

# Analysis of the concentration of vitamin E in erythrocytes of patients with celiac disease

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## Abstract

**Introduction:** Consumption of gluten proteins leads to an enteropathy characterised by lymphocytic infiltration of mucous membrane, crypts hypertrophy, and atrophy of villi. Enteropathy leads to disturbances in the immune system as well as secondary deficiency of vitamin E.

**Aim:** Analysis of the concentration of vitamin E in erythrocytes of patients with celiac disease.

**Material and methods:** Three experimental groups were distinguished among 77 patients with histologically confirmed celiac disease (mean age: 17 years): those who strictly respected gluten-free diet (group I,  $n = 48$ ), patients breaking dietary recommendations (group II,  $n = 22$ ), and those with newly diagnosed disease (group III,  $n = 7$ ). Additionally, a control group consisting of healthy individuals with negative serological markers of celiac disease was formed (group IV,  $n = 20$ ). Vitamin E concentration was determined by high performance liquid chromatography with ultraviolet detector.

**Results:** Significantly lower average concentration of vitamin E was demonstrated in erythrocytes in all examined groups of patients with celiac disease compared to the control group. Among the patients with celiac disease, the highest average concentration of vitamin E in erythrocytes was observed in the group who respected the gluten-free diet, a little lower in patients who violated dietary recommendations, and lowest among patients with newly diagnosed disease. These relationships, however, were not statistically significant.

**Conclusions:** Patients with celiac disease are at risk of vitamin E deficiency irrespective of their diet. Vitamin supplementation should be considered in their case, especially immediately after diagnosis of the disease and in case of breaking a gluten-free diet regime.

## Introduction

Celiac disease is a systemic disorder of the immunological background caused by gluten and related prolamins occurring in genetically predisposed individuals [1]. Gluten proteins consumption leads to an enteropathy characterised by lymphocytic infiltration of mucous membrane, crypts hypertrophy, and atrophy of small intestine villi [2]. Symptoms of gastrointestinal tract disorders: chronic diarrhoea, steatorrhoea, recurrent abdominal pain, nausea and vomiting, as well as sudden or unintended weight loss, are observed in

affected patients. Coexisting enteropathy also leads to disturbances in the immune system, as well as secondary deficiency of micro- and macroelements and fat-soluble vitamins [3]. It is supposed that vitamin E deficiency in patients with celiac disease may be a reason for their increased risk of development of cancer, neurological diseases, and reproductive system disorders [4].

Vitamin E (tocopherol) is present in nature in eight natural forms, and  $\alpha$ -tocopherol is the most active among them. Due to its wide distribution in nature

(plant oils, cereals, eggs, and green leafy vegetables), its deficiency in humans is rare. Chronic and long-term deficiency of vitamin E may accompany malabsorption syndromes, including celiac disease [5]. Tocopherol is an important antioxidant preventing oxidation of the structures comprising cell membranes, impeding the formation of free radicals, and accelerating their disposal [6]. Moreover, it strengthens the walls of blood vessels and prevents erythrocytes from premature decay. Core-cerebellar disorders resulting from intensified oxidation processes in neuron cell membranes may occur in cases of vitamin E deficiency. The literature data often report patients suffering from celiac disease with vitamin E deficiency and concomitant neurological disorders (e.g. cerebellar ataxia, sensory neuropathy, epilepsy, and migraine) [7–10]. It is believed that the best determinant of vitamin E availability at the cellular level is an examination of the  $\alpha$ -tocopherol level directly in erythrocytes (Erbc). Tocopherol concentration in erythrocytes in the range from 2.5 to 5  $\mu\text{M/l}$  is considered the proper value.

## Aim

The aim of the study was to analyse the concentration of vitamin E in erythrocytes of patients with celiac disease.

## Material and methods

Three experimental groups were distinguished among 77 patients with histologically confirmed celiac disease, including 48 women and 29 men, aged from 5 to 31 years (mean age: 17 years): those who strictly respected gluten-free diet (group I,  $n = 48$ ), patients breaking dietary recommendations (group II,  $n = 22$ ), and those with newly diagnosed disease (group III,  $n = 7$ ). Additionally, a control group consisting of healthy individuals with negative serological markers of celiac disease was formed (group IV,  $n = 20$ ). None of the patients was additionally supplemented with vitamin E. The average concentration of vitamin E in erythrocytes in the examined groups was compared in the study. Values from 2.5 to 5  $\mu\text{M/l}$  were assumed to be proper

tocopherol concentrations in the erythrocytes. Vitamin E concentration was determined by high-performance liquid chromatography with an ultraviolet detector (Knauer, Germany) [11].

## Statistical analysis

Statistical analysis was conducted using Statistica 10.0 software (StatSoft®). Examination of the normality of Erbc variable distribution demonstrated that it is close to a normal distribution. The changeability of that parameter was described using an arithmetic mean ( $M$ ) and standard deviation ( $SD$ ). The differences between the examined groups were evaluated using Fisher-Snedecor analysis of variance. A test probability of  $p < 0.05$  was accepted as statistically significant. In the table, the presented results include only statistically significant  $p$ -values with an indication of which comparisons they concerned.

## Results

A significantly lower average concentration of vitamin E was demonstrated in erythrocytes in all examined groups of patients with celiac disease compared to the control group (group I vs. group IV: 2.13 vs. 3.15;  $p < 0.0001$ ) (group II vs. group IV: 1.92 vs. 3.15;  $p < 0.0001$ ) (group III vs. group IV: 1.76 vs. 3.15;  $p < 0.0005$ ) (Table I). Among the patients with celiac disease, the highest average concentration of vitamin E in erythrocytes was observed in the group who respected the gluten-free diet (2.13  $\mu\text{M/l}$ ), a little lower in patients who violated dietary recommendations (1.92  $\mu\text{M/l}$ ), and lowest among patients with newly diagnosed disease (1.76  $\mu\text{M/l}$ ). These relationships, however, were not statistically significant. A proper concentration of vitamin E in the group of patients on a strict gluten-free diet was found in 19 out of 48 patients (39.6%) (Table II). In the patients not respecting the dietary recommendations, proper tocopherol concentration was observed in 6 out of 22 examined patients (27.3%). Lowered concentration of vitamin E in erythrocytes was observed in all the patients with newly diagnosed celiac disease and in 1 patient from the control group.

**Table I.** Analysis of average concentration of vitamin E in all groups of patients

Parameter	Erbc [ $\mu\text{M/l}$ ]				P-value
	Group I (strict gluten-free diet)	Group II (mixed diet)	Group III (newly diagnosed celiac disease)	Group IV (healthy)	
N	48	22	7	20	0.0001 (I vs. IV)
Mean	2.13	1.92	1.76	3.15	0.0001 (II vs. IV)
SD	0.83	0.72	0.26	0.33	0.0005 (III vs. IV)

**Table II.** Concentration of vitamin E in patients with celiac disease

Erbc [ $\mu\text{M/l}$ ]	Group I (strict gluten-free diet) n (%)	Group II (mixed diet) n (%)	Group III (newly diagnosed celiac disease) n (%)
< 2.5	29 (60.4)	16 (72.7)	7 (100)
2.5–5.0	19 (39.6)	6 (27.3)	0 (0)

## Discussion

The clinical picture in the classic form of celiac disease is dominated by diarrhoea, steatorrhoea, weight loss, and growth disorders. An increase in the prevalence of the non-classical form of the disease, characterised by gastrointestinal symptoms other than in the classical form and clinical symptoms beyond the gastrointestinal tract, including the nervous system, has been observed in recent years. Neurological disorders are observed in approximately 6–12% of patients with celiac disease [12]. The main role in the pathogenesis of these disorders is played by a secondary deterioration of malabsorption of vitamins responsible for the development and function of the nervous system, including vitamin E. According to another theory, anti-gliadin antibodies can directly damage cells of the nervous system and cellular defects may further be favoured by accompanying dysfunctions in the immune system activity [13, 14].

In the 1960s and 1970s, some publications reported a shortage of tocopherol in patients with celiac disease, who do not follow a gluten-free diet [15, 16]. In 2003, Hozyasz *et al.* examined vitamin E concentrations in serum and erythrocytes of patients with celiac disease [17]. The study included 18 patients on a strict gluten-free diet and 12 patients not respecting the diet. The authors demonstrated statistically significantly lower concentrations of vitamin E in serum (13.7  $\mu\text{M/l}$  vs. 20  $\mu\text{M/l}$ ;  $p < 0.02$ ) and erythrocytes (1.70  $\mu\text{M/l}$  vs. 2.89  $\mu\text{M/l}$ ;  $p < 0.001$ ) of patients breaking dietary recommendations.

In this study, the highest mean concentrations of vitamin E (2.13  $\mu\text{M/l}$ ) were observed in the group of patients strictly respecting a gluten-free diet; however, no statistical differences were obtained compared to the group breaking the diet and newly diagnosed patients. These results might be the reason of the fact that nowadays many patients have an atypical form of celiac disease. Mucosal damage in atypical cases may be very mild and may lead to some absorption problems, without malabsorption syndrome.

In the study by Hozyasz *et al.* [17],  $\alpha$ -tocopherol concentration in all untreated patients was lower than the lower limits of the standard. Moreover, a slight de-

ficiency of vitamin E was found only in 2 cases in the group of patients respecting a gluten-free diet. Proper concentration of vitamin E in this study was only observed in about 40% of the patients respecting the diet and in about 30% of the patients breaking dietary recommendations.

Szaflarska-Popławska *et al.* [18] demonstrated statistically significantly lower tocopherol concentrations in blood serum of patients not obeying the diet, compared to patients following dietary recommendations in celiac disease. Comparing the patients on a diet with the group of healthy subjects, there were no statistically significant relationships, which is significantly different from the results obtained in this study.

The study by Imam *et al.* [19] examined the frequency of fat-soluble vitamins deficiency in patients with newly diagnosed celiac disease. Tocopherol shortage was only demonstrated in 2 among 83 examined patients. Different results were obtained in this study, since vitamin E deficiency was observed in all the patients at the moment of celiac disease recognition.

## Conclusions

Patients with celiac disease are at risk of vitamin E deficiency irrespective of their diet. Vitamin E supplementation should be considered in their case, especially immediately after diagnosis of the disease and in case of breaking a gluten-free diet regime.

## Conflict of interest

The authors declare no conflict of interest.

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