

Original Article

Inhibition of maintenance hemodialysis related vascular calcification by vitamin K in chronic kidney disease

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Abstract: Vascular calcification is an important factor causing cardiovascular disorders in patients with chronic kidney disease. Vitamin K is involved in vascular calcification. This study analyzed the effect of vitamin K on vascular calcification in patients with chronic kidney disease while receiving maintenance hemodialysis. Patients with chronic kidney disease receiving hemodialysis in our hospital were recruited. Experimental group received vitamin K treatment in addition to maintenance dialysis, whereas, control group only received maintenance dialysis. Levels of blood calcium, phosphorus, vitamin K, ALB, ALP, CRP, AOPP and fetuin A were measured. Artery calcification score was calculated to analyze factors regulating vascular calcification and determine the relationship between vitamin K and clinical indexes. Blood calcium, phosphorus, vitamin K, ALB and fetuin A levels were higher in experimental group, which presented lower ALP, CRP, AOPP and artery calcification score ($p < 0.05$) compared with control group. After 1 or 3 months treatment, elevated blood calcium, phosphorus, vitamin K, ALB and fetuin A levels, reduction of ALP, CRP, AOPP and artery calcification score were shown in experimental group ($p < 0.05$ compared with before treatment). High blood calcium, phosphorus, vitamin K, ALB, ALP, CRP, AOPP and elevated fetuin A and vitamin K levels were risk factors causing vascular calcification in patients with chronic kidney disease while receiving maintenance hemodialysis ($p < 0.05$). Vitamin K was negatively correlated with vascular calcification score ($p < 0.05$). Vitamin K can modulate vascular calcification in patients with chronic kidney disease who receive long-term maintenance hemodialysis.

Keywords: Vitamin K, chronic kidney disease, hemodialysis, vascular calcification

Introduction

Even with the advancement of medical treatment techniques, the mortality of chronic kidney disease (CKD) has not been significantly improved. For CKD patients, the major reason for death is cardiovascular disease (CVD) [1]. Recent study indicated that vascular calcification was a major risk factor for CVD. In those CKD patients who received hemodialysis, the incidence of vascular calcification was significantly elevated, as their mortality rate of CVD was more than 20 fold of healthy people [2]. During the onset and progression of vascular calcification, it initially existed in artery middle/inner membrane. After de-differentiation of vascular smooth muscle cells, it developed into bone-like or cartilage-like cell to form collagen matrix layer for mineralization deposition [3, 4]. Recently, vitamin K has been found to be involved in vascular calcification process, as the

replenishment of vitamin K can affect the onset velocity and severity of vascular calcification [5]. In a study of CVD in elder people, lower serum vitamin K level was observed to be correlated with coronary artery calcification [6]. The exact effect of vitamin K on vascular calcification in CKD patients has not been reported yet. This study recruited CKD patients who received maintenance hemodialysis and investigate the function of vitamin K on vascular calcification.

Materials and methods

General information

A total of 100 CKD patients who received long-term hemodialysis in Yantaishan Hospital from June 2015 to December 2016 were recruited, including 31 diabetic nephritis (DN) patients, 30 nephritis patients, 19 hypertensive nephri-

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Table 1. Clinical characteristics of patients

	Experimental group	Control group	P
Male	28	30	>0.05
Female	22	20	>0.05
Age (Years)	45.3 ± 10.1	46.1 ± 12.2	>0.05
Dialysis length (Months)	30.3 ± 9.2	31.3 ± 7.2	>0.05
Diabetic nephritis	16	15	>0.05
Nephritis	15	15	>0.05
Hypertensive nephritis	9	10	>0.05
Obstructive nephritis	8	7	>0.05
Polycystic kidney	2	3	>0.05

Table 2. Treatment effectiveness

Group	N	CR	PR	Invalid
Experimental	50	20 (40)	19 (38)	11 (22)
Control	50	12 (24)	17 (34)	21 (42)

tis, 15 obstructive nephritis, and 5 polycystic kidney patients. All patients were randomly divided into two groups, experiment group, in which patients received vitamin K treatment besides dialysis, including 50 patients (28 males, 22 females, aged between 29 and 65 years, average age = 45.3 ± 10.1 years, average span of hemodialysis = 30.3 ± 9.2 months), and control group, in which patients received routine dialysis (50 patients including 30 males, 20 females, aging between 30 and 62 years, average age = 46.1 ± 12.2 years, average span of hemodialysis = 31.3 ± 7.2 months). Two groups of patients showed no significant differences regarding gender, age or other parameters ($p > 0.05$) (Table 1).

Inclusive criteria: Being diagnosed as CKD and received long-term hemodialysis treatment. Stabilized disease condition for more than 1 month. No treatment of any vitamin drugs within one month. No replenishment of levocarnitine, vitamin D or calcium.

Exclusive criteria: Severe chronic or acute infectious disease, liver/gall bladder system disease, malignant tumor, autoimmune disorder, or confirmed vascular calcification of coronary artery.

This study has been pre-approved by the ethical committee of Yantai Hospital. All subjects have signed the consent forms before recruitment in this study.

Dialysis approach

Cimino-brescia arteriovenous fistula was prepared at the flow rate of 200~300 ml/min. Three times of dialysis were performed in each week, with duration of 4~4.5 h in each treatment. Anti-coagulation treatment was performed using 60-80 U/kg of low molecular heparin at 30 min before dialysis.

Dialysis buffer preparation

Experimental group: Vitamin K was mixed in buffer A containing 1.5 mmol/L calcium ions, and was diluted into 57 mg/L dialysis buffer at a ratio of 1:35. Bicarbonate buffer was used as solution B at the flow rate of 500 ml/min.

Control group: Buffer A containing 1.5 mmol/L calcium ion was employed along with bicarbonate solution B at the flow rate of 500 ml/min.

Patient sample collection and index measurement

5 ml blood samples were collected before treatment, and at 1 month and 3 months after treatment. Blood samples were centrifuged at 3000 g for collecting the supernatant. Automatic biochemical analyzer was used to quantify the level of calcium, phosphorous, albumin (ALB). Levels of fetuin A and alkaline phosphatase (ALP) were measured by an immune assay. Rate turbidimetry was used to measure C reactive protein (CRP). UV spectrometry was used to test the advanced oxidation protein products (AOPP). High performance liquid chromatography (HPLC) was employed to detect the vitamin K level following manual instructions [6-8].

Measurement of artery calcification score

CT examination was performed using 130HU as the threshold. Calcification lesion was defined as more than 0.5 mm area. Coronary artery calcification areas of all branches were labelled. Calcification score was calculated as the product of CT values of lesion and area. Total score was then calculated by summation of all sub-scores.

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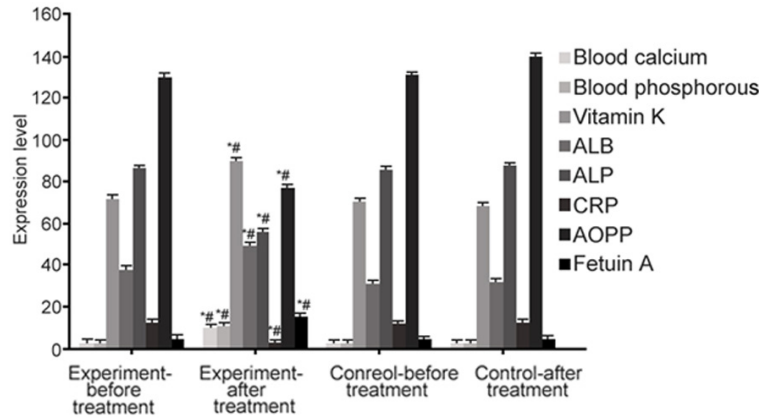


Figure 1. Clinical indexes of all patients before and after treatment. *, $p < 0.05$ compared to control group; #, $p < 0.05$ compared to before treatment.

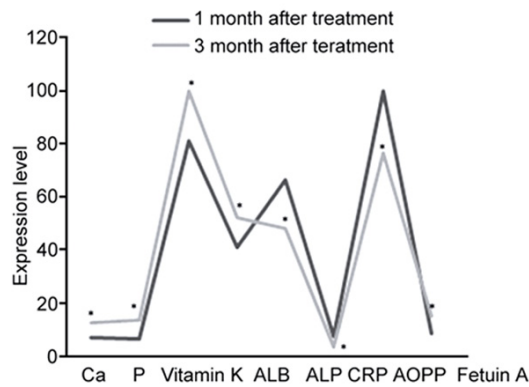


Figure 2. Clinical indexes of experimental group at 1 month and 3 months after treatment. *, $p < 0.05$ compared to 1 month after treatment.

Effectiveness assessment

Complete remission (CR): Disappearance or greatly improvement of clinical symptoms, normal 24 h urine protein level or more than 40% reduction of urine protein; Partial remission (PR): Partial improvement of clinical symptoms, reduction of 24 h urine protein less than 40%; Invalid: No improvement or even worse of clinical symptoms. Effective was defined as CR+PR.

Statistical analysis

SPSS19.0 statistical software was used for statistical analysis. Measurement data were presented as mean \pm standard deviation (SD). Student t-test was used for comparing means between two groups. Logistic regression function was established for vascular calcification. Spearman approach was employed for analyzing

the correlation between vitamin K and other indexes. Multivariate logistic regression was performed to identify the contribution of vitamin K intervention to final outcomes. A statistical significance was defined when $p < 0.05$.

Results

Therapeutic effectiveness after treatment

To evaluate the difference of treatment effectiveness between experimental and control group, we selected 3 months as the endpoint. As shown in **Table 2**, the effectiveness rate was 78% in patients from experimental group and 58% in patients from control group with statistical significance ($p < 0.05$).

Clinical indexes of all patients before and after treatment

To study the effect of vitamin K on CKD patients who received long term dialysis, we measured all clinical indexes including blood calcium, phosphorus, vitamin K, ALB, ALP, CRP, AOPP and fetuin A in all patients before, 3 month and 6 month after treatment. Higher blood calcium, phosphorus, vitamin K, ALB and fetuin A in experimental group after treatment compared with that in control group, whilst ALP, CRP and AOPP levels were lower in experimental group ($p < 0.05$). Furthermore, blood calcium, phosphorus, vitamin K, ALB and fetuin A levels were elevated at 1 or 3 months after treatment, and ALP, CRP and AOPP levels were decreased in experimental group ($p < 0.05$ compared to those before treatment). All clinical indexes at 3 month after treatment showed more significant changes than those at 1 month after treatment ($p < 0.05$, **Figures 1, 2**).

Artery calcification of patients before and after treatment

We also determined artery calcification condition of all patients before and after treatment. Results showed lower vascular calcification score in experimental group before and after treatment ($p < 0.05$ compared with control group). In addition, vascular calcification scores

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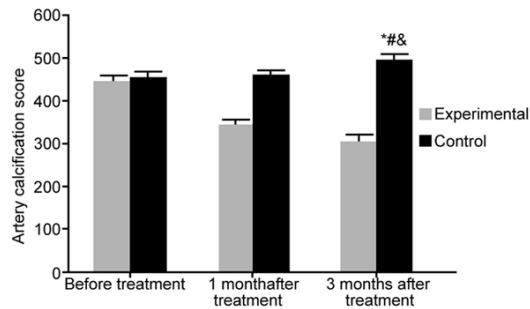


Figure 3. Artery calcification condition before and after treatment. *, $p < 0.05$ compared to control group; #, $p < 0.05$ compared to before treatment; &, $p < 0.05$ compared to 1 month after treatment.

Table 3. Multivariate logistic regression analysis of factors affecting vascular calcification

Item	B	Wald	P
Blood calcium	1.647	6.246	0.005
Phosphorous	1.756	7.356	0.004
Vitamin K	-1.312	5.187	0.035
ALB	1.783	7.105	0.005
ALP	1.821	8.065	0.003
CRP	1.689	7.032	0.004
AOPP	1.779	7.463	0.005
Fetuin A	1.818	8.002	0.003

Table 4. Correlation between vitamin K and clinical indexes in experimental group

Item	r	P
Calcium	0.901	0.06
Phosphorous	0.721	0.07
ALB	0.703	0.08
ALP	0.987	0.06
CRP	0.637	0.09
AOPP	0.744	0.08
Fetuin A	0.925	0.06
Vascular calcification score	-0.276	0.001

of experimental group at 1 month and 3 month after treatment were all lower than those before treatment ($p < 0.05$). At 3 months after treatment, vascular calcification score was significantly decreased compared to that at 1 month after treatment ($p < 0.05$, **Figure 3**).

Multivariate logistic regression analysis of factors governing vascular calcification

To examine the essential factors for vascular calcification, we conducted multivariate logistic

regression analysis by using vascular calcification score as dependent variable, and blood calcium, phosphorus, vitamin K, ALB, ALP, CRP, AOPP and fetuin A as independent variables. Our data demonstrated that high blood calcium, phosphorus, vitamin K, ALB, ALP, CRP, AOPP and elevated fetuin A levels were risk factors of vascular calcification in CKD patients receiving maintenance hemodialysis. Moreover, vitamin K level was also an independent risk factor of vascular calcification ($p < 0.05$, **Table 3**).

Correlation analysis between vitamin K and clinical indexes

This study further analyzed the relationship between vitamin K level and clinical indexes, including blood calcium, phosphorus, vitamin K, ALB, ALP, CRP, AOPP and fetuin A, and vascular calcification score. Results from experimental group showed negative correlation between serum vitamin K level and vascular calcification score ($p < 0.05$), and no correlation with calcium, phosphorus, ALB, ALP, CRP, AOPP or fetuin A ($p > 0.05$, **Table 4**, **Figure 4**).

Discussion

CVD is a major reason for the death of CKD patients at terminal stage, among whom the vascular calcification is the risk factor for CVD pathogenesis [9]. Previous study showed that factors such as parathyroid hormone, parathyroid hormone related polypeptide, calcitriol, glycosylation end products, homocysteine, leptin, calcium/phosphorous, and disrupted lipid metabolism exerted certain roles [10, 11]. Alam et al [12] showed that the protective mechanism in body involving calcification inhibitor could prevent vascular calcification under normal physiological conditions. Current discoveries for calcification inhibitor included fetuin A, pyrophosphate, and osteoprotegerin (OPG) [13]. Previous study reported that vitamin K could also inhibit vascular calcification. However, the exact function of vitamin K on vascular calcification of CKD patients who receiving maintenance hemodialysis remains unclear.

In this study, to illustrate the effect of vitamin K containing dialysis buffer on clinical indexes of CKD patients receiving long term dialysis, we compared various parameters and found that in patients from experimental group who received vitamin K dialysis buffer, blood calcium, phosphorus, vitamin K, ALB and fetuin A

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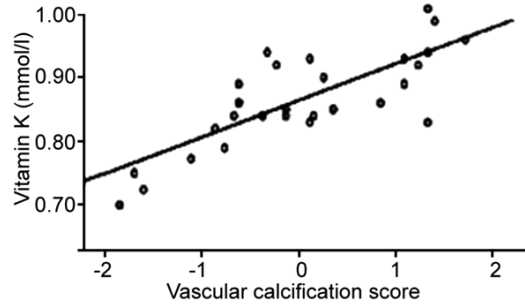


Figure 4. Correlation between vitamin K level and vascular calcification score in experimental group.

were elevated compared with control group, whilst ALP, CRP and AOPP levels were lower. Those clinical indexes showed more potent changes at 3 months post-treatment compared to those after 1 month treatment. Previous study showed that artery vascular calcification in CKD patients were not only attributed to disrupted calcium/phosphorous metabolism, but were also related with oxidative stress or inflammatory response [14]. Fetuin A, an inhibitor against vascular calcification, synthesized in liver, was degraded by proteinase once body was under inflammatory response [15]. CRP is a classical inflammatory response protein and can reduce fetuin A synthesis [16]. AOPP can facilitate the differentiation of smooth muscle cells into osteoblast, and accelerate calcification of such smooth muscle cells [17]. Results of this study showed that vitamin K could elevate serum calcium, phosphorus, ALB and fetuin A levels, and decrease LP, CRP or AOPP levels. The inhibition of oxidative stress or inflammatory response by higher calcium/phosphorous level may impede the vascular calcification in CKD patients.

Our evaluation on conditions of artery calcification in all patients by vascular calcification score indicated that, compared with control group, the score was significantly decreased in experimental group. Of note, the score was gradually reducing from onset to 3 months after treatment. These results also illustrated that vitamin K could inhibit the occurrence of vascular calcification in CKD patients receiving maintenance hemodialysis. As previous evidence showed correlation between reduced vitamin K intake and the occurrence of aorta calcification as well as higher mortality [18], in addition, by using chronic nephritis model mice, warfarin treatment elevated the inci-

dence of vascular calcification, whilst vitamin K intervention relieved vascular calcification [19], these data taken together showed that certain amount of vitamin K intake could reduce the risk of vascular calcification in those patients receiving long-term hemodialysis, which is consistent with our study.

Multivariate logistic regression analysis was performed and determined that vitamin K level was a risk factor causing vascular calcification in CKD patients receiving maintenance hemodialysis. Accumulative findings also exhibited that vitamin K was required for the suppression of vascular calcification, especially in CKD patients, low phosphorous and low potassium food decreased vitamin K intake and some phosphorous binding reagents can bind to vitamin K to accelerate vascular calcification [20-22]. Results of this study indicated that, for the purpose of minimizing oxidative stress and inhibiting body inflammatory response, elevating serum calcium/phosphorous level, vitamin K probably exerted certain inhibiting role on vascular calcification in CKD patients. Moreover, negative correlation between serum vitamin K level and vascular calcification score, but not with blood calcium, phosphorus, vitamin K, ALB, CRP, AOPP or fetuin A levels again validated the certain inhibitory role of vitamin K on the vascular calcification in CKD patients receiving maintenance hemodialysis. In the near future, serum vitamin K level would be elevated by exogenous introduction to resist against oxidative stress, inhibit body inflammatory response, and elevate serum calcium/phosphorous level, thus probably exerting certain inhibitory and treatment functions on vascular calcification in CKD patients.

Conclusion

Vitamin K can elevate blood calcium, phosphorus, ALB and fetuinA levels, and decrease LP, CRP or AOPP levels in CKD patients receiving long term hemodialysis, and suppress vascular calcification, which provides a new evidence and strategy for the treatment of CKD, although detailed mechanisms require further investigation.

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Disclosure of conflict of interest

None.

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