# One-year cholecalciferol supplementation fails to improve bone mineral density or inflammation in patients with vitamin D deficiency undergoing hemodialysis

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**Abstract.** Deficiency in 25-hydroxyvitamin D [25(OH)D] is prevalent among patients undergoing hemodialysis (HD) and is associated with poorer clinical outcomes. The KDIGO guidelines advocate for 25(OH)D supplementation in these patients. In the present study, cholecalciferol was administered to patients undergoing HD exhibiting 25(OH)D deficiency over a period of 1 year. After excluding non-compliant participants, 20 patients completed the study. Bone mineral density (BMD), as well as the T-score and Z-score of the lumbar spine and total hip were assessed pre- and post-supplementation using dual-energy X-ray absorptiometry. Additionally, the serum levels of 25(OH)D, intact parathyroid hormone (iPTH), calcium, phosphorus, C-reactive protein (CRP) and the neutrophil-to-lymphocyte ratio were evaluated. Cholecalciferol supplementation increased the 25(OH)D levels; however, it did not result in significant changes in lumbar spine T-score, Z-score or BMD. However, a decrease in the total hip T-score and BMD was observed by the end of the study period. The serum levels of iPTH, calcium and phosphorus remained stable. Similarly, the levels of inflammation markers, including CRP and the neutrophil-to-lymphocyte ratio, did not exhibit any significant changes. Thus, the present study demonstrates that 1-year cholecalciferol supplementation increases the 25(OH)D levels, but does not improve BMD or inflammation among patients with vitamin D deficiency undergoing HD. Further investigations with larger patient cohorts are required to confirm these findings and to potentially reconsider the relevant KDIGO guidelines.

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## Introduction

Vitamin D is synthesized in the skin or acquired through dietary intake. It is subsequently converted in the liver to 25-hydroxyvitamin D [25(OH)D]. The conversion of 25(OH)D to its active form, 1,25-dihydroxyvitamin D, occurs in the kidneys via the enzyme, 1α-hydroxylase. The active form of vitamin D enhances calcium and phosphorus absorption in the gastrointestinal tract, promotes calcium reabsorption in the distal tubules and stimulates osteoclastic activity (1,2).

In patients undergoing hemodialysis (HD), the impaired ability of the kidneys to activate vitamin D leads to significantly reduced circulating levels of 1,25-dihydroxyvitamin D, and a deficiency in 25(OH)D is also prevalent (3,4). As a result, in this population, active vitamin D receptor (VDR) agonists are administered, instead of non-active forms of vitamin D, primarily for the management of secondary hyperparathyroidism (5). However, clinical data indicate the beneficial effects of maintaining adequate serum 25(OH)D levels in patients undergoing HD. In these patients, decreased serum levels of 25(OH)D are associated with increased overall mortality rates (6), an association on that has also been observed in the general population (7). Based on such observations, the Kidney Disease: Improving Global Outcomes (KDIGO) clinical practice guidelines for the diagnosis, evaluation, prevention and treatment of Chronic Kidney Disease-Mineral and Bone Disorder (CKD-MBD) suggests that in patients with CKD stages 3-5D, 25(OH)D levels should be measured, with the frequency of repeated testing determined by baseline values and therapeutic interventions. The guidelines also suggest that vitamin D deficiency and insufficiency should be addressed using treatment strategies similar to those recommended for the general population. However, this recommendation is classified as level 2, indicating that different choices may be appropriate for different patients, and is assigned a grade C, signifying that the quality of evidence supporting it is low (8).

The beneficial effects of normal 25(OH)D levels may be attributed to the expression of 1α-hydroxylase outside the kidneys, which facilitates the local activation of 25(OH)D. This local activation enables an autocrine or paracrine action at the activation sites (9,10). In bone tissue, both osteoblasts

and osteoclasts express 1α-hydroxylase, allowing 25(OH)D to potentially influence bone metabolism in an autocrine or paracrine manner, independent of kidney-mediated activation (11,12). Bone metabolism may also be indirectly influenced by 25(OH)D through its effects on the parathyroid glands, which also express 1α-hydroxylase (13). As a result, 25(OH)D may suppress parathyroid hormone (PTH) production via autocrine or paracrine mechanisms, a concept supported by some clinical data (14,15). Additionally, dendritic cells, macrophages, and activated T- and B-cells express both VDR and 1α-hydroxylase (16). During inflammation, pro-inflammatory cytokines can upregulate 1α-hydroxylase in these cells, leading to the elevated local conversion of 25(OH)D to 1,25-dihydroxyvitamin D. The anti-inflammatory properties of vitamin D have been substantiated (16,17), and in patients undergoing HD, an inverse association between serum 25(OH)D levels and markers of inflammation has been observed (18).

The present study evaluated the effects of 1-year cholecalciferol supplementation in a cohort of patients with vitamin D deficiency undergoing HD on bone mineral density (BMD), serum intact PTH (iPTH) levels and markers of inflammation. The KDIGO clinical practice guidelines for the diagnosis, evaluation, prevention and treatment of CKD-MBD recommends that in patients with CKD stages 3-5D who exhibit evidence of CKD-MBD and/or have risk factors for osteoporosis, BMD testing should be considered to assess the risk of fractures if the results are likely to influence treatment decisions, particularly when the findings indicate osteoporosis and the physician plans to initiate treatment with anti-osteoporotic agents. This recommendation is classified as level 2, and is assigned a grade B, signifying that the quality of evidence supporting it is moderate (8). The exact KDIGO nomenclature and description for rating guideline recommendations are provided in the KDIGO guidelines (8). The recommendation for BMD testing is based mainly on four prospective cohort studies of dual-energy X-ray absorptiometry (DXA) measures of BMD and incident fractures in adults with CKD G3a to G5D. One of these studies measured BMD annually in 485 patients undergoing HD and found that a lower baseline BMD at the femoral neck and total hip was associated with an increased risk of fractures (19).

## Patients and methods

*Patients*. Initially, 47 patients undergoing HD, who had vitamin D deficiency, defined as serum 25(OH)D levels <20 ng/ml (20), were identified. Patients were recruited between October and November, 2017, and the study was concluded between October and November, 2018. Following the application of exclusion criteria, which disqualified smokers, patients with active infections, malignancies, autoimmune diseases, a history of parathyroidectomy, or those who had been using corticosteroids, cytotoxic drugs, warfarin, anticonvulsants, antidepressants, hormone replacement therapy, or bisphosphonates within the 6 months preceding the study, 36 patients were ultimately enrolled in the study. Over the course of the 1-year study period, 8 patients were withdrawn from the study: Specifically, 3 patients succumbed, 2 patients received kidney transplants, and 3 patients were transferred to another renal unit. Additionally, 8 patients were excluded due to non-compliance with the study protocol, as indicated by the measurements of serum 25(OH)D levels. Consequently, of the initial 36 patients undergoing HD enrolled in the study who were administered cholecalciferol, only 20 patients completed the study. The analysis was conducted on data from these 20 patients undergoing HD who successfully completed the study. The mean age of these patients was 59.4±16.10 years, with 16 of the participants being male. The etiologies of end-stage renal disease among the patients were as follows: Diabetes mellitus in 7 patients, primary glomerulonephritis in 3 patients, hemolytic-uremic syndrome in 1 patient, interstitial nephritis in 1 patient, hypertension in 1 patient, obstructive nephropathy in 1 patient, autosomal dominant polycystic kidney disease in 3 patients, nephrectomy in a patient with a dysplastic contralateral kidney, and an unknown cause in 2 patients.

The patients underwent regular HD using polysulfone dialyzers and a bicarbonate dialysate with a calcium concentration of either 1.25 or 1.5 mmol/l. The HD sessions had a duration of 4 h, were performed three times per week, and had been ongoing for at least 1 year prior to the study. The urea reduction rate (66.7±7.2%), hemoglobin levels (11.62±0.83 g/dl) and albumin levels (3.85±0.27) remained virtually unaltered at the end of the study period compared to those measured at baseline (66.5±8.1%; 11.3±0.98 g/dl and 3.81±0.27, respectively). The laboratory values of interest before and after cholecalciferol administration are presented in Table I. Nephrologists were allowed to independently decide on the use of the phosphate binder sevelamer hydrochloride, the vitamin D analog paricalcitol and the calcimimetic cinacalcet to meet the KDIGO targets for serum PTH, calcium and phosphorus levels (8).

Patients were enrolled in the study within a 2-week period during the winter and completed the study in the same period of the following winter. Each patient received one tablet of cholecalciferol (1,200 IU; D3fix, Uni-Pharma) daily. Cholecalciferol, also known as vitamin D3, is first converted in the liver to 25(OH)D and then to its active form, 1,25-dihydroxyvitamin D in the kidneys (1,2). Compliance was monitored by comparing the serum 25(OH)D levels at 6 months and at the end of the study period with the baseline levels. In the case that the 25(OH)D levels increased by <50% following the initiation of cholecalciferol, non-compliance was suspected, and the patient was excluded from the study. BMD was assessed at both the beginning and the end of the study.

The evaluation and administration of cholecalciferol were carried out following the KDIGO guidelines, which advises that for patients with CKD stages 3-5D, 25(OH)D levels should be measured, and vitamin D deficiency or insufficiency should be managed using treatment approaches similar to those recommended for the general population (8). Similarly, BMD assessment followed the KDIGO guidelines, which recommend that in patients with CKD stages 3-5D who exhibit evidence of CKD-MBD and/or possess risk factors for osteoporosis, BMD testing should be considered to assess fracture risk if the results are likely to influence treatment decisions (8). Informed consent was obtained from each individual enrolled in the study, and the study protocol

Table I. Laboratory values before and after the 1-year cholecalciferol administration.

| Parameter | Before cholecalciferol administration (n=20) | After cholecalciferol administration (n=20) | P-value |
|---|---|---|---|
| URR (%) | 66.5±8.1 | 66.7±7.2 | 0.928 |
| Hemoglobin (g/dl) | 11.3±0.98 | 11.62±0.83 | 0.273 |
| WBC (/μl) | 7,470±2,562 | 8,370±3,008 | 0.119 |
| Neutrophils (/μl) | 5,118±1,969 | 5,835±2,218 | 0.159 |
| Lymphocytes (/μl) | 1,393±640 | 1,550±645 | 0.272 |
| Albumin (g/dl) | 3.81±0.27 | 3.85±0.27 | 0.370 |
| Ca (mg/dl) | 8.99±0.10 | 8.99±0.11 | 1.000 |
| P (mg/dl) | 5.23±0.34 | 5.81±0.24 | 0.059 |
| iPTH (pg/ml) | 300.7±31.4 | 295.3±22.2 | 0.847 |
| 25(OH)D (ng/ml) | 10.1±1.0 | 28.0±1.3 | <0.001 |
| CRP (mg/dl) | 0.69±0.77 | 0.48±0.39 | 0.418 |

UPR, urea reduction rate; WBC, which blood cell; Ca, calcium; P, phosphorus; iPTH, intact parathyroid hormone; 25(OH)D, 25-hydroxyvitamin D.

received approval from the Ethics Committee of the Faculty of Medicine at the University of Thessaly, Larissa, Greece (no. of approval 558/10-2-2017).

*Measurement of serum markers and assessment of BMD and inflammation*. Blood samples were drawn prior to the onset of the second dialysis session of the week. Immunoassays for measuring 25(OH)D and intact PTH (iPTH) were performed in an ELECSYS 2010 automatic analyzer (Roche Diagnostics GmbH). C-reactive protein (CRP) levels were measured using the Cobas Integra 400 automatic analyzer (Roche Diagnostics GmbH). Serum phosphorus, calcium and albumin levels, and complete blood count were determined and documented as part of the routine laboratory assessments.

DXA BMD measurements were conducted using the Hologic Discovery W Bone Densitometer (Hologic Inc.). The BMD, T-score and Z-score of the lumbar spine and the right total hip were documented. After obtaining the relevant consent for publication, a representative measurement from a patient in the study cohort is presented in Fig. 1.

*Statistical analysis*. To assess the normality of the variables, a one-sample Kolmogorov-Smirnov test was employed. All variables, apart from CRP, conformed to a normal distribution, and thus, paired t-tests were utilized for mean comparisons. The results for normally distributed variables are expressed as the mean ± standard error of the mean. For CRP, which did not follow a normal distribution, the Wilcoxon signed-rank test was applied, and the results are presented as the median with interquartile range and total range. A P-value <0.05 was considered to indicate a statistically significant difference. Statistical analyses and graphical representation were performed using IBM SPSS Statistical software (version 29, IBM Corp.), while graphical representation for one figure was generated using GraphPad Prism (Version 10.2.2, Dotmatics).

## Results

*Effect of cholecalciferol on 25(OH)D levels and BMD*. In all patients who completed the study, cholecalciferol supplementation led to a significant increase in serum 25(OH)D levels (Fig. 2A). The serum 25(OH)D concentration increased from 10.1±1.0 ng/ml before cholecalciferol administration to 28.0±1.3 ng/ml at the end of the study period (P<0.001; Fig. 2B).

Cholecalciferol supplementation did not significantly affect the lumbar spine T-score, Z-score, or BMD. The T-score was -1.48±0.36 before treatment and -1.41±0.33 after treatment (P=0.471; Fig. 2C). The Z-score also did not exhibit any significant change, from -0.72±0.33 before treatment to -0.55±0.35 after treatment (P=0.174) (Fig. 2C). The lumbar spine BMD also remained stable, with measurements of 0.92±0.04 g/cm$^2$ before treatment and 0.93±0.04 g/cm$^2$ after treatment (P=0.416; Fig. 2D).

In total hip measurements, both the T-score and BMD exhibited a statistically significant decrease by the end of the study period. The T-score decreased from -1.22±0.30 to -1.48±0.32 (P=0.007; Fig. 2D), and BMD decreased from 0.84±0.05 g/cm$^2$ to 0.80±0.05 g/cm$^2$ (P<0.001; Fig. 2E). The Z-score did not exhibit a statistically significant decrease, changing from-0.61±0.28 before treatment to -0.82±0.32 after treatment (P=0.111; Fig. 2D).

*Effects of cholecalciferol on serum iPTH, calcium and phosphorus levels*. Cholecalciferol supplementation did not significantly affect the serum iPTH, calcium, or phosphorus levels. The serum iPTH levels were 300.7±31.4 pg/ml at the beginning of the study and 295.3±22.2 pg/ml at the end of the study (P=0.847; Fig. 3A). The serum calcium levels remained unaltered, with values of 8.99±0.10 mg/dl at the start and 8.99±0.11 mg/dl at the end of the study (P=1.0; Fig. 3B). The serum phosphorus levels exhibited a non-significant increase from 5.23±0.34 mg/dl before treatment to 5.81±0.24 mg/dl after treatment (P=0.06; Fig. 3C).

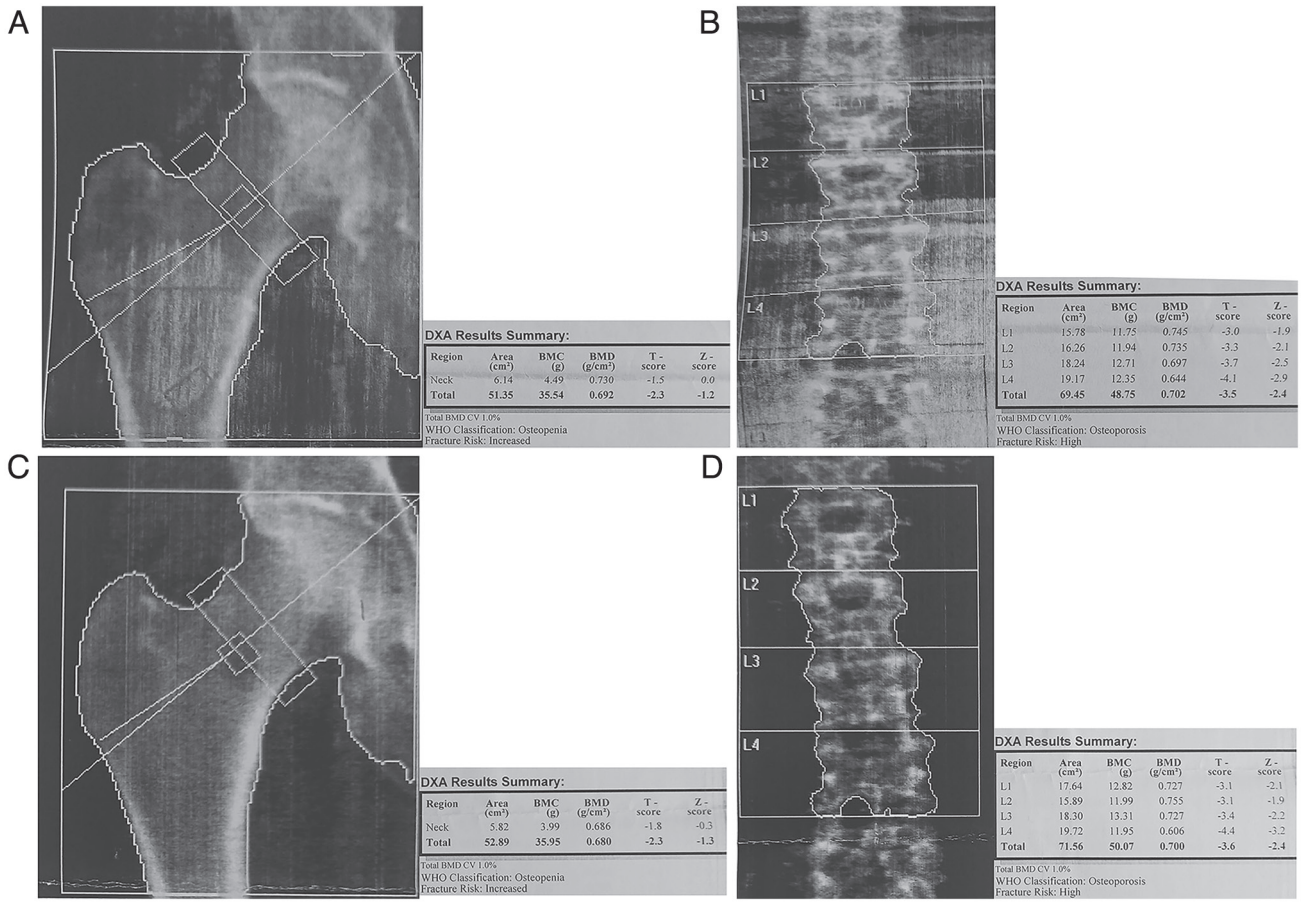


Figure 1. A representative DXA BMD measurement from a patient of the present study. DXA BMD measurements from a 79-year-old male patient: (A) Initial total hip and (B) lumbar spine measurements taken at the start of the study. Follow-up (C) total hip and (D) lumbar spine measurements obtained after 1 year of cholecalciferol administration. DXA, dual-energy X-ray absorptiometry; BMD, bone mineral density.

*Effects of cholecalciferol on CRP levels and the neutrophil-to-lymphocyte ratio.* The administration of cholecalciferol did not significantly affect the inflammatory markers, CRP and the neutrophil-to-lymphocyte ratio. At the initiation of the study, the median CRP value was 0.4 mg/dl (interquartile range, 0.30-0.78 mg/dl, with a minimum of 0.1 mg/dl and a maximum of 2.8 mg/dl), and 0.35 mg/dl (interquartile range, 0.20-0.75 mg/dl, with a minimum of 0.1 mg/dl and a maximum of 1.4 mg/dl) by the end of the study (P=0.418; Fig. 4A). The neutrophil-to-lymphocyte ratio was initially 4.12±1.92 and was slightly altered to 4.05±1.45 by the end of the study (P=0.859; Fig. 4B).

## Discussion

Vitamin D deficiency, characterized by serum 25(OH)D levels <20 ng/ml (20), is prevalent among patients undergoing HD (3,4). While the traditional model of vitamin D metabolism posits that 25(OH)D should be converted to its active form, 1,25-dihydroxyvitamin D, in the kidneys via the enzyme, 1α-hydroxylase (1,2), the KDIGO clinical practice guidelines advise measuring serum 25(OH)D levels and administering 25(OH)D in cases of vitamin D deficiency or insufficiency in patients with CKD at stages 3-5D (8). This recommendation is informed by observational studies, one of which found that in patients with CKD, low serum 25(OH)D levels, but not low 1,25-dihydroxyvitamin D levels, were associated with a higher risk of developing progression to end-stage renal disease and increased mortality (21). In patients undergoing HD, low serum 25(OH)D levels have been linked to an elevated mortality rate (6). The beneficial effects of maintaining normal 25(OH)D levels may be attributed to the expression of 1α-hydroxylase in extrarenal tissues, which facilitates the localized activation of 25(OH)D. This localized activation allows for autocrine or paracrine actions at the sites where 25(OH)D is converted to its active form (9,10).

At the bone level, the active form of vitamin D, 1,25-dihydroxyvitamin D, upregulates the expression of receptor activator of nuclear factor-κB ligand (RANKL) in osteoblasts. RANKL binds to its receptor RANK on preosteoclasts, promoting their maturation into osteoclasts and subsequent bone resorption. However, 1,25-dihydroxyvitamin D also stimulates osteoblasts to produce osteoprotegerin, a decoy receptor that binds to and neutralizes RANKL, thereby inhibiting osteoclastogenesis and inducing osteoclast apoptosis. Both osteoblasts and osteoclasts express 1α-hydroxylase, which allows for the local conversion of 25(OH)D to 1,25-dihydroxyvitamin D, independent of renal activation (11,12). Additionally, PTH upregulates RANKL and promotes osteoclastogenesis, and the role of 1,25-dihydroxyvitamin D in suppressing PTH has been well-established (5). Notably, the parathyroid glands express 1α-hydroxylase, enabling the local conversion of 25(OH)D

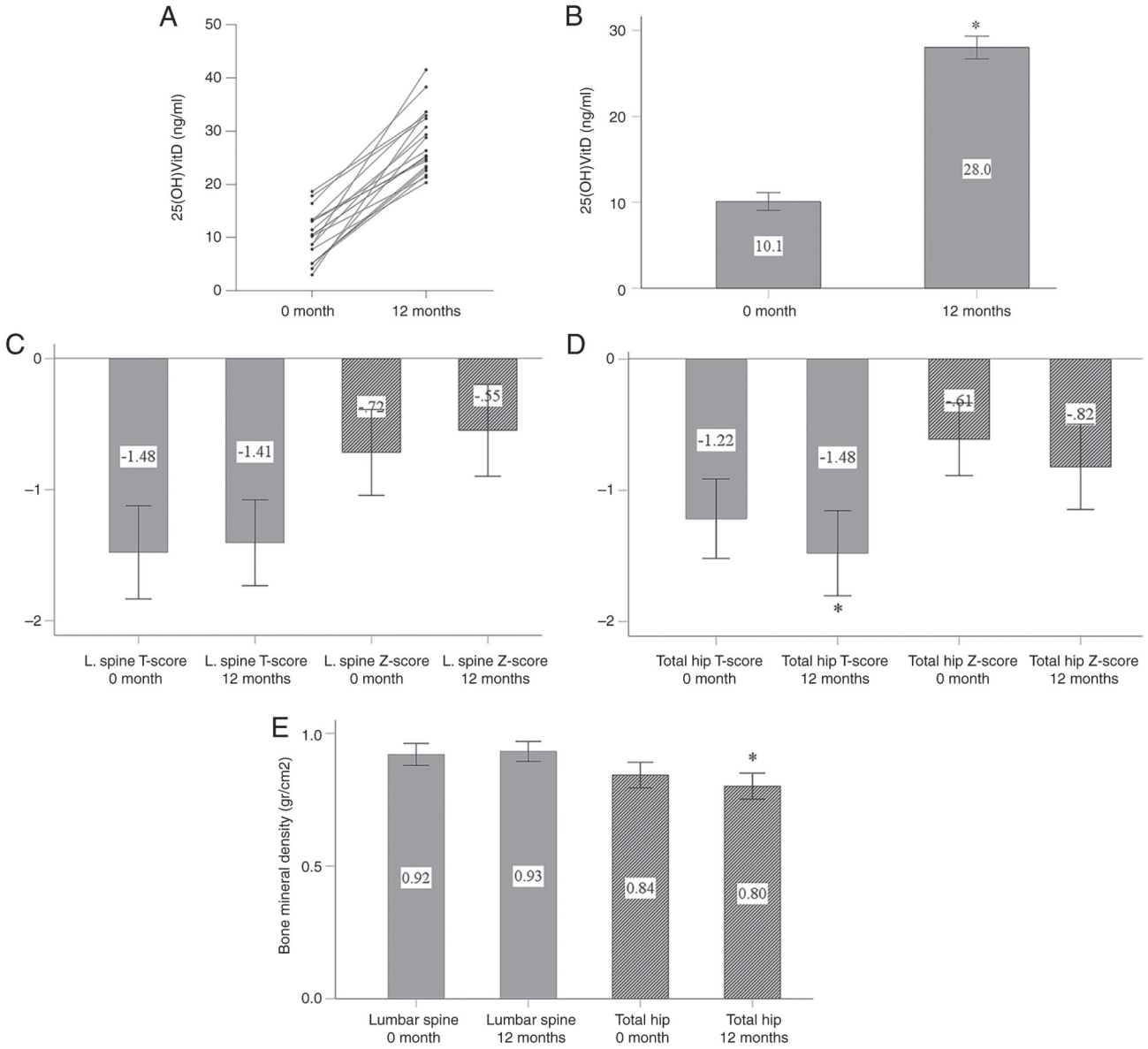


Figure 2. Effects of cholecalciferol on 25(OH)D levels and BMD. The administration of cholecalciferol resulted in an elevation in (A) serum 25(OH)D levels in all subjects, (B) as well as an increase in the mean value. Cholecalciferol had no significant effect on the lumbar spine (C) T-score, (C) Z-score, or (E) BMD. While cholecalciferol did not alter the (D) total hip Z-score, it led to a reduction in both the (D) T-score and (E) BMD. The numbers within the bars indicate the mean values. Error bars represent the standard error of the mean; *P<0.05, vs. 0 months. BMD, bone mineral density; 25(OH)D, 25-hydroxy vitamin D.

to 1,25-dihydroxyvitamin D, which can then function in an autocrine or paracrine manner to suppress PTH production independently of renal activation (13). Some clinical data support this concept (14,15).

However, the data from the present study indicated that although 1 year of cholecalciferol supplementation significantly increased the serum 25(OH)D levels, it did not lead to improvements in BMD in patients undergoing HD. In the general population, the T-score measures the standard deviation of the bone density of a patient relative to the mean of a young, healthy reference population. A T-score <-2.5 indicates the highest risk of fracture, is diagnostic for osteoporosis, and suggests the need for pharmaceutical treatment. Scores between -2.5 and -1.0 indicate an intermediate fracture risk, are diagnostic of osteopenia, and may leave the best therapeutic approach uncertain. A T-score >-1.0 is considered normal. T-scores are reliable for assessing the risk of fractures in untreated post-menopausal females and older males (22). The Z-score, calculated as standard deviations from the mean of a reference group matched by age, ethnicity and sex, should be used to assess fracture risk in children, premenopausal females and in males <50 of age. A Z-score <-2.0 indicates a lower-than-expected bone mass, warranting further investigation when supported by the clinical history of the patient (22). In the present study, DXA revealed no significant changes in the lumbar spine T-score, Z-score, or BMD before and after cholecalciferol administration. Notably, by the end of the study period, there was a trend towards a decline in the total hip Z-score that did not reach statistical significance, while the decline in the T-score was statistically significant. Additionally, total hip BMD exhibited a slight, yet statistically significant decrease.

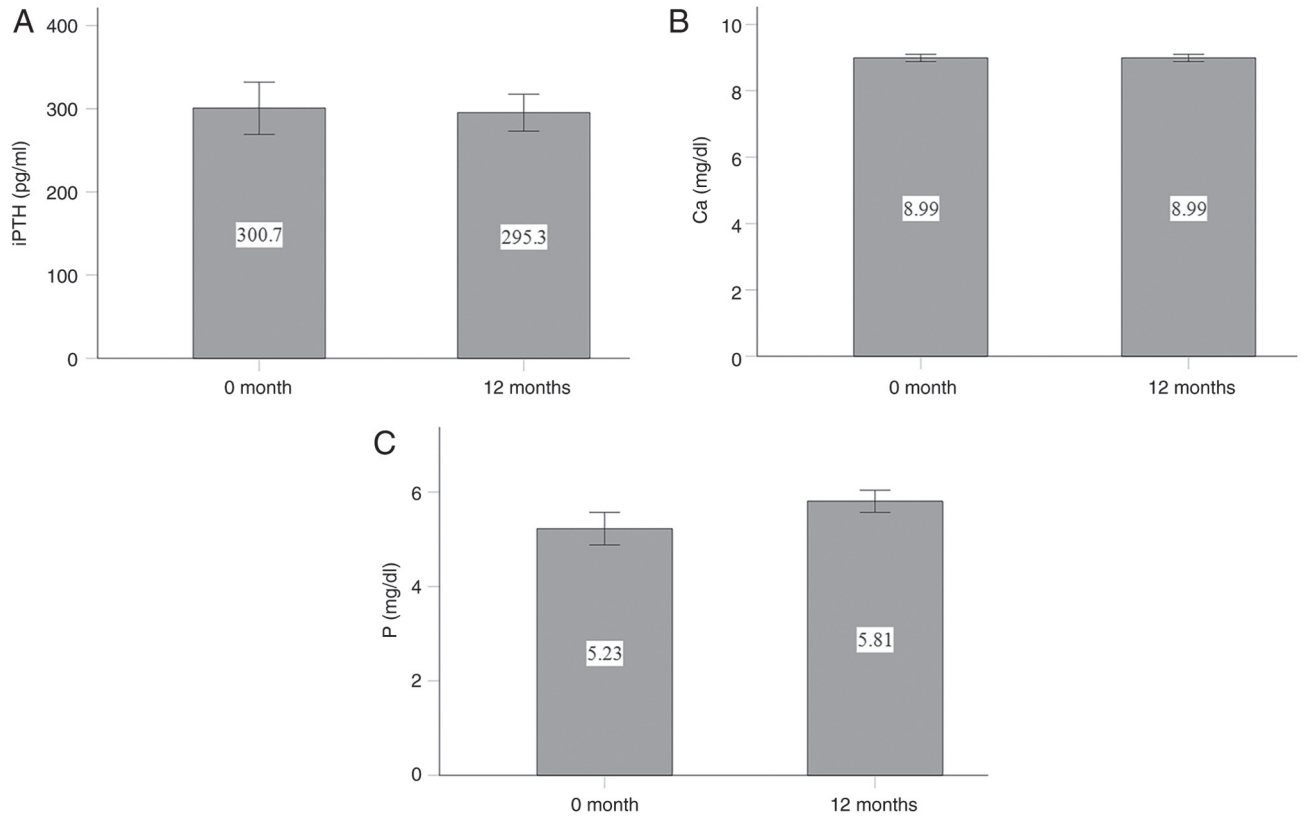


Figure 3. Effects of cholecalciferol on serum iPTH, Ca and P levels. The administration of cholecalciferol did not result in significant changes in serum (A) iPTH, (B) Ca, or (C) P levels. The numbers within the bars indicate the mean values. Error bars represent the standard error of the mean. Ca, calcium; iPTH, intact parathyroid hormone; P, phosphorous.

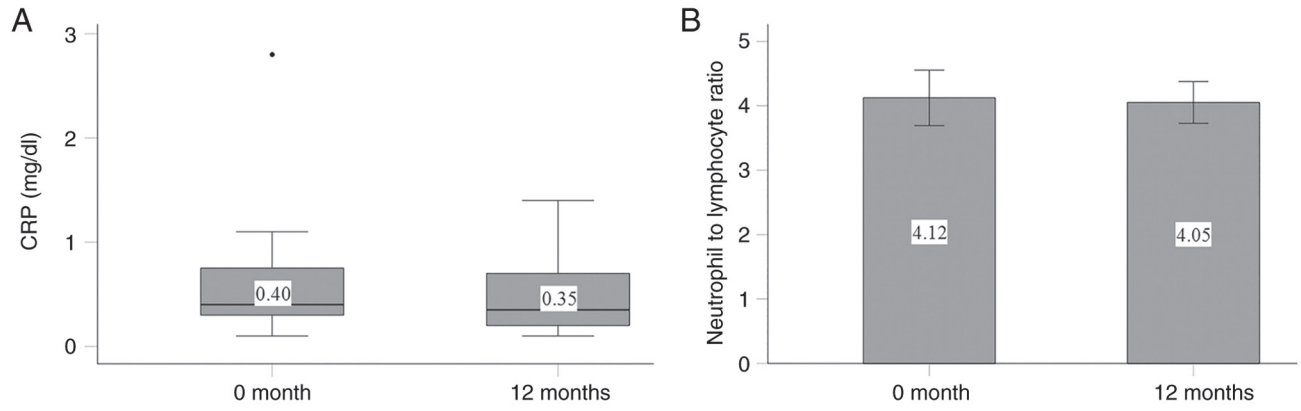


Figure 4. Effects of cholecalciferol on CRP and the neutrophil-to-lymphocyte ratio. (A) The administration of cholecalciferol did not significantly affect the serum CRP levels. The numbers within the bars indicate the median values, with error bars representing the range and box plots showing the interquartile range. (B) Similarly, cholecalciferol did not significantly alter the neutrophil-to-lymphocyte ratio. In this case, the numbers within the bars represent the mean values, and error bars indicate the standard error of the mean. CRP: C-reactive protein.

Of note, a previous study found that in patients undergoing HD and peritoneal dialysis, BMD is not associated with serum 25(OH)D levels (23). A post hoc analysis of the IMPROVE-CKD study, which included patients with stage 3b/4 CKD, also found no association between serum 25(OH)D levels and lumbar spine BMD, as assessed by CT-derived Hounsfield unit values (24). However, the aforementioned findings are not universal. For instance, another study detected a positive association between 25(OH)D levels and BMD Z-scores in patients undergoing HD or peritoneal dialysis (25). In addition to the uncertainty regarding the association between serum 25(OH)D levels and DXA-BMD, there are few studies that have evaluated the effects of cholecalciferol administration, and their results are controversial. In a post hoc analysis of the Vitamin D, Calcium, Lyon Study II (DECALYOS II), which randomized 610 elderly females to receive either cholecalciferol and calcium supplementation or a placebo for 2 years, the participants overall exhibited a decrease in BMD over time with active treatment. However, in a

subgroup of 100 females on cholecalciferol and calcium with an estimated glomerular filtration rate <45 ml/min, the rate of BMD loss appeared slower compared to those on the placebo. It is important to note, however, that women with a serum creatinine level >150 $\mu$mol/l were excluded from the trial (26). As regards patients undergoing HD, a randomized trial involving 19 patients undergoing HD with serum 25(OH)D levels <20 ng/ml found no difference in BMD after 1 year between those who received 2,000 IU of cholecalciferol three times per week and those who received a placebo (27). Similar results were observed in another small trial that included 12 patients undergoing HD (28).

Likewise, in the present study, cholecalciferol supplementation did not alter the serum iPTH, calcium or phosphorus levels. A previous randomized trial reported comparable results, demonstrating that oral ergocalciferol can elevate 25(OH)D levels in patients undergoing HD without causing significant changes in serum calcium, phosphorus, or iPTH levels over a 12-week period (29). Collectively, the findings of the present study indicate that cholecalciferol administration may not significantly enhance bone health in patients undergoing HD.

In addition to CKD-MBD, which is a known contributor to increased mortality rates in patients undergoing HD (30), chronic inflammation is also prevalent in this population and further exacerbates the risk of mortality (31,32). Notably, dendritic cells, macrophages, B-cells and T-cells express both the VDR and 1α-hydroxylase. This enables the conversion 25(OH)D to its active form within these cells, allowing direct effects through the VDR independent of renal activation (16). During inflammatory processes, 1α-hydroxylase expression is upregulated, and in extreme cases of macrophage activation, such as in sarcoidosis, the produced 1,25-dihydroxyvitamin D can have systemic effects, including hypercalcemia (33). VDR activators have demonstrated anti-inflammatory properties (16), and in patients undergoing HD, serum 25(OH)D levels have been inversely associated with inflammation markers (18). However, the data of the present study indicated that 1 year of cholecalciferol supplementation did not significantly affect the serum CRP levels. Similarly, the neutrophil-to-lymphocyte ratio, another sensitive marker of inflammation tested in patients undergoing HD (18,34), was not affected by cholecalciferol. These findings suggest that cholecalciferol supplementation may not effectively mitigate the chronic inflammation characteristic of patients undergoing HD. The data of the present study align with the findings of a large randomized trial involving 746 patients undergoing HD with elevated depressive scores, who were administered cholecalciferol at 50,000 IU/week vs. a placebo. The trial reported no difference in CRP levels over the 12-month duration (35). A previous meta-analysis of 18 trials with 1834 patients also demonstrated that vitamin D supplementation did not exert anti-inflammatory effects in patients with CKD (36).

The present study critically evaluated the KDIGO guidelines concerning the measurement and administration of 25(OH)D in patients undergoing HD. It is important to highlight that this recommendation is classified as level 2 and grade C. Notably, a recent meta-analysis of 128 randomized trials, including 11,270 participants with stage 3-5 CKD or undergoing HD, found that vitamin D supplements and activated vitamin D analogues had no significant effect on the primary outcomes of all-cause mortality, cardiovascular-related mortality, or fractures. The lack of measurable clinical benefit was consistent across subgroup analyses that distinguished between vitamin D supplementation and activated vitamin D analogues (24). Generally, there is a notable discrepancy between studies assessing the impact of serum 25(OH)D levels on parameters, such as inflammation, mortality, or fracture rates in patients undergoing HD and those evaluating the effects of 25(OH)D supplementation. In studies focusing on serum 25(OH)D levels, which often report beneficial outcomes and suggest causality, numerous confounding factors likely influence the results. For example, low serum 25(OH)D levels are associated with increased inflammatory markers and mortality (6,18). However, this association does not necessarily imply that low 25(OH)D levels directly cause inflammation. Inflammation in patients undergoing HD can arise from multiple factors, which in turn may lead to protein-energy wasting (PEW) syndrome and increased mortality rates (37,38). PEW is often characterized by a reduced food intake, resulting in lower vitamin D consumption and subsequently, in reduced serum 25(OH)D levels. In this context, inflammation would be the cause of low serum 25(OH)D levels rather than the consequence. Supporting this finding, patients undergoing HD who have PEW syndrome consistently exhibit low serum 25(OH)D levels (39).

The present study had certain limitations, which should be mentioned. A limitation of the present study is the lack of a placebo control group and the relatively small sample size of patients undergoing HD. Nevertheless, these participants were meticulously selected, and their adherence to the protocol was confirmed through serum 25(OH)D level measurements. Another limitation is that nephrologists had discretion in prescribing the phosphate binder sevelamer hydrochloride, the vitamin D analog paricalcitol, and the calcimimetic cinacalcet, all of which could potentially influence the evaluated outcomes. Despite this, the use of these medications aligns with KDIGO recommendations for managing serum iPTH, calcium and phosphorus levels, thereby reflecting actual clinical practice.

In conclusion, supplementation with cholecalciferol over a 1-year period effectively increases serum 25(OH)D levels. However, this intervention does not lead to significant improvements in BMD or a reduction in inflammation among patients undergoing HD who have vitamin D deficiency. Further research involving larger patient cohorts is thus necessary to validate these findings and, if warranted, to potentially revise the relevant KDIGO guidelines.

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## Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

## Authors' contributions

TE and PM designed the study. MD, PM, GP, MT, CP, MAPK, EL, IS and TE interpreted and analyzed the results, TE and MD wrote the manuscript. TE and PM confirm the authenticity of all the raw data. All authors drafted the manuscript, critically revised the manuscript, agree to be fully accountable for ensuring the integrity and accuracy of the work, and have read and approved the final manuscript.

## Ethics approval and consent to participate

The study protocol received approval from the Ethics Committee of the Faculty of Medicine at the University of Thessaly, Larissa, Greece (no. of approval 558/10-2-2017). Informed consent was obtained from each individual enrolled in the study.

## Patient consent for publication

Relevant consent for publication was obtained from the patient whose representative measurements are presented in Fig. 1.

## Competing interests

The authors declare that they have no competing interests.

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