

Vitamin K status in peritoneally dialyzed patients with chronic kidney disease

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Abnormal vitamin K status was documented in patients with chronic kidney diseases (CKD), especially those undergoing hemodialysis. The data related to patients undergoing peritoneal dialysis (PD) are contradictory. Therefore, in the present study we aimed to evaluate vitamin K status in patients with CKD who are treated with continuous ambulatory PD. Twenty-eight patients entered into the study. Dialysis vintage ranged from 3 to 89 months. Vitamin K status was assessed in all subjects using undercarboxylated prothrombin measurement (PIVKA-II). In addition, total protein and albumin levels, total cholesterol, LDL cholesterol, triglyceride, calcium, urea and creatinine concentrations were determined. PIVKA-II concentrations were abnormal in 13 (46.4%) subjects. BMI values, both total and LDL cholesterol concentrations were significantly higher in patients with than those without vitamin K deficiency. Moreover, PIVKA II levels correlated with BMI values ($r=0.441$, $p<0.019$), LDL cholesterol ($r=0.434$, $p<0.021$) and creatinine ($r=0.406$, $p<0.032$) concentrations. However, through the use of logistic regression analysis and multiple regression analysis, no clinical factor was documented to be the independent risk factor of vitamin K deficiency. In conclusion, vitamin K deficiency is a frequent condition in peritoneally dialyzed patients. Assessment of vitamin K status should become a standard procedure in this group of patients.

Keywords: vitamin K, kidney disease, dialysis

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INTRODUCTION

The term vitamin K denotes a family of fat soluble vitamins responsible for post-translational protein modification (Krueger *et al.*, 2009). Vitamin K is the cofactor of γ -glutamyl carboxylation of proteins by the microsomal enzyme γ -glutamyl carboxylase. This post-translational reaction is necessary for the conversion of coagulation factors II, VII, IX, X and proteins C and S into their bioactive forms in the liver (vitamin K1 and K2). Vitamin K2 is required for γ -glutamyl carboxylation of osteocalcin, which is secreted by osteoblasts and odontoblasts and of Matrix-Gla Protein (MGP), an inhibitor of vascular medial-calcification that occurs in many different tissues (Malyszko *et al.*, 2002; Malyszko *et al.*, 2004; Holden *et al.*, 2008). It is well known that the tunica media is the major site of MGP expression, yet it

still remains unclear by what mechanism MGP prevents arterial calcification. In the general population, vitamin K supplementation can be used as a preventive measure against vascular calcification and the formation of bioactive coagulation factors (Danziger, 2008).

It is important to establish vitamin K status in chronic renal failure patients to prevent its potential deficiency and complications that may be related to it, such as bleeding and an increased risk of cardiovascular disease. Subclinical vitamin K deficiency may not cause any noticeable changes in the levels of coagulation factors, and its detection threshold is very different from that of apparent vitamin K deficiency. The current European guidelines related to nutrition suggest that vitamin K deficiency is not found except for cases of prolonged antibiotic therapy and coagulation abnormalities (Fouque *et al.*, 2007). However, abnormal vitamin K status was documented in patients with chronic kidney diseases (CKD), especially in those subjects undergoing hemodialysis (Pilkey *et al.*, 2007). The data related to patients on peritoneal dialysis (PD) are contradictory (Nagasawa *et al.*, 1998b; Vychytil & Druml, 1998). Therefore, in the present study, we aimed to evaluate vitamin K status in patients with CKD who are treated with continuous ambulatory PD.

MATERIALS AND METHODS

Twenty-eight patients (16 females and 12 males) aged from 27 to 76 years (median [1st–3rd quartile]: 60 [48–68]) with CKD agreed to participate in the study. CKD was of different etiology: 11 patients developed diabetic nephropathy, 11 patients had hypertension related nephropathy, 2 patients had nephropathy of unclear etiology, the rest of the patients developed CKD due to glomerulonephritis, bilateral kidney stone disease, SLE or ischemia. All patients were treated with continuous ambulatory PD due to CKD. Dialysis vintage ranged from 3 to 89 months (24 [7–37]). None of the patients was anticoagulated with coumarin or warfarin. One patient was treated with low-molecular heparin due to dilated cardiomyopathy secondary to hypertension.

Vitamin K status was assessed in all subjects using undercarboxylated prothrombin measurements (pro-

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Abbreviations: CKD, chronic kidney disease; MGP, Matrix-Gla protein; PD, peritoneal dialysis; PIVKA-II, undercarboxylated prothrombin.

thrombin induced by vitamin K absence, PIVKA-II) by an enzyme immunoassay method (Asserachrom PIVKA-II, Roche Diagnostic, Poland) as described earlier (Acta Alimentaria). The cut-off value was set at <2 ng/mL. Higher values were considered to be indicative of vitamin K deficiency. In addition, total protein and albumin levels, total cholesterol, LDL cholesterol, triglyceride, calcium, urea and creatinine concentrations as well as GFR were determined.

The statistical differences in clinical parameters between patients with and without vitamin K deficiency were calculated with the use of the Mann-Whitney test. The relationship between single or all clinical parameters and PIVKA II concentrations were assessed using Spearman correlation and multiple regression analysis, respectively. The influence of clinical parameters on the presence/absence of vitamin K deficiency was determined with the use of logistic regression analysis.

Mathematical analysis of the collected experimental data was also performed using two data mining procedures to define the potential predictor of vitamin K deficiency. In the first step, the standard model of classification and regression trees (CR&T) was used with the following options: equal misclassification costs, Gini measure of the goodness of fit, estimated prior probabilities, stopping rule — prune on misclassification error (stopping parameter — minimum 5 cases), and ten-fold cross validation. Because the splitting value of LDL predictor in the optimal tree (in a generated sequence of four trees) was equal to 100.75, we tried to change the value to exactly 100. That trial was made using the interactive trees procedure. Adoption of this corrected level did not change the structure of the misclassification matrix. The distribution of vitamin K deficiency in patients with LDL concentrations >100 mg/dL and in those with low LDL levels was assessed with the use of Chi²test (with Yates correction).

All calculations were performed using STATISTICA (data analysis software system), version 8.0. StatSoft, Inc. (2008).

If not stated otherwise, measures of location are reported as medians [1st–3rd quartiles]. 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. An informed and written consent was obtained from each of the patients.

RESULTS

PIVKA II concentrations were lower than 2 ng/mL in 15 (53.6%) patients. In the remaining 13 (46.4%) subjects the concentrations were abnormal, which is indicative of vitamin K deficiency. Undercarboxylated prothrombin concentrations in this subgroup of patients ranged from 2.2 to 34.8 ng/mL (4.2 [1st–3rd quartiles] <3.1 – 7.3). However, coagulological parameters (prothrombin time, INR) were normal in all subjects studied.

BMI values, LDL and total cholesterol concentrations were significantly higher in patients with vitamin K deficiency than in those without (Table 1). PIVKA II levels correlated to BMI values ($r = 0.441$, $p < 0.019$), LDL cholesterol ($r = 0.434$, $p < 0.021$) and creatinine ($r = 0.406$, $p < 0.032$) concentrations. No other significant correlations were stated. However, using logistic regression analysis and multiple regression analysis, no clinical fac-

tor was documented to be the independent risk factor of vitamin K deficiency.

Vitamin K deficiency was significantly more frequent in patients with higher LDL concentrations than in those with lower LDL levels ($p < 0.0018$). The sensitivity and negative predictive value of such classification in the differentiation between selected groups was very high (92.3% and 91.7%, respectively), with a moderate specificity and positive predictive value (73.3% and 75.0%, respectively).

DISCUSSION

Our results suggest that peritoneally dialyzed patients with CKD are at high risk of subclinical vitamin K deficiency as defined by the specific and sensitive marker, PIVKA-II. In the first reports on vitamin K status in CKD, it was documented that phylloquinone concentrations in hemodialyzed and peritoneally dialyzed patients, kidney transplant recipients, chronic renal failure patients and healthy subjects did not differ (Malyszko *et al.*, 2002; Malyszko *et al.*, 2004) or that phylloquinone levels in PD patients could be even higher than in healthy controls (Vychytil & Druml, 1998). However, in subsequent studies, frequent vitamin K deficiency was reported in hemodialyzed patients and those not requiring renal replacement therapy (Pilkey *et al.*, 2007; Nerlander *et al.*, 2009; Holden *et al.*, 2010; Schlieper *et al.*, 2011). Pilkey *et al.* (2007) revealed abnormal phylloquinone and undercarboxylated osteocalcin concentrations in 29% and 93% of the hemodialyzed subjects, respectively. Subclinical vitamin K deficiency criteria based upon phylloquinone, undercarboxylated osteocalcin and PIVKA II levels were met respectively by 6%, 60% and 97% of the patients not requiring renal replacement therapy (Holden *et al.*, 2010). Schlieper *et al.* (2011) documented abnormal PIVKA II concentrations in 64% of the hemodialyzed subjects. On the other hand, Nerlander *et al.* (2009) found vitamin K deficiency based upon PIVKA II measurements in 14.6% of patients. However, 60% of them were warfarinized. The obtained data are important, particularly for patients with CKD and subclinical vitamin K deficiency who require treatment with warfarin or coumarins. These patients could have increased risk of bleeding and disturbed bone mineralization secondary to this treatment.

The data obtained in patients undergoing PD are less concordant (Nagasawa *et al.*, 1998b; Vychytil & Druml, 1998; Malyszko *et al.*, 2002; Malyszko *et al.*, 2004; Holden *et al.*, 2008). Vychytil & Druml (1998) documented that the mean vitamin K concentrations in their patients treated with PD were twice as high as those in healthy controls. In two other reports (Malyszko *et al.*, 2002; Malyszko *et al.*, 2004) phylloquinone concentrations in PD patients and healthy subjects did not differ. However, Holden *et al.* (2008) documented frequent vitamin K deficiency; abnormal phylloquinone levels were present in 23.8% of patients, with high percentages ($>20\%$) of undercarboxylated osteocalcin present in all subjects studied. Our results are in favor of the opinion that vitamin K deficiency is frequent in PD patients. Possible reasons for the contradictory results might be related to different methods assessing vitamin K status. One available procedure is to measure vitamin K1 and K2 concentrations (i.e. phylloquinone and menaquinone) levels. On the other hand, it is possible to assess the presence of undercarboxylated proteins (prothrombin and os-

Table 1. Basic clinical features in patients with and without vitamin K deficiency.

Variables	Vitamin K deficiency Median [1 st –3 rd quartiles]		Statistical significance
	No	Yes	
Age (years)	57 [40.5–73.5]	60 [57–66]	0.29
BMI (kg/m ²)	23.6 [22.3–28.6]	31.8 [29.9–38.4]	0.0018
Dialysis vintage (months)	20 [8–37]	27 [7–35]	0.96
eGFR (ml/min)	12 [8.5–13.5]	9 [7–11]	0.13
Total protein (g/dL)	6.5 [6.1–6.8]	7.0 [6.6–7.3]	0.12
Albumin (g/dL)	4.00 [3.60–4.35]	3.80 [3.39–4.30]	0.62
Calcium (mg/dL)	9.55 [8.73–9.85]	8.75 [8.45–9.58]	0.37
Urea (mg/dL)	112 [78–135]	114 [99–127]	0.65
Creatinine (mg/dL)	5.94 [4.97–7.85]	8.07 [6.71–9.83]	0.07
Cholesterol (mg/dL)	177.0 [159.5–197.0]	208 [188–249]	0.037
LDL cholesterol (mg/dL)	95.6 [81.5–106.3]	125.3 [105.3–169.1]	0.006
Triglycerides (mg/dL)	101 [82–130.5]	167 [89–273]	0.11

teocalcin). The other possible explanation is the lack of well-defined and reliable control groups which may result in misleading interpretation of the collected data.

Patients with inadequate vitamin K status seem to have a higher rate of cardiovascular and cerebrovascular diseases due to increased risk of vascular tunica media calcification (Nagasawa *et al.*, 1998a; Krueger *et al.*, 2009). The role of vitamin K-dependent proteins as well as the exact mechanisms of their action are not fully understood. Therefore, further studies should be carried out on larger groups of patients with CKD to elucidate this issue. Vitamin K2 supplementation may be a viable pharmacological treatment option for CKD patients with vitamin K deficiency (Schlieper *et al.*, 2011). The optimal indicated dosage of such supplementation should be further explored as well as the safety of that treatment in patients on long-term warfarin or coumarin therapy (Fusaro *et al.*, 2011).

Although PIVKA II concentrations in the present study were found to be somehow related to nutritional status, renal function, and cholesterol concentrations, we did not identify any independent risk factor for vitamin K deficiency. However, we did not include vitamin K intake within the model, which is an important drawback of the analysis. The small number of patients included is another limitation of the study which results in its limited power. Although it was proven through mathematical analysis that LDL cholesterol could potentially serve as a potential predictor in optimal tree decision, further studies are needed to validate this finding. Vitamin K deficit seems to be rather unlikely in patients with low LDL concentrations (high negative predictive value). Interestingly, Vychytil & Druml (1998) and Nagasawa *et al.* (1998a) documented significant correlations between phylloquinone concentrations and total cholesterol levels which were not observed in our study. However, they assessed vitamin K levels, whereas we searched for the presence of undercarboxylated prothrombin representing vitamin K deficiency. The observed discrepancies might also potentially be related to different nutritional behaviors. However, the small sample size of the studies should be kept in mind.

In other multiple linear regression models with logged values of various measurements of vitamin K status (phylloquinone, undercarboxylated osteocalcin, PIVKA

II) it was documented that the potential significant predictors include: dietary vitamin K, triglycerides, ApoE4 allele, dialysis vintage, levels of phosphates and calcium (Pilkey *et al.*, 2007; Holden *et al.*, 2010). However, the observed effects were minor and different predictors were significant in different models. Moreover, the analysis was conducted in patients not requiring renal replacement therapy (Holden *et al.*, 2010) or in hemodialyzed subjects (Pilkey *et al.*, 2007). Taking into account the frequency of vitamin K deficiency reported in the present study as well as in other publications, the assessment of vitamin K status should become a standard procedure in PD patients and probably in all CKD subjects.

The measurement of PIVKA II concentrations is done with the use of the enzyme immunoassay method (ELISA) whereas vitamin K1 levels are determined using HPLC. Since the availability of ELISA is definitely far more common therefore, at present, we suggest the assessment of PIVKA II. Vitamin K status should be checked as an annual routine control. In case of vitamin K deficiency, the supplementation should be introduced and adjusted in three-month intervals. In the meantime, the significance of vitamin K2 status and supplementation should be elucidated which may potentially imply the change of recommendations. It is especially important since vitamin K2 has a higher affinity for γ -glutamyl carboxylase and has a more widespread tissue distribution (Krueger 2009).

In conclusion, vitamin K deficiency is a frequent condition in peritoneally dialyzed patients. Assessment of vitamin K status should become the standard procedure in this group of patients.

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