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Ferric reducing ability of plasma and assessment of selected plasma antioxidants in adults with celiac disease

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Abstract: Introduction: Oxidative stress with an excessive free radical production and a reduction in the activity of protective antioxidants is considered as one of the mechanisms responsible for gluten toxicity. However, its role in celiac disease (CD) is unclear.
Objectives: Evaluation of plasma nonenzymatic antioxidant capacity in patients with CD (both untreated patients and those receiving gluten-free diet [GFD]) by measuring the ferric reducing ability of plasma (FRAP) as well as assessing selected plasma antioxidants.
Patients and methods: The study included 169 adult patients: 48 patients with untreated active CD, 72 patients with CD on a GFD, and 49 healthy controls. In each group, we measured the serum levels of selected antioxidants (uric acid, bilirubin, albumin, and vitamin E) and used the FRAP assay to assess the total antioxidant capacity (TAC) of plasma. In each patient, serological and histopathological activity of CD was also evaluated.
Results: There were no significant differences in the TAC of plasma measured with the FRAP assay between the study groups. Patients with CD had higher uric acid levels compared with controls (p <0.001), while bilirubin levels were lower in patients with active disease than in controls (p <0.05). Serum vitamin E levels were lower in all patients with CD compared with controls (p <0.01).

Conclusions: The FRAP assay is not the method of choice for assessing the TAC of plasma in patients with CD. Owing to high serum uric acid levels, the FRAP assay results in these patients may be overestimated despite the reduced levels of other plasma antioxidants.

Key words: celiac disease, oxidative stress, FRAP assay, total antioxidant capacity, uric acid.

Introduction

Celiac disease (CD) is a chronic inflammatory disease of the small intestine that occurs in genetically predisposed individuals due to intolerance of proline- and glutamine-rich protein contained in wheat, barley, and rye. Activation of the innate and adaptive immune systems results in histological changes to the small intestine including intraepithelial lymphocytosis, crypt hyperplasia, and villous atrophy. This leads to impaired nutrient absorption from the gastrointestinal tract [1]. Activation of the immune system by gluten peptides is commonly believed to be an important mechanism involved in the pathogenesis and progression of CD [2]. However, recent studies have pointed to another possible pathogenetic mechanism of CD, namely, the direct cytotoxic effect of gluten on enterocytes. This effect is associated with oxidative stress, a phenomenon related to excessive free radical production and reduced activity of protective antioxidants [3]. As a result of reduced antioxidant protection, the inflamed intestinal mucosa may become more vulnerable to oxidative damage and its regenerative capacity may become impaired, which in turn compromises mucosal integrity. Moreover, the impairment of intestinal permeability enhances the toxic effects of environmental factors.

In physiological conditions, the damaging effects of free radicals are counteracted by the antioxidant system, which includes antioxidant enzymes (glutathione peroxidase, glutathione reductase, superoxide dismutase, and catalase), nonenzymatic antioxidants (glutathione, albumin, bilirubin, ceruloplasmin, and uric acid), as well as nutrient antioxidants (carotenoids; vitamins A, C, and E) [4].

There is an increasing body of evidence suggesting a relationship between oxidative stress and CD. Studies have revealed a reduced activity of peroxidase and glutathione reductase, as well as decreased glutathione levels, in the small intestine of patients with CD, while an increased activity of copper- and zinc-containing superoxide dismutase has been shown in patients with active CD [5, 6]. Reduced glutathione peroxidase activity may be partially explained by glutathione deficiency due to chronic inflammation of the small intestine [7]. Moreover, patients with CD show reduced absorption of vitamins, including vitamin E [8]. Finally, these patients have a reduced expression of the antioxidant and anti-inflammatory enzymes paraoxonase 1 and 3 in the small intestine [9].

The assessment of the severity of oxidative stress, including the evaluation of antioxidant capacity, in patients with CD may have therapeutic implications. It should be hypothesized that oral antioxidant supplementation may reduce the toxic effects of peptides contained in gluten on enterocytes and help alleviate histological lesions, thus exerting beneficial effects on the course of the disease. Therefore, antioxidant supplementation, in addition to a lifelong gluten-free diet [GFD], may prove to be an effective additional treatment in patients with CD, especially those who do not fully adhere to GFD. The latter patients are likely those with the most severe oxidative stress, thus antioxidant supplementation may additionally lower the risk of possible complications from CD.

There is currently no standard method of choice for assessing antioxidant activity. The available techniques include all assays for individual antioxidants as well as the assessment of the so called total antioxidant capacity (TAC), which is the measure of the antioxidant capabilities of all individual compounds in a studied sample.

The components of the plasma/serum antioxidant defense system include uric acid (35–65%), plasma protein (10–50%), vitamin C (~14%), and vitamin E (~7%) [10]. According to other authors, the plasma components contributing to its TAC include the -SH groups (52.9%), uric acid (33.1%), vitamin C (4.7%), total bilirubin (2.4%), vitamin E (1.7%), and others (5.2%) [11]. Considering the involvement of numerous plasma components in antioxidant defense, as well as a possible variation in their distribution, it is generally agreed that the assessment of the TAC is more valuable than that of individual antioxidants in pathological conditions that are strongly related to oxidative stress [11]. The evaluation of TAC may provide information on the individual susceptibility to oxidative stress.

There are 2 ways for antioxidants to scavenge free radicals: through hydrogen atom transfer or single atom transfer. Consequently, there are 2 types of TAC assay: 1) hydrogen atom transfer-based assays (e.g. oxygen radical absorbance capacity [ORAC], total reactive antioxidant potential [TRAP]), which measure the antioxidant capacity to scavenge free radicals by donating hydrogen, and 2) single atom transfer-based assays (e.g. ferric reducing ability of plasma [FRAP], cupric reducing antioxidant capacity [CUPRAC]), which measure the antioxidant capacity to *e*– transfer and free radical or metal ion reduction. TAC assays are widely used as they are easy and quick to perform [12]; they are based on assessing the free radical scavenging activity and reducing potential of antioxidants [12, 13]. They were originally developed for assessing the TAC of foods and nutrients. However, biological fluids contain not only nutrient antioxidants, but also different types of endogenous antioxidant enzymes, dietary antioxidants, and even oxidizing products [14, 15]. Therefore, the use of different assays for measuring the TAC of biological samples is being investigated in various diseases.

There have been few studies assessing the TAC with the above assays in patients with CD. Thus, the aim of this study was to assess plasma nonenzymatic antioxidants

in patients with CD, both untreated patients and those on a GFD, by using the FRAP assay. To our knowledge, this is the first such study in this patient group. Additionally, we measured the levels of selected individual components of the plasma antioxidant system: uric acid, albumin, bilirubin, and vitamin E.

Patients and Methods

The study included 169 outpatients of the Clinic and Department of Gastroenterology and Hepatology of the University Hospital in Kraków, Poland. The study group was divided into 3 subgroups. The first subgroup included 48 untreated patients with active CD (39 women and 9 men; mean age, 35.1 ± 11.07), both newly diagnosed individuals and those nonadherent to GFD, who were positive for celiac antibodies. The second subgroup included 72 patients with CD (60 women and 12 men; mean age, 40.74 ± 14.84) who remained on a GFD for at least 2 years (mean disease duration, 10.63 ± 8.3 years). The third subgroup included 49 healthy controls (41 women and 8 men; mean age, 40.13 ± 12.83 years) with functional gastrointestinal disorders, without endoscopic abnormalities of the upper gastrointestinal tract, without duodenal abnormalities on a histological biopsy, and with negative test results for anti-endomysial antibodies (anti-EmAs) or anti-transglutaminase antibodies (anti-TGAs).

CD was diagnosed on the basis of a clinical picture, positive test results for anti-EmAs or anti-TGAs, as well as typical histological findings on duodenal biopsy. All patients underwent an endoscopic examination of the upper gastrointestinal tract, and at least 4 duodenal biopsy specimens were obtained for a histopathological study. The Marsh classification was used to assess the degree of damage to intestinal mucosa [16].

On the day of the endoscopic examination, blood samples were obtained from the antecubital vein to assess the levels of anti-EmAs and anti-TGAs, C-reactive protein (CRP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (AP), γ-glutamyltransferase (GGT), total protein, albumin, bilirubin, uric acid, and vitamin E. Anti-TGA titers were measured using a commercial enzyme-linked immunosorbent assay (ELISA; Aesku Diagnostics GmbH, Germany), and the results were expressed as units (U)/ml of serum. The results exceeding 15 U/mL were considered positive. Anti-EmA titers were measured using an immunofluorescent assay, and the results exceeding 1:10 were considered positive. The exclusion criteria were as follows: diabetes, inflammatory bowel diseases, active infection, history of cancer, chronic hepatic and hepatobiliary diseases, chronic renal failure, alcohol abuse, smoking, chronic use of nonsteroidal anti-inflammatory drugs, as well as the use of antioxidant supplements, oral contraceptives, immunostimulants, and immunosuppressants.

All patients provided written informed consent to participate in the study, and the study protocol was approved by the Ethics Committee of Jagiellonian University

Medical College in Kraków (KBET/174/B/2013). The study was conducted in accordance with the principles laid down by the Declaration of Helsinki.

Blood tests

Blood samples were drawn in the morning in the fasting state. On the same day, the levels of CRP, ALT, AST, AP, GGT, total protein, albumin, bilirubin, and uric acid were measured. For vitamin E level measurement, the blood was centrifuged at 1000 *g* for 15 minutes at a temperature of 4°C. The obtained serum samples were stored at a temperature of –80°C until further analysis.

Measurement of vitamin E levels

Vitamin E levels were measured using the commercial General Vitamin E Elisa Kit E0922Ge, according to the manufacturer's instructions (Wuhan EIAab Science). To assess optic density at a wavelength of 450 nm, a microplate spectrophotometer was used (Stat Fax 2100 Awareness Technology Inc., Palm City, USA).

FRAP assay

The FRAP assay was conducted according to the method by Benzie and Strain [17], with slight modification, using 48-well plates, an automated reader (Synergy-2, BioTek, USA), and syringe rapid dispensers [18]. Each well was filled with 0.4 ml of acetate buffer (pH, 3.6), followed by 50 µl of plasma. The plate was conditioned at a temperature of 37°C for 2 minutes, and then 0.2 ml of reactive mixture (1:1 [vol./vol.] 20 mM ferric (III) chloride [$FeCl_3$·6H2O, POCh] and 10 mM tripyridyltriazine [Sigma] in 40 mM HCl [POCh]) was shaken for 30 seconds. Absorbance at a wavelength of 593 nm was measured with kinetic mode for 15 minutes. The method was calibrated by the use of ferrous sulphate pentahydrate $FeSO_4$·5H2O (POCh) standard solution with a concentration range of 0–1.5 mM. The reducing ability of the sample was expressed in ferrous ion equivalents in plasma (µmol Fe2+/L). Each sample was run in triplicate.

Statistical analysis

Descriptive statistics was used for the analysis of all studied parameters. The normal distribution of variables was assessed using the Kolmogorov–Smirnov test. The study groups were compared using the analysis of variance with the post hoc Tukey test for normally distributed variables and homogeneous variances or the Kruskal-Wallis test with the post hoc Dunn test for all the remaining variables. A *p* value of less than

0.05 was considered statistically significant. The statistical analysis was performed using the commercial software packages: STATISTICA PL v.10 (StatSoft, Tulsa, USA) and GraphPad Prism v.3.02 (GraphPad Software, San Diego, USA).

Results

Blood test results in controls and patients with celiac disease

The laboratory test results are presented in Table 1. The mean CRP levels were higher in patients with treated and untreated CD, as compared with controls ($p<0.01$ and $p<0.001$, respectively). The mean AST and ALT levels were significantly higher in patients with CD than in controls, without significant differences between the subgroups of treated and untreated patients with CD.

Table 1. Laboratory test results in the study subgroups: controls, patients with untreated celiac disease, and patients with treated celiac disease.

	Controls (n = 49)		Patients with CD (n = 120)			
			Treated CD (n = 72)		Untreated CD (n = 48)	
	Mean ± SD	Median	Mean ± SD	Median	Mean ± SD	Median
Albumin (g/L)	42.2 ± 3.9	41.0	44.1 ± 3.0[c]	44.0	43.1 ± 4.3	43.5
AP (U/L)	52.0 ± 20.2	46.0	61.5 ± 25.4[c]	55.0	64.4 ± 26.3[c]	52.0
ALT (U/L)	16.7 ± 6.4	16.0	22.3 ± 12.9[e]	20.0	26.8 ± 21.9[c]	20.0
AST (U/L)	17.1 ± 4.2	17.0	22.2 ± 6.2[d]	22.0	26.9 ± 16.3[d]	22.0
Bilirubin (µmol/L)	9.7 ± 3.7	9.2	9.1 ± 4.1	8.2	8.5 ± 5.4[e]	7.7
CRP	0.7 ±0.6	0.5	1.8 ± 4.2[c]	0.5	2.7 ± 7.1[d]	0.8
GGT (U/L)	20.4 ± 11.5	16.0	17.2 ± 15.3	14.0	36.6 ± 101.6	10.5[e]
Total protein (g/L)	74.1 ± 5.7	76.0	71.2 ± 5.0[e]	72.0	72.3 ± 7.5	73.0
Uric acid (µmol/L)	186.1 ± 37.2	188.0	254.9 ± 46.5[d]	247.0	260.3 ± 56.2[d]	240.0
Vitamin E (µmol/L)	48.3 ± 21.0	50.1	38.2 ± 36.2[c]	28.6	42.0 ± 36.7[c]	31.1

[a]p <0.001 versus treated CD; [b]p <0.05 versus treated CD; [c]p <0.01 versus controls; [d]p <0.001 versus controls; [e]p <0.05 versus controls

Abbreviations: ALT — alanine aminotrasferase; AP — alkaline phosphatase; AST — aspartate aminotrasnferase; CD — celiac disease; GGT — γ-glutamyltransferase

Hypertransaminasemia was observed in 10 patients (20.8%) with untreated active CD and in 9 patients (12.5%) with treated CD, but not in controls. The mean AP levels were lower in controls than in patients with CD ($p<0.05$). The mean GGT levels were higher in patients with active CD than in controls ($p<0.05$).

The mean albumin levels did not differ between the subgroups of patients with CD, but were lower in controls compared with patients on a GFD (p <0.01). The mean total protein levels were lower in patients with CD on a GFD than in controls (p <0.05). Serum uric acid levels were elevated only in patients with CD: in 4 patients (8.3%) with active CD and in 4 patients (5.5%) on a GFD. Uric acid levels were higher in patients with CD irrespective of the treatment status, as compared with controls (p <0.001), while bilirubin levels were lower in patients with active CD than in controls (p <0.05).

Serum vitamin E levels

Serum vitamin E levels were lower in untreated and treated patients with CD, as compared with controls (42.0 ± 36.7 μmol/L vs 48.3 ± 21.0 μmol/L and 38.2 ± 36.2 μmol/L vs 48.3 ± 21.0 μmol/L, respectively, p <0.01).

Vitamin E deficiency, defined as the level below 16.2 μmol/L, was observed for more than 15.4% of patients with CD and 9.5% of controls. Optimal vitamin E levels (>30 μmol/L), which is required for protection against cardiovascular disease and cancer, were shown for less than 46% of patients with CD and more than 83% of controls.

Total antioxidant capacity expressed as the ferric reducing ability of plasma

There were no significant differences between the groups in terms of the TAC measured with the FRAP assay (Table 2).

Table 2. Total antioxidant capacity measured with the FRAP assay in controls, patients with untreated celiac disease, and patients with treated celiac disease.

	Controls (n = 49)		Patients with CD (n = 120)			
			Treated CD (n = 72)		Untreated CD (n = 48)	
	Mean ± SD	Median	Mean ± SD	Median	Mean ± SD	Median
FRAP (μmol Fe2+/L)	787.1 ± 157.9	746.1	824.6 ± 153.1	802.3	824.0 ± 181.7	767.1

Abbreviations: FRAP — ferric reducing ability of plasma; others — see Table 1

Anti-endomysial and anti-transglutaminase antibody titers and intestinal mucosal damage

Patients in the control group tested negative for anti-EmA and anti-TGA titers (Table 3). Significantly higher antibody titers were revealed for untreated patients with CD, while they were significantly lower in patients treated with a GFD compared

with those with untreated disease. The degree of damage to intestinal mucosa was the lowest in the control group, while it was higher in patients on a GFD and the highest in patients with active CD. The differences between the groups were significant (Table 3).

Table 3. Clinical characteristics of the study groups: celiac disease antibody titers and the degree of intestinal mucosal damage on histological examination.

	Controls (n = 49)	Treated CD (n = 72)	Untreated CD (n = 48)
Antibody titer			
0	49 (100%)	70 (97.2%)	2 (4.2%)
1	0	2 (2.8%)	13 (27.1%)
2	0	0	15 (31.3%)
3	0	0	18 (37.5%)
Antibody titer			
mean ± SD	0	0.02 ± 0.2	2.02 ± 0.9[a,b]
median	0	0	2
Degree of intestinal mucosal damage[1]			
Normal mucosa — 0	47 (95.9%)	30 (41.7%)	3 (6.2%)
Marsh 1 — 1	2 (4.1%)	26 (36.1%)	10 (20.8%)
Marsh 2 — 2	0	0	0
Marsh 3a — 3	0	6 (8.3%)	9 (18.8%)
Marsh 3b — 4	0	9 (12.5%)	14 (29.2%)
Marsh 3c — 5	0	1 (1.4%)	12(25%)
Degree of intestinal mucosal damage[1]			
mean ± SD	0.04 ± 0.2	1.2 ± 1.5	3.2 ± 1.6
median	0.0	1.0[a]	4.0[a,b]

Data are expressed as number (percentage) of patients or as mean ± SD and median.

Antibody titer:0, negative; 1, low (anti-TGA <3 × ULN; anti-EmA [+]); 2, high (3 × ULN < anti-TGA <10 × ULN; anti-EmA [++]); 3, very high (anti-TGA >10 × ULN; anti-EmA [+++])

[1]classified according to the Marsh classification; each stage was scored from0 (normal mucosa) to 5 (total villous atrophy)

[a]p <0.001 vs control; [b]p <0.001 vs treated CD

Abbreviations: anti-EmA — anti-endomysial antibodies; anti-TGA — anti-transglutaminase antibodies; ULN — upper limit of normal; others — see Table 1

Discussion

The pathogenesis of CD has not been fully elucidated so far. Inflammation and oxidative stress associated with higher levels of reactive oxygen species and reduced antioxidant defense seem to be involved in the molecular mechanism of the disease. Literature data provide an increasing body of evidence for a significant association between oxidative stress and CD, and particularly for reduced antioxidant activity in patients with CD, independent of age.

In diseases that are significantly associated with oxidative stress, the TAC is usually measured to assess the individual susceptibility to oxidative stress, which may help elucidate the pathogenesis of the disease and implement appropriate treatment. However, there have been few studies assessing the TAC in patients with CD. Ferreti *et al.* [19] showed that the TAC measured with the ORAC assay is significantly lower in patients with CD, irrespective of diet, as compared with controls. Moreover, patients on a GFD showed a significantly higher TAC in comparison with untreated individuals, although the levels did not reach those observed in controls. The results suggest that GFD can only partially improve intestinal mucosal function.

Ersin Sayar *et al.* [20] also showed lower TAC, assessed using the method described by Erel [11], in pediatric patients with CD in comparison with healthy controls. Moreover, the TAC was significantly higher in patients with CD after starting treatment with a GFD, as compared with the baseline values.

To our knowledge, this study is the first to use the FRAP assay to assess the TAC in patients with CD. This method was previously used by Moslemnezhad *et al.* [21] to assess the TAC in patients with Alzheimer disease. The authors showed significantly lower results of the FRAP assay in patients than in controls.

Surprisingly, we did not observe differences in the FRAP assay results between patients with CD and the control group. This finding can be explained by the different composition of individual antioxidants in the FRAP assay in comparison with the other assays for TAC assessment. This is in agreement with the results of Lee *et al.* [22], who compared 4 different assays for assessing the TAC of plasma (including the 2,2'-azino-bis-3-ethylbenzthiazoline-6-sulfonic acid [ABTS] radical scavenging assay, 1,1-diphenyl-2-picrylhydrazyl [DPPH] radical scavenging assay, FRAP, and ORAC), in relation to the 8-isoprostane concentration in urine. They showed a higher correlation of ABTS results with the 8-isoprostane concentration than in the case of the other assays. They also showed a positive correlation between the ABTS and FRAP assay results, which is in line with previous observations [23–25]. This phenomenon can be partially explained by a significant antioxidant contribution of uric acid and ascorbic acid to the TAC measured with the 2 above assays [15, 24, 26]. Not all tests measure the same antioxidants. In humans, ascorbic acid, α-tocopherol, and, most importantly, uric acid are the main components of the TAC measured by the FRAP assay, while

thiols and albumin — of that measured by the CURPAC assay. The FRAP method assumes that if an antioxidant can reduce the ferric ion Fe3+, then it is also capable of reducing the ferric ion Fe2+. Uric and ascorbic acids are typical compounds that show both these activities; therefore, the results of the FRAP assay can be significantly associated with their plasma concentrations [27].

Studies conducted to date have shown reduced vitamin E and ascorbic acid levels in patients with CD [8, 28], which can be explained by impaired absorption of nutrient antioxidants due to intestinal mucosal damage. These observations are corroborated by the results of our study. Unlike Hozyasz *et al.* [29], who reported significantly lower serum levels of α-tocopherol in untreated patients with CD in comparison with those on a GFD, our current and previous studies [30] revealed significantly lower serum vitamin E levels in both subgroups of patients with CD, irrespective of the diet. Importantly, the GFD did not result in a significant increase in vitamin E levels.

In our previous study [30], we evaluated uric acid as a nonenzymatic antioxidant in the serum of adult patients with CD. Both in the previous and in the current studies, we showed significantly higher uric acid levels in patients with CD than in controls despite the lack of metabolic disorders that could have otherwise account for this finding. Uric acid is considered a marker of oxidative stress, but also as a protective factor with antioxidant activity [31, 32]. It is one of the most important antioxidants in human biological fluids, as it is responsible for neutralizing more than 50% of free radicals in blood [33].

Elevation in uric acid levels is a physiological response to oxidative stress [34]. It is possible that hyperuricemia is a compensatory or protective mechanism for the prevention or correction of oxidative damage. Our results seem to suggest that the elevated uric acid levels in patients with CD in comparison with controls are caused by oxidative stress and that uric acid may function here as an antioxidant. Its role in diseases related to oxidative stress has not been fully elucidated, but experimental and clinical studies have confirmed its antioxidant activity in vivo [33]. It is effective in scavenging free radicals but also in stabilizing ascorbic acid levels in serum [35].

The significant contribution of uric acid to the TAC measured by the FRAP assay, as well as the significant increase in uric acid levels in both subgroups of patients with CD in our study, may explain why we did not observe significant differences in the FRAP assay results between patients with CD and controls despite the fact that other active antioxidants in plasma were lower than in controls.

An interesting finding was the fact that the mean AST and ALT levels were significantly higher in both celiac subgroups, as compared with controls. This is in line with the results of a study by Castillo *et al.* [36], who showed that liver enzyme levels were higher at diagnosis in 40% to 50% of adult and pediatric patients with CD. The risk of nonalcoholic fatty liver disease is higher in patients with CD than in the general population not only in the first year but also in the 15 years of diagnosis

[37]. One of the explanations of hypertransaminasemia in patients with CD is altered intestinal permeability, as it is responsible for the passage of intestinal toxins to the liver through the portal venous system. This may produce an inflammatory reaction in the liver due to oxidative stress. However, further studies are needed to explain the pathogenesis of hepatitis related to gluten sensitivity as well as the involvement of oxidative imbalance in this condition.

Unlike transaminase levels, the bilirubin level in active CD was significantly lower in patients with active CD, as compared with controls. Like α-tocopherol, bilirubin is a well-known antioxidant [38]. It has a similar capability of reducing oxidized phospholipids as antioxidant vitamins [39]. Significantly lower bilirubin levels were reported in patients with severe asthma in an Australian study [40], which suggested the modulatory effect of antioxidant vitamins and bilirubin on inflammation in asthma. This finding is in line with our results, suggesting altered bilirubin levels in response to oxidative stress.

In summary, the lack of significant differences between the study groups in terms of the FRAP assay results, as observed in our study, does not translate into the lack of reduced TAC in patients with CD. On one hand, it may suggest a significant role of uric acid as one of the main plasma antioxidants in CD, but on the other hand, it may also suggest significantly reduced activity of the antioxidants that are not measured by the FRAP assay.

Considering the above conclusion, it seems justified to question the appropriateness of the term "total antioxidant capacity" [41], as this parameter never actually reflects the TAC of the body. Plasma or serum TAC does not reflect the antioxidant capacity of the whole blood, as it does not include antioxidant enzymes in blood cells and vascular walls; therefore, "nonenzymatic antioxidant capacity" seems to be a more appropriate term to describe the TAC [42]. Our results also indicate that in patients with CD it is enzymatic antioxidants that play the major role in oxidative imbalance. The reduced activity of these antioxidants in this population has been reported before [5, 6, 9].

In conclusion, the study of antioxidant capacity in patients with CD should primarily include the assessment of the activity of enzymatic antioxidants in addition to plasma nonenzymatic antioxidants. Moreover, the assessment of antioxidant capacity in patients with CD should not be limited to the use of the FRAP assay, as this may result in false results. Studies comparing the different assays for TAC measurement in patients with CD are needed to select the method of choice that would best reflect susceptibility to oxidative stress in these patients. Assays for the assessment of plasma TAC may be particularly useful in clinical practice, because they are easy to perform, quick, and cost-effective. They may prove valuable as an initial diagnostic test or as a tool for therapy monitoring in patients with CD.

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Contribution statement

AP-G and MZ-W conceived the idea of and designed the research; AP-G carried out the literature research and study selection; the qualities of included studies were carried out by MZ-W; AP-G, AP-B, PP, PZ and MK performed the research; PP and PZ analyzed the data; AP-G and MZ-W wrote the paper; TM revised the manuscript for final submission.

Conflict of interest

The authors declare that they have no conflict of interest.

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