Sevelamer use, vitamin K levels, vascular calcifications and vertebral fractures in hemodialysis patients: results from the VIKI study

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Running title: Sevelamer and vitamin K levels in hemodialysis

Word count: Abstract = 317, main text = 3797

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ABSTRACT

BACKGROUND: Hyperphosphatemia is a risk factor for vascular calcifications (VCs) and VCs belong to mineral bone disorders (MBD) in chronic kidney disease (CKD) patients. Vitamin K-dependent proteins such as Matrix Gla Protein (MGP) and Bone Gla Proteins (BGP or osteocalcin) can inhibit VCs and regulate bone mineralization.

OBJECTIVE: To evaluate whether the phosphate binder, sevelamer, could influence vitamin K levels in hemodialysis (HD) patients.

METHODS: In a secondary analysis of the VItamin K Italian (VIKI) study, we evaluated the relationship between vitamin K status, VFs and VCs in 387 HD patients with/without sevelamer. Levels of serum vitamin 25(OH)D, alkaline phosphatase (ALP), vitamin k vitamers: K1 and K2 or menaquinone (MK, including: MK4, MK5, MK6 and MK7), total and undercarboxylated (uc) forms for both BGP and MGP were determined.

RESULTS: No significant differences were observed between sevelamer-treated and untreated patients for main clinical characteristics. Lower MK4 levels (0.45 vs. 0.6 ng/mL, p=0.01) and a higher MK4 deficiency was observed in sevelamer-treated patients (13.5% vs. 5.4%, p=0.005). Multivariate logistic regression revealed that MK4 deficiency was associated with sevelamer use (Odds Ratio; OR: 2.64, 95% CI: 1.25–5.58, p=0.011) and aortic calcification (OR: 8.04, 95% CI: 1.07–60.26, p=0.04). In the same multivariate logistic regression model, sevelamer significantly amplified the effect of total BGP levels on the odds of fractures so that in sevelamer-treated patients, the OR of VFs was about 3 times higher in patients with total BGP <150 μg/L compared to those with total BGP ≥150 μg/L (OR: 3.15, 95% CI: 1.46-6.76, p=0.003), whereas no such effect was found in those untreated

(total BGP <150 µg/L vs. total BGP ≥150 µg/L: OR: 1.21, 95% CI: 0.66-2.23, p=0.54] (p=0.049 for effect modification by sevelamer).

CONCLUSION: These data suggest that sevelamer could interfere with MK4 levels in HD patients and its use in patients with low BGP levels (<150 µg/L) could increase bone fragility in CKD patients.

KEYWORDS: Sevelamer, vitamin K, hemodialysis, vascular calcification, vertebral fractures

INTRODUCTION

The presence of cardiovascular disease (CVD) is a major cause of morbidity and mortality in patients on hemodialysis (HD) (1,2). Vascular calcification (VC), mainly occurs during the late stages of chronic kidney disease (CKD), which is an important contributor to CVD and CKD-related mortality (3). In a recent meta-analysis, Wang et al. showed that a higher degree of cardiovascular calcifications was associated with an increased risk of CV-related mortality and all-cause mortality by 181% and 73% respectively (4). The prevalence of aortic arch calcification in these patients ranged from 23.3% to 57.6% (4).

Among other risk factors, elevated levels of phosphorus (hyperphosphatemia) is also associated with increased VC (5) as well as bone fractures (BFs) (6), necessitating the use of phosphate binders to control serum phosphate (7). Although phosphate binders can improve hyperphosphatemia and may therefore delay the development of calcification, this benefit may be offset by calcium-loading (8). In this regard, even in patients with CKD where phosphate and calcium levels are controlled, VC can still persist (9), pointing towards other factors that may be involved, such as vitamin K. In the case of the latter, its deficiency has gained increasing recognition as being another important risk factor for the development of VCs and BFs, both in the general population and in CKD (10–12).

Vitamin K exists in 3 main forms, K1 and K2 (natural forms), and K3 (or menadione), the synthetic form (13). Vitamin K1, (or phylloquinone, PK), is found in vegetables, while vitamin K2, (also known as menaquinone, MK) is produced by obligate and facultative anaerobic bacteria (14). The most well-known function of vitamin K1 is as an essential cofactor for the function of glutamyl carboxylase (GGCX) enzyme which is responsible for the post-translational modification of vitamin K-dependent proteins (VKDPs) through the conversion of specific glutamic acid (Glu) into calcium binding -carboxyglutamic acid (Gla) residues (15).

VKDPs are categorized as hepatic and extra-hepatic VKDPs. Hepatic VKDPs include coagulation factors II, VII, IX, X, and anticoagulant protein C, protein S, and protein Z; together, these VKDPs are involved in the regulation of blood coagulation (16). Several Extra-hepatic VKDPs are known to improve bone and vascular health, including: Bone Gla Protein (BGP or osteocalcin), Matrix Gla Protein (MGP) (11,12,17,18). Adequate levels of vitamin K are necessary for the activation (by carboxylation) of VKDPs such as BGP or MGP (19).

BGP is secreted by osteoblasts, it undergoes carboxylation for the transformation of the undercarboxylated form (ucBGP) into the fully functional (carboxylated) form (cBGP)(20). BGP is known to be involved in the regulation of bone matrix mineralization. Indeed, in knock-out mice, Ducy et al. demonstrated that osteocalcin-deficient mice develop hyperostosis (excessive bone growth) confirming its role in promoting normal bone mineralization (21). BGP has also been shown to protect against VC through its effect on adiponectin (22), Confavreux and colleagues also observed that higher total BGP concentrations were associated with lower abdominal aortic calcification progression rate and lower mortality (23). In patients with HD, we have also confirmed an association between low levels of BGP and the presence of VCs (24).

MGP is secreted from vascular smooth muscle cells and chondrocytes and requires post-translational serine phosphorylation in addition to gamma-carboxylation (12,25). In particular, phosphorylation occurs at 3 serine residues via the enzyme casein kinase regulating protein secretion into the extracellular environment (12,26,27). The mechanism through which MGP inhibits VC is not well known but it is thought to be associated to the carboxylated active form of MGP that binds calcification crystals in blood vessels forming

vesicles and apoptotic bodies preventing calcium phosphate precipitation in addition to the trans-differentiation of vascular smooth muscles cells into an osteogenic phenotype (12,18). Different forms of MGP can be used to measure vitamin K deficiency (26). Plasma dephosphorylated undercarboxylated MGP form (-dp-ucMGP) levels are also positively correlated with VC and therefore may be used as an early marker of VC in CKD patients (27,28).

Another vitamin K action, that is poorly studied, is as a ligand of the steroid and xenobiotic receptor (SXR) and pregnane X receptor (PXR, murine ortholog) acting to prevent bone disorders (29).

Vitamin K2 has several different vitamers or menaquinones (MK), of which MK4-MK7 can exert important biologic actions. In-vitro, MK4 has been shown to act as an inhibitor of VC in vascular smooth muscle cells (VSMCs) (30,31). Furthermore, in HD patients, Fusaro et al. showed that MK4 deficiency emerged as a significant predictor of aortic calcifications (24). As well as VC-protective effects, MK4 supplementation is also associated with a reduced risk of fracture (32,33).

It is recognised that phosphate binders such as sevelamer can inhibit gastrointestinal uptake of vitamin K by the undesired binding to fat soluble vitamins (34–36).

Considering the limited data available examining the association between sevelamer use and vitamin K status, the aim of the present study was to examine patients treated with sevelamer in the VIKI cohort to determine whether this phosphate binder could influence levels of VKDPs and different vitamin K vitamers.

METHODS

Patients

The present study is a post-hoc analysis of the original VIKI study (24), involving 18 dialysis centres across Italy. Patients were enrolled between November 2008 and November 2009 and follow-up assessment of vital status was performed in December 2011 (24). We included male or female adults who had been on HD for >1 year. Exclusion criteria were patients who had a life expectancy <6 months, cancer (with the exception of basal cell carcinoma), coagulation disorders, or conditions (according to the investigator) that could interfere with the study outcome. All patients gave their informed consent in writing for the use of their medical records for the study. All local ethics committees approved the study, which was conducted according to the regulations for observational studies.

Data collection and study objective

The original VIKI study was primarily designed to assess the prevalence vitamin K1 (phylloquinone) and K2 (i.e., menaquinones; MKs), deficiency in HD patients (24). Secondary objectives evaluated the impact of vitamin K status on VKDPs (e.g. MGP and BGP) and their association with VC and VF. Information on concomitant treatment, including mineral and bone disorders treatment (i.e. oral calcitriol, vitamin D analogues, calcimimetics and phosphate-binding drugs) was collected. Fasted venous blood samples were collected from patients before dialysis session for routine bone biochemistry (total alkaline phosphatase; ALP, albumin, C-reactive protein; CRP, aluminium, parathyroid hormone; PTH, 25-OH vitamin D; 25(OH)D and lipid profile) and vitamin K components (total osteocalcin; BGP; undercarboxylated (uc) BGP, total MGP, and ucMGP determination. Vitamin K components were determined by a simple, sensitive, and selective reversed-phase high performance

liquid chromatography, developed for the simultaneous determination of vitamin K in human plasma as previously described in detail (24). Vitamin K values were corrected according to triglyceride levels (37) and vitamin k deficiency was defined as previously described in the VIKI study (24).

Laboratory assays

PTH

Serum PTH was measured by automated LIAISON® N-Tact® PTH Assay 310910 (DiaSorin Inc., Stillwater MN, USA), a direct, 2-site, sandwich-type chemiluminescence immunoassay (CLIA) carried out on the LIAISON® instrument. Analytical sensitivity was 1 pg/mL and the intra-assay and inter-assay CVs were 3.7-6.3 and 3.5-5.3%, respectively.

25(OH)D

For quantitative determination of total serum 25(OH)D (both D2 and D3 form), we used the automated LIAISON® 25 OH Vitamin D TOTAL Assay 310600, a direct competitive CLIA executed on the LIAISON® instrument. Analytical sensitivity was <10 nmol/L, and the intra-assay coefficients of variation (CV) were between 2.9 and 5.5%, while the inter-assay CV was 6.3-12.9%.

Total and ucBGP

The quantitative determination of total BGP in serum was performed using the automated LIAISON® Osteocalcin Assay 310950, a direct, 2-site, sandwich-type CLIA executed on the LIAISON® instrument. The analytical sensitivity is <0.3 ng/mL and the intra-assay CV is 3-8%, while the inter-assay CV is 4-9%. For the quantitative determination of ucBGP, we used the

Glu-osteocalcin Enzyme Immunoassay (EIA) Kit MK118 (Takara Bio Inc., Otsu, Shiga, Japan), as previously described (38). Analytical sensitivity was 0.25 ng/mL and the intra-assay and inter-assay CVs were 4.4-6.7 and 5.7-9.9%, respectively.

Total and ucMGP

The quantitative determination of MGP was performed using the Human MGP—Matrix Gla Protein ELISA Kit (Biomedica Medizinprodukte GmbH & Co KG, Vienna, Austria). Analytical sensitivity was 0.3 nmol/L, and the intra-assay and inter-assay coefficients of variation (CVs) were 5–6 and 7–9 %, respectively. The measurement of the total ucMGP was performed by VitaK using a competitive ELISA, as described previously (26). The analytical sensitivity was 21 nmol/L, and the intra-assay and inter-assay CVs were 8.9 and 11.4%, respectively.

Assessment of vertebral fracture and vascular calcification

A radiograph of the thoracic and lumbar regions of the spinal column (D5 to L4) in the latero-lateral view with the patient in the lateral recumbent position was obtained. A VF was considered to be present when the height of the vertebral body was reduced by at least 20 % (4 mm), according to Genant (39).

VCs were quantified by measuring the length of calcific deposits along the abdominal aortic wall (mild 0.1–5 cm, moderate 5.1–10 cm and severe [10 cm) as described and validated previously (40). The presence of calcifications of the iliac arteries was evaluated through the same radiograph (mild 0.1–3 cm, moderate 3.1–5 cm, and severe [5 cm) (40). We followed patients for a mean period of 2.7±0.5 years.

Statistical analysis

Data are presented as mean ± standard deviation (SD) for quantitative variables or median and interquartile range (IQR) for not normally distributed asymmetric variables. Discrete or nominal variables were presented as number and percentages. Normal distribution of continuous variables was tested using the Shapiro-Wilk test. For discrete variables, the differential distribution between patients treated with sevelamer and those not treated with sevelamer was analysed by χ^2 test or Fisher's exact method. For quantitative variables, comparison between medians was performed by the Mann–Whitney rank test whereas means were compared by unpaired T-test. Multiple linear and logistic regression models (stepwise approach) were used to assess the strength of the association between MK4 levels and deficiency as well as the presence of VFs and use of sevelamer. In logistic models, data were expressed as odds ratio (OR), 95% confidence interval and P value. In linear models, data were expressed as beta (β) coefficient and P value. When appropriate, each outcome variable underwent logarithmic transformation. Covariates initially included in models were the following: sevelamer use, K1/triglycerides, aortic/iliac calcification, age, myocardial infarction, type of dialysis, ALP, PTH, MGP, BGP, cholesterol, albumin, and the following therapies: aluminium, proton pump inhibitor (PPI), lanthanum, oral vitamin D calcitriol, calcium carbonate and vitamin D analogues. Factors included as independent variables were those associated with the outcome at the univariate level ($p \leq 0.10$) or associated with sevelamer status ($p \leq 0.10$). The effect modification by sevelamer use on the relationship between total BGP levels and the odds of fractures was investigated by simultaneously introducing into the same logistic model, the risk factor (BGP <150 µg/L vs. BGP ≥150 µg/L), the effect modifier (use of sevelamer) and their multiplicative term. The

effect of BGP levels in treated and untreated patients on the odds of fractures was investigated by the standard linear combination method. - statistical analyses were performed using STATA statistical package (version 13, College Station, Texas, USA).

RESULTS

Clinical characteristics

In this post-hoc analysis of the VIKI study, from a total of 387 HD patients, 163 (42.1%) were treated with sevelamer and 224 (57.9%) did not receive sevelamer. Clinical characteristics of HD patients by sevelamer treatment are presented in **Table 1**. The majority of patients were male 63%; N=244), aged 64.2±14.1 years with median vintage dialysis of 4.1 years. Most frequent co-morbidities included hypertension (78.6%; N=304), diabetes (21.9%; N=85), myocardial infarction (18.9%; N=73) and approximately half of patients (55.3%; N=214) had VF. Characteristics were similar between patients with and without sevelamer treatment apart from age (63 vs. 69 years, p=0.002), type of dialysis (p=0.036), and incidence of myocardial infarction (13.5% vs 22.8%, p=0.021) in sevelamer vs. patients not treated with sevelamer, respectively. Concomitant medication by sevelamer status is shown in **Figure 1**. In all patients, PPIs or anti-gastric agents were the most frequently prescribed (concomitant) medication (75.7%; 81.6% in sevelamer treated vs. 71.4% without sevelamer) and approximately 40% of patients were receiving statins, calcium carbonate, beta-blocker or oral calcitriol (**Supplementary Material Table 1**). A significantly lower proportion of sevelamer-treated patients were receiving other phosphate binders such as aluminium salts (12.9% vs. 33.5%, p<0.001), calcium acetate (1.8% vs. 8%, p=0.008) and lanthanum (3.7% vs. 22.3%).

Biochemical measures

A range of biochemical measures were examined in patients treated with and without sevelamer (**Table 2**). While no differences in levels of calcium, phosphorus or 25(OH)D were observed in patients treated with sevelamer, higher levels of ALP (89 U/L vs. 77.5 U/L,

p<0.001), PTH (290 pg/mL vs. 209 pg/mL, p<0.001) and total BGP (210 µg/L vs. 152 µg/L, p=0.002) were observed. In contrast, sevelamer-treated patients had lower levels of total MGP (16.4 nmol/L vs. 20.3 nmol/L, p=0.037) as well as total cholesterol (155 mg/dl vs. 173 mg/dl, p<0.001) and LDL cholesterol (80 mg/dl vs. 96.5 mg/dl, p<0.001) compared to patients not receiving sevelamer.

Vascular calcification

Although approximately 80% of patients presented with aortic and 55% had iliac calcifications respectively, no significant difference was observed in terms of frequency or severity of calcifications by sevelamer treatment (**Figure 1**).

Vitamin K levels and vitamin K deficiency

Serum concentrations of vitamin K1 and range of menaquinones (MK) components of vitamin K2 in sevelamer treated vs. patients not treated with sevelamer are presented in **Table 3**. Among all 10 components analysed, only MK4 emerged as being significantly different in sevelamer treated vs. patients not treated with sevelamer, respectively (0.45 ng/ml vs. 0.6 ng/ml, p=0.01) (**Figure 2**). Confirming these findings, a significantly higher proportion of sevelamer-treated patients were deficient in MK4 (13.5% vs. 5.4%, p=0.005) as well as MK4 adjusted for triglyceride levels (19% vs. 11.2%, p=0.03) (**Figure 3**).

Association between sevelamer use and vitamin K (deficiency and levels)

Stepwise logistic regression analysis revealed that MK4 deficiency was associated with sevelamer-use (OR: 2.64, 95% CI: 1.25-5.58, p=0.011), vitamin K1/triglycerides (OR: 0.35, 95% CI: 0.14-0.92, p=0.032) and aortic calcification (OR: 8.04, 95% CI: 1.07-60.26, p=0.04)

after adjusting for potential confounders (**Table 4**). Considering all data continuously in a separate model, sevelamer use (β= -0.18, 95% CI: -0.68, -0.152, p=0.002) emerged as being significantly (negatively) associated with MK4 levels (**Table 5**).

Association between low BGP levels, sevelamer use and vertebral fracture

Stepwise logistic regression was next applied to evaluate variables associated with the presence of VFs (**Table 6**). Age (OR: 1.02, 95% CI: 1.00-1.04, p=0.026) and gender (female) (OR: 0.56, 95% CI: 0.35-0.89, p=0.015) emerged as predictor variables significantly associated with the presence of VFs. In the same multivariate logistic regression model, the use of sevelamer significantly amplified the role of total BGP levels on the odds of fractures so that in sevelamer-treated patients, the odds ratio of VFs was about 3 times higher in patients with total BGP <150 µg/L (N=56) compared to those with total BGP ≥150 µg/L (N=107: OR: 3.15, 95% CI: 1.46-6.76, p=0.003), whereas no such effect was found in those untreated (total BGP <150 µg/L vs. total BGP ≥150 µg/L: OR: 1.21, 95% CI: 0.66-2.23, p=0.54] (p=0.049 for effect modification)(**Table 7**).

DISCUSSION

Considering the increasingly recognised role of vitamin K in VC (11,19,24,41,42), VF (24,43) and mortality (24,44), in patients with CKD, it is critical that those burdened with co-morbid diseases requiring multiple medications are monitored for the presence of drugs that could interfere with the potential absorption and action of vitamin K and VKDPs.

In the present subanalysis of the VIKI cohort, we examined whether HD patients treated with sevelamer could impact upon levels of VKDPs and different vitamin K vitamers that may be associated with VC or VF risk.

To date, in-vitro binding studies examining the affinity of sevelamer with vitamin K have shown differing results(34,45). Neradova and colleagues recently showed that sucroferric-oxyhydroxide and sevelamer carbonate did not bind vitamin K2, in contrast to other phosphate binders (45). As alluded to by these Authors, the lack of interaction between sevelamer and vitamin K may have been attributed to the low doses of sevelamer used (37 µg/mL) in their study (45). Furthermore, Westenfeld and colleagues examined the effect of vitamin K2 supplementation on functional vitamin K deficiency in HD patients and did not observe any relationship between sevelamer use and circulating vitamin K (menaquinone) levels, although their study was not designed, nor powered (n=53) to explore this interaction (46). Furthermore, only 22% of participants were prescribed sevelamer (46). However, Jansz and colleagues did observe that in HD patients who recently underwent kidney transplantation, sevelamer administered as monotherapy was found to be associated with higher dp-ucMGP levels compared to no phosphate binders, suggesting a worsening in vitamin K status by this phosphate binder (47).

Collectively, based upon findings from these pre-clinical (34,45) and clinical studies (46,47), the hypothesis that sevelamer could interact with fat soluble vitamins has already been

postulated (36). However, no study has specifically examined the association between sevelamer use on vitamin k homeostasis and hard outcome such as VC and VFs in HD patients. Findings from the original VIKI study based on 387 HD patients revealed that the vitamin K system may be important for preserving bone health and avoiding VC (24). In that study, vitamin K1 deficiency emerged as the strongest predictor of VFs (OR: 2.94, 95% CI, 1.38–6.26) while MK4 deficiency was a predictor of aortic calcification. These findings corroborate with earlier observations by Beulens et al., where a high dietary intake of menaquinone (MK4-M10) was associated with a reduced risk of aortic calcification or coronary calcifications respectively (48). In the present study, patients treated with sevelamer actually decreased MK4 levels by approximately 25% (0.45 ng/mL vs. 0.6 ng/mL, $p<0.01$) and bearing in mind the aforementioned detrimental effects of MK4 deficiency (24) coupled with the benefit afforded with high dietary intake of menaquinone (48) our findings should be considered a warning, especially in this setting where patients are already burdened with several comorbidities (including 80% presenting with aortic calcification) increasing the potential risk of mortality.

Furthermore, multivariate regression analysis revealed that MK4 deficiency was associated with sevelamer use (OR: 2.64, 95% CI: 1.25–5.58, p=0.011) and aortic calcification (OR: 8.04, 95% CI: 1.07–60.26, p=0.04). In this same multivariate logistic regression model, sevelamer use significantly amplified the effect of total BGP levels on the risk of VFs. In sevelamer-treated patients, the risk of VFs was approximately 3-fold higher in patients having BGP <150 μg/L vs. those with BGP ≥150 μg/L, whereas this effect was not observed in patients not treated with sevelamer. The observation that MK4 levels and MGP levels were significantly decreased in patients treated with sevelamer is particularly concerning. Multivariate analysis revealed that sevelamer use and the presence of aortic calcification

were significantly associated with MK4 deficiency. Low MK4 levels are a predictor of aortic calcification (24), Furthermore, as previously mentioned, a high dietary intake of menaquinone is associated with reduced risk of VCs (48,49). While we cannot offer a precise explanation of the mechanism by which sevelamer use and the presence of aortic calcification are associated with MK4 deficiency, recent in-vitro evidence by Yang et al. has demonstrated that MK4 accelerates warfarin-induced calcification of aortic valve interstitial cells in high inorganic phosphate medium; this effect being mediated by pregnane X receptor–bone morphogenetic protein 2–ALP signalling (50).

On a separate note, sevelamer use was associated with a significant reduction in levels of total cholesterol and LDL cholesterol and a non-significant increase in HDL cholesterol, with statin treatment remaining the same in both groups (approximately one-third of patients). This is perfectly in line with previous studies (8,51), However, these well-established lipid-lowering effects did not appear to be translated into an improvement in the rate of VC in sevelamer-treated patients, where no difference in the percentage of patients with VCs was observed in those with or without sevelamer.

A key finding that emerged from this subanalysis of the VIKI study revealed that patients treated with sevelamer were not only associated with MK4 deficiency or decreased MK4 levels, but that patients who had low levels of BGP (<150 μg/L) had a 3-fold increase in the risk of VF (OR: 3.15, 95% CI: 1.46–6.76, p=0.003). Indeed, the link between BGP (osteocalcin) and fracture has been well documented (52). In a recent prospective study performed in 126 type 2 diabetic patients with early CKD (stages 2-3) in Portugal, an increased risk of bone fracture and dysregulation in mineral metabolism was associated with low levels of osteocalcin and Klotho and high levels of FGF-23 (53). Furthermore, in EVERFRACT, lower

total BGP (151 µg/L vs. 213 µg/L, p<0.01) levels were observed in HD patients stratified by the presence of VF (54). This study and others (55) confirm the concept that bone fragility and VC share common pathways, of which VKDPs likely play a central role. Although mortality was not assessed in the present subanalysis, it is important to underline that previous studies have reported a significant association between the presence of hip fracture and mortality (56) as well as in female HD patients the presence of both and VC and VF increased the risk of mortality [RR = 3.2 (1.0–10.0) and RR = 4.8 (1.7–13.4), respectively](57).

Taken together, findings from the present study suggest that phosphate binders such as sevelamer should be administered with caution in HD patients with poor vitamin K status.

Study limitations

The main limitation of this study is intrinsic to its observational design. The limited sample size in this sub-analysis (particularly those patients having BGP <150µg/L receiving sevelamer; N=56) in multivariate analysis may limit the generalizability and overall impact of our findings. We stratified patients as either receiving sevelamer or not. However, this study is unique in that the patient cohort comprises a very select study population, an extensive range of laboratory measures encompassing vitamin K components as well VC and VF as hard outcome measures.

CONCLUSION

Results from the present study and our other sub-analyses of the VIKI cohort suggest that HD patients with diabetes (41) and who smoke cigarettes (38) who are treated with

sevelamer should be monitored for VC and VF and supplemented with vitamin K accordingly. Patients treated with sevelamer in the present study were observed to have a higher rate of MK4 deficiency (13.5% vs. 5.4%, p=0.005), that was associated with sevelamer use and aortic calcification. In patients deficient for BGP (<150 µg/L), sevelamer should be administered with caution. Moreover, considering the strong association between MK4, BGP and risk of VC and VFs respectively, screening of these biomarkers prior to treating with sevelamer would be recommended. Further long-term studies examining the use of sevelamer in HD patients on vitamin K profile and hard outcome measures such as mortality are needed to verify our observations.

ACKNOWLEDGEMENTS

AUTHOR CONTRIBUTIONS

COMPLIANCE WITH ETHICAL STANDARDS

Ethics approval and consent to participate

Local ethics committee approval from all participating centres and written informed consent for the anonymous use of personal data was obtained from every patient, in compliance with Legislative Decree 196/2003.

Consent for publication

Not applicable.

Previous publication

The main result of the present study have been published as an abstract and poster at the 2019 American Nephrology Meeting, “Sevelamer Use Is Associated with Decreased Vitamin K Levels in Hemodialysis Patients: Results from the Vitamin K Italian (VIKI) Study”, ABSTRACT: FR-PO146, November 08, 2019, Washington, USA. https://www.asn-online.org/education/kidneyweek/2019/program-abstract.aspx?controlId=3232698

Competing interests

Authors have declared no conflict of interest.

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FIGURE LEGENDS

Figure 1. Frequency and severity of aortic or iliac calcifications in HD patients with and without sevelamer. Data are presented as %.

Figure 2. Levels of MK4 in HD patients with and without sevelamer. Data presented as median, 25th/75th percentiles and maximum/minimum recorded values. P-values denote statistically significant differences between sevelamer and non-sevelamer treated patients.

Figure 3. Deficiency status in vitamin K1 and a range of menaquinones (MK4-MK7) in HD patients with and without sevelamer. Data are presented as %. Total vitamin K1 and MK4-7 levels are also adjusted for triglyceride levels. P-values denote statistically significant differences between sevelamer and non-sevelamer treated patients.

Characteristic	All patients N=387	Sevelamer N=163 (42.1%)	No sevelamer N=224 (57.9%)	p-value
Gender, males, n (%)	244 (63.0)	97 (59.5)	145 (64.7)	0.3
Age, years, median (IQR)	64.2±14.1 (18-89)	63 (51, 72)	69 (60, 76)	**0.002**
BMI, kg/cm^2, median (IQR)	25.1±4.4	24.2 (21.4, 27.5)	24.7 (22.0, 28.3)	0.26
Smoker, n (%) (n=370)				0.44
Yes	51 (13.8)	24 (15.5)	27 (12.6)	
No	234 (63.2)	100 (64.5)	134 (62.3)	
Ex	85 (22.9)	31 (20)	54 (25.1)	
Current/previous alcohol drinker, N (%)	82 (21.2)	35 (22.7)	47 (22.7)	0.99
Medical history				
Dialysis vintage, months, median	49 (30, 95)	49 (30, 95)	49.5 (26, 91.8)	0.58
Type of dialysis, n (%)				**0.036**
Bicarbonate dialysis	189 (48.8)	66 (40.5)	123 (54.9)	
Hemofiltration	32 (8.3)	13 (8)	19 (8.5)	
Hemodiafiltration	102 (26.4)	55 (33.7)	47 (21.0)	
Acetate free biofiltration	54 (13.9)	24 (14.7)	30 (13.4)	
Other types of dialysis	10 (2.6)	5 (3.1)	5 (2.2)	
Previous kidney transplant, n (%)	54 (13.9)	20 (12.3)	34 (15.2)	0.42
Hypertension, n (%) IPA	304 (78.6)	130 (79.8)	174 (77.7)	0.62
Angina, n (%)	64 (16.5)	27 (16.6)	37 (16.5)	0.99
Myocardial infarction, n (%)	73 (18.9)	22 (13.5)	51 (22.8)	**0.021**
Atrial fibrillation, n (%)	51 (13.2)	22 (13.5)	29 (12.9)	0.87
Heart failure, n (%)	39 (10.1)	14 (8.6)	25 (11.2)	0.41
Diabetes Mellitus, n (%)	85 (21.9)	38 (23.3)	47 (21)	0.58
Peripheral vascular disease, n (%)				0.62
No	253 (65.4)	106 (65)	147 (65.6)	
Asymptomatic	98 (25.3)	39 (23.9)	59 (26.4)	
Intermittent claudication	28 (7.2)	15 (9.2)	13 (5.8)	
Amputation	8 (2.1)	3 (1.9)	5 (2.2)	
Cerebrovascular accident, n (%)				0.21
No	346 (8.9)	141 (86.5)	205 (91.5)	
Stroke	20 (5.2)	12 (7.4)	8 (3.6)	
Other type	21 (5.4)	10 (6.1)	11 (4.9)	
Vertebral fractures, n (%)	214 (55.3)	85 (52.1)	129 (57.6)	0.29
Vertebral fractures among men, n (%)	145 (59.4)	57 (58.8)	88 (60.7)	0.76
Vertebral fractures among women, n (%)	69 (48.3)	28 (42.4)	41 (51.9)	0.26

Table 1. Clinical characteristics of HD patients with and without sevelamer

Data are presented as median and interquartile range or mean ± standard deviation. BMI = body mass index.

Table 2. Biochemical measures in HD patients with and without sevelamer treatment

Variable	Sevelamer N=163 (42.1%)	No sevelamer N=224 (57.9%)	p-value
Calcium (mg/dl)	9.1 (8.7, 9.5)	9.1 (8.7, 9.6)	0.78
Phosphorus (mg/dl)	4.7 (3.9, 5.7)	4.5 (3.8, 5.4)	0.23
Magnesium (mg/dL)*	2.5 (2.1, 2.7)	2.3 (2, 2.6)	0.11
Aluminium (µg/L)	10.7 (7.6, 18)	14 (8, 23)	0.24
Alkaline phosphatase (U/L) median	89 (74, 122)	77.5 (61, 105)	**<0.001**
PTH (pg/ml)	290 (171, 446)	208 (117, 348)	**<0.001**
25(OH)D (ng/mL)	29 (18.8, 45.2)	28.4 (19.2, 44.3)	0.6
Albumin (g/dl; mean±SD)	38.87±3.77	37.97±4.63	0.24
CRP (mg/L)	1.7 (0.4, 5.0)	1.7 (0.54, 5.2)	0.46
KT/V (mean±SD)	1.24±0.27	1.26±0.27	0.46
Total cholesterol (mg/dl)	155 (134, 184)	173 (146.5, 204)	**<0.001**
Triglycerides (mg/dl)	140 (109, 203)	153 113, 210)	0.19
HDL-cholesterol (mg/dl)	41 (35, 52)	40 (31, 49)	0.066
LDL-cholesterol, mg/dl	80 (62.8, 104.3)	96.5 (76, 120)	**<0.001**
BGP total (µg/L)	210 (117, 363.2)	152 (83.6, 276)	**0.002**
BGP decarboxylated (µg/L) median	11.88 (6.27, 17.00)	10.57 (4.2, 18.8)	0.29
MGP total (nmol/L)	16.39 (12.0, 27.6)	20.26 (13.6, 33.3)	**0.037**
MGP decarboxylated (nmol/L)	547 (296.9, 920)	584 (265, 944.3)	0.99

Data are presented as median and interquartile range or mean ± standard deviation. BGP = bone Gla proteins; CRP = C-reactive protein; HDL = high-density lipoprotein; LDL = low-density lipoprotein; MGP = matrix Gla proteins; PTH = parathyroid hormone; 25(OH)D; 25-hydroxyvitamin D or calcifediol. * based on 139 patients.

Table 3. Vitamin K levels by sevelamer status.

Vitamers	Sevalmer N=163 (42.1%	No sevalemer N=224 (57.9%)	p-value
K1 (ng/mg)	0.63 (0.31, 0.98)	0.64 (0.35, 1.14)	0.55
K1/triglycerides (ng/mg)	0.46 (0.19, 0.64)	0.40 (0.21, 0.63)	0.96
MK4 (ng/mg)	0.45 (0.15, 0.67)	0.60 (0.27, 0.67)	**0.010**
MK4/triglycerides (ng/mg)	0.36 (0.10, 0.51)	0.37 (0.15, 0.51)	0.088
MK5 (ng/mg)	1.00 (0.52, 1.03)	1.00 (0.44, 1.01)	0.67
MK5/triglycerides (ng/mg)	0.75 (0.35, 0.80)	0.75 (0.30, 0.75)	0.36
MK6 (ng/mg)	0.48 (0.20, 0.63)	0.48 (0.22, 0.63)	0.66
MK6/triglycerides (ng/mg)	0.35 (0.11, 0.51)	0.34 (0.13, 0.51)	0.91
MK7 (ng/mg)	1.15 (0.53, 1.22)	0.98 (0.47, 1.19)	0.21
MK7/triglycerides (ng/mg)	0.84 (0.39, 0.92)	0.61 (0.27, 0.87)	0.073

Data are presented as median and interquartile range. K1 = vitamin K1; MK = menaquinone. Levels of vitamin K1 and MK components were also adjusted for triglyceride levels.

Table 4. Logistic regression with MK4 deficiency as outcome variable.

Variable	Odds Ratio	95% CI	p-value
Sevelamer use	2.64	(1.25, 5.58)	0.011
K1/Triglycerides	0.35	(0.14, 0.92)	0.032
Aortic calcification	8.04	(1.07, 60.26)	0.042

The stepwise analysis identified the following variables as covariates: sevelamer use, K1/triglycerides, aortic calcification. The following variables remained out of the model: age, myocardial infarction, type of dialysis, ALP, PTH, MGP, BGP, total cholesterol, albumin, and the following therapies: aluminum, proton pump inhibitor, lanthanum, calcitriol use, calcium carbonate, calcium acetate, vitamin D analogues.

Table 5. Multiple linear regression of continuous data with MK4 levels as outcome variable.

Variable	β	95% CI	p-value
Sevelamer use	-0.18	(-0.68, -0.15)	0.002
K1/triglycerides	0.16	(0.08, 0.36)	0.002
Aluminium use	0.13	(0.05, 0.60)	0.021
Lanthanum use	-0.11	(-0.69, -0.016)	0.040
Calcitriol use	0.13	(0.06, 0.55)	0.015

Model adjusted for the following covariates: sevelamer use, K1/triglycerides, aortic calcification, age, myocardial infarction, type of dialysis, ALP, PTH, MGP, BGP, total cholesterol, albumin, and the following therapies: aluminum, proton pump inhibitor, lanthanum, calcitriol use, calcium carbonate, calcium acetate, vitamin D analogues.

Table 6. Multiple linear regression with the presence of fracture as outcome variable.

Variable	Odds ratio	95% CI	p-value
Age, 1 year increments	1.02	(1.03, 1.04)	**0.026**
Gender, female	0.56	(0.35, 0.89)	**0.015**
Sevelamer use	0.57	(0.29, 1.1)	0.094
BGP < 150	1.21	(0.66, 2.23)	0.539
BGP <150 µg/L by sevelamer use	2.6	(1.0, 6.73)	**0.049**

Model adjusted for the following covariates: aortic calcification, iliac calcification, myocardial infarction, type of dialysis, ALP, PTH, total MGP, deficit Ki, deficit MK4, total cholesterol, albumin, and the following therapies: aluminum, proton pump inhibitor, aluminum, lanthanum, calcitriol use, calcium carbonate, calcium acetate, vitamin D analogues.

Table 7. Multiple linear regression with the presence of fracture as outcome variable.

Variable	Odds ratio	95% CI	p-value	p-value*
BGP <150 µg/L (no sevelamer)	1.21	(0.66, 2.23)	0.54	0.049
BGP <150 µg/L (sevelamer use)	3.15	(1.46, 6.76)	**0.003**	

Model adjusted for the following covariates: cut-off BGP, sevelamer use, aortic calcification, iliac calcification, total MGP, age, gender, type of dialysis, acute myocardial infarction, ALP, PTH, albumin, total cholesterol, proton pump inhibitor use, aluminum, lanthanum, calcitriol use, calcium carbonate, calcium acetate, vitamin D analogues, deficit K1, deficit MK4.
*Denotes statistical significance between the two odds ratios.

Figure 1

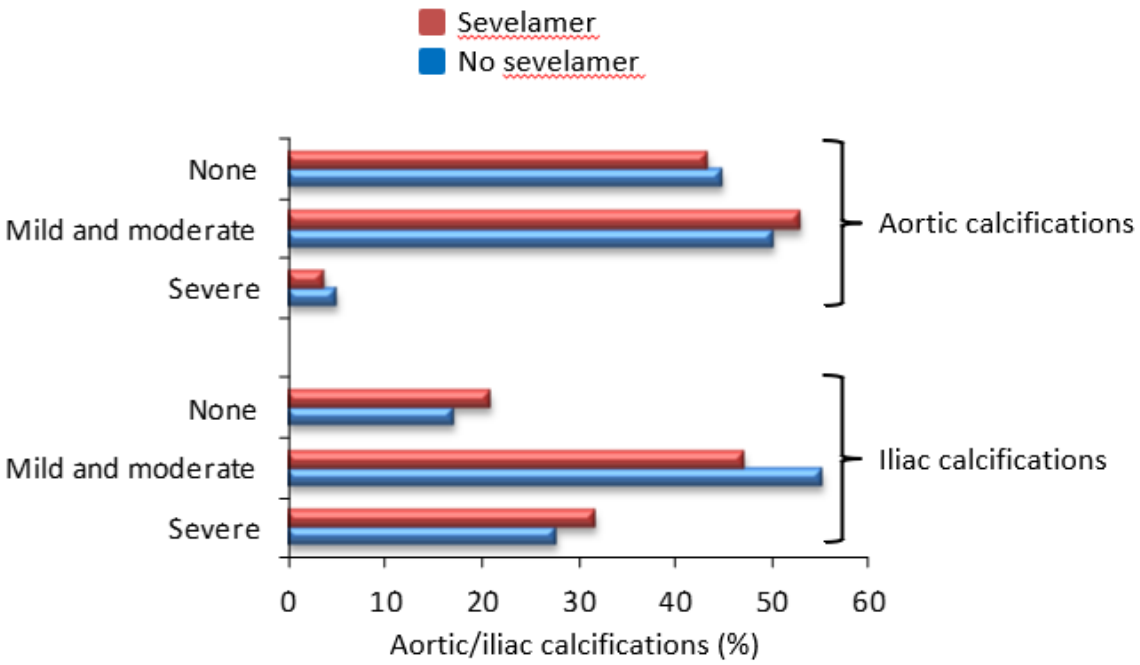

Sevelamer
No sevelamer
None
Mild and moderate
Severe
Aortic calcifications
None
Mild and moderate
Severe
Iliac calcifications
0
10
20
30
40
50
60
Aortic/iliac calcifications (%)

Figure 2

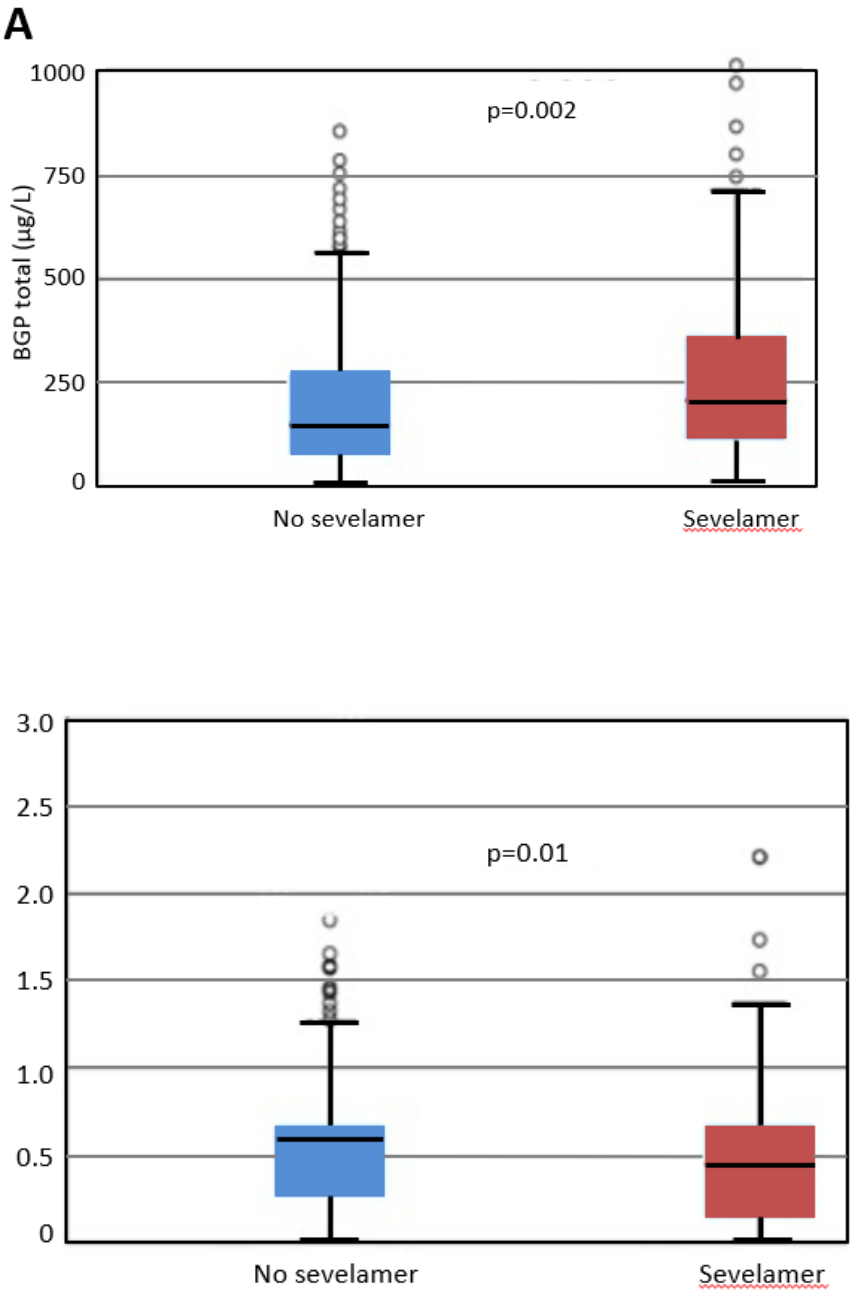
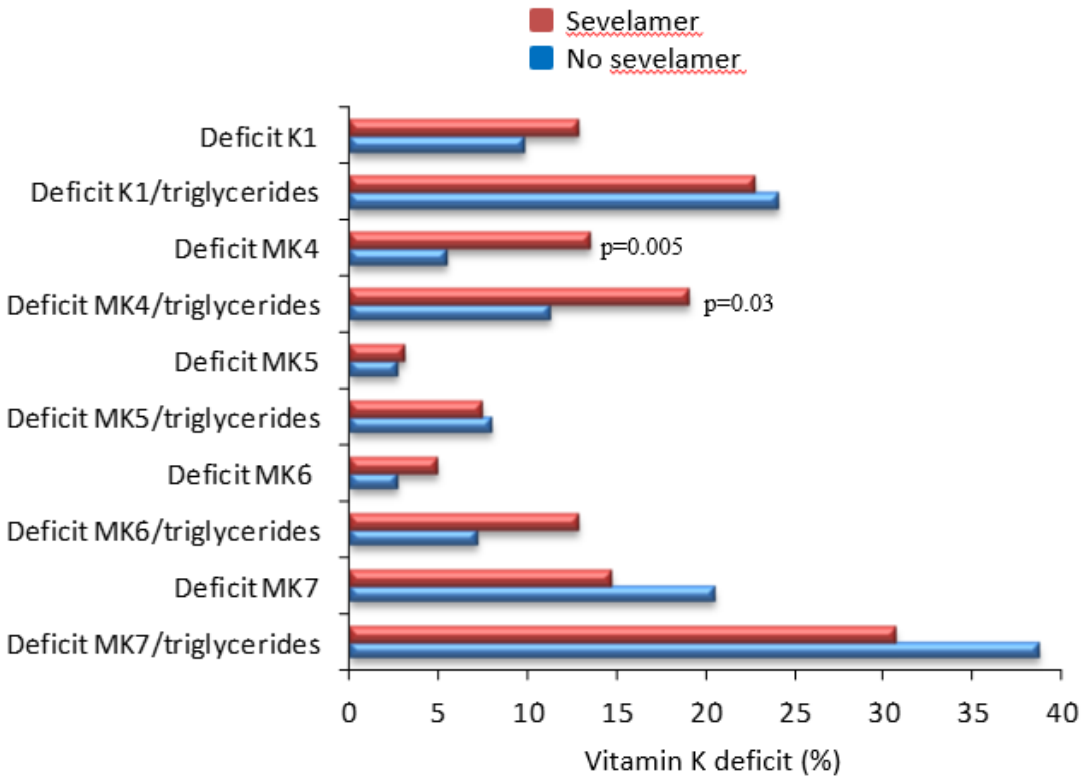

A
1000
750
500
250
0
BGP total (µg/L)
p=0.002
No sevelamer
Sevelamer
B
3.0
2.5
2.0
1.5
1.0
0.5
0
MK4 (ng/ml)
p=0.01
No sevelamer
Sevelamer

Figure 3.


Sevelamer
No sevelamer
Deficit K1
Deficit K1/triglycerides
Deficit MK4
p=0.005
Deficit MK4/triglycerides
p=0.03
Deficit MK5
Deficit MK5/triglycerides
Deficit MK6
Deficit MK6/triglycerides
Deficit MK7
Deficit MK7/triglycerides
0
5
10
15
20
25
30
35
40
Vitamin K deficit (%)