J Med Biochem 2017; 36 (3) DOI: 10.1515/jomb-2017-0017

UDK 577.1 : 61 **ISSN 1452-8258**

J Med Biochem 36: 243–250, 2017

Original paper
Originalni naučni rad

THE ROLE OF OXIDATIVE STRESS IN THE ETIOPATHOGENESIS OF GLUTEN-SENSITIVE ENTEROPATHY DISEASE

ULOGA OKSIDATIVNOG STRESA U ETIOPATOGENEZI GLUTENSKE ENTEROPATIJE

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Summary

Background: The objective here is to examine the role of overall oxidative stress in the etiopathogenesis of gluten-sensitive enteropathy disease and its relationship with gluten free diet and autoantibodies.
Methods: Eighty gluten-sensitive enteropathy patients and 80 control group participants were included in the study. As oxidative stress parameters, we researched total antioxidant status (TAS), total oxidant status (TOS), oxidative stress index (OSI), paraoxonase-1 and arylesterase parameters in the serum samples of gluten-sensitive enteropathy patients.
Results: In comparison to the control group, gluten-sensitive enteropathy patients had lower TAS, paraoxonase-1 and arylesterase levels and gluten-sensitive enteropathy patients had considerable TOS and OSI levels. In contrast, patients who agreed to the gluten free eating routine had a higher OSI proportion and patients who did not conform to the gluten free eating regimen had a lower paraoxonase-1 level. An affirming reciprocation was detected amidst TOS and OSI proportion and gluten-sensitive enteropathy autoantibodies and C-reactive protein levels and a negative correlation was found between arylesterase level and gluten-sensitive enteropathy autoantibodies.
Conclusions: We observed oxidative stress levels to be higher in gluten-sensitive enteropathy patients contrasted with the control group. Oxidative stress level showed differences in gluten-sensitive enteropathy patients depending on gluten diet content and autoantibody positivity. In point

Kratak sadržaj

Uvod: Cilj je ovde bio da se ispita uloga ukupnog oksidativnog stresa u etiopatogenezi glutenske enteropatije i njegov odnos sa bezglutenskom ishranom i autoantitelima.
Metode: Osamdeset pacijenata sa glutenskom enteropatijom i 80 članova kontrolne grupe je uključeno u studiju. Kao parametri oksidativnog stresa, istraživani su ukupni antioksidantni status (UAS), ukupni oksidantni status (UOS), indeks oksidativnog stresa (IOS), paraoksonaza 1 i arilesteraza u uzorcima seruma pacijenata sa glutenskom enteropatijom.
Rezultati: U poređenju s kontrolnom grupom, pacijenti sa glutenskom enteropatijom imali su niže nivoe UAS-a, paraoksonaze-1 i arilesteraze a pacijenti sa glutenskom enteropatijom imali su znatne nivoe UOS-a i IOS-a. Za razliku od toga, pacijenti koji su pristali na bezglutenski režim ishrane imali su višu proporciju IOS-a a pacijenti koji nisu pristali na bezglutenski režim imali su niži nivo paraoksonaze 1. Afirmativan reciprocitet utvrđen je između UOS-a i proporcije IOS-a i autoantitela za glutensku enteropatiju i nivoa C-reaktivnog proteina, a negativna korelacija je pronađena između nivoa arilesteraze i autoantitela za glutensku enteropatiju.
Zaključak: Uočili smo da je nivo oksidativnog stresa bio viši kod pacijenata sa glutenskom enteropatijom u poređenju s kontrolnom grupom. Nivo oksidativnog stresa pokazao je razlike među pacijentima s glutenskom enteropatijom zavisno od sadržaja glutena u ishrani i pozitivnih autoantitela.

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List of abbreviations: AGA-IgA-G: anti-gliadin antibody IgA-G; Anti-t TGA-G: anti-tissue transglutaminase IgA-G; CRP: C-reactive protein; ESR: erythrocyte sedimentation rate; OSI: oxidative stress index; PON1: paraoxonase 1; TAS: total antioxidant status; TOS: total oxidant status.

of fact, C-reactive protein and gluten-sensitive enteropathy autoantibodies are identified with oxidative anxiety parameters resulting in the possibility that oxidative stress might be successful in the gluten-sensitive enteropathy pathogenesis.

Keywords: oxidative stress index, serum paraoxonase 1 / arylesterase, total antioxidant status, total oxidant status

Zapravo, C-reaktivni protein i autoantitela za glutensku enteropatiju identifikuju se sa parametrima oksidativne anksioznosti, što rezultira mogućnošću da oksidativni stres uspešno učestvuje u patogenezi glutenske enteropatije.

Ključne reči: indeks oksidativnog stresa, paraoksonaza 1 / arilesteraza u serumu, ukupni antioksidantni status, ukupni oksidantni status

Introduction

Gluten-sensitive enteropathy is a chronic inflammatory disease connected with excessive sensitivity to gluten and characterized by lymphocyte infiltration in the proximal part of small intestine, villous atrophy, crypt hyperplasia and mucosal harm (1). Although environmental, genetic, immunological and microbial factors are blamed in its etiopathogenesis, in addition to this there have been studies suggesting that oxidative stress may have a role (1, 2). It is thought that especially some gliadin peptides resistant to proteolytic digestion cause small intestinal damage, induce the production of proinflammatory cytokines, and thus increase oxidative stress (1).

Oxidative stress is characterized based on an instability amidst the oxidant and antioxidant mechanisms and the consequent increase in free radicals (2). However, it is difficult to determine the oxidative stress status by measuring free radicals due to the instability of these molecules. Hence, new laboratory techniques have been developed in order to measure stable oxidant and antioxidant substances in the laboratory environment. It is difficult to measure these parameters separately since it is time consuming, laboratory dependent and open to interactions between substances. For this reason, total antioxidant status (TAS) is used to indicate body's overall antioxidant status, total oxidant status (TOS) is used to indicate overall oxidant status of the body and oxidative stress index (OSI) is used to indicate oxidative stress status of the body (3, 4). Also, the antiinflammatory and antioxidant serum arylesterase/paraoxonase 1 (PON1) enzyme, which is encoded by the PON1 gene and responsible for removing oxidized lipids that cause inflammation from the body, is used as an antioxidant marker (5, 6). In our literature review, we have not found a study conducted with these oxidative stress parameters which indicate the general oxidative status in gluten-sensitive enteropathy disease and hold a primary status in antioxidant protection at the same time.

For this reason, we purposed to research the behavior of TAS, TOS, OSI, PON1 and arylesterase levels in the etiopathogenesis of gluten-sensitive enteropathy disease and their relationship with gluten intolerance diet, gluten-free diet and gluten-sensitive enteropathy autoantibodies.

Material and Methods

Research group

Our research was done between May 2015 and September 2015 in Turkey Yuksek Ihtisas Training and Research Hospital and Ankara Numune Training and Research Hospital. The research comprised a sum of 160 attendants over 18 years old. Eighty of the participants were gluten-sensitive enteropathy disease patients followed-up in the Gastroenterology clinic and the other 80 attendants were healthy volunteers. Patients who were diagnosed with gluten-sensitive enteropathy disease using endoscopic biopsy and under regular follow-up in our centre were consolidated in the patient group respectively of the application. The control group attendants were chosen from healthy individuals with demographic characteristics similar to the patient group and no known chronic disease and medication use, who applied to our hospital for check-up.

Participants with documented chronic inflammatory diseases (cardiovascular and cerebrovascular diseases, rheumatic and autoimmune diseases, acute-chronic infections), acute-chronic liver and kidney failure, malignancy, vitamin and antioxidant drug use and tobacco and alcohol use were excluded from the study.

We arranged two subgroups (GCD: patients resistant to a gluten free eating routine, GFD: patients consistent with a gluten free eating regimen) to comprehend the impact of eating regimen on oxidative stress in gluten-delicate enteropathy ailment. Patient compliance with a gluten eating routine was acquired from patient documents and connected surveys.

The present study was designed in accordance with the 2013 Brazil version of the Helsinki Declaration and confirmed by the Institutional Ethics and Research Committee of Turkey Yuksek Ihtisas Training and Research Hospital. Written consent was taken from all participants included in the study.

Biochemical parameters

Venous blood samples of all participants included in the study were taken between 08:00–10:00 AM after 8 hours of fasting in order to measure oxidative stress markers. The blood specimens were centrifuged for ten minutes at 1,500 rpm and then plasma and serum samples were separated. Serum specimens were kept at –80 °C. TAS, TOS, PON1 and arylesterase parameters were studied on the identical serum samples in the same session.

Hemogram, biochemistry, c-reactive protein (CRP), erythrocyte sedimentation rate (ESR) and autoantibody [anti-gliadin antibody IgA-G (AGA-IgA-G) or anti-tissue transglutaminase IgA-G (Anti-t TGA-G)] levels of participants were obtained from patient files and reflect values at the time when participants were included in the study. ELISA method was used to quantify autoantibodies. Rates over 20 U/L were reckoned as positive.

Measurement of TAS, TOS, PON1, arylesterase and calculation of OSI

Commercial kits (TAS, Rel Assay Diagnostics, Gaziantep, Turkey, REF. No: RL0017, LOT No: JE 14042A) were used to measure serum TAS level via a colorimetric method. CV%: 10. Linearity: 0–2.75 mmol/L. The results are expressed in mmol Trolox equivalents/L. Commercial kits (TOS, Rel Assay Diagnostics, Gaziantep, Turkey, REF. No: RL0024, LOT No: JE 14048O g) were used to measure serum TOS level via a colorimetric method. CV%: 10. Linearity: 0–33.5 μmol/L. The results are signified in micromolar H_2O_2 equivalents per liter.

Commercial kits (PON1, Rel Assay Diagnostics, Gaziantep, Turkey, REF. No: RL0031, LOT No: JE14028P) were used to measure serum PON1 level via a colorimetric method. CV%: 5. Linearity: 0–750 U/L.

Commercial kits (Arylesterase, Rel Assay Diagnostics, Gaziantep, Turkey, REF. No: RL0055, LOT No: JR13017AR) were used to measure serum arylesterase level via a colorimetric method.

The proportion of TOS to TAS procured the OSI, an indicator of the rating of oxidative stress.

Statistical analysis

Analyses were performed with Statistical Package for Social Sciences (SPSS) for Windows 20 (IBM SPSS Inc., Chicago, USA). Distribution of data was determined by Kolmogorov-Smirnov test. As the results are presented as mean value±standard deviation (SD) for continuous variables with normal distribution, for continuous variables without normal distribution they are presented as mean. Categorical variables were summarized as numbers and percentages. We compared continuous variables with independent sample t-test, Mann Whitney U test, and Kruskall Wallis H test, where appropiate. We studied the association between the numeric parameters via Pearson and Spearman correlation analysis. When examining the relation between TAS, TOS, OSI, PON1 and arylesterase and gluten-sensitive enteropathy antibodies, effects of demographic and clinical factors were removed using partial correlation. A $p<0.05$ was noted substantial for statistical analyses.

Results

Table I recapitulates the demographic characteristics and laboratory findings related to all groups. The study population consisted of a total of 160 participants, 80 gluten-sensitive enteropathy disease patients (Female/Male: 63/17, age: 44.2±13.1 years, body mass index: 25.6±4.5 kg/m^2) and 80 control group participants (Female/Male: 60/20, age: 43.9±13.5 years, body mass index: 25.7±4.9 kg/m^2). No significant difference was determined among the groups with regards to gender, age, BMI levels and smoking and alcohol use ($p>0.05$). The median disease duration in gluten-sensitive enteropathy disease patients was found to be 7.1 years (min: 1 year, max: 35 years). AGA-IgA (+) was detected in 53.8% of the patients, AGA-IgG (+) in 33.8%, Anti-t TGA (+) in 40% and Anti-t TGG (+) in 15%.

There was no significant difference between the gluten-sensitive enteropathy disease patients and the control group in mean total protein and median ESR level ($p>0.05$). Mean albumin level (4.6±0.6 g/L vs 4.0±0.5 g/L; $p<0.001$) and median CRP level (4.0 mg/L vs 1.9 mg/L; $p=0.001$) were higher in gluten-sensitive enteropathy disease patients compared to the control group.

In comparison to the control group, TAS (1.3±0.2 mmol Trolox equivalent/L vs 1.8±0.2 mmol Trolox equivalent/L; $p<0.001$), PON1 (122.5±36.4 U/L vs 155.8±47.6 U/L; $p<0.001$) and arylesterase (971.06±235.1 U/L vs 1249.2±279.2 U/L; $p<0.001$) levels were found to be lower and TOS (6.9±2.0 micromolar H_2O_2Eq/L vs 4.3±1.9 micromolar H_2O_2 Eq/L; $p<0.001$) and OSI (5.3±1.5 vs 2.4±1.1; $p<0.001$) levels were found to be higher in gluten-sensitive enteropathy disease patients.

The distributions of mean TAS, TOS, OSI, PON1 and arylesterase levels in the gluten-sensitive enteropathy group according to diet compliance and antibody positivity are given in *Table II*. There was no significant difference between the patients whose diet is gluten free and the patients whose diet is incompatible with a gluten free regimen in terms of mean TAS, TOS and arylesterase levels. In comparison to patients with diet compliance, OSI rate was higher in patients non-compliant with gluten free diet

Table I The demographic characteristics and laboratory findings of study population.

Variables	Gluten-sensitive enteropathy disease	Control	p
	n (80)	*n* (80)	
Gender (female), *n* (%)	63 (78.8)	60 (75)	0.574
Age (years)	44.2±13.1	43.9±13.5	0.887
BMI (kg/m^2)	25.6±4.5	25.7±4.9	0.681
Smoking, n (%)	18 (22.5)	20 (25)	0.936
Alcohol, n (%)	3 (3.8)	-	0.245
Duration of disease (years)	6.5 (6.5)	-	-
Total protein (g/L)	7.4±0.7	7.4±0.5	0.896
Albumin (g/L)	4.6±0.6	4.0±0.5	<0.001*
ESR (mm/h)	15 (11.5)	11(9.5)	0.438
CRP (mg/L)	4.0 (6.2)	1.9 (4)	0.001*
TAS (mmol Trolox equivalent/L)	1.3±0.2	1.8±0.2	<0.001*
TOS (micromolar H_2O_2 Eq/L)	6.9±2.0	4.3±1.9	<0.001*
OSI (arbitrary unit)	5.3±1.5	2.4±1.1	<0.001*
PON1 (U/L)	122.5±36.4	155.8±47.6	0.036*
Arylesterase (U/L)	971.06±235.1	1249.2±279.2	<0.001**

p<0.05 statistically significant
Abbreviations: BMI: body mass index, ESR: erythrocyte sedimentation rate, CRP: C-reactive protein, TAS: total antioxidant status, TOS: total oxidant status, OSI: oxidative stress index, PON: paraoxonase

Table II Thiol/disulphide homeostasis level according to diet compliance and antibody positivity.

Variables	TAS (mmol Trolox equivalent/L)	*p*	TOS (micromol H_2O_2 Eq/L)	*p*	OSI (Arbitrary unit)	*p*	PON1 (U/L)	*p*	Arylesterase (U/L)	*p*
Diet										
GCD (*n*=25)	1.3±0.2	0.251	7.1±2.3	0.086	5.8±1.6	0.047*	96.4±37.6	<0.001*	967.2±238.2	0.814
GFD (*n*=55)	1.3±0.2		6.1±2.3		4.9±2.2		132.4±40.7		981.2±232.1	
AGA-IgA										
(-) (*n*=38)	1.3±0.2	0.819	6.1±1.6	0.002*	4.8±1.4	0.012*	128.4±46.4	0.244	936.8±236.8	0.228
(+) (*n*=42)	1.3±0.2		7.5±2.3		6.1±2.8		117.4±37.4		1000.6±232.4	
AGA-IgG										
(-) (*n*=54)	1.4±0.2	0.037*	6.0±2.1	0.015*	4.6±1.6	<0.001*	129.9±40.2	0.021*	1012.9±239.1	0.263
(+) (*n*=26)	1.3±0.2		7.3±2.4		6.0±1.8		108.0±37.6		949.7±232.4	
Anti-t TGA										
(-) (*n*=34)	1.3±0.2	0.824	6.0±1.8	0.001*	4.6±1.4	0.001*	132.6±40.8	0.039*	993.5±233.4	0.300
(+) (*n*=46)	1.3±0.2		7.6±2.3		5.8±1.7		115.8±30.6		937.4±237.4	
Anti-t TGG										
(-) (*n*=69)	1.3±0.2	0.184	5.0±1.6	<0.001*	3.8±1.4	0.004*	124.2±45.6	0.412	1103.7±282.1	0.020*
(+) (*n*=11)	1.4±0.2		7.2±2.2		5.2±2.1		112.8±34.1		900.4±221.2	

*p<0.05 statistically significant
Abbreviations: TAS: total antioxidant status, TOS: total oxidant status, OSI: oxidative stress index, PON: paraoxonase, GCD: gluten-containing diet, GFD: gluten-free diet, AGA-IgA: anti-gliadin antibodies IgA, AGA-IgG: anti-gliadin antibodies IgG, Anti-t TGA: anti-tissue transglutaminase IgA antibodies, Anti-t TGG: anti-tissue transglutaminase IgA antibodies.

Table III Relationship of oxidative stress parameters with other findings in the whole population and in the patient group.

Groups	Variables	TAS		TOS		OSI		PON1		Arylesterase	
		r	*p*	*r*	*p*	*r*	*p*	*r*	*p*	*r*	*p*
Control	Age	0.010	0.901	-0.160	0.143	-0.122	0.125	-0.138	0.082	-0.163	0.139
	BMI	-0.198	0.112	0.112	0.158	0.125	0.116	0.005	0.946	-0.086	0.277
	Total protein	-0.041	0.609	0.002	0.982	-0.011	0.890	0.393	0.001*	0.344	0.002*
	Albumin	0.192	0.115	-0.045	0.569	-0.090	0.260	-0.139	0.079	0.041	0.606
	ESR	-0.051	0.523	0.025	0.749	0.053	0.502	0.390	<0.001*	0.120	0.131
	CRP	0.169	0.132	-0.043	0.585	-0.068	0.393	0.326	0.004*	0.315	0.006*
Gluten-sensitive enteropathy disease	Age	0.073	0.523	-0.158	0.162	-0.174	0.123	-0.072	0.526	-0.067	0.558
	BMI	0.131	0.247	0.420	0.004*	0.398	0.007*	0.004	0.970	-0.063	0.579
	AGA-IGA	0.136	0.228	0.298	0.022*	0.337	0.015*	-0.143	0.205	-0.351	0.039*
	AGA-IGG	0.198	0.078	0.300	0.029*	0.346	0.019*	-0.071	0.531	-0.370	0.016*
	Anti-t TGA	0.146	0.197	0.288	0.031*	0.370	0.022*	0.052	0.648	-0.324	0.035*
	Anti-t TGG	0.176	0.119	0.285	0.024*	0.382	0.028*	-0.047	0.676	-0.322	0.038*
	Total protein	-0.062	0.587	0.078	0.490	0.107	0.343	0.063	0.578	0.046	0.686
	Albumin	0.114	0.312	0.093	0.413	0.072	0.525	-0.061	0.593	-0.086	0.448
	ESR	-0.042	0.712	0.070	0.535	0.105	0.356	0.081	0.477	-0.005	0.968
	CRP	-0.077	0.500	0.292	0.009*	0.423	0.002*	-0.353	0.017*	-0.123	0.278
	AGA-IGA†	0.167	0.184	0.288	0.034*	0.299	0.037*	-0.097	0.444	-0.263	0.034*
	AGA-IGG†	0.095	0.452	0.275	0.042*	0.270	0.033*	-0.051	0.689	-0.266	0.032*
	Anti-t TGA†	0.055	0.662	0.266	0.039*	0.263	0.040*	0.108	0.390	-0.278	0.044*
	Anti-t TGG†	0.130	0.300	0.272	0.037*	0.269	0.036*	0.035	0.781	-0.283	0.046*

†: demographic and laboratory findings have been adjusted
*p<0.05 statistically significant
Abbreviations: TAS: total antioxidant status, TOS: total oxidant status, OSI: oxidative stress index, PON: paraoxonase, BMI: body mass index, ESR: erythrocyte sedimentation rate, CRP: C-reactive protein, AGA-IgA: anti-gliadin antibodies IgA, AGA-IgG: anti-gliadin antibodies IgG, Anti-t TGA: anti-tissue transglutaminase IgA antibodies, Anti-t TGG: anti-tissue transglutaminase IgA antibodies

(5.8±1.6 vs 4.9±2.2, respectively; $p=0.047$), whereas PON1 level was lower (96.4±37.6 U/L vs 132.4±40.7 U/L, respectively; $p<0.001$). Compared to patients whose diet is compatible with gluten free diet, more advanced CRP level was found in patients incompatible with gluten free diet ($p<0.05$). In comparison to AGA-IgA (-) patients, mean TOS level (7.5±2.3 micromolar H_2O_2 Eq/L vs 6.1±1.6 micromolar H_2O_2 Eq/L, respectively; $p=0.002$) and OSI rate (6.1±2.8 vs 4.8±1.4, respectively; $p=0.012$) were found to be higher in AGA-IgA (+) patients. In comparison to AGA-IgG (-) patients, TAS level (1.3±0.2 mmol Trolox equivalent/L vs 1.4±0.2 mmol Trolox equivalent/L, respectively; $p=0.037$) and PON1 level (108.0±37.6 U/L vs 129.9±40.2 U/L, respectively; $p=0.021$) were found to be lower in AGA-IgG (+) patients, whereas mean TOS level (7.3±2.4 micromolar H_2O_2 Eq/L vs 6.0±2.1 micromolar H_2O_2 Eq/L, respectively; $p=0.015$) and OSI rate (6.0±1.8 vs 4.6±1.6, respectively; $p<0.001$) were found to be higher. In comparison to Anti-t TGA (-) patients, mean PON1 level (115.8±30.6 U/L vs 132.6±40.8U/L, respectively; $p=0.039$) was found to be lower, whereas mean TOS level (7.6±2.3 micromolar H_2O_2 Eq/L vs 6.0±1.8 micromolar H_2O_2 Eq/L, respectively; $p=0.001$) and OSI rate

(5.8±1.7 vs 4.6±1.4, respectively; $p=0.001$) were found to be higher in Anti-t TGA (+) patients. In comparison to Anti-t TGG (-) patients, mean arylesterase level (900.4±221.2 U/L vs 1103.7±282.1 U/L, respectively; $p=0.020$) was found to be lower, whereas mean TOS level (7.2±2.2 micromolar H_2O_2 Eq/L vs 5.0±1.6 micromolar H_2O_2 Eq/L, respectively; $p<0.001$) and OSI rate (5.2±2.1 vs 3.8±1.4, respectively; $p=0.004$) were found to be higher in Anti-t TGG (+) patients.

The correlation between TAS, TOS, OSI, PON1 and arylesterase levels and demographic and clinical findings in the patient group and the control group is given in *Table III* in detail. No demographic and clinical findings were found to be associated with TAS levels in gluten-sensitive enteropathy disease patients. A positive correlation was approved between TOS levels and BMI ($r=0.420$, $p=0.004$), AGA-IgA ($r=0.298$, $p=0.022$), AGA-IgG ($r=0.300$, $p=0.029$), Anti-t TGA ($r=0.288$, $p=0.031$), Anti-t TGG ($r=0.285$, $p=0.024$) and CRP ($r=0.292$, $p=0.009$) levels. A positive correlation was found between OSI rate and BMI ($r=0.398$, $p=0.007$), AGA-IgA ($r=0.337$, $p=0.015$), AGA-IgG ($r=0.346$, $p=0.019$), Anti-t TGA ($r=0.370$, $p=0.022$), Anti-t TGG ($r=0.382$, $p=0.028$) and CRP ($r=0.423$, $p=0.002$) levels. There was no relation between PON1 level and demographic findings, whereas among laboratory findings, only CRP level ($r=0.353$, $p=0.017$) had a positive correlation with PON1 level. There was no relation between arylesterase level and demographic findings. Among the clinical findings, arylesterase had a negative correlation with AGA-IgA ($r=-0.351$, $p=0.039$), AGA-IgG ($r=-0.370$, $p=0.016$), Anti-t TGA ($r=-0.324$, $p=0.035$) and Anti-t TGG ($r=-0.322$, $p=0.038$). When the effects of demographic and clinical findings were ignored, it was found that the relation between TOS, OSI and arylesterase levels and AGA-IgA, AGA-IgG, Anti-t TGA and Anti-t TGG levels remained.

Discussion

In the present study, we found the oxidative stress level, in comparison to the control group, to be higher in gluten-sensitive enteropathy patients. In subgroups of the patient group, oxidative stress level was approved to be higher in patients whose diet is incompatible with gluten free diet and in patients with significantly higher autoantibody levels compared to other gluten-sensitive enteropathy disease patients. To the best of our knowledge, the research of ours is preliminary in examining the oxidative stress level in gluten-sensitive enteropathy disease patients according to diet compliance and autoantibody levels.

Gluten-sensitive enteropathy disease is commonly seen all over the world and its nature widely varies, from asymptomatic to serious malabsorption. Although genetic predisposition and environmental factors are generally blamed in its etiology, it has been shown that impaired intestinal flora and autoimmunity may have a role as well (7, 8). However, despite of a large number of studies, there are still major uncertainties about its etiology and behavior. Oxidative stress, mainly characterized by the increase in free radicals depending on environmental factors and the consequent cellular damage, is thought to have an important place in the etiology of gluten-sensitive enteropathy disease (9). Oxidized metabolites and free radicals emerge in gluten-sensitive enteropathy disease as a result of oxidation in structures of proteins and lipids bound to certain gliadin peptides, which leads to oxidative imbalance (10, 11).

In the present study, we found that TAS level was lower and TOS level and OSI ratio were higher in gluten-sensitive enteropathy disease patients compared to the control group. This shows that oxidative stress was significantly higher in the patient group compared to the control group. High oxidative stress level found in the patient group has two possible explanations. The first and most likely cause is chronic inflammation, because we found the CRP level, which is indicative of chronic inflammation, to be higher in gluten-sensitive enteropathy patients compared to the control group. In addition, the positive correlation between CRP level and TOS and OSI ratio and the negative correlation between PON1 level and arylesterase level found as a result of correlation analysis support that increased oxidative stress level in gluten-sensitive enteropathy patients is associated with chronic inflammation. Increased oxidative stress associated with chronic inflammation can be explained with the activation of NF-κB pathway (1).

In addition, we found PON1 and arylesterase levels to be lower in the patient group compared to the control group. We believe that this is associated with high oxidative stress due to increased lipid peroxidation. Because arylesterase is a serum enzyme which protects low density lipoprotein against oxidative damage and is firmly bound to high density lipoprotein, we would expect the arylesterase enzyme level to be lower due to high consumption in case of oxidative stress. When similar studies in the literature are examined, it can be seen that oxidant parameters (lipid hydroperoxides, thiobarbituric acid-reactive substances, 8-hydroxyguanosine) increase (12–14) and antioxidant parameters (glutathione, PON1, PON3...) decrease (15, 16) in gluten-sensitive enteropathy disease. When we partitioned gluten-sensitive enteropathy patients into two as the patients agreeable with a gluten free eating routine and the patients resistant to a gluten free eating regimen, it was found that OSI rate was higher and PON1 level was lower in the patients agreeable with a gluten free eating routine. It is reported in the literature that the iNOS level, which increases in case of oxidative stress, is lower in celiac disease. It is seen that the iNOS level gradually

improves once the patient begins to comply with the gluten diet (17, 18). We believe that this may be due to a higher intestinal inflammation level in the patients resistant to a gluten free eating regimen (19) because it has been shown in previous studies that inflammation is higher and the gliadin toxicity-related oxidative stress level is significantly increased in patients who do not comply with the gluten diet (20). In addition, we found the CRP level, which is indicative of chronic inflammation, to be higher in the patients resistant to a gluten free eating regimen in comparison with patients whose diet is compatible with a gluten free regimen. As mentioned above, oxidative stress level is expected to be higher in case of high inflammation.

It was found in our study that oxidative stress level was higher in autoantibody positive gluten-sensitive enteropathy disease patients compared to autoantibody negative gluten-sensitive enteropathy disease patients. Although we have not found a study which examines the relation between autoantibodies and oxidative stress in gluten-sensitive enteropathy disease, there are studies which examine this relation for other diseases. Nanda et al. determined that oxidant radical rates were more advanced in autoantibody positive hypothyroid patients compared to autoantibody negative hypothyroid patients and it was determined that a positive correlation exists between autoantibodies and oxidant radicals (21). In another study, a negative relation was found between antithyroid peroxidase antibody and PON (22). This relationship may be directly associated with inflammation (22). It may also be related to the fact that increased ROS levels prevent tTG from further fragmentation through the ubiquitin-proteasome system (1). In this case, anti-t TG antibodies may increase together with tTG. In point of fact, a substantial relation was determined between gluten-sensitive enteropathy autoantibodies and TOS, OSI and arylesterase in our research which proposes the possible status of oxidative stress in gluten-sensitive enteropathy pathogenesis.

Our main limitations are the cross-sectional design of the study and lack of investigation on oxidized lipid parameters.

In conclusion, we found the oxidative stress level to be higher in gluten-sensitive enteropathy patients in comparison to the control group. Oxidative stress level showed differences in gluten-sensitive enteropathy patients depending on gluten diet content and autoantibody positivity. In point of fact, CRP and gluten-sensitive enteropathy autoantibodies are associated to oxidative stress parameters suggesting that oxidative stress may be effective in gluten-sensitive enteropathy pathogenesis. Imminent studies that explore the cell reinforcement treatment's impact on autoantibodies in gluten-sensitive enteropathy disease are important so as to understand whether oxidative stress has a role in gluten-sensitive enteropathy disease etiopathogenesis or not.

Acknowledgments: None.

Funding: None.

Conflict of interest statement

The authors stated that they have no conflicts of interest regarding the publication of this article.

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Received: February 6, 2017

Accepted: March 22, 2017