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RESEARCH ARTICLE

Effect of Gluten-Free Diet on Serum Antioxidant Levels in Children with Celiac

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ABSTRACT

Objective: Celiac disease (CD) is an inflammatory condition of the small intestine triggered by the consumption of gluten. A strict gluten-free diet (GFD) is the only treatment that can eliminate CD complications. It was aimed to evaluate the effect of a gluten-free diet on serum total glutathione (tGSH) level, superoxide dismutase (SOD), myeloperoxida (MPO), paroxanase (PON-1) and aryl esterase (ARE) activity in patients with celiac disease, an autoimmune disease.

Materials and Methods: The study was conducted with 68 participants, 39 of whom were celiac and 29 were healthy. Two groups were formed in patients with celiac disease as newly diagnosed and previously diagnosed and following a gluten-free diet. Blood samples were taken from all participants and tGSH, SOD, MPO, PON-1, and ARE measurements were made spectrophotometrically from serum samples.

Results: While no significant change was observed in tGSH, SOD, and ARE levels, MPO activity was observed to be significantly lower in celiac patients compared to healthy controls, while this decrease was found to be higher in the newly diagnosed group. While PON-1 activity was significantly lower in newly diagnosed patients compared to the control group, it was higher in the gluten-compatible diet group.

Conclusion: Low MPO values in celiac patients may be insufficient to function by creating oxidative stress in inflammation. While PON-1 values are significantly lower in newly diagnosed celiacs, it can be said that they reach normal values with adherence to a gluten-free diet.

Keywords: Antioxidant, Celiac, Gluten-free Diet, Paraoxonase, Superoxide dismutase

INTRODUCTION

Celiac disease (CD) is an autoimmune and multifactorial disease caused by environmental and genetic factors. CD is triggered by gluten proteins especially found in different nutrients such as wheat and barley rye.[1] Toxic and immunogenic pathways are caused by mechanisms of gluten intestinal epithelial damage. The conversion of gluten to immunogenic and toxic peptides occurs by proteolysis. These peptides may adversely affect cells and cause oxidative stress in enterocytes, disrupting cell differentiation and death.[2,3] The incidence of CD is about 1-3% of the population worldwide.[4] Although the incidence of the disease is different in varied regions of the world, it is increasing day by day.[5]

Unfortunately, there are not many treatment methods for CD. The exclusively effective treatment used today is a gluten-free diet (GFD). Stringent GFD improves parameters of blood biochemistry, clinical signs, some lesions, and other risk of related disease complications in most patients of CD.[6] Gastrointestinal microbiome treatments have also started to be used as alternative treatment methods.[7]

The high-level creation of reactive oxygen species (ROS), which pass over the ability of biologically functional antioxi-

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dants, causes oxidative stress.[8] It is thought, that the increase of ROS levels of CD is caused by the entry of gliadin peptides into cells, peptide accumulation in lysosomes, and activation of transduction pathways.[9] Oxidative stress affects numerous physiological conditions, causes damage to proteins and lipids, decreases cell membrane fluidity, and is involved in the disease occurrence of different diseases.[10] Oxidative stress and inflammation associated with decreased antioxidant defense systems and elevated levels of reactive oxygen species may be effective in the molecular mechanism of celiac disease.[11]

Effective antioxidant mechanisms, in reaction to oxidative stress, protect the body against free radical damage for instance glutathione (GSH), serum paraoxanase (PON), superoxide dismutase (SOD), and arylesterase (ARE). GSH defends cells from ROS depredation by responding with hydrogen peroxide (H_2O_2) and organic peroxides and removing H_2O_2 from cells.[12] SOD is an antioxidant enzyme that acts as a central component in defending against oxidative stress.[13] PON is an ester hydrolase that catalyzes the hydrolysis of various organic molecules and can protect low-density lipoproteins against peroxidative reactions.[14] Hydrolysis of toxic metabolites can be carried out by the activity of another hydroloase enzyme, ARE.[15] Myeloperoxidase (MPO) is in excess in phagocytes, it catalyzes oxidative species and $H_2O_2$to produce hypochlorous acid (HOCl), and it also reduces nitric oxide activity and increases oxidative stress.[16,17]

Despite being associated with dietary gluten, CD is a genetic disorder and for this reason, keeps going during life. In this way, it is possibly liable for chronic inflammation and oxidative stress. This increases various malignant risks, and oxidative deoxyribonucleic acid damage can cause life-threatening diseases such as cancer.[18] Diets to be applied in daily life are very important in order not to cause more serious problems and to protect living standards.

In the literature, research on children with CD is limited. Due to inflammatory formation and cell damage, oxidant-antioxidant balance is expected to be impaired in patients with CD. In this study, in line with the information between CD and oxidative stress relationship, we aimed to investigate and interpret how a gluten-free diet will affect this situation and its effect on serum GSH, SOD, PON-1, ARE, and MPO levels.

MATERIALS AND METHODS

Patients and Control Group

The present study included 39 children aged 6-15, diagnosed with CD and 29 children without any health problems, who applied to the Pediatrics Diessease polyclinic. The approximative power (1-beta) test value was found as 0.89 with the G-Power program. The diagnosis of CD was made in line with the recommendations of the European Society of Pediatric Gastroenterology, Hepatology, and Nutrition.[19] Newly diagnosed cases and those who have been exposed to a gluten-free diet for at least one year were included in the study group. Individuals with an inflammatory or infectious condition, diabetes mellitus, or any hepatic, metabolic, cardiac, or renal disease were excluded from the study. Moreover, the control group was formed from children of the same age group without any health problems. Informed consent was obtained from the participants. Legal custodian's assent of the children was obtained. The study was approved by the Clinical Ethics Committee of Atatürk University Faculty of Medicine (12/2021 No.B.30.2.ATA.0.01.00/70).

Biochemical Analysis

Blood samples taken for routine biochemistry analysis from patients who agreed to participate in the study were separated into aliquots. Aliquoted samples were transferred to 1.5 mL Eppendorf tubes and stored at -80 °C until the day of analysis.

Serum, SOD, PON-1, ARE, and MPO levels concentrations were analyzed and evaluated using commercial colorimetric kits in a multiplex reader spectrophotometer.

Determination of MPO Activity

The activity of MPO assays is analyzed concerning the method procedures established by Bradley et al.[20] Serum MPO activity was analyzed by transferring 100 mL of serum samples to 1 mL of 1.5 mM o-dianisidine hydrochloride containing 0.0005% (wt/vol) hydrogen peroxide and 1.9 mL of 10 mM phosphate buffer (pH 6.0). Measurements of absorbance changes were made for each sample with an Ultraviolet-vis spectrophotometer at 450 nm.[20]

Determination of SOD Activity

Superoxide Dismutase Assay Kit (Cayman, USA) was performed for analysis of levels of serum SOD. Analyzes were performed according to the directions included in the kit. According to the kit content, superoxide radicals produced by xanthine oxidase and hypoxanthine are determined using tetrazolium salt. The amount of enzyme required to exhibit 50% dismutation of the superoxide radical is defined as one unit of SOD.

tGSH Analysis

tGsh results were analyzed using the methods of Sedlak et al. According to the approach outlined by Sedlak et al.[21] In this method, 5,5'-dithiobis [2-nitrobenzoic acid] disulfide (DTNB), which is chromogenic, is rapidly reduced by sulfhydryl groups. For deproteinization before analysis, 100 µL of meta-phosphoric acid was added to 100 µL of the sample and centrifuged at 1,000 x g for 2 min. A cocktail mixture consisting of 80 mL of 625 U/L Glutathione reductase, 5.85 mL of 100 mM Na-phosphate buffer, 2.8 mL of 1mM DTNB, and 3.75 mL of 1 mM NADPH was prepared for measurement. 150 µL

of the prepared cocktail was mixed with 50 µL of supernatant. At 412 nm, the color formed during reduction is calculated by measuring in the spectrophotometer. A calibration curve was drawn using oxidized L-glutathione (GSSG) and sample results were calculated.

PON-1 and ARE Activity Measurement

Serum PON-1 paraoxonase/ARE activities were analyzed using previously used methods.[22,23] Serum PON-1 activities were analyzed spectrophotometrically in the method with diethyl-p-nitrophenylphosphate as a substrate. 1 nmol 4-nitrophenol/mL serum/min was defined as a unit for PON-1 activity. ARE activity was calculated using phenylacetate as the substrate by measuring the absorbance of the obtained phenol at 270 nm. The activities of PON-1 and ARE were calculated with their absorption coefficients. (17.100 M^{-1} cm^{-1} and 1.310 M^{-1} cm^{-1}, respectively). For ARE activity, as a unit, 1 nmol phenol/mL serum/min was defined.

Statistical Analysis

SPSS for Windows (IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp. IBM Corp. Released 2012) was used to do data analysis. Shapiro Wilk test was used to evaluate the normal distribution of the data. Accordingly, the Independent-T test was used to compare the patient and control groups for normally distributed data, while the ANOVA test was used to compare patient subgroups with more than two. Data were expressed as mean±standard deviation (Mean±SD) and $p<0.05$ was considered significant.

RESULTS

While 21 of 39 celiac children were girls and 18 boys in the celiac group, there were 17 girls and 12 boys in the control group. The mean age of the 2 groups did not differ. Body mass index (BMI) was lower in children with celiac (Table 1).

MPO activity was observed lower in children in the total celiac group than in the control group, and this decrease was statistically significant ($p<0.001$). PON-1 activity was examined to be lower in the total celiac group, yet this decrease wasn't significant ($p>0.05$). When the two groups are compared, no difference was detected in ARE, SOD activity, and tGSH levels ($p>0.05$). The data of the total celiac group and the control group are demonstrated in Figure 1.

When the three groups were compared, a significant difference was observed in MPO activities. MPO activity was lower in newly diagnosed celiac children compared to the control group and GFD-CD group. However, the observed decrease was statistically significant only compared to the control group ($p<0.05$). MPO activity was higher in the GFD-CD group compared to the newly diagnosed group and lower than the healthy control group. On the other hand, there was no statistically significant difference between the control group and the GFD-CD groups. When the PON-1 activity between the groups was compared, it was found that it was statistically lower in the newly diagnosed group and higher in the gluten-free diet group compared to the control group ($p<0.05$).

When tGSH level and SOD activity were evaluated, although it was lower in the gluten-free diet group, no statistically significant difference was observed between the three groups ($p>0.05$). On the other hand, while ARE activity was low in the newly diagnosed group, it was close to control in the gluten-free diet group. However, this decrease in the newly diagnosed group was not significant ($p>0.05$). Table 2 presents the data for all three groups.

DISCUSSION

The consequences of the examination were evaluated in our current study to evaluate the antioxidant levels of newly diagnosed CD patients and celiac patients compatible with a gluten-free diet as well as to define the effect of a gluten-free diet on these parameters. When compared with the control group in the same age group, BMI was found lower in the celiac group. This can be explained by the situation caused by the nutrient absorption of celiac patients. Some differences were observed in the gluten-free diet group compared to the newly diagnosed or control group. While this difference was evident in MPO values, no difference was observed in other parameters.

The gastrointestinal symptoms of CD, such as diarrhea, abdominal pain, weight, fatigue, and bloating, are similar to various irritable bowel syndromes, often referred to as diarrhea variant irritable bowel syndromes.[24] Oxidative stress is caused by an impaired antioxidant system or increased levels of ROS.[25] ROS are very dangerous for metabolism due to their high reactivity and production in cells. Nonenzymatic antioxidants such as vitamins, and glutathione and antioxidant enzymes such as glutathione peroxidase/reductase, and superoxide dismutase are antioxidant defense systems that prevent the detrimental effects of ROS. In some cases, the amount of ROS may exceed the volume of the antioxidant defense system, leading to the occurrence of oxidative stress.[26]

Oxidative stress is related to the pathology of many diseases, and CD is one of them. CD is increasing day by day, especially in developing countries, due to modifications in wheat production and processing, heightened awareness of the disease, and changes in diet fluidity.[27] Celiac disease is a genetic disorder, but is affected by dietary gluten and persists throughout life. Due to these properties, it can be a source of chronic oxidative stress and brings various risks for metabolism.[28] Gluten peptides in enterocytes accumulate in lysosomes, altering certain signal transduction pathways and disrupting the oxidation defense balance by raising ROS levels.[29] A recent study on wheat germ peptides indicates that some peptides (WGP2-P7 and WGP11) significantly increased levels of glutathione reduc-

Table 1. Demographic data of celiac and control groups.

	Total Celiac patients n:39; Mean±SD	Control n:29; Mean±SD	P value
Age (year)	11.2±2.8	11.9±1.9	0.250
BMI	15.1±2.1	18.8±2.6	0.045

BMI: Body mass index

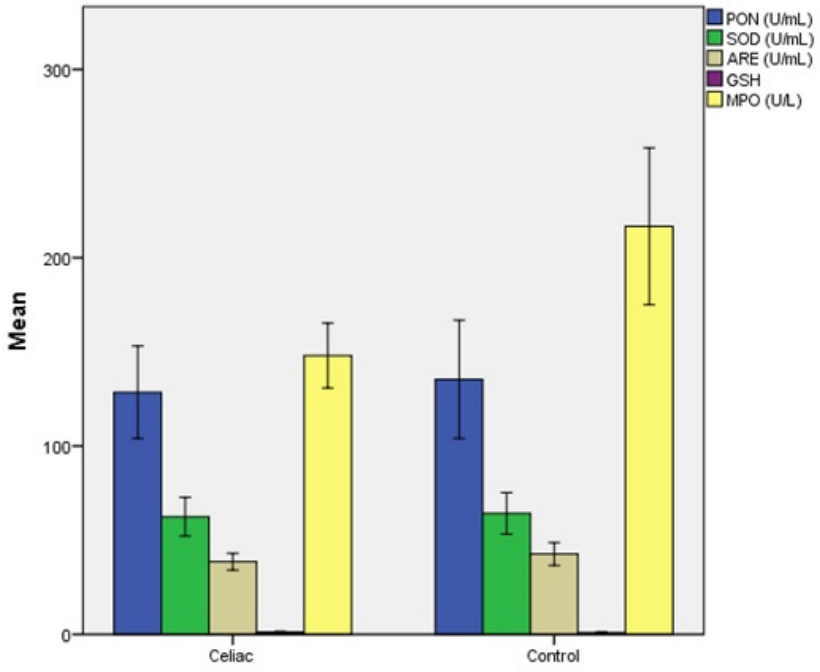

Figure 1. Data of total celiac and control groups.

Table 2. Analysis results of ND-CD, GFD-CD, and control group.

	ND-CD n: 18 Mean±SD	GFD-CD n:21 Mean±SD	Control n:29 Mean±SD	P value
SOD (U/mL)	68.9±36.8	54.9±28.2	64.2±29.7	0.367
MPO (U/mL)	131.5±41.3[a]	167.2±54.4	216.2±112.1	0.003*
tGSH (mol/L)	1.5±1.4	0,98±063	1.3±0.86	0.283
PON-1 (U/mL)	99.1±72.0[a]	162.8±68.4[a,b]	135.4±84.5	0.039*
ARE (U/mL)	35.7±13.3	41.8±14.1	42.6±16.4	0.249

ND-CD: New diagnostic celiac disease group, GFD-CD: Gluten-free diet-celiac disease, SOD: Superoxide dismutase, MPO: Myeloperoxidase, tGSH: Total glutathione, PON-1:Paraoxonase-1, ARE: Arylesterase
*: Statistically significant (among three groups).
a: Statistically significant when compared to the control group.
b: Statistically significant when compared to the ND-CD.

tases and glutathione peroxidase. As a result of this, emphasized that this situation significantly increased the antioxidant level of the cells.[30]

The answer to the question of provided that the use of a gluten-free diet in children with CD is adequate to maintain the balance of serum oxidative/antioxidant in these people is still a subject of research. Rowicka, and et. al. found no differences in the intensity of oxidative/antioxidant between children with celiac disease treated with a gluten-free diet and healthy children.[31]

In their study, Stojiljković et al. found a significant decrease in GSH concentration in the intestinal mucosa of individuals with active CD, and showed that even in patients on a gluten-free diet, the GSH level was 25% lower than in controls. They reported that the GSH concentration was also significantly reduced in the peripheral blood of both active and gluten-free diet patient groups.[32] In our current study, no difference was observed in the newly diagnosed and gluten-free diet patients compared to the control group. Moreover, laboratory studies on the gluten-free diet have also indicated that it allows normalization of bone and mineral metabolism and reverse some abnormalities.[33] GFD is thought to be beneficial not only for people with CD but also for healthy people.[34]

Many functions in the body are adversely affected by ROS-induced oxidative stress. Various studies have been carried out to figure out this process in children with celiac disease. It has been shown that the antioxidant potential of patients newly diagnosed with celiac is lower than that of healthy individuals.[35,36] Studies indicate the importance of a gluten-free diet in CD. Improvements such as improvement of the intestinal mucosa and improvement of clinical features were observed. This situation is considered accompanied by oxidative stress and antioxidant balance.[37] While Rowicka et al. did not find a change in serum total antioxidant capacity levels between CD and control groups, Ferretti et al. found lower compared to controls, Ferretti et al. also showed that it is higher in newly diagnosed patients who don't follow a gluten-free diet.[31,37] Stojiljković et al. showed increased SOD activities and a significant reduction in glutathione content. They stated that antioxidant capacity decreases with consumed glutathione in celiac patients and that dietary antioxidants will be important in the complementary treatment of the disease.[38] In our study, however, no impact of a gluten-free diet on SOD activity was observed in patients with CD.

Previous studies have shown that PON-1, 2, and 3 are expressed in human intestinal cells.[39] In a later study, the relationship of PON-1 with intestinal inflammatory diseases was examined and PON gene expressions were compared in celiac and healthy duodenal tissue biopsies. A severe loss of PON-1 has been reported in celiac patients.[40] Ferretti at all. showed a lower PON-1 and ARE activity in the serum of both groups of CD, (11 at diagnosis, 16 receiving gluten-free diet therapy) compared to control subjects and they thought that this situation might contribute to gastrointestinal cell damage. Kaplan et al. reported that PON-1 and ARE levels were lower in gluten-sensitive enteropathy patients compared to the control group.[41] [37] Similarly, according to our results, PON-1 activity was found low in celiac patients, and a significant increase in PON-1 activity was observed with a gluten-free diet. Moreover, it was found higher in the gluten-free diet group than in the healthy control group. Studies on MPO in celiac patients were limited in the literature. Maluf et al. showed inflammatory marker MPO levels were increased in CD patients compared to controls.[42] However, on the contrary, in our study, a low MPO value was found in newly diagnosed celiac patients, while it was higher in the gluten-free diet-compliant group than in the newly diagnosed group, as well control group had the highest MPO value, which was significant.

One of the limitations of our study is a low number of sample subgroups. Another limitation is that the lipid profile of the sample group could not be included in the study. It would be more meaningful to evaluate the obtained PON-1 values together with HDL.

CONCLUSION

There are many studies in the literature on antioxidant levels in celiac patients, but research on the effects of gluten gluten-free diets on these factors is limited. MPO values in celiac patients may be insufficient to function by creating oxidative stress in inflammation. Although an increase is observed with the gluten-free diet, it remains lower than the control group. Likewise, while PON-1 values are significantly lower in newly diagnosed celiacs, it can be said that they reach normal values with adherence to a gluten-free diet. However, higher PON-1 activity was observed in CD patients on a gluten-free diet compared to the control group, and it is anticipated to investigate the effect of gluten on PON-1 activity in healthy people to better understand this increase.

Ethics Committee Approval: This study was approved by the Clinical Ethics Committee of Atatürk University Faculty of Medicine (12/2021 No.B.30.2.ATA.0.01.00/70).

Informed Consent: Legal custodian's assent of the children participated in the research was obtained.

Peer Review: Externally peer-reviewed.

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