Thiol/Disulphide Homeostasis and Oxidative Stress Parameters in Children and Adolescents with Attention Deficit/ Hyperactivity Disorder

Dikkat Eksikliği Hiperaktivite Bozukluğu Olan Çocuk ve Ergenlerde Tiyol/Disülfit Dengesi ve Oksidatif Stres Parametreleri

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Abstract

Aim: Research investigating association between attention deficit/hyperactivity disorder (ADHD) and oxidative stress have reported conflicting and inconsistent findings. We aimed to investigate a novel oxidative stress marker, thiol/disulphide homeostasis, in patients with ADHD and compare the results with the healthy control group.

Materials and Methods: A total of 47 medication naïve children and adolescents (35 boys and 12 girls) aged 6–17 years with a diagnosis of ADHD were investigated for oxidative stress parameters and results were compared with that of 41 subjects (28 boys and 13 girls) matched for age and gender. The Schedule for Affective Disorders and Schizophrenia for School-Age Children-Present and Lifetime Version (KSADS-PL) was conducted to support ADHD diagnosis and to exclude comorbid psychiatric disorders. Thiols, total antioxidant status (TAS), total oxidant status (TOS), oxidative stress index (OSI) levels were measured in serum samples in addition to myeloperoxidase (MPO) and dynamic thiol/disulphide homeostasis.

Results: TOS, OSI and MPO levels were significantly lower in ADHD group (p=0.001, p=0.022, p=0.007, respectively). Predominantly inattentive type ADHD had significantly higher levels of TAS (p=0.014) and TOS (p=0.01) than those with combined ADHD subtype. Oxidative stress index (OSI) levels decreased with increasing age in both ADHD and control groups as well in the whole sample when control and patient groups were tested together (r=-0.376, p=0.009; r=-0.479, p= 0.002; and r=-0.367, p<0.001, respectively). TAS scores significantly increased with age in ADHD group (r= 0.523, p<0.001). Thiol/disulphide homeostasis showed no difference.

Discussion and Conclusion: The current study reveals no association between pediatric-age ADHD and thiol/disulphide homeostasis. The nature of relationship between oxidative stress and ADHD needs to be clarified with methodologically robust studies.

Keywords: oxidative stress; thiol/disulphide homeostasis; attention deficit hyperactivity disorder; children and adolescents

Özet

Amaç: Bilimsel çalışmalar dikkat eksikliği hiperaktivite bozukluğu (DEHB) ve oksidatif stres arasındaki ilişki ile ilgili farklı ve tutarsız veriler sunmaktadır. Bu çalışmada DEHB hastalarında tiyol/disülfit homeostazı gibi yeni bir oksidatif stres markırını incelemek ve sonuçları sağlıklı kontrol grubu ile karşılaştırmak amaçlanmıştır.

Gereç ve Yöntemler: Çalışmaya psikotrop ilaç kullanım öyküsü olmayan 6–17 yaş aralığında ve DEHB tanılı 47 çocuk (35 erkek ve 12 kız) ve yaş ve cinsiyet olarak benzer 41 sağlıklı çocuk (28 erkek, 13 kız) dâhil edilmiştir. Total antioksidan seviyesi (TAS), total oksidan seviyesi (TOS), oksidatif stres indeksi (OSI), miyeloperoksidaz (MPO) ve dinamik tiyol/disülfit homeostazı gibi oksidatif stres parametreleri bu iki grup arasında karşılaştırılmıştır. Eş tanı durumu Okul Çağı Çocukları için Duygulanım Bozuklukları ve Şizofreni Görüşme Çizelgesi-Şimdi ve Yaşam Boyu Şekli-Türkçe Uyarlaması (ÇDŞG-ŞY-T) kullanılarak değerlendirilmiştir.

Bulgular: TOS, OSI ve MPO değerleri DEHB grubunda anlamlı olarak düşük bulunmuştur (sıra-

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sıyla p=0,001; p=0,022; p=0,007). Dikkat-eksikliği-baskın-tip DEHB grubunda TAS (p=0.014) ve TOS (p=0.01) değerleri kombine alt tipe oranla anlamlı olarak daha düşük çıkmıştır. Oksidatif stres indeksi (OSI), hem hasta ve kontrol gruplarında hem de iki grup birlikte değerlendirildiğinde artan yaş ile birlikte istatistiksel olarak anlamlı bir azalma göstermiştir (sırasıyla r= - 0.376, p=0.009; r= - 0.479, p= 0.002 ve r= - 0.367, p<0.001). TAS değerleri kontrol grubunda, TOS değerleri ise hasta grubunda yaş ile anlamlı bir ilişki göstermiştir. Tiyol/disülfit dengesi iki grup arasında anlamlı bir fark göstermemiştir.

Tartışma ve Sonuç: Mevcut çalışmada, dinamik tiyol/disülfit dengesi ve çocukluk çağı DEHB'si arasında anlamlı bir ilişki bulunamamıştır. Diğer çalışmalarda bildirilen ve bizim çalışmamızda da kısmen desteklenen olası ilişki alanların açığa kavuşturulması ve olası sebep-sonuç ilişkisinin aydınlatılması için metodolojik olarak daha güçlü çalışmalara ihtiyaç vardır.

Anahtar Sözcükler: oksidatif stres; tiyol/disülfit dengesi; dikkat eksikliği hiperaktivite bozukluğu; çocuk ve ergen

INTRODUCTION

Attention deficit hyperactivity disorder (ADHD) is one of the most common neuro-behavioral disorders in children and characterized by symptoms of hyperactivity, impulsiveness and inattention that are beyond the norm for a child's age (1). Existing knowledge about etiopathogenesis of ADHD remains largely speculative; however, possible involvement of multiple factors such as neurochemical deficits, cerebral circulatory impairments and subtle fetal and perinatal brain damage due to toxic, metabolic or physical insult have been advocated (2,3). Oxidative stress has recently been an area of academic interest in terms of its theoretical role in pathogenesis of ADHD. The underlying mechanisms is said to be via "oxygen paradox," which refers to the opposing effects of oxygen on physiological functioning of cells. Oxygen is essential for aerobic life; however, excessive amounts of its free radical metabolic by-products are toxic to the protein structure of cell membranes and inhibit the uptake of enzymes/neurotransmitters. Antioxidant defense systems are in place to prevent formation of these oxidants and their harmful effects. When this redox homeostasis is tipped toward an overbalance of free radicals, then oxidative stress occurs (4–6). Human brain is highly susceptible to oxidative damage caused by free radicals and this imbalance of oxidant and antioxidant parameters are thought to contribute to the development of several childhood psychiatric disorders including autism, developmental disorders, and ADHD. However, research investigating association between ADHD and oxidative stress have reported conflicting and inconsistent findings. For example, while malondialdehyde (MDA), a marker of lipid peroxidation, was found in high levels in children (7) and adults (8,9) with ADHD, lowered concentrations of MDA were reported in other studies (10,11). A recent meta-analysis conducted by Joseph et al. (12) reported results from six studies involving a total of 231 ADHD patients and 207 controls. Association of ADHD with antioxidant status was reported to be not significant and its association with oxidative stress lost significance after correcting for intra-study clustering.

Conversely, ADHD was concluded to be associated with increased oxidative and nitrosative stress (O&NS) in a more recent review article (13). Inconsistency in findings was, at least in part, attributed to differences in O&NS markers tested, samples used, populations examined, and testing and collection protocols utilized to examine relevant markers. Sezen et al. (14) reported total oxidant status (TOS) and the oxidative stress index (OSI) to be higher in children with attention deficit hyperactivity disorder (ADHD) than the control group ($p < 0.001$). Markers of oxidative stress; paraoxonase-1 (PON-1) ($p = 0.002$) and arylesterase (ARE) ($p = 0.010$) activity and total antioxidant status (TAS) ($p < 0.001$) were lower in the patient group than the control group. The authors concluded that there was a significantly increased oxidative stress in children with ADHD.

Similar results were reported by Kul et al. (15) in a pediatric ADHD group. TAS was noted to be significantly lower, and TOS and OSI significantly higher in patients than healthy controls. They also noted that those with comorbid oppositional defiant disorder (ODD) had lower TAS than subjects with no comorbidity, and no difference was found in TOS or OSI among the ADHD subtypes. Alpak et al.(16) found significantly lower thiol levels in adult ADHD patients than control group. They interpreted results to be a reaction to increased catalase (CAT) levels and that thiol acted like a pro-oxidant in their study. Contrasting results were reported in pediatric patients, where MDA and nitric oxide, good indicators of the oxidative

stress, were found higher than controls (7). Thiol levels were found higher than controls, but this finding was not statistically significant. To sum up, there does not seem to be a convincing research-based evidence that firm conclusions with clinical implications –if possible at all– can be drown upon.

MATERIALS AND METHODS

Subjects and study hypotheses

The study group included a total of 47 children and adolescents (35 boys and 12 girls) aged 6–17 years, who had been diagnosed with attention deficit/hyperactivity disorder (ADHD) for the first time at the outpatient department of child and adolescent psychiatry at Bezmialem Vakif University between January 2016 and February 2016. Diagnosis of ADHD was determined with a clinical interview based on the Diagnostic and Statistical Manual of Mental Disorders, 5th edition (1). The Schedule for Affective Disorders and Schizophrenia for School-Age Children-Present and Lifetime Version was conducted to support ADHD diagnosis and to exclude comorbid psychiatric disorders (K-SADS-PL) (17) is a semi-structured interview, Turkish adaptation of which was conducted by Gökler and colleagues (18). The ADHD group patients had not been on any medication when the blood sample was taken or in the preceding 6 weeks. Those with learning disabilities and autism spectrum disorders were excluded based on clinical assessment.

Control group consisted of 41 children and adolescents (28 boys and 13 girls) matched for age and gender, who had attended the pediatric outpatient clinic at Bezmialem Vakıf University and discharged without any diagnosis or treatment. They had no chronic metabolic or neurological conditions or psychiatric disorders to note. The study inclusion criteria for both groups were being aged between 6 and 17 years, having an IQ over 80, having no medication use current or in the previous six weeks, absence of underlying endocrine or metabolic disorders or abnormal results from routine laboratory tests. None of the children were taking nutritional supplements that could be accounted for antioxidant activity.

Based on the previously reported findings we hypothesized that children and adolescents with ADHD will have higher levels of oxidative stress parameters. We also aimed to investigate a novel oxidative stress marker, thiol/disulphide homeostasis, in patients with ADHD and compare the results with healthy controls. The study protocol was approved by Bezmialem Vakıf University ethics committee (date: 28.08.2015; number: 71306642-050.01.04).

Measurement of oxidative and antioxidant stress parameters

A novel automated colorimetric measurement method described by Erel (19) was used to measure the plasma's total oxidative status (TOS) and total antioxidant status (TAS). The ratio of TOS to TAS is compared using the oxidative stress index (OSI). The OSI value was calculated according to the reported formula (19,20): OSI (arbitrary unit) = [TOS (μmol H2O2 Equiv./L) / TAS (mmol Trolox Equiv./L)] x 100. The total thiol concentration of sulfhydryl groups (SH) in the plasma was analyzed by using the methods first described by Ellman (21) and modified by Hu (22). Here, thiols interact with 5.5'-dithiobis-(2-nitrobenzoic acid) (DTNB), causing a highly colored anion with a maximum peak at 412 nm (e412 = 13 600 M -1 cm -1). The results were expressed in μmol/L (23).

In terms of measuring dynamic thiol/diulphide homeostasis, we used the novel automated assay method developed by Erel and Neselioglu (24), in which both sides of thiol/disulphide balance can be measured as opposed to the method developed by Ellman (21), which can measure only one side of this balance. This novel method is based on the sulfhydryl groups of proteins turning into a reversible formation of disulphides under oxidative conditions and reduction of disulphide bonds into thiol groups again. The principle of the new assay is sodium borohydride (NaBH4), which is used to reduce the disulphide bonds to the thiol groups. The sum of existing thiol groups and reduced thiol groups gives the total thiol. The NaBH4 residuals that are not used are completely removed by formaldehyde. Hence, the extra reduction of 5,50-dithio-bis-(2-nitrobenzoic acid) (DTNB) is prevented. The native thiol is measured using a modified Ellman's reagent (the classic Ellman's reagent was modified by adding a formaldehyde solution). The difference between the total thiol and the native thiol is divided by two to obtain the amount of the disulphide bond. Measure-

ments were made using a Cobasc501 (Roche Diagnostics, Mannheim, Germany). Serum thiol/disulphide homeostasis values were presented as mmol/L (25).

Statistical analysis

The Statistical Package for the Social Sciences version 19.0 (SPSS Inc., Chicago, IL, USA) was used to conduct the statistical tests. The normality of continuous variables was tested by the Shapiro-Wilk and Kolmogorov-Smirnov tests. Continuous variables with a normal distribution were reported as mean ± standard deviation. Comparisons were made between groups, using independent-sample t-tests for normally-distributed data and Mann-Whitney U test for the data that disputes normal distribution. Pearson's (for normally distributed data) or Spearman' correlation analysis (for non-normally distributed data) were used to evaluate correlations between the scales and variables. All analyses were conducted as two-tailed tests and $p<0.05$ was deemed statistically significant.

RESULTS

Demographics and comorbidities

There was no statistically significant difference between the mean age and gender distribution of the all subjects in ADHD and control groups (Table 1).

When ADHD subtypes were compared, however, combined group (ADHD-C) patients were younger than both inattentive subtype ($p<0.001$) and control group ($p=0.03$). The most common ADHD subtype was ADHD-combined (ADHD-C, 46.8%, n=22), which was followed by ADHD-inattentive (ADHD-IA, 42.6%, n=20), and ADHD-hyperactive/impulsive (ADHD-H/I, 10.6%, n=5) subtypes. Comorbidity was present in 31.5% (15/47) of the patients and elimination disorders (mostly enuresis) was the most common

Table 1. Demographical values and oxidative stress parameters of the ADHD group and control group

	ADHD sub-groups (Mean ± SD)				Control (Mean ± SD)	*Values**
	ADHD-IA	**ADHD-C**	**ADHD-H/I**	**ADHD-Total**		
N (%)	20 (42.6 %)	22 (46.8 %)	5 (10.6 %)	47 (100%)	41 (100%)	
Age (years)	11.30 ± 2.83	8.27 ± 2.25[D]	8.60 ± 2.70	*9.60 ± 2.91*	*10.22 ± 3.19*	*t = 0.781 p = 0,437*
Sex (m/f)	12/8	18/4	5/0	*35/12*	*28/13*	*X^2= 0.229 p = 0.648*
Total Antioxidant Status (TAS) [mmol TroloxEquiv/L]	1.14 ± 0.14[A]	1.03 ± 0.14	1.14 ± 0.16	*1.09 ± 0.15*	*1.10 ± 0.16*	*t= 0.552 p=0.583*
Total Oxidative Status (TOS) [µmol H2O2 Equiv/L]	11.37 ± 2.41[B]	11.52 ± 2.29[B]	12.14 ± 2.28	*11.52 ± 2.30*	*13.85 ± 2.74*	*t= 4,324* ***p=0.001***
Oxidative Stress Index (OSI) [AU]	10.11 ± 2.32[B]	11.37 ± 2.51	10.69 ± 1.41	*10.76 ± 2.37*	*12.76 ± 3.09*	*t= 3,432* ***p=0.022***
Myeloperoxidase (MPO) [IU/L]	42.76 ± 20.60[C]	42.51 ± 20.56[C]	36.09 ± 8.02[C]	*41.93 ± 19.45*	*62.30 ± 27.07*	*z=3.835* ***p=0.007***
Native thiol, µmol/L, mean ± SD	421.40 ± 22.97	424.39 ± 25.99	422.40 ± 18.30	*422.90 ± 23.61*	*431.19 ± 33.24*	*t=1.330. p=0.188*
Total thiol, µmol/L, mean ± SD	455.22 ± 19.75	461.80 ± 22.65	464.90 ± 25.43	*459.33 ± 21.56*	*464.70 ± 29.04*	*z=0. 993 p=0.324*
Disulphide, µmol/L, mean ± SD	16.91 ± 6.94	18.98 ± 5.86	21.26 ± 7.16	*18.34 ± 6.49*	*16.81 ± 2.64*	*z=0.422 p=0.108*
Disulphide/ Native thiol %, mean ±SD	4.07 ± 1.81	4.52 ± 1.51	5.03 ± 1.68	*4.38 ± 1.65*	*3.96 ± 0.93*	*z=0.640 p=0.522*
Disulphide / Total thiol %, mean ±SD	3.72 ± 1.51	4.12 ± 1.28	4.54 ± 1.37	*3.99 ± 1.39*	*3.66 ± 1.39*	*z=0.648 p=0.517*
Native thiol/ Total thiol %, mean ±SD	92.57 ± 3.03	91.87 ± 2.57	90.37 ± 2.75	*92.07 ± 2.78*	*92.71 ± 1.52*	*z=0.506 p=0.613*

ADHD: Attention deficit/hyperactivity disorder, IA: Inattentive type, C: Combined type, H/I: Hyperactive/impulsive type, AU: Arbitrary Unit; A= Higher than combined group (p=0.01); B= lower than control group (p=0.01); C= lower than control (p=0.02); D= lower than inattentive type (p<0.001) and control (p=0.003).
*Difference between control group and ADHD-total group.

Table 2. Correlation of oxidative stress parameters with age

			TAS	TOS	OSI	Thiol/ Disulphide	MPO
Age	Total (N=88)	Correlation coefficient (r)	**0.363***	-0.194	**-0.367****	-0.043	-0.080
	ADHD (N=47)	Correlation coefficient (r)	**0.523****	-0.067	**-0.376***	0.005	-0.052
	Control (N=41)	Correlation coefficient (r)	0.197	**-0.437***	**-0.479***	-0.100	-0.208

*p < .01. **p < .001, TAS: total antioxidant status, TOS: total oxidative status, OSI: oxidative stress index, MPO: myeloperoxidase

group (8/47, 16.8%), which is followed by oppositional defiant disorder (10.5%) and tic disorders (4.2%).

Comparison of parameters between ADHD total and control groups

Total antioxidant levels (TAS) showed no difference between ADHD patients and control group (1.09 ± 0.15 vs 1.10 ± 0.16; p=0.682); however, TOS score was significantly lower in ADHD group (11.52 ± 2.30 vs 13.85 ± 2.74; p=0.001). We also found oxidative stress index (OSI) significantly lower in ADHD patients as compared to control group (12.76 ± 3.09 vs 10.76 ± 2.37; p=0.02). Myeloperoxidase (MPO) value was also significantly lower in the patient group (62.30 ± 27.07 vs 41.93 ± 19.45; p=0.007). Similar to TAS levels, the other parameters tested did not significantly differ between the two groups (Table 1).

Comparison of parameters between ADHD subtypes and control group

Predominantly inattentive type ADHD patients had significantly higher levels of TAS than those with combined ADHD subtype (1.14 ± 0.14 vs 1.03 ± 0.14; p=0.014). Total oxidative status (TOS) was significantly more elevated in inattentive ADHD type as compared to combined group of ADHD patients (1.14 ± 0.14 vs 1.03 ± 0.14; p=0.01). ADHD subtype group did not differ between themselves and from control group in terms of other oxidative stress parameters.

Correlation of parameters with age

In control group, total oxidative status (TOS) levels showed a decline as age increased (r= -0.437, p=0.004); however, there was no significant relationship in ADHD group. Total antioxidant status (TAS) scores, on the other hand, significantly increased with age in ADHD group (r= 0.523, p<0.001). TAS levels showed no relationship with age in control group however this relationship was significant when patient and control groups were combined (r=0.323; p=0.001)

Oxidative stress index (OSI) levels decreased as age increased in both ADHD and control groups as well as in the whole sample when control and patient groups were tested together (r= -0.376, p=0.009; r= -0.479, p= 0.002; and r= -0.367, p<0.001, respectively) (Table 2).

Correlation of parameters in children and adolescents as separate groups

We also examined the parameters within the ADHD group based on an artificial division of the patients as children (aged 6 to 11 years) and adolescents (aged 12 to 17 years). In those under the age of 12, the levels of TOS (t= -4.736, p<0.001), OSI (t= -3.742, p = 0.001), and myeloperoxidase (MPO) (z= -3.631, p< 0.001) were statistically significantly lower than the group categorized as adolescents. The other parameters revealed no significant difference.

DISCUSSION AND CONCLUSIONS

Although thiol levels as an antioxidant parameter have been previously reported, to our knowledge this is the first study that investigated the dynamic thiol/ disulphide homeostasis and myeloperoxidase in children and adolescents with ADHD. The first major finding is the plasma levels of TAS, TOS, and OSI that appear to be in line with inconsistencies highlighted in the literature. Total antioxidant status (TAS) levels in our study showed no difference between ADHD patients and healthy controls, which is similar to findings in the study reported by Karababa et al. (26). In their adult ADHD population they noted plasma homocysteine and folate levels as statistically significantly different between the groups; but serum vitamin B12, TAS, TOS, and OSI levels showed no significant difference. On the other hand, previous studies had re-

ported higher than normal TAS levels in adults ADHD patients by Selek et al. (27) and lower than normal in children by Kul et al. (15). Reported as antioxidant activity instead of TAS, Ruchi et al. (28) noted lower levels in ADHD children while higher than normal levels were reported by Çelik et al. (29) in a similar age group.

Due to the impracticality of measuring different oxidant and antioxidant molecules, additive effects of oxidants and antioxidants, measured as total oxidant-antioxidant status is more valid and reliable than measuring each of them separately (15). We found TOS and OSI levels lower than control group in our pediatric-age ADHD patients. While some studies had reported increased levels of TOS and OSI as oxidative stress parameters in adults (27) and children with ADHD (15,23), some other researchers had reported no statistically significant difference in TOS and OSI levels in adults (26) or in advanced oxidation protein products in children (10). However, it seems that the oxidative stress parameter tested can give rise to different and sometimes opposing results. For example, when the parameter tested was 8-Oxo-2'-deoxyguanosine or malondialdehyde (MDA) then lower than control levels were found in ADHD children in the same study conducted by Oztop et al. (10). The latter parameter was reported to be decreased in ADHD children in another study (11) and similarly nitrosative stress parameters were noted to be lower in children with disruptive behaviors (30).

Decreased antioxidant activity reported in our study is possibly associated with hypo-dopaminergic activity. Malondialdehyde (MDA), noted to be a good indicator of oxidative stress, was reported to be significantly lower in the ADHD group than in the control group (10), which is consistent with the findings in Spahis et al.'s study (11) where decreased oxidative stress was observed in the ADHD patients, as evidenced by 20% lower MDA concentrations than in the control subjects in ADHD children. Varol et al. (30) found that the levels of another oxidant, NO, were low in ADHD children [17]; and Çelik et al. (29) reported similar results, noting that "no oxidative stress develops in individuals with ADHD in high antioxidant activity and stable catalase activity." These findings might suggest decreased plasma oxidant levels, and it is possible that a decreased oxidant level is associated with the hypodopaminergic state in ADHD. But how do we explain such a wide array of findings in published literature? More specifically, what would be a convincing explanation for the lower than control levels of oxidative stress in patient with ADHD? If the findings are reliable can we then suggest ADHD to be protective against oxidative stress? We do need to take into account the fact that all variables that could possibly be accounted for oxidative stress or imbalanced antioxidant status have not been fully controlled. We surely need more robust studies with methodologically stronger designs and larger sample size to clarify this confusion so that findings can then be presented with clear implications for therapeutic approaches.

We also compared the oxidative and antioxidant stress parameters between the children and adolescents in the ADHD group. Both TAS and TOS levels were lower in those under the age of 12 (children) as compared to adolescents. When oxidative stress is high, antioxidant levels are normally expected to be lower; however, a compensatory increase in oxidants was speculated to be a possible explanation by Selek et al. (27), who had reported higher than control levels of both TOS and TAS in ADHD patients. We found that the parameters that indicate increased oxidative stress (high levels of TOS and OSI) tend to decrease with age while TAS levels shows a statistically significant increase in parallel to age. This comparison should not be confused with those in the paragraph above, in which both TAS and TOS levels were reported to be higher in adolescents.

We looked into how these parameters change among the ADHD subtypes. TAS levels were significantly higher in patients with inattentive type (ADHD-IA) than combined type ($p=0.01$). We can only compare this findings with that of Kul et al. (15), who had reported that their study was the first to have compared oxidative parameters among the ADHD subtypes. They noted lower levels of TAS in the ADHD-C and ADHD-H/I subtypes than those in the ADHD-IA subtype. They speculated that, although it is uncertain, several environmental and biological variables and/or unknown factors may contribute to the development of this condition and that impairment of the antioxidant defense mechanisms in the ADHD-H/I com-

pared with the ADHD-IA subtype should be considered in prospective studies. Our results revealed higher levels of TOS in the inattentive (ADHD-IA) and combined types in comparison to the control group (p=0.01). The oxidative stress index (OSI) is the ratio of TOS to TAS, which reveals how much homeostasis deviates from the normal baseline (19,20), and therefore reflects the current oxidative status. In our study those with inattentive-type ADHD also had higher than control levels of OSI (p=0.001). TOS levels were similar among all subtypes in Kul et al.'s study (15). Different than our findings, they found OSI values lower in the ADHD-IA group in comparison with the other subtypes and concluded that the ADHD-C and ADHD-H/I subtypes did not show excessive oxidant levels and that the oxidant levels were similar across ADHD subtypes.

Although there have been several studies reporting on different antioxidant biomarkers in ADHD as listed by Lopresti (13), our study is the first where myeloperoxidase and thiol/disulphide homeostasis are reported in pediatric ADHD population. Myeloperoxidase (MPO) is an enzyme that is abundantly stored in inflammatory cells such as neutrophils, macrophages, and monocytes, and is involved in diverse oxidation reactions, including lipid peroxidation, by acting as an enzyme in generating multiple reactive oxygen and nitrogen species; and it may promote endothelial dysfunction (31). We found no statistically significant difference between the total ADHD group and control group; however, when ADHD subtypes were compared between themselves and with control group, the difference reached statistically significant levels. Those with ADHD-C, ADHD-IA, and ADHD-C subtypes had lower levels of MPO than the control group (p=0.002). This is a novel finding that has not been previously reported. Myeloperoxidase plays an essential role in the antimicrobial and antiviral system of humans and can be inhibited by natural products (32), thus the role of MPO in etiopathogenesis of ADHD needs further evaluation. Dynamic thiol/disulphide homeostasis has critical roles in antioxidant protection as a novel marker of oxidative stress and is being increasingly implicated in many disorders; and its determination provide valuable information on various normal or abnormal biochemical processes (24). We investigated the serum levels of native thiol, total thiol, disulphide, and disulphide/native thiol, disulphide/total thiol, native thiol/total thiol ratios; however, our findings revealed no statistically significant differences between ADHD patients and control group.

In terms of comorbidities, it needs to be emphasized that elimination disorders have been highly represented in our group (Table 1); however, there was no statistically significant difference in any parameters between those with or without comorbidities. ADHD combined type was the most common subtype in our ADHD group, which is in line with the literature. Although control and total ADHD groups did not differ in terms of age and gender; those with type ADHD were significantly younger than patients with ADHD-IA and healthy controls.

Our study has a list of methodological limitations; therefore the results need to be interpreted with caution. A cross-sectional design, absence of a multicentered representation of the patients and relatively small sample size make the results difficult to be generalized. However the study also have some strengths worth mentioning, such as having a matched control group, controlling variables that may interfere with the findings as much as possible, and using K-SADS-PL to asses comorbidities.

In conclusion, to the best of our knowledge this is the first study to investigate myeloperoxidase as an antioxidant marker and thiol/disulphide homeostasis in pediatric-age patients with ADHD. Instead of categorizing children and adolescents as the same group we also compared the results between these two potentially rather different groups. Findings reported in the current study are in line for some parameters but completely opposite in others, hence methodologically more robust studies are needed to clarify the existing confusion and conflict in the related literature.

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