



Effects of agmatine, glutamate, arginine, and nitric oxide on executive functions in children with attention deficit hyperactivity disorder

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Abstract

In this study, we aimed to investigate the effects of agmatine, nitric oxide (NO), arginine, and glutamate, which are the metabolites in the polyamine pathway, on the performance of executive functions (EF) in attention deficit hyperactivity disorder (ADHD). The ADHD group included 35 treatment-naive children (6–14 years old) who were ewly diagnosed with ADHD. The control group consisted of 35 healthy children with the same age and sex, having no previous psychiatric disorders. In the study groups, Stroop test (ST) and trail making test (TMT) were used to monitor EF, and blood samples were collected to measure agmatine with ultra-high-performance liquid chromatography and NO, glutamate, and arginine with enzyme-linked immunosorbent assay (ELISA). The EFs were significantly impaired in the ADHD group. The agmatine and arginine levels of the ADHD group were significantly higher than their peers. The NO and glutamate levels were also higher in the ADHD group compared to the control group, but these differences did not reach statistical significance. Children with ADHD had more difficulties during EF tasks compared to healthy children. The elevated NO and glutamate levels may be related with the impairment during EF tasks. Therefore, agmatine and arginine may increase to improve EF tasks through its inhibitory effect on the synthesis of NO and glutamate. Further studies are needed about polyamine pathway molecules to shed light on the pathophysiology of ADHD.

Keywords Attention deficit hyperactivity disorder · Executive functions · Agmatine · Nitric oxide · Glutamate · Polyamine pathway

Introduction

Polyamines are aliphatic molecules that have an essential role in many functions, including cell proliferation, post-transcriptional regulation, modulation of synaptic activity,

and modulation of ion channels (Celik et al. 2017). To date, the relationship of polyamines with psychiatric disorders such as schizophrenia, mood disorders, anxiety, and suicidal behavior has been investigated in several studies (Fiori and Turecki 2008).

Polyamines also interact with other molecules such as catecholamines, GABA, nitric oxide (NO), and glutamate (Ozcecin 2015). NO is a reactive oxygen species synthesized from L-arginine by NOS (Stuehr 2004). It regulates various physiological processes, such as neurotransmission, plasticity, and neuronal cell death (Li et al. 2013). Besides, NO is involved in a series of physiological functions such as noradrenaline and dopamine release, modulation of memory, learning, and alertness (Akyol et al. 2002). Glutamate is the major excitatory neurotransmitter involved in many physiological functions in the brain, such as cognition, memory, perception, and learning (Coyle et al. 2002). Agmatine, an endogenous cationic polyamine, is synthesized from L-arginine by Arginine decarboxylase (Tabor and Tabor 1984) and plays a critical role in cognitive functions, emotion

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processing and pain perception (Li et al. 1994). It activates nicotinic, $\alpha 2$ adrenergic, imidazoline, 5-HT_{2A}, and 5-HT₃ receptors. It also antagonizes the *N*-methyl-D-aspartate (NMDA) receptor and controls Nitric Oxide (NO) synthesis endogenously by competitively inhibiting all brain isoforms of the nitric oxide synthase (NOS) (iNOS and nNOS) (Reis and Regunathan 2000). Numerous preclinical studies have revealed the potential therapeutic effects of the administration of exogenous agmatine on functions related to the central nervous system (CNS). These studies have shown that agmatine has beneficial effects on learning and working memory. Mounting data indicates that agmatine levels are high in the brain regions related to learning and memory, suggesting a potential involvement in learning and memory processing (Leitch et al. 2011; Liu and Collie 2009; Liu and Bergin 2009; Rushaidhi et al. 2013).

Agmatine, a member of polyamines, have been investigated mainly in many psychiatric disorders including schizophrenia, major depressive disorder, autism and anxiety disorder to date; however, there is no study regarding their place in the etiopathogenesis of attention deficit hyperactivity disorder (ADHD) which is one of the most common childhood neuropsychiatric disorders, characterized by extreme hyperactivity, inattention, and impulsivity (American Psychiatric Association 2013). In addition to these cardinal symptoms of ADHD, impairment in executive functions (EFs) such as response inhibition, planning, organization, working memory, the ability to shift attention from one direction to another is also commonly reported in children with ADHD, and these poor executive functions are strongly related to clinic manifestation (Kofler et al. 2010). Further, although learning disabilities are not at a level to be diagnosed, the majority of children with ADHD can struggle with learning and schoolwork, since learning requires using the executive functions particularly in paying attention, the ability to focus, engaging with a task, and using working memory, which is impacted in ADHD (Mayes et al. 2000; DuPaul and Volpe 2009).

Previous studies have indicated that children with ADHD have significantly lower scores than their peers in neuropsychological tests evaluating the EFs (Raiker et al. 2012). However, the underlying mechanism of the broad impairment of EFs in ADHD is largely unknown, despite several theories have been proposed to explain the pathology (Uzun Cicek et al. 2020; Diamond and Lee 2011) molecules related to learning and executive functions such as agmatine, NO, arginine and glutamate may be implicated in the pathology of the impaired EFs. Considering the above-mentioned facts, we aimed to investigate whether serum agmatine and arginine, glutamate, and NO have a relationship with the performance of EFs in children with ADHD. Since there is an inverse relationship between NO and glutamate with agmatine, we hypothesized

that NO and glutamate levels could be higher while agmatine and arginine levels could be lower in children with ADHD.

Materials and methods

Thirty-five children aged 6–14 years who were admitted to the outpatient clinic of department of child and adolescent psychiatry Sivas Cumhuriyet University Hospital between November 2017 and November 2018. Patients who had attention deficit and hyperactivity symptoms were included in the study (ADHD group). These children have been newly diagnosed with ADHD and treatment-naive, and also had no comorbid psychiatric disorder. Thirty-five volunteer children with the similar age and sex were included as controls who had no previous psychiatric admission and did not use any psychotropic medication. Ethics committee approval was provided by the Ethics Committee of Cumhuriyet University for the study. Also, written consent was obtained from all of the adolescents and their parents.

The diagnosis of ADHD and its subtypes were based on the Diagnostic and statistical manual of mental disorders (DSM-V) criteria (APA 2013). Also, all children involved in the study were evaluated using a semi-structured psychiatric interview by a child psychiatrist to determine whether the child had any psychiatric disorder. Children with neurologic disorders like epilepsy, mental and motor retardation, autism spectrum disorder, and special learning disorder were excluded. Stroop test and Trail making tests were performed to evaluate the executive functions and attention of all children.

Biochemical tests

Venous fasting blood was taken from all participants and collected in a serology tube; and then the blood sample was centrifuged at 4000 rpm for 15 min. Blood serum was transferred into Eppendorf tubes that were labeled and stored at -80°C until the biochemical analysis.

Agmatine levels were measured on ultra-high-performance liquid chromatography using the method developed by Uzbay et al. (2013) and calculated by comparing with standard chromatograms. The standard curve was drawn from the area versus the concentration graph.

NO, glutamate, and arginine levels were measured by enzyme-linked immunosorbent assay (ELISA) with Human ELISA kits (catalog number SG-11015, SinoGeneClon Biotech Co. Ltd, Hangzhou, China).

Executive function tests

Stroop test

It is a neuropsychological test used to measure the function of sustained attention of individuals by suppressing one of

the two competing stimuli. Also, it is used to evaluate the suppression of distracting stimuli (resistance to disruptive stimulus) and the ability to hold the response to inappropriate stimuli (Golden and Golden 2002).

The form of the Stroop test consists of four white cards of sizes 14.0 cm × 21.5 cm. The first card contains color names printed in black on a white background, the second card contains color names printed in a different color than the word, the third card contains circles printed in different colors, and the fourth card contains neutral words printed in different colors. The test consists of 5 sub-sections with an increasing level of difficulty. The first two sections require reading the words on the cards, and the last three sections require telling the colors of the words or shapes. The second card is the stimulant card of the whole test and is used twice for reading in the second section and for color calling in the fifth section. Other cards are used only once. The response times, the number of errors and corrections obtained from the five sections are recorded on the form. Thus, five different completion time, errors, and corrections are obtained from five sections. The fifth section is the critical section where the disturbing effect is measured (Kilic et al. 2002). We used a Tubitak Basic Sciences Research Group version of the test developed by combining the Stroop test with the Victoria form in this study (Kilic et al. 2002).

Trail making test (TMT)

This test provides information about visual tracking, application speed, attention speed, motor speed, mental flexibility, and executive functions (Spreen and Strauss 1998). It consists of two sub-sections: Trail making A (TMA) and Trail making B (TMB). In TMA, the patient is asked to connect circles numbered 1–25 with a solid line in ascending order, and in TMB, the patient is asked to match circles numbered 1–13 with circles labeled A–L, so that circle numbered 1 corresponds to circled labeled A; and circle numbered 2 to circled labeled B, and so on. TMA tests visual scanning, sequential ranking, and visual-motor coordination speed; on the other hand, TMB includes processes such as distinguishing between numbers and letters, integration of two independent series, learning and systematically applying an organizational principle, syntactic integration, verbal problem solving and planning (Reitan and Deborah Wolfson 2004). The patient is asked to accomplish the tasks as soon as possible in tests.

Statistical analysis

IBM SPSS Statistics for Windows, version 23 (IBM Corp., Armonk, NY, USA) was used for the evaluation of clinical and biochemical data. Before statistical comparisons, normality tests, including the Shapiro–Wilk test, were

performed for numeric data. Mann–Whitney and chi-square tests were used for numeric and categorical data analyses, respectively. Pearson's correlation coefficients were obtained to evaluate the associations of EF and biochemical parameters. Data were presented as median with range or interquartile range and number (%). A *p* value of less than 0.05 was considered statistically significant.

Results

The children with ADHD and controls were similar in terms of the age, gender, parental education and occupation, family type and family income except for school grade that was lower in the ADHD group ($p < 0.001$) (Table 1). Among the ADHD group, 31.4% (11) had an inattentive type, and the remaining 68.6% (24) had a combined type; however, none had a hyperactive-impulsive type.

Figure 1 presents the results of the Stroop test, including the completion time and numbers of error and correction obtained from sections 1–5. The completion time of ADHD group from the sections 1–5 were significantly higher than the controls ($p = 0.005$; $p = 0.006$; $p < 0.001$; $p < 0.001$, and $p = 0.001$, respectively). In sections 3, 4, and 5, the number of error of ADHD group were significantly higher than the controls ($p = 0.011$; $p = 0.003$; $p < 0.001$, respectively). In sections 1 and 2, completion times of ADHD group and controls were found comparable ($p = 0.317$; $p = 0.317$). In sections 2–5, the number of correction of ADHD group were significantly higher than the controls ($p = 0.001$; $p < 0.001$; $p < 0.001$ and $p < 0.001$ respectively). In sections 1, the number of corrections of ADHD group and controls were found comparable ($p = 0.079$).

Figure 2 presents the results of the Trail making test, including the completion time and numbers of error obtained from the sections A1–B. The completion time of ADHD group from sections A1, A2, and B were significantly higher than the controls ($p = 0.001$; $p = 0.001$ and $p < 0.001$ respectively). The number of errors of ADHD group from sections A1, A2, and B were significantly higher than the controls ($p = 0.006$; $p = 0.023$ and $p < 0.001$, respectively).

Figure 3 presents the results of agmatine, glutamate, arginine, and NO levels of controls and ADHD group. The serum agmatine and arginine levels of ADHD group were significantly higher than the controls ($p < 0.001$; $p = 0.049$). Although these significant differences implied a relationship between ADHD and these biomarkers, we found no diagnostic value of them with logistic regression analyses ($p > 0.05$). The serum glutamate and NO levels of ADHD group and controls were found comparable ($p = 0.067$; $p = 0.796$). Also, ADHD subtypes were compared among each other and were found similar in terms of the agmatine, glutamate,

Table 1 Sociodemographic characteristics of children with ADHD and controls

	ADHD (<i>n</i> =35)	Controls (<i>n</i> =35)	<i>p</i> value
Age, year	9.0 (8.0–11.0)	10.0 (9.0–14.0)	0.630
Gender, F/M			
Female	47.1% (8)	52.9% (9)	0.780
Male	50.9% (27)	49.1% (26)	
Maternal education			
Primary	25.7% (9)	22.9% (8)	0.061
Secondary	22.9% (8)	5.7% (2)	
High school	31.4% (11)	25.7% (9)	
University	20.0% (7)	45.7% (16)	
Paternal education			
Primary	11.4% (4)	5.7% (2)	0.559
Secondary	14.3% (5)	8.6% (3)	
High school	40.0% (14)	37.1% (13)	
University	34.3% (12)	48.6% (17)	
Family			
Nuclear	88.6% (31)	94.3% (33)	0.588
Extended	2.9% (1)	2.9% (1)	
Divorced	8.6% (3)	2.9% (1)	
Family income			
Low	17.1% (6)	2.9% (1)	0.136
Moderate	22.9% (8)	28.6% (10)	
High	60.0% (21)	68.6% (24)	
School grade			
Good (> 70)	34.3% (12) ^a	94.3% (33)	<0.001
Medium (50–70)	42.9% (15)	5.7% (2)	
Bad (< 50)	22.9% (8)	0.0% (0)	
Comorbidities			
ODD	48.6% (17)	2.9% (1)	<0.001
CD	17.1% (6)	0.0% (0)	0.012
Enuresis Nocturna	22.9% (8)	8.6% (3)	0.101

Data were expressed as % (number)

ODD oppositional defiant disorder, CD conduct disorder

^a*p*<0.05 vs. controls

arginine and NO levels (*p*<0.820; *p*<0.316; *p*<0.163; and *p*<0.352, respectively).

There were significantly positive moderate correlations between the serum arginine level and glutamate with NO levels in the ADHD group (*r*=0.67, *r*=0.58; *p*<0.001, *p*=0.001, respectively). There was a significantly positive moderate correlation between the serum agmatine and glutamate levels in the controls (*r*=0.46; *p*=0.006). There was no significant correlation among other serum biomarkers in the study groups (*p*>0.05) (Fig. 4). In the ADHD group, analyses of the relationship of the Stroop test and TMT parameters with serum biomarkers revealed no meaningful association (*p*>0.05).

Figure 4 displays the correlation graphs of arginine, glutamate, and NO. Pearson correlation analyses revealed that there were positive significant moderate correlations between glutamate and arginine (*r*=0.67, *p*<0.001), glutamate and NO (*r*=0.52, *p*=0.002), and NO and arginine (*r*=0.82, *p*<0.001).

Discussion

To the best of our knowledge, the relationship between serum agmatine and arginine, glutamate, and NO values with the performance of EFs was investigated in children with ADHD for the first time. Compared to the controls, ADHD group had impaired EF test results with both Stroop and TMT tests. Serum agmatine and arginine meaningfully increased in ADHD group, but the increases of serum NO and glutamate were not prominent. Considering the association of serum parameters with EF test results, we could not find any significant relationship; however, we linked it to the small sample size of the study.

Not surprisingly, we found a significant difference between children with ADHD and their healthy peers in terms of EFs. We observed that it took a long time to complete all subsections of the Stroop test in all ADHD group. Also, they made more errors and more corrections in sections 3, 4, and 5. Similarly, children with ADHD completed the test in a long time and made more mistakes than their healthy peers in all subsections of the Trail making test. Several studies evaluating EFs in children with ADHD indicated that these children showed higher impairment in EF tasks (Kofler et al. 2010; Raiker et al. 2012; Crosbie et al. 2013). This was confirmed by our study.

To date, many studies have investigated the association of agmatine with psychiatric disorders such as schizophrenia, depression, anxiety disorder, and autism; however, no study has been conducted on the relationship between ADHD and agmatine levels. In schizophrenia studies, it was observed that low-dose (20 mg/kg) agmatine reduced symptoms of schizophrenia (Pålsson et al. 2008), but in high doses (160 mg/kg), it caused antipsychotic-resistant schizophrenia symptoms (Uzbay et al. 2010). Agmatine shows its antidepressant-like effect with NMDA receptor blockade and NOS inhibition (Li et al. 2003). It was shown that exogenous agmatine improved the symptoms in depressive patients (Shopsin 2013). Agmatine shows its anxiolytic effect by blocking the NMDA-calcium-NOS pathway and upregulating 5HT receptors. Experimental studies indicated that endogenous agmatine production increased 5-times under stress, and exogenous agmatine showed its anxiolytic effect even at low doses (Gong et al. 2006). In patients with autism spectrum disorder (ASD) endogenous agmatine level was

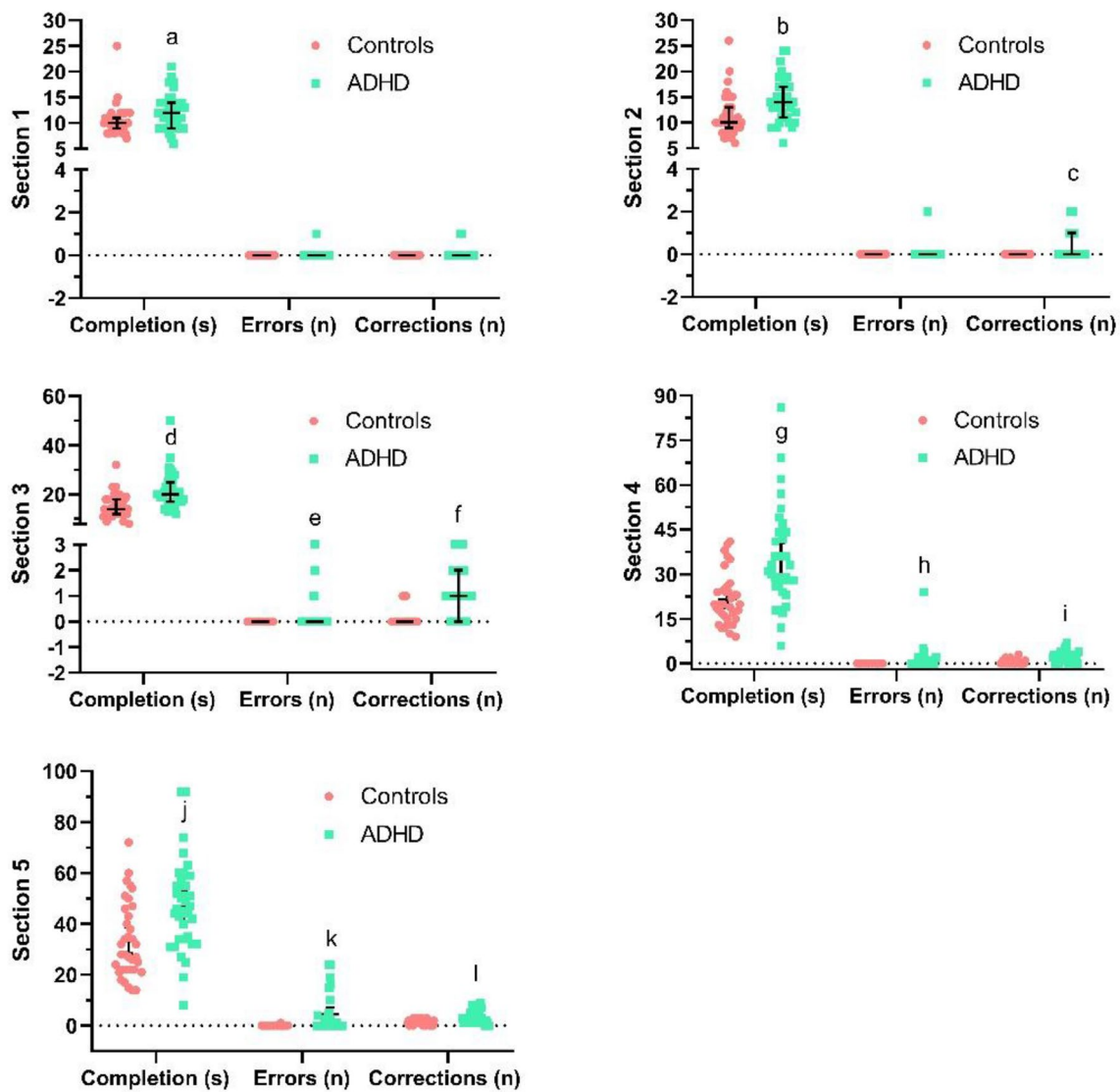


Fig. 1 Data of ADHD group and controls of Stroop test, including completion time and numbers of error and correction obtained from sections 1–5. Data were expressed as median with range and analyzed with the Mann–Whitney test. ^{a,b,d,g,j}Completion time of ADHD group

were higher than controls ($p < 0.05$). ^{e,h,k}Numbers of error of ADHD group were higher than controls. ^{c,f,i,l}Numbers of correction of ADHD group were higher than controls

found to be lower, NO, and glutamate levels were higher than expected (Esnafoglu and Irendo 2018).

Previous studies indicated that agmatine has positive effects on learning and memory by NOS inhibition and NMDA receptor blockage (Leitch et al. 2011; Liu and Collie 2009; Liu and Bergin 2009; Rushaidhi et al. 2013). Liu and his colleagues reported that endogenous agmatine levels increased in CA1 and dentate gyrus regions of the hippocampus, entorhinal cortex, and vestibular nucleus during spatial learning in rats. Also, they showed that the amount of endogenous agmatine decreased in these brain regions depending on age in rats (Liu and Collie 2009; Liu and Bergin 2009). A similar study stated that memory and learning

of young rats improve with agmatine supplementation (Rushaidhi et al. 2013).

In rats, agmatine supplementation has a protective effect on memory and learning impairment induced with scopolamine (Moosavi et al. 2012). It was later shown that this effect is independent of dose (Utkan et al. 2012). In another study, it has been shown that agmatine supplementation has a protective effect on neuroinflammation and memory impairment caused by lipopolysaccharides (Zarifkar et al. 2010). Streptozotocin (STZ)-induced Alzheimer's model in rats has indicated that exogenous agmatine ameliorates cognitive functions and reduce STZ-induced cell death (Song et al. 2014). Furthermore, it has a protective effect against

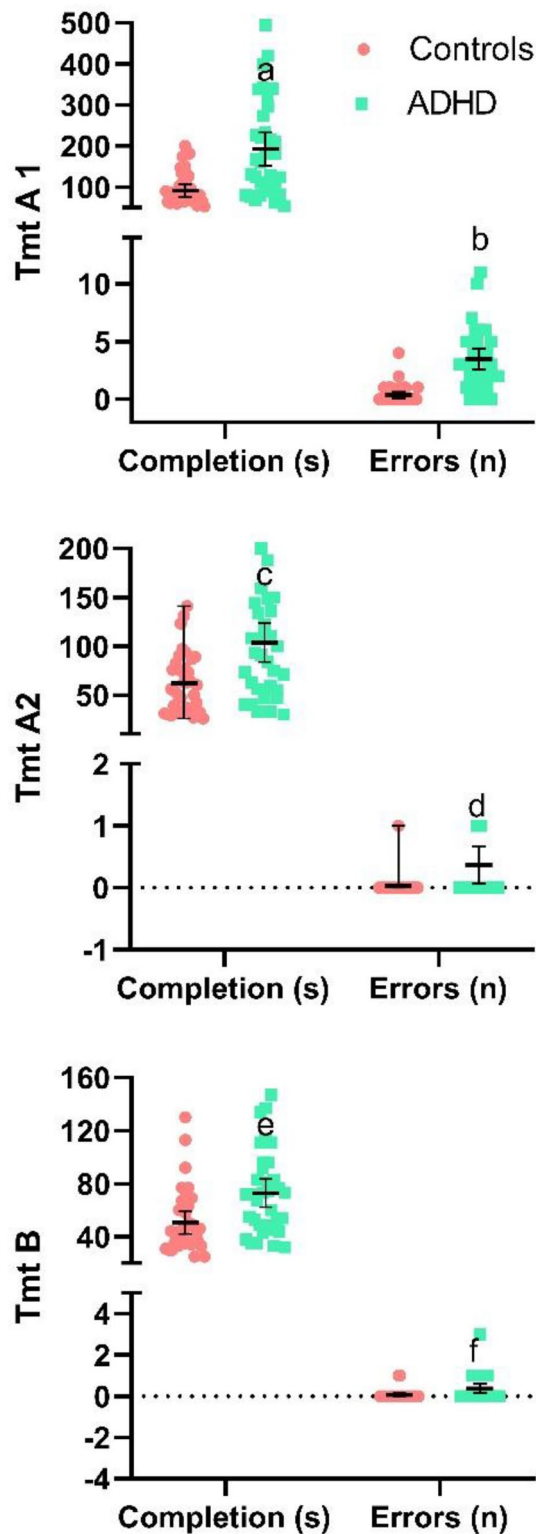


Fig. 2 Data of ADHD group and controls of Trail making test, including completion time and numbers of error obtained from sections A1–B. Data were expressed as median with range and analyzed with the Mann–Whitney test. ^{a,c,e}Completion time of ADHD group were higher than controls ($p < 0.05$). ^{b,d,f}Numbers of error of ADHD group were higher than controls

memory impairment induced by beta-amyloid 25–35 substance at 40 mg/kg IP dose (Liu and Bergin 2009). Based on the hypothesis we created at the outset of the study, we were expecting to find low agmatine and arginine levels in the patient group compared to the control group. However, we found that agmatine was significantly higher in children with ADHD. This may be attributed to the protective effect of agmatine on impaired EFs.

There are several studies examined plasma arginine levels and its metabolites in psychiatric disorders. According to the last five years of literature, two of these studies were conducted on patients with major depressive disorder (Ozden et al. 2020; Hess et al. 2017), and a recent one was studied in the patients with obsessive–compulsive disorder (Yilmaz et al. 2016), and the other one performed on the first-episode psychotic patients (Garip et al. 2019). However, likewise agmatine, there is no study examining arginine levels and its metabolites such as putrescine in patients with ADHD in the literature. In our study, we observed that arginine levels are significantly higher in children with ADHD than those in the control group. This finding suggests that the increase of arginine may be related to the substrate-product relationship, as it is the precursor for agmatine.

It is known that if NO presents in abnormally high concentrations, it acts as free radicals and exerts neurotoxic effects by increasing oxidative stress (Akyol et al. 2004). The studies investigating the relationship between oxidative stress and ADHD revealed that blood levels of NO and oxidative stress are high in patients with ADHD (Ceylan et al. 2010; Joseph et al. 2015). Moreover, it was observed that rats treated with NOS inhibitor improved symptoms of hyperactivity and attention deficit (Aspide et al. 2000). Antioxidant agents such as omega-3 and vitamin C, given as a supplementary treatment, attenuated the symptoms in patients with ADHD (Joshi et al. 2006). S-nitrosothiol (-SNO) formed by nitrosation reaction from NO plays a vital role in biological processes such as posttranslational modifications, cell signal transduction pathways, and neuronal function under normal conditions, and causes cell destruction and neurodegeneration under abnormal conditions. The relationship of this SNO protein with ADHD has not been studied so far; however, it has been found to play a role in the pathogenesis of neurodegenerative diseases such as Parkinson’s disease and Alzheimer’s disease (Nakamura et al. 2013). Shank protein genes are known to play a role in synaptic development and function. Mutation in these genes affects the formation of SNO by disrupting the nitric oxide (NO)—mediated posttranslational modification, which negatively affects the oxidative/nitrosative stress balance, impairing synaptic functions, and causing apoptosis. So, SHANK3 gene mutation has been associated with neuropsychiatric diseases such as autism spectrum disorder (ASD) and intellectual disabilities (Amal et al. 2020). In line

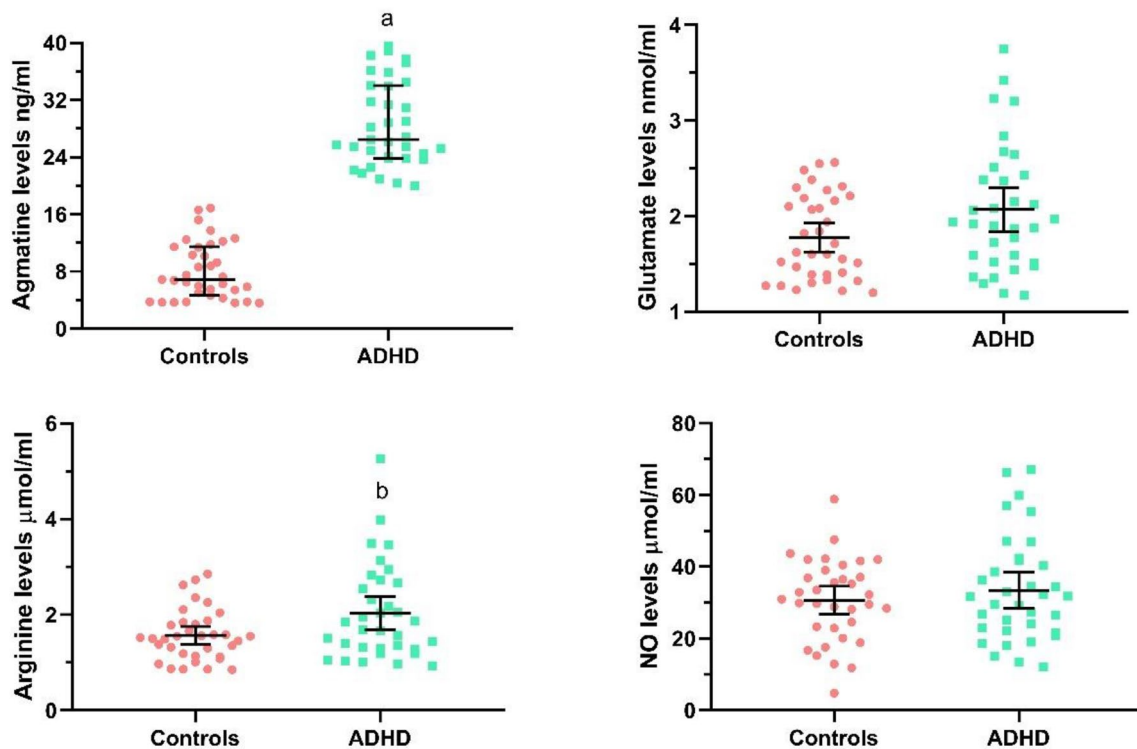


Fig. 3 Data of agmatine, glutamate, arginine, and NO levels of controls and ADHD group. Data were expressed as median with range and analyzed with the Mann–Whitney test. ^aAgmatine levels time of

ADHD group were higher than controls ($p < 0.05$). ^bArginine levels of ADHD group were higher than controls

with our hypothesis, we were expecting to observe a higher level of NO in the patient group; we found that blood levels of NO were higher in children with ADHD. However, this difference was not statistically significant. This may be due to the dramatic increase in the agmatine level in the ADHD group. In fact, the level of agmatine may have increased to compensate the adverse effect of NO.

Glutamate regulates the dopaminergic neurotransmission system (Hawi et al. 2015), which plays an essential role in the etiology of ADHD, especially by NMDA receptor activation. Studies on dopamine and glutamatergic systems showed that NMDA receptors are related to cognitive memory and attention deficit in ADHD (Kotecha et al. 2002). The defect in glutamate signals linked to the underlying pathology in some patients with ADHD (Adler et al. 2012). Decreased prefrontal GABA levels has been shown in a study conducted on children with ADHD (Edden et al. 2012), while another study found increased glutamatergic levels in prefrontal brain areas (MacMaster et al. 2003). Moreover, a genome-wide study showed over-representation of the metabotropic glutamate receptor genes in several ADHD cohorts (Elia et al. 2012). Imaging studies have reported that glutamatergic transmission varies in the prefrontal cortex and related regions in patients with ADHD (Arnsten and Rubia 2012). Furthermore, studies showed

that Atomoxetine, used in the treatment of ADHD, acts as an NMDA receptor blocker in clinically significant doses (Ludolph et al. 2010) and Memantine, NMDA receptor blocker, improves ADHD symptoms in young patients and adults with ADHD (Surman et al. 2013). Relying on our hypothesis, we were expecting to find a higher level of glutamate in the patient group; indeed, we found that the children with ADHD had a higher level of glutamate than controls, but this difference was not statistically significant. This situation may be due to the dramatic increase in agmatine levels in the ADHD group. In other words, agmatine may have increased to compensate for the adverse effect of glutamate on EFs because of the inverse relationship between them.

Our study has some limitations and strengths. The primary limitation is that we used the Stroop test and TMT only to evaluate the EFs in children. However, there are several neuropsychological tests, including the Wisconsin Card Sorting Test, Benton Visual Retention Test, etc. The other limitation is the small size of our study, which was 70 children in total. The main strength of our study is that it is a prospective clinical study conducted on children; whereas most of the studies on agmatine in the literature are animal studies at the preclinical level. Furthermore, to the best of our knowledge, there is no other study examining the possible relationship between agmatine levels and

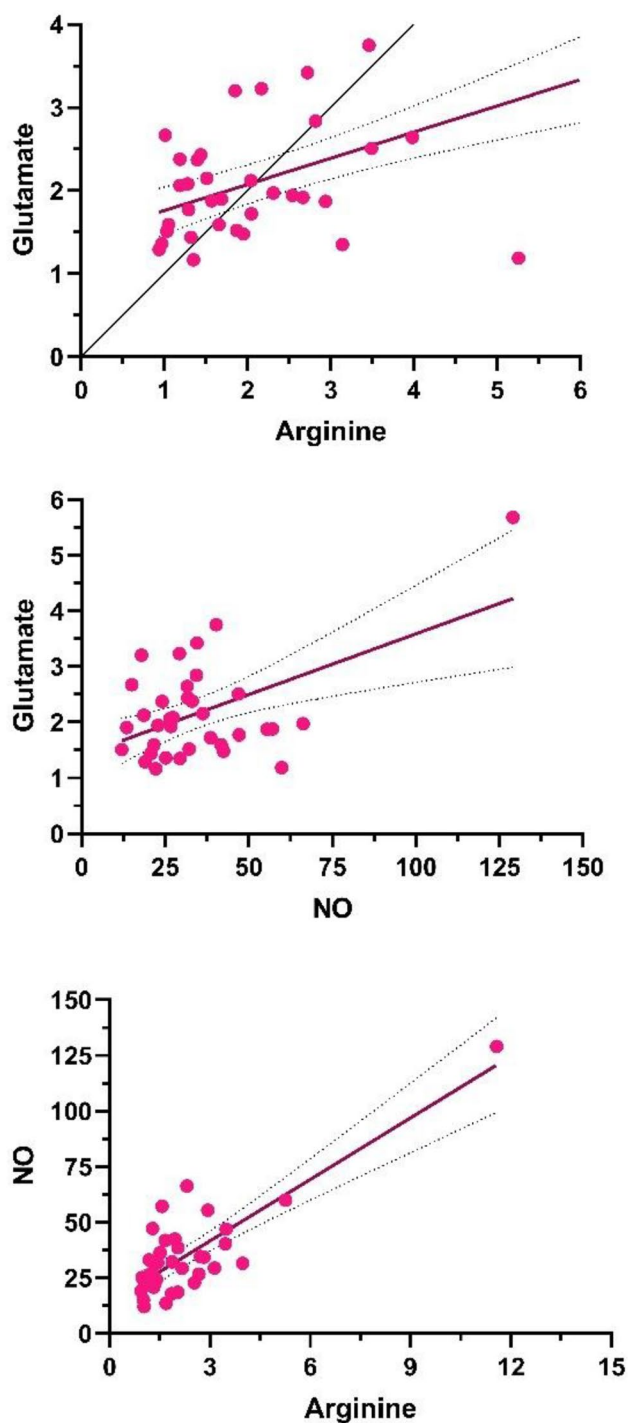


Fig. 4 Correlations of arginine, glutamate, and NO. Correlation coefficients of glutamate and arginine, glutamate and NO, and NO and arginine are $r=0.67$ ($p<0.001$), $r=0.52$ ($p=0.002$), and $r=0.82$ ($p<0.001$), respectively

EFs in children with ADHD. In this respect, our results are noteworthy as it is the first study in the field of neuropsychiatry and biochemistry.

Conclusion

In the present study, we investigated the relationship between the executive functions (EF) and agmatine, arginine, NO, and glutamate levels in children with ADHD for the first time in the literature. As expected, EFs were found to be worse in children with ADHD; but interestingly, the blood agmatine and arginine levels of these children were significantly higher than their non- ADHD peers. NO, and glutamate levels were also higher in children with ADHD. We related the elevation in NO and glutamate levels in the study group to impaired EFs of children with ADHD. It may be; therefore, agmatine increased to compensate or to suppress the adverse effects that may occur due to the elevation in NO and glutamate. However, the differences between the patient and control group in terms of NO and glutamate levels were not found to be statistically significant, which may be related to the inhibitory effect of agmatine on NO and glutamate synthesis. In addition, as agmatine levels are already high in children with ADHD, we propose not to give external agmatine for therapeutic purposes in ADHD. Since there is no similar study in the literature about the subject that can support or discuss the findings of our study, we cannot say that the parameters we compared and the results we obtained are strictly related to each other. We believe that the results obtained from this study are highly valuable and make essential contributions to the literature, but our results are required to be supported in the future by similar studies with large sample size or with animal studies.

Author contributions The study was planned by SAS. All children who participated in the study were evaluated in terms of EFs by SAS and AUC. Blood samples were collected by SAS. Blood samples were measured with HPLC in terms of agmatine by SE. Blood samples were measured with ELISA in terms of arginine, NO, and glutamate by DU and DB. The interpretation of data was made by SAS and DU. The manuscript was written by SAS. Statistical analysis of the study and proofreading of the manuscript was made by FI.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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