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VITAMIN K STATUS IN YOUNG CHILDREN WITH CYSTIC FIBROSIS*

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Introduction. Cystic fibrosis (CF) patients are at risk of developing vitamin K deficiency. However, there is no clinical data clearly describing vitamin K status in the youngest age group. Therefore, in the present study we aimed to assess body resources of vitamin K in children aged up to 3 years and to correlate vitamin K status with selected clinical factors.
Material and methods. The study comprised 52 CF patients receiving and not receiving vitamin K supplementation. In all subjects, the concentration of the undercarboxylated prothrombin (PIVKA-II), as a marker of vitamin K deficiency, was determined.
Results. PIVKA-II concentrations were pathological in 24 (46.2%) CF children, in remaining 28 (53.8%) patients vitamin K status was found to be normal. No statistical differences in clinical parameters (*Z-score* for body height and weight, number of hospitalizations and sweat chloride concentrations) neither in distribution of *Pseudomonas aeruginosa* colonization nor in pancreatic status between selected subgroups with normal and abnormal PIVKA-II concentrations were documented. Normal vitamin K status was more frequent in patients receiving proper vitamin K supplementation ($p < 0.0078$). However, vitamin K deficiency appeared in 5 out of 21 patients receiving at least 2.5 mg vitamin K/week. In logistic regression model, no clinical parameter was proven to be a risk factor for vitamin K deficiency.

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Conclusion. Vitamin K deficiency is frequent in CF infants and toddlers, and may also appear in those receiving recommended supplementation. There is no strong relationship between clinical expression of the disease and vitamin K status.

Key words: cystic fibrosis, vitamin K deficiency, PIVKA-II

INTRODUCTION

Cystic fibrosis (CF) is the most common life-limiting recessive genetic disease [Huyghebaert et al. 2007, McKay 2007]. It is caused by mutations in the cystic fibrosis transmembrane regulator gene (*CFTR*), which is allocated on chromosome 7 [McKay 2007]. Most CF infants develop exocrine pancreatic insufficiency in early life [Kalnins et al. 2007, Walkowiak et al. 2005 a]. It leads in turn to intestinal malabsorption of fat and fat-soluble vitamins, including vitamin K [Huyghebaert et al. 2007, Walkowiak et al. 2005 b].

Vitamin K dependent proteins play an important role in haemostasis, bone mineralization and calcium homeostasis, growth control and signal transduction [Berkner 2008]. Vitamin K is an essential cofactor in the posttranslational γ-carboxylation of glutamic acid residues to form γ-carboxyglutamic acid residues which are able to bind calcium [Van Hoorn et al. 2003, Mosler et al. 2003]. In vitamin K deficient subjects these proteins are functionally defective since they cannot bind calcium [Wilson et al. 2001]. The presence of circulating proteins in their undercarboxylated forms seems to be highly related to vitamin K status. Undercarboxylated form of prothrombin called PIVKA-II (prothrombin inducted by vitamin K absence or antagonism) is one of these proteins [Conway 2004] and is believed to be a sensitive marker of vitamin K deficiency [Urquhart et al. 2007, Krzyżanowska and Walkowiak 2010].

Available evidence strongly suggest that all CF patients are at risk of developing vitamin K deficiency [Van Hoorn et al. 2003] because of inadequate dietary intake, maldigestion and malabsorption, and antibiotic therapy [Verghese and Beverley 2003]. However, vitamin K supplementation doses in CF have not been well established [Kalnins et al. 2007]. Vitamin K deficiency was clearly documented in CF patients in a wide age range but there is no clinical data clearly describing vitamin K status in the youngest age group. Interestingly, Sokol et al. failed to detect any case of vitamin K deficiency in a group of 36 infants despite previously reported cases of prolonged prothrombin time and coagulopathy [Sokol et al. 1989].

AIM

In the present study we aimed to assess body resources of vitamin K in CF children aged up to 3 years. We also made an attempt to correlate vitamin K status with several clinical factors.

MATERIAL AND METHODS

The study comprised 52 CF patients aged 2 months and 3 years receiving (n = 31) and not receiving (n = 21) vitamin K supplementation. Diagnosis of CF was based upon history, clinical manifestation, increased sweat chloride concentration (Gibson and Cooke method) and confirmed by the *CFTR* gene analysis.

The genotypes of the studied patients were as follows: F508del/F508del (n = 22), F508del/- (n = 14), F508del/CFTRdel2,3(21kb) (n = 6), F508del/2184insA (n = 2), F508del/1717-1G-A (n = 1), F508del/F1286C (n = 1), F508del/2183AA>6 (n = 1), F508del/N1303K (n = 1), F508del/1898+1G-A (n = 1), 2183AA>A/- (n = 1), CFTRdel2,3(21kb)/Y1092X (n = 1), G542X/N1303K (n = 1). The clinical characteristics of studied subjects have been summarised in Table 1.

Table 1. Clinical characteristics of studied patients

Studied parameter	Body height (Z-score)	Body weight (Z-score)	Vitamin K dose mg/week	Vitamin K dose mg/kg/week	Number of hospitalizations	Sweat chloride concentration mmol/l
Median	–0.64	–1.06	0.7	0.1	2	90
1st to 3rd quartile	–1.98-0.64	–1.56-–0.13	0-2.8	0-0.4	1-3	72-102

Eleven (21.2%) patients were pancreatic sufficient, while the remaining 41 (78.8%) subjects presented with steatorrhea. Similarly, eleven (21.2%) patients were colonized with *Pseudomonas aeruginosa*, while in 41 (78.8%) patients bacterial colonization was not demonstrated.

Vitamin K status was estimated by the measurement of PIVKA-II concentrations using an enzyme immunoassy method (Asserachrom PIVKA-II, Roche Diagnostic, Switzerland). Plate was coated with mouse monoclonal F(ab')2 fragments specific for PIVKA-II. Calibration plasmas and samples were introduced in the coated microwells and the PIVKA-II was connected by its specific antigenic determinant. In the next stages, the horseradish peroxidase-labelled anti-prothrombin antibodies, substrate solution and sulfuric acid were added respectively. The absorbance of each well was detected by Microstrip Reader (Behring EL 301) at 492 nm. Concentrations of PIVKA-II were calculated directly from the standard curve. The cut-off value was set at 2 ng/ml. Higher concentrations were considered to be abnormal (vitamin K deficiency).

The differences in *Z-score* for body height and weight, vitamin K dose, number of hospitalizations and sweat chloride concentrations between subgroups of patients with normal and abnormal vitamin K status were assessed using Mann-Whitney test (non-paired data). The differences in *Pseudomonas aeruginosa* colonization, distribution of pancreatic sufficiency/insufficiency and vitamin K supplementation in selected subgroups were analysed with the use of chi^2-test. The potential influence of all studied parameters on the occurrence of vitamin K deficiency was assessed with the use of multivariable logistic regression. The level of significance was set at $p < 0.05$.

The protocol of the investigation was approved by the Bioethical Committee of the Poznań University of Medical Sciences, Poland and Lviv Cystic Fibrosis Centre.

RESULTS

PIVKA-II concentrations were pathological in 24 (46.2%) CF children, abnormal values ranged from 2.3 to 190.4 ng/ml (mean: 26.2 ±47.4, median: 5.8). In the remaining 28 (53.8%) patients vitamin K status was found to be normal.

The clinical data of selected subgroups with normal and abnormal PIVKA-II concentrations has been summarised in Table 2.

Table 2. Clinical parameters of subgroup with normal and abnormal PIVKA-II

Studied parameter <1st to 3rd quartile (Median)>	Body height (Z-score)	Body weight (Z-score)	Vitamin K dose mg/week	Vitamin K dose mg/kg/week	Number of hospitaliza-tions	Sweat chloride concentration mmol/l
CF subgroup with PIVKA-II being						
normal	–1.10-0.64	–1.56- –0.11	0-2.8	0-0.45	1-3	76-101
	(–0.47)	(–0.89)	(2.8)	(0.31)	(2)	(90)
abnormal	–2.11-0.40	–1.56-–0.32	0-1.3	0-0.13	1-4	60-103
	(–0.88)	(–1.13)	(0)	(0)	(3)	(86)
Statistical significance	n.s.	n.s.	$p < 0.036$	$p < 0.070$	n.s.	n.s.

Vitamin K doses were significantly higher in patients with normal vitamin K status. However, no significant relationship between PIVKA-II level and vitamin K dose was stated ($r = 0.087$). Clinical characteristics of patients (*Z-score* for body height and weight, number of hospitalization and sweat chloride concentration) in selected subgroups did not significantly differ. The distribution of vitamin K deficiency in patients with and without pancreatic insufficiency and *Pseudomonas aeruginosa* colonization was not different either (Table 3).

Table 3. *Pseudomonas aeruginosa* colonization, pancreatic exocrine function and vitamin K supplementation in CF subgroups with normal and abnormal of PIVKA-II concentration

Studied parameter		PIVKA-II		Statistical significance
		normal n, %	abnormal n, %	
Pseudomonas aeruginosa colonization	yes	5 (45.4)	6 (54.6)	n.s.
	no	23 (56.1)	18 (43.9)	
Pancreatic sufficiency	yes	7 (63.6)	4 (36.4)	n.s.
	no	21 (51.2)	20 (48.8)	
Vitamin K supplementation*	yes	16 (76.2)	5 (23.8)	$p < 0.0078$
	no	12 (38.7)	19 (61.3)	

*Dose ≥ 2.5 mg/week.

Patients receiving vitamin K in a dose of at least 2.5 mg/week more frequently had normal vitamin K status ($p < 0.0078$). However, vitamin K deficiency appeared in 5 out of 21 patients receiving such a supplementation. Four patients with severe vitamin K deficiency (PIVKA-II > 50 ng/ml) did not receive any supplementation. The subject with the most severe vitamin deficit (PIVKA-II = 190.4 ng/ml) was using the dose of 0.7 mg/week.

The multivariable analysis of logistic regression comprising all clinical parameters failed to detect any risk factor of vitamin K deficiency.

DISCUSSION

There are no reliable clinical studies directly assessing vitamin K status in infants and toddlers with CF. The existing reports comprise few young CF children. However, published results refer to whole groups (median age ≥ 10 years) not allowing for age-specific conclusions in the youngest included subjects [Montalembert et al. 1992, Mosler et al. 2003, Rashid et al. 1999, Wilson et al. 2001]. High frequency of vitamin K deficiency was detected, despite reasonable supplementation implemented in the majority of subjects. Therefore, we aimed in the present study to assess the frequency of vitamin K deficiency in CF children up to 3 years.

The major CF symptoms are related to respiratory and gastrointestinal systems [Wagener and Headley 2003]. Almost 90% CF patients have pancreatic insufficiency. It may lead to malabsorption of dietary fat and consequently results in a loss of vitamins that are co-absorbed with fat [Borowitz et al. 2002]. Therefore, CF patients are at high risk of developing vitamin K deficiency [Van Hoorn et al. 2003]. Other factors potentially negatively influencing vitamin K status comprise the long-term use of antibiotics and bowel resection [Conway et al. 2005]. Available evidence strongly suggests that liver disease is also a significant risk factor. It results in worse fat absorption, reducing the bile salt pool, and also may decrease the synthesis of clotting factors [Rashid et al. 1999]. It has been proven that children with chronic cholestatic liver disease frequently have vitamin K deficiency despite regular supplementation [Mager et al. 2006]. We have failed in the present study to determine any clinical risk factor for the occurrence of vitamin K deficiency. However, no child underwent bowel resection neither was documented as having significant liver involvement. Although none of the assessed clinical parameters allowed for the selection of patients having vitamin K deficiency, its high frequency points to a need of early implementation of monitoring.

Vitamin K dose in the present study ranged from 0 to 6.7 mg/week. Significant difference in vitamin K dose (mg/week) between subgroups with normal and abnormal PIVKA-II concentrations was stated ($p < 0.036$). Based on PIVKA-II concentrations, supplementation of vitamin K with a dose of at least 2.5 mg/week was effective in 16 (76.2%) out of 21 patients. In remaining 5 (23.8%) patients such supplementation was insufficient. In 19 out of 31 (61.3%) CF children not receiving or receiving lower vitamin K dose, its abnormal status was detected. However remaining, 12 (38.7%) patients in this subgroup were categorized as having normal vitamin K status.

According to available data vitamin K deficiency may occur in CF patients with and without supplementation. Nevertheless, the requirements for vitamin K supplementation in CF patients have not been well established [Kalnins et al. 2007]. Beker et al. [1997]

examined 18 CF patients aged 13 to 35 years and reported that supplementary dose of 5 mg vitamin K_1/week improved vitamin K parameters (undercarboxylated osteocalcin and PIVKA-II), however normal levels were not achieved. In a group of 32 CF patients aged 7 month to 25 years with or without oral vitamin K_1 supplementation, Mosler et al. [2003] documented that seven patients who received between 6 and 20 mg vitamin K_1 per week had supranormal vitamin K plasma concentrations. On the other hand, PIVKA-II levels were normal. One year of vitamin K supplementation (10 mg vitamin K_1/week), resulted in an increase of the levels of carboxylated osteocalcin in a group of schoolchildren CF subjects [Nicolaidou et al. 2006]. Since the assessment of vitamin K status was not the major aim of the study the available data on the effectiveness of vitamin K supplementation is limited. In turn, Drury et al investigated 14 children of a similar age receiving either 1 mg/day or 5 mg/day vitamin K_1 for one month. Such supplementation resulted in normalization of undercarboxylated osteocalcin levels in 3 patients. No trend towards a difference between doses was observed. The authors concluded that a longer period of supplementation might be needed to normalize vitamin K status [Drury et al. 2008].

CONCLUSIONS

In conclusion, vitamin K deficiency is frequent in CF infants and toddlers and may also appear in those receiving recommended supplementation. There is no strong relationship between clinical expression of the disease and vitamin K status. Early monitoring of vitamin K deficiency should be implemented and doses should be adjusted individually.

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USTROJOWE ZASOBY WITAMINY K U MAŁYCH DZIECI CHORYCH NA MUKOWISCYDOZĘ

Wstęp. Chorzy na mukowiscydozę (ang. *cystic fibrosis* – CF) są szczególnie narażeni na występowanie niedoborów witaminy K. Jednakże nie ma jednoznacznych danych określających status powyższej witaminy w najmłodszej grupie wiekowej. Dlatego celem badania była ocena ustrojowych zasobów witaminy K u dzieci do 3 roku życia oraz próba korelacji statusu witaminy K z wybranymi parametrami klinicznymi.
Materiał i metody. Badaniem objęto 52 pacjentów z CF suplementownych i niesuplementowanych witaminą K. U wszystkich chorych oceniono stężenie niekarboksylowanej protrombiny (PIVKA-II) jako markera niedoboru witaminy K.
Wyniki. U 24 (46,2%) chorych na CF stwierdzono nieprawidłowe stężenia PIVKA-II, ustrojowe zasoby witaminy K były prawidłowe u pozostałych 28 (53,8%) pacjentów. Nie wykazano istotnych statystycznie różnic pomiędzy parametrami klinicznymi (standary-

zowana wysokość i masa ciała, liczba hospitalizacji i stężenie chlorków w pocie, kolonizacja *Pseudomonas aeruginosa*, wydolność trzustki) w podgrupach z prawidłowymi i nieprawidłowymi stężeniami PIVKA-II. Prawidłowy status witaminy K stwierdzono częściej u pacjentów otrzymujących właściwą dawkę suplementacyjną ($p < 0,0078$). Jednakże niedobór witaminy K pojawił się u 5 z 21 pacjentów otrzymujących przynajmniej 2,5 mg witaminy K na tydzień. W modelu regresji logistycznej, żaden z parametrów klinicznych nie okazał się czynnikiem ryzyka występowania niedoboru witaminy K.
Wnioski. Niedobór witaminy K u niemowląt oraz małych dzieci z CF jest częsty i może się pojawić pomimo stosowania zalecanej suplementacji. Brak jest istotnej zależności między kliniczną ekspresją choroby a ustrojowymi zasobami witaminy K.

Słowa kluczowe: mukowiscydozy, niedobór witaminy K, PIVKA-II

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