*Hemodialysis International* 2017; **21**:97–106

# Effect of vitamin D supplementation on endothelial dysfunction in *hemodialysis* patients

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**Abstract**

**Introduction:** Patients with chronic kidney disease (CKD) commonly experience 25-hydroxyvitamin D3 (25-OH-D3) deficiency, and these patients have a higher incidence of cardiovascular diseases (CVDs) due to endothelial dysfunction (ED). The aim of our study was to investigate the effect of 25-OH-D3 deficiency and its supplementation on ED in patients with CKD. **Methods:** Twenty-nine uremic patients on dialysis and 20 healthy controls were evaluated for ED by high-resolution Doppler ultrasonography of the brachial artery. In addition, 25-OH-D3-deficient patients (25-OH-D3 $< 30$ nmol/L) with CKD and healthy controls were evaluated for ED before and after 8 weeks of oral vitamin D (cholecalciferol, 50,000 units) treatment. All subjects were evaluated for percent flow-mediated dilatation (%FMD), percent endothelium-independent nitroglycerin-induced vasodilatation (%NID), and bilateral carotid intima-media thickness (CIMT). **Findings:** Patients on dialysis had lower %FMD and %NID 6.11 [2.27–12.74] and 10.96 [5.43–16.4], respectively, than controls 15.84 [8.19–22.49] and 21.74 [12.49–29.4], respectively ($P < 0.05$). Patients on dialysis had higher left and right CIMT ($0.79 \pm 0.15$ and $0.78 \pm 0.14$, respectively) than controls ($0.60 \pm 0.09$ and $0.59 \pm 0.09$, respectively; $P < 0.05$). In 25-OH-D3-deficient patients with CKD, after vitamin D treatment, %FMD was significantly increased in dialysis patients (10.25 [7.8–12.8]) compared to before supplementation (5.4 [2.77–6.15]; $P < 0.001$). **Discussion:** These results indicated that dialysis patients had significantly lower blood 25-OH-D3 levels and higher CIMT than healthy subjects. In addition, vitamin D supplementation improved ED and increased %FMD in dialysis patients. Our findings suggest that vitamin D supplementation in dialysis patients might prevent CVD.

**Key words:** 25-Hydroxyvitamin D3, carotid intima-media thickness, cholecalciferol, endothelial dysfunction, flow-mediated dilatation

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This study was presented as a poster at the 50th European Renal Association-European Transplant and Dialysis Association (ERA-EDTA) Congress.

*Conflict of Interest*: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

*Disclosure of grants or other funding*: No external funding was obtained for this study.



DOI:10.1111/hdi.12439

## INTRODUCTION

During the last 20 years, researchers have found an overwhelming number of important functions of the endothelium including control of coagulation, fibrinolysis, vascular tone, growth, and immune responses.[1] The endothelium modulates vascular tone by releasing various vasoactive substances, including nitric oxide (NO). It is now clear that endothelial dysfunction (ED) disturbs the physiological protective regulatory balance, a critical factor in atherosclerotic disease progression. Thus, ED is now recognized to occur in a wide variety of disorders with an increased cardiovascular risk, such as essential hypertension,[2] dyslipidemia,[3] smoking,[4] and mental stress.[5]

Cardiovascular events occur more commonly in patients with chronic kidney disease (CKD) than in the general population.[1] The significant burden of atherosclerotic cardiovascular disease (CVD) in end-stage renal disease (ESRD) has been recognized since the early 1990s,[6] and is most likely due to the high prevalence of both traditional risk factors, such as hypertension, dyslipidemia, and smoking, and nontraditional risk factors, such as inflammation, oxidative stress, advanced glycation end products, and homocysteine. C-reactive protein (CRP), a sensitive and objective marker of inflammation, was shown to be associated with CVD in both predialysis[7] and dialysis[8] patients. Recent studies showed that impaired endothelium-dependent vasodilation is a prominent feature in patients with moderate renal impairment,[9] as well as in patients with advanced renal impairment treated by either hemodialysis (HD)[10] or peritoneal dialysis (PD).[11] The underlying mechanisms of ED in ESRD patients are not fully understood but are most likely multifactorial.

Recent observations have indicated that CKD might be associated with high incidences of nutritional vitamin D insufficiency and deficiency, as manifested by decreased levels of 25-hydroxyvitamin D (25-OH-D3), which in turn contributes to the inability to maintain the levels of 1, 25-dihydroxyvitamin D3.[12] In addition, vitamin D has been proposed to regulate numerous cellular functions.[13] The vitamin D receptor is universally expressed in nucleated cells; thus, the spectrum of vitamin D activity is much broader than just the calcium/bone homeostasis.[13–15] Therapy with vitamin D and its analogs have shown multiple beneficial effects in both HD[16] and nondialysis CKD patients,[17] leading to significant survival advantage in treated patients; importantly, these therapeutic and protective effects were independent of suppression of parathyroid hormone (PTH). The mechanism underlying the survival advantage of vitamin D therapy is a subject of intense investigation; however, given the pleiotropic nature of vitamin D activity, it may be due to vitamin D's effect on multiple targets.[18]

Therefore, we herein investigated the effect of 25-OH-D3 deficiency and vitamin D supplementation on ED in patients with CKD undergoing dialysis using complementary imaging and laboratory evaluations.

## MATERIALS AND METHODS

### Patients and methods

In this prospective single center study, we analyzed data from 44 patients with HD and 24 healthy at the Osmangazi University in Turkey between 2011 and 2013. Eligibility criteria for patients included HD for at least 6 months, aged 18 or older and both patients and control group do not have any disease or medication that can effect on endothelial function. Twenty-nine patients and 20 healty controls individuals enrolled in study. The causes of ESRD were hypertension, polycystic kidney disease, ureteral stricture, and chronic glomerulonephritis in 16 (52.2%), four (13.8%), three (10.3%), and two (6.9%) patients, respectively; the etiology of ESRD could not be determined in four (13.8%) patients. Patients with ESRD were treated three times a week with standard bicarbonate HD for 4 hours, Kt/V values were higher than 1.2.

### Exclusion criteria

Patients with acute or chronic infection, systemic disease, diabetes mellitus, amyloidosis, liver disease, acute myocardial infarction, CVD, congestive heart failure (New York Heart Association [NHYA] classes III–IV with an ejection fraction of <50%), and peripheral arterial disease were excluded. Patients on medications that could affect endothelial function were also excluded. Patients were not taking lipid lowering agent, acetylsalicylic acid (ASA), and other antioxidants agent during the study period.

Blood samples for laboratory tests were taken in the morning after 12 hours of fasting. Blood samples and ultrasonographic measurements were performed on days without dialysis session. In both dialysis and control subjects, those with 25-OH-D3 below 30 nmol/L (12.5 ng/mL) were determined to be 25-OH-D3 deficient and were given vitamin D supplementation (cholecalciferol) at 50,000 units per week for 8 weeks, and all recipients were closely monitored against hypercalcemia risk during supplementation. Other medications for both patients

and control subjects, and dialysis schedules of patients were not changed during the supplementation period.

Ethical Research Committee approval was from Osmangazi University Scholl of Medicine obtained for our study, and informed consent was obtained from all subjects after information on the study was provided.

## Laboratory analyses

Hemoglobin, triglyceride, total cholesterol, high-density lipoprotein, low-density lipoprotein (LDL), blood urea nitrogen (BUN), creatinine, calcium (Ca), phosphorus (P), PTH, and albumin were evaluated in all subjects. Biochemical indices of uremic osteodystrophy included serum levels of intact PTH, which was measured with electrochemiluminescence immunoassay using a Roche Elecsys module (Roche Diagnostics, Mannheim, Germany). Following centrifugation of samples at 300×*g* for 7 minutes, supernatants were collected and 25-OH-D3 levels were measured by an enzyme immunoassay kit from Immundiagnostik (Bensheim, Germany). Serum Ca, P, cholesterol, and albumin levels were measured by standard clinical chemistry methods.

## Measurement of brachial artery and carotid intima-media thickness

In all participants, a Toshiba SSA-240 high-resolution ultrasonograph (Toshiba, Tokyo, Japan) with a 7.5-MHz linear transducer was used for all imaging studies. Brachial artery endothelium-dependent flow-mediated dilatation (FMD) was measured in all patients. In addition, in all participants, carotid intima-media thickness (CIMT) was measured with a 7.5-MHz high-resolution probe. After baseline measurements, 25-OH-D3-deficient patients were evaluated using the same criteria, both before and after 8 weeks of oral cholecalciferol (50,000 units). In addition, patients were assessed before and after treatment for reactive ischemia, and in response to sublingual glyceryl trinitrate (0.4 mg) to assess endothelium-independent nitroglycerin-induced vasodilatation (NID).

FMD and NID were assessed according to the guidelines described by Celermajer et al.[19] For FMD, the right arm was placed in extension at the elbow, and the hand was placed in supination. In HD patients, FMD and NID measurements were performed from the arm that did not have a fistula. An optimal longitudinal image of the brachial artery just above the elbow with clear anterior and posterior intimal interfaces between the lumen and vessel wall was established and kept stable. A sphygmomanometer cuff was placed distally from the elbow. A baseline rest image was obtained; thereafter, arterial occlusion was created by cuff inflation to 50 mmHg above the systolic blood pressure for 4 minutes. After deflation, the longitudinal image of the artery was recorded continuously starting from 30 seconds before until 2 minutes after deflation, and the lumen diameter was measured at about 60 seconds. Lumen diameter was defined as the distance between medial and adventitial interfaces of the vessel wall. After a 10-minute resting interval, a 0.4-mg nitroglycerine spray was administered sublingually, and vascular relaxation was measured on the fifth minute. %FMD was defined as the percent change in brachial artery diameter within 1 minute after ischemia compared to baseline. %NID was defined as the percent change within 5 minutes after nitroglycerine administration. Briefly, the following formulas were used for %FMD and %NID: Delta(Δ)FMD (mm) = FMD vessel size − baseline vessel size, ΔNID (mm) = NID vessel size − baseline vessel size, %FMD = ΔFMD/baseline vessel size × 100, and %NID = ΔNID/baseline vessel size × 100.

CIMT measurement was performed by ultrasonography of the distal (posterior) and proximal (anterior) from the right and left main carotid artery, internal carotid artery, external carotid artery, and bulbus of subjects in the supine position. After the carotid bifurcation was located

**Table 1** Demographic and clinical characteristics of subjects

| | Patients (n = 29) | Controls (n = 20) |
|---|---|---|
| Age (y) | 45.8 ± 11.7 | 39.9 ± 7.1 |
| Sex (Males/Females) | 13/16 | 11/9 |
| Height (cm) | 165.7 ± 7.8 | 169.7 ± 6.9 |
| Weight (kg) | 66.3 ± 12.1 | 74.7 ± 8.2 |
| Body mass index (kg/m$^2$) | 24 ± 3.9 | 26 ± 3.3 |
| Blood pressure(mmHg) | | |
| Systolic | 118 ± 17 | 120 ± 11 |
| Diastolic | 78 ± 12 | 78 ± 9 |
| MAP | 91 ± 13 | 91 ± 9 |
| Medication | | |
| Active vitamin D | 5 (17.2% | - |
| Calcium-containing phosphate-binding | 22 (75.9%) | - |
| No medication | 2 (6.9%) | 20 (100%) |
| Cause of ESRD | | |
| Hypertension | 16/29 | - |
| Glomerulonephritis | 2/29 | - |
| Polycystic kidney disease | 4/29 | - |
| Chronic pyelonephritis | 3/29 | - |
| Other/unknown | 4/29 | - |

ESRD = end-stage renal disease; MAP = mean arterial pressure; - =none.

**Table 2** Laboratory findings and endothelial function of subjects

| | Patients (n = 29) | Controls (n = 20) | P |
|---|---|---|---|
| Hb (g/dL) | 10.4 ± 1.8[a] | 14.5 ± 1.5 | <0.05 |
| Platelet ($10^3$/μL) | 240 ± 114 | 261 ± 68 | NS |
| Glucose (mg/dL) | 94 ± 20 | 88 ± 10 | NS |
| BUN (mg/dL) | 49 ± 13 | 12 ± 2 | <0.05 |
| Creatinine (mg/dL) | 8.1 ± 2.7 | 0.8 ± 0.1 | <0.05 |
| Total protein (g/dL) | 6.7 ± 0.7 | 7.3 ± 0.3 | <0.05 |
| Albumin (g/dL) | 3.8 ± 0.4 | 4.6 ± 0.2 | <0.05 |
| Triglycerides (mg/dL) | 158 ± 82 | 112 ± 44 | <0.05 |
| LDL cholesterol (mg/dL) | 134 ± 47 | 117 ± 35 | NS |
| HDL cholesterol (mg/dL) | 46 ± 13 | 50 ± 10 | NS |
| Total cholesterol (mg/dL) | 191 ± 63 | 181 ± 35 | NS |
| 25 (OH) vit D (nmol/L) | 30 ± 13.4 | 34.26 ± 8.7 | NS |
| PTH (pg/mL) | 173 (13.10–1198)[b] | 63.6 (29–112) | <0.05 |
| CRP (mg/dL) | 1.12 (0.39–1.94) | 0.32 (0.3–0.34) | <0.05 |
| Sedimantation (mm/h) | 46.8 ± 27.8 | 5.8 ± 5.15 | <0.05 |
| CIMT left (mm) | 0.79 ± 0.15 | 0.60 ± 0.09 | <0.05 |
| CIMT right (mm) | 0.78 ± 0.14 | 0.59 ± 0.09 | <0.05 |
| ΔFMD% | 6.11 (2.27–12.74) | 15.84 (8.19–22.49) | <0.05 |
| Δ NID% | 10.96 (5.43–16.4) | 21.74 (12.49–29.4) | <0.05 |

BUN = blood urea nitrogen; CIMT = carotid intimae media thickness; CRP = C-reactive protein; FMD = flow mediated dilatation; HDL = high density lipoprotein; LDL = low density lipoprotein; NID = nitroglycerin-induced vasodilatation; NS = non significant; PTH = parathormone.
[a]Mean ± SD.
[b]Median (Q1,Q3).

in the transverse screening, transducer was rotated 90°, and longitudinal views showing the anterior (proximal) and posterior (distal) walls were obtained. CIMT measurements were collected from the posterior wall about 1.5 cm distal to the bifurcation from where it was observed as thickest. Distance between the lumen intima and media/adventitia was accepted as intima-media thickness (IMT). Each measurement was separately recorded, and measurements from four points were collected to calculate the proximal, distal, right, and left total averages and were recorded as CIMT.

The all measurements were performed by the same physician. Measurements were not taken from areas with plaques.

## Statistical analysis

The vitamin D deficiency is observed in approximately 40% of normal population, and 90% of patients with renal insufficiency. When an effect size of treatment on improvement of ultrasound measures was considered to be 0.8, and when the difference of prevalance of vitamin D insuffiency was 50%, samples size calculations under a two-tailed study design, a 5% of type-I error, and a group allocation of 1.5:1 revealed that 25 patients in study group and 17 participants in control group were sufficient to achieve an estimated power of 80%. With an estimated 10% of lost-to-follow-up ratio was considered, 29 patients and 20 controls were included in the study.

IBM SPSS Statistics 21.0 for Windows were used for data analysis. The distribution of variables was analyzed by the Shapiro-Wilk test. Parametric tests were used for data with normal distribution, whereas nonparametric tests were used for data with non-normal distribution. Independent samples t test and Kruskal-Wallis test were used to determine difference between independent two groups. Paired samples t test and Wilcoxon test were used to determine difference between pretreatment and post-treatment. The chi-square test was applied for categorical variables. The relationships between variables were evaluated using Pearson's and Spearman's rho correlation coefficients. Results were expressed as mean ± standard deviation (SD) or median (interquartile range), and P values of <0.05 were considered as statistically significant.

# RESULTS

There were no demographic differences between the patients and control subjects, as shown in Table 1.

**Table 3** Acording to 25(OH) vitamin D levels; laboratory findings and endothelial function of subjects

| | 25(OH) vitamin D deficiency (<30 nmol/L) | | | 25(OH) vitamin D deficiency without (>30 nmol/L) | | |
|---|---|---|---|---|---|---|
| | Patients (n = 17) | Controls (n = 8) | P | Patients (n = 12) | Controls (n = 12) | P |
| 25(OH) vit D (nmol/L) | 20.3 ± 4.9[a] | 2.,5 ± 3.1 | <0.05 | 43.7 ± 8.26 | 39.4 ± 7.2 | NS |
| Hb (g/dL) | 10.2 ± 1.9 | 14.3 ± 1.6 | <0.01 | 10.7 ± 1.8 | 14.64 ± 1.5 | <0.05 |
| Triglycerides (mg/dL) | 159.9 ± 94.0 | 118.2 ± 35.7 | NS | 156.1 ± 64.8 | 108.6 ± 49.4 | NS |
| Total Cholesterol (mg/dL) | 193.7 ± 59.1 | 199.6 ± 33.9 | NS | 187.6 ± 71.6 | 169.2 ± 31.2 | NS |
| HDL (mg/dL) | 44.5 ± 12.3 | 50.37 ± 6.2 | NS | 47.2 ± 13.3 | 50.4 ± 11.6 | NS |
| LDL (mg/dL) | 136.9 ± 44.5 | 134.9 ± 32.5 | NS | 128.8 ± 52.2 | 105.1 ± 32.4 | NS |
| CRP (mg/dL) | 1.1 (0.47–1.96) | 0.3 (0.3–0.35) | <0.05 | 0.37 (0.3–0.55) | 0.31 (0.3–0.33) | NS |
| Sedimentation (mm/h) | 54 ± 28 | 8 ± 6 | <0.001 | 36.7 ± 24.7 | 4.17 ± 4.17 | <0.05 |
| Serum phosphorus (mg/dL) | 5.3 ± 1.2 | 3.4 ± 0.2 | <0.05 | 5.01 ± 1.18 | 3.48 ± 0.42 | <0.05 |
| Serum calcium (mg/dL) | 9.0 ± 0.8 | 9.3 ± 0.4 | NS | 8.53 ± 0.85 | 9.26 ± 0.17 | <0.05 |
| PTH (pg/mL) | 191.7 (103.15–595.79)[b] | 66.15 (42.6–85.4) | <0.05 | 148 (96–390) | 63.6 (43.47–81.7) | <0.05 |
| CIMT left (mm) | 0.78 ± 0.15 | 0.62 ± 0.07 | <0.05 | 0.79 ± 0.16 | 0.58 ± 0.10 | <0.05 |
| CIMT right (mm) | 0.79 ± 0.15 | 0.61 ± 0.08 | <0.01 | 0.77 ± 0.14 | 0.58 ± 0.10 | <0.05 |
| ΔFMD% | 5.4 (2.77–6.15) | 15.34 (11.27–19.8) | <0.001 | 6.25 (2.17–13.83) | 16.82 (7.25–23.18) | <0.05 |
| ΔNID% | 11.9 (6.96–13.1) | 21.82 (16.58–29.3) | <0.05 | 10 (4.9–17.94) | 23.25 (10.72–37.17) | <0.05 |

CIMT = carotid intimae media thickness; CRP = C-reactive protein; FMD = flow mediated dilatation; HDL = high density lipoprotein; LDL = low density lipoprotein; NID = nitroglycerin-induced vasodilatation; NS = nonsignificant; PTH = parathormone.
[a]Mean ± SD.
[b]Median (Q1,Q3).

Compared to the control group, the patients exhibited statistically significant differences in hemoglobin, albumin, total protein, BUN, creatinine, triglyceride, PTH, CRP, sedimentation rate, %FMD, %NID, and bilateral CIMT values ($P < 0.05$).The levels of 25-OH-D3were nonsignificantly lower in patients than in controls (Table 2).

When subgroup analysis according to vitamin D levels; patients and controls were divided in to two groups who has more or less than 30 nmol/L. Seventeen patients and 8 controls with low vitamin D levels were observed while 12 patients and 12 controls with normal vitamin D levels were seen (Table 3).

The deficiency in 25-OH-D3 was more profound in patients than in controls. When both groups were stratified according to %FMD, neither group exhibited adequate dilatation; however, control subjects had a significantly higher %FMD than the patients ($P < 0.001$). Similarly, stratification of low vitamin D patients and subjects according to %NID following nitroglycerin challenge showed that while both groups achieved dilatation, it was significantly higher in controls in patients ($P < 0.05$). Furthermore, as seen in Table 3, patients with low 25-OH-D3 levels had significantly higher bilateral CIMT, PTH, P, sedimentation rate, and CRP values than controls ($P < 0.05$). The patients with normal vitamin D level had significantly higher bilateral CIMT, PTH, phosphorus, sedimentation, and CRP values than controls ($P < 0.05$) (Table 3).

When the patient with low vitamin D level compared to normal, percentages of FMD were lower in the patients. This suggests that, endotel dysfunction is not only dependent to chronic renal failure. The lack of vitamin D may have an effect on ED.

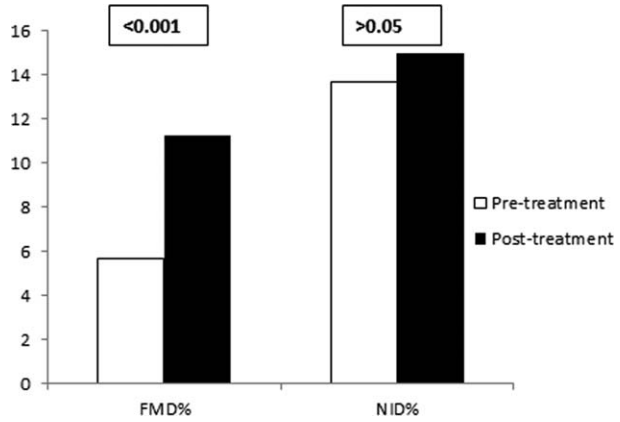


**Figure 1** FMD% and NID% change before and after Vitamin D in dialysis patients. (Maximal change in brachial artery (BA) diameter).

Following vitamin D supplementation in patients and control subjects with low 25-OH-D3 levels, P, PTH, CRP, sedimentation, and total cholesterol values were significantly decreased ($P < 0.05$). Although vitamin D supplementation did not lead to a change in CIMT values in these subjects, there was a significant increase in %FMD after treatment ($P < 0.001$). Finally, vitamin D treatment did not lead to a significant change in % NID in neither patients nor controls ($P > 0.05$) (Table 4; Figures 1 and 2).

## DISCUSSION

We herein showed that impaired endothelial function could be corrected by vitamin D supplementation in vitamin D-deficient CKD patients. This effect may occur associated with preventing atherosclerosis and inflammation by vitamin D replacement.

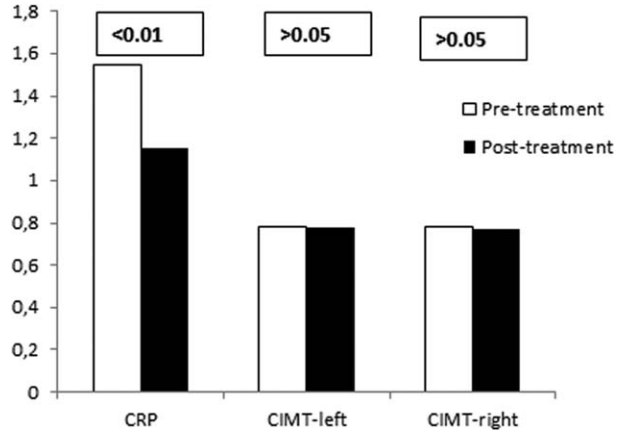


**Figure 2** C-reactive protein, carotid intima-media thickness (CIMT)-left and CIMT right measures before and after Vitamin D in dialysis patients.

The most frequent cause of death in patients receiving dialysis is CVD.[6,20,21] Studies predict that patients older than 45 years receiving dialysis have a 100-fold increase in CVD risk compared with the general population.[22–25]

Serum vitamin D levels, assessed by serum 25-OH-D3, are usually lower in patients with CKD, as recently demonstrated in studies including predialysis,[26] HD,[27] and PD patients.[28–30] Patients with severe proteinuria and those receiving PD particularly have very low levels of vitamin D.[30] However, the most common causes of vitamin D deficiency are insufficient sunlight and malnutrition. In an observational study, vitamin D deficiency approached 50% of the normal population.[29]

The studies in recent years showed the relationship between vitamin D deficiency and CVD risk. Overall, these studies suggest vitamin D replacement might reduce CVD and cardiovascular risk.[31–35]

Unfortunately, these studies that showing effects of vitamin D supplementation in CKD patients are very rare. High proinflammatory cytokine levels, known to occur in ESRD patients[25] and hypertriglycemia, are commonly seen in patients with chronic renal failure and are associated with atherosclerosis.[36] In our study, the levels of triglycerides, LDL, and total cholesterol were decreased after vitamin D supplementation in those with low 23-OH-D3, suggesting that vitamin D replacement might contribute to prevention of atherosclerosis.

The patients and controls are evaluated according to vitamin D, both of group have low vitamin D but difference not significant.[26–30,37]

In a meta-analysis by Kandula et al., PTH levels decreased after vitamin D replacement; however, the highest decrease was observed in ESRD patients. In addition, hypercalcemia and hyperphosphatemia occurred less frequently than predicted following vitamin D replacement.[38] Our findings were in agreement with these results.

Atherosclerosis was demonstrated to be more common in HD patients compared not only to healthy individuals but also to patients with classical CVD risk factors in the absence of renal failure.[39] Increased CIMT is a marker of atherosclerosis and an early precursor of coronary artery disease.[40] As such, CIMT measurement has become a widely used test and is suggested for use as a screening test in select patients.[41] In our study, CIMT values in patients with renal failure were higher and did not change with vitamin D supplementation. As any change in CIMT is likely a slow process, the evaluation of CIMT following a 2-month vitamin D supplementation, which was done

**Table 4** Pretreatment and post-treatment laboratory values and endothelial function characteristics of subjects with 25-OH-D3 deficiency

| | Patients | Controls |
|---|---|---|
| Hb (g/dL) | | |
| Pretreatment | 10.2 ± 1.9[a] | 14.3 ± 1.6 |
| Post-treatment | 10.2 ± 1.5 | 14.46 ± 1.3 |
| Fosfor (mg/dL) | | |
| Pretreatment | 5.28 ± 1.24* | 3.42 ± 0.17 |
| Post-treatment | 5.04 ± 1.07 | 3.4 ± 0.01 |
| Kalsiyum (mg/dL) | | |
| Pretreatment | 8.96 ± 0.82 | 9.3 ± 0.4 |
| Post-treatment | 8.90 ± 0.66 | 9.2 ± 0.2 |
| PTH (pg/mL) | | |
| Pretreatment | 191.7 (103.15–595.79)[b,*] | 66.15 (42.6–85.4)** |
| Post-treatment | 166 (91–488) | 46 (25.95–62.85) |
| Total kolesterol (mg/dL) | | |
| Pretreatment | 193.7 ± 14.35* | 199.62 ± 33.86 |
| Post-treatment | 178.05 ± 42.69 | 213.6 ± 35.97 |
| LDL (mg/dL) | | |
| Pretreatment | 136.9 ± 44.46 | 134,87 ± 32,51 |
| Post-treatment | 133.4 ± 32.9 | 142,1 ± 36,6 |
| HDL (mg/dL) | | |
| Pretreatment | 44.5 ± 12.33 | 50,37 ± 6,16 |
| Post-treatment | 44.59 ± 11.5 | 50,0 ± 9,18 |
| Trigliserid (mg/dL) | | |
| Pretreatment | 159.9 ± 94 | 118.25 ± 35.74 |
| Post-treatment | 150.3 ± 59.1 | 120.4 ± 57.17 |
| CRP (mg/dL) | | |
| Pretreatment | 1.1 (0.47–1.96)** | 0.3 (0.3–0.35) |
| Post-treatment | 0.80 (0.32–1.55) | 0.34 (0.31–0.49) |
| Sedimantasyon (mm/h) | | |
| Pretreatment | 54 ± 28** | 82 ± 6 |
| Post-treatment | 45 ± 23 | 81 ± 4 |
| CIMT left (mm) | | |
| Pre-treatment | 0.78 ± 0.15 | 0.62 ± 0.07 |
| Post-treatment | 0.79 ± 0.16 | 0.61 ± 0.06 |
| CIMT right (mm) | | |
| Pre-treatment | 0.78 ± 0.14 | 0.61 ± 0.08 |
| Post-treatment | 0.77 ± 0.14 | 0.61 ± 0.06 |
| ΔFMD% | | |
| Pretreatment | 5.4 (2.77–6.15)*** | 15.34 (11.27–19.8) |
| Post-treatment | 10.25 (7.8–12.8) | 17.80 (15.05–19.40) |
| ΔNID% | | |
| Pretreatment | 11.9 (6.96–13.1) | 21.82 (16.58–29.3) |
| Post-treatment | 14.5 (13.8–21.01) | 22.04 (20.03–26.81) |

CIMT = carotid intimae media thickness; CRP = C-reactive protein; FMD = flow mediated dilatation; HDL = high density lipoprotein; LDL = low density lipoprotein; NID = nitroglycerin-induced vasodilatation; PTH = parathormone.

*$P < 0.05$ Pretreatment compared vs. Post-treatment.

**$P < 0.01$ Pretreatment compared vs. Post-treatment.

***$P < 0.001$ Pretreatment compared vs. Post-treatment.

[a]Mean ± SD.

[b]Median (Q1,Q3).

in this study, may not have been ideal to conclusively evaluate the effect of vitamin D on CIMT. Thus, patient evaluation after a longer treatment regimen at a maintenance dose will more clearly assess this outcome. However, decreases in several markers of inflammation that were observed in our study suggested that vitamin D might indeed change CIMT by controlling inflammation.

ED, defined by the impairment of regulatory functions of endothelium (including vasodilatation, smooth muscle cell proliferation, and fibrinolysis), plays a pivotal role in the pathogenesis of CVD.[42] FMD of brachial arteries is a validated and useful marker to confirm the presence and extent of ED.[19,43] Numerous studies previously demonstrated ED in CKD patients using FMD.[44] In addition, increased asymmetric dimethylarginine, a NO synthetase inhibitor, provides additional evidence for ED in CKD patients.[45,46] The most important outcome of our study was low %FMD in end-stage CKD patients, which was also reported by Yilmaz et al.[45] Additional, we were able to show significant increases in %FMD after vitamin D supplementation in CKD patients. In contrast, our results indicated that there was no significant change in %NID despite supplementation.

The short vitamin D supplementation of 2 months and a relatively small cohort are two major limitations of our study. Future long-term studies involving larger patient populations are necessary to evaluate the effect of vitamin D on endothelial function.

In summary, vitamin D might exhibit beneficial effects on endothelial function and might reduce the risk of CVD, the most important cause of morbidity and mortality in CKD patients.

## CONCLUSION

We herein showed that impaired endothelial function could be corrected by vitamin D supplementation in vitamin D-deficient CKD patients. Our results further suggested that vitamin D supplementation might provide protection against atherosclerosis through reductions in inflammatory parameters, including CRP and sedimentation rate. Thus, we suggest that vitamin D therapy can reduce the incidence of CVDs in patients with renal failure and that determination of 25-OH-D3 levels with vitamin D supplementation may be appropriate in this patient population.

## ACKNOWLEDGMENTS

No external funding was obtained for this study.The authors declare no industrial links.

## AUTHOR CONTRIBUTIONS

YK and GS selected patients and designed and wrote the manuscript; YK, GS, EFU and NAD performed the FMD and CIMT measurements; YK, GS and BS performed and evaluated laboratory tests; CB, YK, and GS performed the statistical tests. All authors interpreted the results and approved the final version of the manuscript.

Manuscript received February 2016; revised April 2016.

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