Research Article

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Comparison of nitric oxide and adrenomedullin levels of children with attention deficit hyperactivity disorder and anxiety disorder

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Abstract

Objectives: Many studies show that adrenomedullin (ADM) is associated with nitric oxide (NO) and various mechanisms and is involved in the etiopathogenesis of schizophrenia, bipolar disorder and autism by oxidative stress and HPA axis dysregulation. The aim of this study comparison of nitric oxide and adrenomedullin levels in children with ADHD, AD and healthy control included in our study, especially due to their effect mechanisms as they may predict anxiety symptom, was to investigate the relationship between nitric oxide and adrenomedullin levels and anxiety symptoms in children with ADHD, AD and healthy control.

Methods: The study included 27 ADHD, 27 AD and 23 healthy children without any previous drug use, without comorbid disease. The semi-structured interview was conducted by the researcher in all the children attending the study. Sociodemographic information form, Conner's Parent and Teacher Rating scale and State-Trait Anxiety Inventory (STAI) were evaluated. NO level measured by spectrophotometer, ADM levels were measured by ELISA.

Results: There was no statistically significant difference in the serum NO and ADM levels of the children included in the sampling group according to age and sex. There was no statistically significant difference between NO and ADM levels between ADHD, AD and control groups. There was no statistically significant relationship between serum NO and ADM levels and ADHD, AD and control group children of state-trait anxiety scores.

Conclusions: These findings may suggest that NO and ADM levels in children with ADHD, AD do not show these diseases and that these parameters are not associated with anxiety symptoms.

Keywords: adrenomedullin; anxiety disorder; attention deficit hyperactivity disorder; child; nitric oxide.

Introduction

It is stated that free radicals and antioxidant defense systems play a role in the pathophysiology of neuropsychiatric disorders [1]. There are studies showing that oxidative stress is increased in attention deficit hyperactivity disorder (ADHD) [2–5]. In a meta-analysis study, it was reported that oxidative balance was impaired in individuals diagnosed with ADHD [6]. On the other hand, there are studies suggested that oxidative stress is not affected in patients with ADHD [7]. There are studies showing an increase in oxidative stress in anxiety disorders (AD) as well as research that does not support this result [8–12].

In studies evaluating oxidative stress in ADHD, it has been shown that the activity of nitric oxide synthase (NOS) enzyme, which enables the synthesis of both nitric oxide (NO) and nitric oxide from L-arginine, was increased and these changes may play a role in ADHD etiopathogenesis. It has been stated that the increase in NO levels is aimed at compensating oxidative stress [10, 11]. On the other hand, in a study in hyperactive rats, it has been reported that mobility decreases with the administration of NOS-1

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inhibitors [13]. Also, abnormal neuronal NOS variants were detected in patients with ADHD [14].

NOS enzyme activity was found to be high in brain regions associated with the limbic system, such as the hypothalamus, hippocampus, and amygdala, which play an important role in anxiety disorders [15]. An anxiolytic role of NO is suggested in some studies, whereas in other studies, its role in psychopathology is described. There are ongoing studies that are intended to explain the role of NO in anxiety disorders [16].

There is a complex relationship between adrenomedullin (ADM) and NO. The stimulation of ADM gene expression by inflammatory cytokines is regulated by NO [17]. NO plays a role in ADM signal transmission by providing ADM receptor activation [18]. ADM has been shown to increase NOS and therefore NO levels [19]. ADM has been shown to help reduce superoxide production, thereby protecting neurons from ischemia, and this effect is related to high NO levels [20]. Moreover, in rats with ADM gene destroyed, excessive mobility and anxiety behaviors were detected [21].

In some studies, it has been suggested that ADM and NO may play a role in the pathogenesis of psychiatric diseases such as bipolar disorder, schizophrenia, major depressive disorder, schizophrenia and autism spectrum disorder [22–25].

In this study, it was aimed to determine the NO and ADM levels associated with oxidative stress in ADHD and AD, which are common disorders in childhood. In this context, serum NO and ADM levels of children with ADHD, AD and healthy control group were compared, and the relationship between state-trait anxiety scores and serum NO and ADM levels and that between serum NO and ADM levels were evaluated.

Materials and methods

Sampling

In total, 77 children, aged between 6 and 12 years, who were admitted to Ankara University Faculty of Medicine, Child and Adolescent Psychiatry Outpatient Clinic were included in the study. The Schedule for Affective Disorders and Schizophrenia for School-Age Children-Present and Lifetime version (K-SADS-PL) interview was conducted to diagnose pure ADHD and AD and to exclude patients with ADHD + AD and other comorbid psychiatric disorders (such as psychotic disorders, bipolar disorder, tic disorder, and behavior disorders). Children who were found to have autism spectrum disorder, language development delays, intellectual disability, and learning disabilities in the clinical evaluation performed according to DSM 5 were also excluded from the study. In clinical interviews, the Wechsler Intelligence Scale

for Children (WISC-R) was applied to patients suspected of intellectual disability, and WISC-R and Learning Disorder Battery were applied to those suspected of specific learning disorder. Four patients who were diagnosed with intellectual disability and six who were diagnosed with specific learning disorder were not included in the study. The patient group comprised children who were newly diagnosed with ADHD and AD and did not receive any medication, and the control group comprised 23 healthy children who were admitted to the Ankara University Faculty of Medicine, Child and Adolescent Psychiatry Outpatient Clinic without any psychiatric disorders, including ADHD and AD.

Criteria for inclusion in the study for all three groups; being between the ages of 6–12, having a normal intelligence level, not having chronic medical diseases such as kidney pathologies, cardiovascular pathologies, epilepsy, not having an infection table and related drugs in the last 1 week and not entering puberty. To exclude possible medical diseases, all children underwent clinical examinations by the pediatrician, and the results of routine laboratory tests, including biochemical, hematological and thyroid function measurements were evaluated. None of the children was taking dietary supplements, such as antioxidants.

Procedure

A K-SADS-PL interview was conducted with all children and their parents to diagnose and to exclude patients with accompanying psychiatric disorders. The Conners' Parent Rating Scale-Revised Long Form (CPRS-R:L) was applied to the parents, the State-Trait Anxiety Inventory (STAI) was applied to the children in three groups, and the Conners' Teacher Rating Scale-Revised Long Form (CTRS-R:L) was applied to the teachers. The study was approved by the Ankara University Faculty of Medicine Ethics Committee (25.07.2016 decision no. 13-611-16). All families and children were informed verbally and in writing about the study, and they signed informed consent forms prepared by the rules outlined in the Helsinki Declaration.

Venous blood samples in the amount of 10 ml were collected from 9:00 am to 11:00 am full stomach in the morning from the participants of all three groups who were approved to participate in the study. The collected blood samples were centrifuged at 1,500g relative centrifugal force for 10 min in order to separate the plasma from the cells, the obtained plasmas were stored in eppendorf tubes at -80°C until biochemical analysis. NO levels measured by spectrophotometer. Adrenomedullin levels were determined at same day by working with Enzyme Linked Immunosorbent Assay (ELISA) method after all samples were collected in the Medical Biochemistry Department Laboratory.

NO levels were studied with the Cayman Colorimetric Assay kit (Cayman Chemical Company, MI, USA). NO measurement is a colorimetric method based on the formation of purple colored compound with Griess reagent and total nitrate and nitrite levels. The purple-colored compound formed was measured on 540 nm (nm) wavelength μ Quant Biotek (USA) brand spectrophotometer. The absorbance values obtained according to the given standard concentrations were calculated and reported in $\mu\text{mol/L}$. The assay range of the NO kit was 5–35 $\mu\text{mol/L}$. The analytical centivity of the NO kit was 2 $\mu\text{mol/L}$. Inter-assay CV 3.4% included in the kit insert. Intra-assay CV was 2.7%.

Adrenomedullin levels were studied with SunRed (Shanghai, China) ELISA kit. Sample ADM levels were measured by double antibody sandwich enzyme-linked immunosorbent method at 450 nm wavelength μ Quant Biotek (USA) brand spectrophotometer. The absorbance values obtained were calculated according to the

given standard concentrations graph and reported as ng/L. The lower and upper reading limits of the ADM kit were in the range of 7–1,500 ng/L SunRed assay range. Analytical sensitivity of the ADM kit was: 5.12 ng/L.

The budget of the research was met by the researcher and no financial support was received.

Statistical analysis

After the data were obtained, statistical evaluations were made in the “SPSS (Statistical Package for Social Sciences) 11.5” package program. Shapiro–Wilk test was used to test whether the variables obtained from the data are suitable for normal distribution. ADHD, AD and control groups Conners’ Teacher and Parent Rating Scale forms total scores and subscale scores, and STAI scores and age from socio-demographic data were evaluated by Kruskal–Wallis test. Post-hoc analyzes were carried out to find out which group or groups the difference was for the sub-scales and total scores of the scale that showed a significant difference in STAI, CPRS-R:L, CTRS-R:L scales with Kruskal–Wallis test. One-Way ANOVA test was used to compare WISC-R scores. Fisher’s Exact test was used for the gender parameter.

Kruskal–Wallis test was used to compare serum adrenomedullin and nitric oxide levels of ADHD, AD and control group. Spearman Correlation test was applied to the data to examine the relationship between ADHD, AD and control group serum adrenomedullin and nitric oxide levels. The Spearman Correlation test was used to examine the relationship between trait anxiety scale scores and state anxiety scale scores, and serum adrenomedullin and nitric oxide levels in all three groups. Statistical significance level (p) was accepted as 0.05.

Results

There was no significant difference ($p>0.05$) between ADHD ($n=27$), AD ($n=27$) and control ($n=23$) groups in terms of age (9.1 ± 2 ; 9 ± 1.7 ; 9.6 ± 1.9 , respectively). The gender distribution was similar between the groups ($p=0.263$). Patients and control groups did not differ significantly in terms of education and income levels of parents or any medical status or adverse life events. Similarly, no significant difference was found between the groups in terms of way of birth or postpartum trauma, surgical operation and seizure. In addition, no difference was observed a history of psychiatric disease in parents.

There was no significant difference between the total intelligence scores of children in the ADHD ($n=17$), AD ($n=12$), and control ($n=11$) groups who were administered

WISC-R (101 ± 14.9 , 96 ± 13.5 , 96 ± 13.7 , respectively; $p>0.05$). Furthermore, in the ADHD, AD, and control groups, the State Anxiety Inventory (SAI) scale scores of 45.5 ± 6.7 , 48.5 ± 7.3 , 48 ± 4.4 , respectively, and the Trait Anxiety Inventory (TAI) scale scores of 45.7 ± 8.8 , 51.8 ± 7 , 48 ± 7.6 , respectively, were determined. Although the TAI scale scores were higher in the AD group, no significant difference was found between the three groups ($p>0.05$).

In the ADHD, AD, and control groups, the total CPRS-R:L scale scores of the parents were 33 ± 10.2 , 8.4 ± 8.6 , 4.4 ± 4.6 , respectively, and the total CTRS-R:L scale scores of the teachers were 70 ± 26.3 , 23.3 ± 30.1 , 18 ± 15.7 , respectively. The total scores of both scales of the ADHD group were higher than the other two groups and the statistically significant difference was between ADHD and the other two groups ($p<0.05$). No significant difference was found between the scores of the AD and the control group. The “Anxiety-Shyness” subscale scores of the scale based on parental evaluations were found to be 7.4 ± 4 for the ADHD group, 8.4 ± 3.9 for the AD group, and 4.8 ± 3.7 for the control group; the difference between the scores was statistically significant ($p=0.012$). In post hoc analysis performed using Kruskal–Wallis test, it was noted that the “Anxiety-Shyness” subscale scores differed between the AD and control groups ($p=0.010$), whereas those between the ADHD and AD groups were not significantly different. There was no significant difference between the three groups in the “Anxiety-Shyness” subscale scores of the scale that the teachers scored.

There was no significant difference between serum ADM and NO levels between the three groups ($p>0.05$) (Table 1). There was no significant correlation between serum ADM and NO levels ($r=-0.048$; $p>0.05$). In all children included in the study and the ADHD, AD, and control groups separately, no correlation was found between the SAI and TAI scale scores and the serum ADM and NO levels ($p>0.05$) (Table 2).

Discussion

In this study, a statistically significant difference was not observed between patients with ADHD and AD and healthy

Table 1: Serum adrenomedullin and nitric oxide levels of ADHD, AD and control groups.

	ADHD Median (Q_1 – Q_3)	AD Median (Q_1 – Q_3)	Control Median (Q_1 – Q_3)	p-Value*
Serum ADM, ng/L	61.6 (26.9–206.7)	45.5 (13.3–191)	45.3 (7.5–82.4)	0.251
Serum NO, μ mol/L	16.3 (13.6–21.7)	14 (12–16.7)	16.6 (14.1–17.3)	0.076

*Kruskal–Wallis Test

Table 2: Relationship of serum adrenomedullin and nitric oxide levels with state-trait anxiety scale.

		Serum ADM	Serum NO
State anxiety Scale (total)	r	0.158	0.126
	p	0.170	0.274
Trait anxiety Scale (total)	r	-0.036	-0.049
	p	0.757	0.670
ADHD-state anxiety Scale	r	0.197	0.139
	p	0.324	0.488
ADHD-trait anxiety Scale	r	0.293	0.107
	p	0.138	0.595
AD-state anxiety Scale	r	0.169	-0.066
	p	0.399	0.745
AD-trait anxiety Scale	r	0.026	-0.242
	p	0.899	0.224
Control-state anxiety Scale	r	0.164	0.362
	p	0.453	0.089
Control-trait anxiety Scale	r	-0.254	0.300
	p	0.243	0.164

Spearman correlation test, r =correlation coefficient.

controls of similar age and sex in terms of serum ADM and NO levels, which are indicators of oxidative stress and HPA axis. In addition, when patients with ADHD and AD and healthy controls were evaluated according to the state-trait anxiety scale, there was no statistically significant relationship between anxiety symptoms and serum NO and ADM levels.

In the study where Özgür et al. compared the NO and ADM levels between children with ADHD and the control group, no significant difference was found between the two groups NO and ADM levels [26]. In the thesis study carried out by Işıldar, the role of NO and ADM in ADHD etiopathogenesis was investigated and both of them were found to be decreased compared to the control group [27]. In the studies comparing NO levels in individuals with ADHD in adult age group, NO levels were found to be higher than the control group. However, we did not encounter a study evaluating ADM levels in adult ADHD studies in the literature.

In rats whose ADM gene was destroyed, excessive mobility and anxiety behaviors were detected [21]. Adrenomedullin has been shown to help reduce superoxide production, thereby protecting neurons from ischemia, and this effect is associated with high NO levels [20]. In a

study conducted by Kong et al., it compared those with left ventricular hypertrophy due to hypertension and those with anxiety in addition to these and reported that ADM levels of the group of patients with anxiety increased [28]. In a study conducted by Meyer et al. on cardiovascular risk factors in patients, it was reported that pro-ADM levels had an inverse relationship with anxiety and no association with depression [29]. In the literature, we did not find any studies in adult or pediatric age group with no comorbid cardiovascular system disease in which only AD and NO-ADM levels were compared. For this reason, we could not compare the results of serum NO and ADM levels of the group with anxiety disorder and the relationship between NO-ADM levels and anxiety symptoms with similar studies.

While Savaş et al. found a significant relationship between these two parameters in patients diagnosed with bipolar disorder, but no relation was found between serum ADM and NO in the study conducted by Zoroğlu et al. in individuals with schizophrenia [22, 30]. According to the data obtained in our study, no correlation was found between ADM and NO levels.

It has been demonstrated that adrenomedullin stimulates HPA axis activity by activating central neuroendocrine and autonomic pathways [31]. Although central adrenomedullin has stimulating effects on the HPA axis and the sympathetic system, it has inhibitory effects on the pituitary and vessels. These inhibitory effects are thought to be compensated by NO activated by ADM [31, 32]. In the literature, we did not encounter any study evaluating the HPA axis with NO-ADM levels in the AD and ADHD group in the pediatric age group. Therefore, we could not compare our results with similar studies.

It has been demonstrated that adrenomedullin stimulates HPA axis activity by activating central neuroendocrine and autonomic pathways [31]. Although central adrenomedullin has stimulating effects on the HPA axis and the sympathetic system, it has inhibitory effects on the pituitary and vessels. These inhibitory effects are also thought to be compensated by NO activated by ADM [31, 32]. In the literature, we did not encounter any study evaluating the HPA axis with NO and ADM levels in the AD and ADHD group in the pediatric age group. Therefore, we could not compare our results with similar studies.

The contradictory results obtained in the studies might be owing to the facts that there are many parameters affecting oxidative stress, the factors affecting oxidative stress cannot be fully evaluated, the relationship between psychiatric disorders such as ADHD and AD and serum NO and ADM levels are too complex to be explained by oxidative stress alone, and oxidative stress is compared with data obtained with cross-sectional evaluation. In

addition, the difference in the results in the literature might be owing to the facts that the relationship between psychiatric disorders such as ADHD and AD and serum NO and ADM levels is too complex to be explained by HPA axis dysregulation and the HPA axis cannot be fully evaluated with cross-sectional data without including all the factors affecting the HPA axis.

When information in the literature was reviewed, no studies were found to correlate serum NO and ADM levels with the severity of anxiety symptoms in children. In this respect, our study is a pioneer in literature for the identification of endophenotypes by evaluating the relationship between symptoms and markers. The result indicating that there is no difference between serum ADM and NO levels and the STAI scores of children in the ADHD, AD, and control groups may be owing to the fact that anxiety symptoms are caused by mixed mechanisms that cannot be associated only with oxidative stress or HPA axis.

Limitations of our study; due to the small number of individuals included in the study, the results could not be generalized, included single center data, the parameters such as cortisol, corticotropic releasing hormone, adrenocorticotrophic hormone which are indicators of HPA axis and antioxidant substances and other oxidant parameters were not included in the study. In addition, it is the strengths of the study that the diagnoses of the individuals included in the study were made through a semi-structured interview, not including the adolescent age group, individuals with medical and psychiatric comorbidities, children with intellectual disability.

Conclusion

In our study, no significant difference was found between serum NO and ADM levels between the ADHD, AD and control groups. There was no correlation between anxiety symptoms and serum NO and ADM levels in all three groups. Our study is the first in the literature to evaluate the relationship of inter-related biochemical parameters involved in many-body systems, such as NO and ADM, with anxiety, which is a psychiatric symptom, in the pediatric age group. Because there are conflicting results regarding serum NO and ADM levels for ADHD and AD patients among children and adults, we believe that further studies should be conducted by associating these parameters with symptoms in various age groups. Our study is important in terms of drawing attention to the investigation of biochemical markers in pediatric psychiatry in the future by bringing new questions to mind for identification of endophenotypes with biochemical markers. In the future,

there is a need for studies with large sample sizes to compare children, adolescent, and adult age groups regarding oxidant and antioxidant parameters and other parameters related to the HPA axis.

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