RESEARCH LETTER

Oral vitamin E supplementation in reducing nitrosative stress in adults treated for celiac disease

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Introduction The pathogenesis of celiac disease (CD) is complex and has not been fully elucidated yet. It has been postulated that oxidative stress, because of an increase in the concentration of reactive oxygen species and antioxidant capacity reduction, is one of the processes possibly involved in gliadin toxicity.[1] Oxidative imbalance induced by gliadin peptides in enterocytes activates the transcription of proinflammatory cytokines and enzymes, such as inducible nitric oxide synthase, which in turn increases the production of nitric oxide (NO) metabolites favoring oxidative stress.[1]

Based on our previous study,[2] we hypothesized that persistent nitrosative stress despite gluten-free diet (GFD) may be responsible for persistent histopathologic changes and that GFD is only partially able to improve oxidative imbalance. Hence, serum NO levels seem to be useful as a marker of treatment efficacy, and alterations in these levels could indicate CD activity. It is possible that oral antioxidant supplementation may decrease the toxic effects of peptides. In the current study, we aimed to evaluate the effect of treatment with oral vitamin E on oxidative imbalance in adult patients with CD on GFD. For that purpose, we assessed the fasting plasma levels of nitrate as a marker of endogenous NO production and oxidative stress. Moreover, we monitored the individual components of antioxidant capacity. To the best of our knowledge, this is the first study evaluating the effects of oral vitamin E supplementation on oxidative imbalance in patients with CD.

Patients and methods The study included 18 patients (mean [SD] age, 49.1 [13.7] years) with CD with negative celiac antibody titers. There were 16 women (88.9%; mean [SD] age, 46.8 [12.8] years) and 2 men (11.1%; mean [SD] age, 67 [2.8] years). All patients were on GFD for at least 2 years and were additionally supplemented with oral vitamin E at a dose of 400 mg twice daily for 3 months. Each patient underwent blood tests twice: before vitamin E supplementation and after 3 months of treatment. Blood was collected by venipuncture to assess the concentrations of NO, complete blood count, iron, alanine aminotransferase (ALT), aspartate aminotransferase, alkaline phosphatase, γ-glutamyltransferase, antioxidants: vitamins D and E, glutathione peroxidase-3 (GPx3), uric acid, albumin, ferritin, and bilirubin.

All patients provided written informed consent to participate in the study and the study protocol was approved by the Ethical Committee of Jagiellonian University Medical College in Kraków, Poland (KBET/174/B/2013).

Nitric oxide, vitamin E, and glutathione peroxidase-3 assays The levels of NO and vitamin E were evaluated using commercially available enzyme-linked immunosorbent assay kits according to the manufacturer's protocol (for NO, Parameter Total Nitric Oxide and Nitrate/Nitrite KGE001, R&D Systems, Minneapolis, Minnesota, United States; for vitamin E, General Vitamin E Elisa Kit E0922Ge, Wuhan EIAab Science, Wuhan, China). A spectrophotometric microplate reader (Stat Fax 2100, Awareness Technology, Inc., Palm City, Florida, United States) was used to determine the optical density at 540 nm and 450 nm, respectively. Plasma GPx3 activity was evaluated with hydrogen peroxide as the substrate, as described elsewhere.[3]

Statistical analysis Descriptive statistics were calculated for all parameters. The normality

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of parameters distribution was checked by the Kolmogorov–Smirnov and Lilliefors tests. The differences between repeated measurements were tested using either the paired *t* test (for parameters with normal distribution) or the Wilcoxon signed-rank test (for parameters with nonnormal distribution). Differences with a *P* value of less than 0.05 were considered significant. Statistical analysis was performed using the Statistica software, version 12 (Statsoft, Tulsa, Oklahoma, United States).

Results The mean levels of ferritin, uric acid, bilirubin, and albumin did not differ between baseline and follow-up at 3 months. None of the participants had vitamin E deficiency. Interestingly, there was also no significant difference in the levels of vitamin E between baseline and follow-up at 3 months. The white blood cell count was significantly lower at 3 months compared with baseline values. On the other hand, vitamin E supplementation did not result in any differences in platelet count, red blood cell count, hemoglobin concentrations, mean corpuscular volume, or serum iron levels. The mean serum NO level, GPx3 activity, and ALT levels were significantly lower at 3 months compared with baseline. Unlike ALT, the mean aspartate aminotransferase, alkaline phosphatase, and γ-glutamyltransferase levels did not differ between the 2 timepoints. Finally, serum vitamin D levels were significantly elevated after supplementation. Laboratory test results showing significant differences are presented in TABLE 1.

Discussion Nitrosative stress seems to be involved in the pathogenesis of CD. A higher production of NO metabolites and the resultant nitrosative stress promote the impairment of tight junctions in the small intestine of patients with CD, perhaps by downregulating the expression of zonula occludens 1.[4] In our previous study,[2] we reported significantly higher serum NO levels in patients with active CD compared with controls. We also showed a positive correlation between the degree of intestinal mucosal damage and serum NO levels in patients with CD.[2] These results are in line with the findings from another study.[5] Hence, we suggested that serum NO levels might be a marker of treatment efficacy. On the other hand, we reported that nitrosative stress in these patients persists despite GFD as well as serological and clinical remission and it may be responsible for persistent histopathologic changes.[2] The results of the current study demonstrated that vitamin E supplementation in treated patients with CD reduced the concentration of NO, which shows that it may have a beneficial effect on oxidative imbalance.

To our knowledge, this is the first study to assess the effect of vitamin E supplementation in treated patients with CD. In the previous study,[2] we found significantly reduced serum concentrations of vitamin E in patients with CD regardless of compliance with the diet.

Assuming that increased serum levels of NO are probably a marker of an ongoing inflammation in the small intestine in untreated patients with CD, the reduction of its concentration by vitamin E supplementation can provide a favorable effect on the degree of intestinal damage. This finding is particularly important in the light of a study by Dickey et al,[6] who reported that more than 80% of patients with persistent villous atrophy had normal levels of serological markers. It is also important considering that, in general, the rates of mucosal healing on GFD were reported to be only 57% to 76%.[7] We observed a significant increase in vitamin D concentrations after vitamin E supplementation. The improvement of the intestinal barrier may facilitate the absorption of fat-soluble vitamin D.

In contrast to vitamin D, we observed no increase in the concentration of other nonenzymatic antioxidants tested.

The results of previous studies demonstrated a reduced serum and intestinal activity of GPx in patients with CD when compared with healthy controls.[2,8] In our study, we showed decreased serum concentrations of GPx3 in patients with CD after vitamin E supplementation. There is strong evidence that GPx3 is transcriptionally upregulated by oxidative stimuli and as part of oxidative stress response.[9] In this case, it can be assumed that the reduction of GPx3 activity may be secondary to less severe nitrosative stress associated with reduced NO concentrations.

Excessive production of NO may lead to increased mucosal permeability facilitating the passage of intestinal toxins to the liver and may produce an inflammatory reaction in the liver caused by oxidative stress. This mechanism appears to be crucial in the pathogenesis of nonalcoholic fatty liver disease, the risk of which increases in CD. A systematic search of randomized controlled trials suggested that adjuvant vitamin E therapy offers a significant biochemical and histologic improvement in adult patients with nonalcoholic fatty liver disease.[10] These observations are in line with the results of our study. The use of vitamin E decreased the level of ALT in treated patients with CD. Noteworthy, a high dose of vitamin E was associated with an increase in the risk of high blood pressure, hemorrhagic stroke, all-cause mortality, cerebral infarction,[11] and prostate cancer.[12]

A limitation of our study is a small sample size and the fact that we did not assess the pattern of concomitant alterations in the intestinal mucosa. Therefore, future studies of a larger number of patients with CD are needed, including tissue examination and analysis of more parameters of oxidative stress. The optimal safe and effective dose of vitamin E remains to be determined.

In summary, our results indicate that supplementation with antioxidant vitamin E in patients with CD may reduce nitrosative stress and can

TABLE 1 Blood test results of the study patients at baseline and after 3 months of vitamin E supplementation

Parameter	Baseline		At 3 months		Difference between the mean values of 2 measurements (3 months – baseline)	*P* value
	Mean (SD)	Median (IQR)	Mean (SD)	Median (IQR)		
NO, µmol/l	99.3 (47.4)	80.1 (64.6–147)	66.5 (46)	62.8 (33.3–87.2)	–32.8	0.02
WBC, × 10^3/µl	5.15 (1.06)	5.37 (4.10–5.92)	4.65 (0.77)	4.50 (4.12–5.25)	–0.5	0.04
RBC, × 10^6/µl	4.54 (0.24)	4.6 (4.28–4.76)	4.52 (0.24)	4.53 (4.35–4.72)	–0.02	0.06
Hemoglobin, g/dl	13.6 (0.7)	13.4 (13.1–14.2)	13.5 (0.7)	13.4 (13.1–14.3)	0	0.72
Platelets, × 10^3/µl	216.4 (37.9)	221 (182–259)	214 (40.2)	220.5 (179–242)	–2.4	0.63
Fe, µmol/l	20.3 (7.5)	19.7 (14.3–21.2)	17.7 (7.1)	13.7 (12.1–22.9)	–2.7	0.13
ALT, U/l	23.7 (13.1)	19.5 (17–22)	20.1 (11.2)	16.5 (14–22)	–3.6	0.02
AST, U/l	20.6 (5.6)	20.5 (17–23)	19.8 (5.3)	19.5 (17–23)	–0.7	0.48
Alkaline phosphatase, U/l	46.3 (9.7)	46 (41–51)	47.1 (8.1)	48 (40–50)	0.7	0.6
γ-Glutamyltransferase, U/l	20.1 (17.9)	11.5 (9–26)	20.1 (16.8)	14 (9–24)	–0.1	0.13
GPx3, U/l	652.4 (68)	659.2 (604.5–694.5)	589.2 (69.9)	562.7 (553–610.9)	–63.1	0.01
Total vitamin D, ng/ml	23.9 (8.6)	22.1 (17–31)	26 (8)	27.2 (18.2–31.2)	2.1	0.01
Vitamin E, µmol/l	66.2 (8)	66.6 (61.4–72.7)	68.7 (8.7)	67.3 (63.6–75.5)	2.5	0.39
Uric acid, µmol/l	235.1 (62.5)	226 (205–280)	222.1 (61.8)	203.5 (188–274)	–12.9	0.18
Albumin, g/l	45.3 (2.1)	44.8 (44–47.3)	45.1 (2.7)	44.7 (43–47.6)	–0.2	0.67
Ferritin, µg/l	36.4 (21.1)	32 (24–48)	33.8 (20.8)	30 (20–45)	–2.6	0.06
Bilirubin, µmol/l	8.2 (2.4)	8.8 (6.3–9.7)	7.5 (2.6)	7.1 (5.7–9.1)	–0.71	0.26

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; Fe, iron; GPx3, glutathione peroxidase 3; IQR, interquartile range; NO, nitric oxide; WBC, white blood cells; RBC, red blood cells

have a positive effect on the regression of inflammation of the small intestine, which persists despite GFD. By enhancing the intestinal barrier, vitamin E may have a beneficial effect on other concomitant diseases, including fatty liver.

ARTICLE INFORMATION

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CONFLICT OF INTEREST None declared.

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