

Araştırma / Original article**Low HO-1 and high KEAP1 serum levels in children with attention deficit hyperactivity disorder: the first clinical study**

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ÖZ

Objective: The purpose of this study was therefore to evaluate serum heme oxygenase-1 (HO-1), kelch-like ECH-associated protein 1 (KEAP1), and nuclear factor erythroid 2 related factor 2 (NRF2) levels in children with attention deficit hyperactivity disorder (ADHD) and to reveal their association with the severity of ADHD. **Methods:** The patient group consisted of 41 children aged 6–12 years, first diagnosed with ADHD and the control group consisted of 34 age- and gender- matched children. ADHD was diagnosed by child psychiatrists during clinical interviews based on the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5). The parents of the children in the ADHD group were also given the Turgay DSM-IV-based Disruptive Behavior Disorders Child and Adolescent Rating and Screening Scale (T-DSM-IV-S). Serum HO-1, KEAP1, and NRF2 levels were determined in the biochemistry laboratory using the ELISA technique. **Results:** HO-1 levels in the patient group were significantly lower than in the control group, while KEAP1 levels were significantly higher, but no significant difference was observed between the groups in terms of NRF2 ($p=0.041$, $p<0.001$, $p=0.290$, respectively). Spearman's correlation analysis revealed no significant relation between inattention scores and HO-1, KEAP1 or NRF2, ($r=-0.197$, $p=0.224$; $r=0.005$, $p=0.976$; $r=0.274$, $p=0.088$, respectively). **Discussion:** Low HO-1 and high KEAP1 levels in the patient group suggest that oxidative stress increased in this group, but that HO-1 levels were unable to balance this. (*Anadolu Psikiyatri Derg* 2020; 21(3):319-326)

Keywords: attention deficit hyperactivity disorder, oxidative stress, heme oxygenase-1, NRF2, KEAP1

Dikkat eksikliği hiperaktivite bozukluğu olan çocuklarda düşük HO-1 ve yüksek KEAP1 serum düzeyleri**ABSTRACT**

Amaç: Bu çalışmanın amacı, dikkat eksikliği hiperaktivite bozukluğu (DEHB) olan çocuklarda serum heme oksijenaz-1 (HO-1), kelch benzeri ECH ile ilişkili protein 1 (KEAP1) ve nükleer faktör eritroid 2 ile ilişkili faktör 2 (NRF2) düzeylerini değerlendirip, bozukluğun şiddeti ile ilişkilerini ortaya koymaktır. **Yöntem:** Hasta grubu 6-12 yaşları arasında 41 DEHB'li çocuktan, kontrol grubu ise yaş ve cinsiyet açısından eşleştirilmiş 34 çocuktan oluşmaktadır. Zihinsel Bozuklukların Tanısal ve İstatistiksel El Kitabı, Beşinci Baskı (DSM-5) temel alınarak yapılan klinik görüşmeler sırasında DEHB tanısı çocuk psikiyatristleri tarafından konmuştur. DEHB'li çocukların ailelerine de Turgay DSM-IV Tabanlı Yıkıcı Davranış Bozuklukları Çocuk ve Ergen Derecelendirme ve Tarama Ölçeği (T-DSM-IV-Ö) verilmiştir. ELISA tekniği kullanılarak biyokimya laboratuvarında serum HO-1, KEAP1 ve NRF2 düzeyleri belirlendi. **Bulgular:** Hasta grubundaki HO-1 düzeyleri kontrol grubuna göre anlamlı derecede düşüken, KEAP1 düzeyleri anlamlı derecede yüksek bulundu, ancak NRF2 açısından gruplar arasında anlamlı fark gözlenmedi ($p=0.041$, $p<0.001$,

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$p=0.290$, sırasıyla). Spearman korelasyon analizi dikkatsizlik puanları ile HO-1 veya KEAP1 arasında anlamlı bir ilişki olmadığını ortaya koymuştur, ($r=-0.197$, $p=0.224$; $r=0.005$, $p=0.976$; $r=0.274$, $p=0.088$, sırasıyla). **Tartışma:** Hasta grubundaki düşük HO-1 ve yüksek KEAP1 düzeyleri bu grupta oksidatif stresin arttığını, ancak HO-1 düzeylerinin bunu dengelemediğini göstermektedir. (*Anatolian Journal of Psychiatry* 2020; 21(3):319-326)

Anahtar sözcükler: Dikkat eksikliği hiperaktivite bozukluğu, oksidatif stres, hem oksijenaz-1, NRF2, KEAP1

INTRODUCTION

Attention deficit hyperactivity disorder (ADHD) is the most common childhood developmental disorder. Although genetic factors play a dominant role, the etiology is known to be complex, involving the interaction of several genetic and environmental biological factors (such as problems during pregnancy, toxins, head trauma, and various drugs).¹ Free radicals that form due to an electron leaving or being added to an atom or molecule, and reactive oxygen species (ROS) are the most important free radicals in living organisms.² ROS damage polyunsaturated fatty acids, proteins, carbohydrates and nucleic acids.³ Antioxidant defense systems have developed in the body to prevent the formation of ROS and the damage they cause.⁴ Impairment of the homeostasis between ROS and antioxidants in favor of the former is known as oxidative stress.³

Since both genetic and environmental risk factors can increase the probability of oxidative stress, the last few decades have seen increased research into the relation between ADHD and oxidative stress.

Decrease plasma total antioxidant status (TAS)⁵⁻⁷ and serum glutathione peroxidase (GSH-Px),⁸ paraoxonase-1(PON-1), arylesterase (ARE),⁶ and glutathione S-transferase (GST) activities⁹ and 8-hydroxy-2'-deoxyguanosine (8-OHdG) (a marker of DNA oxidative stress)¹⁰ level in children with ADHD than in control subjects have been reported. ADHD patients exhibited higher serum nitric oxide synthase (NOS), xanthine oxidase (XO) and adenosine deaminase (ADA) (a marker of cellular immunity) activities,⁹ and nitric oxide (NO) and malondialdehyde (MDA) (an indicator of lipid peroxidation) levels,⁸ plasma total oxidant status (TOS)⁶ and oxidative stress index (OSI).^{6,7,11} In the light of these studies, there appears to be an imbalance between the oxidant and antioxidant systems in children with ADHD.

The activity of the antioxidant enzyme heme oxygenase-1 (HO-1) mediates the catabolism of toxic heme to carbon monoxide (CO), free ferrous iron (Fe^{++}), biliverdin and bilirubin.^{12,13} The products that form following this activity then contribute to protection against oxidative dam-

age, apoptosis regulation, inflammation regulation and angiogenesis.^{14,15}

Nuclear factor erythroid 2 related factor 2 (NRF2) is a cytoprotective transcription factor that regulates cytoprotective genes such as HO-1.¹⁴ NRF2 is normally present in the form of an inactive complex with kelch-like ECH-associated protein 1 (KEAP1). KEAP1 is a negative regulator of the cytoplasmic redox signaling protein NRF2.¹⁶ The KEAP1-NRF2 signaling pathway serves to preserve redox homeostasis through the transcriptional induction of cytoprotective genes.¹⁷

While oxidative stress has been investigated in the etiology of ADHD, HO-1, NRF2, and KEAP1 serum levels have not been previously studied. The purpose of this study was therefore to evaluate HO-1, NRF2, and KEAP1 levels in children with ADHD and to reveal their association with the severity of ADHD.

METHODS

Study population

The study group consisted of 41 children (34 boys and seven girls) aged 6-12 years, first diagnosed with ADHD at the outpatient department of our Child and Adolescent Psychiatry Clinic between April 2017 and January 2018. ADHD was diagnosed by child psychiatrists during clinical interviews based on the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5).¹⁸ The Schedule for Affective Disorders and Schizophrenia for School-Age Children-Present and Lifetime Version - Turkish Version (K-SADS-PL-T) was applied to corroborate the diagnosis of ADHD and in order to exclude comorbid psychiatric conditions.¹⁹ The parents of the children in the ADHD group were also given the Turgay DSM-IV-based Disruptive Behavior Disorders Child and Adolescent Rating and Screening Scale(T-DSM-IV-S).²⁰ Severity (mild, moderate, severe) of ADHD was determined based on DSM-5. Mild; few, if any, symptoms in excess of those required to make the diagnosis are present, and symptoms result in no more than minor impairments in social or occupational functioning. Moderate, symptoms or functional impairment between 'mild' and 'severe' are pre-

sent. Severe, many symptoms in excess of those required to make the diagnosis, or several symptoms that are particularly severe, are present, or the symptoms result in marked impairment in social or occupational functioning.¹⁸

Patient group exclusion criteria; insufficient intellectual capacity to understand the questions, use of any drugs within the previous two weeks (including antioxidant medication), presence during evaluation of any comorbid mental disorder (except for oppositional defiant disorder and conduct disorder) or physical, neurological, genetic, or other medical diseases (autism spectrum disorder, fragile-X syndrome, tuberous sclerosis, phenylketonuria, Lesch-Nyhan syndrome, fetal alcohol syndrome, history of head trauma, cardiovascular disease, autoimmune disease, infection, renal and hepatic diseases, malignancy, obesity, history of atopic eczema and/or allergy) and a history of maternal substance use during pregnancy. Patients underwent psychological examination by a child and adolescent psychiatrist and physical and neurological examination by a pediatric neurologist. Complete blood counts, function tests (thyroid, liver and kidney), and routine biochemical tests were requested for all patients. Electroencephalography and magnetic resonance imaging were also requested in cases involving clinical uncertainty.

Following psychological and medical assessment of the 58 patients we planned to include in the study, 17 cases were eliminated (due to anxiety and/or depressive disorder in 15, absence epilepsy in one, and obesity in one), and 41 cases were enrolled in the study.

The control group consisted of 34 age- and gender- matched children (26 boys, 8 girls) admitted to the outpatient healthy child follow-up clinic over the 9-month study period. The children in the control group underwent psychiatric and physical examinations. The children in this group had no psychiatric and neurological problems, no known genetic syndromes, metabolic or endocrine diseases or infections, or allergic reactions, and were not receiving any antioxidant therapy or medications.

Sociodemographic data were elicited at the first interview with those children who agreed to be included in the study and their parents, using a sociodemographic data form prepared by the authors.

Ethics

Written informed consent was obtained from all children and their parents after the study purpose and procedure had been explained. The study was approved by the Faculty Ethics Committee (No.12, Session no. 04, dated 06.04.2017) and was conducted in conformity with the ethical standards set out in the revised Declaration of Helsinki (2013). The research was supported by the Harran University Scientific Research Coordination Office (protocol No. 17187, dated 08-12-2017).

Blood specimen collection and biochemical analysis

Five-milliliter blood samples were collected from the antecubital vein from both the patient and control groups following fasting of at least 8 h and were placed into gel-containing biochemistry tubes. These were immediately centrifuged at 4000 rpm for 10 min at 4°C for serum separation. This part was collected, divided into two separate halves, and stored in Eppendorf tubes at -80°C until required. Serum HO-1, KEAP1, and NRF2 levels were determined in the biochemistry laboratory using the Enzyme-Linked Immunosorbent Assay (ELISA) technique.

Human HO-1, human KEAP1, and human NRF2 (NFE2L2) measurements

Human HO-1 (ng/ml) was studied with an ELISA kit (Fine Test Catalogue No (CN): EH3234), Human KEAP1 (pg/ml) was studied using an ELISA kit (Fine Test CN: EH4240), and Human NRF2 (ng/ml) was studied using an ELISA kit (Fine Test CN: EH3417), and were read at 450 nm using a Thermo, Multiskan GO device (Thermo Fisher Scientific).

Statistical analysis

Statistical analyses were performed on SPSS 23.0 software (SPSS Inc., Chicago, IL, USA). The Pearson's chi-square test was used to compare categorical variables. The Shapiro-Wilk normality test was used to check normal distribution assumptions. Since the data obtained were not normally distributed, non-parametric tests were subsequently employed. Data were expressed as median values (interquartile range). The Mann-Whitney U test was employed for constant and binary comparisons, and Spearman's correlation analysis for correlation analysis. The Kruskal Wallis test was used to compare three or more non-parametric groups. *p* values <0.05 were regarded as statistically significant.

RESULTS

The median ages of the ADHD and control groups were 9 and 7.5 years, respectively. No

statistically significant difference was observed between the patient and control groups in terms of sociodemographic parameters ($p>0.05$) (Table 1).

Table 1. Sociodemographic characteristics and HO-1, KEAP1, and NRF2 serum levels of the attention deficit hyperactivity disorder and control groups

Parameters	Patients (n=41)	Controls (n=34)	p	z
Gender n (%) (female/male) ^a	7 (17.1%) / 34 (82.9%)	8 (23.5%) / 26 (76.5%)	0.487	
Age, median (min-max) ^b	9 (6-12)	7.5 (6-12)	0.453	-0.751
Mother's age median (min-max) ^b	34 (25-49)	33 (23-42)	0.394	-0.853
Mother's education (years) median (min-max) ^b	5 (0-16)	2.5 (0-16)	0.557	-0.588
Father's age median(min-max) ^b	39 (30-61)	37 (28-53)	0.064	-1.850
Father's education (years) median (min-max) ^b	5 (0-16)	5 (0-16)	0.240	-1.175
Number of siblings median (min-max) ^b	3 (0-8)	3 (1-6)	0.064	-1.855
Rank among siblings median (min-max) ^b	2 (1-8)	2 (1-6)	0.772	-0.290
Parents living together/separated ^a n (%)	38 (92.7%) / 3 (7.3%)	34 (100%) / 0(0%)	0.107	
BMI, median (min-max) ^b	16.79(14.31-22.37)	15.43 (14.12-22.89)	0.051	-1.953
HO-1 (ng/ml), median (min-max) ^b	0.43 (0.09-1.92)	0.55 (0.22-19.98)	0.041	-2.044
KEAP1 (pg/ml), median (min-max) ^b	33.83 (10.26-56.16)	25.06 (15.34-47.08)	<0.001	-3.933
NRF2 (ng/ml), median (min-max) ^b	1.18 (0.85-7.98)	1.14 (0.16-8.20)	0.290	-1.059

^a: Chi-square; ^b: Mann-Whitney U; IQR= inter quartile range

Table 2. Comparison of the patient subgroups (according to the presence or absence of comorbidity) in terms of HO-1 (ng/ml), KEAP1 (pg/ml), and NRF2 (ng/ml) levels

Parameters	ADHD (n=41) Median (min-max))			p^1	p^2	p^3
	with ODD(n=15)/ without ODD(n=26)	with CD(n=5)/ without CD(n=36)	with ODD and/or CD(n=16) without ODD and/or CD(n=25)			
HO-1	0.34 (0.09-1.92) 0.45 (0.20-1.53)	0.29 (0.25-1.44) 0.43 (0.09-1.92)	0.36 (0.09-1.92) 0.45 (0.20-1.53)	0.38 z=-0.867	0.37 z=-0.897	0.68 z=-0.401
KEAP1	37.16 (22.82-53.72) 33.25 (10.26-56.16)	32.67 (10.26-56.16) 34.26 (22.82-53.72)	34.91 (22.82-53.72) 33.83 (10.26-56.16)	0.87 z=-0.162	1 z=0.000	0.89 z=-0.134
NRF2	1.16 (0.85-2.99) 1.21 (0.86-7.98)	1.16 (0.85-2.04) 1.19 (0.86-7.98)	1.16 (0.85-2.99) 1.20 (0.86-7.98)	0.49 z=-0.677	0.56 z=-0.578	0.68 z=-0.401

Mann-Whitney U; ¹: ADHD with ODD vs. without ODD; ²: ADHD with CD vs. without CD; ³: ADHD with ODD and/or CD vs. without ODD and/or CD; ODD: Opposition Defiant Disorder; CD: Conduct Disorder

The predominantly hyperactive/impulsive presentation (ADHD-H) constituted 2.4% (n=1) of the ADHD group, the predominantly inattentive presentation (ADHD-I) 17% (n=7), and the combined presentation (ADHD-I/H) 80.4% (n=33).

Severity of ADHD determined based on DSM-5 was mild in three cases (1 boy, 2 girls (7.3%), moderate in 21 (17 boys, 4 girls) (51.2%), and severe in 17 (16 boys, 1 girl) (41.4%).

Opposition defiant disorder (ODD) was determined in 15 patients (36.5%), conduct disorder (CD) in 5 (12.1%), and ODD and/or CD in 16

(39%).

Comparison of the patient and control groups in terms of HO-1, KEAP1, and NRF2 levels revealed that HO-1 levels in the patient group were significantly lower than in the control group, while KEAP1 levels were significantly higher, but no significant difference was observed between the groups in terms of NRF2 ($p=0.041$, $p<0.001$, and $p=0.290$, respectively) (Table 1).

No significant difference was determined in terms of HO-1, KEAP1 and NRF2 between the mild, moderate and severe ADHD groups based

Table 3. Comparison of the patient subgroups symptom severity in terms of HO-1 (ng/ml), KEAP1 (pg/ml), and NRF2 (ng/ml) levels

Severity	HO-1 Median (min-max)	KEAP1 Median (min-max)	NRF2 Median (min-max)	p^1	p^2	p^3
ADHD (n)				0.896	0.723	0.343
Mild (3)	0.45 (0.43-0.45)	28.18 (25.7-34.70)	1.20 (1.18-3.26)			
Moderate (21)	0.42 (0.2-1.09)	35.04 (10.26-56.16)	1.16 (0.86-5.92)			
Severe (17)	0.34 (0.09-1.92)	33.83 (22.82-53.72)	1.34 (0.85-7.98)			
ADHD-I/H (n)				0.892	0.427	0.760
Mild (2)	0.455 (0.45-0.46)	31.44 (28.18-34.70)	1.19 (1.18-1.20)			
Moderate (15)	0.43 (0.21-1.09)	38.24 (24.40-56.16)	1.16 (0.86-5.92)			
Severe (16)	0.38 (0.25-1.92)	33.25 (22.82-53.72)	1.37 (0.85-7.98)			
ADHD-I (n)				0.314	0.314	0.243
Mild (1)	No data	No data	No data			
Moderate (5)	0.26 (0.20-0.98)	24.35 (10.26-35.37)	1.22 (0.89-1.92)			
Severe (1)	No data	No data	No data			

Kruskal Wallis; ¹: The patient subgroups (ADHD or ADHD-I/H or ADHD-I) symptom severity (mild/moderate/severe) in terms of HO-1; ²: Patient subgroups (ADHD or ADHD-I/H or ADHD-I) symptom severity (mild/moderate/severe) in terms of KEAP1; ³: Patient subgroups (ADHD or ADHD-I/H or ADHD-I) symptom severity (mild/moderate/severe) in terms of NRF2; ADHD-I/H: the ADHD group, the combined presentation, ADHD-I: the ADHD group, the predominantly inattentive presentation

on DSM-5 ($p=0.896$, 0.723 , and 0.348 , respectively).

No significant difference was determined in terms of HO-1, KEAP1 and NRF2 among the ADHD-I/H mild ($n=2$), moderate ($n=15$) and severe ($n=16$) subgroups based on DSM-5 ($p=0.892$, 0.427 , and 0.760 , respectively).

No significant difference was observed in terms of HO-1, KEAP1 and NRF2 between the ADHD-I mild ($n=1$), moderate ($n=5$), and severe ($n=1$) subgroups based on DSM-5 ($p=0.314$, 0.314 , 0.243 , and respectively).

Spearman's correlation analysis revealed no significant relation between inattention scores determined using T-DSM-IV-S and HO-1, α KEAP1 or NRF ($r=-0.197$, $p=0.224$; $r=0.005$, $p=0.976$; and $r=0.274$, $p=0.088$, respectively).

Spearman's correlation analysis revealed no significant relation between hyperactivity scores determined using T-DSM-IV-S and HO-1, KEAP1 or NRF2 ($r=-0.114$, $p=0.520$; $r=-0.033$, $p=0.852$; and $r=0.065$, $p=0.716$, respectively).

DISCUSSION

The purpose of our study was therefore to investigate HO-1, NRF2, and KEAP1 levels in children with ADHD, and to reveal any relations between these and the severity of the disorder. Our study showed that low HO-1 and high KEAP1 serum levels in children with ADHD.

Ceylan et al.⁸ determined a decrease in activity of the antioxidant enzyme GSH-Px in children with ADHD, but observed no changes in catalase (CAT) or superoxide dismutase (SOD) activity. Another study reported lower GST and PON-1 activities in children with ADHD, but higher NOS, XO, and ADA activities.⁹ Higher levels of NO (a reactive oxygen species) and MDA have also been reported in children with ADHD compared to controls.⁸ High levels of oxidants and low GSH-Px activities suggest an oxidative imbalance in pediatric patients with ADHD.⁸ Sezen et al.⁶ observed decreased PON-1 and ARE activity in children with ADHD. However, other studies have reported no difference in PON-1,²¹ serum GPx, and serum SOD¹⁰ levels between children with ADHD and control subjects, and no relation has been determined between GPx and serum SOD levels and ADHD symptom severity.¹⁰ Indeed, in another study, MPO were significantly low in the ADHD group.²² Lower serum PON and ARE levels,²³ and serum SOD activity²⁴ have also been determined in adults with ADHD compared to control subjects. These studies of adults and children with ADHD indicate an impairment in antioxidant enzyme levels/activity and/or oxidative balance. We also determined low levels of the antioxidant enzyme HO-1 in children with ADHD compared to the controls. However, we determined no correlation between the mild, moderate and severe ADHD groups based on DSM-5 and HO-1 levels. We determined no correlation between HO-1 levels and

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inattention or hyperactivity scores. These findings suggest an insufficiency in the antioxidant system in subjects with ADHD, although this is not related to severity of the disease.

Apart from enzymes, studies have also evaluated oxidant and antioxidant status in subjects with ADHD. Sezen et al.⁶ reported decreased TAS, and increased TOS and TOS/TAS ratios in children with ADHD. TAS in children with ADHD has also been reported to decrease in comparison with reference values.⁵ Lower TAS⁷ and higher TOS and OSI^{7,11} values have been determined in patients with ADHD than in healthy controls. Higher²⁵ and lower¹¹ plasma thiol levels, and also no difference²¹ have all been reported in children with ADHD, while abnormal thiol/disulfide homeostasis has been reported,²⁵ while another study reported no difference.²² Low plasma TOS, OSI,²² serum NO,²⁶ serum 8-OHdG²¹ and serum MDA,^{21,27} no difference from control values in terms of serum CoQ, and 8-OHdG levels¹⁰ have also been reported in children with ADHD. High serum MDA,²³ NO²⁴ and plasma MDA²⁸ have been reported in adults with ADHD. These studies suggest an imbalance in oxidant and antioxidant parameters in subjects with ADHD. In our study, we determined higher KEAP1 levels in children with ADHD compared to the controls. However, we observed no significant difference in NRF2 levels. KEAP1 is a negative regulator of NRF2.¹⁶ No significant relation was determined between severity of ADHD and NRF2 and KEAP1 levels. High KEAP1 levels in patients with ADHD in our study may have caused negative NRF2 regulation, and NRF2 may not have responded sufficiently to oxidative stress as a result. Whether KEAP1 level elevation is a cause or a result is unknown, although it suggested that the response mechanism to oxidative stress may be impaired in children with ADHD, and that oxidative stress may play a role in the pathogenesis of the disorder. In addition, HO-1 levels in patients with ADHD may have decreased in association with oxidative stress, or NRF2 (which also functions as a HO-1 regulator against oxidative stress) may not have reached a level capable of compensating for oxidative stress as a result of not increasing sufficiently. In addition, dopamine is very sensitive to auto-oxidation when antioxidant defense is weak,²⁹ and the dopamine cytotoxicity that emerges can lead to attention and activity-re-

lated problems, in the prefrontal cortex and basal ganglia for example.³⁰ Since dopamine is an important neurotransmitter for ADHD pathogenesis, any condition that may cause dopamine dysfunction is important for ADHD. Therefore, low HO-1 levels fail to balance the oxidative stress and may be associated with dopamine dysfunction in ADHD. In association with this, activation of the Nrf2-dependent HO-1 pathway has been shown to ameliorate the neurotoxicity in oxidative stress-induced human dopaminergic SH-SY5Y cells (as an in vitro cellular Parkinson's disease (PD) model).³¹⁻³³ In addition, NRF2 knockout mice have been reported to be more disposed to striatal damage,³⁴ and a greater loss of dopamine transporter levels in the striatum of NRF2 knockout mice than in wild-type mice has even been reported in a mouse PD model.³⁵ The finding that adenoviral overexpression of HO-1 protects dopaminergic neurons from toxicity in a rat model of PD in another study shows the neuroprotective importance of HO-1 in striatal neurons.³⁶ A very recent study showed that pioglitazone attenuated neurobehavioral impairments and dopaminergic neuronal loss in the substantia nigra by promoting HO-1 enzyme and NRF2 expression and activity in mice.³⁷ Low HO-1 levels or an insufficient NRF2 reaction therefore fail to balance oxidative stress and may be associated with dopamine dysfunction in ADHD. In the light of dopaminergic dysfunction in ADHD, although the above preclinical studies may provide some indication concerning the pathophysiology of ADHD, similar ADHD-model animal studies may be more enlightening.

The limitations of this study include the small sample number, and the fact that parameters were measured in serum, rather than directly in cerebrospinal fluid. The presence of a control group is the strength of our study.

In conclusion, low HO-1 and high KEAP1 levels in the patient group suggest that oxidative stress increased in children with ADHD, but that HO-1 levels were unable to balance this. These findings suggest an increase in oxidative stress in children with ADHD, although this is not related to severity of the disease. In the light of the existing literature and our own findings, there is clearly a need for wider-ranging studies concerning oxidative stress and ADHD.

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