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Vitamin K, Vertebral Fractures, Vascular Calcifications, and Mortality: VItamin K Italian (VIKI) Dialysis Study

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ABSTRACT

Vitamin K (vitamin K1 or phylloquinone and vitamin K2, a series of menaquinones [MKs]) is involved in the production of bone and matrix amino acid γ-carboxy-glutamic acid (Gla) proteins, regulating bone and vascular calcification. Low vitamin K concentrations are associated with increased risks of fractures and vascular calcification, and frequent complications in hemodialysis patients. We carried out an observational study to establish the prevalence of vitamin K deficiency and to assess the relationship between vitamin K status, vertebral fractures, vascular calcification, and survival in 387 patients on hemodialysis for ≥1 year. We determined plasma levels of vitamin K compound, bone-Gla-protein, matrix-Gla-protein, and routine biochemistry. Vertebral fractures (reduction in vertebral body height by ≥20%) and aortic and iliac calcifications were also investigated in a spine (D_5–L_4) radiograph. Three-year patient survival was analyzed. Important proportions of patients had deficiency of MK7 (35.4%), vitamin K1 (23.5%), and MK4 (14.5%). A total of 55.3% of patients had vertebral fractures, 80.6% had abdominal aorta calcification, and 56.1% had iliac calcification. Vitamin K1 deficiency was the strongest predictor of vertebral fractures (odds ratio [OR], 2.94; 95% confidence interval [CI], 1.38–6.26). MK4 deficiency was a predictor of aortic calcification (OR, 2.82; 95% CI, 1.14–7.01), whereas MK5 deficiency actually protected against it (OR, 0.38; 95% CI, 0.15–0.95). MK7 deficiency was a predictor of iliac calcification (OR, 1.64; 95% CI, 1.03–2.60). The presence of vertebral fractures was also a predictor of vascular calcifications (OR, 1.76; 95% CI, 1.00–3.08). Increased alkaline phosphatase and C reactive protein (CRP), age, and cerebrovascular events were predictors of mortality. Our study suggests that the vitamin K system may be important for preserving bone mass and avoiding vascular calcification in hemodialysis patients, pointing out a possible role of vitamin K in bone and vascular health. Based on our results, we suggest that the general population should also be studied for vitamin K deficiency as a possible cause of both vertebral fractures and vascular calcification.

KEY WORDS: DIALYSIS; MENAQUINONE; PHYLLOQUINONE; BONE; CALCIFICATION

Introduction

Vitamin K includes a series of 2-methyl-1,4-naphthoquinone derivatives: vitamin K1 (phylloquinone), which occurs naturally in food, mainly green leafy vegetables and fruits (avocado, kiwi), and vitamin K2; ie, several menaquinones (MKs), which are mainly synthesized by bacteria in the intestinal tract and occur in cheese, meat, and fermented soya derivatives.

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Because very little vitamin K is stored, it needs to be continually replenished.[1]

Vitamin K acts as the coenzyme of a carboxylase that determines carboxylation of glutamic acid residues, resulting in the formation of the amino acid γ-carboxy-glutamic acid (Gla). In the liver this reaction controls the production of coagulation factors, whereas in extrahepatic tissues vitamin K controls also the production of bone and matrix Gla proteins (BGP and MGP). BGP is a small protein produced by osteoblasts under the control of vitamin D, which modulates the expression of its gene. It contains three GLA residues that enable its binding to hydroxyapatite in bone.[2] BGP knockout mice develop hyperostosis, showing that it has a role in promoting normal bone mineralization.[3] Vitamin K deficiency in bone can be measured indirectly by measuring uncarboxylated BGP (ucBGP). MGP is a larger protein produced by osteoclasts, chondrocytes and vascular smooth muscle cells (VSMC), which is a potent inhibitor of arterial calcification.[4] MGP knockout mice experience pathological fractures due to severe osteoporosis and widespread vascular calcification.[5] MGP undergoes not only vitamin K-dependent γ-glutamate carboxylation, which determines its bioactivity as calcification inhibitor, but also serine phosphorylation. Thus, there are two possible states: an extracellular phosphorylated fraction and a nonphosphorylated fraction inside matrix vesicles or apoptotic bodies, implying that nonphosphorylated MGP may predict local VSMC stress.[4]

In chronic kidney disease (CKD) mineral and bone disorder is very common and associated with major adverse outcomes: in CKD patients the risk of hip fracture is four times higher than in the general population and the Dialysis Outcomes and Practice Patterns (DOPPS) study conducted in 12 countries in 2002 to 2004 reported an incidence of 8.9 new hip fractures and of 25.6 new fractures of any kind per 1000 patient-years.[6,7] Another common disorder in CKD patients is vascular calcification. The prevalence of calcification of the aorta is twice as high as in the general population and vascular calcifications have been found to be associated with a higher prevalence of vertebral fractures.[8] The pathogenesis of vascular calcification in CKD patients is multifactorial, and phosphate overload is probably one of the most relevant inducing factors.[9] In fact, inorganic phosphate induces arterial calcification directly through a real "ossification" of the arterial tunica media, and control of serum phosphate in CKD patients is crucial in preventing vascular calcification. Calcification inhibitors are also important determinants of vascular calcification,[10] and the vitamin K-dependent MGP is one of the most relevant of them.

What is more, in women on hemodialysis, both severe vascular calcifications and vertebral fractures are risk factors for mortality ($RR = 3.2$ and 4.8, respectively).[8] Aortic calcification is also associated with osteoporotic fractures in the general population.[11]

Low intake of vitamin K1 has recently been associated with an increase in the risk of hip fracture in the general population, and treatment with vitamin K may reduce the relative risk for nonvertebral and hip fractures.[12,13] Moreover, consumption of natto, a local Japanese food rich in vitamin K, is associated with significantly higher bone mineral density (BMD) in elderly Japanese men, and high dietary intake of vitamin K is associated with higher BMD in elderly subjects of both genders.[14,15] Conversely, low dietary vitamin K intake is associated with low BMD in women of all ages.[16] Few studies have investigated the role of vitamin K in cardiovascular disease. In the Rotterdam study high dietary intake of vitamin K2 was associated with a reduction in the risk not only of severe aortic calcification (odds ratio [OR], 0.48; 95% confidence interval [CI], 0.32–0.71), but also of the relative risk of CHD mortality (OR, 0.43; 95% CI, 0.24–0.77).[17]

We carried out an observational study, with follow-up to assess vital status, primarily to assess the prevalence of deficiency of vitamin K1 and vitamin K2 (menaquinones) in dialysis patients. The secondary objectives were to assess the impact of vitamin K status on vitamin K–dependent proteins related to bone turnover, vertebral fractures, vascular calcification, and survival.

Patients and Methods

This was an observational study conducted at 18 dialysis centers in Italy. All the local ethics committees approved the study, which was conducted according to the regulations in force related to observational studies. Approval dates ranged from July 14, 2008 to October 26, 2009. Patient enrollment took place between November 2008 and November 2009, and follow-up to assess vital status was performed in December 2011.

Patient population

We included adult patients of both genders who had been on hemodialysis for more than 1 year, provided that they gave their informed consent in writing to the use of their medical records for the study.

We excluded patients who had a life expectancy of less than 6 months, who had any evidence of cancer (with the exception of basalioma), who had coagulation disorders, or who had any condition that, according to the investigator, could interfere with the outcome of the study.

Healthy controls ($n = 62$) were blood donors matched by age (56.8 ± 3.9; range, 51–65 years) and gender (males: 43 [69.4%]; females: 19 [30.6%]) to dialysis patients. They were recruited on a voluntary basis during the study period.

Procedures

We collected the following information: demographic data (initials or ID number, gender, age); renal failure history (cause, type of hemodialysis, duration of hemodialysis in months, transplantation history); lifestyle (smoking status, alcohol consumption); medical history; and body mass index (BMI).

We drew venous blood samples when the patients were fasting for the determination of serum concentrations of vitamin 25(OH)D, vitamin K components: osteocalcin (bone Gla Protein, or BGP) and matrix Gla protein (MGP), routine biochemistry, including in particular total alkaline phosphatase (ALP), albumin, C-reactive protein (CRP), aluminum, and lipid profile. The Laboratory in Perugia determined vitamin K components by a simple, sensitive, and selective reversed-phase high-performance liquid chromatography (HPLC) method, developed

for the simultaneous determination of vitamin K in human plasma. Clear and well-separated chromatographic PK and MKs profiles were obtained in healthy human and uremic plasma. Uremic plasma is characterized by an increased level of plasma lipids and lipoproteins, which are interfering factors to chromatography. Thus, we adopted a liquid-liquid extraction and then a solid-phase extraction of human plasma using Polymeric Reversed Phase cartridges, achieving a good reproducibility. The vitamers were measured by an electrochemical detector after postcolumn reduction with platinum on alumina powder and using the MK8 form as internal standard. Quantitative recovery was obtained in a range of 80% to 96% for PK and MK vitamers. Vitamin K values were corrected according to triglyceride levels.[18] The Laboratory in Padua determined the following: total 25-OH vitamin D and total BGP using the LIASON 25 OH Vitamin D and Osteocalcin Assays 310600 and 310950 (DiaSorin, Inc., Stillwater, MN, USA); undercarboxylated osteocalcin (uc-OC) using the Glu-OC EIA Kit MK118 (Takara Bio, Inc., Otsu, Shiga, Japan), MGP using the Human MGP–Matrix GLA Protein Kit (Biomedica Medizinprodukte GmbH & Co KG, Wien, Austria); and undercarboxylated MGP using by the method provided by VitaK BV (Maastricht, The Netherlands).[19] The other parameters were determined according to standard laboratory methods.

We obtained a radiograph of the thoracic and lumbar regions of the spinal column (D_5–L_4) in the latero-lateral view with the patient in the lateral recumbent position. The following recommendations were made: the X-ray was always to be performed by the same technician using the same film distance (100 cm) and the same focus for the central ray, namely D_7 for the dorsal region and L_3 for the lumbar region. The radiographs were sent to CNR for blinded evaluation in duplicate.

We considered a vertebral fracture to be present when the height of the vertebral body was reduced by at least 20% (4 mm), according to Genant and colleagues[20] and Guglielmi and colleagues.[21]

We quantified vascular calcifications by measuring the length of calcific deposits along the anterior and posterior wall of the aorta (mild 0.1–5 cm, moderate 5.1–10 cm, and severe >10 cm). This methodology has been used and validated by previous publications.[22] With a similar approach, we also determined the presence or absence of calcifications of the iliac arteries in the same radiograph (mild 0.1–3 cm, moderate 3.1–5 cm, and severe >5 cm).

We followed patients for survival for a mean period of 2.7 ± 0.5 years.

Statistical analysis

We determined the sample size to study the prevalence of vitamin K deficiency. Based on the finding of a prevalence of 24% setting a vitamin K cutoff of 0.5 nmol/L in Framingham's study in the general population and of 29% in 142 hemodialysis patients, we assumed that the prevalence of vitamin K deficiency would be about 30% and that therefore 400 patients would be needed to achieve a 95% CI of ± 4.[23,24]

We defined vitamin K deficiency considering the values included between the 5th and the 95th percentile of the distribution of healthy control subjects as normal reference values.[25]

We conducted the identification of any predictors of vertebral fractures (model a) or vascular calcification (model b) using univariate logistic regression. Any significant predictors with $p \leq 0.20$ were introduced into a multivariate model using the stepwise selection method. In the multiple regression models we included variables that met criteria to be confounders (ie, variables that were related to both the exposure [vitamin K deficiency] and to study outcomes), which were not an effect of exposure and which were not in the causal pathway between the exposure and the study outcomes,[26] as well as all univariate correlates of study outcomes. For quantitative variables, linearity assumption was evaluated considering the analysis of quartiles. Possible interactions among predictors were also assessed. OR and corresponding 95% CI were calculated for each variable.

We checked the vital status of patients through a follow-up telephone interview. The differential distribution of ALP, CRP, MGP (total, undercarboxylated [uc]), BGP (total, undercarboxylated [uc]), vitamin K deficiency, and aortic and iliac calcifications between survivors and nonsurvivors was analyzed by χ^2 or Fisher's exact test, or the Wilcoxon rank sum test. Survival curves to determine the association of mortality and aortic or iliac calcifications were estimated using Kaplan-Meier analyses; the log-rank test was also performed. Cox proportional hazards models were defined with outcome overall mortality, and the assumption of proportionality was assessed through the analysis of Schoenfeld residuals of the covariates introduced in the model. Hazard ratio (HR) and 95% CIs were calculated.

We performed all statistical analyses using the SAS statistical package (version 9.2, SAS Institute, Inc., Cary, NC, USA).

Results

A total of 387 adult patients and 62 healthy controls were included. The patients were mainly men (63%). Substantial proportions of patients had cardiovascular risk factors, such as hypertension (78.6%) and diabetes mellitus (22%). Some patients were suffering from concomitant diseases that could interfere with vitamin K status (liver disease 14%, malabsorption syndrome 1%). A minority had a history of fractures (6.7% before dialysis, 10.4% after) (Table 1).

The serum concentrations of phylloquinone (PK) and the various menaquinones (MK) components of vitamin K2 are shown in Table 2. Important proportions of patients had deficiency of MK7 (35.4%), PK (23.5%), and MK4 (14.5%). The distribution of the most deficient component, MK7, is shown in Fig. 1*A* and *B*. All the subgroups of patients with vitamin K component deficiency had significant increases in triglycerides ranging from 26% to 59.8% ($p < 0 \cdot 0001$ except MK6, $p = 0.004$).

The comparison between dialysis patients and healthy controls (Table 3) disclosed a number of significant differences: in dialysis patients ucBGP was 11-fold higher and total BGP was 12-fold higher, whereas ucMGP was threefold lower and total MGP was more than twice as high. In addition, the median vitamin D level was lower by 27.5% in dialysis patients. There were no differences in total and/or ucBGP and/or MGP between

Table 1. Main Characteristics of the Study Cohort

Parameter	Study cohort ($n = 387$)
Age (years), mean ± SD (range)	64.2 ± 14.1 (18–89)
Gender, *n* (%)	
Males	244 (63.0)
Females	143 (37.0)
BMI (kg/m^2), mean ± SD	25.1 ± 4.4
Type of dialysis, *n* (%)	
Bicarbonate	189 (48.7)
Hemodiafiltration	102 (26.4)
Acetate free biofiltration	54 (14.0)
Hemofiltration	32 (8.3)
Other	10 (2.6)
Smoking (both smokers and ex-smokers)	136 (36.8)
Alcohol drinkers (active and former)	82 (22.7)[a]
Diabetes mellitus	85 (22.0)
Arterial hypertension	304 (78.6)
Angina	64 (16.5)
Myocardial infarction	73 (18.9)
Atrial fibrillation	51 (13.2)
Heart failure	39 (10.1)
Peripheral arterial disease	134 (34.6)
Cerebrovascular events [stroke]	41 (10.6) [20 (5.2)]
Parathyroidectomy	26 (6.7)
Fractures before dialysis	26 (6.7)
After dialysis	40 (10.4)
Malabsorption syndrome	4 (1.0)
Liver disease	54 (14.0)

Values are *n* (%), mean ± SD, or mean ± SD (range).
BMI = body mass index.
[a]Data on alcohol consumption available for 361 patients.

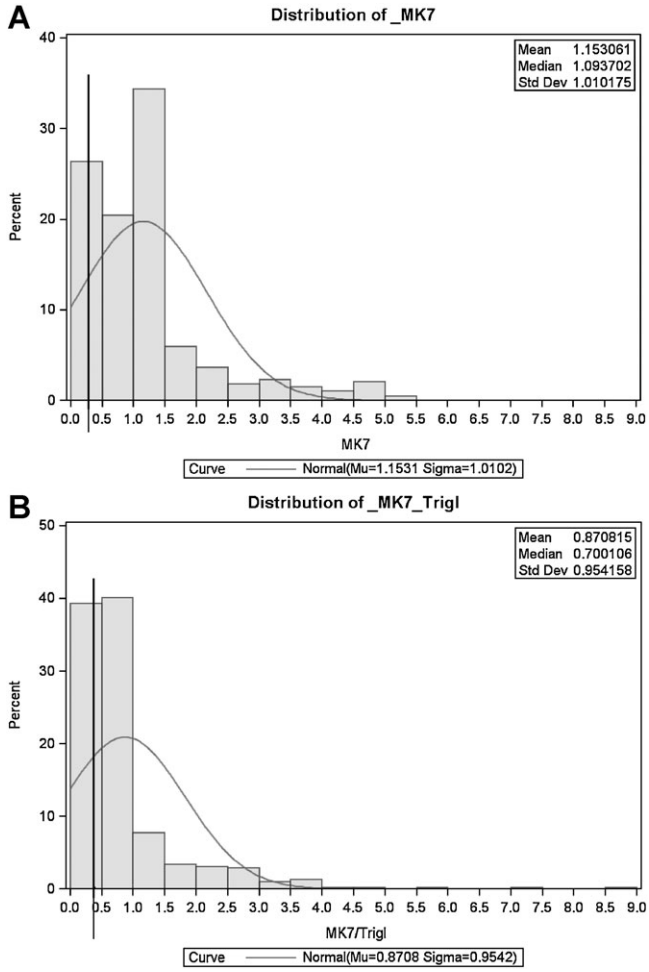

Fig. 1. Distribution of plasma levels of MK7, the vitamer which showed the most remarkable deficiency. Concentrations are expressed in ng/mL. (*A*) Unadjusted. (*B*) adjusted for triglycerides levels.

Table 2. Vitamin K Status in 387 Adult Patients on Dialysis and 62 Healthy Controls

	Healthy controls			Dialysis patients			Literature[22]	Patients with
Vitamers	Mean ± SD	Median	Range	Mean ± SD	Median	Range	normal range	deficiency (%)
PK (Vit K1)	1.36 ± 1.08	1.13	0.14–4.06	0.98 ± 1.00	0.63	0.03–5.12	0.17–3.05	11.1
Adjusted PK[a]	1.80 ± 1.77	1.26	0.11–7.22	0.70 ± 0.80	0.43	0.01–5.07	0.21–6.23	23.5
MK4	0.91 ± 0.85	0.84	0.03–3.42	0.67 ± 0.74	0.51	0.01–4.86	0.07–2.68	8.8
Adjusted MK4[a]	1.20 ± 1.28	0.75	0.06–5.23	0.51 ± 0.67	0.37	0.01–5.19	0.06–4.39	14.5
MK5	1.29 ± 1.12	1.13	0.09–5.12	1.00 ± 0.77	1.00	0.02–4.99	0.10–3.07	2.8
Adjusted MK5[a]	1.56 ± 1.35	1.40	0.08–5.95	0.75 ± 0.66	0.75	0.01–5.52	0.10–3.79	7.8
MK6	1.10 ± 1.14	0.73	0.04–5.09	0.63 ± 0.67	0.48	0-01–4.79	0.04–3.33	3.6
Adjusted MK6[a]	1.26 ± 1.30	0.62	0.05–5.43	0.47 ± 0.59	0.34	0.003–5.31	0.05–3.87	9.6
MK7	1.95 ± 1.37	1.43	0.22–4.82	1.15 ± 1.01	1.09	0.02–5.15	0.33–4.48	18.1
Adjusted MK7[a]	2.53 ± 2.19	1.93	0.23–9.46	0.87 ± 0.95	0.70	0.004–8.89	0.44–6.46	35.4

The vitamers concentrations are expressed in ng/mL. Vitamin K deficiency was defined considering the values included between the 5th and the 95th percentile of the healthy control subjects distribution as normal reference values.[22]
MK = menaquinone; PK = phylloquinone.
[a]Adjusted for triglycerides.

Table 3. Healthy Controls Versus Dialysis Patients

Variables	Healthy controls ($n = 62$)	CKD patients ($n = 387$)	p
25-OH-D (ng/mL)			<0.0001
Median	40.0	29.0	
25th percentile	36.2	27.2	
75th percentile	44.5	31.5	
Total BGP (μg/L)			<0.0001
Median	15.3	182	
25th percentile	12.8	96.3	
75th percentile	18.2	318.8	
ucBGP (ng/mL)			<0.0001
Median	0.88	11.00	
25th percentile	0.54	4.62	
75th percentile	1.60	17.20	
Total MGP (nmol/L)			<0.0001
Median	8.0	18.8	
25th percentile	7.0	12.7	
75th percentile	9.0	30.9	
ucMGP (nmol/L)			<0.0001
Median	1682.9	569.0	
25th percentile	1286.00	288.0	
75th percentile	2147.5	933.0	

BGP = bone Gla protein; CKD = chronic kidney disease; MGP =Gla protein; uc = undercarboxylated.

patients with and without vitamin K deficiency, except for the patients with vitamin K1 deficiency, who had significantly lower median ucBGP (8.7 versus 11.7 ng/mL, $p = 0.01$) and significantly higher ucMGP (683 versus 519.5 nmol/L, $p = 0.03$), and patients with MK6 deficiency, who had significantly higher total median MGP (22.5 versus 17.9 nmol/L, $p = 0.01$).

More than one-half of the patients (55.3%) had vertebral fractures. The comparison between patients with and without vertebral fractures suggested that the presence of vertebral fractures was associated with vitamin K1 deficiency (15.4% versus 5.8%, $p < 0.01$) (Table 4), but not with MKs. This was confirmed by logistic regression, which identified vitamin K1 deficiency as the strongest predictor of vertebral fractures that increased the probability threefold (OR, 2.94). In contrast, albumin was a protective factor: an increase by 1 g/dL was associated with a 40% reduction in the probability of fracture (OR, 0.6) (Table 4).

Most patients had vascular calcification: 80.6% had calcification of the abdominal aorta, which was moderate in 29.7% and severe in 30%, and 56.1% had iliac calcification, which was moderate in 29.7% and severe in 14%. Only 16.5% of patients did not have any vascular calcification and more than one-half the patients (53.2%) had calcification of both the abdominal aorta and the iliac arteries.

The proportion of patients with MK4 deficiency was significantly higher among the patients with aortic calcification (10.6% versus 1.3%, $p = 0.01$). On the contrary, MK5 deficiency was significantly lower (6.4% versus 13.3%, $p = 0.04$). Logistic regression showed that MK4 deficiency was a predictor that increased the probability of aortic calcification threefold, whereas MK5 deficiency actually protected against it, reducing the probability by 67% (Table 5). Among the patients with iliac calcifications the proportion of patients with MK7 deficiency was significantly higher than in those without (41% versus 28.2%, $p = 0.009$) and logistic regression showed that MK7 deficiency is a predictor of iliac calcification (OR, 1.61), together with age (OR, 1.86), atrial fibrillation (OR, 3.10), and vertebral fractures (OR, 2.10) (Table 6). MK4/triglyceride deficiency and vertebral fractures were found to be predictors of calcification of both the abdominal aorta and the iliac arteries (OR, 3.99; 95% CI, 1.23–12.93, and OR, 2.80; 95% CI, 1.46–5.37, respectively).

A total of 77 patients died during follow-up (19.9%). Mean follow-up amounted to 2.7 ± 0.5 years. Most patients died of cardiovascular events ($n = 51$); other causes were infections ($n = 11$), cancer ($n = 5$), and miscellaneous ($n = 10$). Aortic and iliac calcifications were more common in nonsurvivors (90.9% versus 78.1%, $p = 0.01$, and 66.2% versus 53.6%, $p = 0.04$, respectively); the differences were more pronounced when only severe calcifications were considered (48.1% versus 25.5%, $p = 0.0001$; 23.4% versus 11.6%, $p < 0.01$). The course of survival probability of patients with and without severe aortic calcifications is shown in Fig. 2. There were no significant differences in vitamin K1 or MKs between nonsurvivors and survivors. The same was true for other laboratory variables, except for median alkaline phosphatase (ALP) and C reactive protein (CRP), which were significantly higher in nonsurvivors (99.0 versus 80.0 pg/dL, $p = 0.0002$; 1.85 versus 1.54 mg/L, $p = 0.03$) and median MGP, which was significantly lower (15.0 versus 19.7 nmol/L, $p = 0.02$). Nonsurvivors had more vertebral fractures (62.3% versus 53.6%,

Table 4. Logistic Regression With the Presence of Vertebral Fractures as Outcome

	Odds ratio	95% CI	p
Sex (male)	1.75	1.13–2.69	0.0118
Age ≥67 years	1.80	1.18–2.76	0.0064
Vitamin K1 deficiency	2.94	1.38–6.26	0.0053
Albumin (g/dL)	0.60	0.38–0.94	0.0264
Steroid therapy	2.41	0.89–6.54	0.0840

CI = confidence interval.

Table 5. Logistic Regression With the Presence of Abdominal Aortic Calcification as Outcome

	Odds ratio	95% CI	p
Age (years)	1.05	1.03–1.07	<0.0001
Arterial hypertension	2.00	1.07–3.75	0.0307
Myocardial infarction	2.78	1.10–7.05	0.0310
Vertebral fractures	1.81	1.03–3.18	0.0389
MK4/triglycerides deficiency	2.82	1.13–7.05	0.0266
MK5/triglycerides deficiency	0.33	0.13–0.85	0.0217

CI = confidence interval.

Table 6. Logistic Regression With the Presence of Iliac Calcification as Outcome

	Odds ratio	95% CI	*p*
Age ≥67 years	1.86	1.21–2.87	0.0048
Triglycerides (>206 mg/dL)	1.67	1.00–2.79	0.0521
Atrial fibrillation	3.10	1.49–6.42	0.0024
Vertebral fractures	2.10	1.67–3.22	0.0007
MK7/triglycerides deficiency	1.61	1.02–2.54	0.0426

CI = confidence interval.

Table 7. Logistic Regression With Mortality as Outcome

	HR	95% CI	*p*
Age (increase by 1 year)	1.047	1.024–1.070	<0.0001
CRP (increase by 1 Unit)	1.029	1.017–1.042	<0.0001
ALP (increase by 10 Units)	1.049	1.012–1.082	0.0056
Cerebrovascular event	1.917	1.046–3.515	0.0354
Angina	1.602	0.943–2.723	0.0813

ALP = alkaline phosphatase; CI = confidence interval; CRP = C reactive protein; HR = hazard ratio.

$p = 0.16$) and lower ucMGP levels (477 versus 584 ng/mL, $p = 0.14$). ALP and CRP were predictors of mortality (Table 7).

Discussion

In this observational study substantial proportions of hemodialysis patients had deficiency of vitamin K compounds (up to 35%), vertebral fractures (55%), and vascular calcification (aortic calcification 80%, iliac calcification 56%). Vitamin K compound deficiency was found to be a predictor of both vertebral fractures and vascular calcification. Mortality was associated with lower MGP and higher ALP levels.

To our knowledge, this is the first study that provides a comprehensive assessment of vitamin K status, including most vitamin K vitamers; ie, phylloquinone (PK) and menaquinones (MK4, MK5, MK6, and MK7), adjusting their values for triglycerides levels. Previously, only vitamin K1 levels were measured in the few large studies that assessed vitamin K deficiency.[18,23,24] These studies found that vitamin K1 deficiency amounted to 24% in the general population and to 29% in hemodialysis patients,[23,24] which is consistent with our finding of a rate of 23.5% vitamin K1 deficiency. Vitamin K2 has several different vitamers: MK7 is better known and often the only one considered, but others, such as MK4, MK5, and MK6, may have important biologic actions. Moreover, the adjustment for triglyceride concentration is particularly relevant for the assessment of vitamin K status. Vitamin K components are all liposoluble compounds that become part of chylomicrons after absorption from the gut and as such are transported to the liver. Vitamin K1 remains partly in the liver, whereas vitamin K2 is transferred to VLDL and LDL for transport and there is close correlation ($r = 0.99$) between triglyceride concentrations and vitamin K1.[1,18]

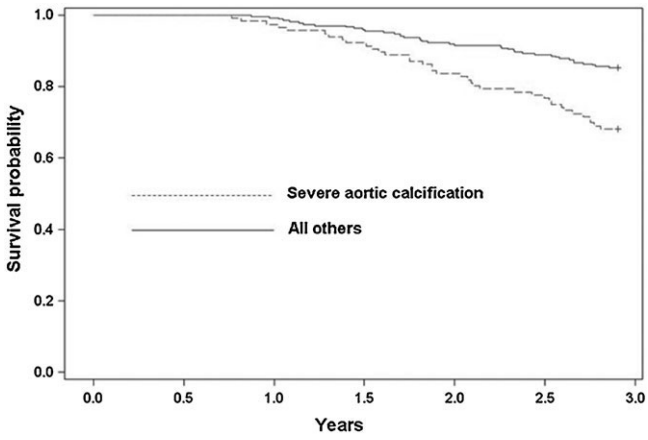

Fig. 2. Survival curves in patients with severe aortic calcifications (dotted line) and in all the remaining patients (without calcifications or with mild to moderate calcification, log rank test $p < 0.0001$).

Previous publications reported that vitamin K1 had no effect on bone mineral density or bone markers of skeletal turnover.[27,28] However, in both cases an association with a reduced incidence of fractures was found for a low dietary vitamin K intake[27] and for warfarin-induced vitamin K deficit.[28]

This is the first study to relate vitamin K1 and K2 deficiency directly both to vertebral fractures and vascular calcification. Vitamin K1 deficiency was the strongest predictor of vertebral fractures. This is consistent with the findings of Cheung and colleagues,[29] who found that 5 mg vitamin K1 protected postmenopausal women from fractures, despite a lack of effect on BMD and bone resorption. It is also consistent with the findings of Nakano and colleagues,[13] who found that plasma concentrations of vitamin K1 and albumin were significant determinants of hip fracture in the general population, whereas MK-7 was not. Indeed, our results show that satisfactory albumin status protects against vertebral fractures (OR, 0.60), whereas MK7 does not. These findings are also supported by the outcome of the Hordaland Health Study,[12] in which low dietary intake of vitamin K1, but not of vitamin K2, was associated with hip fracture in 2800 Norwegian subjects, monitored for 10 years.

The other important result was that MK4 deficiency is a predictor of aortic calcification in humans. This finding, reported for the first time in humans, is consistent with the experimental suggestion that MK4 acts as an inhibitor of vascular calcification in the vessel wall, based on the downregulation of osteoprotegerin gene expression and osteoprotegerin protein secretion from MK4-treated cells.[30]

MK7 was not a predictor of aortic calcification, whereas it was a predictor of iliac artery calcifications (OR, 1.61). The different effect of menaquinones on different vascular compartments can not be explained with the available data. Our findings need to be confirmed and expended by further studies. Similarly and surprisingly, MK5 *deficiency* appeared to be protective against aortic calcification (OR, 0.33). To our knowledge this is the first time that deficiency of a component of the vitamin K2 system is found to actually be protective against vascular calcification. The fact that two MKs appear to exert opposite effects suggests that vitamin K2 should not be studied as a single entity.

Findings reported in Table 3 also need to be mentioned. We confirmed that 25(OH)-vitamin-D levels in CKD patients are lower than healthy control and the same applies to ucMGP levels. Accordingly, Cranenburg and colleagues[19] have highlighted in four different patient populations (patients who underwent angioplasty, patients with aortic stenosis, hemodialysis patients, and calciphylaxis patients) significantly lower circulating ucMGP levels in comparison with healthy controls. In particular, in the hemodialysis and calciphylaxis populations, virtually all subjects had ucMGP levels below the normal adult range. On the other hand, dialysis patients in our study showed higher levels of total MGP, total BGP, and ucBGP. However, total MGP, including active, calcification-inhibiting carboxylated MGP, was lower in the subgroup of nonsurvivors. Recently, Schlieper and colleagues[31] reported that dephosphorylated, carboxylated MGP levels are lower in dialysis patients than in normal subjects, with an increased risk of all-cause (HR, 2.2) and cardiovascular mortality (HR, 2.7). Our finding of lower total MGP in the subgroup of nonsurvivors is similar. However, we did not measure dephosphorylated, carboxylated MGP levels. The increased levels of total MGP are also a noteworthy finding, as it can not be considered a marker of calcification. The finding, in patients with vitamin K1 deficiency, of significantly lower ucBGP and significantly higher ucMGP, as well as, in patients with MK6 deficiency, of significantly higher total MGP is contradictory and needs to be confirmed by further studies.

Another significant finding was that ALP increase is a predictor of mortality. The association between high ALP and mortality has already been reported in dialysis patients.[32] We did not measure the ALP bone isoenzyme, but only total ALP. This could determine an influence of liver impairment to the results obtained in our study. However, liver disease was among the variables considered in our study (54 patients, 14%; see Table 1), but it was associated neither with K2 vitamers nor with bone biochemical data.

The main limitation of this study is its observational design. The prognostic impact of a condition, such as vitamin K deficiency, is difficult to study with an experimental design. Nevertheless, the possibility of potential bias or imprecision in data collection should be kept in mind.

At present, guidelines for CKD patients do not make any particular recommendations regarding vitamin K.[33] In view of the potential consequences of vitamin K–deficiency vertebral fractures, which are associated with higher mortality, and vascular calcification, which is a risk factor for cardiovascular morbidity and mortality, we believe that adequate intake of vitamin K, together with calcium and vitamin D, should be recommended. The addition may preserve bone calcification and avoid harmful vascular calcification in CKD patients. The recommendations should be formulated in terms of intake of both vitamin K1 and MKs, namely MK4 and MK7 and not vitamin K2 in general or just MK7. At present, no recommendations can be made about vitamin K supplements, an issue that needs to be clarified in randomized controlled trials.

Moreover, we suspect that the role of vitamin K deficiency in bone and vascular disease may not be confined to CKD patients. Calcium supplements (with or without vitamin D) have been found to increase the risk of cardiovascular events in postmenopausal patients.[34] Additional studies should be performed to investigate the role of vitamin K in bone fractures and vascular calcification in the general population.

Disclosures

All authors state that they have no conflicts of interest.

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Authors' roles: Study design: MF, MN, GT, VV, FG, NV, MP, MZ, AD, DMiotto, SG, GG, DMiozzo, and MG. Study conduct: MF, AD, DMiozzo, and SG. Data collection: MF, VV, FG, NV, MP, MZ, GG, DMiotto, and LD. Data analysis: MN and GT. Data interpretation: MF, VV, FG, NV, MP, MZ, AD, DMiozzo, SG, GG, DMiotto, LD, and MG. Drafting and reviewing the manuscript: MF and MG. Approval of the final manuscript: all authors. MF is the guarantor who takes responsibility for the integrity of the data analysis.

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