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Amino acid levels in children with celiac disease

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Abstract

Background & aim: **plasma amino acid levels may show differences in regard to physiological changes, diet and diseases. The aim of the study is to measure the amino acid levels in children with celiac disease and compare them with the controls.**

Material and methods: **sixty-two children with classic celiac disease and 62 age and sex matched healthy control were enrolled in this study. Plasma amino acid levels of the children were measured by using tandem mass spectrometry.**

Results: **celiac children had significant lower plasma levels of citrülline, glutamine and cystine than control ($p<0.05$). The alanine, asparagine, glutamic acid, hydroxyproline, isoleucine, leucine, phenylalanine, proline, serine, threonine and valine were significantly higher in celiac children than in controls ($p<0.05$). On the other hand there were no significant difference in levels of arginine, argininosuccinate, aspartic acid, glycine, homocysteine, hydroxylysine lysine, methionine, ornithine, tryptophan, tyrosine, histidine levels between celiac children and healthy controls ($p>0.05$).**

Conclusions: **this study indicated that plasma amino acid levels can be variable in the celiac disease. Further studies with a large number size are needed whether plasma amino acids assays help to reflect of the intestinal mucosal damage and for following compatibility of gluten free diet in the celiac patients.**

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Key words: *Amino acids*. *Citrulline*. *Glutamine*. *Cystine*. *Celiac disease*.

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NIVELES DE AMINOÁCIDOS EN NIÑOS CON ENFERMEDAD CELÍACA

Resumen

Antecedentes y objetivo: **los niveles de aminoácidos en plasma pueden mostrar diferencias en lo que se refiere a los cambios fisiológicos, la dieta y las enfermedades. El objetivo del estudio es medir los niveles de aminoácidos en los niños con enfermedad celíaca y compararlos con los controles.**

Material y métodos: **en este estudio se inscribieron 62 niños con enfermedad celíaca clásica emparejados por edad y sexo con 62 controles sanos. Los niveles de aminoácidos en plasma de los niños se midieron utilizando la espectrometría de masas.**

Resultados: **los niños celíacos tenían niveles significativamente inferiores plasmáticos de citrulina, glutamina y cistina que el grupo control ($p<0,05$). Alanina, asparagina, ácido glutámico, hidroxiprolina, isoleucina, leucina, fenilalanina, prolina, serina, treonina y valina fueron significativamente mayores en los niños celíacos que en los controles ($p<0,05$). Por otro lado, no hubo ninguna diferencia significativa en los niveles de arginina, argininosuccinato, ácido aspártico, glicina, homocisteína, lisina, hidroxilisina, metionina, ornitina, triptófano, tirosina, histidina entre los niños celíacos y los controles sanos ($p>0,05$).**

Conclusiones: **este estudio mostró que los niveles de aminoácidos en plasma pueden ser variables en la enfermedad celíaca. Se necesitan estudios con un tamaño mayor para conocer si los ensayos de aminoácidos en plasma ayudan a reflejar la lesión de la mucosa intestinal y para el seguimiento de la compatibilidad de la dieta libre de gluten en los pacientes celíacos.**

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Palabras clave: *Aminoácidos*. *Citrulina*. *Glutamina*. *Cistina*. *Enfermedad celíaca*.

Introduction

Celiac disease (CD) is a chronic inflammatory proximal small intestinal disease caused by permanent intolerance to gluten and gluten like proteins in wheat, barley and rye in the genetically predisposed persons[1]. CD is clinically atypical or silent in most patients. The incidence is between 0.3-1 % in different countries[2].

The disease is diagnosed with increased intra-epithelial lymphocytes, crypt hyperplasia and villous atrophy in the small intestinal biopsy. However, before a small intestinal biopsy is done, the detection of anti-gliadin antibodies (AGA), anti-tissue transglutaminase antibody (anti-dTG) and/or endomysium antibodies (EMA) is important as the first step of investigation[3,4]. After initiation of strict gluten-free diet (GFD), antibody levels are expected to decrease or even disappear. The lifelong gluten elimination from the diet is the only treatment modality available[5,6].

Recently, a small number of studies showed that there is a relationship between intestinal mucosal damage and plasma amino acid levels. Such studies have emphasized that plasma citrulline level might be a useful parameter for the clinical progress of CD as well as necrotizing enterocolitis and short bowel syndrome[7,8]. In this study, we measured whole plasma amino acid levels in a group celiac patients and compared them with the controls.

Material and methods

This study was performed at the Department of Pediatric Gastroenterology of Erciyes University Medical Faculty in Kayseri, Turkey, from October 2012 to December 2013. Sixty-two children with classic CD, aged 18 months-18 years, were included in the presented study. Sixty-two age and sex matched healthy children were included as the control group. The diagnosis of CD was based on the criteria of European Society for Paediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN)[2]. The patients with chronic disorders such as IgA deficiency, Turner sydrome, Diabetes Mellitus type 1, Down syndrome were excluded. The study was approved by the Erciyes University noninvasive clinical research ethics board and performed according to the Declaration of Helsinki.

Analysis of amino acids profile

After acceptance of informed consent, 2 cc morning fasting venous blood was obtained from all patients and controls into the ethylenediaminetetraacetic acid tubes (EDTA) to determine the plasma amino acid levels. Samples were centrifuged at 5000 rpm for 5 minutes using a Sigma centrifuge device. The plasma portion was separated in to eppendorf tubes and kept at -20 °C until the next step. Then, the samples were removed from the freezer for melting in room temperature. Further centrifugation was applied at 4000 rpm for 3 minutes. 200 μL was taken from the upper phase and nitrogen has been removed by using a blow up tube. 10 μL from the acquired sample was studied for plasma amino acids by the tandem mass spectrometry method using a zivak commercial amino acids liquid chromatography–mass spectrometry (LC-MS/MS) analysis kit.

Statistical analysis

Results are expressed as means±SD or median (range). Shapiro Wilk test was carried out to determine normality of data distribution. Shapiro-Wilk test revealed abnormal data distribution for values of alanine, argininosuccinate, aspartic acid, cystine, citrulline, glutamine, glutamic acid, glycine, homocysteine, hydroxylysine, hydroxyproline, lysine, methionine, tyrosine, and EMA ($p<0.05$). Because of abnormal data distribution of these values, median values (Interquartile range) were determined and compared with Mann-Whitney U test between groups. For age, height, and body weight values, total protein, albumin, asparagines, isoleucine, leucine, phenylalanine, proline, serine, threonine, and valine were determined and compared with independent t test because of normal data distribution between groups ($p>0.05$). Correlation analyses were evaluated with Spearman correlation test. A p value of less than 0.05 was considered significant.

Results

The mean age of 62 celiac patients (39 male, 63 %) and 62 controls (31 male, 50 %) were 9.46 ± 4.42 and 8.70 ± 3.98 years respectively. The mean follow-up was 3.94 ± 1.90 years in the celiac patients. There were no significant differences between two groups with respect to age, height, weight, body mass index, serum total protein or albumin ($p > 0.05$). Serum EMA level was negative in eight (12.9 %) of the celiac patients and was positive in 54 of them (87.1 %) while it was negative in all (100 %) control patients. Serum EMA level was significantly higher in patients with celiac patients ($p < 0.05$, Table I).

The average plasma citrulline (25 vs 58 μmol/l, $p<0.05$), glutamine (815 vs 986 μmol/l, $p<0.05$), cystine (20 vs 52.5 μmol/l, $p<0.05$), levels were significantly lower in patients with celiac disease than in the controls, while the average alanine (658.5 vs 49 μmol/l, $p<0.05$), asparagines (92.62 ± 28.35 vs 74.85 ± 27.26 μmol/l, $p<0.05$), glutamic acid (75 vs 58 μmol/l, $p<0.05$), hydroxyproline (24 vs 19.5 μmol/l, $p<0.05$), isoleucine (111.26 ± 38.15 vs 79.64 ± 28.16 μmol/l, $p<0.05$), leucine (185.16 ± 63.12 vs 146.06 ± 42.29 μmol/l, $p<0.05$), phenylalanine (101.39 ± 28.11 vs 90.88 ± 28.699 μmol/l, $p<0.05$), proline (429.35

Table I
Anthropometric characteristics in patients with celiac and control group

	Celiac patients N= 62	*Control group N=62*	*p value*
Age (yr)**	9.46 ± 4.42	8.70 ± 3.98	> 0.05
Body height (cm)**	127.95 ± 21,78	132.51 ± 19.90	> 0.05
Body weight (kg)**	28.72 ± 12.62	31.83 ± 11.85	> 0.05
Body mass index (kg/m2)**	16.67 ± 2.68	17.39 ± 2.27	> 0.05
Serum total protein (g/dl)**	7.06 ± 0.38	7.07 ± 0.35	> 0.05
Albumin (g/dl)**	3.90 ± 0.27	3.87 ± 0.33	> 0.05
EMA (RU/ml)*	119.35 (0-200)	0.00	< 0.05[a]

*Median (Interquartile range), **Mean ± Standard Deviation (SD), [a]Mann Whitney U test [b]Independent samples test.

± 148.95 vs 358.25 ± 140.25 μmol/l, p<0.05), serine (239.97 ± 66.05 vs 170.50 ± 41.75 μmol/l, p<0.05), threonine (188.22 ± 63.51 vs 143.90 ± 41.79 μmol/l, p<0.05), and valine (484.19 ± 164.54 vs 394.38 ± 127.61 μmol/l, p<0.05) were significantly higher in celiac children than in controls (Table II).

The average arginine (67 vs 75.5 μmol/l, p>0.05), argininosuccinate (0.7 vs 0.6 μmol/l, p>0.05), aspartic acid (35 vs 26 μmol/l, p>0.05), glycine (325.5 vs 320.5 μmol/l, p>0.05), homocysteine (0.19 vs 0.14 μmol/l, p>0.05), hydroxylysine (0.14 vs 0.2 μmol/l, p>0.05), lysine (98.5 vs 100 μmol/l, p>0.05), methionine (35 vs 30 μmol/l, p>0.05), ornithine (0.5 vs 69.17 μmol/l, p>0.05), tryptophan (65.68 ± 20.36 vs 64.58 ± 19.03 μmol/l, p>0.05), tyrosine (50 vs 62.5 μmol/l, p>0.05), histidine (64 vs 63.5 μmol/l, p>0.05) levels were not significantly different between celiac children and healthy controls (Table II).

Correlation analysis between the EMA levels and plasma amino acids levels of patients with CD have revealed no significant relation (p>0.05). Plasma citrülline concentration was positively correlated to glutamine (r=0.357, p=0.04) in patient with CD (Figure 1).

Discussion

In this study we have investigated plasma amino acid levels in patients with CD compared to age and sex matched controls. It has not been reported the measuring of whole plasma amino acid levels in celiac patients so far, thus, we compared our results with a small number of the previously reported studies in the literature.

We observed lower plasma levels of citrulline, glutamine and cystine in the celiac patients than in the control. Citrulline is a non protein amino acid produced by enterocytes from conversion of glutamine[9].The recent studies showed a relation between the level of plasma citrülline and mucosal damage in the celiac patients. This study also showed that patients with CD had decreased citrülline levels (p<0.05). This result is to similar the literature. Ioannou *et al.*[7], observed that the mean plasma citrulline level of children with CD (24.5 μmol/l) was statistically lower than those of the controls (32.5 μmol/l). Furthermore Hozyasz *et al.*[10], conducted a study with 61 celiac children. They reported significantly lower citrulline levels in untreated celiac children (24.9 μmol/l) compared to celiac patients on a GFD (32.2 μmol/l). More recently Miceli *et al.*[11] reported that the mean plasma citrulline levels in the utreated celiac children (12.4 μmol/l) was statistically lower than in the controls (24.7 μmol/l). These results support plasma citrulline concentration is reliable marker of villous atrophy in the celiac patients.

Glutamine is a nonessential amino acid and preferential substrate for enterocytes[12]. Many researchers have investigated the effect of glutamine on intestinal mucosal demage. Eden hold *et al.*[13], showed that glutamine decreases the permeability changes caused by indomethacin. More recently Sun j *et al.*[14], determined glutamine could reduce the duration of diarrhea. In this study, plasma glutamine levels of the patients with CD were significantly lower in contrast to the controls (p<0.05). These results were in agreement with Blasco Alonso *et al.*[15], who found that celiac children had significantly lower plasma glutamine levels. We think that the low concentration of plasma glutamine in CD

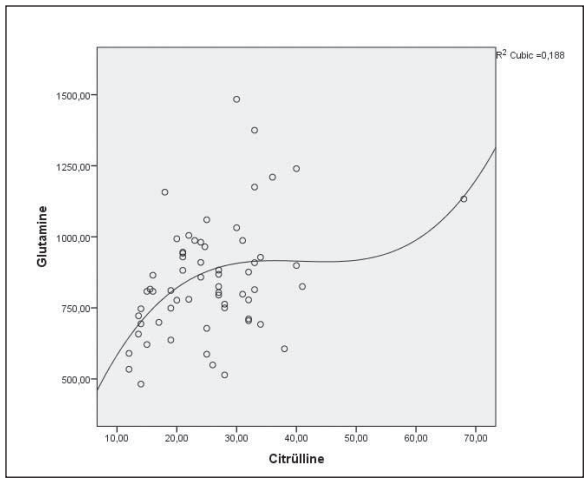

Fig. 1.—Plasma citrülline concentration was positively correlated with glutamine (r=0.357, p=0.04) in patient with CD.

Table II
Mean plasma amino acids levels in patients with celiac and control group

Amino acids (µmol/l)	*Celiac patients*	*Control group*	*p value*
Alanine *	658.5 (294.00 - 1430.00)	497 (200 - 995)	<0.05[a]
Arginine*	67 (13 - 187)	75.5 (25 - 150)	>0.05[a]
Argininosuccinate*	0.7 (0.01 - 16)	0.6 (0.2 - 14)	>0.05[a]
Asparagine**	92.62±28.35	74.85 ± 27.26	< 0.05[b]
Aspartic acid*	35 (11 - 132)	26 (2 - 497)	>0.05[a]
Cystine*	20 (9 - 69.9)	52.5 (21 - 250)	<0.05[a]
Citrulline*	25 (12 - 68)	38.5 (9 - 221)	<0.05[a]
Glutamine*	815 (482 - 1484)	986 (515 - 1896)	<0.05[a]
Glutamic acid*	75 (25 - 250)	58 (32 - 136)	<0.05[a]
Glycine*	325.5 (176 - 687)	320.5 (155 - 454)	>0.05[a]
Homocysteine*	0.19 (0.01 - 0.9)	0.14(0.01 - 0.6)	>0.05[a]
Hydroxylysine*	0.14 (0.01 - 0.89)	0.2 (0.1 - 0.7)	>0.05[a]
Hydroxyproline*	24 (11 - 85)	19.5 (6 - 68)	<0.05[a]
İsoleucine**	111.26 ± 38.15	79.64 ± 28.16	< 0.05[b]
Leucine**	185.16 ± 63.12	146.06 ± 42.29	< 0.05[b]
Lysine*	98.5 (41 - 358)	100 (30 - 418)	>0.05[a]
Methionine*	35 (11 - 86)	30 (14 - 127)	>0.05[a]
Ornithine**	70.50 ± 29.28	69.17 ± 26.83	>0.05[b]
Phenylalanine**	101.39 ± 28.11	90.88 ± 28.69	<0.05[b]
Proline **	429.35 ± 148.95	358.25 ± 140.25	<0.05[b]
Serine**	239.97 ± 66.05	170.50 ± 41.75	< 0.05[b]
Threonine**	188.22 ± 63.51	143.90 ± 41.79	< 0.05[b]
Tryptophan**	65.68 ± 20.36	64.58 ± 19.03	> 0.05[b]
Tyrosine*	50 (20 - 169)	62.5 (19 - 212)	>0.05[a]
Valine**	484.19 ± 164.54	394.38 ± 127.61	< 0.05[b]
Histidine*	64 (26 - 150)	63.5 (28 - 122)	>0.05[a]

*Median (Interquartile range), **Mean ± Standard Deviation (SD), [a]Mann Whitney U test, [b]Independent samples test.

might be a marker of intestinal mucosal injury and needs further studies

In the present study there was a significant positive correlation between citrülline and glutamine in the celiac patients (r=0.357, p=0.04). This result supports Marini *et al*.[16], have found that glutamine was a precursor for citrülline synthesis.

Our findings indicated that the decreased plasma citrülline and glutamine levels in patients with CD was consistent with Blasco Alonso *et al*.[15], who detected lower concentrations of citrülline and glutamine in the children with celiac disease. We believe that the low concentration of glutamine could negatively affect citrülline biosynthesis in intestinal mucosa.

Compliance to GFD varies from 45% to 81% in the celiac children, as reported by the North American Society of Pediatric Gastroenterology Hepatology, and Nutrition[1]. Ig A type of antitissue transglutaminase-2, antiendomysium (EMA), and antideaminated forms of gliadin peptide antibodies are widely used as follow-up of the celiac patients[17]. Ozgenç *et al*.[18], have also reported that EMA positivity alone was significantly related to severe mucosal damage in the celiac patients. In present study, the serum EMA level was positive in (87.1 %) of patients with CD. This high level of EMA could be due to low compliance of GFD by our patients. Also, there was a no significant correlation between EMA and amino acids in patients with CD. However, we share belief that a low plasma, citrulline, glutamine and cystine levels which is associated with high serum EMA level in CD could be reflected to villous atrophy or impaired absorption.

Proline and hydroxyproline are a non-essential amino acid found in structures of prolamines. Prolamines are responsible for the gluten toxicity in celiac patients[19]. In this study plasma proline and hydroxyproline levels were significantly higher in patients with CD than in controls ($p<0.05$). We think that measuring plasma proline, hydroxyproline may help to show gluten toxicity in the celiac patients.

In this study we found various amino acid levels between patients with CD and control group. Our results were in agreement Sılk *et al*.[20], have shown that there is considerable variation in the ability of individual patients with adult CD to absorb the amino acids.

The essential amino acids concentrations are easily affected with diet and protein intake[21]. In the present study we determined the some essential amino acids as valine, isoleucine, leucine, threonine, and phenylalanine were significantly increased in the celiac patients than in the controls. These differences may be due to children's dietary habits. On the other hand there were no significant difference in levels of arginine, argininosuccinate, aspartic acid, glycine, homocysteine, hydroxylysine lysine, methionine, ornithine, tryptophan, tyrosine, histidine levels between celiac children and healthy controls. Our findings showed that these amino acids might not be affected absorption or transport in intestinal mucosa in CD.

In conclusion, so far few researchers have focused on the levels of some amino acids in celiac disease. In this study underlined that there were some variation of plasma amino acid levels between celiac patients and healthy controls. Large number size further studies are needed whether plasma amino acids assays help to reflect of the intestinal mucosal damage and for following compatibility of gluten free diet in the celiac patients.

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