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Vitamin E status and its determinants in patients with cystic fibrosis

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ABSTRACT

Purpose: The risk of vitamin E deficiency is of primary concern in cystic fibrosis patients. However, early diagnosis and routine vitamin E supplementation can lead to its normal or even high levels. In the present study, we assessed vitamin E status in a large group of cystic fibrosis patients. Moreover, we also aimed to establish determinants of its body resources in cystic fibrosis patients.

Material and methods: The study group comprised 211 cystic fibrosis patients aged from 1 month to 48 years. In all of them serum α-tocopherol concentration was analyzed using high-performance liquid chromatography.

Results: Median vitamin E concentration was 9.9 μg/ml (1st–3rd quartile: 7.5–13.5). Vitamin E deficiency was found in 17 (8.0%) and high levels were documented in 24 (11.4%) participants. Patients with and without vitamin E deficiency did not differ significantly with respect to age, standardized body weight and height, FEV1, albumin concentration and vitamin E supplementation dose. However, vitamin E deficiency appeared more frequently in participants without vitamin E supplementation. Moreover, in multiple linear regression analysis pancreatic insufficiency, severe *CFTR* gene mutation and vitamin E dose, were potentially defined as determinants of vitamin E concentration.

Conclusions: Vitamin E deficiency in cystic fibrosis patients is rather rare nowadays. Excessive vitamin E levels seem to be more frequent. Vitamin E status wasn't documented to be strictly related to clinical determinants. Beyond vitamin E supplementation, exocrine pancreatic function and *CFTR* gene mutations may have had an impact on the vitamin E body resources in cystic fibrosis patients.

1. Introduction

Cystic fibrosis (CF) is the most frequent autosomal recessive disease which is caused by mutations in *CFTR* gene [1,2]. The membrane protein is a product of *CFTR* gene transcription and functions as an ion channel for sodium, chloride and water [3]. Dysfunctional protein leads to the accumulation of mucus on epithelial surfaces in many organs of respiratory, digestive and reproduction systems [4]. Accordingly, pancreatic insufficiency is one of the many consequences occurring in 85–90% CF patients and leads to fat-soluble vitamins deficiency (A, D, E, K) [5,6].

Vitamin E is an umbrella term for a group of tocopherols and tocotrienols – the fat-soluble compounds [7]. The main sources of vitamin E include plant oils, margarines, nuts, eggs, whole meal cereal and some fruit and vegetables (e.g. broccoli) [8]. Vitamin E has antioxidant activity and prevents oxidative stress [9]. Moreover, it plays a role in improving nerve conduction, maintaining an integration of hemoglobin membrane, and in connection with vitamin A it is important for normal vision [10]. In addition, vitamin E is used in preventing cardiovascular diseases, cancer, cataracts and Alzheimer's Disease [11].

Dietary intake of vitamin E cannot prevent its deficiency in people with CF. For that reason supplementation is recommended [12]. Current guidelines on the treatment of CF suggest regular vitamin E supplementation and serum monitoring at least annually and 3–6 months after a dosage change [13]. The recommended dosage is 50 IU per day for infants and between 100 UI–400 IU per day in older patients

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[13,14]. Supplementation in CF is usually oral and the vitamin needs to be taken with food containing fat and enzymes [13]. Based on the available evidence, vitamin E deficiency in CF patients seems to be rather rare. However, the risk of vitamin E deficiency increases during inflammation (especially of the digestive and respiratory systems) [15]. On the other hand, routine supplementation of vitamin E can lead to a high level of serum α-tocopherol and potential toxicity [16]. It is worth mentioning that the available evidence documenting the potential predictors of vitamin E concentration in CF patients is scarce.

The aim of the present study was to assess vitamin E status and determinants of its body resources in CF patients.

2. Materials and methods

2.1. Material

Two hundred and eleven patients with CF – 101 (47.9%) females and 110 (52.1%) males, aged from 1 month to 48 years were recruited. The diagnosis was based on accepted guidelines [17].

Mutations in one or both alleles of *CFTR* gene were documented in 204 patients (96.7%). The genotype could not be identified in 7 (3.3%) patients. The *CFTR* mutations of the studied group (presented in the legacy nomenclature) were as follows: F508del/F508del (n = 78); F508del/- (n = 23); F508del/3849 + 10kbC > T (n = 7); F508del/CFTRdele2,3(21kb) (n = 7); F508del/2143delT (n = 7); F508del/1717-1G > A (n = 5); F508del/2183AA > G (n = 5); F508del/N1303K (n = 4); F508del/2184insA (n = 3); F508del/3272-26A > G (n = 3); F508del/R553X (n = 3); 3849 + 10kbC > T/3600 + 1G > T (n = 2); A155P/3171insC (n = 2); F508del/3659delC (n = 2); F508del/K710X (n = 2); F508del/G542X (n = 2); F508del/1078delT (n = 1); F508del/2721del11 (n = 1); F508del/296 + 1G > C (n = 1); F508del/3121-2A > G (n = 1); F508del/3171insC (n = 1); F508del/3600 + 2insT (n = 1); F508del/4374 + 1G > T (n = 1); F508del/D1152H (n = 1); F508del/dup1716 + 51- > 61 (n = 1); F508del/G27V (n = 1); F508del/G551D (n = 1); F508del/IVS2 + 1G > T (n = 1); F508del/L467F (n = 1); F508del/R1158X (n = 1); F508del/R334W (n = 1); F508del/R347P (n = 1); F508del/R851X (n = 1); F508del/W1282X (n = 1); F508del/Y1092X (n = 1); F508del/C524X (n = 1); F508del/G85E (n = 1); G542X/- (n = 1); G542X/G542X (n = 1); C524X/G542X (n = 1); G542X/H954P (n = 1); G542X/R553X (n = 1); N1303K/- (n = 1); N1303K/3272-26A > G (n = 1); N1303K/3849 + 10kb (n = 1); N1303K/CFTRdele2,3(21kb) (n = 1); N1303K/G551D (n = 1); Q1313X/- (n = 1); R553X/1717-1G-A (n = 1); R347P/R347P (n = 1); S1196X/Q1382X (n = 1); T582I/2721del11 (n = 1); W1282X/CFTRdele2,3(21kb) (n = 1); 3849 + 10kbC > T/384910kbC > T (n = 1); 3849 + 10kbC > T/W1282X (n = 1); CFTRdele2,3(21kb)/F1052V (n = 1); 1524 + 1G > A/E585X (n = 2); 1717-1G-A/1717-1G-A (n = 1); 2183AA > G/- (n = 1); 2183AA > G/R117H (n = 1); 2184insA/2789 + 2insA (n = 1); 2184insA/622-1G > A (n = 1); 3659delC/- (n = 1); 3849 + 10kbC > T/1717-2A > G (n = 1).

The nutritional status was analyzed using anthropometric parameters – standardized body height and weight (Z-score) and albumin concentration. Moreover, clinical expression of disease (lung function using spirometry- FEV1; biochemical markers of liver function- ALT, AST, GGT; respiratory tract colonization by *Pseudomonas aeruginosa*; diabetes; liver diseases – cirrhosis, non-cirrhotic liver disorders, pancreatic function – elastase-1 concentration in stool), and vitamin E supplementation were assessed. Clinical parameters in the study group are presented in Table 1.

Body weight and height – two standard deviations below the mean values for all subjects – were documented in 16 (7.6%) and 21 (10.0%) subjects, respectively. Hypoalbuminemia was found in 39 (18.5%) CF patients. Abnormal activity of liver enzymes ALT, AST and GGT were documented in 33 (15.6%), 22 (10.4%), and 18 (8.5%) patients, respectively.

Table 1
Clinical parameters in CF patients group.

Clinical parameters	Median (1st–3rd quartile)
Body weight (*Z-score*)	−0.63 (−1.28 to −0.03)
Body height (*Z-score*)	−0.38 (−1.25 to 0.37)
Albumin [g/dl]	3.89 (3.60–4.20)
AST [U/l]	30.0 (23.0–39.0)
ALT [U/l]	24.0 (16.0–33.5)
GGT [U/l]	13.0 (9.0–19.5)
FEV1 [%][a]	79.5 (56.8–94.0)
INR	1.06 (1.00–1.14)
Vitamin E dose [mg/day][b]	145.8 (55.0–242.0)

[a] FEV1 was assessed in 155 patients. Age of the participants (under 6 years old) determined the possibility of performing the test.
[b] Median and 1st–3rd quartile for vitamin E dose were calculated for all CF patients (receiving and not receiving vitamin E).

One hundred and seventy five (82.9%) patients had pancreatic insufficiency. Liver cirrhosis was documented in 9 (4.3%) studied patients. *Pseudomonas aeruginosa* were isolated from the sputum at least once within a 6-month period prior to the study in 78 (37.0%) patients. Fourteen (6.6%) patients had diabetes.

One hundred and seventy four (82.5%) patients had vitamin E supplementation. The dose ranged from 24.3–400 mg per day (mean ± SD: 195.9 ± 112.4 mg/day; median:181.0; 1st–3rd quartile: 100.0–300.0). Seventeen (8.0%) subjects received vitamin E from multivitamin preparations in a very low dose (≤15 mg/day) not recommended in CF and 20 (9.5%) were not supplemented.

The study was conducted in accordance with the Declaration of Helsinki. Written, informed consent from patients (> 16 years old) and the patients' parents (patients under 16 years old) was collected. The project was approved by the Bioethical Committee at Poznan University of Medical Sciences, Poland (decision no. 244/2012).

2.2. Method

Vitamin E (α-tocopherol) concentration was analyzed by high-performance liquid chromatography (HPLC) using Hewlett Packard 1100 Series HPLC System (Wladbronn, Germany). Supelco C18 column (4.6 mm × 150 mm; 5 µm) was used for separating vitamin E. The mobile phase flow rate (methanol-butylated hydroxytoluene) was 1.4 ml/min. Detection with a UV detector was carried out at 292 nm.

In the first stage, serum samples were deproteinized by an equal volume of ethanol. After protein precipitation, samples were extracted with a 10-fold increase in the volume of hexane and centrifuged. The hexane layer was drawn, then evaporated to dryness and dissolved in 100 µl methanol. 20 µl of solution was applied to a column. The comparison of peak area in the sample with the surface of the peak standard containing a known concentration of the vitamin E in a 20 µl volume (taking into dilution ratio) was used to calculate the final content of α-tocopherol in 1 ml of serum.

Stock standard solution of vitamin E (0.02 µg/ml) was prepared by dissolving 60 mg of vitamin E (Merck, Warsaw, Poland) in 10 ml of ethanol absolute (≥99.8%). Working standard solution was prepared by diluting 10 µl of stock standard solution in10 ml of ethanol absolute.

Stock internal standard of α-tocopherol acetate was prepared by dissolving 40 mg of α-tocopherol acetate (Sigma-Aldrich, Poznan, Poland) in 10 ml of ethanol absolute. Working internal standard solution was prepared by diluting 1.6 ml of stock internal standard in 10 ml ethanol absolute.

Reference values for vitamin E concentration were as follows: for 1-year-old patients 3.8–16.0 µg/ml, for patients aged 4–12 years old 4.0–16.0 µg/ml, for those over 12 years old 5.0–20.0 µg/ml [18].