Advances in Medical Sciences 63 (2018) 341–346

Contents lists available at ScienceDirect

Advances in Medical Sciences

journal homepage: www.elsevier.com/locate/advms

Original research article

Vitamin E status and its determinants in patients with cystic fibrosis

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ARTICLE INFO

Keywords:
Cystic fibrosis
Tocopherol
Fat-soluble vitamins
Pancreatic disease
Liver cirrhosis

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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https://doi.org/10.1016/j.advms.2018.04.001
Received 31 July 2017; Accepted 4 April 2018
Available online 03 August 2018

[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].

2.3. Statistical analysis

The Mann-Whitney test and chi^2-test were used to assess differences between patients with and without vitamin E deficiency. Multiple linear regression analysis, multiple forward and backward stepwise logistic regression analysis were used for assessing the potential influence of the studied parameters on the vitamin E status in two models depending on the classifications of the *CFTR* gene mutations (F508del/F508del vs. other/other and severe/severe vs. other/other). The classification of *CFTR* gene mutations was based on consensus concerning the use and interpretation of cystic fibrosis mutation analysis in clinical practice [19]. Independent variables in the regression models were as follows: age, Z-score for body weight and height, FEV1, albumin concentrations, diabetes, liver cirrhosis, pancreatic sufficiency/insufficiency, *Pseudomonas aeruginosa* colonization, *CFTR* gene mutations, vitamin E dose and vitamin E preparation – no or yes (AquADEKs and other than AquADEKs – Vitaminum E – tablets and liquid, Vitaminum A + E, Capivit A + E, ADEK, Multi Sanostol, Kinder Biovital, Vitaral, Centrum Junior, Centrum, Multivitamin, Vigor, Multi-Tabs, Vita-miner, Falvit). The level of significance was set at $p < 0.05$. Statistical analyses were carried out using StatSoft. Inc (2014) STATISTICA (data analysis software system version 12).

3. Results

Median vitamin E concentration in the study group was 9.9 μg/ml (1st–3rd quartile: 7.5–13.5). Vitamin E deficiency (< 3.8 μg/ml for infants, < 4.0 μg/ml for children aged 4–12 years, < 5.0 μg/ml for children over 12 years old and adults) was found in 17 (8.0%) subjects and high levels of vitamin E (> 16 μg/ml for infants and children aged 4–12 years, > 20 μg/ml for children over 12 years old and adults) 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 dose (Table 2). However, vitamin E deficiency appeared more frequently in CF patients without the vitamin E supplementation (Table 3).

In multiple linear regression analysis pancreatic insufficiency, *CFTR* gene mutation and vitamin E dose were potentially defined as determinants of vitamin E concentration in all regression models (Table 4). In multiple forward and backward stepwise logistic regression analysis, the independent variables were added or removed according to their statistical contribution in explaining the variance of vitamin E. The results of multiple forward and backward stepwise logistic regression analysis are summarized in Tables 5 and 6, respectively.

Table 2
Clinical parameters in CF patients with and without vitamin E deficiency.

Parameter Median (1st–3rd quartile)	Vitamin deficiency[a]	
	Yes	No
Number of patients (%)	17 (8.0)	194 (92.0)
Age (year)	16.6 (11.7–18.1)	10.8 (4.8–17.0)
Z-score for body weight	−0.57 (−1.64 to −0.08)	−0.63 (−1.27 to −0.06)
Z-score for body height	−0.07 (−0.90 to 0.33)	−0.41 (−1.30 to 0.37)
FEV1 [%][b]	69.00 (58.61–84.50)	80.25 (57.05–94.00)
Albumin [g/dl]	3.9 (3.6–4.2)	3.9 (3.6–4.2)
Vitamin E dose [mg/day][c]	242.0 (10.0–300.0)	145.8 (55.0–220.0)

[a] For 1 year old – < 3.8 μg/ml, 4–12 years old – < 4 μg/ml, over 12 years and adults – < 5 μg/ml.
[b] FEV1 was assessed in 155 patients. Age of the participants (under 6 years old) determined the possibility of performing the test.
[c] Median and 1st–3rd quartile for vitamin E dose were calculated for all CF patients (receiving and not receiving vitamin E).

Table 3
The distribution of vitamin E deficiency depending upon clinical parameters in CF patients.

Clinical parameter		Vitamin E deficiency		*p*
		Yes[a]	No	
Number of patients (%)		17 (8.0)	194 (92.0)	–
Z-score for body weight	< −1	5 (7.1)	65 (92.9)	0.7311
	≥ −1	12 (8.5)	129 (91.5)	
Z-score for body height	< −1	3 (4.5)	63 (95.5)	0.2061
	≥ −1	14 (9.6)	131 (90.4)	
FEV1 [%][b]	< 80	7 (9.0)	71 (91.0)	0.7906
	≥ 80	6 (7.8)	71 (92.2)	
Albumin [g/dl]	< 3.5	1 (2.6)	38 (97.4)	0.1628
	≥ 3.5	16 (9.3)	156 (90.7)	
Diabetes	Yes	2 (14.3)	12 (85.7)	0.3755
	No	15 (7.6)	182 (92.4)	
Liver cirrhosis	Yes	0 (0.0)	9 (100.0)	0.3641
	No	17 (8.4)	185 (91.6)	
Pancreatic sufficiency	Yes	1 (2.8)	35 (97.2)	0.2013
	No	16 (9.1)	159 (90.9)	
Ps. aeruginosa colonization	Yes	10 (12.8)	68 (87.2)	0.0515
	No	7 (5.3)	126 (94.7)	
CFTR gene mutation	F508del/ F508del	3 (3.8)	75 (96.2)	0.0853
	other/other	14 (10.5)	119 (89.5)	
	severe/severe	10 (7.3)	127 (92.7)	0.5822
	other/other	7 (9.5)	67 (90.5)	
Vitamin E supplementation	Yes	11 (6.3)	163 (93.7)	0.0446
	No[c]	6 (16.2)	31 (83.8)	

[a] For younger than 1 year – < 3.8 μg/ml, 4–12 years old – < 4 μg/ml, over 12 years and adults – < 5 μg/ml.
[b] FEV1 was assessed in 155 patients older than 6 years.
[c] CF patients not receiving and receiving vitamin E below recommended dose.

4. Discussion

The risk of vitamin E deficiency is of primary concern in CF patients. However, early diagnosis and routine vitamin E supplementation can lead to its normal or even high levels. Therefore, vitamin E body resources should be monitored regularly and the dose should be adjusted accordingly [20]. In the present study, we assessed vitamin E status in a large group of CF patients. Moreover, we also established determinants of its body resources in CF patients.

In the present study, we found low, normal and high concentrations of α-tocopherol in 17 (8.0%), 170 (80.6%), 24 (11.4%) CF patients, respectively. The majority of CF patients with vitamin E deficiency and half of the subjects with high levels of vitamin E had pancreatic insufficiency. Varied proportions of CF patients with vitamin E deficiency have been reported [16,21,22]. Feranchak et al. and Rana et al. found 22 (23%) out of 96 infants and 105 (20%) out of 523 older children with vitamin E deficiency, respectively [21,23]. Back et al. found deficient or suboptimal vitamin E status in 1 (14.3%) out of 7 patients aged 6–11 years, 4 (57.1%) out of 7 patients aged 12–17 years and all 6 (100%) patients over 18 years of age. In total, there were 55% of CF patients with deficiency and suboptimal vitamin E status [22]. In the present study, the highest percentage of vitamin E deficiency was found in patients aged 12–17 years (14.8%) and over 18 years (10.2%). Huang et al. documented vitamin E deficiency only in 9 (4%) out of 222 children. However, the authors referred not to α-tocopherol concentrations but to α-tocopherol:cholesterol ratio [16].

The percentage of CF patients with vitamin E deficiency in the present study was small. Interestingly, in our group of patients vitamin E excess was more common than its deficiency. A very frequent occurrence (48%) of high vitamin E levels in CF patients was described earlier by Huang et al. [16], who referred, however, to 95th percentile of vitamin concentrations from NHANES III as a cut-off level. Using this value (1138 μg/dl), excessive vitamin status should be attributed to 81

Table 4
The multiple linear regression analysis.

Clinical parameters	First model[b]	Second model[c]
	Vitamin E [μg/ml]	
p model	0.00001	< 0.00000
R^2 for model	0.27000059	0.27439250
Adjusted R2 for model	0.20874190	0.21350236
	p {beta ± standard error}[a]	
Age	0.359874 {0.06375 ± 0.069399}	0.701226 {0.02808 ± 0.073032}
Body weight (*Z-score*)	0.786706 {−0.19096 ± 0.704385}	0.850020 {−0.13270 ± 0.700524}
Body height (*Z-score*)	0.533541 {−0.30562 ± 0.489672}	0.581102 {−0.26988 ± 0.487998}
Albumin [g/l]	0.764933 {0.27415 ± 0.915127}	0.705014 {0.34567 ± 0.911293}
FEV1 [%]	0.748863 {−0.00655 ± 0.020434}	0.447792 {−0.01530 ± 0.020105}
Diabetes	0.885173 {−0.24306 ± 1.680042}	0.888236 {0.23969 ± 1.702509}
Liver disease	0.233648 {−2.22633 ± 1.861370}	0.230999 {−2.22874 ± 1.852790}
PI/PS[d]	0.000000 {6.54846 ± 1.225905}	0.000008 {5.94261 ± 1.284606}
Pseudomonas aeruginosa colonization	0.403643 {−0.82058 ± 0.979661}	0.419811 {−0.79064 ± 0.977206}
CFTR gene mutation	0.049626 {1.87359 ± 0.946274}	0.029918 {2.33189 ± 1.063301}
Vitamin E dose [mg/day]	0.024796 {0.00818 ± 0.003607}	0.036062 {0.00756 ± 0.003572}
Vitamin E preparation[e]	0.274598 {1.97447 ± 1.800308}	0.239698 {2.11511 ± 1.791466}

[a] *p* {regression slope coefficient ± standard error of regression slope coefficient}.
[b] *CFTR* gene mutations were divided as follows: F508del/F508del vs. other/other.
[c] *CFTR* gene mutations were divided as follows: severe/severe vs. other/other.
[d] Pancreatic insufficiency/sufficiency.
[e] Vitamin E preparation were divided as follows: no or yes (AquADEKs and other than AquADEKs preparation – Vitaminum E – tablets and liquid, Vitaminum A+E, Capivit A+E, ADEK, Multi Sanostol, Kinder Biovital, Vitaral, Centrum Junior, Centrum, Multivitamin, Vigor, Multi-Tabs, Vita-miner, Falvit).

Table 5
Multiple forward stepwise logistic regression analysis.

p model	R^2	Adjusted R2 for model	Dependent variable	Independent variable	beta ± standard error[a]	*p*
< 0.0000[b]	0.26194595	0.23222565	Vitamin E [μg/ml]	PI/PS[d]	6.71272 ± 1.188434	0.000000
				Vitamin E dose [mg/day]	0.00815 ± 0.003512	0.021622
				CFTR gene mutation	2.12800 ± 0.893535	0.018503
				Vitamin E preparation	2.03391 ± 1.694188	0.231842
				Body height (*Z-score*)	−0.39145 ± 0.355396	0.272476
				Liver cirrhosis	−1.92723 ± 1.814565	0.289913
< 0.0000[c]	0.26637574	0.23683383	Vitamin E [μg/ml]	PI/PS	5.98530 ± 1.261331	0.000005
				Vitamin E dose [mg/day]	0.00739 ± 0.003480	0.035434
				CFTR gene mutation	2.48196 ± 0.965681	0.011146
				Vitamin E preparation	2.28762 ± 1.686958	0.177131
				Liver cirrhosis	−2.05614 ± 1.803349	0.256041
				Body height (*Z-score*)	−0.37269 ± 0.353378	0.293292

[a] regression slope coefficient ± standard error of regression slope coefficient.
[b] *CFTR* gene mutations were divided as follow: F508del/F508del vs. other/other.
[c] *CFTR* gene mutations were divided as follow: severe/severe vs. other/other.
[d] Pancreatic insufficiency/sufficiency.

Table 6
Multiple backward stepwise logistic regression analysis.

p model	R^2	Adjusted R2 for model	Dependent variable	Independent variable	beta ± standard error[a]	*p*
< 0.0000[b]	0.17403157	0.16866814	Vitamin E [μg/ml]	PI/PS[d]	6.375112 ± 1.119168	< 0.00000
< 0.0000[c]	0.17403157	0.16866814	Vitamin E [μg/ml]	PI/PS	6.375112 ± 1.119168	< 0.00000

[a] regression slope coefficient ± standard error of regression slope coefficient.
[b] *CFTR* gene mutations were divided as follow: F508del/F508del vs. other/other.
[c] *CFTR* gene mutations were divided as follow: severe/severe vs. other/other.
[d] Pancreatic insufficiency/sufficiency.

(38.4%) of our patients. Taking into account these findings, not only vitamin E deficiency but also its excessive level in CF patients should be taken into consideration.

Hypervitaminosis E is definitely not a frequent phenomenon in clinical practice [24]. With more effective CF care and proper patients' compliance, vitamin E deficiency seems to disappear or to be less frequent. On the other hand, excessive vitamin E levels may become more common, as documented in the present study and previous reports [16]. We did not observe any symptoms of vitamin E toxicity in our patients. A meta-analysis summarizing long-term non-CF supplementation studies suggests an increase in mortality and in the incidence of heart failure, especially in patients with chronic diseases [25]. However, significant effects were observed for high-dosage trials (500–2000 IU/day). There are no data in this respect for CF patients and those patients who receive high doses should be observed. Routine monitoring of vitamin E levels in CF patients is mandatory, with not only increased

doses in case of vitamin E deficiency but also decreased regimens in the patients with its excessive levels.

In the present study, vitamin E deficiency appeared more frequently in CF patients not receiving vitamin E. Siwamogsatham et al. in their study did not find a correlation between serum α-tocopherol concentration and vitamin E supplementation dose [26]. Similarly, Woestenenk et al. did not prove any significant association between serum α-tocopherol levels and total vitamin E intake (diet and supplementation) [27]. We failed to document a statistically significant relation between vitamin E deficiency and *Pseudomonas aeruginosa* colonization which was borderline ($p = 0.0515$). Previous studies suggested that in CF patients the balance between antioxidant and prooxidant processes is impaired [28,29]. Chronic inflammation in CF patients leads to oxidative stress and damage of pulmonary tissue [28]. Hakim et al. documented that reduced serum level of vitamin E even in the reference value is associated with increasing rates of pulmonary exacerbations [29]. However, Woestenenk et al. found that higher levels of vitamin E did not correlate with higher FEV1 [27]. In the present study, we have not found any differences in FEV1 between patients with and without vitamin E deficiency.

In the present study, vitamin E deficiency did not appear more frequently in CF patients with pancreatic insufficiency. Available data are contradictory. Some authors did not find a correlation between exocrine pancreatic function and fat-soluble vitamins levels including vitamin E [30–32], whereas others documented such an association [23,29]. Interestingly, Hakim et al. found lower vitamin E concentration in CF patients with pancreatic insufficiency both at and after exacerbation than in those with pancreatic sufficiency [29]. However, in none of the studies the multiple analysis was carried out.

Based on the multiple linear regression analysis (models with different classifications of the *CFTR* gene mutations), it seems that pancreatic insufficiency, *CFTR* gene mutations (F508del or severe mutations) and low vitamin E dose were potential risk factors of vitamin E deficiency. Based on multiple forward stepwise logistic regression analysis in both models endo- and exogenous clinical determinants could explain about 26% of the variation of vitamin E concentration. Using multiple backward stepwise logistic regression analysis, pancreatic exocrine function was documented to be a determinant of vitamin E concentration. CF patients with pancreatic insufficiency have worse vitamin E status compared to those with pancreatic sufficiency. Moreover, patients with two severe *CFTR* gene mutations show lower serum vitamin E concentrations than those carrying two mild mutations. It is worth mentioning that the presence of mild mutations does not exclude pancreatic insufficiency [33]. Therefore, patients with for example mild mutations of *CFTR* gene and pancreatic insufficiency could also have vitamin E deficiency.

The main limitation of this study is the evaluation of vitamin E status using only α-tocopherol concentration. The available evidence suggests that vitamin E circulates in the blood bound to lipoprotein. Therefore, it seems that vitamin E levels should be estimated using the α-tocopherol to the total lipid (cholesterol, triacyglycerol, phospholipid) ratio [20,34]. Unfortunately, the ratio α-tocopherol:total lipid has been rarely clinically available [34]. Therefore, it is possible to use α-tocopherol:cholesterol ratio instead of α-tocopherol:total lipid for estimating vitamin E status [16]. The evaluation of α-tocopherol:total lipid ratio may be important when serum lipid levels are low (as it is frequently in CF) because of falsely decreased α-tocopherol concentrations [16]. In the present study we did not measure total cholesterol concentrations at the same time as vitamin E levels. Therefore, we could not estimate vitamin E:cholesterol ratio. If vitamin E:cholesterol ratio had been determined, the percentage of CF patients with deficit and excess of vitamin E would be probably respectively lower and higher.

5. Conclusions

Vitamin E deficiency in cystic fibrosis patients is rather rare nowadays. Excessive vitamin E levels seem to be more frequent. Vitamin E status was not documented to be strictly related to clinical determinants. Beyond vitamin E supplementation, exocrine pancreatic function and *CFTR* gene mutations may have an impact on the vitamin E body resources in CF patients. Vitamin E status should be routinely monitored and the supplementation doses should be adjusted (both in case of deficiency and high levels).

Conflicts of interest

The authors declare no conflict of interest.

Financial disclosure

Supported by a grant from the Poznan University of Medical Sciences (No. 502-0101103115-07588).

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