Effect of vitamin K₁ 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 K₁-dependent γ-carboxyglutamate.

Aim
We aimed to investigate the effect of vitamin K₁ 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 K₁ 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

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 K₁ or phylloquinone and vitamin K₂, 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 patients.
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 K1 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 K1. 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 ~1000g. The serum was stored at ~8°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 K1) (P<0.037) accompanied by a decrease in serum cholesterol level,
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
menequinone-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 K1 and a diet low in phosphorus also is low in vitamin K2 content. Because vitamin K1 can be converted to vitamin K2 after ingestion, both diets may affect vitamin K2 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 K1 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 K1 or K2.

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 D3 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 D3. Moreover, Shea et al. [26] showed that the supplementation of vitamin K1 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 K2, and regression of

| 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; GMP, Matrix Gla protein; LT, left; RT, right.
this vascular pathology in this rat model was found under high intake of both vitamins K1 and K2. 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 K2 in patients with CKD stages 3–5, concluding that supplementation with vitamin K2 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 K1 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 K1 had an increase in the serum level of MGP in the vitamin K1 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 (EFSAN) [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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Nil.

Conflicts of interest
There are no conflicts of interest.

References
1 Verberckmoes SC, Persy V, Behets GJ, Neven E, Huikens A, Zebeger-Gong H, et al. Uremia-related vascular calcification: more than apatite deposition. Kidney Int 2007; 71:298–303.
2 Jablonski KL, Chonchol M. Vascular calcification in end-stage renal disease. Hemodial Int 2013; 17:S17–S21
3 Toussaint ND, Kerr PG. Vascular calcification and arterial stiffness in chronic kidney disease: implications and management. Nephrology 2007; 12:500–509.
4 Fusaro M, D’Angelo A, Gallieni M. Consequences of vitamin K2 deficiency in hemodialysis patients. Am J Kidney Dis 2012; 60:168–171.
5 D’Nicolantonio JJ, Bhutani J, O’Keefe HJ. The health benefits of vitamin K. Open Heart 2015; 2:e000300.
6 Schurgers LJ, Cranenburg EC, Vermeer C. Matrix Gla-protein: the calcification inhibitor in need of vitamin K. Thromb Haemost 2008; 100:593–603.
7 Wei FF, Drummen NEA, Schulte AE, Thijs L, Jacobs L, Petit T, et al. Vitamin K dependent protection of renal function in multi-ethnic population studies. ElBio Med 2016; 4:162–169.
8 Schousboe JT, Wilson KE, Hangartner TN. Detection of aortic calcification during vertebral fracture assessment (VFA) compared to digital radiography. PLoS One 2007; 2:e715.
9 Okano T, Shimomura Y, Yamane M, Suhara Y, Kamao M, Sugira M, Nakagawa K. Conversion of phylloquinone (vitamin K1) into menaquinone-4 (vitamin K2) in mice. Two possible routes for menaquinone-4 accumulation in cerebra of mice. J Biol Chem 2008; 283:11270–11279.
10 Hirota Y, Tsugawa N, Nakagawa K, Suhara Y, Tanaka K, Uchino Y, et al. Menadione (vitamin K3) is a catalytic product of oral phylloquinone (vitamin K1) in the intestine and a circulating precursor of tissue menaquinone-4 (vitamin K2) in rats. J Biol Chem 2013; 288:33071–33080.
11 Shearer MJ, Newman P. Recent trends in the metabolism and cell biology of vitamin K with special reference to vitamin K cycling and MK-4 biosynthesis. J Lipid Res 2014; 55:345–362.
12 Schurgers LJ, Teunissen KJ, Knappen MH, Kwajilaal M, van Diest R, Appels A, et al. Novel conformation-specific antibodies against matrix gamma-carboxyglutamic acid (Gla) protein: undercarboxylated matrix Gla protein as marker for vascular calcification. Artheroscler Thromb Vasc Biol 2005; 25:1629–1633.
13 Ellen CMC, Schurgers LJ, Uiterwijk HH, Beulens JWJ, Dalmeijer GW, Westerhuis R, et al. Vitamin K intake and status are low in hemodialysis patients. Kidney Int 2012; 82:605–610.
14 Holden RM, Morton AR, Garland JS, Pavlov A, Day AG, Booth SL. Vitamins K and D status in stages 3–5 chronic kidney disease. Clin J Am Soc Nephrol 2010; 5:590–597.
15 Stankowiak-Kulpa H, Krzyżanowska P, Kozioł L, et al. Vitamin K status in peritoneally dialyzed patients with chronic kidney disease. Acta Biochim Pol 2011; 58:617–620.
16 Wyskida K, Zakgoła A, Wajda J, Klein D, Witkowicz J, Ficek R, et al. Functional deficiency of vitamin K in hemodialysis patients in Upper Silesia in Poland. Int Urol Nephrol 2016; 48:765–771.

17 Kidney Disease Improving Global Outcomes (KDIGO) CKD-MBD Work Group. KDIGO clinical practice guideline for the diagnosis, evaluation, prevention, and treatment of Chronic Kidney Disease-Mineral and Bone Disorder (CKD-MBD). Kidney Int Suppl 2009 113:S1–130.

18 Braam LA, Dissel P, Gijsbers BL, Spronk HM, Hamulyák K, Soute BA, et al. Assay for human matrix Gla protein in serum potential applications in the cardiovascular field. Arterioscler Thromb Vasc Biol 2000; 20:1257–1261.

19 Pilkey RM, Morton AR, Boffa MB, Noordhof C, Day AG, Su Y, et al. Subclinical vitamin K deficiency in hemodialysis patients. Am J Kidney Dis 2007; 49:432–439.

20 Schlieper G, Westenfeld R, Krüger T, Cranenburg EC, Magdeleyns EJ, Brandenburg VM, et al. Circulating non-phosphorylated carboxylated matrix Gla protein predicts survival in ESRD. J Am Soc Nephrol 2011; 22:387–395.

21 Zheng S, Liu Z, Wang D. The efficacy of vitamin K on vascular calcification for chronic renal failure patient receiving dialysis. Int J Clin Exp Med 2016; 9:18092–18097.

22 Beulens JW, Bots ML, Atsma F, Bartelink ML, Prokop M, Geleijnse JM et al. High dietary menaquinone intake is associated with reduced coronary calcification. Atherosclerosis 2009; 203:489–493.

23 Khalil A, Youssef AG. Effect of aerobic exercise, vitamin K and vitamin D on bone metabolism in ovariectomized adult rats. Nat Science 2015; 13:1–11.

24 Kaesler N, Magdeleyns E, Herfs M, Schettgen T, Brandenburg V, Fliser D, et al. Impaired vitamin K recycling in uremia is rescued by vitamin K supplementation. Kidney Int 2014; 86:286–293.

25 Braam LA, Hoeks AP, Brouns F, Hamulyák K, Gerichhausen MJ, Vermeer C. Beneficial effects of vitamins D and K on the elastic properties of the vessel wall in postmenopausal women: a follow-up study. Thromb Haemost 2004; 91:373–380.

26 Shea MK, O’Donnell CJ, Hoffmann U, Dallal GE, Dawson-Hughes B, Ordovas JM, et al. Vitamin K supplementation and progression of coronary artery calcium in older men and women. Am J Clin Nutr 2009; 89:1799–1807.

27 Neven E, D’Haese PC. Vascular calcification in chronic renal failure what have we learned from animal studies? Circ Res 2011; 108:249–264.

28 Kurnatowska I, Grzelak P, Masajtis-Zagajewska A, Kaczmanska M, Stefaričzyk L, Vermeer C, et al. Effect of vitamin K2 on progression of atherosclerosis and vascular calcification in nondialyzed patients with chronic kidney disease stages 3–5. Pol Arch Med Wewn 2015; 125:631–640.

29 Curiel MD. Action of vitamin K on bone health. Rev Osteoporos Metab Miner 2015; 7:33–38.

30 Beulens JW, van der ADL, Grobbee DE, Slujs I, Spijkerman AM, van der Schouw YT. Dietary phylloquinone and menaquinones intakes and risk of type 2 diabetes. Diabetes Care 2010; 33:1699–1705.

31 European Food Safety Authority (EFSA). EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA) 3. Scientific Opinion on the substantiation of a health claim related to monacolin K in SYLVAN BIO red yeast rice and maintenance of normal blood LDL-cholesterol concentrations pursuant to Article 13(5) of Regulation (EC) No 1924/2006. EFSA J 2013; 11:3084.

32 Westenfeld R, Krueger T, Schlieper G, Cranenburg EC, Magdeleyns EJ, Heidenreich S, et al. Effect of vitamin K2 supplementation on functional vitamin K deficiency in hemodialysis patients: a randomized trial. Am J Kidney Dis 2012; 59:186–195.