Original Article
https://doi.org/10.9758/cpn.2023.21.1.88
Clinical Psychopharmacology and Neuroscience 2023;21(1):88-98
pISSN 1738-1088 / eISSN 2093-4327
Copyright© 2023, Korean College of Neuropsychopharmacology

The Effect of Drug Use, Body Mass Index and Blood Pressure on Oxidative Stress Levels in Children and Adolescents with Attention Deficit and Hyperactivity Disorder

Dilşad Yıldız Miniksar[1], Mehmet Akif Cansız[1], Ayşe Yeşim Göçmen[2], Mahmut Kılıç[3], Ökkeş Hakan Miniksar[4]

Departments of [1]Child and Adolescent Psychiatry, [2]Biochemistry, [3]Public Health, [4]Anesthesiology and Reanimation, Yozgat Bozok University, Yozgat, Turkey

Objective: The aim of this study was to determine the correlation between clinical variables such as body mass index, blood pressure, drug use and oxidative stress level in children and adolescents with attention deficit and hyperactivity disorder (ADHD).
Methods: Total oxidant status (TOS), total antioxidant status (TAS), oxidative stress index (OSI), malondialdehyde (MDA) and superoxide dismutase (SOD) activity were measured in the serum of 51 patients (38 male, 13 female) diagnosed with ADHD according to DSM-5 diagnostic criteria and 32 control subjects (12 male, 20 female). The Kiddie Schedule for Affective Disorders and Schizophrenia Present and Lifetime Version was applied to all participants.
Results: The mean TOS, OSI, MDA values were determined to be significantly higher ($p < 0.001$) and the mean SOD value was lower in the ADHD group ($p < 0.001$). Multivariate regression analysis indicated significantly lower SOD and diastolic blood pressure values and significantly higher MDA in the ADHD group compared to the control group ($p < 0.01$). Low SOD (sensitivity 90.2%, specificity 78.0%) and high MDA (sensitivity 86.3%, specificity 81.2%) were determined to be predictive parameters for diagnosing ADHD. In univariate analysis, the mean TOS, OSI and SOD values were higher in ADHD patients under medication, while the mean TAS was higher in patients not using medication ($p < 0.01$). Only TOS was significant in multivariate logistic regression analysis ($p < 0.01$).
Conclusion: The results of this study demonstrate that impaired oxidative balance may play a role in the etiology of ADHD.

KEY WORDS: Attention deficit hyperactivity disorder; Oxidative stress; Malondialdehyde; Superoxide dismutase.

INTRODUCTION

Attention deficit and hyperactivity disorder (ADHD) is the most common neurodevelopmental disorder with an early childhood onset prevalence of 5−10% [1]. It is more common in boys than girls and is associated with symptoms of impulsiveness, difficulty concentrating, and hyperactivity [2]. Although the interaction of various neurobiological factors is thought to play a role in the etiology of ADHD, the mechanisms underlying this disorder are still largely unknown [3]. One of the areas investigated in studies on ADHD etiology is oxidative stress. Free radicals that are produced by the reduction reactions are highly toxic and can cause cell damage or even death by affecting the basic structures of cells [4].

Increase in free radicals such as nitric oxide (NO) and malondialdehyde (MDA), and a decrease in antioxidant molecules such as glutathione peroxidase and superoxide dismutase (SOD) have been observed in many psychiatric diseases such as schizophrenia, bipolar affective disorder, depression, autism spectrum disorders and ADHD. Thus, disturbance in the balance between the production of free radicals and antioxidant defences is involved. It has been hypothesized that the effect of oxidative stress arises from the reaction of oxidants with cell membrane proteins. This may lead to deterioration in neuronal cell membrane

Received: July 28, 2021 / **Revised:** October 16, 2021
Accepted: November 1, 2021
Address for correspondence: Dilşad Yıldız Miniksar
Department of Child and Adolescent Psychiatry, Yozgat Bozok University, Atatürk Road 7. Km Azizli/Yozgat 66100, Turkey
E-mail: dr_dilsad1984@hotmail.com
ORCID: https://orcid.org/0000-0002-6389-4377

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.

structure, which may also affect the neuronal receptors, thus causing deterioration of neuron functions. Disruptions in neuronal cell membranes may cause adverse effects on neurotransmitters, especially norepinephrine and dopamine [5-9]. At the same time, inflammation in the brain occurs as a result of the production of reactive oxygen radicals. Neuroinflammation is known to have a significant role in the pathophysiology of ADHD [10]. Therefore, in the neuroinflammation developing associated with the increase in oxidative stress in ADHD, another pathophysiological hypothesis has emerged between ADHD and oxidative stress [11]. ADHD is a neurodevelopmental disorder, in which genetic and environmental factors together with exposure to toxins play a role in the etiology. Genetic and environmental factors and risk factors in pregnancy may contribute to an increase in oxidative stress in ADHD [12,13]. However, the reports of studies on oxidative stress and ADHD are conflicting and inconsistent.

In adult ADHD patients, total antioxidant status (TAS), total oxidant status (TOS) and oxidative stress index (OSI) (TOS / TAS) have been found to be higher than in the control group, but despite this increase in TAS, the oxidant-antioxidant balance was still disturbed. The increase in TAS level might be related to the attempt to balance the increased oxidative load [14]. The level of MDA, a marker of lipid peroxidation, has been shown to be high in some studies and low in others in both adult and adolescent ADHD groups [8,15-18].

One of the most common causes of obesity is nutritional obesity, which increases oxidative stress by leading to lipid peroxidation associated with dietary intake of free fatty acid exceeding the antioxidant capacity [19]. There is a high risk of obesity in individuals with ADHD, and it is assumed that together with obesity, oxidative stress, which plays a role in the etiopathogenesis of ADHD, will increase further [20]. Free oxygen radicals and oxidative stress are also known to increase blood pressure [21]. As a result of the accumulation in the body of the drugs used for several different diseases, free radicals form and oxidative stress increases [22]. Therefore, the aim of this study was to evaluate these parameters that affect oxidative stress.

To the best of our knowledge, there is no study in the literature that has examined the correlation between oxidative stress and body mass index (BMI), blood pressure, and drug use in children and adolescent ADHD patients. The primary goal of this study was to investigate the relationship between BMI, blood pressure and the oxidant-antioxidant balance in children and adolescents with ADHD, and the secondary goal was to determine the factors affecting oxidative stress parameters.

METHODS

The study included 51 children (7－18 years), who were diagnosed with ADHD according to the Diagnostic and Statistical Manual of Mental Disorders 5th edition (DSM-5) criteria (American Psychiatric Association [APA] 2013) and semi-structured interviews (via K-SADS-PL-Turkish) and who gave verbal assent with parental written informed consent for participation. Patients were excluded from the study if they had any intellectual disability, autism spectrum disorder, medical comorbidity (with pediatric consultation), or comorbid psychopathology other than oppositional defiant disorder (ODD, via K-SADS-PL). The diagnostic phase of the study was based on the Turgay DSM-IV and Disruptive Behavior Disorders Rating Scale (T-DSM-IV-S) completed by the parents. The Wechsler Intelligence Scale for Children-Revised (WISC-R) was applied to all the participants to eliminate any patients with intellectual disability. BMI was evaluated according to height and weight, and the BMI percentiles of all the participants were within the normal range for age according to World Health Organization parameters (https://www.who.int/growthref/who2007_bmi_for_age/en/, accessed on September 9, 2018). Blood pressure was measured for each participant. A control group was formed of 32 mentally and physically healthy children and adolescents in the age range of 7－18 years, with similar sociodemographic characteristics. Written informed consent was obtained from the parents or legal guardians of all the participants. The research protocol was approved by the Research Ethics Committee of the University of Yozgat Bozok. All study procedures were conducted in accordance with the principles of the Declaration of Helsinki and local laws and regulations.

Instruments

Kiddie Schedule for Affective Disorders and Schizophrenia for School Age Children-Present and Lifetime Version (K-SADS-PL)-Turkish

This semi-structured interview was developed by

Kaufman *et al.* [23] to evaluate present and lifetime psychopathology in children and adolescents according to DSM-IV criteria. The reliability and validity study of the Turkish translation was conducted by Gökler *et al.* [24]. The K-SADS-PL-Turkish version was used in this study for the ADHD diagnoses and to exclude those with comorbid psychopathologies (except ODD).

The Turgay DSM-IV-Based Child and Adolescent Disruptive Behavioral Disorders Screening and Rating Scale (T-DSM-IV-S)

This scale was developed by Turgay [25] to screen and rate disruptive behavior disorders. A validity and reliability study for the Turkish population was carried out by Ercan *et al.* [26].

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

The WISC-R scale measures the intelligence levels of children aged between 6 years and 16 years 11 months 30 days. It was adapted to Turkish by Uluç *et al.* [27].

Blood Collection and Preparation

Following a 12-hour overnight fast, 3−5 ml venous blood was drawn and transferred into a biochemistry tube. The sera were separated with centrifugation at 4,000 rpm for 10 minutes. All samples were stored at −80°C until analysis.

Determination of total antioxidant status and total oxidant status

Serum TAS and TOS were determined with commercial kits (Rel Assay Diagnostics kit; Mega Tıp, Gaziantep, Turkey) developed by Erel and OSI values were calculated.

Serum TAS was measured by generation of 2,2′-azino-di-(3-ethylbenzthiazoline sulphonate) radical cation using the commercial kit according to the manufacturer's manual.

TOS was measured as described by the manufacturer's protocol. In this method, the oxidants present in the sample oxidized the ferrous ion-o-dianisidine complex to ferric ion. Ferric ion produces a colored complex with xylenol orange in an acidic medium. The color intensity, which can be measured spectrophotometrically, is related to the total amount of oxidant molecules present in the sample. The assay was calibrated with hydrogen peroxide and the results were expressed in terms of μmol H_2O_2 equivalent/L of serum.

Calculation of oxidative stress index

The TOS: TAS ratio was used as the OSI, and was calculated as follows: OSI (arbitrary units) = [(TOS, μmol H_2O_2/L) / (TAS, μmol Trolox equiv./L)].

Determination of superoxide dismutase activity

Total SOD activity was determined using the SOD Activity Assay kit (Rel Assay Diagnostics kit; Mega Tıp), according to the manufacturer's instructions.

Determination of malondialdehyde

MDA levels were measured using a colorimetric kit (Cayman Chemical, Ann Arbor, MI, USA).

Statistical Analyses

Data obtained in the study were analyzed using statistics software (IBM Co., Armonk, NY, USA). Descriptive tables of the data were formed. The arithmetic means of the variables in the ADHD and control group were compared using the Independent Samples *t* test. Non-parametric tests were used in the analysis of variables with non-homogeneous variance. The Chi-square test was applied in the comparisons of frequencies. Correlations between continuous variables were established using Spearman's correlation analysis since the weight percentile (WP), height percentile (HP) and body mass index percentile (BMI-P) were ordinal data. The effects of systolic blood pressure (SBP), diastolic blood pressure (DBP), TAS, TOS, MDA, SOD markers, the presence of ADHD (dummy) and other variables were analyzed using the linear regression (LR) forward LR model. The presence of ADHD and sex, which are categorical variables, were set as dummy variables in the LR analysis. The factors affecting the probability of being in the ADHD group, being in the ADHD combined type and the probability of using ADHD drugs were analyzed using the binary logistic regression (BLR) forward model. Measurement units of continuous variables included in the BLR analysis were standardized by conversion to a Z score. In the BLR analysis, the goodness of fit was evaluated using the Hosmer and Lemeshow test and the effect size with the Nagelkerke R Square. Before the analysis, the homogeneity of the model was examined. The variables found to be significant in the analyses are shown in the tables.

RESULTS

The rate of males was 74.5% (n = 38) in the ADHD group and 37.5% (n = 12) in the control group. The mean age in the control group (13.69 ± 3.26 years) was higher than in the ADHD group (10.08 ± 2.72 years). The weight, height, and BMI percentile, SBP and DBP values were also higher in the control group. The mean TAS, TOS, OSI, and MDA values were higher, the mean SOD value was lower in the ADHD group compared to the control group. With the exception of TAS, these differences were statistically significant ($p < 0.001$) (Table 1). A significant correlation was found between oxidative stress parameters (TAS, TOS, OSI, MDA, and SOD) and age, height percentile, weight percentile, BMI percentile, SBP, and DBP parameters in both the ADHD and control groups (excluding MDA). A weak positive correlation ($r = 0.424$) was found between the MDA values of the control group and DBP ($p < 0.05$).

When the affecting factors were analyzed with BLR by setting ADHD = 1 and control group = 0, the increase in MDA value and the decrease in SOD and DBP values increased the probability of ADHD. In comparison with the control group, MDA was higher and the SOD and DBP values were lower in the ADHD group. These factors were seen to be predictors for ADHD and explained 93.9% of the disease presence (Nagelkerke $R^2 = 0.939$). The factors of age, SBP, WP, HP (borderline insignificant), TOS and OSI, which differed significantly between the ADHD and control groups in the univariate tests, were not included in the table because they were not statistically significant when analyzed with the BLR forward model (Table 2).

Table 1. Mean and standard deviation values of variables in the ADHD group and control group

Variable	ADHD (n = 51)		Control (n = 32)		p value
	Value	Range	Value	Range	
Male	38 (74.5)		12 (37.5)		0.001[a]
Female	13 (25.5)		20 (62.5)		
Age (yr)	10.08 ± 2.72	7−18	13.69 ± 3.26	7−18	< 0.001[b]
Weight percentile	47.76 ± 33.64	3−97	66.25 ± 32.26	3−97	0.036[b]
Height percentile	47.94 ± 32.99	3−97	62.22 ± 34.32	3−97	0.062[b]
BMI percentile	53.00 ± 31.65	3−97	64.00 ± 30.71	3−97	0.123[b]
SBP (mmHg)	94.80 ± 8.18	80−110	106.09 ± 13.36	80−150	< 0.001[b]
DBP (mmHg)	54.90 ± 5.70	45−70	65.16 ± 7.13	60−85	< 0.001[b]
TAS (μmol Trolox Eq/L)	0.72 ± 0.08	0.57−0.95	0.70 ± 0.06	0.60−0.80	0.303[b]
TOS (μmol H_2O_2 Eq/L)	9.23 ± 1.43	7.21−12.77	7.44 ± 0.74	5.96−9.88	< 0.001[c]
OSI (arbitrary unit)	13.10 ± 3.07	7.84−22.02	10.64 ± 1.11	8.35−12.93	< 0.001[c]
MDA (μmol/L)	0.33 ± 0.03	0.26−0.40	0.28 ± 0.03	0.25−0.37	< 0.001[b]
SOD (U/ml)	15.81 ± 1.29	13.46−19.23	18.30 ± 1.04	15.06−19.84	< 0.001[b]

Values are presented as number (%) or mean ± standard deviation.
ADHD, attention deficit hyperactivity disorder; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; TAS, total antioxidant status; TOS, total oxidant status; OSI, oxidative stress index; MDA, malondialdehyde; SOD, superoxide dismutase.
[a]Chi-square test, [b]Independent samples t test, [c]Mann−Whitney U test.

Table 2. Analysis of factors affecting children with ADHD with the binary logistic regression forward LR model

Variable	B	p value	Exp (B)	95% CI for Exp (B)	
				Lower	Upper
MDA-Z score	4.943	0.014	140.132	2.707	7,254.256
SOD- Z score	−3.331	0.008	0.036	0.003	0.423
DBP- Z score	−7.669	0.020	0.000	< 0.001	0.299
Constant	4.620	0.024	101.538		

ADHD, attention deficit hyperactivity disorder; LR, linear regression; MDA, malondialdehyde; SOD, superoxide dismutase; DBP, diastolic blood pressure; CI, confidence interval.
Independent variables Z score entered model. Hosmer and Lemeshow test $p = 1.000$, Nagelkerke $R^2 = 0.939$.

The MDA and SOD cut-off values were determined with a receiver operating characteristic (ROC) curve and the area under the curve values were 0.915 (95% confidence interval [CI] 0.851−0.960) for SOD and 0.868 (95% CI, 0.777−0.958) for MDA ($p < 0.001$) (Figs. 1, 2). When the cut-off value for SOD was ≤ 17.65, sensitivity was 90.2% and specificity was 78.0%. When the cut-off

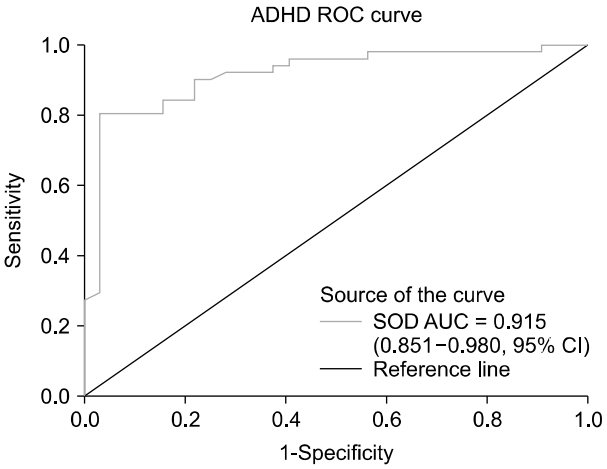

Fig. 1. Receiver operating characteristic (ROC) curve analysis of the effect of superoxide dismutase (SOD) values in the detection of attention deficit hyperactivity disorder (ADHD). The SOD area under the curve (AUC) value was smaller, which indicates a more positive effect on the detection of ADHD.
CI, confidence interval.

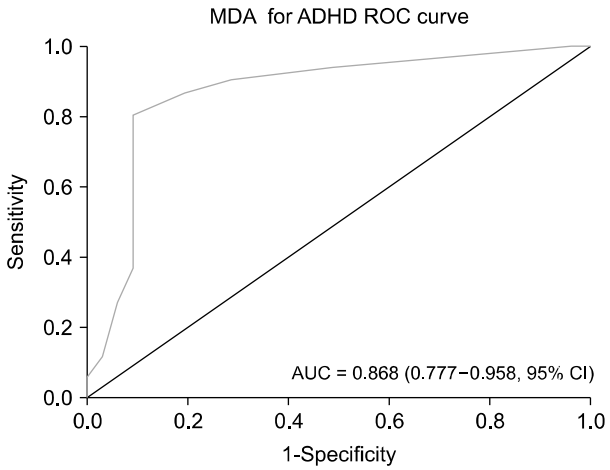

Fig. 2. Receiver operating characteristic (ROC) curve analysis of the effect of malondialdehyde (MDA) values in the detection of attention deficit hyperactivity disorder (ADHD).
AUC, area under the curve; CI, confidence interval.

Table 3. Analysis of factors affecting TAS, TOS, MDA, and SOD values with the linear regression stepwise model

Variable	Unstandardized coefficients	95% confidence interval for B		Standardized coefficients	*t*	*p* value
	B	Lower bound	Upper bound	β		
TAS (Adj.R^2 = 0.169)						
(constant)	1.097	0.893	1.301		10.706	< 0.001
SOD	−0.017	−0.026	−0.008	−0.399	−3.658	< 0.001
TOS	−0.015	−0.026	−0.005	−0.320	−2.950	0.004
BMI-P	0.000	0.000	0.001	0.217	2.139	0.036
TOS (Adj.R^2 = 0.452)						
(constant)	6.762	3.090	10.435		3.665	< 0.001
MDA	12.972	4.663	21.282	0.334	3.107	0.003
TAS	−4.290	−7.739	−0.840	−0.207	−2.475	0.015
ADHD = PRESENT	1.218	0.564	1.873	0.400	3.704	< 0.001
OSI (TOS / TAS) (Adj.R^2 = 0.270)						
(constant)	12.156	11.639	12.673		46.785	0.000
MDA	1.463	0.943	1.983	0.528	5.597	0.000
MDA (Adj.R^2 = 0.495)						
(constant)	0.142	0.066	0.219		3.701	< 0.001
ADHD = PRESENT	0.044	0.027	0.062	0.564	4.988	< 0.001
TOS	0.009	0.004	0.014	0.365	3.748	< 0.001
DBP	0.001	0.000	0.002	0.230	2.275	0.026
SOD (Adj.R^2 = 0.530)						
(constant)	21.235	18.678	23.793		16.522	< 0.001
ADHD = PRESENT	−2.418	−2.947	−1.890	−0.694	−9.105	< 0.001
TAS	−4.182	−7.782	−0.582	−0.176	−2.312	0.023

TAS, total antioxidant status; TOS, total oxidant status; MDA, malondialdehyde; SOD, superoxide dismutase; ADHD, attention deficit hyperactivity disorder; DBP, diastolic blood pressure; BMI-P, body mass index percentile.

value for MDA was ≥ 0.295, sensitivity was 86.3% and specificity was 81.2%. These two parameters can be used as predictors for the diagnosis of ADHD.

When factors that affect oxidative stress parameters (TAS, TOS, OSI, MDA, and SOD) were analyzed with linear regression (Table 3), the decrease of SOD (β = −0.399) and TOS (β = −0.320) values (in the order of importance) and the increase of BMI-P (β = 0.217) value affected TAS. These 3 parameters explained 16.9% (Adj.R^2 = 0.169) of the change in TAS. TOS was affected by the presence of ADHD (β = 0.400), an increase in MDA (β = 0.334), and a decrease in TAS (β = −0.207) (in order of importance). These 3 parameters explained 45.2% (Adj.R^2 = 0.452) of the change in TAS. The increase in MDA (β = 0.528) only affected the OSI (TOS / TAS) value and explained 27.0% of the change in OSI (Adj.R^2 = 0.270). MDA was affected by presence of ADHD (β = 0.564), and an increase in TOS (β = 0.365) and DBP (β = 0.230) values (in order of importance). These 3 parameters explained 49.5% (Adj.R^2 = 0.495) of the change in MDA. SOD was affected by the presence of ADHD (β = −0.694) and a decrease in TAS (β = −0.176). These 2 parameters explained 53.0% (Adj.R^2 = 0.530) of the change in SOD.

In the ADHD group, 41.2% (21/51) of the patients used medication; 19 used methylphenidate and 2, atomoxetine, sex, age, weight, height and BMI percentile, SBP and DBP values were found to be significantly different according to the drug use status in the ADHD group. The oxidative stress parameters (TAS, SOD, TOS, and OSI) were significantly higher in patients who did not use medication. The MDA levels, ADHD disease duration, and ADHD type were not different according to drug usage ($p > 0.05$). The average dose of drug was 24.57 mg/g, and the average duration of use was 18 (median 5) months (Table 4). When the factors found to be significant at $p < 0.2$ in univariate tests were analyzed with BLR, only the increase in TOS value was found to be significant, while the changes in TAS, MDA, and SOD were not significant.

In the ADHD group, 74.5% (38/51) of the patients were combined type ADHD, and the remainder were ADHD-inattentive type 25.5% (13/51). There were no patients with hyperactive-impulsive type. When the factors affecting the ADHD type were analyzed with the BLR forward model, only the reduction in age was significant. Younger patients were more likely to be in the ADHD-combined type, and older patients in the ADHD-inattentive type (Table 5). No significant relationship was determined between ADHD types and the parameters of TAS, TOS, OSI,

Table 4. Mean and standard deviation values of variables according to medication use in children with ADHD

Variable	No medication used (n = 30)	Medication (n = 21)	Total (n = 51)	*t* test[b] *p* value
Male	21 (55.3)	17 (44.7)	38 (100.0)	0.518[a]
Female	9 (69.2)	4 (30.8)	13 (100.0)	
Age (yr)	10.00 ± 2.84	10.19 ± 2.60	10.08 ± 2.72	0.808
Weight percentile	48.50 ± 34.90	46.71 ± 32.56	47.76 ± 33.64	0.854
Height percentile	44.33 ± 31.85	53.10 ± 34.68	47.94 ± 32.99	0.356
BMI percentile	54.80 ± 29.77	50.43 ± 34.75	53.00 ± 31.65	0.632
SBP (mmHg)	94.83 ± 7.93	94.76 ± 8.73	94.80 ± 8.18	0.976
DBP (mmHg)	55.00 ± 5.57	54.76 ± 6.02	54.90 ± 5.70	0.885
TAS (μmol Trolox Eq/L)	0.74 ± 0.07	0.68 ± 0.08	0.72 ± 0.08	0.010
TOS (μmol H_2O_2 Eq/L)	8.40 ± 0.88	10.41 ± 1.22	9.23 ± 1.43	< 0.001
OSI (arbitrary unit)	11.43 ± 1.68	15.50 ± 3.04	13.10 ± 3.07	< 0.001[c]
MDA (μmol/L)	0.33 ± 0.03	0.34 ± 0.03	0.33 ± 0.03	0.177
SOD (U/ml)	15.55 ± 1.53	16.19 ± 0.74	15.81 ± 1.29	0.006[c]
ADHD duration	33.33 ± 19.63	26.00 ± 29.25	30.31 ± 24.06	0.289
ADHD-Inattentive type	8 (61.5)	5 (38.5)	13 (100.0)	1.000[a]
ADHD-Combined type	22 (57.9)	16 (42.1)	38 (100.0)	
Treatment dose (mg/g)		24.57 ± 12.24	Min−Max (10.0−54.0)	
Treatment duration (mo)		17.95 ± 27.32	Min−Max (1.0− 120.0)	

Values are presented as number (%) or mean ± standard deviation.
ADHD, attention deficit hyperactivity disorder; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; TAS, total antioxidant status; TOS, total oxidant status; OSI, oxidative stress index; MDA, malondialdehyde; SOD, superoxide dismutase.
[a]Fisher's Exact test, [b]Independent Samples *t* test, [c]Mann−Whitney *U* test.

Table 5. Analysis of factors affecting drug use of children with ADHD with the binary logistic regression forward LR model

Variable	B	*p* value	Exp (B)	95% CI for EXP (B)	
				Lower	Upper
Drug use					
TOS	1.792	< 0.001	6.003	2.262	15.934
Constant	−17.103	< 0.001	0.000		
ADHD-Combined					
Age	−0.298	0.018	0.742	0.580	0.949
Constant	4.198	0.002	66.525		

ADHD, attention deficit hyperactivity disorder; LR, linear regression; TOS, total oxidant status; CI, confidence interval.
Hosmer and Lemeshow test $\chi^2 = 4.70$, $p = 0.789$. Nagelkerke $R^2 = 0.622$.

MDA, SOD, height, weight, BMI percentile, SBP, and DBP, included in the BLR analysis forward model.

DISCUSSION

In this study, the correlation of oxidative stress parameters with BMI, blood pressure, ADHD subtypes, and drug use was investigated in children and adolescents with ADHD. The mean values of oxidative stress parameters (TAS, TOS, OSI, and MDA) were higher, while the mean SOD value was lower in the ADHD group compared to the control group (Table 1). The power of determining the presence of ADHD from elevated MDA and low DBP and SOD values was 93.9% (Table 2). High MDA and low SOD were determined as predictive parameters in diagnosing ADHD. In univariate tests, while SOD, TOS, and OSI levels were high in drug users, TAS levels were high in patients not using drugs (Table 4). However, only TOS levels were significant in multivariate binary logistic regression ($p < 0.01$) (Table 5).

Although there are inconsistent findings in literature about the relationship between ADHD and oxidative stress, there are studies showing that oxidative stress increases in ADHD, which is consistent with the current study. Guney *et al.* [28] reported high TAS, TOS, and OSI values in the ADHD group. Even though the TAS (antioxidant parameter) value was not significant in the current study, it was higher than in the control group. Some studies have shown that ADHD patients have higher antioxidant activity than normal [14]. This can be explained by the fact that ADHD patients have normal/high levels of antioxidant production, but as their response to oxidative stress is insufficient, the expected oxidative damage levels are higher [13,14]. A previous study reported that TOS and OSI were higher and TAS was lower in ADHD patients than in the control group [29]. There are also studies that have reported high TAS, TOS, and OSI in adult ADHD patients [14]. However, in those studies, the SOD and MDA parameters were not examined. In the current study, 4 oxidative stress parameters were evaluated together with SBP and DBP using multivariate regression, and the results showed that changes in TOS and TAS were not significant, while the decrease in SOD and DBP and increase in MDA were significant.

An important finding of this study is that according to the ROC analysis, high MDA level and low SOD activity are predictors of ADHD diagnosis in children and adolescents (Figs. 1, 2). In parallel with these findings, Joshi *et al.* [30] reported high lipid peroxidation in children with ADHD and stated that their symptoms improved with antioxidant treatment. In contrast, other studies have reported low MDA levels in children with ADHD [30]. In a study conducted with adult ADHD patients, it was found that the oxidant parameters MDA and NO increased, and SOD, which showed antioxidant activity, decreased [15,31]. While MDA levels have been found to be high in studies conducted with both adult and pediatric ADHD groups at the same time, there are also studies that have reported low MDA. However, it is obvious that MDA, which is the breakdown product of the main chain reactions leading to the oxidation of polyunsaturated fatty acids, is a reliable indicator of oxidative stress [8,15-18]. Consistent with the current study, there are studies in which SOD activity was low in the ADHD group, as well as studies where there was no significant difference from the control group [31,32]. Since SOD enzymatic activity is the first and most important defence mechanism against oxidants, the low level in the ADHD group can be consid-

ered a strong indicator that oxidative stress may be increased in the ADHD group in the current study [31].

Considering that oxidative stress may be affected not only by the presence of ADHD but also by some possible clinical variables, age, BMI and blood pressure were also evaluated in all participants. It is known that oxidative stress increases with age [33]. It is also known that BMI can be affected by oxidative stress, and DNA damage increases and antioxidant capacity decreases in obese patients [34,35]. Studies have shown that increased blood pressure is also associated with increased oxidative stress. Rodrigo *et al.* [36] showed that the increase in blood pressure values was in a linear relationship with decreased antioxidant capacity. Low levels of antioxidants such as glutathione, SOD, and vitamin E have been reported in hypertension [37]. In the current study, although the mean age, weight, height, BMI percentile, SBP and DBP values were lower in the ADHD group, their oxidative stress levels were higher. Thus, even low levels of some clinical variables known to increase oxidative stress (BMI, SBP, DBP, and high age) did not reduce oxidative stress in the ADHD group. In conclusion, the presence of ADHD alone was found to be a strong predictor of increases in oxidative stress. However, sex may also have affected the high oxidative stress parameters in the ADHD group, since 74.5% of ADHD children and only 37.5% of the control group were male. Studies have shown that estrogen has an antioxidant role and protects against oxidative stress [38].

The results of this study showed high TOS, OSI, and SOD activity in ADHD patients using medication (methylphenidate [n = 19] and atomoxetine [n = 2]), and high TAS levels in those not using medication (Table 4). In an experimental study, chronic methylphenidate use has been shown to increase oxidative stress. It was also found that methylphenidate dose and duration of drug exposure are also important in increasing oxidative stress [39-41]. In the current study, drug dose and duration were not found to be associated with oxidative stress. In another study, oxidative stress levels were compared in children whose treatment did not include medication at first, but methylphenidate was added later and a significant increase in plasma antioxidant levels and a significant decrease in oxidative stress levels were found in these children after methylphenidate was initiated [28]. Another study found that chronic methylphenidate exposure increased SOD activity, which was consistent with the current study results [42]. It is not surprising that the level of TAS was high in the ADHD patients that did not use medication. Some studies have emphasized that the antioxidant capacity is actually sufficient in ADHD, but there may be an insufficient response to oxidative stress [13,14,43]. Another view on this issue is that stimulants can increase dopamine levels that tend to affect both antioxidants and oxidants, and have a paradoxical effect [44,45]. It has been suggested that methylphenidate transforms into a methylphenidyl radical inside the cell and oxidizes its own microenvironment, while outside the cell (plasma) it gives electrons in the serum without being metabolized due to the electron-rich phenyl ring, thus showing antioxidant activity and creating a neuroprotective effect [39,42]. In the current study, only 2 of the 21 patients that used drugs were taking atomoxetine. A previous study at the cellular level has shown that atomoxetine may play a protective role against oxidative DNA damage [46]. One of the reasons for higher TOS and OSI in ADHD patients using medication may be due to insufficient use of atomoxetine compared to methylphenidate. Therefore, there is a need for further comprehensive studies with titration of different drugs for treatment of ADHD.

When the relationship between ADHD subtypes and oxidative stress was examined, no significant relationship was found between subtypes and oxidative stress. In some studies, TAS values of the ADHD-inattentive type, combined, and hyperactive-impulsive type have beeb reported to be higher or lower, and the results are not consistent [14,29]. Factors such as duration of illness and the age of patients may have affected the results, because in the current study, combined type ADHD was predominant, and the mean age was lower than in the ADHD-inattentive type.

Genetic, environmental and biochemical factors play a role in the etiopathogenesis of ADHD. The number of studies of ADHD involving neuroimaging and genetics has increased in recent years [47,48]. Although there are conflicting results in the field of ADHD and oxidative stress, ADHD, which is a neurodevelopmental brain disease, can be considered to exacerbate the risk of oxidative stress. ADHD is a disease related to the developing brain. It has been shown that obstetric complications causing damage to the developing brain could be another source of oxidative stress [49]. The fact that children and adoles-

cents with ADHD benefit from antioxidant treatments also supports the idea that oxidative stress increases in ADHD. In a meta-analysis, moderate improvements in ADHD symptoms were seen after treatment with antioxidant omega-3 fatty acids. Increased lipid peroxidation (high MDA) in ADHD patients may be the cause of fatty acid deficiency in these individuals. In addition to the findings of omega-3 fatty acids, N-Acetyl Cysteine (NAC), a precursor and NO inhibitor of glutathione, a potent antioxidant, has been shown to reduce inattention and hyperactivity in ADHD rat models [50,51]. In addition to animal models, NAC and Pycnogenol, which are both antioxidants, have been shown to be effective in the treatment of ADHD in humans [52,53]. In a meta-analysis by Bloch *et al*, omega-3 fatty acids (especially formulations including Eicosapentaenoic acid at higher doses) were shown to significantly reduce ADHD symptoms in individuals diagnosed with ADHD [52]. The low levels of zinc, which act as cofactors in many antioxidative reactions, and ferritin, which limits the formation of iron free radicals in ADHD, and increased exposure to lead, which increases oxidative stress by consuming glutathione, increases the risk of ADHD [54-56]. In a brain imaging study, the iron levels in the thalamus of ADHD patients were found to be low [57]. However, rather than iron deficiency of low serum ferritin, this was thought to be a parameter showing oxidative stress in ADHD [56].

The main limitation of this study was the small sample size. Homogeneity could not be achieved between the patient and control groups in respect of clinical variables. The mean age and mean SBP and DBP values of the control group were significantly higher than those of the ADHD group. Venous blood samples were able to be taken as there was a low number of very young children and the older age group are more co-operative and willing to give blood. Another limitation of the study was that there were only two patients using atomoxetine, which was not sufficient to investigate the effects of atomoxetine on oxidative metabolism.

However, this study also had several strengths, including the evaluation of BMI and blood pressure, which are thought to affect oxidative stress parameters, the comparison of variables with those of the healthy control group, the use of K-SADS-PL to exclude patients with psychiatric comorbidities other than ODD, evaluation with WISC-R to exclude intellectual disability, and the exclusion of patients with autism spectrum disorders. Moreover, despite the increasing knowledge about the etiology of ADHD, it is not yet fully known how the drugs used in treatment affect the neurobiology and neuroadaptation process of the disease. The inclusion of patients both using and not using medication is therefore another strong aspect.

In conclusion, the results of this study demonstrated that oxidative stress increased in patients with ADHD. TAS and TOS, which have been shown to be important oxidative parameters in previous studies, were not found to be significantly different as a result of multivariate analysis. The main factors that increase oxidative stress were determined to be high MDA and low SOD levels. Therefore, MDA and SOD levels may be effective biomarkers in diagnosing ADHD. Nevertheless, further comprehensive studies are needed to better understand this negative role of oxidative stress in ADHD.

■ Funding

None.

■ Acknowledgments

The authors would like to thank the children and their families who participated in this study on a voluntary basis.

■ Conflicts of Interest

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

■ Author Contributions

Conceptualization: Dilşad Yıldız Miniksar, Mehmet Akif Cansız. Data acquisition: Dilşad Yıldız Miniksar, Mehmet Akif Cansız. Supervision: Dilşad Yıldız Miniksar, Ayşe Yeşim Göçmen, Mahmut Kılıç, Ökkeş Hakan Miniksar. Writing—original draft: Dilşad Yıldız Miniksar. Writing—review & editing: Dilşad Yıldız Miniksar, Ökkeş Hakan Miniksar, Mahmut Kılıç, Ayşe Yeşim Göçmen.

■ ORCID

Dilşad Yıldız Miniksar
https://orcid.org/0000-0002-6389-4377
Mehmet Akif Cansız
https://orcid.org/0000-0003-1102-4676
Ayşe Yeşim Göçmen
https://orcid.org/0000-0002-8511-639X

Mahmut Kılıç https://orcid.org/0000-0002-8921-1597
Ökkeş Hakan Miniksar
https://orcid.org/0000-0001-5645-7729

REFERENCES

1. Scahill L, Schwab-Stone M. *Epidemiology of ADHD in school-age children. Child Adolesc Psychiatr Clin N Am 2000;9:541-555.*
2. American Psychiatric Association. *Diagnostic and statistical manual of mental disorders (DSM-IV-TR). 4th ed. Washington, D.C.:American Psychiatric Association;2000.*
3. Schuch V, Utsumi DA, Costa TV, Kulikowski LD, Muszkat M. *Attention deficit hyperactivity disorder in the light of the epigenetic paradigm. Front Psychiatry 2015;6:126.*
4. Kannan K, Jain SK. *Oxidative stress and apoptosis. Pathophysiology 2000;7:153-163.*
5. Yorbık Ö, Cansever A, Söhmen T. *Otizmde nöronal görüşler. Turk J Child Adolesc Ment Health 2002;9:118-127.*
6. Mahadik SP, Mukherjee S. *Free radical pathology and antioxidant defense in schizophrenia: a review. Schizophr Res 1996;19:1-17.*
7. Reddy RD, Yao JK. *Free radical pathology in schizophrenia: a review. Prostaglandins Leukot Essent Fatty Acids 1996;55:33-43.*
8. Ceylan M, Sener S, Bayraktar AC, Kavutcu M. *Oxidative imbalance in child and adolescent patients with attention-deficit/hyperactivity disorder. Prog Neuropsychopharmacol Biol Psychiatry 2010;34:1491-1494.*
9. Ng F, Berk M, Dean O, Bush AI. *Oxidative stress in psychiatric disorders: evidence base and therapeutic implications. Int J Neuropsychopharmacol 2008;11:851-876.*
10. Alvarez-Arellano L, González-García N, Salazar-García M, Corona JC. *Antioxidants as a potential target against inflammation and oxidative stress in attention-deficit/hyperactivity disorder. Antioxidants (Basel) 2020;9:176.*
11. Anand D, Colpo GD, Zeni G, Zeni CP, Teixeira AL. *Attention-deficit/hyperactivity disorder and inflammation: what does current knowledge tell us? A systematic review. Front Psychiatry 2017;8:228.*
12. Banerjee TD, Middleton F, Faraone SV. *Environmental risk factors for attention-deficit hyperactivity disorder. Acta Paediatr 2007;96:1269-1274.*
13. Archana E, Pai P, Prabhu BK, Shenoy RP, Prabhu K, Rao A. *Altered biochemical parameters in saliva of pediatric attention deficit hyperactivity disorder. Neurochem Res 2012;37:330-334.*
14. 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.*
15. Bulut M, Selek S, Gergerlioglu HS, Savas HA, Yilmaz HR, Yuce M, *et al. Malondialdehyde levels in adult attention-deficit hyperactivity disorder. J Psychiatry Neurosci 2007;32:435-438.*
16. Bulut M, Selek S, Bez Y, Cemal Kaya M, Gunes M, Karababa F, *et al. Lipid peroxidation markers in adult attention deficit hyperactivity disorder: new findings for oxidative stress. Psychiatry Res 2013;209:638-642.*
17. Oztop D, Altun H, Baskol G, Ozsoy S. *Oxidative stress in children with attention deficit hyperactivity disorder. Clin Biochem 2012;45:745-748.*
18. Spahis S, Vanasse M, Bélanger SA, Ghadirian P, Grenier E, Levy E. *Lipid profile, fatty acid composition and pro- and antioxidant status in pediatric patients with attention-deficit/hyperactivity disorder. Prostaglandins Leukot Essent Fatty Acids 2008;79:47-53.*
19. Khan NI, Naz L, Yasmeen G. *Obesity: an independent risk factor for systemic oxidative stress. Pak J Pharm Sci 2006;19:62-65.*
20. Albayrak Ö, Pütter C, Volckmar AL, Cichon S, Hoffmann P, Nöthen MM, *et al. Common obesity risk alleles in childhood attention-deficit/hyperactivity disorder. Am J Med Genet B Neuropsychiatr Genet 2013;162B:295-305.*
21. Wilcox CS. *Reactive oxygen species: roles in blood pressure and kidney function. Curr Hypertens Rep 2002;4:160-166.*
22. Wong HS, Dighe PA, Mezera V, Monternier PA, Brand MD. *Production of superoxide and hydrogen peroxide from specific mitochondrial sites under different bioenergetic conditions. J Biol Chem 2017;292:16804-16809.*
23. 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.*
24. Gökler B, Ünal F, Pehlivantürk 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)]. Turk J Child Adolesc Ment Health 2004;11:109-116. Turkish.*
25. Turgay A. *Disruptive behavior disorders: child and adolescent screening and rating scales for children, adolescents, parents and teachers, West Bloomfield:Michigan Integrative Therapy Institute Publication;1994.*
26. Ercan ES, Amado S, Somer O, Çikoğlu S. *Development of a test battery for the assessment of attention deficit hyperactivity disorder. Turk J Child Adolesc Ment Health 2001;8:132-144.*
27. Uluc S, Oktem F, Erden G, Gencoz T, Sezgin N. *Wechsler intelligence scale for children-IV: a new era for Turkey in evaluation of intelligence in the clinical context. Turk Psychol Artic 2011;14:49-57.*
28. Guney E, Cetin FH, Alisik M, Tunca H, Tas Torun Y, Iseri E, *et al. Attention Deficit Hyperactivity Disorder and oxidative stress: a short term follow up study. Psychiatry Res 2015;229:310-317.*
29. Kul M, Unal F, Kandemir H, Sarkarati B, Kilinc K, Kandemir SB. *Evaluation of oxidative metabolism in child and adolescent patients with attention deficit hyperactivity disorder. Psychiatry Investig 2015;12:361-366.*

30. Joshi K, Lad S, Kale M, Patwardhan B, Mahadik SP, Patni B, *et al. Supplementation with flax oil and vitamin C improves the outcome of Attention Deficit Hyperactivity Disorder (ADHD). Prostaglandins Leukot Essent Fatty Acids 2006;74:17-21.*
31. Selek S, Savas HA, Gergerlioglu HS, Bulut M, Yilmaz HR. *Oxidative imbalance in adult attention deficit/hyperactivity disorder. Biol Psychol 2008;79:256-259.*
32. Ceylan MF, Sener S, Bayraktar AC, Kavutcu M. *Changes in oxidative stress and cellular immunity serum markers in attention-deficit/hyperactivity disorder. Psychiatry Clin Neurosci 2012;66:220-226.*
33. Andreazza AC, Frey BN, Valvassori SS, Zanotto C, Gomes KM, Comim CM, *et al. DNA damage in rats after treatment with methylphenidate. Prog Neuropsychopharmacol Biol Psychiatry 2007;31:1282-1288.*
34. Demirbag R, Yilmaz R, Gur M, Celik H, Guzel S, Selek S, *et al. DNA damage in metabolic syndrome and its association with antioxidative and oxidative measurements. Int J Clin Pract 2006;60:1187-1193.*
35. Galili O, Versari D, Sattler KJ, Olson ML, Mannheim D, McConnell JP, *et al. Early experimental obesity is associated with coronary endothelial dysfunction and oxidative stress. Am J Physiol Heart Circ Physiol 2007;292:H904-H911.*
36. Rodrigo R, Prat H, Passalacqua W, Araya J, Guichard C, Bächler JP. *Relationship between oxidative stress and essential hypertension. Hypertens Res 2007;30:1159-1167.*
37. Parıldar Z, Parıldar M, Kabaroğlu C, Turgan N, Mutaf I, Memiş A, *et al. Glutathione related enzymes in renovascular hypertension effect of endovascular treatment. Türk Klinik Biyokimya Derg 2003;1:41-46.*
38. Ruiz-Larrea MB, Leal AM, Martín C, Martínez R, Lacort M. *Antioxidant action of estrogens in rat hepatocytes. Rev Esp Fisiol 1997;53:225-229.*
39. Comim CM, Gomes KM, Réus GZ, Petronilho F, Ferreira GK, Streck EL, *et al. Methylphenidate treatment causes oxidative stress and alters energetic metabolism in an animal model of attention-deficit hyperactivity disorder. Acta Neuropsychiatr 2014;26:96-103.*
40. Martins MR, Reinke A, Petronilho FC, Gomes KM, Dal-Pizzol F, Quevedo J. *Methylphenidate treatment induces oxidative stress in young rat brain. Brain Res 2006;1078:189-197.*
41. Kawatani M, Tsukahara H, Mayumi M. *Evaluation of oxidative stress status in children with pervasive developmental disorder and attention deficit hyperactivity disorder using urinary-specific biomarkers. Redox Rep 2011;16:45-46.*
42. Schmitz F, Scherer EB, da Cunha MJ, da Cunha AA, Lima DD, Delwing D, *et al. Chronic methylphenidate administration alters antioxidant defenses and butyrylcholinesterase activity in blood of juvenile rats. Mol Cell Biochem 2012;361:281-288.*
43. Joseph N, Zhang-James Y, Perl A, Faraone SV. *Oxidative stress and ADHD: a meta-analysis. J Atten Disord 2015;19:915-924.*
44. Yen GC, Hsieh CL. *Antioxidant effects of dopamine and related compounds. Biosci Biotechnol Biochem 1997;61:1646-1649.*
45. Miyazaki I, Asanuma M. *Dopaminergic neuron-specific oxidative stress caused by dopamine itself. Acta Med Okayama 2008;62:141-150.*
46. Schmidt AJ, Clement HW, Gebhardt S, Hemmeter UM, Schulz E, Krieg JC, *et al. Impact of psychostimulants and atomoxetine on the expression of 8-hydroxyguanine glycosylase 1 in human cells. J Neural Transm (Vienna) 2010;117:793-797.*
47. Sharp SI, McQuillin A, Gurling HM. *Genetics of attention-deficit hyperactivity disorder (ADHD). Neuropharmacology 2009;57:590-600.*
48. Tripp G, Wickens JR. *Neurobiology of ADHD. Neuropharmacology 2009;57:579-589.*
49. Shim SY, Kim HS. *Oxidative stress and the antioxidant enzyme system in the developing brain. Korean J Pediatr 2013;56:107-111.*
50. Bloch MH, Qawasmi A. *Omega-3 fatty acid supplementation for the treatment of children with attention-deficit/hyperactivity disorder symptomatology: systematic review and meta-analysis. J Am Acad Child Adolesc Psychiatry 2011;50:991-1000.*
51. 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.*
52. Chovanová Z, Muchová J, Sivonová M, Dvoráková M, Zitnanová I, Waczulíková I, *et al. Effect of polyphenolic extract, Pycnogenol, on the level of 8-oxoguanine in children suffering from attention deficit/hyperactivity disorder. Free Radic Res 2006;40:1003-1010.*
53. Garcia RJ, Francis L, Dawood M, Lai ZW, Faraone SV, Perl A. *Attention deficit and hyperactivity disorder scores are elevated and respond to N-acetylcysteine treatment in patients with systemic lupus erythematosus. Arthritis Rheum 2013;65:1313-1318.*
54. Scassellati C, Bonvicini C, Faraone SV, Gennarelli M. *Biomarkers and attention-deficit/hyperactivity disorder: a systematic review and meta-analyses. J Am Acad Child Adolesc Psychiatry 2012;51:1003-1019.e20.*
55. Jomova K, Valko M. *Advances in metal-induced oxidative stress and human disease. Toxicology 2011;283:65-87.*
56. Orino K, Lehman L, Tsuji Y, Ayaki H, Torti SV, Torti FM. *Ferritin and the response to oxidative stress. Biochem J 2001;357(Pt 1):241-247.*
57. Cortese S, Azoulay R, Castellanos FX, Chalard F, Lecendreux M, Chechin D, *et al. Brain iron levels in attention-deficit/hyperactivity disorder: a pilot MRI study. World J Biol Psychiatry 2012;13:223-231.*