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Original Article

Correlation of serum 25-hydroxyvitamin D level with vascular calcification in hemodialysis patients

Fang Wang, Shukun Wu, Yizhe Ruan, Li Wang

Department of Urology, Sichuan Academy of Medical Science, Sichuan Provincial People's Hospital, Chengdu 610072, China

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Abstract: Objective: The aim of this study was to analyze the correlation of serum 25-hydroxyvitamin D level with vascular calcification in patients treated with hemodialysis. Methods: As a cross-sectional study, 126 patients receiving maintenance hemodialysis (MHD) in our hospital were enrolled in this study. According to the serum 25-hydroxyvitamin D level, the patients were divided into 25-hydroxyvitamin D deficiency group (30 ng/ml or less than 30 ng/ml) and 25-hydroxyvitamin D normal level group (>30 ng/ml). All of the subjects underwent lateral lumbar, pelvis and hands X-ray examination to score the degree of calcification (Kauppila score). Results: Among the 126 patients treated with MHD, there were 110 patients with 25-hydroxyvitamin D deficiency and 16 patients with normal 25-hydroxyvitamin D level. There was no significant difference found in gender, age, age of dialysis, active vitamin D treatment, blood calcium, blood phosphorus, blood parathyroid hormone (PTH) and other related indicators between the two groups. The incidence of vascular calcification in patients with 25-hydroxyvitamin D deficiency was significantly higher than that in patients with normal 25-hydroxyvitamin D level ($P = 0.001$). Serum 25-hydroxyvitamin D level had a negative correlation with the calcification score ($r = 0.193$, $P = 0.193$). Logistic regression showed that 25-hydroxyvitamin D was not a risk factor for vascular calcification in MHD patients. Serum 25-hydroxyvitamin D level is generally low in patients with MHD. Conclusions: Patients with 25-hydroxyvitamin D deficiency have a higher incidence of vascular calcification with a markedly negative correlation. Thus, for the patients treated with MHD, vitamin D deficiency should be actively treated.

Keywords: Maintenance hemodialysis, 25-hydroxyvitamin D, calcification score, vascular calcification

Introduction

Vitamin D is an important hormone which can regulate the calcium and phosphorus metabolism balance in body and affect the immune system and cardiovascular system [1]. In general population and patients with chronic kidney disease, the deficiency of active vitamin D is very common [2, 3]. Low level of 25-hydroxyvitamin D is more obvious in hemodialysis patients, and is associated with lower long-term survival rate [4].

Cardiovascular diseases are the most common cause of death in patients with hemodialysis. The vascular calcification is the most common complication in hemodialysis patients, and is the most common cause for occurrence and progression of cardiovascular disease [5]. The previous view believes that, vitamin D can increase the calcium and phosphorus absorption in the gastrointestinal tract, thereby increasing the calcium-phosphorus product and vascular calcification, but on the other hand, vitamin D has positive direct effect on the vascular wall [6]. It is found that the serum vitamin D level in hematodialysis patients is negatively correlated with vascular sclerosis [7] and vascular calcification degree [8, 9]. This suggests that, vitamin D may be a calcification inhibitory factor, and low level of 25-hydroxyvitamin D is closely related with cardiovascular events and cardiovascular mortality in hemodialysis patients [10, 11].

In clinic, the active vitamin D is often used in hemodialysis patients on the basis of high parathyroid hormone levels, and the level of 25-hydroxyvitamin D is not routinely monitored. Therefore, the effect of 25-hydroxyvitamin D level on vascular calcification is not very clear.

Table 1. Clinical data of the patients in VD-deficiency group and VD-normal group

Parameters	VD-deficiency group (n = 110)	VD-normal group (n = 16)	*P* value
Age (years)	52.8±5.1	60.1±17.8	>0.05
Male (%)	56 (50)	8 (49.1)	>0.05
Mean 25-hydroxyvitamin D level (ng/ml)	16.6±5.8	49.9±9.3	0.001*
Systolic pressure (mmHg)	133±18	132±22	>0.05
Diastolic pressure (mmHg)	74±16	74±12	>0.05
Age of dialysis (months)	66.2±49.5	72.9±54.2	>0.05
Active vitamin D treatment (%)	75 (68.2)	6 (85.7)	>0.05
Diabetes (%)	35 (31.8)	2 (12.5)	>0.05
Haemoglobin (g/L)	109.2±15.3	108.7±16.3	>0.05
ALB (g/L)	42.3±3.4	41.4±2.4	>0.05
IL-6 (pg/ml)	4.9±8.2	3.9±2.2	>0.05
Cholesterol (mmol/l)	3.3±1.3	3.6±1.0	>0.05
Blood calcium (mmol/l)	2.0±0.3	2.1±0.1	>0.05
Blood phosphorus (mmol/l)	1.7±0.6	1.3±0.3	>0.05
ALP (U/L)	128.8±85.5	101.5±31.6	>0.05
Blood iPTH (pg/ml)	507.1±453.0	395.3±141.4	>0.05

Data were shown as mean ± SD; *$P < 0.05$, compared with the normal group.

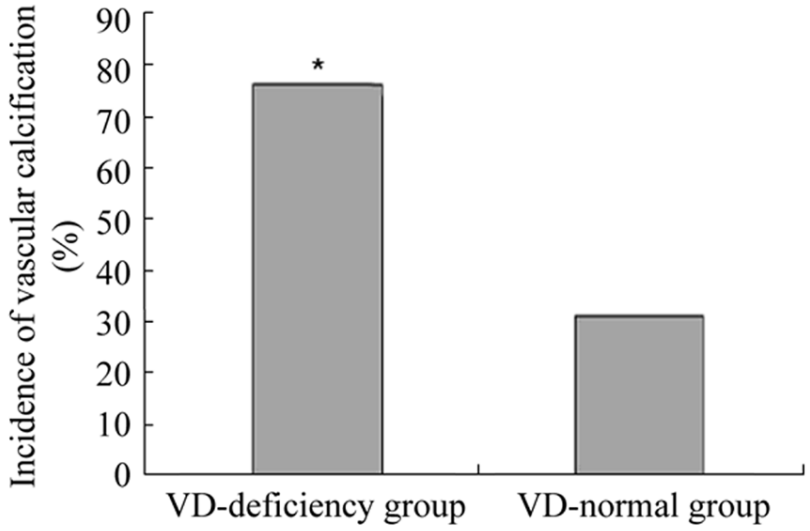

Figure 1. Incidence of vascular calcification in VD-deficiency group and VD-normal group. *$P < 0.05$ compared with VD-normal group.

In this study, the 25-hydroxyvitamin D level in hemodialysis patients was monitored to understand the situation of active vitamin D deficiency. Kauppila scoring was used to understand the vascular calcification in patients, and the related analysis was performed to investigate the effect of 25-hydroxyvitamin D deficiency on vascular calcification in hemodialysis patients.

Materials and methods

Subjects

126 hemodialysis patients at the Blood Purification Center of the People's Hospital of Sichuan Province were enrolled in this study, which was conducted in September. Inclusion criteria: 1) Patient's informed consent had been obtained. 2) Receiving long-term maintenance hemodialysis (MHD) in our Blood Purification Center. 3) With a dialysis frequency of three times per week. 4) With normal diet and normal daily life activities. 5) Monitoring of serological indexes, such as routine blood examination, blood biochemistry, electrolyte, immunoreactive parathyroid hormone (iPTH), ferritin (FER) and so on. 6) With an age higher than 18 years. Serum 25-hydroxyvitamin D level of each patient was determined. In addition, the patients' gender, age, dialysis vintage, blood pressure, primary disease, dialysis adequacy, use of blood active vitamin D treatment, blood calcium, phosphorus, PTH, alkaline phosphatase (ALP), hemoglobin and blood lipid levels were all recorded. According to the guidelines of Kidney Disease Outcomes Quality Initiative (KDOQI) [12], the patients were divided into two groups basing on their blood 25-hydroxyvitamin D level: 25-hydroxyvitamin D deficiency group (30 ηg/ml or less than 30 ηg/ml) and 25-hydroxyvitamin D normal level group (> 30 ηg/ml). X-ray examinations of lat-

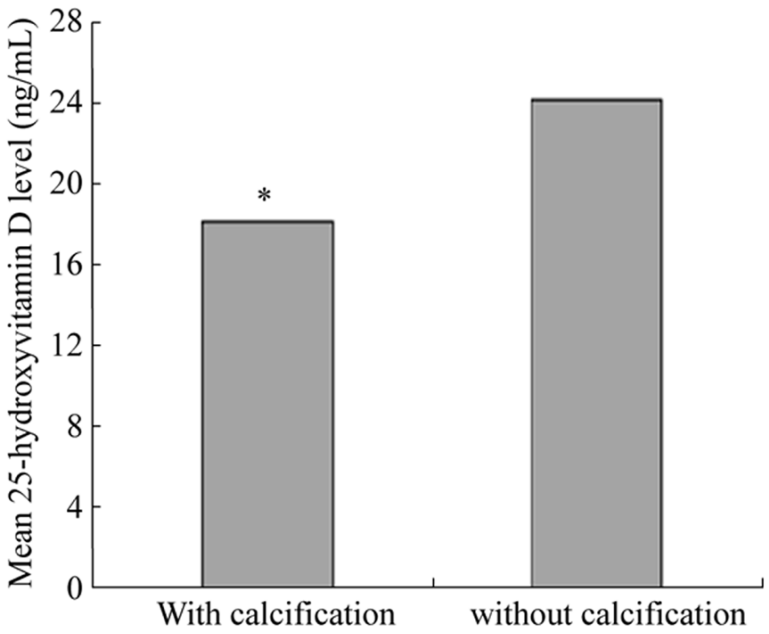

Figure 2. Mean 25-hydroxyvitamin D levels in patients with calcification and without calcification. *P < 0.05 compared with patients without calcification.

eral lumbar, pelvis and hands were carried out on each patient for calcification score. This study was conducted in accordance with the declaration of Helsinki. This study was conducted with approval from the Ethics Committee of Sichuan Provincial People's Hospital. Written informed consent was obtained from all participants.

Serological examination

Blood of each patient was collected in the early morning when the patient was in fasting and then serum was isolated and stored at -80°C before use. Turbidimetric method was used to determine the serum phosphorus and calcium levels with the kits purchased from the BioSino Technology Co., Ltd and performed at the Center of Medical Laboratory. IPTH determination was performed on a chemiluminescence analyzer (Immulite 1000, dpc Company, USA) at the Department of Nuclear Medicine using the corresponding reagents supplied by dpc Company. The 25-hydroxyvitamin D level was measured using automatic radioimmunoassay with the kit offered by Tianjin Union Medicine and Technology Co., Ltd and completed the Department of Nuclear Medicine.

Calcification scoring

X-ray examinations of lateral lumbar, pelvis and hands were carried out on all of the subjects when they were at fasting using Fuji X-ray machine (Model XG-1, Japan). According to Kauppila score, calcification scores of the abdominal aorta, femoral artery, iliac artery, radial artery and finger web artery were calculated [13]. Briefly, pelvic X-ray plain film was divided into four parts by two mutually vertical lines, in which horizontal line was positioned on the tangent plane of the bilateral femoral heads and the vertical line was positioned on the vertebral column. Normotopia film of hands was also divided into four parts by a horizontal line above the metacarpal of each hand; abdominal lateral plain film was divided into upper abdomen and lower abdomen by a horizontal line through the intervertebral space between L2 and L3 [14]. The calcifications in the 10 parts were counted according to the following scoring method: each part could be scored 1 or 0 due to with or without calcification and each patient could get a final score from 0 to 10. Scoring from 0 to 3 was regarded as mild calcification, from 4 to 6 was regarded as moderate calcification and from 7 to 10 was regarded as severe calcification. All films were blinded interpreted by two experienced radiologists and recorded by a renal physician.

Statistical analysis

SPSS v17.0 software was used for statistical analysis. Measurement data were showed as mean ± standard deviation (SD) and compared using t test. Numeration data was compared using Chi-square test. Correlation analysis was performed using Pearson correlation and Logistic regression was used to analyze the risk factor. P < 0.05 was considered statistically significant.

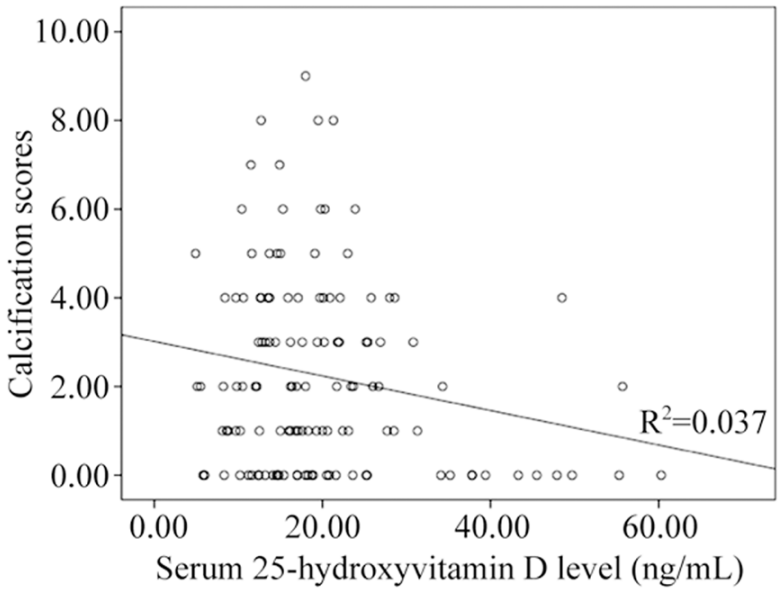

Figure 3. Serum 25-hydroxyvitamin D level in MHD patients was negatively correlated with the calcification scores (r = 0.193, *P* = 0.031).

Table 2. Logistic regression analysis on the risk factors for vascular calcification

Parameters	OR	*P* value	95.0% CI for EXP (B)	
			Upper limit	Lower limit
Gender	1.030	0.955	0.365	2.9
Age	1.057	0.006*	1.016	1.099
25-hydroxyvitamin D	0.971	0.402	0.908	1.040
Active vitamin D	0.536	0.294	0.167	1.717
Age of dialysis	1.009	0.184	0.996	1.022
Systolic pressure	1.019	0.322	0.981	1.059
Diastolic pressure	0.980	0.405	0.935	1.027
Ca	0.299	0.303	0.030	2.973
P	0.386	0.046	0.152	0.984
PTH	1.001	0.119	1.000	1.002
Diabetes	1.904	0.297	0.568	6.39

*$P < 0.05$, statistically significant.

Results

Calcification scores in patients treated with MHD

The 126 MHD patients enrolled in this study included 64 males and 62 females, with a mean age of 53.3±15.3 years and mean age of dialysis of 66.6±49.5 months. Among the 126 patients, there were 37 patients with diabetes and 81 patients received oral administration of active vitamin D with a daily dose of 0.25 μg to 0.75 μg. There were 110 patients with 25-hdroxyvitamin D deficiency (VD-deficiency group), accounting for 87.3%, with a mean 25-hydroxyvitamin D level of 16.6±5.8 ηg/ml, among whom there were 84 cases with vascular calcification. There were 16 patients with normal 25-hydroxyvitamin D level (49.9±9.3 ηg/ml, VD-normal group), among whom there were 5 cases of calcification. The patients' gender, age, age of dialysis, active vitamin D treatment, blood calcium, blood phosphorus, blood PTH and other related indicators showed no significant statistical significance between the two groups (**Table 1**). However, the incidence of vascular calcification in VD-deficiency group was obviously higher than that in the normal group ($P < 0.05$, **Figure 1**). The mean 25-hydroxyvitamin D level in the patients with calcification was 18.2±8.2 ηg/ml, significantly lower than that in the patients without calcification (24.2±14.6 ηg/ml, $P < 0.0001$; **Figure 2**).

Relationship between 25-hydroxyvitamin D levels and the calcification scores

Pearson correlation analysis showed that 25-hydro-xyvitamin D level was negatively correlated with the calcification scores (r = 0.193, *P* = 0.031). Namely, the degree of vascular calcification in patients increased with the down-regulation of the serum 25-hydroxyvitamin D level (**Figure 3**). In addition, Logistic regression showed that age and high phosphorus were two risk factors for vascular calcification in MHD patients (**Table 2**).

Discussion

A growing number of studies in recent years have shown that there is a widespread insufficiency and deficiency of vitamin D in the patients with chronic kidney disease (CKD). It even exists in the early stage of CKD and aggravates with the decrease of glomerular filtration rate (GFR). Wolf and Bansal et al. found that vitamin D insufficiency and deficiency is common in MHD patients, with a prevalence of 78% [15, 16]. Many researchers have shown that the prevalence of vitamin D deficiency in the patients with advanced CKD was 81.5%, and its severity is associated with the progress of kidney function [17]. Our results that the prevalence of 25-hydroxyvitamin D deficiency in the MHD patients was 87.3% were consistent with the above reports.

Vascular calcification is a common complication of maintenance hemodialysis in patients, which is also an important factor influencing the prognosis of the patients with ESRD. X-ray examination is highly susceptible to the moderate and severe calcification, but is difficult to detect the mild or a small amount of calcification [18-20]. Garcia-Canton et al. [21] made a cross-sectional observation on 210 patients with stage IV and V CKD before dialysis. They analyzed the pelvis, hands and lateral lumbar films using Adragao and Kauppila score and determined the serum 25-hydroxyvitamin D level of each patient and then found that low 25-hydroxyvitamin D concentration is an independent predictor for adragao scores higher than 3 and Kauppila scores higher than 7. In this study, we analyzed the economic and simple films of pelvis, hands and lateral lumbar using Kauppila score and found that there were 89 patients with vascular calcification among the 126 MHD patients, accounting for 70.6%.

Recently, a body of evidences shows that serum vitamin D level in patients with ESRD is negatively correlated with the degree of calcification, indicating that vitamin D is an inhibitor for calcification. Patricia et al. [22] analyzed the serum 25-hydroxyvitamin D concentration and X-ray calcification scores in 223 MHD patients and found that 25-hydroxyvitamin D concentration is negatively correlated with calcification scores. Gerard et al. [7] reported that the blood 25-hydroxyvitamin D level in the patients receiving blood dialysis is negatively correlated with pulse wave velocity (PWV); suggesting vitamin D deficiency may be associated with atherosclerosis and endothelial dysfunction. Similarly, Chitalia et al. [23] evaluated the endothelial function of CKD patients without dialysis by measuring the blood flow regulating diastole (FMD) and found that the lower the levels of 25-hydroxyvitamin D is, the lower FMD is and the more seriously the endothelial function is impaired. Thus, vitamin D deficiency is believed to lead to endothelial dysfunction and thereby aggravate or induce the occurrence of vascular calcification. At present, the possible mechanisms for vitamin D deficiency causing or worsening the vascular calcification may be: 1) Vitamin D inhibits vascular calcification by inhibiting the production of type I collagen and the core binding factor α1 (type I collagen promotes the deposition of calcium, while the core binding factor α1 enhance the deposition of type I collagen). 2) Vitamin D promotes the product of Matrix Gla protein, a vascular calcification inhibitor, to inhibit the vascular calcification. 3) Vitamin D deficiency leads to vascular endothelial dysfunction and thereby causes vascular calcification. 4) Active vitamin D can stimulate the expression of Klotho protein and osteopontin in the arterial wall, both of which have preventive effect on vascular calcification [24]. Our findings showed that the incidence of vascular calcification in the patients with 25-hydroxyvitamin D deficiency was obviously higher than that in the patients with normal 25-hydroxyvitamin D level (76.4% vs. 31.3%, $P = 0.001$) and 25-hydroxyvitamin D level had a negative correlation with the calcification scores ($r = 0.193$, $P = 0.193$). These were all consistent with the results mentioned above. However, Logistic regression analysis did not show 25-hydroxyvitamin D deficiency is a risk factor for vascular calcification in MHD patients, which was not consistent with the results of some researches. This may be associated with the small sample size, but it can be enlarged in the future study. Logistic regression showed that age and hyperphosphatemia were two risk factors for vascular calcification in MHD patients, consistent with the results reported previously.

Active vitamin D treatment has its advantages and disadvantages. On one hand, the increasing absorption of calcium and phosphorus induced by active vitamin D can elevate the calcium and phosphorus load and may promote the development of vascular calcification. On the other hand, vitamin D has a positive effect on the blood vessel walls. Although the use of

active vitamin D improves the vitamin D level in patients, some studies report it may aggravate the progress of vascular calcification due to the deposition of calcium and phosphate but not the local effect on the arterial wall [11]. Low dose of active vitamin D may have a protection effect on vascular calcification in case of no increase in calcium and phosphorus load [25]. In this study, about more than 60% of the patients treated with active vitamin D3, mainly with moderate doses of calcitriol, but the incidence of 25-hydroxyvitamin D deficiency remains as high as 87.3%, indicating the treatment got very low success rate, which may due to the frequency of the detection and the contradiction and effectiveness of the treatment. The results also showed that vascular calcification had no obvious correlation with the administration of active vitamin D. The benefits of active vitamin D treatment are not very clear yet, but some retrospective and observational studies have shown that active vitamin D treatment has survival benefit in hemodialysis patients. Both the all-cause mortality and cardiovascular mortality during PTH period decrease significantly [26, 27].

In conclusion, among the patients treated with maintenance hemodialysis, 25-hydroxyvitamin D level is generally low even in the patients receiving active vitamin D treatment. Moreover, there is a high incidence of vascular calcification among them. Patients with 25-hydroxyvitamin D deficiency have a higher incidence of vascular calcification, and the severity of vascular calcification increases with the decrease of 25-hydroxyvitamin D levels. Thus, 25-hydroxyvitamin D deficiency should be actively corrected in MHD patients. At present, prospective randomized controlled study is still needed to evaluate the impact of active vitamin D treatment on the survival of the patients with ESRD and receiving dialysis.

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Disclosure of conflict of interest

None.

Address correspondence to: Dr. Li Wang, Department of Urology, Sichuan Academy of Medical Science, Sichuan Provincial People's Hospital, Chengdu 610072, China. Tel: +86 28 87393339; Fax: +86 28 87393196; E-mail: cnliwang@126.com

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