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The evaluation of α-tocopherol concentration instead of α-tocopherol:cholesterol ratio in adult cystic fibrosis patients results in the overestimation of vitamin E deficiency

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ABSTRACT

Introduction. It has been suggested that serum vitamin E concentrations in CF patients may not properly reflect the body resources of vitamin E. Therefore, we aimed to assess vitamin E status in CF adults relating it to reference values for healthy subjects, also taking into account the α-tocopherol:cholesterol ratio.
Material and Methods. The study group was composed of 33 CF patients – 18 (54.5%) females and 15 (45.5%) males – aged from 18 to 47 years. In all CF patients nutritional status and clinical expression of disease – lung function; *Pseudomonas aeruginosa* colonization; diabetes; cirrhosis; exocrine pancreatic function and vitamin E supplementation were analyzed. Vitamin E (α-tocopherol) concentration was assessed by high-performance liquid chromatography (HPLC).
Resuls. CF adults have lower vitamin E concentrations (median: 830 μg/dl vs. 1132 μg/dl, $p = 0.00174$) and higher vitamin E: cholesterol ratio (median: 7.2 mg/g vs. 6.7 mg/g, $p < 0.00001$) than healthy subjects. The underlying factor that determines this phenomenon is related to low cholesterol levels observed in CF patients. The percentage of low vitamin E concentrations defined in comparison to healthy Polish peers is considerably higher than low vitamin E:cholesterol ratios (39.4% vs. 21.2%, $p = 0.0011$).
Conclusions. The evaluation of α-tocopherol concentration instead of α-tocopherol:cholesterol ratio in CF adults results in the overestimation of vitamin E deficiency. Moreover, there is also potentially a large percentage of individuals with excessive vitamin E body resources. However, this aspect demands further studies.

Keywords: fat-soluble vitamins, gastrointestinal diseases, pancreatitis, high-performance liquid chromatography.

Introduction

It is difficult to determine the optimal markers of vitamin E body resources useful in the clinical care of cystic fibrosis (CF) patients. The vitamin E:total lipid ratio may be preferable, but it is rarely available. Some authors suggested that vitamin E concentration and vitamin E:cholesterol ratio in comparison with the healthy peer group may be the appropriate way to evaluate the vitamin

E status in CF patients [1]. According to the current data, it is unclear whether to assess vitamin E concentration or vitamin E:cholesterol ratio as biomarker for vitamin E status in CF [2]. Moreover, the available evidence shows that age-dependent reference ranges of vitamin E concentrations are very different, which additionally hinders the correct classification of patients with a deficiency or excessive levels of vitamin E [3–6].

Therefore, in the present study, we aimed to assess the body resources of vitamin E in CF adults relating them to reference values for healthy subjects, also taking into account the α-tocopherol:cholesterol ratio.

Material and Methods

Material

The study group was composed of 33 patients with CF — 18 (54.5%) females and 15 (45.5%) males — aged from 18 to 47 years. The diagnosis was based on accepted guidelines [7, 8]. Mutations in one or both alleles of the CFTR gene were found in 31 patients (93.9%). The genotype could not be identified in 2 (6.1%) patients. Eight CF patients were homozygous for the mutation F508del. In the other CF patients the following CFTR gene mutations were identified: F508del/CFTRdele2,3(21kb) (n = 2), F508del/3849+10kbC>T (n = 4), F508del/1717–1G>A (n = 1), F508del/2184insA (n = 1), F508del/3659delC (n = 1), F508del/2183AA>G (n = 1), F508del/3121–2A>G (n = 1), F508del/G551D (n = 1), F508del/R334W (n = 1), F508del/R347P (n = 1), A155P/3171insC (n = 2), 3849+10kbC>T/3600+1G>T (n = 2), 3849+10kbC>T/W1282X (n = 1), N1303K/3849+10kb (n = 1), F508del/- (n = 2), Q1313X/- (n = 1).

In all CF patients nutritional status (standardized body weight and height, serum albumin concentration), clinical expression of the disease and vitamin E supplementation were analyzed. Clinical assessment included: lung function (spirometry), biochemical markers of liver function (aspartate transaminase — AST, alanine transaminase — ALT, gamma-glutamyl transferase — GGT), respiratory tract colonization by *Pseudomonas aeruginosa*, diabetes, liver cirrhosis [9], exocrine pancreatic function (fecal elastase-1 concentration) [10,11] and presented in **Table 1**.

Table 1. Clinical parameters in CF patients

Clinical parameters	Median (1st-3rd quartile)
Age [years]	22.8 (19.7–27.8)
Body weight (Z-score)	-0.65 (-1.00– -0.11)
Body height (Z-score)	-0.24 (-1.00–0.25)
BMI (Z-score)	20.0 (18.3–22.3)
Albumin [g/dl]	3.9 (3.7–4.1)
FEV1 [%]	67.0 (43.0–83.0)
ALT [U/L]	19 (14–30)
AST [U/L]	22 (17–26)
GGT [U/L]	15 (10–22)
Vitamin E dose [mg/day][a]	100.0 (5.0–200.0)

[a] Median and 1st-3rd quartile for vitamin E dose were calculated for all CF patients (receiving and not receiving vitamin E)
FEV1 — forced expiratory volume in 1 second; GGT, gamma-glutamyl transferase; ALT, alanine transaminase; AST, aspartate transaminase

Seventy-nine healthy subjects — 54 (68.4%) females and 25 (31.6%) males — aged 18.5–29 years constituted the comparative group.

Twenty-four (72.7%) CF adults were pancreatic insufficient. Liver cirrhosis was documented in 3 (9.1%) studied patients. *Pseudomonas aeruginosa* had been isolated from the sputum at least once within a 6-month period before the study in 24 (72.7%) patients. Four (12.1%) subjects had diabetes.

Eighteen (54.5%) CF adults were receiving vitamin E according to existing recommendations [2]. The dose ranged from 100.0–400.0 mg per day (mean±SD: 250.2 ± 109.9 mg/day; median: 181.0; 1st-3rd quartile: 181.0–362.0). Seven (21.2%) patients took vitamin E in very low doses (≤15mg/day), not recommended in CF, and 8 (24.3%) patients did not receive any supplementation.

The study was conducted in accordance with the Declaration of Helsinki. Written, informed consent from patients (>16 years old) and patients' parents (for patients under 16 years old) was collected. The project was approved by the Bioethical Committee at Poznan University of Medical Sciences (decisions no. 244/2012 and 200/2018).

Methods

Vitamin E (α-tocopherol) concentration was analyzed by high-performance liquid chromatography (HPLC). Total cholesterol concentration was determined in human serum using the Beckman Coulter AU analyzer.

Normal vitamin E concentrations and α-tocopherol:cholesterol ratios were defined by using 5th to 95th percentile of the studied com-

parative group (healthy adult subjects). These values were used for a comparison with CF subjects. In addition, we used existing reference values of vitamin E concentration for adults in Poland (5.0–20.0 µg/ml / 500–2000 µg/dl) [3] and Great Britain [5].

Statistical analysis

The Mann-Whitney U test was used to assess differences between CF adults and healthy subjects regarding α-tocopherol concentrations, α-tocopherol:total cholesterol ratio and cholesterol levels. The Fisher's exact test was used to estimate the accordance of the distribution of vitamin E and vitamin E:cholesterol ratio. 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).

Results

CF adults have lower vitamin E concentrations and higher vitamin E:cholesterol ratio than healthy subjects. The underlying factor that determines this phenomenon is related to low cholesterol levels observed in CF patients (**Table 2**).

The distribution of vitamin E concentrations and of α-tocopherol:cholesterol ratios in CF adults has been presented in **Figure 1**. Depending on the reference values used [3,5, own data from the present study], low vitamin E concentrations were found in 4 (12.1%), 3 (9.1%) and 13 (39.4%) CF

Table 2. Comparison of α-tocopherol and cholesterol concentrations, and α-tocopherol:cholesterol ratio between CF patients and healthy subjects

Median (1st-3rd quartile)	CF adults (N = 33)	Healthy adults (N = 79)	p
α-tocopherol [µg/dl]	830 (640–1300) (274–2570)*	1132 (987–1251) (781–1510)*	0.00174
α-tocopherol:cholesterol [mg/g]	7.2 (5.6–9.7) (2.3–14.4)*	6.7 (6.0–7.3) (5.3–7.9)*	<0.00001
Cholesterol [mg/dl]	128 (114–153)	171 (155–187)	<0.00001

*<5th-95th percentile>

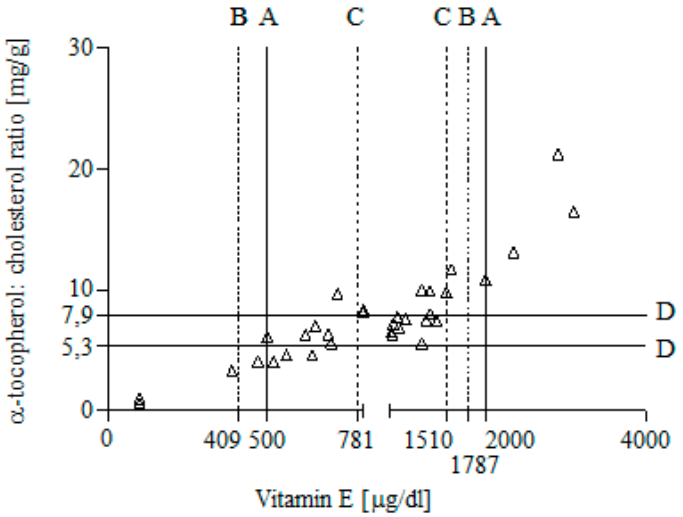

Figure 1. Vitamin E concetrations and α-tocopherol:cholesterol ratios in CF adults. A – Polish reference values of vitamin E concentration for adults in Poland (Prószyńska et al., 1991); B – British reference values (Ford et al., 2006); C, D – normal vitamin E concentrations and normal α-tocopherol:cholesterol ratios defined by using 5th to 95th percentile of the comparative group (healthy adult subjects)

patients, respectively. Similarly, high values were documented in 3 (9.1%), 4 (12.1%) and 5 (15.2%) CF patients, respectively.

What is worth noting, the percentage of low vitamin E concentrations defined in comparison to healthy Polish peers is considerably higher than low vitamin E/cholesterol ratios (39.4% vs. 21.2%, $p = 0.0011$). The distribution of low/normal/high results of both parameters has been presented in **Table 3**. The assessment of vitamin E body resources based upon its concentrations in CF seems to result in the overestimation of occurring deficits. We have found 7 (21.2%) CF adults with low vitamin E concentration and low values of α-tocopherol:cholesterol ratios. However, 6 (18.2%) CF patients with low α-tocopherol levels have normal or high α-tocopherol:cholesterol ratios.

Table 3. The distribution of low/normal/high results of α-tocopherol concentration and α-toco-pherol:cholesterol ratio

		α-tocopherol:cholesterol ratio [mg/g]			p
		Low	Normal	High	
α-tocopherol concentration [μg/dl]	Low	7 (21.2)	5 (15.2)	1 (3.0)	0.00008
	Normal	0 (0)	9 (27.2)	6 (18.2)	
	High	0 (0)	0 (0)	5 (15.2)	

Discussion

In the current study, we documented that the measurement of α-tocopherol, instead of α-tocopherol:cholesterol ratio in adults with CF may overestimate vitamin E deficiency. Vitamin E concentrations were significantly higher in healthy subjects. However, after the correction for cholesterol level this phenomenon appeared to be apparent. In fact vitamin deficiency in CF adults patients was less frequent that one could assess based upon vitamin E levels exclusively.

We intentionally selected patients with varied vitamin E supplementation to have the possibility to assess patients with different vitamin E body resources. In the studied group, there were subjects not receiving vitamin E, and receiving it in non-recommended doses. This study group does not reflect the typical population of CF adults. However, it allowed us to the reach the objective of the study.

According to the current data, the proper way to assess vitamin E status in CF is still uncertain [2]. 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, triacylglycerol, phospholipid) ratio [12, 13]. Unfortunately, the ratio α-tocopherol:total lipid has rarely been clinically available [13]. Therefore, instead of α-tocopherol:total lipid it is possible to use α-tocopherol:cholesterol ratio for estimating vitamin E status [4]. The evaluation of α-tocopherol:total lipid ratio may be relevant when serum lipid levels are low (as it is frequently in CF) because of falsely decreased α-tocopherol concentrations [4]. Therefore, we can expect that some patients may have low α-tocopherol concentration and normal vitamin E: lipid ratio or normal vitamin E concentration and high α-tocopherol:lipid ratio. Ford et al. found that 32 (56%) out of 57 subjects studied with vitamin E deficiency had low α-tocopherol concentrations and normal α-tocopherol:cholesterol ratio. They also documented two out of 457 non-CF patients with normal vitamin E levels and low vitamin E:cholesterol ratio in their study. Both of these patients (a 20-week-old child and a 53-year-old male) had cholestasis. In the past, normal vitamin E concentration and low vitamin E:cholesterol ratio were described in patients with chronic cholestasis and neurological symptoms of vitamin E deficiency [14]. In the present study, we have not documented any of CF adults with normal α-tocopherol levels and low α-tocopherol:cholesterol ratios. Another important issue is that normal values of vitamin E concentration differ between countries and publications. According to three different normal ranges considered in the present study, vitamin deficiency could be diagnosed in 12.1%, 9.1% and 39.4%, respectively (**Figure 1**).

The consistency of α-tocopherol concentrations and α-tocopherol:cholesterol ratio in CF is doubtful, as has been documented in the present study. Ford et al. documented high concordance — 92% and 99.5% respectively — of results between α-tocopherol concentrations and α-tocopherol:cholesterol ratio in all subjects aged 11 days to 90 years and participants with normal vitamin E status, respectively. However, the percentage of concordant results in subjects with vitamin E deficiency was only 42.0% [5].

In conclusion, the evaluation of α-tocopherol concentration not α-tocopherol: cholesterol ratio in CF adults results in the overestimation of vitamin E deficiency. Moreover, there is also potentially a large percentage of individuals with excessive vitamin E body resources, when using α-tocopherol:cholesterol ratio for estimating vitamin E status. However, this aspect demands further studies.

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Conflict of interest statement

The authors declare no conflict of interest.

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Specific author contributions

E.S. designed the study, performed the statistical analysis, analyzed and interpreted data and drafted the manuscript, P.K-J performed the statistical analysis and revised the manuscript, D.W., SZ.K. provided the data and revised the manuscript, J.W. designed the study, coordinated data acquisition, analyzed and interpreted data, drafted and revised the manuscript. All authors read and approved the final manuscript.

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