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. 2005a]. It leads in turn to intestinal malabsorption of fat and fat-soluble vitamins, including vitamin K [Huyghebaert et al. 2007, Walkowiak et al. 2005b].

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, malabsorption, and antibiotic therapy [Vergheese 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), CFTR-del2,3(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

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

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 ch²-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

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

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

<table>
<thead>
<tr>
<th>Studied parameter</th>
<th>PIVKA-II</th>
<th>Statistical significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>normal n, %</td>
<td>abnormal n, %</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa colonization</td>
<td>yes</td>
<td>5 (45.4)</td>
</tr>
<tr>
<td></td>
<td>no</td>
<td>23 (56.1)</td>
</tr>
<tr>
<td>Pancreatic sufficiency</td>
<td>yes</td>
<td>7 (63.6)</td>
</tr>
<tr>
<td></td>
<td>no</td>
<td>21 (51.2)</td>
</tr>
<tr>
<td>Vitamin K supplementation*</td>
<td>yes</td>
<td>16 (76.2)</td>
</tr>
<tr>
<td></td>
<td>no</td>
<td>12 (38.7)</td>
</tr>
</tbody>
</table>

* 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 \((\text{PIVKA-II} > 50 \text{ ng/ml})\) did not receive any supplementation. The subject with the most severe vitamin deficit \((\text{PIVKA-II} = 190.4 \text{ ng/ml})\) 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 \((\text{median age} \geq 10 \text{ years})\) not allowing for age-specific conclusions in the youngest included subjects \([\text{Montalembert et al. 1992, Mosler et al. 2003, Rashid et al. 1999, Wilson et al. 2001}]\). 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 \([\text{Wagener and Headley 2003}]\). 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 \([\text{Borowitz et al. 2002}]\). Therefore, CF patients are at high risk of developing vitamin K deficiency \([\text{Van Hoorn et al. 2003}]\). Other factors potentially negatively influencing vitamin K status comprise the long-term use of antibiotics and bowel resection \([\text{Conway et al. 2005}]\). 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 \([\text{Rashid et al. 1999}]\). It has been proven that children with chronic cholestatic liver disease frequently have vitamin K deficiency despite regular supplementation \([\text{Mager et al. 2006}]\). 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 \((\text{mg/week})\) 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 \([\text{Kalnins et al. 2007, Beker et al. 1997}]\)
examined 18 CF patients aged 13 to 35 years and reported that supplementary dose of 5 mg vitamin K1/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 K1 supplementation, Mosler et al. [2003] documented that seven patients who received between 6 and 20 mg vitamin K1 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 K1/week), resulted in an increase of the levels of carboxylated osteocalcin in a group of schoolchildren CF subjects [Nicolaïdou 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 K1 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.

REFERENCES


USTROJOWE ZASOBY WITAMINY K U MAŁYCH DZIECI CHORYCH NA MUKOWISCYDOZĘ


Material i metody. Badaniem objęto 52 pacjentów z CF supplementowanych 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 stężeńiami 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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