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Cholecalciferol effect on oxidative stress and novel predictors of inflammation in hemodialysis patients: a prospective randomized trial

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Abstract

Background Emerging evidence links vitamin D deficiency to oxidative stress (OS) and inflammation, posing ongoing risks to cardiovascular outcomes in hemodialysis (HD) patients. Despite this, current data are lacking regarding the optimal approach or schedule for administering vitamin D in this population. This study investigated the effectiveness of oral weekly versus oral monthly cholecalciferol supplementation on 25-hydroxy vitamin D (25(OH)D) levels, oxidative stress, inflammatory indicators, and secondary hyperparathyroidism in HD population. HD patients ($N=50$) were randomly allocated to Group A (oral weekly 50,000 IU cholecalciferol) or Group B (oral monthly 200,000 IU cholecalciferol) for a 3 months duration. Serum levels of 25(OH)D, malondialdehyde (MDA), superoxide dismutase (SOD), high sensitivity C-reactive protein (HsCRP), neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), and intact parathyroid hormone (iPTH) were assessed at baseline and upon completion of the study.

Results A notable increase in serum 25(OH)D levels observed in both groups, with Group A showing a notably greater increase ($p=0.003$). Group A demonstrated significant reductions in serum MDA and increases in SOD, along with declines in hsCRP and NLR levels, which were not observed in Group B. Moreover, Group A exhibited a greater drop in iPTH (ΔiPTH = −30 pg/mL vs. −3 pg/mL) compared to Group B. Clinicaltrial.gov: NCT05460338, registered 13/07/2022.

Conclusions Weekly oral 50,000 IU cholecalciferol supplementation emerges as a tolerable, safe and effective approach for restoring vitamin D levels in HD patients, while concurrently mitigating inflammation, OS, and secondary hyperparathyroidism. This finding suggests that the more frequent the administration of oral cholecalciferol, the higher the efficiency observed.

Keywords Cholecalciferol, Oxidative stress, Inflammation, IPTH, Hemodialysis

Background

Vitamin D action seems to extend beyond its traditional reputation as a steroid hormone affecting skeletal tissues and regulating bone mineral metabolism. Recent studies have uncovered a range of diverse effects attributed to vitamin D, beyond its well-established functions [1, 2]. Growing evidence indicates that vitamin D may play a

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significant role in reducing both oxidative stress (OS) and inflammation in the body [3].

Over the last 20 years, researchers have identified oxidative stress as an emerging, non-conventional risk factor contributing to the advancement of chronic kidney disease (CKD) [4]. OS was shown to be prominent in CKD patients because of the increased production of pro-oxidants molecules, accompanied with the insufficient clearance oxidative products [5]. Hence, Hemodialysis (HD) patients in particular are in a chronic status of inflammation and OS. This is mostly due to the continuous loss of antioxidants throughout HD sessions and the activation of white blood cells, which triggers the release of additional reactive oxygen species (ROS) [6].

Indeed, cardiovascular diseases are responsible for nearly half of the death rates in the HD population, with systemic inflammation being one of the major leading mechanism underlined [7]. HD patients are commonly believed to experience chronic systemic inflammation, with a significant majority being vitamin D deficient [8]. Vitamin D deficiency in CKD is linked to elevated risks of both all-cause and cardiovascular mortality [9]. In fact, the HD population are known to be more vulnerable to higher cardiovascular morbidity and mortality rates [10]. Suboptimal concentrations of serum levels of 25(OH)D fail to mitigate OS and increases intracellular oxidative damage as well as the speed of cell apoptosis. Evidence suggests that inflammation is a known complication in HD population for which there is no established treatment strategy. However, inflammation has also been demonstrated to independently predict death in end-stage renal disease (ESRD) patients [11]. The chronic inflammatory status in the HD population maybe due to multiple factors, such as the uremic milieu, the effect of genetics and epigenetics, infections and the dialysis process itself [12]. Indeed, Vitamin D appears to regulate both inflammation and OS [13, 14]. The cardiovascular protection afforded by vitamin D intake can be attributed to its ability to downregulate parathyroid hormone [15]. However, other studies have shown that the pleiotropic roles of vitamin D on OS are beyond its effect on parathyroid hormone [16]. Experts define a deficient level of vitamin D as a serum 25(OH)D level below 20 ng/mL; while, insufficient levels of vitamin D are typically defined as 21 ng/mL to 29 ng/mL. However an optimal target of more than 30 ng/mL is recommended [17]. Serum levels which are less than 15 ng/mL are associated not only with increased mortality risk but also with progression to dialysis in pre-dialysis CKD patients [18]. Although both Kidney Disease Outcomes Quality Initiative (KDOQI) [19] and Kidney Disease Improving Global Outcomes (KDIGO) [20], have recommended screening for low serum 25(OH)D levels in CKD and dialysis patients, data regarding appropriate treatment protocols or regimens for vitamin D supplementation in HD population remain limited.

This current study aimed to evaluate the impact of weekly *vs.* monthly regimens of native vitamin D (cholecalciferol) supplementation, on replenishing the 25(OH) D levels, managing hyperparathyroidism, and if either regimen can possess an effect on HD-derived OS and inflammation.

Methods

Study design

This study utilized a prospective, single-blinded, block randomized design and was conducted at an institutional hospital, specifically within the HD units. The research adhered to Good Clinical Practice Guidelines and was carried out in accordance with the ethical principles outlined in the Declaration of Helsinki (revised in 2013). All patients provided written informed consent from the principal investigator prior to participating in this trial. Intention-to-treat analysis was conducted.

Patient eligibility

Patient eligibility criteria comprised individuals of both genders, aged 18 to 70 years, undergoing regular HD for a duration exceeding 3 months. Additionally, eligible candidates were required to maintain a stable clinical condition (controlled chronic condition), without hospitalization (no inpatient admission history) within the preceding 3 months, and exhibit 25(OH)D levels below 30 ng/ml.

We have excluded patients with previous or current hypersensitivity to cholecalciferol, current or recent cholecalciferol intake, liver failure, any digestive malabsorption disease, or who had participated in another clinical trial 4 weeks prior to enrollment. Pregnant or breastfeeding females were also excluded.

Treatment intervention

Treatment intervention involved screening all HD patients at the unit to determine eligibility. Fifty qualifying HD patients were randomly allocated to two groups. Each of the fifty patients was assigned a code from 1 to 50. Subsequently, patients were then randomly assigned to either Group A or Group B in blocks of four patients. Six distinct blocks were assigned (AABB, BBAA, BABA, ABBA, BAAB). Random list of numbers was created using SPSS, to aid in the selection and allocation process across the six blocks.

Fifty HD patients were randomly assigned into the two following groups

Group A 25 HD patients (47.52 ± 12.87 years, 15 males:10 females) were on Oral 50.000 IU cholecalciferol, once in a week, for 3 months.

Group B 25 HD patients (47.2 ± 11.06 years, 14 males:11 females) were on Oral 200.000 IU cholecalciferol, once in a month, for 3 months.

A 3 months' period of follow-up was done by the principal investigator.

All patients received their routine medications without any change throughout the 3 months' study duration. Cholecalciferol 50.000 IU (Vidrop®) and cholecalciferol 200.000 IU (Devarol®) were both manufactured locally.

Both study groups had weekly follow-ups and were monitored weekly by the principal investigator for any adverse effects or side effects. A designated nurse at the HD unit was providing the patients with cholecalciferol at the end of the mid-week HD session. Patients in both groups had no change in their routine medications provided by the unit. Medications impacting Ca levels or PO4 levels such as PO4 chelators were monitored throughout the study period.

A complete patient history was documented at the initial visit by the principal investigator. All patients in both groups underwent comprehensive clinical testing before participating in the study, by the attending physician. Demographic data, baseline characteristics of patients, and their laboratory results were collected and recorded during the recruitment phase.

Sample size

The sample size was calculated based on 25(OH)D serum levels. Based on a prior study involving HD patients, it was hypothesized that a significant difference of 7.48 ng/mL could be achieved (assuming a difference of 9.99 ng/mL in Group A compared to 2.51 ng/mL in Group B), utilizing the Two-group T-test with a power of 95% and a 5% as alpha error. Ideally, the study should have included a total of 40 patients. However, 50 patients were enrolled in this study (25 patients in Group A and 25 patients in Group B), considering an anticipated dropout rate of at least 25% [21]. The sample size calculation was calculated using the GPower program.

Laboratory measurements

To assess baseline parameters, endpoint study parameters, and routine laboratory data, a 4 ml blood sample was collected from each patient before the mid-week HD session and prior to the administration of heparin. Patient sera were then subjected to centrifugation at 3000 rpm for a minimum of 10 min at 4 °C. Subsequently, the sera were frozen at − 80 °C.

The primary outcome was the increase in 25(OH)D serum levels.

Secondary outcomes: an increase in sodium dismutase (SOD) serum levels, a decrease in intact parathyroid hormone (iPTH) serum levels, a decrease in malondialdehyde (MDA) serum levels, a decrease in high sensitivity C-reactive Protein (HsCRP) serum levels, a decrease in the neutrophil to lymphocyte (NLR) ration, a decrease in platelet to lymphocyte ration (PLR), an increase in calcium serum levels (Ca), and an increase in phosphate serum levels (PO4).

Blood samples were subjected to biochemical analysis at the Central labs of the institution before and after the study. The assessment of intact parathyroid hormone (iPTH) levels utilized the Electrochemiluminescence immunoassay (ECLIA) technique. Serum levels 25(OH)D, MDA, HsCRP and SOD were all determined using the Enzyme Linked Immunosorbent Assay (ELISA) technique.

The NLR was computated by dividing the absolute neutrophil count by the absolute lymphocyte count, and same for the PLR, by dividing the absolute platelet count by the absolute lymphocyte count.

Statistical analysis

Continuous variables were reported either by their mean and standard deviation (Mean ± SD) or by median and their interquartile range (Median (IQR)). Categorical data were illustrated as counts and corresponding percentages. A 95% confidence interval was employed with a margin of error set at 5%. Statistical analyses included paired or unpaired T tests for the data distributed normally. For nonnormally distributed data, paired samples were analyzed using the Wilcoxon signed-rank test; while, unpaired samples were analyzed using the Mann–Whitney *U* test.

Results

A total of 216 maintenance HD patients were assessed for their eligibility to the study, at the HD units of the hospital. Fifty patients fulfilled the inclusion criteria and were randomized into the study groups. Enrollment and allocation data are presented in Fig. 1.

Baseline

Baseline data show no significant differences noted between the two study groups (Tables 1, 2).

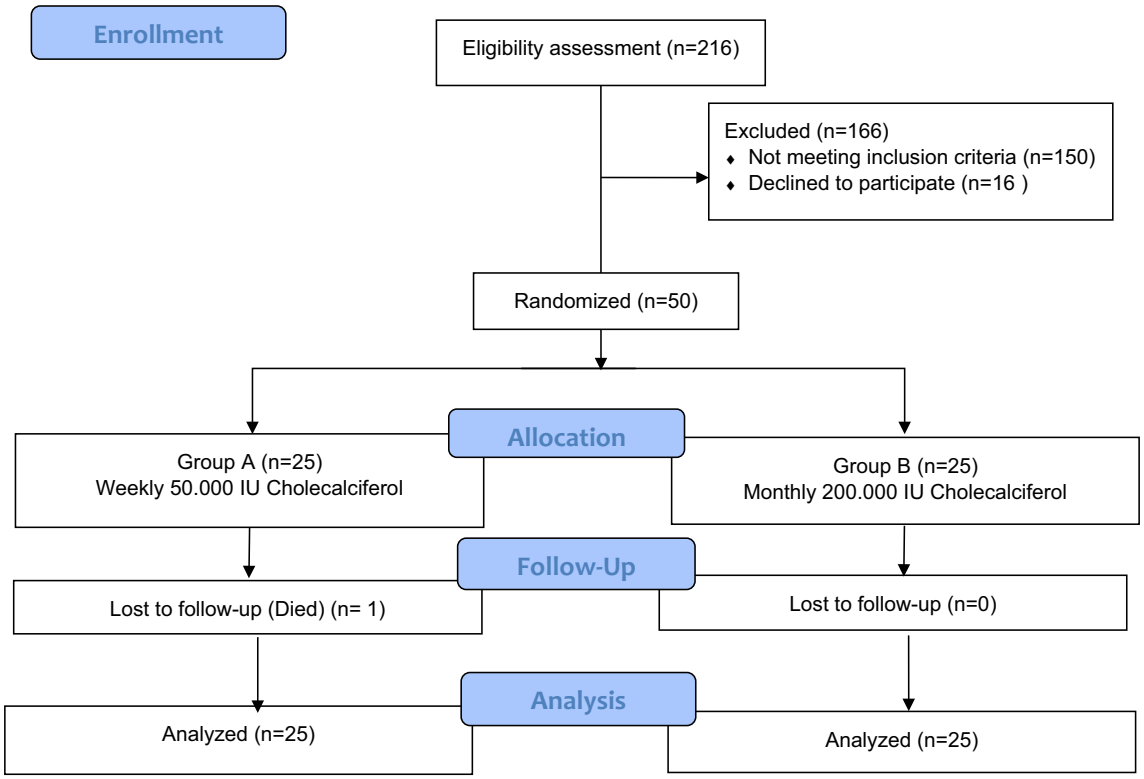

Fig. 1 Flow diagram summarizing enrollment, allocation, follow-up, and analysis processes

Endpoint assessment

Cholecalciferol efficacy

Following the 3-month period, cholecalciferol administration provided a significant increase in 25(OH)D serum levels in both study groups. Group A exhibited a significantly greater increase compared to Group B ($p=0.003$).

Group A exhibited a significantly larger increase in serum levels compared to Group B during the study period ($p<0.001$); specifically, increases of +16.23 ng/mL versus +13.75 ng/mL, respectively (Table 3).

Regarding OS markers, Group A showed a greater significant decline in MDA serum levels ($p<0.001$) overtime (a within group difference). After the completion of the study, and when comparing both groups, a significant decline in MDA levels was noticed in Group A, together with a significantly higher increase in serum SOD levels noted in Group A vs. Group B ($p=0.011$). The baseline change in MDA serum levels was significant only in Group A, as well as the change in serum levels of SOD. For inflammatory markers, serum levels of HsCRP significantly declined in Group A overtime ($p<0.001$). After the completion of the study, a significant decrease in HsCRP levels was observed exclusively in Group A ($p=0.019$). The change from baseline in serum HsCRP levels was significantly greater in Group A than group B ($p<0.001$).

NLR significantly declined throughout the study period in both groups. However, at endpoint assessment, a significant decrease in NLR was noted in Group A ($p=0.021$) only. The change from baseline was greater in Group A (−0.44 vs. −0.11, $p<0.001$). As for the PLR, no significant change was detected in both groups neither between groups nor overtime. However, a declining trend was noticed in both study groups, but did not attain statistical significance (Table 3).

Regarding serum iPTH levels, Group A showed a significant decrease over time ($p=0.001$). While at endpoint, a significant reduction was noticed in Group A when compared to Group B ($p=0.02$). Group A showed a significantly greater change from baseline ($p=0.006$) compared to Group B (Table 3).

Cholecalciferol safety

Both dosage regimens of cholecalciferol, 50,000 IU per week and 200,000 IU per month, were well-tolerated by the patients, with no reports of adverse effects or safety concerns associated with the drugs. At the study's conclusion, no significant difference was observed in

Table 1 Baseline assessments and clinical data

Baseline evaluation	Group A (*n* = 25)	Group B (*n* = 25)	*P*-value
A-Demographic data			
Age in years; mean ± SD	47.52 ± 12.87	47.2 ± 11.06	0.925
Male sex; n (%)	15 (60%)	14 (56%)	0.774
Female sex; n (%)	10 (40%)	11 (44%)	
Weight (Dry); mean ± SD (Kg)	77.88 ± 13.45	81.6 ± 13.84	0.34
HD vintage in years			
mean ± SD	7.2 ± 1.63	7.84 ± 1.62	0.17
KT/V; mean ± SD	1.23 ± 0.06	1.22 ± 0.07	0.691
SBP mean ± SD (mmHg)	128.8 ± 17.25	133 ± 25.12	0.299
DBP mean ± SD (mmHg)	80.8 ± 13.8	88 ± 16.83	0.11
B. Clinical data			
Etiology: n (%)			
Hypertension	10 (24%)	9 (36%)	0.952
Chronic Glomerulonephritis	5 (20%)	4 (16%)	
Diabetes mellitus and Hypertension	5 (20%)	3 (12%)	
Diabetes mellitus	3 (12%)	3 (12%)	
Reflux Nephropathy	2 (8%)	3 (12%)	
Chronic Pyelonephritis	3 (12%)	2 (8%)	
Unknown	1 (4%)	1 (4%)	
Comorbidities: n (%)			
Hypertension	4 (16%)	9 (36%)	0.740
Diabetes mellitus	4 (16%)	5 (20%)	
Ischemic cardiomyopathy	3 (12%)	2 (8%)	
Tobacco use	2 (8%)	2 (8%)	
No-comorbidities	12 (48%)	7 (28%)	
Drugs: n (%)			
Calcium-based phosphate chelator	7 (28%)	9 (36%)	0.544
Non-Calcium phosphate chelator	5(20%)	6 (24%)	0.733
Alpha-calcidol (0.25 µg each other day)	13 (52%)	12 (48%)	0.777

n: number of patients Hemodialysis (HD), Systolic Blood Pressure (SBP), Diastolic Blood Pressure (DBP)

serum Ca and PO4 levels between the groups, as shown in Table 2.

In both groups, all patients maintained 25(OH)D levels within the safe range of < 100 ng/ml, and there were no reports of hypervitaminosis D. No adjustments to the dosages were necessary throughout the study (Table 3).

At endpoint, no significant differences were noted in serum Ca and serum PO4 levels between the groups (Table 2).

Additionally, all patients have maintained serum 25(OH)D levels within the safe range of less than 100 ng/ml, with no reported cases of hypervitaminosis D. Furthermore, there was no need for modification of vitamin D dosage during the study period, as detailed in Table 3.

Discussion

This study aimed to showcase the cholecalciferol's pleiotropic effects, efficacy and safety of the two dosing schedules on OS and inflammation among the HD population. Thankfully, both dosages utilized in this study demonstrated no adverse effects on either serum Ca or PO4 levels. Additionally, none of the patients approached toxic levels of 25(OH)D, indicating the safety of the chosen dosing regimens.

At the end of study, both groups had a significant elevation in their 25(OH)D serum levels. However, the weekly regimen (Group A) was more favorable. A significant reduction of MDA, HsCRP, iPTH serum levels and NLR was noted only in the weekly regimen. Adding to the greater change from baseline (ΔiPTH = − 30

Table 2 Baseline and endpoint assessment of routine laboratory parameters

Parameter	Group A ($n=25$)	Group B ($n=25$)	*P*-value
Ca[a] (mg/dL)	Mean ± SD	Mean ± SD	
At Baseline	8.22 ± 0.51	8.23 ± 0.46	0.930
At Endpoint	8.4 ± 0.7	8.32 ± 0.56	0.659
P-value	0.56	0.431	
Albumin (g/dL)	Median (IQR)	Median (IQR)	
At Baseline	3.3 (3.05–4.0)	3.4 (3.1–3.75)	0.703
At Endpoint	3.4 (3.0–4.0)	3.5 (3.2–3.7)	0.937
P-value	0.515	0.735	
Cr.Ca[b] (mg/dL)	Median (IQR)	Median (IQR)	
At Baseline	8.8 (8.4–9.3)	8.7 (8.35–9)	0.267
At Endpoint	8.8 (8.3–9.25)	8.7 (8.4–9.25)	0.907
P-value	0.798	0.354	
PO4[c] (mg/dL)	Mean ± SD	Mean ± SD	
At Baseline	6.21 ± 0.32	6.25 ± 0.35	0.739
At Endpoint	6.26 ± 0.37	6.22 ± 0.25	0.689
P-value	0.675	0.771	

n: number of patients, a) Calcium (b) Corrected calcium, c) Phosphate

(−0.92 to −0.5), $p=0.001$) that was noticed only in the weekly regimen as well.

The initial observation of the two groups in this study revealed that 94% of the patients were deficient in vitamin D; while, the remaining 6% were insufficient. Specifically, all patients in Group A, receiving weekly cholecalciferol intake, were initially 100% vitamin D deficient. However, over the course of the 12-week study period, these patients experienced a remarkable increase in their serum levels of 25(OH)D. Till the study completion, none of the patients in Group A remained deficient or insufficient in vitamin D.

Conversely, patients assigned to the monthly cholecalciferol regimen (Group B) began the study with 94% of them being deficient in vitamin D. However, at the endpoint, none of the patients in Group B remained deficient in vitamin D, marking a decrease from 94 to 0% deficiency. Additionally, only 6% of patients in Group B were classified as insufficient in vitamin D after the completion of the study. Yet, both study groups were well-matched concerning baseline 25(OH)D serum levels, α-calcidol doses, as well as Ca-based and non-Ca-based PO4 chelators.

This increase in 25(OH)D serum levels was in line with the previously published results [22–25]. Even with various dosing regimens and duration, none of these studies has reported toxicity.

It's notable to mention that even the utilization of cholecalciferol (100,000 IU/week) did not result in any toxicity concerning serum 25(OH)D, Ca, or PO4 levels [26, 27]. Monitoring cholecalciferol levels can be effectively accomplished through the assessment of serum 25(OH)D, which acts as the primary circulating form of vitamin D in the body[28].

This finding reinforces the widely recognized excellent safety profile of cholecalciferol utilization in the literature, as previously documented in literature [29, 30]. Monitoring cholecalciferol levels can be effectively accomplished through the assessment of serum 25(OH)D, which acts as the primary circulating form of vitamin D [31]. As cholecalciferol has long half-life; approximately 2 months [32], taking cumulative doses of cholecalciferol intermittently on a weekly or monthly basis results in similar 25(OH)D levels as taking the equivalent daily dosage when the body reaches a stable state [33, 34].

The two regimens used in this study were suggested because of their affordability and availability in the market. They are both locally manufactured and widely distributed and are often used in HD patients [35, 36], for the sake of better compliance and less frequent intake. Indeed, in the current study, both weekly and monthly doses were given by a designated nurse, which has resulted in a 100% adherence rate. The two doses adopted in this study have proved excellent results, with no cases of hypercalcemia reported. However, no study has figured out the optimum dose regimen in terms of the pleotropic actions of vitamin D on inflammation or OS.

In ESRD, the triad of secondary hyperparathyroidism, OS and inflammation is well-established [37]. This study has found that different dose regimens of cholecalciferol intake may affect secondary hyperparathyroidism differently. By assessing the iPTH levels at the end of the 12 weeks, there was a significant change between the two regimens ($p=0.02$), favoring Group A. Adding to this, a better significant change from baseline (ΔiPTH = −30 (−0.92 to −0.5) vs, −3.0 (−13.5 to 10), $p=0.006$) over time, was also noted among patients in Group A only. This indicates that the weekly regimen of cholecalciferol is evidently superior to the monthly regimen. Indeed, cholecalciferol intake has been proved to elevate not only 25(OH)D serum levels, but also 1,25 $(OH)_2D$ levels [38–41]. This effect contributes to the reduction in PTH levels and bone markers [38]. While the 1α-hydroxylated vitamin D derivatives are more effective at controlling PTH levels, the main adverse effects are increased risk of hypercalcemia and hyperphosphatemia [42], and the consequences on cardiovascular diseases. In the current study, the slight decrease in iPTH levels may be overshadowed by the elevated levels of ionized calcium, phosphate, fibroblast growth factor-23 and the possible development of parathyroid nodular hyperplasia [43].

Table 3 Baseline and endpoint assessment of 25(OH)D, oxidative stress, and inflammatory parameters

Parameter	Group A (*n* = 25)	Group B (*n* = 25)	*P*-value
25(OH)D[a] (ng/mL)	Mean ± SD	Mean ± SD	
At baseline	17.69 ± 1.3	18.23 ± 1.91	0.248
At endpoint	33.92 ± 1.62	31.98 ± 2.59	0.003*
P-value	< 0.001 **	< 0.001 **	
Change; Mean ± SD	+ 16.23 ± 1.46	+ 13.75 ± 1.400	< 0.001 **
MDA[b] (nmol/ml)	Mean ± SD	Mean ± SD	
At Baseline	28.7 ± 3.3	27.8 ± 2.39	0.181
At Endpoint	26.1 ± 1.77	27.66 ± 2.32	0.011*
P-value	< 0.001 **	0.76	
Change; Median IQR	− 2.57 (− 4.2 to − 0.8)	+ 0.06 (+ 0.017 to 0.26)	< 0.001**
SOD[c] (U/mL)	Mean ± SD	Mean ± SD	
At Baseline	5.62 ± 1.00	5.66 ± 0.93	0.873
At Endpoint	6.44 ± 0.74	5.82 ± 0.9	0.011*
P-value	< 0.001**	0.473	
Change; Median IQR	+ 0.06 (+ 0.26 to 1.37)	+ 0.02 (− 0.4 to 0.6)	0.002*
HsCRP[d] (mg/L)	Mean ± SD	Mean ± SD	
At Baseline	12.53 ± 1.7	12.52 ± 1.65	0.936
At Endpoint	11.68 ± 1.16	12.6 ± 1.55	0.019*
P-value	< 0.001**	0.087	
Change; Mean ± SD	− 0.85 ± 1.05	+ 0.13 ± 0.37	< 0.001**
iPTH[e] (pg/ml)	Mean ± SD	Mean ± SD	
At Baseline	555.6 ± 117.74	574.36 ± 104.45	0.773
At Endpoint	474 ± 121.4	522.2 ± 113.43	0.338
P-value	0.001*	0.062	
Change; Median IQR	− 30 (− 92 to − 0.5)	− 3 (− 13.5 to 10)	0.006*
NLR[f]			
At Baseline	1.85 (1.54–2.31)	1.847 (1.455–2.3)	0.698
At Endpoint	1.5 (1.13–1.67)	1.678 (1.44–2.03)	0.021*
P-value	< 0.001**	0.001*	
Change	− 0.44 (− 0.2 to 0.735)	− 0.11 (− 0.005 to 0.19)	< 0.001**
PLR[g]			
At Baseline	109.57 (91.45–152.05)	123.03 (89.7–157.28)	0.9
At Endpoint	108.66 (90.67–151.56)	117.62 (95.29–159.26)	0.41
P-value	0.236	0.53	
Change	− 1.6 (− 5.7 to 2.59)	− 2.37 (− 8.8 to 5.95)	0.906

n: number of patients. (a) 25-hydroxy-Vitamin D, (b) Malondialdehyde, (c) Superoxide dismutase, (d) High sensitive C-reactive protein, (e) intact parathyroid hormone, (f) Neutrophils to Lymphocytes ration, (g) Platelet to Lymphocyte ratio

*($p \leq 0.05$) is considered significant, **($p \leq 0.001$) is considered highly significant

However, other clinical studies have did not show any significant decrease in iPTH levels [22, 26].

A well-known marker of oxidative stress; Malondialdehyde (MDA), is a short chain aldehyde that acts by stimulating the expression of expression of white blood cell adhesion and other inflammatory molecules, leading to their accumulation in the sub-endothelial area. The process of taking up the oxidized molecules in the arterial walls by macrophages, forming foamy cells, is the key first step for developing atherosclerosis [44]. Higher levels of MDA indicate more systemic oxidation [45, 46]. On the other side, one of the most effective enzymatic antioxidants is Superoxide dismutase (SOD); the one that defends against oxidative damage by enzymatically converting molecules of O_2 into H_2O_2. It's worthy to mention that SOD is s the primary antioxidant enzyme that regulates OS during the progressive renal injury [47].

The initial findings of this study identified an initial elevation in baseline MDA levels, and a baseline reduction in SOD among both groups. This baseline increase in the MDA levels among HD patients was also shown previously [48–51], as well as the decrease in the SOD baseline levels [50–52]. It seems that ESRD is a chronic status of elevated OS [53].

Vitamin D plays a crucial role in downregulating nitric oxide (NO); a potent regulator of ROS, along with its role in upregulating the SOD. The utilization of vitamin D analogs and their effect on OS and inflammation were studied previously [37, 54, 55]. However, in this study, we are documenting that the weekly intake of 50.000 IU of the native form of vitamin D3; cholecalciferol, had a significant improvement on OS status among the HD population. This is noted through the reduction observed in MDA levels, and the elevation in SOD levels after its intake for 12 constitutive weeks. This was in agreement with Tmadon et al., who documented a statistical decrease in MDA levels (− 0.1 vs. + 0.1 µmol/l; $p = 0{\cdot}009$), after the use of a bi-monthly 50.000 IU of vitamin D3 in diabetic HD patients, compared to a placebo group [35]. However, in our study, the monthly intake of cholecalciferol was not shown to be effective in dropping the MDA levels.

Several circulating markers and indicators of inflammation are used in order to monitor and follow-up this chronic inflammatory status. C-reactive protein (CRP), Interlukin-1 (IL-1), interlukin-6 (IL-6), tumor necrosis alpha (TNF-alpha), paracalcitonin, ferritin and even cholesterol levels [12, 56, 57]. However, it is widely recognized that CRP is considered the gold standard among these inflammatory markers due it its proven accuracy and its low cost and availability [58]. HsCRP; being strongly linked to vascular diseases, has also been demonstrated to independently predict mortality in patients with ESRD [11].

Recently, there has been increasing evidence supporting that both NLR and PLR ratio act as inexpensive and readily available indicators of systemic inflammation across various disorders [59–61], and especially in HD patients [62, 63]. These not only indicate inflammation but also serve as indicators of worse prognosis in various conditions (eg. malignancies, CKD and myocardial function) [64].

As per the results of our study, elevated levels of HsCRP were noticed at the baseline assessment in both study groups. This is supporting previous studies assessing the inflammatory status in HD patients [12, 65, 66]. Adding to this, elevated baseline levels of NLR and PLR were noticed as well. This study confirms the strong relationship of inflammation with ESRD patients, and especially HD patients. Whether inflammation reduces vitamin D levels, or vitamin D can suppress inflammation is still an area of clinical controversy [14, 67–70]. In 2016, Akbas et al., has documented a significant association between PLR and NLR and with 25(OH)D in 4120 patients [71]. Two years later, in 2018, a high inverse correlation between decreased serum levels of 25(OH)D and inflammatory markers including HsCRP, NLR and PLR was stated by Haddad et al. [14]. The latter study has paved the way for us to assess the effect of cholecalciferol supplementation with two dosing regimens on such inflammatory markers. Our study has demonstrated a significant reduction in HsCRP levels following the administration of the weekly dose of cholecalciferol for 12 weeks. When it comes to NLR, both groups (Group A) and (Group B), had a significant difference after the 12 weeks' study period. However, the change from baseline was higher in the weekly regimen group (ΔNLR − 0.44 vs − 0.11, $p < 0.001$). Lastly, for the effect of cholecalciferol intake on PLR, there was no statistical difference in both study groups.

Adding vitamin D to iron sulfate in female patients with iron deficiency anemia has significantly decreased PLR [72]; however, another recent study with vitamin D intake in diabetic patients did not exhibit a significant difference in both NLR and PLR, but a decreasing trend was documented [73].

The ameliorative beneficial effect of vitamin D on PLR may not have been detected in the current study, possibly due to the characteristics of the study population. Patients on HD are much more prone to have worse inflammatory status than other patients. Moreover, the 3 months' study period was considered to be an insufficient period to promote a significant change in PLR. As several studies have pointed to the association between 25(OH)D levels, HsCRP, NLR and PLR, as the best of our knowledge, this is the first study to assess the impact of native vitamin D on such inflammatory markers (NLR and PLR) in the HD population.

The efficiency of the weekly regimen of cholecalciferol over the monthly regimen is presumably due to the pharmacokinetics and pharmacodynamics of cholecalciferol itself. This is resembled in different involvement of enzymatic compartment. In other words, the higher the efficiency, the lower the saturation of 25-hydoxylase enzyme or the lesser the induction of 24-hydroxylase enzymes. Both enzymes are vitamin D catabolizing enzymes. Moreover, greater production of 24,25 $(OH)_2D$ was shown to be related to the bolus based regimens, more than the daily regimens [74]. All together, these findings suggest that the more frequent the administration of oral cholecalciferol, the higher the efficiency observed. Longitudinal studies are needed to determine whether the observed benefits are

sustained over time and how they impact long-term health outcomes.

Conclusion

In conclusion, weekly and monthly cholecalciferol seem to be safe and effective options to replenish serum levels of 25(OH)D in HD. However, weekly 50.000 IU cholecalciferol intake for 12 weeks has offered an amelioration of OS markers and inflammatory markers in our HD population. Same applies for secondary hyperparathyroidism, where a slight reduction in iPTH levels was shown only in Group A. Both NLR and PLR Both NLR and PLR are cost-effective and readily accessible indicators of inflammation that may be taken into consideration more often to assess and follow-up inflammation in HD patients.

Limitations

The study is not without limitations. First, the study period was limited only to 3 months. Longer duration studies are warranted in order to confirm the long-term effects of cholecalciferol. Second, the study took place in a public institutional tertiary setting, where a lot of resources such as calcimimetic agents and phosphate chelators are not covered by the standard public health insurance system. The need for better controlling options for persistent secondary hyperparathyroidism and hyperphosphatemia are warranted.

Abbreviations

CKD	Chronic kidney disease
CRP	C-Reactive protein
ESRD	End stage renal disease
HD	Hemodialysis
HsCRP	High sensitivity C-reactive protein
iPTH	Intact parathormone
IL-1	Interlukin-1
IL-6	Interlukin-6
KDIGO	Kidney disease improving global outcomes
KDOQI	Kidney disease outcomes quality initiative
MDA	Malondialdehyde
NLR	Neutrophil-to-lymphocyte ratio
NO	Nitric oxide
OS	Oxidative stress
PO4	Phosphate
PLR	Platelet-to-lymphocyte ratio
ROS	Reactive oxygen species
Ca	Serum calcium
SPSS	Statistical package for social science
TNF-alpha	Tumor necrosis alpha
SOD	Superoxide dismutase
25(OH)D)	Vitamin D

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Author contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by MA. The first draft of the manuscript was written by MA and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Availability of data and material

Data are available upon request.

Declarations

Ethics approval and consent to participate

ClinicalTrials.gov registration number: NCT05460338. A written informed consent was provided to the patients, by the principal investigator, prior to their participation in the trial. The ethics committee for experimental and clinical studies at Faculty of Pharmacy, Ain Shams University, Cairo, Egypt revised and approved the study protocol (REC: RHDIRB2020110301, 03/17/2022).

Consent for publication

Not applicable.

Competing interests

All authors declare they have no financial interests. All authors declare no conflicts of interest.

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