Total oxidant status, total antioxidant capacity and ischemia modified albumin levels in children with celiac disease

Ersin Sayar[1], Sebahat Özdem[2], Gülbahar Uzun[2], Ali İşlek[3], Aygen Yılmaz[3], Reha Artan[3]

[1]Department of Pediatric Gastroenterology, Konya Training and Research Hospital, Konya, [2]Department of Biochemistry and [3]Division of Pediatric Gastroenterology, Department of Pediatrics, Akdeniz University Faculty of Medicine, Antalya, Turkey. E-mail: ersinsayar@hotmail.com

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In our study, we aimed to investigate ischemia modified albumin (IMA) as an oxidative stress marker, as well as other oxidant and antioxidant markers that have not been evaluated in children with celiac disease. A total of 37 pediatric patients who were diagnosed with celiac disease (CD) and 29 healthy children were enrolled in this prospective study. We evaluated the IMA, total oxidant status, total antioxidant capacity, sulfhydryl, and advanced oxidation protein products in all of the subjects. We also compared the levels at the time of the diagnosis, and following a gluten-free diet (GFD) in the children with CD. While the IMA and the other oxidant marker levels were significantly higher in the patient group compared to the control group, the antioxidant marker levels were found to be significantly lower in the patient group, compared to the control group. We also determined that the tissue transglutaminase IgA showed a highly positive correlation, and that the IMA showed a moderately positive correlation with the Marsh-Oberhuber histopathological stage. Additionally, the IMA and other oxidant marker levels were significantly lower, while the antioxidant marker levels were significantly higher after the GFD, compared to the pre-diet period. We detected that oxidative stress played a role in the pathogenesis of CD, and that this could be evaluated using oxidative stress markers, which would regress after the GFD. We also detected that IMA is a marker that shows a correlation with the histopathological stage, and may be used in the diagnosis.

Key words: ischemia modified albumin, children, celiac disease, oxidative stress.

Celiac disease (CD) is an autoimmune inflammatory disease of the small bowel, characterized by the complex interactions of genetic and environmental factors. Gluten is the key environmental factor leading to intestinal epithelial injury in genetically susceptible individuals[1], which develops via two mechanisms: toxic and immunogenic. Gluten is converted to immunogenic and toxic peptides through proteolysis; the latter leads to oxidative stress in enterocytes, resulting in apoptosis, the breakdown of cell differentiation, and disruptions in the tight junctions[2].

Ischemia, hypoxia, acidosis, and oxidative stress may alter the structure of the terminal amino side of serum albumin, which binds metals like cobalt, copper, and nickel. This albumin with a low capacity for metal binding is defined as "ischemia modified albumin" (IMA)[3,4]. IMA is generally accepted as a reliable oxidative stress marker, and has been previously studied in different diseases related to oxidative stress, such as ischemic heart disease, diabetes mellitus, hyperlipidemia, chronic renal failure, obesity, and thalassemia[5-10].

In our study, we aimed to investigate IMA in children with CD, at the time of diagnosis and after a gluten-free diet (GFD). We also aimed to investigate the levels of oxidant and antioxidant markers [total oxidant status (TOS), advanced oxidation protein products (AOPP), total antioxidant capacity (TAC), and

sulfhydryl (SH)] which were not previously studied in CD (like IMA).

Materials and Methods

A prospective study was planned at the Pediatric Gastroenterology Outpatient Clinic of the Akdeniz University School of Medicine, from March, 2011 through March, 2013. Ethics committee approval was obtained from the local ethics committee of Akdeniz University. Serological screening [tissue transglutaminase (tTG) IgA, IgG, and total IgA] was done for the patients who were admitted with both typical and atypical signs and symptoms of CD, and those who had diseases in which the CD prevalence is relatively high. The patients who had chronic diseases which are known to exhibit the pathogenesis of oxidative stress and alter the IMA levels (chronic cardiovascular diseases, chronic renal failure, diabetes mellitus, obesity, and thalassemia major) were excluded from the study.

Upper gastrointestinal endoscopies were performed to the subjects whose tTG antibodies were detected to be positive and at least three duodenal biopsies were obtained. The diagnosis of CD was made according to the diagnostic criteria of the European Society for Pediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN), and all the patients were put on a gluten-free diet after the diagnosis[11]. The serum samples of the 37 patients were obtained at the time of the diagnosis, and in the third month on the GFD. Control group included 29 healthy children, whose serological screening detected to be negative and who had no history of any chronic disease. The serum samples from the control group were also obtained. All the samples were stored at -80 ^{o}C, and the levels of IMA, TAC, TOS, AOPP, and SH were measured. Written informed consent was obtained from the patients and parents for all of these procedures.

The serum IMA level was measured using an albumin-cobalt binding test in the serum samples obtained in plain jelly biochemistry tubes. For the serum IMA measurement, 95 μL of patient serum was mixed with 5 μL of cobalt chloride, and incubated for 5 min[12]. The cobalt chloride concentration was adjusted to 0.58 mmol/L during incubation. A very small part of cobalt is known to bind to albumin in the presence of ischemia and oxidative stress[12]. In order to determine that the cobalt was not bound to the albumin, 25 μL of dithiothreitol (final concentration: 1.67 mmol/L) was added to the measurement power after incubation, and the dithiothreitol formed a colored complex with the cobalt not bound to the albumin. The formed colored complex was measured spectrophotometrically at a 500 nm wavelength. After plotting a five-point calibration curve, the absorbance values were evaluated in the 5-180 U/ml range in the calibration curve[12,13].

The level of TAC developing against the potent free radicals was measured using a method developed by Erel[14]. The TOS was measured with the automatic calorimetric method, using another method developed by Erel[15]. The total sulfhydryl groups (SH) were measured spectrophotometrically using Ellman reactive 5,5'-ditiobis-2-nitrobenzoic acid[16], and the level of AOPP was measured spectrophotometrically using the Witko-Sarsat method[17].

A statistical analysis was done using SPSS software version 16.0. The demographic characteristics of the patients and the controls were recorded, and the complaints of the patients, tTG levels, and histopathological stages of the duodenal biopsies were transferred to the SPSS program. The levels of IMA, TOS, TAC, AOPP, and SH were also recorded. Additionally, the Mann-Whitney U test or Student's t-test was used for the comparison of the averages of the groups, and Spearman's correlation test was used for the determination of the relationship between two measurements. A p level of <0.05 was accepted as being statistically significant.

Results

All 37 patients who were diagnosed with CD during the study period were included in the study, and the control group was composed of 29 healthy children. While females made up 56.8% (21/37) of the CD group, they made up 55.2% (16/29) of the control group (p=0.898). The mean ages were 7.49±4.54 for the CD group, and 7.29±4.83 (p=0.791) for the control group. The clinical signs and symptoms of the CD patients are given in Table I. Accordingly, while 26 (70.3%) patients were admitted with the typical clinical findings of CD, 9 (24.3%) were admitted with atypical findings. Two

patients (5.4%) with Down syndrome, in which the CD prevalence is relatively high, were diagnosed using a serological screening. When the duodenal biopsies of the patients were evaluated with the Marsh-Oberhuber staging system, 3 cases (8.1%) showed the findings of stage 2, 4 cases (10.8%) had stage 3a, 11 cases (29.7%) had stage 3b, and 19 cases (51.4%) had stage 3c (Table II).

While the levels of the oxidant markers IMA, TOD, and AOPP were significantly higher in the serum samples obtained from the CD patients before the gluten-free diet (p=0.001), the levels of the antioxidant markers TAC, and SH were significantly lower than those of the control group (p=0.001) (Table III).

When the correlations of the tTG IgA levels and the IMA, TOS, AOPP, TAC, and SH levels were evaluated, the tTG IgA was seen to be moderately correlated with the IMA levels (r=0.653, p=0.001), and no correlation was detected between the other markers. Similarly, when potential correlations between the Marsh-Oberhuber histopathological stage and the tTG IgA and other markers were evaluated, a highly positive correlation was detected between the histopathological stage and the dTG IgA (r=0.761, p=0.001); additionally, a moderately positive correlation was detected between the histopathological stage and the IMA (r=0.647, p=0.001). There was no positive or negative correlation between the histopathological stage and the other markers.

After the GFD, while the IMA, TOS, and AOPP levels were significantly lower when compared to the levels before the GFD in the children with CD (p=0.001), the TAC and SH levels were significantly higher when compared to the levels before the GFD (p=0.001) (Table IV).

Discussion

Conditions like ischemia, hypoxia, acidosis, and oxidative stress could transform albumin via the alteration of an amino acid terminal, which is defined as "ischemia modified albumin" (IMA)[3,4]. Previously, IMA was used as an early marker of myocardial ischemia and acute coronary syndrome. Recent studies have shown that it is also an indicator of diabetes mellitus, hyperlipidemia, chronic renal failure, obesity, and thalassemia (besides cardiac diseases), suggesting that IMA is a good marker of oxidative stress[6-10]. We did not encounter any studies evaluating the status of IMA in individuals with CD in the literature.

There is still no knowledge of any precise therapy method, other than GFD, for CD, although the prevalence of the disease has been increasing each year. Therefore, there exists an increase of interest in the etiopathogenesis in order to increase the number of potential novel therapies[18,19]. It has been reported that some α-gliadin peptides of gluten, especially P31-43, enter into the cell through endocytic uptake, are stored in the lysosomes, and increase the amount of free radicals like reactive oxygen and

Table I. Clinical Signs and Symptoms and Histopathological Staging of Duodenum Biopsies of Children with CD.

Clinical signs and symptoms	n (%)
Growth retardation	20 (54.1)
Chronic diarrhea	6 (16.2)
Iron deficiency anemia	4 (10.8)
Abdominal pain-dyspeptic complaints	3 (8.1)
Growth retardation + iron deficiency anemia	1 (2.7)
Chronic diarrhea + iron deficiency anemia	1 (2.7)
Down syndrome	2 (5.4)
Histopathological staging of duodenum biopsies	n (%)
Stage 2	3 (8.1)
Stage 3a	4 (10.8)
Stage 3b	11 (29.7)
Stage 3c	19 (51.4)

nitrogen radicals by activating certain signaling pathways[20,21]. Therefore, oxidative stress is assumed to be one of the key mechanisms playing a role in gliadin toxicity.

In our study, we also compared the levels of oxidant (TOS, AOPP) and antioxidant (TAC, SH) markers in the CD patients with the control group. However, in the literature, we did not encounter a research report investigating these markers in CD. In our study, the IMA, TOS, and AOPP levels were detected to be significantly higher in the children with CD when compared to the control group, and the TAC and SH levels were found to be significantly lower. These findings support the role of oxidative stress in CD.

The relationship between oxidative damage and CD has been supported with some studies, especially in adults[22-31]. An increase was shown in the prostaglandin E2 levels of the intestinal biopsy samples in the patients with CD; while, a reduction was detected in the antioxidant enzymes (glutathione peroxidase, glutathione reductase) and glutathione levels [22-25]. Lavö et al.[24] showed a higher PGE2 concentration in the jejunal secretion samples of 7 adults when compared to healthy controls. Stojilkovic et al.[25] compared glutathione, lipid hydroperoxides, and antioxidant enzyme activity with a healthy control group, and showed that while the superoxide dismutase activity increased in CD, the glutathione, glutathione peroxidase, and glutathione reductase activity were seen to decrease. Similarly, in the study of Stahlberg et al.[26] a significant reduction was detected in the glutathione peroxidase and epoxide hydrolase activities in the intestinal biopsies of 41 children with CD, when compared to the healthy controls.

The nitric oxide synthase (iNOS) activity was shown to increase in the duodenal biopsies of the celiac patients in two studies[27,28]. Among them, in the study of Daniels et al.,[27] the mRNA expression of the iNOS was detected to increase in the duodenal enterocytes of 22 celiac patients. In the study of Becket et al.[28] the intestinal biopsy samples of 11 untreated and 10 treated adults with CD were compared with 9 controls, and the iNOS activity was detected to increase in the biopsy samples of the untreated individuals, when compared to the treated and healthy subjects. Ter Seege et al.[29] reported that the NOS activity was evaluated using nitrotyrosine staining levels in duodenal biopsies, and this nitrotyrosine staining increased in 11 celiac patients compared to the control group. In the studies of Murray et al.[30] and Högberg et al.[30,31] high levels of nitric oxide were present in the serum and urine of children with CD, and this was shown to be correlated with increased iNOS expression in the small intestine.

In our study, we evaluated the potential correlation between the Marsh-Oberhuber histopathological stage and the tTG IgA and other markers. There was a highly positive correlation between the histopathological stage and the tTG IgA (r=0.761); while, a moderately positive correlation was detected between the histopathological stage and the IMA (r=0.647). There was no positive or negative correlation between the histopathological stage and the other markers. In two recent studies investigating the relationship between

Table II. Comparison of Control Group and CD Groups in Terms of Oxidant and Antioxidant Markers

	CD (-)	CD(+) Before diet	p*	CD(+) After diet	p**
IMA	72.66 ±13.46	92.90 ±12.12	0.001	77.94 ±8.37	0.001
TOS	39.72 ±45.83	120.40 ±39.12	0.001	23.47 ±22.51	0.001
TAC	2.92 ±0.04	2.88 ±0.05	0.001	2.93 ±0.02	0.001
AOPP	17.59 ±19.60	31.92 ±23.33	0.001	11.18 ±9.31	0.001
SH	229.50 ±53.65	157.12 ±59.89	0.001	241.50 ±31.76	0.001

IMA: ischemia modified albumin, TOS: total oxidant status, TAC: total antioxidant capacity, AOPP: advanced oxidation protein products, SH: sulfhydryl

* p value for CD(-) vs CD(+) patients before diet

** p value for CD(+) patients before and after diet

the histopathological findings of the duodenal biopsies of celiac patients and the tTG IgA levels, a highly positive correlation was detected between the antibodies and the histopathological stage. Allesio et al.[32] conducted a study with 412 celiac patients aged between 10 months and 72 years, and Zanini et al.[33] conducted a study with 945 adult celiac patients, and both studies revealed that the histopathological stage and the tTG IgA levels were highly correlated.

In addition, the detection of a relationship between the histopathological stage and IMA level, although not as high as with the tTG IgA, may suggest the possible usage of IMA in the diagnosis of CD. However, larger studies are required to support this finding. In the study of Ertekin et al.,[34] the serum nitric oxide levels were found to be higher in 41 children with CD than in those of the control group, and these levels were found to be correlated with the histopathological findings. We did not encounter any other studies evaluating the correlation between the histopathological stage and any oxidant marker, although there are studies available with regard to the relationship between CD and the markers of oxidative stress.

In our study, we also evaluated the effects of the GFD on the status of oxidative stress, which we did not often encounter in studies investigating the relationship between oxidative stress and CD. For this purpose, we compared the levels before and after the diet, and we detected that the IMA, TOS, and AOPP levels were significantly lower after the GFD. However, the TAC and SH levels were observed to be significantly higher when compared to the values before the diet. These findings indicated that the oxidative stress regressed after the gluten-free diet in the celiac patients. In the study of Ertekin et al.,[34] the serum nitric oxide levels were found to be higher in 41 children with CD than in those of the control group, and these levels were found to regress after the GFD. In contrast, in a study by Szaflarska-Poplawska et al.,[35] 8-oxodG, a marker of oxidative stress, was found to increase significantly in children with CD; however, these values were not found to regress with the GFD.

In conclusion, we detected that oxidative stress played a role in the pathogenesis of CD, which could be evaluated with oxidative stress markers, and should regress after a GFD. We also found that IMA is a marker showing a correlation with the histopathological stage, and may be used in the diagnosis.

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