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Plasma amino acid profile in autism spectrum disorder (ASD)

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Abstract. – OBJECTIVE: In our study, we aimed to reveal pathophysiologic mechanisms in ASD by comparing plasma amino acid levels between patients and healthy controls while considering vitamin B12 and D levels.

PATIENTS AND METHODS: The study included 21 patients aged 2-18 years-old who were followed with a diagnosis autism spectrum disorder (ASD) and 21 age and sex-matched healthy children from our outpatient clinic as control group.

RESULTS: The study included 42 children and adolescents aged 2-18 years-old (19 girls and 23 boys). There were no significant differences in terms of body weight and height between the groups. We found significant differences in levels of ammonium, phosphoethanolamine, histidine, homocysteine, carnosine, methionine, cystathionine, cyste0ine, threonine, 3-methyl histidine and phenylalanine/tyrosine ratio between patient and control groups. Both vitamin B12 and D were significantly lower in the ASD group compared to controls. In the variance analysis with vitamin B12 and D as covariates, significant differences persisted for only phosphoethanolamine (p=0.04), cystathionine (p<0.001), cystine (p=0.006) and threonine (p=0.02).

CONCLUSIONS: Further studies are needed on the amino acids that show variations in children with ASD in order to reveal their role in the etiology and therapeutic use in ASD.

Key Words:
Plasma amino acids, Autism spectrum disorder, Children, Adolescent.

Introduction

ASD is a heterogeneous group of neurodevelopmental disorders with biological origin. Its incidence has been reported to be 1:88 children worldwide and it is more common in boys. It has a complex etiology including genetic and environmental factors[1]. It is associated with a variety of known risk factors such as mutant or variant genes, advanced paternal age, prematurity and birth complications[2-4]. Higher prevalence rates in siblings and monozygotic twins suggest genetic origin while phenotypical heterogeneity among family members suggests that inheritance may be polygenic and influenced by environmental factors. In these children, ASD can be accompanied by abnormalities or disorders of other systems[5]. For instance, behavioral disorders and gastrointestinal disorders are more prevalent in children with ASD when compared to normal children. This leads to impairment in intake or absorption of dietary content. As a result, changes in levels of some amino acids as well as vitamin or mineral deficiencies are seen[6]. These deficiencies can either play a role in the etiology or occur as a result of disease[4]. There are many studies about changes in plasma amino acid levels in children with ASD. Most of these studies made efforts to find a physiopathological mechanism in children with ASD. However, in most studies, changes in vitamin levels were disregarded while assessing plasma amino acids. Thus, different results were obtained in different studies, leading conflicting results such as high or low levels of a certain amino acid across studies.

In our study, it was aimed to shed light on pathophysiologic pathways in ASD by measuring and comparing plasma amino acid levels with healthy controls while taking vitamin B12 and D levels into consideration.

Patients and Methods

The study included 21 patients aged 2-18 years-old who were diagnosed with ASD in Van

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Region Education and Research Hospital. These ASD patients referred to Van YYU Department of Pediatrics. The diagnosis of ASD was made based on ASD diagnostic interview according to both Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-V) and Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision (DSM-IV-TR). Childhood Autism Rating Scale (CARS) was used to categorize participants as not autism, mild-moderate autism and severe autism. Twenty-one age- and sex-matched children who admitted to YYU Outpatient Clinic of Pediatrics and did not diagnose with any illness were employed as a control group. Neither any diagnostic criteria for ASD nor history of vitamin supplement intake existed in the control group.

Body weight and height were recorded and then blood samples were drawn for assessment of routine hemogram and blood biochemistry, amino acid and vitamin level from patients and healthy controls. The patients with any comorbid disease, known genetic/metabolic disease, head injury or previous surgery, those with an additional chronic disease, and those with a history of chronic drug use were excluded. Subjects with abnormal hepatic or renal functions were excluded.

Measurements of calcitriol and vitamin B12 were performed at our central laboratory of YYU University, School of Medicine by using the Architect CI-16200 (Abbott Diagnostics, Abbott Park, IL, USA) with the chemiluminescent method. Plasma amino acid levels were measured by using Aracus amino acid analyzer (membraPure GmbH, Neuendorfstraße 20a; 16761 Hennigsdorf/Berlin, Germany) with the ion exchange chromatographic method.

The study was approved by Ethics Committee of YYU, School of Medicine. The study was conducted in accordance by Helsinki Declaration. All parents and children, if possible, gave written informed consent before participation.

Table I. Comparison of the groups in terms of age, weight, height and gender.

	ASD Mean±SD (min-max)	Control Mean±SD (min-max)	p
Age (year)	8.38±5.35 (2-18)	9.80±4.25 (3.5-17)	>0.05
Weight (kg)	39.20±2.35 (11.9-83)	29.04±14.64 (15-59)	>0.05
Height (m)	1.27±0.30 (0.88-1.7)	1.27±0.27 (0.93-1.65)	>0.05
Gender	n (%)	n (%)	
Female	8 (38.1)	11 (52)	>0.05
Male	13 (61.9)	10 (48)	

ASD: Autism Spectrum Disorders; SD: Standard deviation; Min: Minimum, Max: Maximum.

Statistical Analysis

Statistical analyses were performed by using SPSS for Windows version 13.0 (SPSS Inc., Chicago, IL, USA). Values were expressed as mean, standard deviation and minimum maximum. Continuous variables with normal distribution were compared using Student's *t*-test, while skewed data were compared with Mann-Whitney U Test. Chi-Square tests were used where appropriate. $p<0.05$ was considered to be statistically significant.

Results

The study included 42 children and adolescent aged 2-18 years (19 girls and 23 boys). Of these, 21 were in the ASD group while 21 healthy children and adolescents were employed as a control group. Table I presents the distribution of age, gender, body weight and height in the groups. There were no significant differences in age, gender, body weight and height between groups.

There were significant differences in ammonium, phosphoethanolamine, histidine, homocysteine, carnosine, methionine, cystathionine, threonine, 3-methyl histidine and phenylalanine/tyrosine ratios between patient and control groups. Ammonium, phosphoethanolamine, histidine, cystathionine, cystine, 3-methyl histidine and phenylalanine/tyrosine ratio were found to be higher while homocysteine, carnosine, methionine, cystine and threonine were found to be lower in ASD group compared to control group (Table II).

There were significant differences in vitamin B12 and D levels between groups. Both vitamins were significantly lower in ASD group compared to controls (Table III). In variance analysis with vitamin B12 and D as covariates, for both groups significant differences persisted

Table II. Comparison of the groups in terms of plasma amino acid levels.

	ASD Mean±SD (min-max)	Control Mean±SD (min-max)	p
□-aminoadipic acid	1.49±1.95	2.44±3.02	>0.05
□-amino butyric acid	12.94±8.57	11.56±3.94	>0.05
Alanine	386.88±152.45	599.00±1044.50	>0.05
Arginine	70.29±22.90	70.68±26.06	>0.05
Asparagine	37.69±10.67	32.75±11.95	>0.05
Aspartic acid	5.26±3.13	5.07±1.44	>0.05
B-alanine	20.83±7.92	27.23±18.09	>0.05
Ethanolamine	7.58±7.97	4.25±3.75	>0.05
Phenylalanine	55.50±17.63	52.41±16.55	>0.05
Phosphoethanolamine	12.50±12.40	6.42±4.53	<0.05
Phosphoserine	15.64±12.31	11.63±5.09	>0.05
Glycine	204.87±51.94	243.36±60.96	>0.05
Glutamic acid	32.14±10.01	31.36±14.91	>0.05
Hydroxylysine	0.92±0.64	3.83±14.12	>0.05
Hydorxyproline	18.35±10.88	20.68±24.81	>0.05
Histidine	**83.38±21.25**	**66.08±17.98**	**<0.05**
Homocysteine	**2.40±1.39**	**3.48±2.01**	**=0.05**
Isoleucine	54.14±21.63	57.55±24.59	>0.05
Carnosine	**0.74±1.72**	**2.77±1.81**	**<0.01**
Lysine	119.93±49.94	127.00±53.28	>0.05
Leucine	82.50±27.29	90.50±40.67	>0.05
Methionine	**12.71±5.73**	**19.09±7.71**	**<0.01**
Ornithine	72.56±19.69	69.16±33.28	>0.05
Proline	262.33±111.06	236.36±81.34	>0.05
Serine	131.36±50.87	132.00±25.93	>0.05
Cystathionine	**4.86±2.33**	**1.00±0.64**	**<0.001**
Cystine	**12.44±8.88**	**30.28±10.37**	**<0.001**
Citrulline	24.19±10.42	22.93±5.89	>0.05
Taurine	43.20±22.92	40.95±35.05	>0.05
Tyrosine	**47.50±14.23**	**60.82±25.58**	**=0.05**
Threonine	**76.94±43.26**	**121.77±52.24**	**<0.01**
Tryptophan	21.72±9.12	23.75±9.39	>0.05
Valine	197.93±65.64	195.59±59.78	>0.05
GABA	24.40±34.44	22.03±17.24	>0.05
1-methyl histidine	**22.68±19.26**	**15.20±4.93**	**>0.05**
3-methyl histidine	**15.24±5.81**	**9.98±3.42**	**<0.01**
Phenylalanine/tyrosine	**1.19±0.25**	**0.94±0.42**	**<0.05**

ASD: Autism Spectrum Disorders; SD: Standard deviation; Min: Minimum, Max: Maximum.

Table III. Comparison of vitamin B12 ve D levels in the groups.

	ASD Mean±SD	Control Mean±SD	p
Vitamin B12	233.62 ± 60.73424	428.50 ± 173.8589	<0.001
25 OH Vitamin D	14.94 ± 7.652305	29.42 ± 9.069202	<0.001

ASD: Autism Spectrum Disorders; SD: Standard deviation, Min: Minimum, Max: Maximum.

for only phosphoethanolamine (p=0.04), cystathionine (p<0.001), cystine (p=0.006) and threonine (p=0.02) (Table IV). When correlation between plasma amino acids, vitamin B12 and D were assessed, it was found that there was a positive correlation between vitamin D and aminoadipic acid while there was a negative correlation between vitamin D and alanine, arginine, aspartic acid, phosphoserine, hydroxylysine, homocysteine, carnosine, ornithine, cystathionine, citrulline, taurine. However, vitamin B12 positively correlated with ethanolamine only. When partial correlation analysis was performed between vitamin D and

Table IV. The correlation curve between plasma amino acids and vitamin B12, vitamin D in the groups.

	ASD				Control			
	Vitamin B12		Vitamin D		Vitamin B12		Vitamin D	
	c.c.	p	c.c.	p	c.c.	p	c.c.	p
A-aminoadipic acid	0.009	0.976	0.895 (*)	0.04	0.192	0.417	0.174	0.45
A-amino butyric acid	0.292	0.311	-0.158	0.800	-0.16	0.501	-0.128	0.581
Alanine	0.243	0.402	-1.000(**)	0.000	0.187	0.431	-0.178	0.44
Arginine	-0.031	0.916	-0.895(*)	0.040	-0.132	0.579	-0.346	0.125
Asparagine	-0.150	0.608	-0.368	0.542	-0.049	0.838	-0.177	0.442
Aspartic acid	-0.097	0.741	-0.895(*)	0.040	0.202	0.392	0.515(*)	0.017
B-alanine	0.199	0.495	-0.368	0.542	-0.146	0.539	0.078	0.737
Ethanolamine	0.898(**)	0.000	0.053	0.933	-0.42	0.065	0.107	0.645
Phenylalanine	-0.075	0.798	-0.158	0.800	-0.139	0.558	0.1	0.666
Phosphoethanolamine	0.142	0.629	-0.579	0.306	-0.102	0.667	0.004	0.987
Phosphoserine	0.491	0.075	-1.000(**)	0.000	0.083	0.727	0.286	0.209
Glycine	0.242	0.405	-0.579	0.306	0.243	0.302	-0.129	0.578
Glutamic acid	0.004	0.988	-0.368	0.542	-0.05	0.835	0.042	0.856
Hydroxylysine	-0.239	0.411	-0.895(*)	0.040	0.078	0.742	0.108	0.64
Hydroxyproline	0.062	0.833	-0.684	0.203	0.105	0.661	0.143	0.536
Histidine	0.341	0.233	-0.579	0.306	-0.028	0.907	0.049	0.831
Homocysteine	0.062	0.833	-0.895(*)	0.040	0.067	0.778	0.071	0.759
Isoleucine	0.235	0.419	-0.158	0.800	-0.151	0.524	-0.257	0.26
Carnosine	0.127	0.665	-1.000(**)	0.000	0.155	0.515	0.021	0.929
Lysine	0.190	0.515	-0.158	0.800	-0.15	0.527	-0.038	0.871
Leucine	0.235	0.420	-0.158	0.800	-0.156	0.512	-0.06	0.795
Methionine	-0.166	0.569	0.368	0.542	-0.277	0.236	-0.211	0.358
Ornithine	-0.018	0.952	-0.895(*)	0.040	0.122	0.607	-0.068	0.771
Proline	-0.264	0.361	0.368	0.542	0.047	0.845	-0.188	0.414
Serine	0.195	0.505	-0.579	0.306	-0.179	0.45	-0.213	0.354
Cystathionine	0.137	0.640	-0.895(*)	0.040	-0.076	0.75	-0.163	0.48
Cystine	0.358	0.208	-0.368	0.542	-0.297	0.203	-0.418	0.059
Citrulline	-0.296	0.303	-0.895(*)	0.040	0.032	0.892	-0.17	0.461
Taurine	0.296	0.303	-1.000(**)	0.000	0.455(*)	0.044	0.127	0.582
Tyrosine	-0.164	0.576	-0.368	0.542	-0.255	0.277	-0.24	0.295
Threonine	0.314	0.274	-0.158	0.800	-0.307	0.188	-0.286	0.209
Tryptophan	-0.097	0.741	-0.158	0.800	-0.312	0.18	-0.309	0.173
Valine	0.235	0.420	-0.158	0.800	-0.166	0.484	-0.178	0.44
GABA	-0.321	0.263	-0.108	0.863	0.353	0.127	0.051	0.827
1-methyl histidine	0.531	0.051	-0.579	0.306	-0.12	0.616	0.228	0.321
3-methyl histidine	-0.109	0.712	0.684	0.203	0.119	0.619	0.337	0.135
Phenylalanine/tyrosine	0.173	0.555	0.158	0.800	0.364	0.115	0.679(**)	0.001

ASD: Autism Spectrum Disorders; SD: Standard deviation; Min: Minimum, Max: Maximum.

variables with significant correlation by taking vitamin B12 levels as a control variable, statistical significance didn't persist for any of the amino acids. When the same analysis was performed for vitamin B12, it was seen that there was no significant correlation between vitamin B12 and any of the plasma amino acid levels.

Discussion

In recent years, abnormalities in plasma amino acid levels have been reported in various disease groups, particularly in patients with ASD[7,8]. In patients with ASD, some changes demonstrated in amino acid levels are attributed to vitamin deficiencies. Moreover, it is proposed that vitamin supplementation has beneficial effects in the management of ASD in many studies. It is reported that these beneficial effects result from normalization of increased amino acid levels due to vitamin deficiency. The demonstration of the fact that decreased methionine and increased homocysteine as a result of vitamin B12 deficiency can lead psychiatric disorders and even worsen course of the disease is a good example[9-11]. The relationship between vitamin B12 deficiency and homocysteine in children with ASD was demonstrated in a previous research and suggested that high homocysteine levels could be related to increased oxidative stress and ASD development[12]. Subsequent years, it was seen that vitamin B12 supplementation resulted in improvement in the severity of ASD complete recovery couldn't be achieved. It was also attempted to investigate amino acid profiles in children with ASD and establish a link between amino acid metabolism disorders and ASD previously[13].

Shimmura et al[14] evaluated levels of 25 amino acids in children with ASD and found that plasma glutamate level was high while glutamine level was low in children ASD. Moreno-Fuenmayor et al[15] found that levels of glutamic acid, aspartic acid and taurine were high while glutamine and asparagine levels were low. Similarly, Aldred et al[13] found levels of glutamic acid, phenylalanine, asparagine, tyrosine, alanine, lysine and glutamine as high in children ASD. Finally, Triouvanziam et al[16] found that glutamate level was high while glutamine, threonine, asparagine, citrulline, serine, tyrosine and leucine levels were low in patients with ASD. Considering all these studies, it is apparent that there are controversial results in different studies.

In our study, we found ammonium, phosphoethanolamine, histidine, cystathionine, 3-methyl histidine and phenylalanine/tyrosine ratio higher while homocysteine, carnosine, methionine, cystine and threonine lower in children with ASD compared to controls. Our results are only similar with Aldred et al's and Triouvanziam et al's results. Because we found phenylalanine levels higher and threonine levels lower in ASD children compared to controls as them[13,16]. Thus, our results were consistent with some studies while inconsistent with others in the literature. We think that the results might be due to vitamin deficiencies and adjusted according to vitamin D and B12 levels which were found to be significantly lower in ASD group compared to controls. In the variance analysis using vitamin levels as covariates, it was found that there were significant differences in phosphoethanolamine (high), cystathionine (high), cystine (low) and threonine (low) levels.

Phosphoethanolamine (PE) is a phosphomonoester metabolite of the phospholipid metabolism. PE is a precursor of phospholipid synthesis and a product of phospholipid breakdown. Phosphomonoesters are present at much higher levels in brain than in other organs. In developing brain, phosphomonoesters are normally elevated during the period of neural proliferation. This also coincides with the occurrence of normal programmed cell death and synaptic pruning in developing the brain. These findings are consistent with the role of phosphomonoesters in membrane biosynthesis. PE shows a strong structural similarity to the inhibitory neurotransmitter, GABA, and the GABA-B receptor partial agonist, 3-amino-propylphosphonic acid. PE is a phosphomonoester which is shown to be decreased in the brain of patients with Alzheimer's disease (AD) in autopsy studies[17-21]. Therefore, we think that it can be involved in pathophysiology of ASD disorders. In our study PE levels were found to be high in children with ASD.

Threonine is an essential amino acid in human. It is abundant in human plasma, particularly in the newborn. Threonine promotes normal growth by helping to maintain the proper protein balance in the body. Threonine also supports cardiovascular, hepatic, central nervous, and immune system function. Threonine is needed to synthesize glycine and serine, two amino acids that are necessary for the production of collagen, elastin, and muscle tissue.

Threonine helps to keep connective tissues and muscles throughout the body strong and elastic, including the heart, where it is found in significant amounts. Threonine combines with the amino acids aspartic acid and methionine to help the liver with lipotrophic function, or the digestion of fats and fatty acids. It supports the immune system by aiding in the production of antibodies, and because it is found largely in the central nervous system, may be helpful in treating some types of depression. Severe deficiency of threonine causes neurological dysfunction and lameness in experimental animals. Threonine supplementation may also be useful in the treatment of Amyotrophic Lateral Sclerosis (ALS), also known as Lou Gherig's Disease, because it increases glycine levels in the central nervous system (CNS) (administering glycine is ineffective, since it cannot cross the blood brain barrier). Research indicates that symptoms of Multiple Sclerosis (MS), another disease that affects the nerve and muscle function, may be treated with threonine supplementation[22]. Therefore, we think that this amino acid can be considered in the pathophysiology and treatment of ASD. Similar to our study, threonine levels were found to be low in children with ASD in a recent study[23].

Conclusions

In variance analysis with vitamin B12 and D as covariates, for both groups significant differences persisted for only in phosphoethanolamine (high), cystathionine (high), cystine (low) and threonine (low) in children with ASD compared to healthy controls. It was thought that changes in remaining amino acids were influenced by vitamin B12 and D deficiency. Thus, we think that further studies are needed to on the amino acids that showed variation in children with ASD in order to investigate their role in the etiology and their potential implications for the treatment of ASD.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

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