Effect of vitamin K_1 supplementation on matrix Gla protein level and vascular calcification in hemodialysis patients

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Background
Matrix Gla protein (MGP) is a potent calcification inhibitor of the arterial wall. Its activity depends on vitamin K-dependent γ-carboxyglutamate.
Aim
We aimed to investigate the effect of vitamin K_1 on MGP levels after 3 months' supplementation and the relationship between MGP level and vascular calcification.
Patients and methods
A prospective case–control pilot study was conducted over a period of 3 months. The study included 57 long-term hemodialysis patients in stable conditions who were divided into two groups and were compared with 27 healthy age-matched controls. Group I consisted of 28 hemodialysis patients who received vitamin K_1 at 10 mg three times per week for a successive period of 3 months. Group II consisted of 29 hemodialysis patients who did not receive vitamin K. Group III consisted of 27 healthy participants as controls. The serum level of MGP was measured by radioimmunoassay; in addition, hemoglobin%, parathyroid hormone, calcium, phosphorus, sodium, potassium, alkaline phosphatase, total cholesterol, triglyceride, and carotid intima–media thickness were also measured. Plain radiograph of the abdomen in lateral view was acquired to determine the abdominal aortic calcification score at the start of the study, which was reassessed after 3 months in groups I and II.
Results
We found a significant increase in blood pressure, body mass index (BMI) with elevation in the serum level of MGP in patient groups than control from the start. A significant elevation in MGP level was observed in group I accompanied by a decrease in serum cholesterol level, compared with group II. We did not find any significant change in carotid intima–media thickness or abdominal aortic calcification score in either group after 3 months. There was no significant correlation between elevated MGP level and vascular calcification either. No significant difference was found in other parameters.
Conclusion
Vitamin K supplementation may be essential for End Stage Kidney Disease (ESKD) patients on hemodialysis. Vitamin K can increase the level of MGP and decrease cholesterol level, but its beneficial effect on the vascular bed needs a long-term study.

Keywords:
hemodialysis, matrix Gla protein, vascular calcification, vitamin K

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Introduction

Vascular calcification is an important risk factor and predictor for cardiovascular mortality in ESKD patients [1,2]. Even with the absence of traditional cardiovascular risk factors, vascular calcifications can occur in young to middle-aged hemodialysis patients [3]. Vitamin K (vitamin K_1 or phylloquinone and vitamin K_2, one of the menaquinones) acts as the coenzyme for carboxylation of glutamic acid residues, which leads to formation of the amino acid γ-carboxyl-glutamic acid (Gla). Matrix Gla protein (MGP) is a larger protein produced by osteoclasts, chondrocytes, and vascular smooth muscle cells [4]. Low vitamin K concentrations are associated with increased risks for fractures and vascular calcification, which is a frequent complication in hemodialysis

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patients [5,6]. Recently, vitamin K was found to have protective effects on kidney functions [7].

Patients and methods

Patients

A prospective case–control pilot study was conducted over a period of 3 months at the Nephrology Unit of El-Hussein University Hospital on 60 adult patients with ESKD who had been on dialysis for at least 1 year. These patients were divided into two groups and compared with 27 age-matched apparently healthy controls. Of the 60 patients enrolled, 57 completed the study. One patient discontinued hemodialysis because of travels to Morocco; another one underwent a kidney transplant; and the last one died because of myocardial infarction. All hemodialysis patients were adults and on hemodialysis for at least 1 year. They were being treated by three sessions weekly for 4 h each using a Fresenius 4008S machine (Fresenius Medical care AG & CO.KGaA, D-61346 bad Homburg, Made in Germany) with bicarbonate buffer.

Group I included 28 hemodialysis patients (26 men and two women) aged between 35 and 60 years, with a mean (SD) age of 49.79 (11.12) years. This group had been receiving 10 mg of vitamin K_1 orally for 3 months. Group II included 29 hemodialysis patients (11 men and 18 women) aged between 35 and 60 years, with a mean (SD) age of 50.86 (10.41) years. They did not receive vitamin K_1. Group III included 27 healthy volunteers (20 men and seven women) of a mean (SD) age of 45.74 (5.24) years as a control group.

Methods

After providing informed consent and ethical Committee approval, all participants were subjected to history taking and complete physical examination. Laboratory investigations included measurement of hemoglobin%, parathyroid hormone (PTH), calcium, phosphorus, sodium, potassium alkaline phosphatase, total cholesterol, triglycerides, and MGP. Eight milliliters of venous blood were collected from each control and patient after overnight fasting between 8 and 10 a.m. in a serum separator tube and allowed to clot at room temperature for half an hour before centrifugation for 15 min at ~1000*g*. The serum was stored at −80°C for human matrix GMP protein assay by quantitative enzyme immunoassay using a kit from Wuhan ElAab Science Co. (China; www.eiaab.com). Laboratory investigations were carried out at the start for all groups and after 3 months for group I and group II only.

Carotid duplex ultrasound for right and left common carotid arteries was taken to assess intima–media thickness and the presence or absence of atheromatous plaques at the start of the study for all groups, and after 3 months for group I and group II only. The examination was mapped using Xaote Lab 50 linear probe; SonoSite vascular probe (Toshiba® machine, Japan) 7.5 MHz imaging/5 MHz Doppler probe or higher.

Plain radiograph of the abdomen in lateral view was taken to detect aortic calcification at the start of the study for all groups, and after 3 months for group I and group II. The radiograph was taken in standing position using standard radiographic equipment. Abdominal aortic calcification was assessed using a previously validated 24-point scale [8]. Patients with a history of thrombosis or coagulation disorder, diabetic patients, pregnant women, and patients on steroid therapy were excluded from the study.

Statistical analysis

Statistical analysis was carried out using SPSS program (IBM Corp., Released 2011, IBM SPSS Statistics for Windows, Version 20.0, Armonk, NY: IBM Corp. Chicago, USA) (version 20). Descriptive data were expressed as mean (SD) for quantitative values and as a percentage for qualitative data. Paired sample *t*-test and one-way analysis of variance (including a post-hoc test) were performed to compare mean values as regards two or more intervals of time within the same group. Bivariate correlations were ascertained to study the correlations between different parameters. Survival analysis including Kaplan–Meir test and Cox's regression analysis was conducted to assess the predictive effect of different factors. *P* values more than 0.05 were considered nonsignificant and *P* values less than 0.05 were considered significant.

Results

There was a significant difference in sex distribution ($P<0.000$) among patients when compared with controls (Table 1), with increase in blood pressure ($P<0.000$), BMI ($P<0.003$), and elevation in the serum level of MGP in patients than in controls at the start of the study ($P<0.001$) (Table 2). After 3 months, we found significant elevation in MGP level in group I (after 3 months of vitamin K_1) ($P<0.037$) accompanied by a decrease in serum cholesterol level,

Table 1 Demographic data of 57 patients and 27 controls

	Group I (*N*=27)	Group II (*N*=29)	Control group III (*N*=27)	Test	*P*-value
Age [mean (SD)] (years)	49.79 (11.12)	50.86 (10.41	45.74 (5.24)	2.304	0.106
Sex [*N* (%)]					
Female	2 (7.10)	18 (62.10)	7 (25.90)	20.409	0.000
Male	26 (92.90)	11 (37.90)	20 (74.10)		
Duration of dialysis [mean (SD)]	6.04 (3.14)	7.55 (3.58)	–	1.696	0.096

Table 2 Some clinical data and laboratory parameters at baseline of patients and controls

	Group I (*N*=28)		Group II (*N*=29)		Control group (III) (*N*=27)		One-way ANOVA		Post-hoc analysis		
	Mean	SD	Mean	SD	Mean	SD	*F*	*P*-value	P_1	P_2	P_3
Systolic BP	138.75	14.51	135.86	16.80	110.56	8.92	34.196	0.000	0.000	0.000	0.435
Diastolic BP	88.39	9.91	86.90	11.05	73.15	5.74	22.645	0.000	0.000	0.000	0.543
BMI	27.38	5.59	26.39	4.28	23.61	1.08	6.120	0.003	0.001	0.014	0.365
Hb (g/dl) (%)	10.62	2.37	9.85	2.12	12.49	1.14	13.283	0.000	0.000	0.000	0.190
Ca (mg/dl)	10.00	3.32	10.59	3.70	10.15	0.66	0.310	0.734	0.851	0.577	0.451
PO_4 (mg/dl)	5.44	2.24	5.47	1.55	3.85	0.53	9.122	0.000	0.001	0.000	0.955
ALP (IU/dl)	189.10	158.74	247.97	233.26	60.15	12.51	9.427	0.000	0.000	0.000	0.258
Cholesterol (mg/dl)	182.90	42.83	176.47	46.18	122.11	23.83	20.170	0.000	0.000	0.000	0.578
TG (mg/dl)	166.97	92.42	173.37	75.13	97.63	14.48	9.933	0.000	0.000	0.000	0.770
PTH (mg/dl)	415.97	539.39	286.76	227.65	29.67	9.25	9.198	0.000	0.000	0.000	0.232
MGP (pg/ml)	31.29	13.83	29.31	11.94	20.15	3.69	8.248	0.001	0.000	0.002	0.493
Na (mg/dl)	135.90	4.09	134.63	4.73	138.56	1.78	7.786	0.001	0.003	0.000	0.272
K (mg/dl)	5.09	0.71	4.78	0.71	4.19	0.44	14.526	0.000	0.000	0.000	0.101

ALP, alkaline phosphatase; ANOVA, analysis of variance; BP, blood pressure; Hb, serum hemoglobin%; MGP, matrix Gla protein; PTH, parathyroid hormone; TG, triglyceride. P_1, comparison between control group and group I. P_2, comparison between control group and group II. P_3, comparison between group I and group II.

Table 3 Laboratory parameters after 3 months of treatment with vitamin K (group I) and without vitamin K (group II)

	Group I (*N*=28)		Group II (*N*=29)		Independent *t*-test	
	Mean	SD	Mean	SD	*t*	*P*-value
Systolic BP	131.43	16.27	138.79	19.16	–1.562	0.124
Diastolic BP	80.89	11.31	85.17	13.06	–1.321	0.192
BMI	27.39	5.60	26.39	4.28	0.765	0.447
MGP (pg/ml)	35.93	8.15	30.31	9.21	2.437	0.018
Cholesterol (mg/dl)	148.61	38.42	189.48	54.76	3.251	0.002
Hb (g/dl)	9.96	2.20	10.41	1.83	0.841	0.404
Ca (mg/dl)	10.61	1.52	11.90	5.65	1.168	0.248
PO_4 (mg/dl)	5.00	2.23	5.97	2.93	1.403	0.166
ALP (IU/dl)	210.0	165.2	285.42	281.77	1.227	0.225
TG (mg/dl)	138.75	75.42	155.03	66.65	0.864	0.391
PTH (mg/dl)	367.81	453.50	281.45	219.62	0.920	0.361
Na (mg/dl)	135.32	3.50	134.34	3.91	0.996	0.323
K (mg/dl)	5.04	0.73	4.56	0.49	2.924	0.005

ALP, alkaline phosphatase; BP, blood pressure; Hb, serum hemoglobin%; MGP, matrix Gla protein; PTH, parathyroid hormone; TG, triglyceride.

compared with group II ($P<0.002$) (Table 3). We did not find any significant change in carotid intima–media thickness (CIMT) or abdominal aortic calcification score (AACS) in the two groups after 3 months (Table 4). Moreover, no correlation between MGP level and vascular calcification was found at the start or after 3 months in both groups of patients (Table 5). There was no significant difference in blood pressure, BMI, serum levels of PTH, calcium, phosphorus, sodium, potassium, hemoglobin%, and alkaline phosphatase between group I and group II either at the start or after 3 months (Table 3).

Discussion

Menadione (K_3) is released from phylloquinone in the intestine after oral intake and converted to

Table 4 Carotid intima–media thickens and abdominal aortic calcification score at baseline and after 3 months in group I (treated with vitamin K) and group II (without treatment)

	At the start of the study		After 3 months		Paired *t*-test	
	Mean	SD	Mean	SD	*t*	*P*-value
Group I (*N*=28)						
CIMT (RT) (mm)	0.75	0.23	0.78	0.22	–0.548	0.588 (NS)
CIMT (LT) (mm)	0.71	0.21	0.74	0.21	–0.545	0.590 (NS)
AACS	5.04	2.03	5.14	1.93	0.917	0.368
Group II (*N*=29)						
CIMT (RT) (mm)	0.89	0.21	0.98	0.20	–1.659	0.108 (NS)
CIMT (LT) (mm)	0.88	0.18	0.97	0.19	–1.867	0.072 (NS)
AACS	7.14	2.33	7.15	2.23	0.131	0.897

AACS, abdominal aortic calcification score; CIMT, carotid intima–media thickens; LT, left; RT, right.

Table 5 Correlation between matrix Gla protein level and vascular calcification in group I and group II at baseline and after 3 months

	MGP							
	Group I (*N*=28)				Group II (*N*=29)			
	At the start		After 3 months		At the start		After 3 months	
	r	*P*-value	*r*	*P*-value	*r*	*P*-value	*r*	*P*-value
CIMT (LT) (mm)	0.151	0.442	0.092	0.641	–0.133	0.493	0.130	0.503
CIMT (RT) (mm)	0.146	0.459	0.073	0.714	–0.125	0.517	0.225	0.241
AACS	0.200	0.308	–0.075	0.704	–0.128	0.508	–0.154	0.425

AACS, abdominal aortic calcification score; CIMT, carotid intima–media thickens; MGP, Matrix Gla protein; LT, left; RT, right.

menaquinone-4 in tissues after being reduced [9,10] by UbiA prenyltransferase-containing domain 1 (UBIAD1) [11].

Many researchers proved that vitamin K was suboptimal and low in Hemodialysis (HD) patients [12,13], in patients with stage 3–5 chronic kidney disease [14], and in peritoneal dialyzed patients [15]. The possible explanation for those findings is the low dietary intake [16] as low-potassium diet (green leafy vegetables) recommended for hemodialysis patients is low in vitamin K_1 and a diet low in phosphorus also is low in vitamin K_2 content. Because vitamin K_1 can be converted to vitamin K_2 after ingestion, both diets may affect vitamin K_2 levels [9]. KDIGO [17] recommend studies that can determine the efficacy of calcification inhibitors in the prevention or delay of arterial calcification, specifically those trials evaluating the administration of vitamin K in Chronic Kidney Disease (CKD) stages 4–5 D (dialysis).

In the situation where vitamin K is deficient, MGP is not activated and accumulates in the areas of vascular calcification and is associated with both intimal and medial calcification [12]. At the start of the study, we found that all hemodialysis patients (group I and group II) had a significant increase in MGP level compared with the control group, as observed by other researchers [13,18–21].

After 3 months, hemodialysis patients who received oral vitamin K_1 10 mg 3 times per week showed a significant increase in the level of MGP compared with that in patients who did not receive vitamin K. Beulens *et al*. [22] found that high dietary vitamin K intake is associated with significant increase in MGP, which reduces coronary calcification. Also, Khalil and Youssef [23] observed an increase in MGP level in 40 ovariectomized rats after vitamin D and K supplementation. Moreover, Kaesler *et al*. [24] showed reduced γ-glutamyl carboxylase enzyme activity on the basis of uremia in the animal model, which was restored by high intake of vitamin K_1 or K_2.

Braam *et al*. [25] observed that postmenopausal women who were randomized to receive a supplement containing 1 mg/day phylloquinone in addition to minerals and 320 IU vitamin D_3 had better carotid artery distensibility, compliance, and elasticity after 3 years, compared with women who received the mineral supplement alone or the mineral supplement with vitamin D_3. Moreover, Shea *et al*. [26] showed that the supplementation of vitamin K_1 for 3 years decreased the progression of vascular calcification in elderly people with pre-existing calcification. In addition, Neven and D'Haese [27] stated that prevention of warfarin-induced medial calcification in rats could be obtained by vitamin K_2, and regression of

this vascular pathology in this rat model was found under high intake of both vitamins K_1 and K_2. Also, Kurnatowska *et al.* [28] obtained a significant change in serum levels of calcification promoters and inhibitors MGP, Osteocalcin (OC), and osteoprotegerin (OPG) after 270 days of supplementation with vitamin K_2 in patients with CKD stages 3–5, concluding that supplementation with vitamin K_2 may reduce the progression of atherosclerosis but does not significantly affect the progression of calcification.

Furthermore, we did not find any significant difference between CIMT and AACS parameters before and after 3 months of supplementation with vitamin K_1 or a significant correlation between the elevated serum level of MGP in group I with CIMT and AACS. This can be attributed to the short duration of the study compared with previous studies. Shea *et al.* [26] found that the patients who received vitamin K_1 had an increase in the serum level of MGP in the vitamin K_1 group with 6% less progression of coronary artery calcification (CAC) compared with those in the control group ($P<0.04$), but neither baseline nor change in MGP can predict the change in CAC, which may indicate that the benefit of vitamin K on CAC progression is not related to increases in serum MGP.

Women on hemodialysis are under great risk for both severe vascular calcifications and vertebral fractures, which increase mortality [29]. We found a significant reduction in serum cholesterol level in the group that received vitamin K. That finding is in concordance with the results of Stankowiak-Kulpa *et al.* [15], who found that both total and low-density lipoprotein-cholesterol concentrations were significantly higher in patients with vitamin K deficiency than in those without. Further, Joline *et al.* [30] found high dietary menaquinone intake to be associated with lower C-reactive protein concentrations and an improved blood lipid profile. Moreover, The European Food Safety Authority (EFSA) [31]concluded that a relationship exists between the consumption of menaquinone K in red yeast rice preparations and maintenance of normal blood low-density lipoprotein-cholesterol concentrations.

Finally, this study did not find a significant difference in blood pressure, BMI, serum levels of calcium, phosphorus, hemoglobin%, PTH, sodium, potassium, and alkaline phosphatase between group I and group II at the start of the study and after 3 months. This finding was similar to the results of Westenfeld *et al.* [32], who gave vitamin K to 53 hemodialysis patients for 6 weeks and did not found any significant changes in some clinical and laboratory parameters among the three patient groups.

Conclusion

Vitamin K supplementation may be essential for ESKD patients on hemodialysis. Vitamin K can increase the level of MGP and decrease cholesterol level, but its beneficial effect on the vascular bed needs a long-term study.

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Conflicts of interest

There are no conflicts of interest.

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