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RESEARCH ARTICLE/ARAŞTIRMA MAKALESİ

High KEAP1, NRF2 and Low HO-1 Serum Levels in Children with Autism

Otizmli Çocuklarda Yüksek KEAP1, NRF2 ve Düşük HO-1 Serum Düzeyleri

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ABSTRACT

Introduction: The purpose of our study was to investigate heme oxygenase-1 (HO-1), nuclear factor erythroid-2-related factor 2 (NRF2), and kelch-like ECH-associated protein 1 (KEAP1) levels in children with autism spectrum disorder (ASD) and to reveal their association with the severity of autism.

Methods: This study measured serum HO-1, KEAP1, and NRF2 levels in 43 patients with ASD (aged 3–12 years) and in 41 age– and gender-matched healthy controls. ASD severity was rated using the Childhood Autism Rating Scale (CARS). HO-1, KEAP1, and NRF2 levels were determined in the biochemistry laboratory using the ELISA technique.

Results: HO-1 levels were significantly lower in patients aged 3–12 years compared to controls aged 3–12, while KEAP1 and NRF2 levels were significantly higher (p=0.020, p<0.001, and p=0.017, respectively). No correlation was determined between ASD severity on the basis of total CARS scores and HO-1, KEAP1 or NRF2 (p>0.05).

Conclusion: This study suggests that oxidative stress is higher in children with ASD and that HO-1 levels are insufficient to achieve oxidative balance.

Keywords: Autism spectrum disorder, oxidative stress, heme oxygenase-1, KEAP1, NRF2

ÖZ

Amaç: Çalışmamızın amacı otizm spektrum bozukluğu (OSB) tanılı çocuklarda heme oksijenaz-1 (HO-1), nükleer faktör eritroid-2 ile ilişkili faktör 2 (NRF2) ve kelch benzeri ECH ile ilişkili protein 1 (KEAP1) düzeylerini araştırmak otizmin ciddiyeti ile ilişkilerini ortaya koymaktır.

Yöntem: Bu çalışmada, OSB tanılı 43 hasta (yaş 3-12 yaş) ve yaş, cinsiyet uyumlu 41 sağlıklı kontrolde serum HO-1, KEAP1 ve NRF2 düzeyleri ölçülmüştür. OSB şiddeti, çocukluk otizm değerlendirme ölçeği (ÇODÖ) kullanılarak derecelendirildi. ELISA tekniği kullanılarak biyokimya laboratuarında HO-1, KEAP1 ve NRF2 seviyeleri belirlendi.

Bulgular: 3-12 yaş arası hastalarda HO-1 düzeyleri 3-12 yaş arası kontrollere göre anlamlı derecede düşükken, KEAP1 ve NRF2 düzeyleri anlamlı olarak yüksek bulundu (sırasıyla p = 0.020, p <0.001 ve p = 0.017). Toplam ÇODÖ skorlarına göre belirlenen OSB şiddeti ile HO-1, KEAP1 veya NRF2 seviyeleri arasında bir ilişki bulunmadı (p> 0.05).

Sonuç: Bu çalışma, OSB'li çocuklarda oksidatif stresin daha yüksek olduğunu ve HO-1 seviyelerinin oksidatif dengeyi sağlamak için yetersiz olduğunu göstermektedir.

Anahtar Kelimeler: Otizm spektrum bozukluğu, oksidatif stres, hem oksijenaz-1, KEAP1, NRF2

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INTRODUCTION

Autism Spectrum Disorder (ASD) is a pediatric neurodevelopmental disorder (1). Although genetic factors occupy an important place in the development of ASD, the condition has been shown to occur as the result of interaction between multiple genetic, environmental, immunological, and neurological factors (2, 3). Oxidative stress, mitochondrial dysfunction, immune dysregulation, inflammation, and exposure to environmental toxicants have also been linked to ASD (4).

Reactive oxygen species (ROS) are known to damage polyunsaturated fatty acids, carbohydrates, proteins and nucleic acids (5), and antioxidant defense mechanisms have developed in the body to prevent that damage. Oxidative stress occurs when the balance between free radicals and antioxidants is compromised in favor of the former (5). Oxidative stress has been recognized as a contributing factor in the progression of numerous neurodegenerative diseases, including Alzheimer's disease (AD) (6). Psychiatric disorders such as schizophrenia, bipolar disorder, major depression and attention deficit hyperactivity disorder have also been shown to be linked to oxidative stress (7–10). There has also been increasing evidence in the last decade showing that oxidative stress undergoes alteration in ASD and that the antioxidant system is unable to redress the balance. Glutathione (GSH)/oxidized glutathione (GSSG) redox ratios in plasma (11) serum (12) and brain tissue (13) have been

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shown to decrease in patients with ASD, and various different redox abnormalities have been reported in children with the condition (14, 15). High plasma levels of the lipid peroxidation marker malondialdehyde (MDA) have been determined in autistic children compared to controls (16, 17). Another study of autistic patients reported low urinary total antioxidant concentration (TAC) and total thiol molecule (TTM) values compared to healthy siblings (18). In addition, increased serum and/or, plasma and/or, erythrocyte oxidative stress and inflammation markers (19–26) have been reported in patients with autism.

Low blood antioxidant enzyme glutathione peroxidase (GSH-Px), and superoxide dismutase (SOD) (27), and of erythrocyte catalase (CAT) (25) and serum paraoxonase 1 arylesterase (28) activities have been determined in autistic children. Low levels of serum SOD (29), and of erythrocyte SOD and GSH-Px (16) have been observed in autistic patients under the age of 6. In another study, although blood SOD activity increased in autistic patients, GSH-Px and CAT activities decreased (30). In contrast to these studies, no change in plasma SOD activity, but increased GSH-Px (23) and CAT activity (18) have also been reported in children with autism. One recent study even reported high erythrocyte SOD and CAT activity (17). Increased heme oxygenase-1 (HO-1) levels in response to oxidative stress have also been reported in the brains of children with autism (31).

HO-1 mediates the catabolism of carbon monoxide (CO), free ferrous iron (Fe^{++}), biliverdin and bilirubin. Its activity, and the resulting products, contribute to protection against oxidative damage, the regulation of apoptosis and inflammation, and angiogenesis (32). Nuclear factor erythroid-2-related factor 2 (NRF2) is a cytoprotective transcription factor that regulates the expression of genes responsible for coding antioxidant, anti-inflammatory and detoxifying proteins, and regulates cytoprotective genes including HO-1 (32). Under normal conditions, NRF2 exists in an inactive complex state with kelch-like ECH-associated protein 1 (KEAP1). Conformational changes occur in KEAP1 in the event of oxidative stress, and it becomes unable to hold onto NRF2. NRF2 then separates from KEAP1 and is translocated to the nucleus (33). The KEAP1-NRF2 signaling pathway serves to preserve redox homeostasis (34).

Previous studies have examined the relationship between oxidative stress and autism, but serum HO-1, NRF2, and KEAP1 levels have not previously been investigated in ASD. The purpose of our study was to investigate serum HO-1, NRF2, and KEAP1 levels in children with ASD and to reveal their association with the severity of autism.

METHODS

Study population

Forty-three children (30 boys, 13 girls) aged 3–12 years presenting to the Harran University Medical Faculty Child and Adolescent Psychiatry Polyclinic, Turkey, between 12/09/2017 and 02/04/2018 and diagnosed with ASD were included in the study. ASD was diagnosed through a clinical interview based on the Diagnostic and Statistical Manual of Mental Disorders, 5th edition (1). Severity of ASD symptoms was evaluated on the basis of Childhood Autism Rating Scale (CARS) scores (35). The validity and reliability of the Turkish version of CARS was confirmed by Gassaloglu et al. (36). The scale consists of 15 domains developed for the differential diagnosis of autism. CARS is particularly effective in differentiating children with autism from those with trainable intellectual disability. Possible total scores range between 15 and 60. Children scoring 15–29.5 are not regarded as exhibiting autistic symptoms, while those scoring 30–36.5 are regarded as mildly-moderately autistic, and those scoring 37–60 as severely autistic (36). The sociodemographic data form elicits data such as the subject's name, age, and sex, number of siblings, rank among siblings, and the age and education levels of both parents.

Physical and neurological examinations were performed following pediatric neurology department consultation, and electroencephalography (EEG) and cerebral magnetic resonance imaging (MRI) were applied in order to identify cases of secondary autism. Patient group exclusion criteria were use of any drug (including antioxidant medication) in the previous two weeks, presence of any comorbid mental disorder (except for intellectual disability) or presence of any physical, neurological, genetic or other medical disease (history of head trauma, atopic eczema and/or history of allergy) at evaluation, and a history of substance, alcohol and tobacco use by the mother during pregnancy.

The control group (CG) consisted of 41 age- and gender-matched children (31 male, 10 female) admitted to the healthy child outpatient clinic over a period of 7 months. These children underwent psychiatric and physical examinations, and their sociodemographic data were recorded. The control group children had no psychiatric and neurological problems, known genetic syndromes, metabolic or endocrine diseases or infections, or allergic reactions, and were not receiving any antioxidant therapy or medications, and there was no history of substance, or alcohol and tobacco use by the mother during pregnancy.

Ethics

The study was approved by the Harran University Medical Faculty Ethics Committee (No. 07, dated 12/09/2017).

All the parents or legal guardians of the participating children gave written informed consent prior to inclusion in the study, in accordance with the Declaration of Helsinki as amended by the World Medical Association Declaration of Helsinki (World Medical Association, 2013). This research was supported by the Harran University Scientific Research Coordination Office (protocol No. 17187 dated 08/12/2017).

Blood Specimen Collection and Biochemical Analysis

Following fasting of at least 8 h, 5 mL blood samples were collected from the antecubital vein from the children in the patient and control groups and placed into gel-containing biochemistry tubes. The blood specimens were immediately centrifuged at 4000 rpm for 10 min at 4°C. The serum part was removed, divided into two halves, and stored at -80°C in Eppendorf tubes until analysis. HO-1, KEAP1, and NRF2 levels in serum were measured in the biochemistry laboratory using the Enzyme-Linked Immunosorbent Assay (ELISA) method and read at 450 nm on a microplate reader (Multiskan GO; Thermo Fisher Scientific, Waltham, MA, USA). Human HO-1 was investigated using an ELISA kit (Fine Test Catalogue No (CN): EH3234). HO-1 levels were expressed as ng/ml. Human KEAP1 was investigated using an ELISA kit (Fine Test CN: EH4240). KEAP1 levels were expressed as pg/ml. Human NRF2 (NFE2L2) was measured using an ELISA kit (Fine Test CN: EH3417). NRF2 levels were expressed as ng/ml.

Statistical Analysis

Statistical analyses were performed on SPSS 23.0 software (SPSS Inc., Chicago, IL, USA). The Pearson chi-square test was used to compare categorical variables. The Shapiro-Wilk normality test was applied to test for normal distribution. Non-parametric tests were employed since the data were not normally distributed. Data were expressed as median values (interquartile range). The Mann-Whitney U test was used for constant and two-group comparisons, and Spearman's correlation analysis for correlation analysis. Linear regression analysis was also performed. P values <0.05 were regarded as statistically significant.

RESULTS

Median ages were six years in the ASD group and seven in the control group. No statistically significant difference was determined between

Table 1. Sociodemographic characteristics and HO-1, KEAP1, and NRF2 levels of the autism spectrum disorder and control groups

Parameters	Patients (n=43)	Controls (n=41)	p
Gender n(%)(F/M)[a]	13 (30.2%) / 30 (69.8%)	10 (24.4%) / 31 (75.6%)	0.548
Age, median(IQR)[b]	6 (3)	7 (3)	0.217
Mother's age median(IQR)	34 (11)	32 (8)	0.713
Mother's education (years) median(IQR)[b]	5 (12)	5 (6,5)	0.335
Father's age median(IQR)[b]	37 (11)	37 (6)	0.527
Father's education (years) median(IQR)[b]	5 (7)	5 (3)	0.248
Number of siblings median(IQR)[b]	2 (3)	3 (2)	0.038
Rank among siblings median(IQR)[b]	2 (2)	2 (3)	0.372
Parents living together/separated[a] n(%)	42 (97%) / 1 (3%)	41 (100%) / 0(0%)	0.326
BMI, median(IQR)[b]	15.97 (3.13)	15.52 (2.87)	0.378
HO-1(ng/ml), median(IQR)[b] KEAP1(pg/ml), median(IQR)[b] NRF2(ng/ml), median(IQR)[b]	0.43 (0.27) 42.6 (26.28) 1.18 (3.14)	0.54 (2.58) 25.02 (8.86) 1.12 (0.50)	0.02 <0.001 0.017

[a]Chi-square p, [b]Mann-Whitney U p. F= female, M=male, IQR= interquartile range

Table 2. Spearman's rho correlation and lineer regression analysis data

Parameteres		HO-1	KEAP-1	NRF2
Age	*(r)*	-0.016	-1.46	-0.504
	(p^1)	*0.920*	*0.350*	*0.001*
(Beta regression coefficient)				*-0.480*
	(p^2)			*0.001*
BMI	*(r)*	0.300	0.386	0.025
	(p^1)	*0.051*	*0.011*	*0.873*
(Beta regression coefficient)			*0.317*	
	(p^2)		*0.039*	
CARS	*(r)*	0.104	0.262	-0.190
	(p^1)	*0.506*	*0.090*	*0.222*

p^1= Spearman's rho correlation *p*, p^2= lineer regression analysis *p*.

the groups in terms of age, gender, mother's age and education, father's age and education, rank among siblings, BMI or parental marital status (*p*>0.05) (Table 1). However, numbers of siblings were significantly higher in the control group (Table 1). Based on CARS scores, 12 (27.9%) patients (all boys) were diagnosed with mild-moderate ASD and 31 (72.1%) (18 boys and 13 girls) with severe ASD. The level of severe autism was significantly higher among girls, (*p*=0.007). The median CARS median (IQR) score in the ASD group was 41 (8.5).

HO-1 levels were significantly lower in patients aged 3–12 years compared to controls aged 3–12, while KEAP1 and NRF2 levels were significantly higher (*p*=0.020, *p*<0.001, and *p*=0.017, respectively) (Table 1).

Spearman's rho correlation analysis revealed negative correlation between age and NRF2, and positive correlation between BMI and KEAP1 (*r*=-0.504, *p*=0.001; and *r*=0.386, *p*=0.011, respectively). No correlation was determined between ASD severity on the basis of total CARS scores and HO-1, KEAP1 or NRF2 (*p*>0.05) (Table 2).

DISCUSSION

Although genetic factors predominate in the etiology of ASD, the last few decades have seen an increase in studies investigating the relationship between oxidative stress and the etiology of ASD by evaluating antioxidant and oxidant parameters. To the best of our knowledge, no previous studies have investigated peripheral HO-1, KEAP1, and NRF2 levels in ASD. The purpose of our study was therefore to assess HO-1, KEAP1, and NRF2 levels in children with ASD and to elaborate their relationship with severity of autism. Our findings showed low HO-1 and high KEAP1 and NRF2 serum levels in children with ASD.

NRF-2 is a transcription factor responsible for the regulation of cellular redox balance in eukaryotic organisms, by regulating the expression of genes that possess the antioxidant response elements (ARE). It also plays a key role in the activities of phase II detoxification enzymes and stress proteins. The various well-characterized antioxidant genes/proteins under this regulation include thioredoxin reductase 1, glutathione-S-transferase, GSH-Px, SOD, CAT, ferritin, and HO-1 (37). Although peripheral HO-1, KEAP1, and NRF2 levels in patients with ASD have not previously been evaluated, various antioxidant markers (in plasma, serum, and erythrocytes) whose expression is regulated by NRF 2 have been investigated in studies in recent decades. Low plasma levels of the antioxidant enzyme GSH-Px and low erythrocyte GSH-Px and SOD activation have been determined in children with autism (27). Increased oxidative stress and decreased CAT (38), and serum paraoxonase 1 arylesterase activity (39) have also been shown in children with ASD (25). In another study, however, despite increased SOD activity in autistic patients, GSH-Px and CAT activities decreased, while no change was determined in plasma and erythrocyte lipid peroxidation and GSH levels (30). In contrast to these studies, other research has reported no change in plasma SOD activity and increased GSH-Px and CAT activity (18) in children with autism. Indeed, one recent study reported high erythrocyte SOD and CAT activity and also increased plasma MDA levels in children with ASD aged 3–6 (17). This suggests that CAT and SOD activities increase in a compensatory manner as a response to increased oxidative stress. Wang et al. reported lower serum SOD levels in autistic children aged 2–6 years than in controls, and even that these were negatively correlated with severity of autism (29). Studies have also determined a decrease in the plasma (11), cerebellum and temporal cortex (13) GSH/GSSG redox ratio. Primary immune cells have been shown to possess a more oxidized extracellular and intracellular microenvironment and to exhibit loss of glutathione redox homeostasis in children with autism compared to control subjects (40). One recent study showed higher serum levels of 8-OHdG, a marker of oxidative stress-related DNA damage, in patients diagnosed with ASD compared to controls (41). These findings indicate differences in oxidant/antioxidant status in patients with ASD. Studies have also suggested that ASD may be linked to dopaminergic dysfunctions (42–44). Decreased release of dopamine in the prefrontal cortex and reduced neural response in the nucleus accumbens have also been shown, in addition to signaling changes in the mesocorticolimbic dopaminergic pathway (42, 43). However, the stereotypic behaviors observed in children with ASD may also arise from a dysfunction of the nigrostriatal dopamine pathway, which has been shown to be involved in mediating stereotypies (45, 46). Pharmacological studies have also revealed the importance of the nigrostriatal dopamine pathway in the formation of stereotypies (47). In addition, dopamine is highly sensitive to auto-oxidation when antioxidant defense is weak, (48) and the dopamine cytotoxicity that emerges can lead to stereotyped behaviors and other ASD-related symptoms in children with ASD. Since dopamine is an important neurotransmitter for the pathogenesis of ASD, any condition that may cause dopamine dysfunction is important for the ASD. The low HO-1 levels shown in this study therefore fail to balance the oxidative stress and may be associated with dopamine dysfunction in ASD. To the best of our knowledge, this is the first clinical study to examine the relationship between peripheral HO-1, KEAP1 and NRF2 levels and ASD. However, preclinical studies have investigated the relationship between the NRF2-dependent HO-1 pathway and dopaminergic cells. Studies have shown that activation of the NRF2-dependent HO-1 pathway ameliorates the neurotoxicity in oxidative stress-induced human dopaminergic SH-SY5Y cells (in an in vitro cellular Parkinson's disease (PD) model) (49–51). One study showed that NRF2-deficient cells and NRF2 knockout mice are significantly more vulnerable to Complex II inhibitors (3-nitropropionic acid and malonate) which cause striatal damage reminiscent of Huntington's disease (52). In addition, a greater decrease in dopamine transporter has been shown in the striatum of NRF2 knockout mice than in wild-type mice in a mouse PD model (53). Another study of PD in a rat model reported that adenoviral overexpression of HO-1 protects dopaminergic neurons against toxicity, thus showing the neuroprotective importance of HO-1 in striatal dopaminergic neurons (54). Moreover, another recent study showed that pioglitazone attenuated neurobehavioral impairments and dopaminergic neuronal loss in the substantia nigra by promoting HO-1 and NRF2 expression and activity in mice (55). In addition, another recent clinical study examining the relationship between the peripheral NRF2/HO-1 pathway and attention deficit hyperactivity disorder (ADHD) (another neurodevelopmental disorder) accompanies by dopaminergic dysfunction, showed that serum HO-1 levels were lower in children diagnosed with ADHD than in healthy controls (56). Only one study reported increased oxidative stress at cerebral examination of autistic patients. Those authors also determined a significant increase in HO-1 levels in the autistic brain as a response to increased oxidative stress (31), although the increase in HO-1 was probably not sufficient to ensure oxidative homeostasis. HO-1 serum levels in our patient group were lower and KEAP1 and NRF2 higher than those in the control group. These findings suggest that HO-1 levels may decrease in an effort to achieve oxidative balance in patients with ASD. Additionally, an increase in NRF2 levels shows the important of increased oxidative stress in patients with ASD. Insufficient HO-1 levels despite the increased NRF2 levels observed in the present study suggested that the oxidative stress balance was not adequately achieved in patients with ASD. Failure to balance oxidative stress may therefore be associated with dopamine dysfunction in children with ASD. Considering this dopaminergic dysfunction in ASD, although the above preclinical studies may provide some indication concerning the pathophysiology of ASD, similar animal model-based ASD studies may be more enlightening.

Low erythrocyte SOD and GSH-Px levels and high levels of MDA, a lipid peroxidation marker, have also been observed in autistic children under six compared to controls. However, no difference was determined in these parameters between the cases and controls aged over 6 (16). A significant finding of our study that may be associated with the above findings was the negative correlation between increased NRF2 serum levels and age. This may suggest that oxidative stress is more intensive in younger children with autism. Our study thus supports the importance of an early therapeutic approach in patients with autism. In a study positive correlation was also determined between CARS scores and MDA (17). However, a study showed no correlation with ASD severity and 8-OhdG serum level (41). Additionally, we determined no correlation between CARS scores and the parameters HO-1, KEAP1 and NRF2. KEAP1 and NFR2 levels were significantly high in our study in children with autism compared to controls. These findings suggested that oxidative stress increases in children with autism. Moreover, low HO-1 serum levels situation suggested an insufficient response to oxidative stress and/or that HO-1 may have decreased in an effort to establish redox homeostasis.

There are a number of limitations to this study, including the low sample number, and parameters being assessed in serum and not directly in cerebrospinal fluid.

In conclusion, elevated serum levels of KEAP1 and NRF2 suggest increased oxidative stress in our patient group. Furthermore, low levels of HO-1 suggest that HO-1 may cause an insufficient response to the KEAP1-NRF2 transcriptional signal or decrease in the process of compensating oxidative stress. We also observed negative correlation between NRF2 and age in patients with ASD. This also suggests that oxidative stress is more intensive in the young age group. Since NRF2 acts as a transcription factor in several antioxidant enzymes in case of oxidative stress, we think that investigation of NRF2 and antioxidant enzymes associated with NRF2 in patients with ASD and/or ASD in a mouse model is important in terms of our understanding of the relationship between oxidative stress and ASD. In the light of the existing literature and our own findings, there is clearly a need for more extensive studies concerning oxidative stress and ASD.

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Ethics Committee Approval: The study was approved by the Harran University Medical Faculty Ethics Committee (No. 07, dated 12/09/2017).

Informed Consent: All the parents or legal guardians of the participating children gave written informed consent prior to inclusion in the study.

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REFERENCES

1. American Psychiatric Association (APA). Diagnostic and statistical manual of mental disorders (DSM-5®): American Psychiatric Pub; 2013.
2. Grice DE, Buxbaum JD. The genetics of autism spectrum disorders. Neuromolecular Med 2006;8:451–460. [Crossref]
3. Rutter M. Genetic Influences and Autism. In: Volkmar FR, Paul R, Klin A, Cohen D, editors. Handbook of autism and pervasive developmental disorders: Diagnosis, development, neurobiology, and behavior (3rd ed.). Hoboken, NJ, USA: John Wiley & Sons; 2005. p.425–453. [Crossref]
4. Rossignol DA, Frye RE. A review of research trends in physiological abnormalities in autism spectrum disorders: immune dysregulation, inflammation, oxidative stress, mitochondrial dysfunction and environmental toxicant exposures. Mol Psychiatry 2012;17:389–401. [Crossref]
5. Gutteridge JM, Halliwell B. Antioxidant Protection and Ixygen Radical Signaling. In: Colton CA, Gilbert DL, editors. Reactive oxygen species in biological systems. Boston, MA. Springer; 2002. p.189–218.
6. Tonnies E, Trushina E. Oxidative Stress, Synaptic Dysfunction, and Alzheimer's Disease. J Alzheimers Dis 2017;57:1105–1121. [Crossref]
7. 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. [Crossref]
8. Camkurt MA, Findikli E, Izci F, Kurutas EB, Tuman TC. Evaluation of malondialdehyde, superoxide dismutase and catalase activity and their diagnostic value in drug naive, first episode, non-smoker major depression patients and healthy controls. Psychiatry Res 2016;238:81–85. [Crossref]
9. Oztop D, Altun H, Baskol G, Ozsoy S. Oxidative stress in children with attention deficit hyperactivity disorder. Clin Biochem 2012;45:745–748. [Crossref]
10. Smaga I, Niedzielska E, Gawlik M, Moniczewski A, Krzek J, Przegalinski E, Pera J, Filip M. Oxidative stress as an etiological factor and a potential treatment target of psychiatric disorders. Part 2. Depression, anxiety, schizophrenia and autism. Pharmacol Rep 2015;67:569–580. [Crossref]
11. James SJ, Cutler P, Melnyk S, Jernigan S, Janak L, Gaylor DW, Neubrander JA. Metabolic biomarkers of increased oxidative stress and impaired methylation capacity in children with autism. Am J Clin Nutr 2004;80:1611–1617. [Crossref]
12. Han Y, Xi QQ, Dai W, Yang SH, Gao L, Su YY, Zhang X. Abnormal transsulfuration metabolism and reduced antioxidant capacity in Chinese children with autism spectrum disorders. Int J Dev Neurosci 2015;46:27–32. [Crossref]
13. Rose S, Melnyk S, Pavliv O, Bai S, Nick TG, Frye RE, James SJ. Evidence of oxidative damage and inflammation associated with low glutathione redox status in the autism brain. Transl Psychiatry 2012;2:e134. [Crossref]
14. Frye R, Delatorre R, Taylor H, Slattery J, Melnyk S, Chowdhury N, James S. Redox metabolism abnormalities in autistic children associated with mitochondrial disease. Transl Psychiatry 2013;3:e273. [Crossref]
15. Ghezzo A, Visconti P, Abruzzo PM, Bolotta A, Ferreri C, Gobbi G, Malisardi G, Manfredini S, Marini M, Nanetti L, Pipitone E, Raffaelli F, Resca F, Vignini A, Mazzanti L. Oxidative stress and erythrocyte membrane alterations in children with autism: correlation with clinical features. PLoS One 2013;8:e66418.
16. Meguid NA, Dardir AA, Abdel-Raouf ER, Hashish A. Evaluation of oxidative stress in autism: defective antioxidant enzymes and increased lipid peroxidation. Biol Trace Elem Res 2011;143:58–65. [Crossref]
17. Altun H, Şahin N, Kurutaş EB, Karaaslan U, Sevgen FH, Fındıklı E. Assessment of malondialdehyde levels, superoxide dismutase, and catalase activity in children with autism spectrum disorders. Psychiatry Clin Psychopharmacol 2018;28:408–415. [Crossref]
18. Ranjbar A, Rashedi V, Rezaei M. Comparison of urinary oxidative biomarkers in Iranian children with autism. Res Dev Dis 2014;35:2751–2755. [Crossref]
19. Essa MM, Guillemin GJ, Waly MI, Al-Sharbati MM, Al-Farsi YM, Hakkim FL, Ali A, Al-Shafaee MS. Increased markers of oxidative stress in autistic children of the Sultanate of Oman. Biol Trace Elem Res 2012;147:25–27. [Crossref]
20. Ghanizadeh A, Berk M, Farrashbandi H, Villagonzalo KA. Targeting the mitochondrial electron transport chain in autism, a systematic review and synthesis of a novel therapeutic approach. Mitochondrion 2013;13:515–519. [Crossref]
21. Kern JK, Geier DA, Sykes LK, Geier MR. Evidence of neurodegeneration in autism spectrum disorder. Transl Neurodegener 2013;2:17. [Crossref]
22. Napoli E, Wong S, Giulivi C. Evidence of reactive oxygen species-mediated damage to mitochondrial DNA in children with typical autism. Mol Autism 2013;4:2. [Crossref]
23. Söğüt S, Zoroğlu SS, Özyurt H, Yılmaz HR, Özuğurlu F, Sivaslı E, Yetkin Ö, Yanık M, Tutkun H, Savaş HA, Tarakçıoğlu M, Akyol Ö. Changes in nitric oxide levels and antioxidant enzyme activities may have a role in the pathophysiological mechanisms involved in autism. Clinica Chimica Acta 2003;331:111–117. [Crossref]
24. Zhang QB, Gao SJ, Zhao HX. Thioredoxin: a novel, independent diagnosis marker in children with autism. Int J Dev Neurosci 2015;40:92–96. [Crossref]
25. Zoroglu SS, Armutcu F, Ozen S, Gurel A, Sivasli E, Yetkin O, Meram I. Increased oxidative stress and altered activities of erythrocyte free radical scavenging enzymes in autism. Eur Arch Psychiatr Clin Neurosci 2004;254:143–147. [Crossref]
26. Zoroğlu SS, Yürekli M, Meram İ, Söğüt S, Tutkun H, Yetkin Ö, Sivasli E, Savaş HA, Yanik M, Herken H, Akyol Ö. Pathophysiological role of nitric oxide and adrenomedullin in autism. Cell Biochem Function 2003;21:55–60. [Crossref]
27. Yorbik O, Sayal A, Akay C, Akbiyik D, Sohmen T. Investigation of antioxidant enzymes in children with autistic disorder. Prostaglandins Leukot Essent Fatty Acids 2002;67:341–343. [Crossref]
28. Paşca SP, Nemeş B, Vlase L, Gagyi CE, Dronca E, Miu AC, Dronca M. High levels of homocysteine and low serum paraoxonase 1 arylesterase activity in children with autism. Life Sci 2006;78:2244–2248. [Crossref]
29. Wang L, Jia J, Zhang J, Li K. Serum levels of SOD and risk of autism spectrum disorder: a case-control study. Int J Dev Neuroscience 2016;51:12–16. [Crossref]
30. László A, Novak Z, Szőllősi-Varga I, du Hai Q, Vetró A, Kovács A. Blood lipid peroxidation, antioxidant enzyme activities and hemorheological changes in autistic children. Ideggyogyaszati Szemle 2013;66:23–28.
31. Evans TA, Siedlak S, Lu L, Fu X, Wang Z, McGinnis W, Fakhoury E, Castellani R, Hazen S, Walsh W, Lewis AT, Salomon RG, Smith MA, Perry G, Zhu X. The autistic phenotype exhibits a remarkably localized modification of brain protein by products of free radical-induced lipid oxidation. Am J Biochem Biotechnol 2008;4:61–72. [Crossref]
32. Loboda A, Damulewicz M, Pyza E, Jozkowicz A, Dulak J. Role of Nrf2/HO-1 system in development, oxidative stress response and diseases: an evolutionarily conserved mechanism. Cell Mol Life Sci 2016;73:3221–3247. [Crossref]
33. Levonen A-L, Hill BG, Kansanen E, Zhang J, Darley-Usmar VM. Redox regulation of antioxidants, autophagy, and the response to stress: implications for electrophile therapeutics. Free Radic Biol Med 2014;71:196–207. [Crossref]
34. Sihvola V, Levonen AL. Keap1 as the redox sensor of the antioxidant response. Arch Biochem Biophys 2017;617:94–100. [Crossref]
35. Schopler E, Reichler RJ, DeVellis RF, Daly K. Toward objective classification of childhood autism: Childhood Autism Rating Scale (CARS). J Autism Dev Disord 1980;10:91–103. [Crossref]
36. İncekaş Gassaloğlu S, Baykara B, Avcil S, Demiral Y. Validity and Reliability Analysis of Turkish Version of Childhood Autism Rating Scale. Turkish Journal of Psychiatry 2016;27. [Crossref]
37. Habtemariam S. The Nrf2/HO-1 Axis as Targets for Flavanones: Neuroprotection by Pinocembrin, Naringenin, and Eriodictyol. Oxid Med Cell Longev 2019;2019:4724920. [Crossref]
38. Zoroglu SS, Armutcu F, Ozen S, Gurel A, Sivasli E, Yetkin O, Meram I. Increased oxidative stress and altered activities of erythrocyte free radical scavenging enzymes in autism. Eur Arch Psychiatry Clin Neurosci 2004;254:143–147. [Crossref]
39. Pasca SP, Nemes B, Vlase L, Gagyi CE, Dronca E, Miu AC, Dronca M. High levels of homocysteine and low serum paraoxonase 1 arylesterase activity in children with autism. Life Sci 2006;78:2244–2248. [Crossref]
40. Rose S, Melnyk S, Trusty TA, Pavliv O, Seidel L, Li J, Nick T, James SJ. Intracellular and extracellular redox status and free radical generation in primary immune cells from children with autism. Autism Res Treat 2012;2012:986519. [Crossref]
41. Kilicaslan F, Ayaydin H, Celik H, Kutuk MO, Kandemir H, Koyuncu I, Kirmit A. Antineuronal antibodies and 8-OHdG an indicator of cerebellar dysfunction in autism spectrum disorder: a case-control study. Psychiatry Clin Psychopharmacol 2019;29:840–846. [Crossref]
42. Scott-Van Zeeland AA, Dapretto M, Ghahremani DG, Poldrack RA, Bookheimer SY. Reward processing in autism. Autism Res 2010;3:53–67. [Crossref]
43. Ernst M, Zametkin AJ, Matochik JA, Pascualvaca D, Cohen RM. Low medial prefrontal dopaminergic activity in autistic children. Lancet 1997;350:638. [Crossref]
44. Dichter GS, Felder JN, Green SR, Rittenberg AM, Sasson NJ, Bodfish JW. Reward circuitry function in autism spectrum disorders. Soc Cogn Affect Neurosci 2012;7:160–172. [Crossref]
45. Lewis M, Kim SJ. The pathophysiology of restricted repetitive behavior. J Neurodev Disord 2009;1:114–132. [Crossref]

46. Grossberg S, Kishnan D. Neural Dynamics of Autistic Repetitive Behaviors and Fragile X Syndrome: Basal Ganglia Movement Gating and mGluR-Modulated Adaptively Timed Learning. Front Psychol 2018;9:269. [Crossref]
47. Lewis MH, Bodfish JW. Repetitive behavior disorders in autism. Ment Retard Dev Disabil Res Rev 1998;4:80–89. [Crossref]
48. Iuga C, Alvarez-Idaboy JR, Vivier-Bunge A. ROS initiated oxidation of dopamine under oxidative stress conditions in aqueous and lipidic environments. J Phys Chem B 2011;115:12234–12246. [Crossref]
49. Sheng XJ, Tu HJ, Chien WL, Kang KH, Lu DH, Liou HH, Lee MJ, Fu WM. Antagonism of proteasome inhibitor-induced heme oxygenase-1 expression by PINK1 mutation. PloS One 2017;12:e0183076. [Crossref]
50. He Q, Song N, Jia F, Xu H, Yu X, Xie J, Jiang H. Role of alpha-synuclein aggregation and the nuclear factor E2-related factor 2/heme oxygenase-1 pathway in iron-induced neurotoxicity. Int J Biochem Cell Biol 2013;45:1019–1030.
51. Kwon SH, Lee SR, Park YJ, Ra M, Lee Y, Pang C, Kim KH. Suppression of 6-Hydroxydopamine-Induced Oxidative Stress by Hyperoside Via Activation of Nrf2/HO-1 Signaling in Dopaminergic Neurons. International Journal of Molecular Sciences 2019;20. [Crossref]
52. Calkins MJ, Jakel RJ, Johnson DA, Chan K, Kan YW, Johnson JA. Protection from mitochondrial complex II inhibition in vitro and in vivo by Nrf2-mediated transcription. Proc Natl Acad Sci U S A 2005;102:244–249. [Crossref]
53. Innamorato NG, Jazwa A, Rojo AI, Garcia C, Fernandez-Ruiz J, Grochot-Przeczek A, Stachurska A, Jozkowicz A, Dulak J, Cuadrado A. Different susceptibility to the Parkinson's toxin MPTP in mice lacking the redox master regulator Nrf2 or its target gene heme oxygenase-1. PloS One 2010;5:e11838. [Crossref]
54. Hung SY, Liou HC, Kang KH, Wu RM, Wen CC, Fu WM. Overexpression of heme oxygenase-1 protects dopaminergic neurons against 1-methyl-4-phenylpyridinium-induced neurotoxicity. Mol Pharmacol 2008;74:1564–1575. [Crossref]
55. Zakaria A, Rady M, Mahran L, Abou-Aisha K. Pioglitazone Attenuates Lipopolysaccharide-Induced Oxidative Stress, Dopaminergic Neuronal Loss and Neurobehavioral Impairment by Activating Nrf2/ARE/HO-1. Neurochem Res 2019. [Crossref]
56. Ayaydın H, Koyuncu İ, Çelik H, Kirmit A, Akaltun İ, Takatak H. Low HO-1 and high KEAP1 serum levels in children with attention deficit hyperactivity disorder: the first clinical study. Anadolu Psikiyatri Derg 2020;21:319–326. [Crossref]