

## Original Article

# The efficacy of vitamin K on vascular calcification for chronic renal failure patient receiving dialysis

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**Abstract:** Vascular calcification is the main factor of inducing cardiovascular diseases in renal failure patients. It was found that vitamin K can inhibit vascular calcification by regulating matrix  $\gamma$ -carboxy-glutamic acid protein (MGP) carboxylation and gene expression. There is still lack of reports about vitamin K application in improving chronic renal failure patient vascular calcification. This study intends to explore vitamin K effect on vascular calcification. A total of 244 cases of renal failure patients with mean age at  $47.6 \pm 6.8$  years old were selected and divided into dialysis group and dialysis-vitamin K group for three months' continuous treatment. Another 86 healthy volunteers were enrolled as normal control. Serum MGP, osteoprotegerin, and osteopontin concentrations were detected by ELISA. Serum MGP, osteoprotegerin, and osteopontin concentration in dialysis group and dialysis-vitamin K group were significantly higher than that in normal control before treatment. After three months' treatment, serum MGP, osteoprotegerin, and osteopontin concentration obviously elevated compared with control ( $P < 0.01$ ). Serum MGP, osteoprotegerin, and osteopontin concentration slightly decreased in dialysis-vitamin K group after treatment ( $P > 0.05$ ), but still higher than that in normal control ( $P < 0.05$ ). In conclusion, Vitamin K can alleviate vascular calcification in renal failure patient. Conventional dialysis combined vitamin K can effectively restrain vascular calcification related protein elevation.

**Keywords:** Renal failure, dialysis, vitamin K, vascular calcification

## Introduction

Cardiovascular disease is a major cause of death in renal failure patients, of which cardiovascular calcification is an important factor that causes cardiovascular disease in chronic renal failure [1, 2]. Local or circulating calcium concentration elevation may cause the calcium ion in the extracellular matrix gradually deposited, eventually leading to vascular calcification and blood vessels effective space narrowing. Meanwhile, it can increase the brittleness of local blood vessels, further inducing cardiovascular disease. Vascular calcification occurs in more than half of chronic renal failure patients, which mainly appears in the large and medium size arteries [3, 4]. It seems that vascular calcification is caused by dynamic imbalance of intravascular calcium and phosphorus. However, more and more studies pointed out that vascular calcification were related with local cell's function and biological characteristics. Vascular smooth muscle cells and endo-

thelial cells transdifferentiate to osteoblastic-like cells, which further secrete organic matters and appear calcium phosphate deposits [5]. Under normal circumstances, vascular calcification inhibiting factors exist in blood vessel, so as to avoid the occurrence of vascular calcification and keep vessel's normal morphology and function [6-8]. The above balance is damaged in chronic renal failure patients, resulting in excess calcium and phosphorus cannot be discharged in time, and eventually inducing deposition [9, 10].

It is revealed that a variety of calcification inhibiting proteins concentration decreased in the process of vascular calcification, such as fetuin A and matrix Gla protein. Matrix Gla protein is a type of vitamin K dependent protein that may be affected by vitamin K deficiency or metabolic disorder. There are more than 17 kinds of vitamin K dependent proteins in the body that participate in coagulation, calcification inhibition, and cell apoptosis [11, 12].

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Vitamin K, especially vitamin K<sub>2</sub>, was firstly considered to alleviate vascular calcification mainly through maintaining vitamin dependent factors' function to restrain calcification. At present, in vitro supplement of vitamin K has been applied in clinic to restore the function of vitamin dependent factor in chronic renal failure patients.

This study detected serum MGP, osteoprotegerin, and osteopontin concentrations in chronic renal failure patients receiving dialysis combined vitamin K to explore the curative effect of vitamin K in renal chronic failure patients.

## Materials and methods

### General information

A total of 244 cases of renal failure patients were recruited from January 2015 to January 2016 in the First Affiliated Hospital of Zhengzhou University, with mean age at  $47.6 \pm 6.8$  years old were selected. Gray-scale ultrasound was applied to test calcification in carotid arteries, abdominal aorta, iliac artery, and lower limb artery. The patients were divided into calcification group ( $n = 198$ ) and non-calcification group ( $n = 46$ ) according to the presence of vascular calcification. The patients were randomly divided into dialysis group and dialysis-vitamin K group for three months' treatment. Another 86 healthy volunteers were enrolled as normal control. Serum was collected before and after treatment. Human osteoprotegerin ELISA detection kit was from Boyao. Human osteopontin ELISA kit was from Biolab. Low temperature high-speed centrifuge was from Thermo. Human matrix  $\gamma$ -carboxy-glutamic acid protein (MGP) ELISA kit was purchased from Shanghai micro biological technology co., LTD. Full-automatic microplate reader was from Bio-Rad. Pipette and spearhead were from Sartorius Stedim Biotech GmbH.

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

## Methods

### Therapeutic schedule

All the renal failure patients in dialysis group and dialysis-vitamin K group received regular

hemodialysis at 3-4 times/week. Each treatment sustained for 4 h, the dialysate volume was 500 ml, and the blood flow velocity was 250-350 ml/min. Fasting blood was collected at three months later. The patients in dialysis-vitamin K group received vitamin K<sub>2</sub> oral administration at 5 mg/time and 2-3 times/day for continuous three months.

### Blood sample collection and separation

A total of 2 ml venous blood was collected in tube containing coagulation accelerator. After placed at room temperature for 5-10 min, the superstratum serum was moved to a 1.5 ml EP tube. After centrifuged at 4°C and 3,500 rpm for 10 min, the serum was stored at -20°C.

### ELISA

Elisa assay was performed according to the manual to test serum MGP, osteoprotegerin, and osteopontin concentrations. Specially, a total of 50  $\mu$ l sample was diluted to five times and added to the plate at 50  $\mu$ l/well. After incubated at 37°C for 30 min, the plate was washed for 5 times. Next, a total of 50  $\mu$ l standard substance was added to the plate and incubated at 37°C for 30 min. After washed for 3-5 times, the plate was added with 50  $\mu$ l color developing agent A and 50  $\mu$ l color developing agent B at 37°C for 15 min. The reaction was terminated by 50  $\mu$ l stop buffer. The plate was detected by microplate reader at 450 nm within 15 min.

### Data analysis

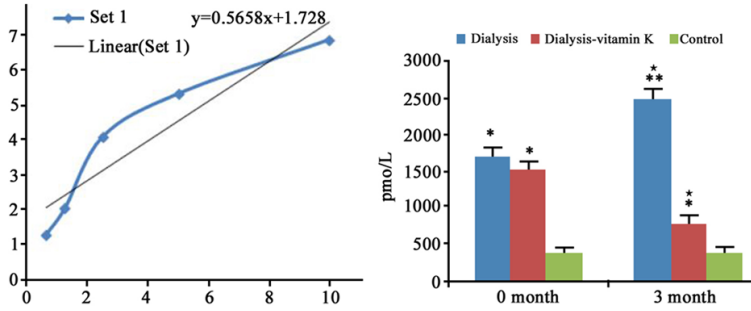
The data analysis was performed on SPSS 16.0 software. Measurement data was depicted as mean  $\pm$  standard deviation. T test was applied for group comparison. Regression equation was constructed by Logistic method.  $P < 0.05$  was considered as statistical significance.

## Results

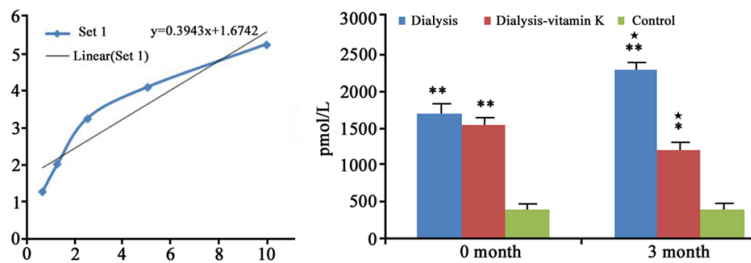
### Serum MGP levels

ELISA assay was applied to detect serum MGP concentration. Standard substance was used to draw the regression curve, and the regression equation was  $y = 0.5658x + 1.728$ . The test result of each sample was substituted to the equation to calculate the MGP concentration. As shown in **Figure 1**, serum MGP concentration in dialysis group and dialysis-vitamin K group before treatment was  $1786.25 \pm 525.32$

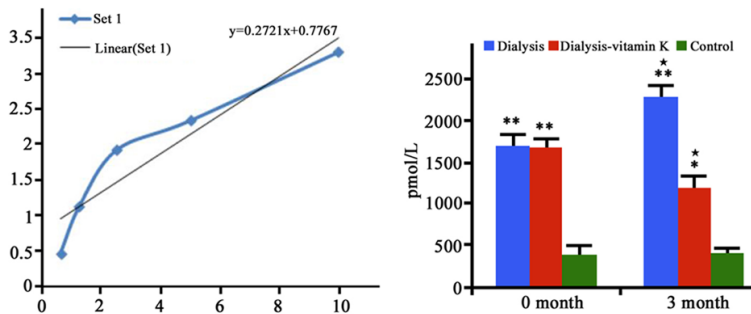
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**Figure 1.** Serum MGP concentration comparison. \* $P < 0.05$ , compared with before treatment. \* $P < 0.05$ , compared with normal control. \*\* $P < 0.01$ , compared with normal control.



**Figure 2.** Serum osteoprotegerin concentration comparison. \* $P < 0.05$ , compared with before treatment. \* $P < 0.05$ , compared with normal control. \*\* $P < 0.01$ , compared with normal control.



**Figure 3.** Serum osteopontin concentration comparison. \* $P < 0.05$ , compared with before treatment. \* $P < 0.05$ , compared with normal control. \*\* $P < 0.01$ , compared with normal control.

pmol/L and  $1698.32 \pm 498.73$  pmol/L, respectively. They were obviously higher than that in normal control ( $468.33 \pm 48.42$  pmol/L,  $P < 0.01$ ). After treatment, MGP concentration in dialysis-vitamin K group was  $998.32 \pm 63.74$  pmol/L, which was significantly lower than that in dialysis group as  $2345.34 \pm 234.33$  pmol/L ( $P < 0.05$ ). MGP concentration in dialysis-vitamin K group slightly reduced, while elevated in dialysis group after treatment ( $P < 0.05$ ). They

were still higher than that in normal control as  $446.32 \pm 36.42$  pmol/L ( $P < 0.05$ ).

### Serum osteoprotegerin levels

ELISA assay was adopted to test serum osteoprotegerin concentration. Standard substance was used to calculate the regression equation as  $y = 0.3943x + 1.6742$ . The test result of each sample was substituted to the equation to calculate the osteoprotegerin concentration. As shown in **Figure 2**, serum osteoprotegerin concentration in dialysis group and dialysis-vitamin K group before treatment was  $1656.25 \pm 325.32$  pmol/L and  $1498.32 \pm 378.53$  pmol/L, respectively. They were markedly higher than that in normal control ( $389.32 \pm 47.43$  pmol/L,  $P < 0.01$ ). After treatment, osteoprotegerin concentration in dialysis-vitamin K group was  $1098.32 \pm 68.64$  pmol/L, which was obviously lower than that in dialysis group as  $2242.34 \pm 254.32$  pmol/L ( $P < 0.05$ ). Osteoprotegerin concentration in dialysis-vitamin K group slightly reduced, while elevated in dialysis group after treatment ( $P < 0.05$ ). They were still higher than that in normal control as  $456.32 \pm 33.46$  pmol/L ( $P < 0.05$ ).

### Serum osteopontin levels

ELISA assay was performed to determine serum osteopontin concentration. Standard substance was used to calculate the regression equation as  $y = 0.5658x + 1.728$ . The test result of each sample was substituted to the equation to calculate the osteopontin concentration. As shown in **Figure 3**, serum osteopontin concentration in dialysis group and dialysis-vitamin K group before treatment was  $1686.25 \pm 527.46$  pmol/L and  $1698.32 \pm 468.78$

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pmol/L, respectively. They were markedly higher than that in normal control ( $488.33 \pm 52.42$  pmol/L,  $P < 0.01$ ). After treatment, osteopontin concentration in dialysis-vitamin K group was  $1184.32 \pm 68.74$  pmol/L, which was obviously lower than that in dialysis group as  $2253.14 \pm 254.71$  pmol/L ( $P < 0.05$ ). Osteopontin concentration in dialysis-vitamin K group slightly reduced, while elevated in dialysis group after treatment ( $P < 0.05$ ). They were still higher than that in normal control as  $456.31 \pm 32.36$  pmol/L ( $P < 0.05$ ).

### Discussion

Vascular calcification is caused by imbalance of calcium and phosphorus metabolism, leading to excessive calcium phosphate compound deposition in the vascular wall. It further results in a series of passive processes of disease. It was showed that vascular calcification was directly related to various diseases, such as chronic renal insufficiency, type 2 diabetes, and cardiovascular diseases [13]. It was indicated that vascular calcification was associated with a variety of proteins abnormal expression, including MGP, osteoprotegerin, osteopontin, and fetuin-A, etc. MGP even has been considered as an indicator of vascular calcification in clinic. Therefore, detection of serum MGP, osteoprotegerin, and osteopontin concentration can effectively predict the risk of vascular calcification [14, 15]. It has been confirmed that vitamin K dependent protein has significant inhibitory effect on vascular calcification [16, 17].

Since more and more vascular calcification appeared in patients with chronic renal failure, vitamin K has been applied in clinic to prevent the occurrence of vascular calcification. However, how vitamin K affect the occurrence of vascular calcification and what related proteins were changed was still controversy. Most researches focused on MGP [18-20], while osteoprotegerin and osteopontin received less attention. Because of the complexity of the vascular calcification inducement in chronic renal failure patients, more related proteins are needed to investigated collectively.

This study selected chronic renal failure patients with vascular calcification diagnosed by gray-scale ultrasound on carotid arteries, abdominal aorta, iliac artery, and lower limb

artery. The patients were randomly divided into dialysis group and dialysis-vitamin K group. Two groups received the same and regulatory hemodialysis treatment at 3-4 times/week. Each treatment sustained for 4 h, the dialysate volume was 500 ml, and the blood flow velocity was 250-350 ml/min. Fasting blood was collected at three months later. The patients in dialysis-vitamin K group received vitamin K2 oral administration at 5 mg/time and 2-3 times/day for continuous three months. Another 86 healthy volunteers were treated as normal control. Serum MGP, osteoprotegerin, and osteopontin concentrations were determined by ELISA.

The results showed that Serum MGP, osteoprotegerin, and osteopontin concentrations in dialysis group and dialysis-vitamin K group were significantly higher than that in normal control before treatment. No statistical significance was observed between dialysis group and dialysis-vitamin K group. After three months' treatment, serum MGP, osteoprotegerin, and osteopontin concentration obviously elevated compared with control. Serum MGP, osteoprotegerin, and osteopontin concentration slightly decreased in dialysis-vitamin K group after treatment, but still higher than that in normal control. Furthermore, there were 198 cases of patients with vascular calcification (81.1%), which was much higher than that of other reports at 50%. Only 46 cases did not appear vascular calcification (18.9%), suggesting that high possibility of vascular calcification in chronic renal failure patients. It is urgently needed to prevent vascular calcification in chronic renal failure patients.

### Conclusion

To sum up, though vitamin K supplement cannot effectively reduce vascular calcification related protein level in chronic renal failure patients during conventional dialysis, but can restrain vascular calcification deterioration.

### Disclosure of conflict of interest

None.

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