

## Original Article

# Plasma glutamine and cystine are decreased and negatively correlated with endomysial antibody in children with celiac disease

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**Background and Objectives:** Glutamine is a nonessential amino acid which improves intestinal mucosal regeneration and absorption. Glutathione is a vital molecule for antioxidant reactions and is synthesized from cystine. The first aim of the study is to measure the plasma glutamine and cystine in children with celiac disease (CD) and compare them with controls. The second aim of this study is to investigate whether these amino acids are correlated with endomysial antibody (EMA) or not. **Methods and Study Design:** Fifty children with CD were compared to 50 healthy, age, and sex matched normal children as control. Plasma glutamine and cystine levels of the children were measured by using tandem mass spectrometry. **Results:** Plasma glutamine (808 vs 870  $\mu\text{mol/L}$ ) and cystine (19 vs 48.5  $\mu\text{mol/L}$ ) were significantly lower in the celiac group than the controls ( $p < 0.05$ ). The levels of plasma glutamine (797 vs 928  $\mu\text{mol/L}$ ,  $n=42$ ) and cystine (18 vs 31.5  $\mu\text{mol/L}$ ,  $n=8$ ) were lower ( $p < 0.05$ ) in the EMA-positive than the EMA-negative celiac patients. We could not find any statistically significance between EMA-negative celiac patients and controls for the plasma glutamine (928 vs 870  $\mu\text{mol/L}$ ) and cystine (31.5 vs 48.5  $\mu\text{mol/L}$ ) ( $p > 0.05$ ). Serum EMA was negatively correlated with plasma cystine ( $r = -0.321$ ,  $p = 0.023$ ), glutamine ( $r = -0.413$ ,  $p = 0.003$ ). **Conclusions:** Our study indicated that plasma glutamine and cystine were significantly lower in the celiac children than the controls. Also, these amino acids were negatively correlated with EMA.

**Key Words:** cystine, glutamine, celiac disease, endomysial antibody

## INTRODUCTION

Celiac disease (CD), which also known as gluten-sensitive enteropathy, is a chronic inflammatory proximal small intestinal disease that leads to gliadin intolerance. The incidence of CD is between 0.3-1% worldwide.<sup>1</sup> CD is classified as follows; classic, atypical, silent, and latent. The diagnosis is based on duodenal mucosal biopsy. In these patients, lifelong gluten free diet (GFD) is currently the only effective treatment option. Endomysial antibody (EMA) is commonly used evaluate response to GFD in the celiac patients. Strict compliance with a GFD is important for prognosis.<sup>2</sup>

Glutamine is a nonessential amino acid which preferential substrate by enterocytes. The effect of glutamine on intestinal barrier functions has been investigated so far.<sup>3,4</sup> Hond et al<sup>5</sup> reported that glutamine can reduce indomethacin induced permeability changes and intestinal mucosal cell apoptosis. Furthermore, some researchers report that glutamine can help protect the gut mucosal lining in patients with Crohn's disease. Also, some previously re-

ported studies emphasize that glutamine could have a protective effect on the gut.<sup>6,7</sup>

Cystine is a nonessential amino acid which consists of two cysteine molecules. It is also required to the biosynthesis of glutathione (GSH) that acts as an antioxidant against oxidative stress. Cystine and cysteine are the major rate limiting factors in GSH biosynthesis and vital for the maintenance of circulating and tissue levels of GSH.<sup>8,9</sup> Some researchers report lower plasma and intestine GSH levels in celiac children than the controls.<sup>9,10</sup> In this study, we investigated plasma glutamine and cystine in celiac patients and compared them with the controls.

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To our knowledge, this study is the first report of plasma cystine in celiac patients.

## SUBJECTS AND METHODS

### Study population

This study was performed at the Department of Pediatric Gastroenterology, Erciyes University Medical Faculty, in Kayseri, Turkey between October 2012 and December 2013. Fifty children with classic CD, aged 5-15 years, were included in the presented study. Fifty age and sex matched healthy children were included as control group. The diagnosis of CD was based on the criteria of European Society for Pediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN).<sup>11</sup> The patients with chronic disorders such as IgA deficiency, diabetes mellitus type 1, Down syndrome were excluded in this study. The study was approved by the Erciyes University Non-invasive Clinical Research Ethics Board and performed according to the Declaration of Helsinki. Informed consent was obtained from the all participants.

### Amino acid profile

The morning fasting venous blood (2 cc) with EDTA tubes was obtained from the all participants to determine plasma amino acid concentrations. 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, samples were removed from the freezer for melting in room temperature. Further centrifugation was applied at 4000 rpm for 3 minutes. Two hundred µL was taken from the upper phase and nitrogen has been removed by using a blow up tube. Ten µL from the acquired sample was studied for plasma amino acids by the liquid chromatography-tandem mass spectrometry (LC-MS/MS).

### Statistical analysis

Results are expressed as mean ± SD or median. Shapiro Wilk test was carried out to determine normality of data distribution. Shapiro-Wilk test revealed abnormal data distribution for values of cystine, glutamine, serum total

protein, albumin 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, weight values, and body mass index 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 the 50 celiac patients (35 women, 70 %) and 50 controls (30 men, 60%) were  $9.5 \pm 3.57$  and  $9.46 \pm 3.47$  years respectively. The mean follow-up was  $3.94 \pm 1.90$  years in the celiac patients. There were no statistically significance between two groups with respect to age, height, weight, body mass index ( $p > 0.05$ ) (Table 1). Serum EMA was significantly higher in the celiac patients than the controls ( $p < 0.05$ ) (Table 2). The average plasma glutamine ( $808$  vs  $870$  µmol/L) and cystine ( $19$  vs  $48.5$  µmol/L) were significantly lower in the celiac patients than the controls ( $p < 0.05$ ) (Table 2). Plasma glutamine ( $797$  vs  $928$  µmol/L,  $n=42$ ) and cystine ( $18$  vs  $31.5$  µmol/L,  $n=8$ ) were lower ( $p < 0.05$ ) in the EMA-positive than the EMA-negative celiac patients ( $p < 0.05$ ) (Table 3). There were no statistically significance between EMA-negative celiac patients and controls for plasma glutamine ( $928$  vs  $870$  µmol/L) and cystine ( $31.5$  vs  $48.5$  µmol/L) ( $p > 0.05$ ). Also, plasma cystine was positively correlated with glutamine ( $r=0.434$ ,  $p=0.000$ ) in the celiac patients (Figure 1). In addition to these data, serum EMA was negatively correlated with plasma cystine ( $r=-0.321$ ,  $p=0.023$ ), glutamine ( $r=-0.413$ ,  $p=0.003$ ), serum total protein ( $r=-0.470$ ,  $p=0.001$ ) and albumin ( $r=-0.371$ ,  $p=0.008$ ) in the celiac patients (Table 4).

## DISCUSSION

In the present study we found that the celiac children had lower plasma cystine and glutamine than the controls. No studies have been reported to evaluate plasma cystine levels in the celiac children so far. In this study, we com-

**Table 1.** Patients characteristics.

	Celiac patients (n=50)	Control group (n=50)	<i>p</i> value*
Age (yr) <sup>†</sup>	9.5±3.57	9.46±3.47	>0.05
Body height (cm) <sup>†</sup>	126±17.6	135±17.4	>0.05
Body weight (kg) <sup>†</sup>	26.9±12.6	31.8±11.9	>0.05
BMI (kg/m <sup>2</sup> ) <sup>†</sup>	14.7±2.38	15.4±2.37	>0.05

<sup>†</sup>Mean±standard deviation (SD).

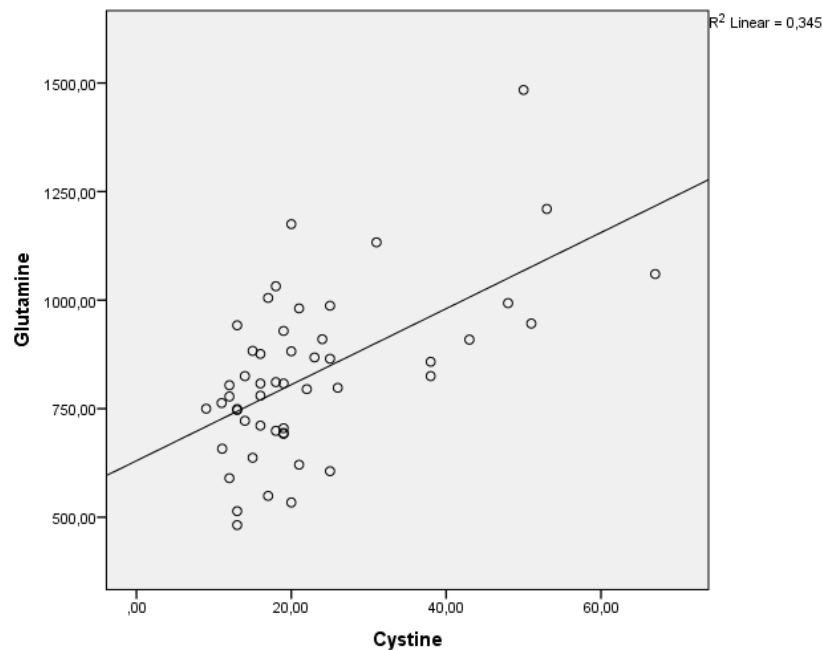
\*Independent samples *t* test.

**Table 2.** The levels of plasma glutamine and cystine in celiac patients and controls

	Celiac patients	Control group	<i>p</i> value*
Cystine (µmol/L) <sup>†</sup>	19 (9-67)	48.5 (21-250)	<0.05
Glutamine (µmol/L) <sup>†</sup>	808 (482-1484)	870 (515-1896)	<0.05
Serum total protein (g/dL) <sup>†</sup>	7 (6.3-8)	7 (6.3-8.4)	>0.05
Albumin (g/dL) <sup>†</sup>	4 (3.3-4.6)	4 (3-4.8)	>0.05
EMA (RU/mL) <sup>†</sup>	120 (0-200)	0.00	<0.05

<sup>†</sup>Median (P<sub>25</sub>-P<sub>75</sub>).

\*Mann Whitney U test.



**Figure 1.** Positive correlation of plasma cystine with glutamine in the celiac patients

pared our results with previously published a small number studies in the medical literature.

Oxidative stress is an important factor in the pathogenesis of CD. Gliadin leads to increased levels of free radicals such as reactive oxygen species in the gut.<sup>10</sup> Stojiljkovic et al<sup>12</sup> observed that the antioxidant enzyme glutathione peroxidase and reductase activities were decreased in biopsies of celiac patients with consequent decreased GSH activity. It is known that GSH synthesis is limited by sulfur-containing amino acid such as cystine and cysteine.<sup>9</sup> Kurihara et al<sup>13</sup> reported that adding cystine and threonine to the diet suppresses the decrease in the GSH level due to illness. The results of previous studies suggest that the plasma cystine positively affect the levels of GSH.<sup>12,13</sup> In this study, we observed lower plasma cystine in the celiac patients than the controls. But, we did not measure GSH in both groups. However, with this study, it was shown that lower plasma cystine might be a supportive marker for oxidative damage in the celiac children.

As mentioned in the introduction, glutamine improves intestinal mucosal regeneration and absorption. It has effects on reducing intestinal mucosal cell apoptosis and increasing enterocyte proliferation.<sup>14</sup> Sun et al<sup>15</sup> reported that glutamine could reduce the duration of diarrhea. Also, Swaid et al<sup>16</sup> reported a study in rats with damaged intestinal mucosa and detected a rapid recovery on intestinal mucosal injury after glutamine-rich diet. In this study, the levels of plasma glutamine were significantly lower in the celiac children than the controls ( $p < 0.05$ ). These results were similar to the results of Blasco Alonso et al<sup>17</sup> who observed that children with CD had significantly lower plasma glutamine levels. These results indicate that lower plasma glutamine might be a biomarker for intestinal mucosal injury in the celiac patients.

GFD means a strict and lifelong elimination of gluten in celiac patients.<sup>18</sup> EMA and Ig A type of anti-tissue transglutaminase-2 antibody are commonly used to eval-

uate response to GFD in celiac patients.<sup>19</sup> Ozgenc et al<sup>20</sup> also reported that EMA positivity alone was significantly related to villous atrophy in celiac patients. Kurppa et al<sup>21</sup> also underlined that EMA positivity was strongly related to severe mucosal damage in patients with CD. In the present study, the levels of serum EMA were positive in 84% of the celiac patients. The high EMA could be due to low compliance with GFD in our patients. In the presented study we found a significant negative correlation between EMA and cystine or glutamine. In addition we observed that plasma cystine was positively correlated with glutamine in the celiac children. The lower plasma glutamine and cystine as well as elevated serum EMA might be markers not only of lack of compliance with GFD, but also of oxidative damage and enterocyte loss in children with CD.

Our study has some limitations. Firstly, with a limited number of studies of plasma amino acids in celiac disease, our results can only be compared with limited data. Secondly, and most importantly, we could not measure plasma cystine and glutamine before and after GFD. However, there was no statistically significant difference between EMA-negative celiac patients and controls for plasma glutamine and cystine levels. On the other hand, plasma glutamine and cystine were significantly different between EMA-positive celiac patients and controls. Thus plasma cystine and glutamine levels might be affected by the GFD used in CD.

In conclusion, plasma glutamine and cystine were lower in celiac children than in their controls. Furthermore, these amino acids were negatively correlated with EMA. Further studies with larger numbers of participants are needed to show whether plasma cystine and glutamine reflect intestinal oxidative damage and for the assessment of GFD in celiac patients.

#### **AUTHOR DISCLOSURES**

The authors declare that there is no conflict of interest.

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## Original Article

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### 腹腔疾病儿童血浆谷氨酰胺和胱氨酸水平下降与肌内 膜抗体有关

**背景与目的：**谷氨酰胺是一种非必需氨基酸，具有提高肠黏膜再生和吸收的功能。谷胱甘肽是人体一种具有抗氧化的重要分子，由胱氨酸合成。该研究的主要目的是检测腹腔疾病（CD）患儿血浆谷氨酰胺和胱氨酸水平，并与对照组比较。其次是探讨这些氨基酸与肌内膜抗体（EMA）是否相关。**方法与研究设计：**选择 50 名 CD 患儿，另选年龄和性别相匹配的 50 名健康儿童作为对照。采用串联质谱法测定儿童血浆谷氨酰胺和胱氨酸的水平。**结果：**CD 患儿血浆谷氨酰胺（808 vs 870  $\mu\text{mol/L}$ ）和胱氨酸水平（19 vs 48.5  $\mu\text{mol/L}$ ）显著低于对照组儿童（ $p < 0.05$ ，EMA 阳性的患儿血浆谷氨酰胺（797 vs 928  $\mu\text{mol/L}$ ,  $n=42$ ）和胱氨酸水平（18 vs 31.5  $\mu\text{mol/L}$ ,  $n=8$ ）显著低于 EMA 阴性的 CD 患儿。我们未发现 EMA 阴性的 CD 患儿和健康对照组儿童血浆谷氨酰胺（928 vs 870  $\mu\text{mol/L}$ ）及胱氨酸水平（31.5 vs 48.5  $\mu\text{mol/L}$ ）之间存在差异（ $p > 0.05$ ）。血清 EMA 与血浆胱氨酸水平（ $r = -0.321$ ,  $p = 0.023$ ）和谷氨酰胺（ $r = -0.413$ ,  $p = 0.003$ ）呈显著负相关。**结论：**该研究表明，CD 患儿血浆谷氨酰胺和胱氨酸含量明显低于健康对照组。另外，这些氨基酸的含量与 EMA 水平呈显著负相关。

**关键词：**胱氨酸、谷氨酰胺、腹腔疾病、肌内膜抗体