehension, learning, and neuromotor development according to their age were evaluated, and the participants considered to have normal mental development level as a conclusion of the evaluation were included in the study.

Afterwards, all participants included in the study were evaluated in terms of serum arginine, NO, ADMA, and adrenomedullin levels. All procedures described to this point for the ADHD group were also performed on the healthy control group.

Subsequently, OROS methylphenidate treatment was planned for patients. The starting dose was 18 mg/day. It was titrated up to a maximum of 54 mg/day (average dose 1 mg/kg/day). After the drug treatment was initiated, the cases were evaluated every two weeks. The interviews at weeks 0, 4, 8, and 10 were conducted face-to-face at the clinic. The evaluations in the second and the sixth weeks were performed by phone. The cases were controlled in terms of the severity of the disease symptoms, the level of benefit from the treatment, and possible drug side effects. At the tenth week, ADHD cases were reevaluated in terms of serum arginine, NO, ADMA, and adrenomedullin levels.

Assessment tools

Sociodemographic Data Form: This form was developed by the researchers to determine the sociodemographic characteristics of the children participating in the study. In this form, questions about the age, sex, developmental stages, literacy learning time, school success, and medical history information as well as the ages, and educational status of the parents, living place, the average monthly income level of the family, and psychiatric/medical genealogical information were asked.

Schedule for Affective Disorders and Schizophrenia for School Age Children-Present and Lifetime Version (K-SADS-PL): K-SADS-PL, a semi-structured interview form developed by Kaufman *et al.*, is used to determine the past and present psychopathology of children and adolescents according to DSM-IV diagnostic criteria [30]. The validity and reliability study of the scale was conducted for the Turkish sample [31].

Conners' Parent Rating Scale-Revised: Long Form (CPRS-R: L): This form was developed by Conners to evaluate parents' observations about the child's behavior in out-of-school settings [32]. The Turkish validity and reliability study of the scale was performed by Kaner *et al.* [33]. It consists of a total of 80 items and 14 subscales. The questions are answered by the parents on a 4-point Likert scale. Each item is scored as 0, 1, 2, or 3 based on symptom severity.

Conners' Teacher Rating Scale-Revised: Long Form (CTRS-R: L): This is a scale developed to evaluate students' classroom behaviors [34]. The Turkish validity and reliability study of the scale was performed by Kaner *et al.* [35]. The scale consists of 59 items and 14 subscales. The questions are answered by the teacher on a 4-point Likert scale. Each item is scored as 0, 1, 2, or 3 based on symptom severity.

Children's Depression Inventory (CDI): This is a measurement tool that can be applied to children between the ages of 6 and 17 years and used to determine the level of depression of the child [36]. It is a 27-item scale. Each item, according to the severity of the symptoms, gets 0, 1, or 2 points. The maximum score is 54 and the higher the score, the heavier the depression. The validity and reliability study in Turkey was conducted by Öy [37], and the pathological cut-off point was determined as 19 points.

The State-Trait Anxiety Inventory for Children (STAI-C): This scale, developed by Spielberger *et al.* [38], has two subscales with 20 questions for state and trait anxiety. Each item is scored as 1, 2, or 3 according to the severity of the symptom. The validity and reliability study of the scale in Turkey was conducted by Özusta (1995) [39].

Clinic Global Impression Scale-Improvement (CGI-I): This scale allows the clinician to assess the level of improvement in the patient's clinical condition as the patient continues to be followed up. It is scored between 1 and 7 points. Scores: 1 = very much improved since the initiation of treatment; 2 = much improved; 3 = minimally improved; 4 = no change from baseline (the initiation of treatment); 5 = minimally worse; 6 = much worse; 7 = very much worse since the initiation of treatment [40].

Biochemical analysis

After the clinical evaluations, all participants included in the study were directed to the Firat University Hospital Biochemistry Laboratory. After 12 hours of fasting, 5 ml of

venous blood samples were taken into anticoagulant tubes and straight biochemistry tubes. After blood samples were centrifuged at 2,500–3,000 rpm for 10–15 minutes, serum and plasma were separated and were stored at -80°C under suitable conditions until the analysis was performed.

In the study samples, total NO and adrenomedullin levels were measured with commercial ELISA kits (Shanghai Coon Koon Biotech Ltd., Shanghai, China) using ELx50 (washer) and ELx800 (reader) devices based on the Enzyme-Linked Immunosorbent Assay (ELISA) method. The samples were colored according to their concentrations by following the steps described in the protocol. After the color formation was observed, the absorbance values of the wells were read at 450 nanometers (nm) using the ELx800 reader, and the results were printed. Concentrations were calculated using the serum absorbance values found. The values found are micromole/liter

($\mu\text{mol/L}$) for NO and picogram/milliliter (pg/ml) for adrenomedullin.

Arginine and ADMA were measured with Shimadzu brand HPLC using kit (Eureka Lab Division, Ancona, Italy) and materials based on high performance liquid chromatography (HPLC) method. Plasma samples were first extracted with the solid phase column included in the kit. Then, 200 μl reagent N (buffer solution), 150 μl reagent O (buffer solution), 30 μl reagent J (starting solution), 30 μl reagent L (derivatization solution) were added to the extract obtained. After vortexing, it was left to incubate for 30 minutes at 20°C . HPLC grade water (150 μl) was added to HPLC system and the results were obtained by chromatographic measurement. The values found for arginine and ADMA are in units of micromole/liter ($\mu\text{mol/L}$).

Statistical Analysis

The analyses were performed using the SPSS (Statistical

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

Variable	ADHD (n = 30)	Control (n = 30)	Statistics	Statistics	<i>p</i> value
Age (yr)	10.03 \pm 1.61	9.87 \pm 0.97	MWU = 403.0	$z = -0.720$	0.471 ^a
Sex			$\chi^2 = 0.271$	df = 1	0.602 ^b
Male	18 (60.0)	16 (53.3)			
Female	12 (40.0)	14 (46.7)			
Body mass index (kg/m ²)	18.88 \pm 4.17	17.34 \pm 2.96	MWU = 330.0	$z = -1.774$	0.076 ^a
Mother age (yr)	36.03 \pm 4.63	37.10 \pm 5.48	MWU = 403.5	$z = -0.693$	0.488 ^a
Father age (yr)	40.63 \pm 6.72	41.55 \pm 6.45	MWU = 377.0	$z = -0.881$	0.378 ^a
Mother education			Fisher's exact = 1.606		0.849
No education	5 (16.7)	3 (10.0)			
Elementary school	10 (33.3)	12 (40.0)			
Secondary school	5 (16.7)	4 (13.3)			
High school	8 (26.7)	7 (23.3)			
University	2 (6.7)	4 (13.3)			
Father education			$\chi^2 = 7.097$	df = 4	0.131 ^b
No education	2 (6.7)	0 (0.0)			
Elementary school	2 (6.7)	8 (26.7)			
Secondary school	7 (23.3)	6 (20.0)			
High school	14 (46.7)	9 (30.0)			
University	5 (16.7)	7 (23.3)			
Family income monthly			Fisher's exact = 1.699		0.666
0–1,500 TL	6 (20.0)	6 (20.0)			
1,501–2,500 TL	12 (40.0)	16 (53.3)			
2,501–3,500 TL	6 (20.0)	3 (10.0)			
> 3,500 TL	6 (20.0)	5 (16.7)			
Living place			Fisher's exact = 2.289		0.329
City	24 (80.0)	21 (70.0)			
Town	4 (13.3)	3 (10.0)			
Village	2 (6.7)	6 (20.0)			

Values are presented as number (%) or mean \pm standard deviation.

ADHD, attention deficit hyperactivity disorder, TL, Turkish Lira; MWU, Mann–Whitney *U*.

^aMann–Whitney *U* test, ^bchi-square.

Package for Social Sciences; IBM Co., Armonk, NY, USA) 21 package program. Chi-square analysis (pearson chi-square) was used to compare categorical variables between groups, and if more than 20% of the expected values were less than 5, the Fisher's exact test was taken into consideration. The suitability of continuous variables to the normal distribution was evaluated with the Kolmogorov–Smirnov test. Independent samples *t* test was used for the comparison of variables that were compatible with normal distribution between the two groups, and the Mann–Whitney *U* test was used for the comparison of variables that were not normally distributed between the two groups. In examining the relationship between continuous variables, Pearson correlation test was used for those with normal distribution, and Spearman correlation test was used for those that did not show normal distribution. Paired samples *t* test was used to compare normally distributed variables at two different times, and the Wilcoxon test was used to compare non-normally distributed variables at two different times. The significance level in the analyses was accepted as $p < 0.05$.

RESULTS

Sociodemographic Data and Scale Scores

A total of 60 participants, 30 ADHD and 30 healthy

controls, were included in our study. In 33.3% ($n = 10$) of the ADHD cases, ODD was also present. The mean age of the ADHD group was 10.03 ± 1.61 years, and the mean age of the control group was 9.87 ± 0.97 years ($p = 0.471$). While 40.0% ($n = 12$) of the ADHD group were female and 60.0% ($n = 18$) were male, 46.7% ($n = 14$) of the control group were female and 53.3% ($n = 16$) were male. No significant difference was found between the ADHD group and the control group in terms of sex ($p = 0.602$).

There were no significant differences between the groups in terms of body mass index, age of parents, parent education, family income level, or living place. Sociodemographic data of the groups are presented in Table 1.

In our study, depressive disorder and anxiety disorder diagnoses were excluded. In the scales we use to evaluate subthreshold symptoms, the mean CDI score was 10.67 ± 4.23 in the ADHD group and 5.80 ± 4.69 in the control group (MWU = 199.5, $z = -3.711$, $p < 0.001$). The mean STAI-C/State score was 33.13 ± 9.00 in the ADHD group and 26.43 ± 6.00 in the control group (MWU = 211.5, $z = -3.534$, $p < 0.001$). The mean STAI-C/Trait score was 36.23 ± 6.15 in the ADHD group and 30.60 ± 6.44 in the control group ($t = 3.466$, $df = 58$, $p = 0.001$).

Clinical symptoms as evaluated with CGI-I improved with methylphenidate treatment in all ADHD cases. In the evaluation at the 10th week of the treatment, 13.3% of

Table 2. Pre-treatment arginine, NO, ADMA, and adrenomedullin levels

Molecules	ADHD (n = 30)	Control (n = 30)	Statistics	Statistics	<i>p</i> value
Arginine ($\mu\text{mol/L}$)	104.25 ± 19.26	63.12 ± 23.59	MWU = 71.5	$z = -5.598$	$< 0.001^a$
NO ($\mu\text{mol/L}$)	39.82 ± 11.68	48.89 ± 11.72	$t = -3.003$	$df = 58$	0.004^b
ADMA ($\mu\text{mol/L}$)	1.21 ± 0.36	1.06 ± 0.22	$t = 2.068$	$df = 47.818$	0.044^b
Adrenomedullin (pg/ml)	527.19 ± 255.25	655.96 ± 215.51	MWU = 298.5	$Z = -2.241$	0.025^a

Values are presented as mean \pm standard deviation.

ADHD, attention deficit hyperactivity disorder; ADMA, asymmetric dimethylarginine; NO, nitric oxide; MWU, Mann–Whitney *U*.

^aMann–Whitney *U* test, ^bindependent samples *t* test.

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

Molecules	Pre-treatment (n = 30)	Post-treatment (n = 30)	Statistics	Statistics	<i>p</i> value
Arginine ($\mu\text{mol/L}$)	104.25 ± 19.26	69.11 ± 11.68		$z = -4.597$	$< 0.001^a$
NO ($\mu\text{mol/L}$)	39.82 ± 11.68	36.52 ± 10.06	$t = 1.286$	$df = 29$	0.208^b
ADMA ($\mu\text{mol/L}$)	1.21 ± 0.36	0.63 ± 0.26	$t = 7.447$	$df = 29$	$< 0.001^b$
Adrenomedullin (pg/ml)	527.19 ± 255.25	618.23 ± 272.15		$z = -1.200$	0.230^a

Values are presented as mean \pm standard deviation.

ADHD, attention deficit hyperactivity disorder; ADMA, asymmetric dimethylarginine; NO, nitric oxide; MWU, Mann–Whitney *U*.

^aWilcoxon test, ^bpaired sample *t* test.

ADHD cases ($n = 4$) were “very much improved”, 70.0% ($n = 21$) were “much improved”, and 16.7% ($n = 5$) were “minimally improved”.

Biochemical Data

In the pre-treatment evaluation, serum arginine and ADMA levels in the ADHD group were significantly higher than in the control group ($p < 0.001$, $p = 0.044$, respectively). Serum NO and adrenomedullin levels were significantly lower in ADHD cases compared to controls ($p = 0.004$, $p = 0.025$, respectively). The pre-treatment data of the groups are presented in Table 2.

In the evaluation performed after 10 weeks of OROS methylphenidate treatment in the ADHD group, a significant decrease was found in serum arginine and ADMA levels compared to pre-treatment ($p < 0.001$, $p < 0.001$, respectively). Serum NO and adrenomedullin levels did

not change significantly ($p = 0.208$, $p = 0.230$, respectively). The data before and after treatment in the ADHD group are presented in Table 3.

In correlation analyses performed in the ADHD group, no significant correlation was found between the CPRS-R: L and CTRS-R: L scores and the pre-treatment blood parameters. The correlation analyses performed are presented in Tables 4 and 5.

DISCUSSION

Pre-Treatment Evaluation

The pre-treatment findings of our study can be summarized as follows: 1) serum arginine levels were significantly higher in the ADHD group compared to the control group; 2) serum NO levels were significantly lower in the ADHD group compared to the control group; 3) serum

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

CPRS-R: L		Arginine ^a	NO ^b	ADMA ^b	Adrenomedullin ^a
Oppositional	<i>r</i>	-0.268	-0.345	0.217	0.309
	<i>p</i>	0.151	0.062	0.249	0.096
Cognitive problems/ inattention	<i>r</i>	0.002	0.037	-0.116	-0.337
	<i>p</i>	0.990	0.844	0.540	0.069
Hyperactivity	<i>r</i>	0.125	-0.331	-0.256	0.055
	<i>p</i>	0.511	0.074	0.172	0.771
Anxious/shy	<i>r</i>	-0.123	-0.330	-0.066	0.237
	<i>p</i>	0.518	0.075	0.730	0.208
Perfectionism	<i>r</i>	0.151	-0.200	-0.112	0.201
	<i>p</i>	0.425	0.289	0.556	0.288
Social problems	<i>r</i>	-0.051	-0.099	-0.136	-0.305
	<i>p</i>	0.789	0.602	0.473	0.101
Psychosomatic	<i>r</i>	-0.184	0.081	0.207	-0.034
	<i>p</i>	0.330	0.670	0.274	0.857
ADHD index	<i>r</i>	-0.076	-0.030	0.172	-0.114
	<i>p</i>	0.689	0.876	0.364	0.549
Conners' global index- restless/impulsivity	<i>r</i>	0.268	-0.299	0.026	-0.046
	<i>p</i>	0.152	0.108	0.890	0.810
Conners' global index- emotional lability	<i>r</i>	-0.203	-0.118	-0.224	0.045
	<i>p</i>	0.283	0.533	0.234	0.815
Conners' global index-total	<i>r</i>	0.108	-0.294	-0.088	0.014
	<i>p</i>	0.569	0.115	0.644	0.943
DSM-IV-inattention	<i>r</i>	-0.083	0.074	0.182	0.349
	<i>p</i>	0.661	0.698	0.337	0.059
DSM-IV-hyperactivity/ impulsivity	<i>r</i>	-0.133	0.255	0.191	0.299
	<i>p</i>	0.484	0.175	0.312	0.109
DSM-IV-total	<i>r</i>	-0.144	0.193	0.207	0.351
	<i>p</i>	0.449	0.307	0.273	0.057

ADHD, attention deficit hyperactivity disorder; ADMA, asymmetric dimethylarginine; CPRS-R: L, Conners' Parent Rating Scale-Revised: Long Form; NO, nitric oxide; *r*, correlation coefficient; DSM-IV, the Diagnostic and Statistical Manual of Mental Disorders 4th edition.

^aSpearman correlation test, ^bpearson correlation test.

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

CTRS-R: L		Arginine ^a	NO ^b	ADMA ^b	Adrenomedullin ^a
Oppositional	r	0.051	-0.156	0.268	0.003
	p	0.788	0.411	0.152	0.985
Cognitive problems/ inattention	r	-0.016	-0.299	-0.006	-0.086
	p	0.933	0.108	0.975	0.651
Hyperactivity	r	0.033	0.073	-0.186	0.168
	p	0.862	0.700	0.325	0.375
Anxious/shy	r	-0.116	-0.299	0.065	0.115
	p	0.540	0.109	0.734	0.546
Perfectionism	r	0.145	-0.245	-0.157	0.267
	p	0.444	0.192	0.408	0.154
Social problems	r	0.166	0.007	-0.104	-0.225
	p	0.380	0.972	0.584	0.231
ADHD index- inattention	r	-0.079	-0.293	-0.059	0.024
	p	0.677	0.116	0.758	0.900
ADHD index- hyperactivity	r	-0.021	-0.099	0.008	-0.198
	p	0.913	0.603	0.965	0.293
Conners' global index- restless/impulsivity	r	0.083	-0.237	0.207	-0.052
	p	0.662	0.208	0.274	0.786
Conners' global index- emotional lability	r	-0.002	-0.094	0.027	0.309
	p	0.992	0.621	0.887	0.097
Conners' global index-total	r	-0.003	-0.187	0.128	0.215
	p	0.986	0.323	0.502	0.253
DSM-IV-inattention	r	-0.015	0.051	0.232	0.225
	p	0.938	0.790	0.217	0.232
DSM-IV-hyperactivity/ impulsivity	r	-0.092	0.332	0.144	0.259
	p	0.630	0.073	0.448	0.168
DSM-IV-total	r	-0.091	0.230	0.213	0.256
	p	0.634	0.222	0.258	0.173

ADHD, attention deficit hyperactivity disorder; ADMA, asymmetric dimethylarginine; CTRS-R: L, Conners' Teacher Rating Scale-Revised: Long Form; NO, nitric oxide; r, correlation coefficient; DSM-IV, the Diagnostic and Statistical Manual of Mental Disorders 4th edition.

^aSpearman correlation test, ^bpearson correlation test.

ADMA levels in the ADHD group were significantly higher than in the control group; 4) serum adrenomedullin levels were significantly lower in the ADHD group compared to the control group. The findings of our study suggest a decrease in NO synthesis in diagnosed ADHD cases not receiving treatment. Since NO synthesis cannot be performed at a sufficient level, it can be argued that arginine levels may be increased in these cases. The reason for this decrease in NO synthesis cannot be definitively stated, but it can be said that the high ADMA levels we detected in ADHD cases may have inhibited NO synthesis by inhibiting NOS. In addition, the low levels of adrenomedullin we detected may also contribute to the decrease in NO synthesis by causing a decrease in NOS activation.

When the studies evaluating NO levels in children and adolescents with ADHD in the literature are reviewed, it is observed that the results are contradictory. In some studies, while NO levels in the ADHD group were found to be

significantly higher than in the control group [12,15], no significant differences were found between the groups in other studies [13,14]. In only one study, in accordance with ours, NO levels were found to be significantly lower in children with ADHD compared to controls [16]. There are also some animal studies that indicate low NO in ADHD. In one study, it was found that ADHD-like behaviors developed in mice with induced neuronal NOS1 deficiency [41].

Some researchers interpreted the role of NO in ADHD etiopathogenesis mostly through oxidative stress imbalance in studies that determined higher levels of NO in ADHD than healthy controls, inconsistent with our study. It has been stated that oxidant-mediated neuronal damage may play a role in the pathophysiology of psychiatric disorders such as ADHD, and cases with ADHD may have defects in coping with oxidative stress [15,42]. However, there are studies in the literature that do not support this

assumption and do not detect a direct relationship between oxidative stress and ADHD [43]. In the literature, it has been suggested that NO may also have the ability to reduce oxidative stress caused by reactive oxygen species or NO-derivative oxidants such as nitrogen oxide/peroxynitrite [44]. From a different point of view, it has been stated that nitrosative stress may be an indicator of antioxidant activity [45]. Taken together, these results suggest that there may be a more complex interplay between ADHD, NO, and oxidative stress mechanisms.

Possible mechanisms of the relationship between ADHD and low NO levels can be summarized as follows: 1) as known, dopamine and noradrenaline are very important neurotransmitters in ADHD. Noradrenaline and dopamine, which regulate prefrontal circuit function, play key roles in functions such as working memory and attention. Both noradrenaline and dopamine play important roles in the prefrontal functions by effects on synaptic transmission, intracellular signaling, and neuronal integration [46]. Animal studies have shown that NO has a stimulating effect on the release of neurotransmitters such as dopamine and noradrenaline. It has been reported that NO stimulates dopamine release in the striatum and noradrenaline release in the hippocampus of rats [47-49]; 2) there may be a bidirectional relationship between NO and dopamine. It has been shown that there is a relationship between NO-releasing neurons and dopaminergic neurons in the striatum of rats, and it has been suggested that the activity of striatal neurons that secrete NO may be regulated by direct synaptic inputs from dopaminergic neurons [50]. In this case, as low NO levels detected in our study may contribute to dopaminergic dysfunction in ADHD; dopaminergic dysfunction, which is blamed in the etiology of ADHD, may also cause low NO levels; 3) it has been reported that NO has an effect on glutamatergic modulation in addition to dopaminergic and noradrenergic systems [51]. In animal studies, NO modifies complex network areas that regulate attention and motivational areas in the brain. It has been shown to perform this function through its effect on cholinergic and glutamatergic neurotransmission [52]. In recent years, it has been stated that the glutamatergic system can also play an important role in ADHD [53,54]. Low NO may play a role in ADHD pathophysiology by causing glutamatergic dysfunction; 4) there are studies indicating a decrease in cerebral perfusion in ADHD [55,56]. Attention

is drawn to the decreased blood flow in ADHD, especially in the prefrontal cortex [57,58]. Considering the vasodilatory role of NO, it suggests that the low NO levels detected in ADHD cases in our study may be associated with decreased cerebral perfusion in ADHD. Low levels of NO may contribute to hypofrontality in ADHD, both through changes in dopamine and noradrenaline release and by decreasing prefrontal cortex blood flow; 5) there are studies indicating that there is HPA axis dysfunction in ADHD. In a study, baseline cortisol levels in patients with ADHD were found to be lower than in controls [59]. NO has been shown to stimulate adrenocorticotrophic hormone secretion in rats and to stimulate the activity of paraventricular nucleus neurons that control the HPA axis [60]. Low NO levels in the ADHD group detected in our study may also contribute to HPA axis dysfunction; 6) NO has been reported to play a role in the sleep-wake cycle [61]. In one study, short sleep time was found to be associated with vasodilator dysfunction, partially due to decreased NO bioavailability [62]. There are studies reporting sleep-wake cycle disorders in patients with ADHD [63,64]. Possible low levels of NO may also contribute to sleep disorders reported in ADHD. Further studies are needed to determine the cause-effect relationship clearly.

There are two studies in the literature evaluating arginine levels in children and adolescents with ADHD [12,13]. Only one study evaluating ADMA levels was found [12]. Sari *et al.* [13], in accordance with our study, reported that arginine levels in the ADHD group were found to be higher in the control group [13]. Jansen *et al.* [12] reported that plasma arginine levels did not differ significantly between ADHD and control subjects, and ADMA levels were significantly lower in the ADHD group. In the study by Jansen *et al.* [12], children who underwent elective surgery were included as the control group. However, as far as we understand, no detailed information was given about the reason for surgery. The results of the study may have been affected by the stressor process experienced by the control subjects related to the surgical application. In our study, we are of the opinion that enrolling completely healthy control subjects increases the strength of our study.

As far as it can be observed when the literature is reviewed, there is only one study evaluating adrenomedullin levels in children and adolescents with ADHD [14]. Gürbüz Özgür *et al.* [14] found no significant differ-

ence between the ADHD group and healthy controls in terms of adrenomedullin levels. Adrenomedullin acts as a vasodilator in the central nervous system. It achieves its vasodilator activity by decreasing vascular resistance with both an endothelium-dependent and an endothelium-independent mechanism. Studies report that it activates CGRP receptors, independent of the endothelium, and as a result, stimulates cAMP production [65]. Adrenomedullin also causes endothelium-dependent vasodilation through its specific receptors on endothelial cells. Adrenomedullin is known to have a vasodilatory effect by using the NO/cGMP pathway [26].

Adrenomedullin has been shown to activate endothelial NOS by two mechanisms: 1) it increases intracellular calcium levels that stimulate NOS activity; and 2) it activates the phosphatidylinoside 3-kinase/Akt pathway, which increases the activity of NOS and phosphorylates NOS even at low calcium concentrations [26-28]. In addition to these, it has been shown that adrenomedullin can inhibit the production of vasoconstrictors such as endothelin-1 [66]. In cerebral ischemia, it helps maintain the homeostasis of the brain by providing collateral circulation and blood supply [67]. When neuroimaging studies in the literature on ADHD are examined, there are studies indicating decreases in cerebral blood flow, especially in the prefrontal cortex [55-58]. The low level of adrenomedullin detected in our study may directly contribute to cerebral hypoperfusion, which is indicated in ADHD. In addition, in the light of the above information from the literature, it would not be wrong to presume that NO production will decrease, as NOS activation will be negatively affected when adrenomedullin levels are low; this situation may contribute to hypoperfusion. In our study, NO levels were found to be significantly lower in the ADHD group. It is thought that low adrenomedullin levels may also have an effect on this low level of NO. Adrenomedullin is known to stimulate hypothalamic NO production and HPA axis activity by activating central neuroendocrine and autonomic pathways [29]. Low adrenomedullin levels in the ADHD group detected in our study may also be related to HPA axis dysfunction. Further research is needed to clarify the findings.

In our study, depressive disorder and anxiety disorder diagnoses were excluded. However, subthreshold symptoms of depression and anxiety in the ADHD group were higher than in the control group. These subthreshold

symptoms may be related to various difficulties arising from the nature of ADHD, such as cases with ADHD are exposed to more negative stimuli, their self-esteem can be affected, the possibility of triggering anxiety by attention problems, and the higher possibility of being exposed to anxiety-provoking situations cases with ADHD than without ADHD.

Post-Treatment Evaluation

The post-treatment findings of our study can be summarized as follows: 1) after treatment, arginine levels in the ADHD group showed a significant decrease compared to pre-treatment; 2) Post-treatment and pre-treatment NO levels did not differ significantly in the ADHD group; 3) ADMA levels after treatment in the ADHD group showed significant decreases compared to pre-treatment; and 4) post-treatment and pre-treatment adrenomedullin levels did not differ significantly in the ADHD group.

In our study, with methylphenidate treatment, ADMA levels decreased significantly in ADHD cases. In a pre-clinical study, it was shown that methylphenidate analogues interact with guanido groups of arginine residues in the dopamine transporter in the rat striatum [68]. For ADMA formation, arginine residues of proteins must be methylated [17]. Methylphenidate may interact with arginine residues in proteins, reducing their methylation and thus the formation of ADMA. In their preclinical study, Li *et al.* [69] stated that DDAH1 expression increased in the striatum and frontal cortices of rats after stimulant (methamphetamine) administration. Similarly in our study, stimulant treatment may have affected DDAH levels. The possible increase in DDAH levels, the enzyme that degrades ADMA, may have reduced the ADMA levels after treatment.

It is expected that the inhibition of NOS will decrease and NO levels will increase with decreasing levels of ADMA after treatment. However, in our study, it was observed that post-treatment NO levels did not differ significantly from pre-treatment levels. Although it is not possible to make a clear comment about the exact cause of this situation, it is possible that the NO produced is used rapidly in various biochemical processes and should be investigated in further studies. On the other hand, in our study, post-treatment arginine levels were found to be significantly lower than pre-treatment. This may be related to the use of more arginine for NO synthesis, which

is expected to increase with the decrease in ADMA levels. In this study, adrenomedullin levels did not show a significant change after treatment. This can be interpreted as the absence of a direct relationship between adrenomedullin levels and the use of methylphenidate. Long-term follow-up studies are needed to clarify the issue.

As far as we can see in the literature, there are no studies that evaluate the relationship between serum arginine, NO, ADMA, and adrenomedullin levels in children and adolescents with ADHD treatment with a methodology similar to our study. There are two studies in which the effect of methylphenidate on blood levels of molecules was evaluated in different cases, as stated in introduction [12,16]. However, as in our study, it was thought that evaluating the blood levels before and after treatment in the same cases was more important in terms of evaluating the direct effect of methylphenidate.

Correlation Analysis

In our study, no significant correlations were found between the CPRS-R: L and CTRS-R: L scores, and the arginine, NO, ADMA, and adrenomedullin levels. As far as we can see, there is no study in the literature evaluating the relationship between serum arginine and ADMA levels and ADHD symptom severity in children with ADHD. Gürbüz Özgür *et al.* [14] evaluated ADHD symptoms with Turgay DSM-IV-Based Child and Adolescent Behavior Disorders Screening and Rating Scale (Parent) in their study, and reported that there was no correlation between scale scores and NO and adrenomedullin levels. Studies conducted with adult ADHD cases also support these findings [42,70]. The changes in arginine, NO, ADMA, and adrenomedullin levels we detected in our study are possibly related to the presence of ADHD rather than its severity.

Our study should be interpreted together with its strengths and limitations. One of the strengths of our study is that it is a controlled study and that the changes in blood parameters according to treatment were examined. It is thought that the inclusion of ADMA and adrenomedullin in the study together with arginine and NO increases the strength of our study. Another strength is the exclusion of confounding factors as methodologically as possible. The small sample size and the inability to evaluate ADHD subtypes are limitations of our study. Another limitation is that the effect of treatment in the ADHD group was eval-

uated after ten weeks. Longer treatment times may be required to better observe the changes in some parameters. In addition, looking at these parameters in other body fluids such as cerebrospinal fluid and urine and in different tissues may give more valid results. In addition, simultaneous evaluation of other metabolites of arginine such as agmatine, putrescine, and ornithine may provide more holistic findings for the etiology. The absence of the use of a standard test to evaluate the intelligence levels of the cases was considered as a limitation. Lastly, since ODD is frequently comorbid with ADHD, we could not exclude ODD.

In conclusion, the results of our study point to low NO and high arginine levels, which are thought to be associated with high ADMA levels and low adrenomedullin levels, in children diagnosed with ADHD. After ten weeks of methylphenidate treatment, there was a decrease in ADMA and arginine levels, but NO and adrenomedullin levels did not change significantly. Further research is needed on the subject.

Funding

This study was supported by the decision of Firat University Scientific Research Projects Coordination Unit TF.18.43.

Acknowledgments

The authors thank the cases and their parents who participated in this study.

This article is produced from the thesis entitled "Evaluation of the levels of arginine/nitric oxide and associated molecules and orexin and the factors that may be related to them in children aged 6–12 years diagnosed with attention deficit hyperactivity disorder" defended by Dr. Ebru Doneray.

Conflicts of Interest

No potential conflict of interest relevant to this article was reported.

Author Contributions

All authors contributed to the study conception and design. Data collection: Ebru Doneray, Kemal Utku Yazici, and Ipek Percinel Yazici. Biochemical material preparation and biochemical analysis: Bilal Ustundag. Writing—first draft: Ebru Doneray, Kemal Utku Yazici,

and Ipek Percinel Yazici, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

■ ORCID

Ebru Doneray <https://orcid.org/0000-0001-9668-5486>
 Kemal Utku Yazici <https://orcid.org/0000-0001-8659-6340>
 Ipek Percinel Yazici <https://orcid.org/0000-0002-6807-655X>
 Bilal Ustundag <https://orcid.org/0000-0001-6621-2450>

REFERENCES

- Moriyama TS, Cho AJM, Verin RE, Fuentes J, Polanczyk GW. Attention deficit hyperactivity disorder. In: Rey JM, editor. *IACAPAP e-textbook of child and adolescent mental health*. Geneva:International Association for Child and Adolescent Psychiatry and Allied Professions;2012. p.4-13.
- Soeters PB, Hallemeesch MM, Bruins MJ, van Eijk HM, Deutz NE. Quantitative in vivo assessment of arginine utilization and nitric oxide production in endotoxemia. *Am J Surg* 2002;183:480-488.
- Laube G, Bernstein HG. Agmatine: multifunctional arginine metabolite and magic bullet in clinical neuroscience? *Biochem J* 2017;474:2619-2640.
- Yilmaz ED, Üstündağ MF, Gençer AG, Kivrak Y, Ünal Ö, Bilici M. Levels of nitric oxide, asymmetric dimethyl arginine, symmetric dimethyl arginine, and L-arginine in patients with obsessive-compulsive disorder. *Turk J Med Sci* 2016;46:775-782.
- Guix FX, Uribesalgo I, Coma M, Muñoz FJ. The physiology and pathophysiology of nitric oxide in the brain. *Prog Neurobiol* 2005;76:126-152.
- Herken H, Gurel A, Selek S, Armutcu F, Ozen ME, Bulut M, et al. Adenosine deaminase, nitric oxide, superoxide dismutase, and xanthine oxidase in patients with major depression: impact of antidepressant treatment. *Arch Med Res* 2007;38:247-252.
- Wiesinger H. Arginine metabolism and the synthesis of nitric oxide in the nervous system. *Prog Neurobiol* 2001;64:365-391.
- Zoroglu SS, Herken H, Yürekli M, Uz E, Tutkun H, Savaş HA, et al. The possible pathophysiological role of plasma nitric oxide and adrenomedullin in schizophrenia. *J Psychiatr Res* 2002;36:309-315.
- Hess S, Baker G, Gyenes G, Tsuyuki R, Newman S, Le Melleo JM. Decreased serum L-arginine and L-citrulline levels in major depression. *Psychopharmacology (Berl)* 2017;234:3241-3247.
- Sweeten TL, Posey DJ, Shankar S, McDougale CJ. High nitric oxide production in autistic disorder: a possible role for interferon-gamma. *Biol Psychiatry* 2004;55:434-437.
- Lu YR, Fu XY, Shi LG, Jiang Y, Wu JL, Weng XJ, et al. Decreased plasma neuroactive amino acids and increased nitric oxide levels in melancholic major depressive disorder. *BMC Psychiatry* 2014;14:123.
- Jansen K, Hanusch B, Pross S, Hanf E, Drabert K, Bollenbach A, et al. Enhanced nitric oxide (NO) and decreased ADMA synthesis in pediatric ADHD and selective potentiation of NO synthesis by methylphenidate. *J Clin Med* 2020;9:175.
- Sari SA, Ulger D, Ersan S, Bakir D, Uzun Cicek A, Ismailoglu F. Effects of agmatine, glutamate, arginine, and nitric oxide on executive functions in children with attention deficit hyperactivity disorder. *J Neural Transm (Vienna)* 2020;127:1675-1684.
- Gürbüz Özgür B, Aksu H, Yılmaz M, Karakoç Demirkaya S. The probable role of adrenomedullin and nitric oxide in childhood attention deficit hyperactivity disorder. *Nord J Psychiatry* 2017;71:521-524.
- Avci S, Uysal P, Yenisey Ç, Abas BI. Elevated melatonin levels in children with attention deficit hyperactivity disorder: relationship to oxidative and nitrosative stress. *J Atten Disord* 2021;25:693-703.
- Varol Tas F, Guvenir T, Tas G, Cakaloz B, Ormen M. Nitric oxide levels in disruptive behavioral disorder. *Neuropsychobiology* 2006;53:176-180.
- Teerlink T, Luo Z, Palm F, Wilcox CS. Cellular ADMA: regulation and action. *Pharmacol Res* 2009;60:448-460.
- Vallance P, Leone A, Calver A, Collier J, Moncada S. Endogenous dimethylarginine as an inhibitor of nitric oxide synthesis. *J Cardiovasc Pharmacol* 1992;20 Suppl 12:S60-S62.
- Celik C, Cayci T, Ozdemir B, Akgul EO, Zincir S, Balikli A, et al. Plasma asymmetric dimethylarginine (ADMA) concentrations in patients with first and multiple episode schizophrenia. *Psychiatry Res* 2011;190:177-180.
- Das I, Khan NS, Puri BK, Hirsch SR. Elevated endogenous nitric oxide synthase inhibitor in schizophrenic plasma may reflect abnormalities in brain nitric oxide production. *Neurosci Lett* 1996;215:209-211.
- Selley ML. Increased (E)-4-hydroxy-2-nonenal and asymmetric dimethylarginine concentrations and decreased nitric oxide concentrations in the plasma of patients with major depression. *J Affect Disord* 2004;80:249-256.
- Zincir S, Zincir SB, Doruk A, Erdem M, Celik C, Ak M, et al. Asymmetric dimethylarginine (ADMA) and treatment response relationship in male patients with first-episode schizophrenia: a controlled study. *Psychiatry Res* 2014;220:76-80.
- Baranyi A, Amouzadeh-Ghadikolai O, Rothenhäusler HB, Theokas S, Robier C, Baranyi M, et al. Nitric oxide-related biological pathways in patients with major depression. *PLoS One* 2015;10:e0143397.
- Kato J, Tsuruda T, Kitamura K, Eto T. Adrenomedullin: a possible autocrine or paracrine hormone in the cardiac ventricles. *Hypertens Res* 2003;26 Suppl:S113-S119.
- Serrano J, Alonso D, Fernández AP, Encinas JM, López JC,

- Castro-Blanco S, et al. Adrenomedullin in the central nervous system. *Microsc Res Tech* 2002;57:76-90.
26. Hayakawa H, Hirata Y, Kakoki M, Suzuki Y, Nishimatsu H, Nagata D, et al. Role of nitric oxide-cGMP pathway in adrenomedullin-induced vasodilation in the rat. *Hypertension* 1999;33:689-693.
 27. Nishimatsu H, Suzuki E, Nagata D, Moriyama N, Satonaka H, Walsh K, et al. Adrenomedullin induces endothelium-dependent vasorelaxation via the phosphatidylinositol 3-kinase/Akt-dependent pathway in rat aorta. *Circ Res* 2001;89:63-70.
 28. Shimekake Y, Nagata K, Ohta S, Kambayashi Y, Teraoka H, Kitamura K, et al. Adrenomedullin stimulates two signal transduction pathways, cAMP accumulation and Ca²⁺ mobilization, in bovine aortic endothelial cells. *J Biol Chem* 1995;270:4412-4417.
 29. Shan J, Krukoff TL. Intracerebroventricular adrenomedullin stimulates the hypothalamic-pituitary-adrenal axis, the sympathetic nervous system and production of hypothalamic nitric oxide. *J Neuroendocrinol* 2001;13:975-984.
 30. Kaufman J, Birmaher B, Brent D, Rao U, Flynn C, Moreci P, et al. 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* 1997;36:980-988.
 31. Gökler B, Ünal F, Pehlivan Türk B, Kültür EÇ, Akdemir D, Taner Y. [Reliability and validity of schedule for affective disorders and schizophrenia for school age children--present and lifetime version--Turkish version (K-SADS-PL-T)]. *Çocuk ve Gençlik Ruh Sağlığı Dergisi* 2004;11:109-116. Turkish.
 32. Conners CK, Sitarenios G, Parker JD, Epstein JN. The revised Conners' Parent Rating Scale (CPRS-R): factor structure, reliability, and criterion validity. *J Abnorm Child Psychol* 1998;26:257-268.
 33. Kaner S, Büyüköztürk Ş, İseri E, Ak A, Özaydın L. [Conners' parent rating scale long form-revised: factor structure, reliability and validity studies]. *Turk J Child Adolesc Ment Health* 2011;18:45-58. Turkish.
 34. Conners CK, Sitarenios G, Parker JD, Epstein JN. Revision and restandardization of the Conners Teacher Rating Scale (CTRS-R): factor structure, reliability, and criterion validity. *J Abnorm Child Psychol* 1998;26:279-291.
 35. Kaner S, Buyukozturk S, Iseri E, Ak A, Ozaydin L. The validity and reliability study of the Turkish version of Conners' Teacher Rating Scale-Revised (CTRS-R). In: *Proceedings of World Psychiatric Association International Congress; Jul 12-16, 2006; Istanbul, Turkey*.
 36. Kovacs M. Rating scales to assess depression in school-aged children. *Acta Paedopsychiatr* 1981;46:305-315.
 37. Öy B. [The children's depression inventory: validity and reliability study]. *Turk J Psychiatry* 1991;2:132-136. Turkish.
 38. Spielberger C, Edwards CD, Lushene J. State-trait anxiety inventory for children. Palo Alto:Consulting Psychologists Press;1973.
 39. Ozusta HS. [Turkish standardization, reliability and validity of the state-trait anxiety inventory for children]. *Turk Psikoloji Dergisi* 1995;10:32-44. Turkish.
 40. Guy W. ECDEU assessment manual for psychopharmacology, revised. US Department of Health, Education, and Welfare Publication (ADM). Rockville:National Institute of Mental Health;1976. p.218-222.
 41. Gao Y, Heldt SA. Lack of neuronal nitric oxide synthase results in attention deficit hyperactivity disorder-like behaviors in mice. *Behav Neurosci* 2015;129:50-61.
 42. Selek S, Savas HA, Gergerlioglu HS, Bulut M, Yilmaz HR. Oxidative imbalance in adult attention deficit/hyperactivity disorder. *Biol Psychol* 2008;79:256-259.
 43. Oztop D, Altun H, Baskol G, Ozsoy S. Oxidative stress in children with attention deficit hyperactivity disorder. *Clin Biochem* 2012;45:745-748.
 44. Espey MG, Miranda KM, Thomas DD, Xavier S, Citrin D, Vitek MP, et al. A chemical perspective on the interplay between NO, reactive oxygen species, and reactive nitrogen oxide species. *Ann N Y Acad Sci* 2002;962:195-206.
 45. Pacher P, Beckman JS, Liaudet L. Nitric oxide and peroxynitrite in health and disease. *Physiol Rev* 2007;87:315-424.
 46. Xing B, Li YC, Gao WJ. Norepinephrine versus dopamine and their interaction in modulating synaptic function in the prefrontal cortex. *Brain Res* 2016;1641(Pt B):217-233.
 47. Satoh S, Kimura T, Toda M, Miyazaki H, Ono S, Narita H, et al. NO donors stimulate noradrenaline release from rat hippocampus in a calmodulin-dependent manner in the presence of L-cysteine. *J Cell Physiol* 1996;169:87-96.
 48. Stewart TL, Michel AD, Black MD, Humphrey PP. Evidence that nitric oxide causes calcium-independent release of [3H] dopamine from rat striatum in vitro. *J Neurochem* 1996;66:131-137.
 49. Ohkuma S, Katsura M. Nitric oxide and peroxynitrite as factors to stimulate neurotransmitter release in the CNS. *Prog Neurobiol* 2001;64:97-108.
 50. Fujiyama F, Masuko S. Association of dopaminergic terminals and neurons releasing nitric oxide in the rat striatum: an electron microscopic study using NADPH-diaphorase histochemistry and tyrosine hydroxylase immunohistochemistry. *Brain Res Bull* 1996;40:121-127.
 51. Dawson TM, Dawson VL. Nitric oxide signaling in neurodegeneration and cell death. *Adv Pharmacol* 2018;82:57-83.
 52. Grammatikopoulos G, Pignatelli M, D'Amico F, Fiorillo C, Fresiello A, Sadile AG. Selective inhibition of neuronal nitric oxide synthesis reduces hyperactivity and increases non-selective attention in the Naples High-Excitability rat. *Behav Brain Res* 2002;130:127-132.
 53. Carrey NJ, MacMaster FP, Gaudet L, Schmidt MH. Striatal creatine and glutamate/glutamine in attention-deficit/hyperactivity disorder. *J Child Adolesc Psychopharmacol* 2007;17:11-17.
 54. Huang X, Wang M, Zhang Q, Chen X, Wu J. The role of gluta-

- mate receptors in attention-deficit/hyperactivity disorder: from physiology to disease. *Am J Med Genet B Neuropsychiatr Genet* 2019;180:272-286.
55. Kim BN, Lee JS, Shin MS, Cho SC, Lee DS. Regional cerebral perfusion abnormalities in attention deficit/hyperactivity disorder. Statistical parametric mapping analysis. *Eur Arch Psychiatry Clin Neurosci* 2002;252:219-225.
56. da Silva N Jr, Szobot CM, Anselmi CE, Jackowski AP, Chi SM, Hoexter MQ, et al. Attention deficit/hyperactivity disorder: is there a correlation between dopamine transporter density and cerebral blood flow? *Clin Nucl Med* 2011;36:656-660.
57. Spalletta G, Pasini A, Pau F, Guido G, Menghini L, Caltagirone C. Prefrontal blood flow dysregulation in drug naive ADHD children without structural abnormalities. *J Neural Transm (Vienna)* 2001;108:1203-1216.
58. Kim BN, Kim JW, Kang H, Cho SC, Shin MS, Yoo HJ, et al. Regional differences in cerebral perfusion associated with the alpha-2A-adrenergic receptor genotypes in attention deficit hyperactivity disorder. *J Psychiatry Neurosci* 2010;35:330-336.
59. Ma L, Chen YH, Chen H, Liu YY, Wang YX. The function of hypothalamus-pituitary-adrenal axis in children with ADHD. *Brain Res* 2011;1368:159-162.
60. Lee S, Kim CK, Rivier C. Nitric oxide stimulates ACTH secretion and the transcription of the genes encoding for NGF-B, corticotropin-releasing factor, corticotropin-releasing factor receptor type 1, and vasopressin in the hypothalamus of the intact rat. *J Neurosci* 1999;19:7640-7647.
61. Virarkar M, Alappat L, Bradford PG, Awad AB. L-arginine and nitric oxide in CNS function and neurodegenerative diseases. *Crit Rev Food Sci Nutr* 2013;53:1157-1167.
62. Bain AR, Weil BR, Diehl KJ, Greiner JJ, Stauffer BL, DeSouza CA. Insufficient sleep is associated with impaired nitric oxide-mediated endothelium-dependent vasodilation. *Atherosclerosis* 2017;265:41-46.
63. Cortese S, Konofal E, Yateaman N, Mouren MC, Lecendreux M. Sleep and alertness in children with attention-deficit/hyperactivity disorder: a systematic review of the literature. *Sleep* 2006;29:504-511.
64. Konofal E, Lecendreux M, Cortese S. Sleep and ADHD. *Sleep Med* 2010;11:652-658.
65. Figueira L, Israel A. Cerebellar adrenomedullinergic system. Role in cardiovascular regulation. *Adv Exp Med Biol* 2017;956:541-560.
66. Kohno M, Kano H, Horio T, Yokokawa K, Yasunari K, Takeda T. Inhibition of endothelin production by adrenomedullin in vascular smooth muscle cells. *Hypertension* 1995;25:1185-1190.
67. Dogan A, Suzuki Y, Koketsu N, Osuka K, Saito K, Takayasu M, et al. Intravenous infusion of adrenomedullin and increase in regional cerebral blood flow and prevention of ischemic brain injury after middle cerebral artery occlusion in rats. *J Cereb Blood Flow Metab* 1997;17:19-25.
68. Volz TJ, Bjorklund NL, Schenk JO. Methylphenidate analogs with behavioral differences interact differently with arginine residues on the dopamine transporter in rat striatum. *Synapse* 2005;57:175-178.
69. Li X, Wang H, Qiu P, Luo H. Proteomic profiling of proteins associated with methamphetamine-induced neurotoxicity in different regions of rat brain. *Neurochem Int* 2008;52:256-264.
70. Selek S, Bulut M, Ocak AR, Kalenderoğlu A, Savaş HA. Evaluation of total oxidative status in adult attention deficit hyperactivity disorder and its diagnostic implications. *J Psychiatr Res* 2012;46:451-455.