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Assessment of Serum Amino Acid Chromatography in Children with Inflammatory Bowel Diseases

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Abstract

Background: Given the important role of amino acids in regulating many metabolic pathways of the body and considering the scarcity of markers for the diagnosis of inflammatory bowel disease (IBD) and its differentiation, we aimed to investigate the status of serum amino acids chromatography in children with IBD.

Materials and Methods

This case-control study was conducted among children with primary diagnosis of IBD who referred to Mofid Children's hospital in Tehran, Iran. Children with a definite diagnosis of chronic IBD on the basis of endoscopy and biopsy were enrolled. In addition, 100 children without any history of predisposing, chronic, or inflammatory disease who referred to the same hospital during the period of the study were also selected. All samples underwent serum amino acids chromatography via HPLC method.

Results

Of all the patients in the IBD group, 18 patients (18%) suffered from Crohn's disease and 82 patients (82%) had ulcerative colitis; the disease was active in 54 patients (54%). The results of serum amino acids chromatography showed that several amino acids were significantly higher in patients with IBD. Considering the normal serum levels of amino acids, only the levels of two amino acids of histidine and tryptophan were significantly different in the IBD group compared the control group. In total, of all the subjects, 30 children (15%) had abnormal amino acid serum chromatography; hence, its prevalence was significantly higher in the IBD group (P=0.048).

Conclusion

The current study showed that serum amino acid chromatography in children with IBD were different from that in healthy children. More specifically, the decrease in tryptophan level was more observed in patients with active disease.

Key Words: Inflammatory bowel disease, Children, Chromatography, Serum amino acids.

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1- INTRODUCTION

Inflammatory bowel disease (IBD) is a chronic bowel disease that has two main subtypes, namely Crohn's disease and ulcerative colitis. The incidence of these diseases is increasing in the general population (1, 2) and up to 25% of patients with the disease are diagnosed before the age of 20 years old (3). According to some researchers, the increase in the prevalence of these diseases, especially in Eastern communities, is due to lifestyle changes and the use of western diets (4, 5). Despite extensive research, the precise etiology of IBD is unclear, and the disease is considered as a multi-factorial disease whose pathogenesis is affected by genetic, environmental factors and immunological diseases. Since the human body is composed of a highly structured metabolic network, the study of metabolites plays a significant role in determining the health status of a person; in addition, dysregulated metabolites has a significant role in the likely pathogenesis of chronic inflammatory diseases and malignancies.

Amino acids are a group of important metabolites in the body. Approximately 20% of the whole body consists of amino acids or their metabolites, which play a very important role as base substrates and regulators in many metabolic pathways of the body (6, 7). As reported by some studies, the plasma levels of amino acids are disturbed in some diseases, including fibrotic liver disease (8, 9). In addition, plasma amino acid profiles are also used as a proxy for screening non-small cell lung carcinoma (10). In addition, it has recently been reported that amino acids directly play a role in suppressing inflammation of the bowel (5). IBD is also a chronic disease that, according to some studies, is associated with a number of clinical biomarkers, such as amino acids (4). In some studies, it has even been observed that the use of a diet containing amino acids and peptides has had a positive effect on immune function and inflammatory bowel disease, which may play a functional role in the treatment of IBD (11). As reported, some amino acids are effective in healing intestinal inflammation in animal models of colitis. For example, glutamine has a protective effect against inflammations caused by certain substances such as indomethacin, acetic acid, and dextran sodium sulfate (12-15), and the prophylactic glycine diet reduces the production of pre-inflammatory cytokines and Chemokines, and treats inflammatory lesions in rats with colitis caused by dextran sodium sulfate (16). In addition, a dietary regimen containing histidine can reduce inflammatory colon lesions in mice with the symptoms of inflammation of the intestine (17). These results show the probable changes in the level of amino acids and their role in IBD. However, few studies have been conducted in this field and each of them have reported the higher or lower levels of a number of amino acids in patients with IBD, as compared with the healthy subjects (4, 5, 18-25). Given the role of these metabolites in inflammatory bowel disease and because of the availability of a limited number of studies on the status of metabolites in children with IBD, this study was conducted to determine the status of serum amino acid chromatography in children with IBD and to use the results of the study for promoting their health and quality of life.

2- MATERIALS AND METHODS

This cross-sectional study was conducted in Mofid pediatrics hospital in Tehran (Iran) during the years 2015 to 2017. Taking into consideration the estimated prevalence of disorder in serum amino acid in patients with IBD, the sample size was determined to include 200 persons (100 patients and 100 children as the control group). Children under the age of 18 years old with a definite diagnosis of chronic inflammatory bowel disease on the

basis of endoscopy and biopsy were enrolled into the study. In addition, 100 children without any history of predisposing, chronic, or inflammatory disease who referred to the same hospital during the period of the study were also selected and enrolled into the control group. Initially, the objectives of the study were explained to the subjects and their parents, and if they agreed to participate in the study, a written informed consent was obtained from them. The study received ethical approval from Shahid Beheshti University of Medical Sciences (code: IR.SBMU.SM.REC.1394.195). Using a checklist the researchers collected data on patients including age, sex, patient group, type of disease (ulcerative colitis, Crohn's disease), and disease status (active, remission) based on the Pediatric Ulcerative Colitis Activity Index (PCDAI) (26); also 5 ml venous blood sample was taken by nurse of ward to measure the erythrocyte sedimentation rate (ESR) and C-Reactive Protein (CRP), and a stool sample was collected to measure level of calprotectin in stool. Afterwards, patients were referred to the laboratory of Tehran Pediatrics Medical Center to undergo amino acids chromatography using high-performance liquid chromatography (HPLC) method. Chromatography was carried out after four to six hours of fasting. Using a mixed solution containing methanol, sodium acetate, and base, HPLC was performed by mean of Knauer device made in Germany. The SPSS software version 22.0 was used for data entry and analysis. Frequency (percentage) and mean (standard deviation) were used to describe the variables. Chromatography results were assessed quantitatively and qualitatively based on the normal values set in the kit, and was compared using Chi-square test and independent t-test. $P<0.05$ was considered as the significant level.

3- RESULTS

Of the 200 children studied, 125 persons (63%) were girl, and most of the children were in the age group 6-10 years old (117 persons, 59%). There was no significant difference between the two groups in terms of age and sex (**Table.1**). Based on the results of laboratory tests, the inflammatory parameters of ESR, CRP and fecal calprotectin were significantly higher in the IBD group (**Table.1**). Of the 100 children in the IBD group, 18 persons (18%) had Crohn's disease and 82 persons (82%) had ulcerative colitis. In addition, 54 patients (54%) were in the active phase of the disease, which did not show any significant difference between the two types of diseases (10 cases (56%) in Crohn's disease, and 44 (54%) in ulcerative colitis, $p=0.884$). From a quantitative point of view, the results of serum amino acid chromatography were slightly different between the two groups, since the amino acids of aspartic acid, threonine, taurine, alanine, and leucine were significantly higher in patients with IBD while glutamine, histidine, tryptophan, α-aminobutyric acid, methionine, phenylalanine, isoleucine, and ornithine were significantly lower in patients with IBD than in the control group (**Table.2**). However, considering the normal serum levels of amino acids, after categorizing them into three groups with a level lower than the normal, normal, and higher than the normal, only the levels of two amino acids of histidine and tryptophan were significantly different in the IBD group, as compared with those in the control group (**Table.3**). In the IBD group the histidine was higher than the normal level in two patients and lower than the normal level in five patients while it was normal in all the patients in the control group ($p=0.027$). Moreover, the level of tryptophan amino acid was lower than the normal level in six patients, while it was normal in all the patients in the control group ($p=0.013$). Of the total number of children studied, 30 children

(15%) had abnormal serum amino acids chromatography. In three patients three amino acids, in six patients two amino acids, and in 21 patients one amino acid had an abnormal level. In the IBD group, the number of children with abnormal serum amino acids chromatography was significantly higher than that in the control group (20 persons (20%) and 10 persons (10%), respectively, p=0.048). The results of serum chromatography of two amino acids of histidine and tryptophan showed no significant difference between the two sexes, between the age groups, and between ulcerative colitis and Crohn's disease (p>0.05). In addition, serum histidine chromatography did not show a significant difference between the two groups of patients with active disease or in remission (p=0.337), but all the cases of abnormal serum tryptophan chromatography were observed in patients with active IBD, which was significantly higher (p=0.020) (**Table.4**).

Table-1: Comparison of baseline and laboratory characteristics in IBD children and control group

Variables		IBD, (n=100)	Control, (n=100)	P- value
Gender	Girl	58 (58%)	67 (67%)	0.189
	Boy	42 (42%)	33 (33%)	
Age group,year	<2	3 (3%)	6 (6%)	0.083
	2-6	10 (10%)	20 (20%)	
	6-10	60 (60%)	57 (57%)	
	10-17	27 (27%)	17 (17%)	
ESR	< 30 mm/h	57 (57%)	75 (75%)	0.007
	>= 30 mm/h	43 (43%)	25 (25%)	
CRP	< 6 mg/L	68 (68%)	89 (89%)	<0.001
	>= 6 mg/L	32 (32%)	11 (11%)	
Calprotectin	< 60 µg/g	9 (9%)	59 (59%)	<0.001
	60-200 µg/g	47 (47%)	30 (30%)	
	>= 200 µg/g	44 (44%)	11 (11%)	
CRP: C-Reactive Protein; ESR: Erythrocyte sedimentation rate; IBD: Inflammatory bowel disease.				

Table-2: Comparison of serum amino acid chromatography in IBD children and control group

Amino acid	Normal range, (µmol/L)	IBD, (n=100)	Control, (n=100)	P value*
Aspartic acid	0-20	13.1 ± 5	11.3 ± 5.4	0.017
Glutamic acid	10-120	67.3 ± 34.9	68 ± 30.9	0.880
Asparagine	24-60	44.2 ± 10	45.9 ± 9.2	0.211
Serine	60-200	120.6 ± 42.7	115.2 ± 37.9	0.344
Glutamine	396-746	522.9 ± 113.2	557.6 ± 105.5	0.026
Histidine	50-130	84.6 ± 25.9	92.1 ± 21.1	0.027
Glycine	140-490	285.8 ± 100.8	310.7 ± 108.1	0.093
Threonine	40-240	163.6 ± 55.5	147.1 ± 57.2	0.039
Citruline	8-47	27.9 ± 9.9	27.8 ± 10.2	0.950
Arginine	40-160	102.7 ± 34.5	94.6 ± 33.1	0.093
Taurine	19-216	125.8 ± 59.1	94.9 ± 48.2	<0.001
Tryptophane	15-73	37.9 ± 16.1	42.2 ± 11.6	0.028
Tyrosine	30-120	70 ± 21.6	72.4 ± 21.5	0.418
α-aminobutyric Acid	6-38	19.3 ± 7.8	23.5 ± 7.7	<0.001
Alanine	240-600	437.7 ± 93.6	410.1 ± 86.8	0.032
Methionine	6-49	20.4 ± 8.3	30.6 ± 11.3	<0.001
Valine	140-350	235 ± 57.9	223.5 ± 57.4	0.161
Phenylalanine	48-109	70.9 ± 15.6	78 ± 18.6	0.004
Iso Leucine	30-130	73.4 ± 21.4	82.6 ± 20.4	0.002
Leucine	60-230	153.7 ± 48.3	132.9 ± 51.6	0.004
Ornithine	20-135	77.4 ± 30.7	101.3 ± 21.6	<0.001
Lysine	80-250	165.4 ± 49.8	164.8 ± 47.8	0.926
IBD: Inflammatory bowel disease' * Independent sample t test.				

Table-3: Comparison of the qualitative results of serum amino acid chromatography between two groups

Amino acid status	IBD, (n=100)			Control, (n=100)			P value*
	Low	Normal	High	Low	Normal	High	
Aspartic acid	-	100 (100%)	-	-	100 (100%)	-	-
Glutamic acid	-	99 (99%)	1 (1%)	-	98 (98%)	2 (2%)	0.561
Asparagine	-	100 (100%)	-	-	100 (100%)	-	-
Serine	-	100 (100%)	-	-	99 (99%)	1 (1%)	0.316
Glutamine	8 (8%)	92 (92%)	-	4 (4%)	96 (96%)	-	0.234
Histidine	5 (5%)	93 (93%)	2 (2%)	-	100 (100%)	-	0.027
Glycine	-	100 (100%)	-	1 (1%)	99 (99%)	-	0.316
Threonine	-	100 (100%)	-	-	99 (99%)	1 (1%)	0.316
Citruline	-	100 (100%)	-	-	100 (100%)	-	-
Arginine	-	99 (99%)	1 (1%)	-	99 (99%)	1 (1%)	1.000
Taurine	-	100 (100%)	-	-	100 (100%)	-	-
Tryptophane	6 (6%)	94 (94%)	-	-	100 (100%)	-	0.013
Tyrosine	-	100 (100%)	-	1 (1%)	99 (99%)	-	0.316
α-aminobutyric Acid	-	100 (100%)	-	-	100 (100%)	-	-
Alanine	-	100 (100%)	-	-	100 (100%)	-	-
Methionine	-	100 (100%)	-	-	100 (100%)	-	-
Valine	2 (2%)	98 (98%)	-	1 (1%)	98 (98%)	1 (1%)	0.513
Phenylalanine	-	100 (100%)	-	-	100 (100%)	-	-
Iso Leucine	1 (1%)	99 (99%)	-	-	99 (99%)	1 (1%)	0.368
Leucine	-	100 (100%)	-	-	99 (99%)	1 (1%)	0.316
Ornithine	-	100 (100%)	-	-	100 (100%)	-	-
Lysine	-	100 (100%)	-	1 (1%)	99 (99%)	-	0.316

IBD: Inflammatory bowel disease; * Chi square test.

Table-4: Comparison of the results of serum histidine and tryptophan chromatography between the two groups by different characteristics of children under the study

Variables		Histidine			Tryptophan		
		Normal	Abnormal	P value*	Normal	Abnormal	P value*
Gender	Girl	40 (95%)	2 (5%)	0.455	41 (98%)	1 (2%)	0.195
	Boy	53 (91%)	5 (9%)		53 (91%)	5 (9%)	
Age group, year	<2	3 (100%)	0 (0%)	0.520	3 (100%)	0 (0%)	0.151
	2-6	10 (100%)	0 (0%)		10 (100%)	0 (0%)	
	6-10	54 (90%)	6 (10%)		58 (97%)	2 (3%)	
	10-17	26 (96%)	1 (4%)		23 (85%)	4 (15%)	
IBD type	Crohn's disease	17 (94%)	1 (6%)	0.791	16 (89%)	2 (11%)	0.313
	Ulcerative colitis	76 (93%)	6 (7%)		78 (95%)	4 (5%)	
IBD status	Active	49 (91%)	5 (9%)	0.337	48 (89%)	6 (11%)	0.020
	Remission	44 (96%)	2 (4%)		46 (100%)	0 (0%)	

IBD: Inflammatory bowel disease; * Chi square test.

4- DISCUSSION

The aim of this study was comparison of the status of serum amino acid chromatography in children with IBD with healthy children. The findings of this study, which compared the two groups of children with IBD and control group, showed that serum amino acid chromatography had a significant quantitative difference between the two groups. However, taking into account the normal levels of amino acids, only the two amino acids of histidine and tryptophan were significantly different in the IBD group, as compared with the control group.

In the control group, both of the amino acids had a normal serum chromatography; while in the IBD group, the histamine acidwas higher than the normal level in two patients and lower than the normal level in five patients; in addition, the tryptophan amino acid was lower than the normal level in six patients. In total, 15% of children had abnormal amino acid chromatography, which was significantly higher in the IBD group. Although serum histidine chromatography did not show a significant difference between the two phases of active disease and in remission, all the cases of abnormal serum tryptophan chromatography were observed in patients with active IBD, which was significantly higher. Both types of IBD cause changes in the metabolism of amino acids, since some studies have reported an increase in serum levels of isoleucine, methionine, lysine, glycine, arginine, and proline and a decrease in serum levels of valine, tyrosine, and serine (23).

Other studies have also reported changes in the serum levels of some other amino acids, such as histidine and homocysteine (19, 21). Kolho et al. reported that serum metabolite profiles in pediatric IBD patients, especially newly diagnosed, are different from healthy children (25). In addition, the use of some diets containing amino acids is reported to be effective in improving the condition of these patients (27, 28). Our study also showed that the serum level of amino acids of aspartic acid, threonine, taurine, alanine, and leucine were significantly higher in patients with IBD while the serum levels of other amino acids including amino glutamine, histidine, tryptophan, α-aminobutyric acid, methionine, phenylalanine, isoleucine, and ornithine were significantly lower in patients with IBD than in the control group. However, considering the normal serum levels of amino acids, only the levels of two amino acids of histidine and tryptophan were significantly different between the two groups. In addition, the amino acid of tryptophan was significantly lower in patients with IBD in the active phase. Hisamatsu et al. showed that the plasma concentrations of histidine and tryptophan in adults with IBD were lower than that in healthy subjects, and in patients with active disease were lower than that in patients at the recovery stage (4). In another study in 2015, they also noted that the low level of histidine in adults with ulcerative colitis can be used as a predictor of relapse (21). According to Xin et al. the metabolic profile of amino acids in adults with ulcerative colitis is different from that in healthy people (24).

Ooi et al., compared the aminograms of IBD patients and the healthy group and have shown differences between the two groups which can reflect the nutritional status, inflammation, and pathogenesis of the disease. In their study, the plasma levels of histidine and tryptophan were specifically decreased in patients with IBD and had an inverse relationship with serum CRP and disease activity (5). In a study by Nikolaus et al., the level of tryptophan was significantly lower in adult patients with IBD than in the control group and there was a significant relationship between tryptophan serum levels and disease activity (22). Therefore, it is suggested that

the reduction of these two amino acids can be indicative of chronic inflammatory conditions; thus, the therapeutic use of them may be considered as a new strategy for the treatment of the patients. Studies have also shown that tryptophan metabolism can be used as an immunological regulator (29-31). Although our study confirms some of the results of other previous studies, almost all of the abovementioned studies, except Kolho et al. study (25), have been conducted in adults with IBD. Therefore, our study is different from the previous studies mainly because of the study population, i.e. the children with the disease. The prevalence of this diseases in more developed communities is different from that in developing countries, which might be attributed to the over consumption of saturated oils and their lifestyle; in this study we did not consider the effects of such factors affecting the nutritional status of the patients, and thus it is the main drawback of this study.

Therefore, considering the results of this study as well as other studies in this field, it is necessary to carry out further comprehensive multicenter studies with a larger sample size while taking into account the factors affecting the nutrition and intestinal health of patients; the results of such studies can help to determine whether the evaluation of the status of amino acids can be used as an indicator of diagnosis or follow-up of the treatment in the patients or not.

5- CONCLUSION

The findings of this study showed that the serum amino acid chromatography in children with IBD were slightly different from that in the control group. From a qualitative point of view, one-fifth of the children with IBD had abnormal chromatography. However, only the levels of two amino acids of histidine and tryptophan in the IBD group were lower than the normal level. Although serum histidine chromatography did not show a significant difference between the two phases of active disease and in remission, however, all the cases with abnormal serum tryptophan chromatography were observed in patients with active IBD, which was significantly higher.

6- CONFLICT OF INTEREST: None.

7- ACKNOWLEDGMENT

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