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Reduced levels of plasma strong antioxidant uric acid in children with Autism Spectrum Disorder and Attention deficit-hyperactivity disorder

Erman Esnafoglu[1](ID) Emine Yurdakul Ertürk[2](ID)

[1]Department of Child and Adolescent Psychiatry, Faculty of Medicine, Ordu University, Ordu, Turkey.
[2]Department of Pediatry, Faculty of Medicine, Ordu University, Ordu, Turkey.

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Abstract

Objective: Uric acid (UA) is one of the most powerful antioxidants in human body fluids, as well as being the end product of purine metabolism. UA alone constitutes half of the scavenging effect of oxidant substances in the plasma.It has proinflammatory and metal chelation effects. In this study, UA levels and UA situation according to the normal range were investigated in patients with Autism Spectrum Disorder (ASD), and Attention deficit-hyperactivity disorder (ADHD).

Methods: Eighty-two ASD, 28 ADHD patients and 66 healthy control subjects were compared and serum UA levels were measured. ASD and ADHD severity were determined by CARS and Atilla Turgay ADHD scale. K-SADS-PL was administered to all subjects over the age of 6 years.

Results: UA levels were found to be significantly lower in the ASD and ADHD groups. UA levels were found to be lower than the normal range in nearly half of the ASD and ADHD groups. This rate was found to be 18.2% in the control group.

Conclusion: it was determined that UA levels were low in ASD and ADHD patients. It can be suggested that it may play a role in the pathophysiology of ASD and ADHD. UA may be a potential treatment target.

Key Words: uric acid, antioxidant capacity, oxidative stress, autism, attention deficit-hyperactivity disorder

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Address for correspondence/reprints:

Erman Esnafoglu

Telephone number: +90 (533) 772 46 34

E-mail: ermanesnafoglu@yahoo.com.tr

INTRODUCTION

Uric acid (UA) emerges as the final metabolic product of adenine and guanine purines from animal proteins, destroyed, damaged and dying cells. Adenine and Guanine are converted to inosine and guanosine by deamination and dephosphorylation. These are then converted to hypoxanthine and guanine by purine nucleoside phosphorylase. These two molecules are converted to Xanthine and then to UA by Xanthine oxidase. UA is mainly produced in the liver, intestine, and vascular endothelium and excreted by the kidneys (1). Although UA excess is associated with some pathological conditions, UA has been shown to have extremely important physiological functions. In fact, some hypotheses have suggested that there is relative hyperuricemia only high ape and humans in mammals, which, besides its many advantages such as increase of life span and decrease cancer rates, stimulates the cerebral cortex and may affect the formation of superior intellectual powers (2-4).

UA accounts for at least half of the antioxidant capacity in plasma. UA has strong antioxidant properties with its reactive oxygen specifies (ROS) and peroxynitrite scavenging effect. Peroxynitrite is formed by the reaction of nitric oxide with superoxide radicals and is associated with many pathologies (5). However, UA contributes to tissue repair and healing by initiating inflammatory processes, scavenging free oxygen radicals, and activating progenitor endothelial cells (6).

UA has been found to be effective in the pathogenesis of some neurodegenerative diseases. For example, UA contributes to the protection of myelin by scavenging peroxynitriteand ROS, which in crease the destruction of myelin. Therefore, Multiple Sclerosis (MS) is almost never seen in gout patients. UA was also found to be low in MS patients (7-10).Similarly, UA levels were found to be generally low in Alzheimer and Parkinson diseases. It has been found that high UA levels are associated with decreased Alzheimer risk (11). Similarly, high UA prevents cognitive decline in Parkinson's patients, and low UA levels are associated with worse cognitive performance (12). Experimental cerebral ischemia studies have shown that UA protects neurons against excitotoxic and metabolic damage (13).

The brain is more exposed to oxidative stress and more susceptible to damage due to its high metabolic activities and using half of the oxygen consumed daily. Especially the high lipid content of the brain makes it sensitive to lipid peroxidation. Mechanisms to prevent lipid peroxidation are of great importance. UA against lipid peroxidation with its strong antioxidant effect reflects the neuroprotective effect of UA (14). Apart from these effects, UA acts as a chelator of metal ions such as iron and copper and transforms them into poorly

reactive forms, making them unable to catalyze free radical reactions (6,15,16).

There are publications that low UA plays a role in the pathogenesis of schizophrenia (17). In first episode schizophrenia patients, UA was found to be low as an antioxidant (18). In a recent meta-analysis study, it was suggested that increased oxidative stress response accompanied by first-episode Sch patients and decreased UA may be a risk factor for schizophrenia, especially in males (19). In patients with depression, low UA is a characteristic of depression and UA returns to normal with antidepressant treatment (20).

Considering the above-mentioned physiological effects of UA and its effects on the pathogenesis of some neuropsychiatric-neurodegenerative diseases, a hypothesis was formed in this study that UA levels may play a role in the pathogenesis of Autism Spectrum Disorder (ASD) and Attention Deficit-Hyperactivity Disorder (ADHD).

METHODS

Participants

Participants of the patient group were formed from those who applied to the Child and Adolescent Psychiatry Department of Ordu University Medical Faculty Training and Research Hospital. Patient groups were formed from subjects diagnosed with ASD and ADHD according to DSM 5 criteria. The subjects were examined by a specialist child psychiatrist and detailed information was obtained from their families. While the severity of the disease in the ASD group was measured with the Childhood Autism Rating Scale (CARS), the severity of the disease in the ADHD group was determined with the AtillaTurgay (AT) DSM-IV Based Child and Adolescent Behavior Disorders Screening and Rating Scale.The healthy group consisted of volunteers who applied to the general pediatric outpatient clinic of our hospital during the well-child visit, did not have any psychiatric diagnosis and did not have a systemic disease in their medical history. K-SADS-PL, a structured psychiatric interview, was applied to all subjects over the age of 6 and a sociodemographic form created by the authors was filled (21). All subjects' medical conditions and histories were evaluated by a specialist pediatrician (3th author). The study was approved by the Ethics Committee of Ordu University Faculty of Medicine (Decision no: 2019/02). Biochemical tests including hemogram, thyroid function tests, kidney and liver function tests were applied to the subjects for general medical screening. These biochemical parameters were measured between 08:00 and 11:00 in the morning after overnight fasting. During these routine measurements, uric acid levels were also measured routinely. Exclusion criteria were systemic diseases, neurological deficits, epilepsy, inflammatory diseases, hypertension, thyroid disorders, gout, obesity, acute or

chronic infections, and those taking psychiatric medication in the last 6 month.

Evaluation Tools

Sociodemographic Data form

Subjects' age, disease duration, birth history, birth weight, duration of breastfeeding, medical disease history, height, weight, BMI, medication history were available.According to the normal range of UA values, groups were divided into three categories as low, normal and high.

Childhood Autism Rating Scale (CARS)

The scale, which consists of 15 items, is graded from 1 to 4 in each item. If the total score is below 30, it is considered normal, 30-36 as mild-moderate autism, 37 and above as severe autism. Turkish validity and reliability were established (22).

Atilla Turgay (AT) DSM-IV Based Child and Adolescent Behavior Disorders Screening and Rating Scale

This scale was developed by Atilla Turgay (AT) according to DSM-IV criteria. This scale consists of 41 items. While 9 of these items measure inattention, 9 evaluate hyperactivity and impulsivity. While the remaining 8 items evaluate oppositionality, 15 items evaluate Conduct Disorder. The Turkish reliability and validity of the AT scale was established (23).

Uric acid measurement

It was routinely measured spectrophotometrically with Roche 701 device from the blood taken from the subjects in the morning after fasting overnight. According to the KIT package insert, the normal range is accepted as 3.3-7.0 mg/dL.

Statistical Analysis

SPSS 22.0 (IBM Corporation, Armonk, New York, USA) was used for data analysis. Whether the numerical variables were normally distributed or not was determined by the Kolmogorov-Smirnov test. Normally distributed variables are indicated as mean±SD. Data that do not show normal distribution are shown as median and IQR values. Categorical variables were expressed as percentages. One-way ANOVA test was used when examining the differences between the three groups of normally distributed numerical variables. Homogeneity of variances was evaluated with Levene's test. In cases where there was significant difference between groups (for UA), pairwise post-hoc comparisons were made using the Tukey test.The Kruskal Wallis test was used when examining the difference between the three groups of numerical variables that did not show normal distribution.Chi-square test was used to determine the difference in categorical variables between the groups. Pearson and Spearman tests were performed in the correlation analyzes between the variables. Since there was a significant correlation between BMI and uric acid levels, the effect of BMI had to be purged. ANCOVA test was performed upon providing the necessary assumptions. Uric acid differences between the

groups were re-evaluated, with BMI as a cofactor. The P value was below 0.05 were considered statistically significant.

RESULTS

Our study included 82 ASD patients, 28 ADHD patients and 66 healthy subjects. There was a gender distribution of 24 females (29.3%) and 58 males (70.7%) in the ASD group, 5 females (17.9%) and 23 males (82.1%) in the ADHD group. The gender distribution in the healthy control group was 20 females (30.3%) and 46 males (67.7%). There was no difference between the groups in terms of gender (p=0.434). Since the age distribution did not show normal distribution, the median value was 6 in the ASD group, 7 in the ADHD group and 5 in the control group.Since the BMI distribution was also not normal, the median value was 17.16 in the ASD group, 17.06 in the ADHD group and 16.72 in the healthy control group. There was no difference between the groups in age and BMI distribution (p values 0.069 and 0.413, respectively). The median value for the CARS score, which indicates the severity of the disease in the ASD group, was 52, and the median value for the Atilla Turgay ADHD scale score was 55.5 in the ADHD group. The distribution of these parameters between the groups is given in Table 1 in detail.

UA values showed a normal distribution among the groups. Therefore, it was shown as mean±SD value between groups. The distribution of UA as mg/dL was found to be 3.42±0.75 in the ASD group, 3.25±0.49 in the ADHD group and 3.73±0.85 in the healthy control group. A significant difference was found in the distribution of UA between the groups (p=0.008). When the groups were compared in pairs in pot-hoc analyzes, a significant difference was found between the ASD and control groups and ADHD and control groups (p values 0.036 and 0.016, respectively). There was no significant difference between the ASD and ADHD groups (p=0.535). When UA values were divided into three categories as low, normal and high according to the normal range, no high value (above 7 mg/dL) was found in all groups.Therefore, the groups were divided into two categories as low and normal. While UA was found to be low (below 3.3 mg/dL) in 38 (46.3%) of the patients in the ASD group, UA was found to be low in 13 (46.4%) of the patients in the ADHD group and in 12 (18.2%) of the subjects in the healthy control group. When the low UA between the groups was evaluated categorically, a significant difference was found between the three groups (p=0.001). When the percentage values were taken into account, it was seen that the low UA percentages of both the ASD and ADHD groups were found to be significantly higher than the healthy control group. It was observed that the percentage values were very close to each other between the ASD and ADHD groups. The distribution of UA values among

the groups and their low-normal percentages are presented in detail in Table 2. In addition, the distribution of UA values between the groups is shown in Figure 1.

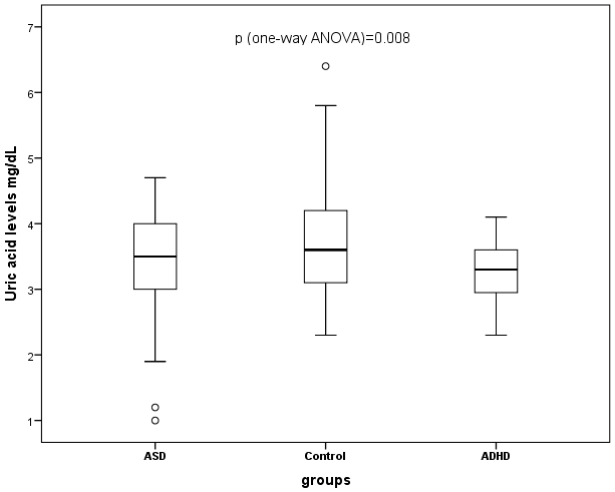

Figure 1. Distribution of Uric acid levels

When the correlation analyzes were examined, no significant results were found. No statistically significant results were found between the CARS score, which indicates the severity of the disease, and UA in the ASD group, and between the Atilla Turgay ADHD scale score and UA in the ADHD group (p values>0.05). When it was examined whether there was any correlation between UA and age and BMI values in all subjects, there was no significant correlation between age and UA, but a significant correlation was found between UA and BMI (r=0.347 and p<0.001).

Since the relationship between UA and BMI has been demonstrated, we wanted to refine the effect of BMI on UA between groups. For this reason, as a result of the evaluation of BMI as a cofactor (ANCOVA), UA values were again found to be significant between the groups (p<0.05).

Table 1. Distribution of the characteristic features of the groups

		ASD group (n=82)	ADHD group (n=28)	Healthy control group (n=66)	P value
Gender	F	24(29.3%)	5(17.9%)	20(30.3%)	0.434[a]
	M	58(70.1%)	23(82.1%)	46(69.7%)	
Age (years)					
	median (IQR)	6 (3.23)	7 (1,5)	5 (3.11)	0.069[b]
BMI	median (IQR)	17.16 (3.15)	17.06 (1.69)	16.72 (4.31)	0.413[b]
CARS score					
	Median (IQR)	52 (9.50)	-	-	-
AT score					
	Median (IQR)	-	55.5 (21.50)	-	-

BMI=Body Mass Index, CARS=Childhood Autism Rating Scale

AT=Atilla Turgay DSM-IV Based Child and Adolescent Behavior Disorders Screening and Rating Scale

IQR=Inter quartile range

a=Chi-square test, b=Kruskal-Wallis test

Table 2. Distrubition of Uric acid values

	ASD group (n=82)	ADHD group (n=28)	Healthy control group (n=66)	P value
Uric acid mg/dL mean±SD	3.42±0.75	3.25±0.49	3.73±0.85	0.008[a]
Uric acid High	-	-	-	0.001[b]
Normal	44 (53.7%)	15 (53.6%)	54 (81.8%)	
Low	38 (46.3%)	13 (46.4%)	12 (18.2%)	

a=One-Way ANOVA; b= Chi-square test

DISCUSSION

In this study, UA levels compared in neurodevelopmental disorders such as ASD and ADHD with a healthy control group. According to the results we obtained, UA levels differed remarkably between ASD, ADHD and healthy control groups, which did not differ significantly in terms of age, BMI and gender, that is, they had similar characteristics. On the other hand, when UA levels were evaluated as low, normal and high compared to the normal range, UA elevation could not be found in all groups, but significantly lower UA percentage was found in the ASD and ADHD groups compared to the healthy control group. In other words, almost half of the subjects in the ASD and ADHD groups show lower UA levels than the normal range. The importance of these findings should be evaluated in the light of the information we have mentioned in the introduction.

Besides being the end product of purine metabolism, its most important physiological function of UA is to constitute half of the antioxidant capacity in human biological fluids. However, there areevidences that UA has pro-inflammatory effects and stimulates the synthesis of some cytokines (6). There is evidence that the oxidation load is high in the pathogenesis of both ASD and ADHD. Numerous studies have associated both diseases with increased oxidative stress and insufficient defensive antioxidant systems (24-25).

It has been shown that especially ASD patients are under oxidative stress. These patients are more prone to neurotoxicity due to oxidative stress (26). Due to the main reasons such as the brain metabolism rate is very high and it uses a high amount of O2, the neuron membrane contains high polyunsaturated fatty acids, high membrane surface area and the release of iron ions that catalyze free radical reactions in the whole brain, Manivasagam et al. (2020) explained in detail in seventeen items that neurons are much more sensitive to oxidative stress than other tissues (27). UA's chelation of metal ions such as iron and copper keeps these ions away from free radical-forming reactions.Therefore, with these effects

of UA, it can be thought that it protects the brain tissue (6, 15, 16).

Oxidative stress is mainly referred to as an imbalance between prooxidants and antioxidants, resulting in reactive oxygen species (ROS) and reactive nitrogen species (RNS). ROS and RNS are composed of superoxide (O2−), hydroxyl, peroxyl, alkoxy, hydrogen peroxide, and peroxynitrite free radicals. This balance is disrupted in the pathogenesis of ASD. As a result, increased lipid peroxidation, mitochondrial damage, neuroinflammation and brain tissue damage, protein and DNA oxidation, altered immune response occur. In addition, with the decrease of methylation capacity, DNA methylation decreases and epigenetic dysregulation occurs. All these factors contribute to the pathogenesis and clinical development of ASD (28). It has been shown that oxidative stress and neuroinflammation coexist in the pathogenesis of ADHD, and antioxidant capacity is decreased (29).

Considering that UA is the most important scavenging antioxidant in body fluids, it can be suggested that the decrease in UA may play a role in the pathogenesis of many neurodegenerative and neuropsychiatric disorders, as well as in the pathogenesis of ASD and ADHD. This situation makes UA a potential treatment target as well as the fact that UA may play an important role in the pathogenesis of ASD and ADHD. A randomized placebo-controlled double-blind study of UA administration was conducted on healthy volunteers. Accordingly, a significant increase in serum free radical scavenging effect was observed in the group receiving UA. Moreover, no side effects were reported (30). It has been reported that adding UA to the treatment in acute stroke patients is beneficial, reduces lipid peroxidation, and increases the effect of recombinant tissue plasminogen activator (rt-PA) treatment (31). In another study, it was reported that administration of UA during acute aerobic physical exercise in healthy individuals was associated with an increase in serum antioxidant capacity and decreased oxidative stress (32). In another clinical study, a negative relationship was found between UA levels and oxidative stress during exhaustive exercise (33). As a result, the additive effect of UA on treatment should be investigated in ASD and ADHD patients and experimental studies in future. This study may be a trigger in this regard. Perhaps trying to keep UA in the upper limits of normal range or normalizing the level at low UA levels in patients with ASD and ADHD without causing urolithiasis, hypertension, and gout may work.

So far, UA levels have been evaluated in ASD patients treated with risperidone. In this study, ASD patients who received and did not receive risperidone were compared. Accordingly, it has been suggested that UA levels are higher in ASD patients receiving

risperidone, and this may be effect on the metabolic side effects of risperidone. However, in this study, ASD patients were not compared with the healthy control group (34).

This study has some strengths and weaknesses. First of all, the greater the number of subjects will allow us to reach more accurate results. Especially in the ADHD group, the number of subjects is quite insufficient. However, the fact that the subjects did not receive any treatment in the last 6 months, which is one of the strengths of this study, prevented us from recruiting a sufficient number of subjects. Because drugs can affect UA levels. It is also a positive value that the two most important neurodevelopmental disorders, ASD and ADHD, were evaluated together. In addition, the acceptance of medical conditions that may affect UA levels as exclusion criteria strengthens the study. In ASD and ADHD, there is a need for further studies such as evaluating it together with other oxidative stress and antioxidant capacity parameters besides UA.

As a result, it was determined that UA levels were low in ASD and ADHD patients. In addition, the percentage of low UA according to normal range was found to be higher in ASD and ADHD patients.

CONCLUSION

Accordingly, it can be suggested that UA may play a role in the pathophysiology of ASD and ADHD, especially through oxidative stress-antioxidant capacity. In addition, metabolic pathways related to UA may be a new target in the treatment of ASD and ADHD.

Ethics Committee Approval: Ethics committee approval was received for this study from Clinical Research Ethics Committee of Ordu University (21/02/2019- 2019-40)

Author Contributions:

Concept: E.E, Design: E.E; Literature search: E.E,E.Y.E, Data Collection and Processing: E.E, E.Y.E; Analysis and/or Interpretation: EE, EYE; Literature Review: E.E,E.Y.E; Writing: E.E, E.Y.E.

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