Evaluation of 25-hydroxy Vitamin D and 1,25-dihydroxy Vitamin D Levels in Maintenance Hemodialysis Patients

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Introduction. Dysregulated vitamin D metabolism is one of the most important issues in chronic kidney disease- mineral and bone disorder (CKD-MBD). Patients with end-stage kidney disease (ESKD) receive large amounts of calcitriol, i.e., 1,25 –dihydroxy vitamin D [1-25$(OH)_2$D], for suppression of parathyroid hormone (PTH). The aim of this study was to evaluate the 1-25$(OH)_2$D status in maintenance hemodialysis patients and its correlation with 25(OH) D level and calcitriol consumption and to determine whether the usual practice of administrating large amounts of calcitriol for suppression of PTH may lead to toxic serum levels.

Methods. One hundred and fifty-six maintenance hemodialysis patients were enrolled. Demographic data, comorbid conditions and history of medication use (cumulative and current doses) were retrieved from Hemodialysis Data Processor Software previously designed for our center. Predialysis serum samples were measured for serum levels of 25(OH)D and 1-25$(OH)_2$D accompanying by markers of mineral bone metabolism and inflammation.

Results. Of 156 patients, 66% were male and the mean age was 56.5 ± 16.3 years. There was no significant correlation between serum level of 25(OH)D and 1,25$(OH)_2$D (r = 0.12, $P > .05$). Only current ingestion of vitamin D was correlated with both 25(OH) D (r = 0.324, $P < .001$) and 1,25$(OH)_2$D serum levels (r = 0.334, $P < .001$). There was no significant relationship between current or cumulative calcitriol consumption and 1,25$(OH)_2$D serum level. 1,25(OH)2D/25(OH)D ratio which, represents the degree of vitamin D hydroxylation efficiency was 0.9 pg/ng (expected value in no CKD > 2.2 pg/ng).

Conclusion. Calcitriol consumption was not correlated with increased serum 1,25$(OH)_2$D level and the practice of hyperparathyroidism treatment with calcitriol may be safely continued, though we are not yet aware of the 1,25$(OH)_2$D status at the cellular level.

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INTRODUCTION

Chronic kidney disease–mineral and bone disorder (CKD-MBD) is very common in CKD and end- stage kidney disease (ESKD) patients, and vitamin D regulation is one of the most important and challenging issues in this area. Due to the important role of 25-hydroxyvitamin D [25(OH) D] and its active metabolite, dihydroxyvitamin D [1-25$(OH)_2$D] in the suppression of parathyroid hormone (PTH), most epidemiologic studies and guidelines, such as those of Kidney Disease: Improving Global Outcomes (KDIGO) and the

Kidney Disease Outcome Quality Initiative (KDOQI), recommend administration of vitamin D supplements to keep circulating 25(OH)D, calcidiol, and its active metabolite, calcitriol, in normal range for efficient suppression of PTH.[1,2] Consequently, a large number of ESKD patients are exposed to high doses of calcitriol and calcidiol for a long time. Nevertheless, these studies have only measured the serum level of 25(OH)D and the level of 1, $25(OH)_2D$ has rarely been measured. On the other hand 1, $25(OH)_2D$ and 25(OH)D have different biological features and their serum levels are poorly matched.[3] The complicated mechanism of mineral homoeostasis has caused uncertainty in the compensation of vitamin D deficiency with vitamin D supplements and its active analogues. In a number of patients, the serum level of 25(OH)D is within the normal range, but its conversion to 1, $25(OH)_2D$ is impaired and in others 1, $25(OH)_2D$ level is normal despite reduced 25(OH)D level; the patients are at risk of vitamin D deficiency and hypervitaminosis respectively.[3]

In hemodialysis patients conversion of 25(OH) D to 1, $25(OH)_2D$ is probably deregulated for two reasons; first one is the well-known deficiency of 1-alpha-hydroxylase that may cause reduced level of active 1-$25(OH)_2D$ and second is the possible increased production of 1,$25(OH)_2D$ due to inflammatory nature of the disease. In a number of inflammatory T helper 1-mediated diseases, such as sarcoidosis and rheumatoid arthritis, 1-$25(OH)_2D$ serum level is increased due to production of the vitamin in the mitochondria of active macrophages and the serum level of 25(OH)D is decreased.[4,5] In such circumstances low serum concentrations of 25(OH)D may lead to unnecessary prescription of vitamin D supplement. Several kidney diseases that ultimately lead to CKD or ESKD have an inflammatory and immunological nature, and Th1, Th2 imbalances in such situations have been mentioned in a number of studies.[6,7] Overall, studies point to cautious prescription of vitamin D in chronic diseases such as kidney failure as long as a degree of vitamin D dysregulation is discovered, and it has been recommended to evaluate the level of 1, $25(OH)_2D$ in addition to 25(OH)D.[5,8]

The aim of our study was to measure the serum levels of 25(OH)D and 1-$25(OH)_2D$ in a cohort of maintenance hemodialysis patients and to examine the correlation between these two vitamins and the surrogates of mineral metabolism [FGF-23, PTH, calcium (Ca) and phosphorus (P)], as well as inflammatory markers, related medications, duration of dialysis and underlying diseases. In addition, 1-$25(OH)_2D$ pg / 25(OH)D ng ratio was considered as an index of vitamin D hydroxylation efficacy and calculated for determination of the degree of dysregulation of vitamin D metabolism. The expected ratio has been as previously defined as more than 2.2 pg/ng by Pasquali *et al*.[3]

MATERIALS AND METHODS

One hundred and fifty-six out of 166 patients undergoing maintenance hemodialysis in Hasheminejad Kidney Center were enrolled. Patients with history of parathyroidectomy, active malignancy, sarcoidosis and those who did not sign the consent form to participate in the study were excluded. Demographic data including age, sex, dialysis vintage, underlying cause of ESKD and comorbidities were extracted from the Hemodialysis Data Processor Software (HDPS), designed for our ward (AIP Company, 2010, Tehran, Iran). History of the current and past medications were collected from the medical charts and confirmed through interview with the patients. Peripheral blood samples were tested for serum levels of 25(OH) D, 1-$25(OH)_2D$, intact PTH (iPTH), FGF23, alkaline phosphatase, albumin, ESR, CRP, and ferritin before dialysis. All laboratory tests, including 25(OH)D, FGF23, and iPTH were conducted in the laboratory of Hasheminejad Kidney Center and 1-$25(OH)_2D$ level was performed in the laboratory of National Nutrition and Food Technology Research Institute of Shahid Beheshti University of Medical Sciences. Quantitative values were presented as mean ± SD. Pearson correlation coefficient was used for investigating the relationship between quantitative continuous variables. To determine difference between the means of two quantitative sets of data t-test was performed. *P* value was considered significant if < .05. IBM SPSS statistics version 21.0 was used for statistical analysis of data. Code (date) of the ethical approval was IR.IUMS. REC1396.30807 (2018-1-2).

Assays

Serum samples were immediately frozen after blood withdrawal at -30°C for up to one month until analysis and the tests were performed according

to the kit manufacturer's protocol.

Measurement of the serum level of 1, 25$(OH)_2$D was performed by enzyme immunoassay (EIA) using commercial kit (ZellBio GmbH, Germany). Typical range of the kit was 50 to 1600 pmol/L with a detection limit of 2.5 pmol/L. The normal range was considered as 48 to 108 pmol/L.[9] Measurement of 25(OH)D serum level was performed by high performance liquid chromatography (HPLC) using the Chrom Abzar Parse Co., Iran, kit. Typical linearity range of the kit was 3 to 130 ng/mL. Normal range was defined as 30 to 100 ng/mL, insufficient as 20 to 30 ng/mL, deficient as < 20 ng/mL, and toxic level as higher than 100 ng/mL.[10]

FGF23 level was measured using EIA (ZellbioGmb,Germany). Typical linearity range of the kit was 5 to 1600 pg/mL, with a detection limit of 2.5 pg/mL. The normal range of FGF23 was < 200 pg/mL.[11,12] Serum level of intact parathyroid hormone (iPTH) was evaluated using EIA assay kit (IBL International Company, UK). Typical linearity range of the kit was 0.1 to 1000 pg/mL and in cases of out of range values, the samples were diluted 2 to 3 times. The detection limit was 1.57 pg/mL.

RESULTS

One hundred and fifty-six patients were enrolled in this study. Of these 104 (66.03%) and 52 (33.97%) were male and female, respectively (Table 1). Etiology of ESKD was unknown in 37.2%. Among patients with known underlying diseases for ESKD, diabetes mellitus (20.5%) was the most prevalent cause, followed by autosomal dominant polycystic kidney disease (9%), hypertension (8.3%), glomerulonephritis (5.7%), multiple myeloma (5.1%), and reflux nephropathy (3.2%). Mean serum level of 1, 25$(OH)_2$D was higher in females than in males (63.9 ± 92 vs. 49.4 ± 35.8 pmol/L, $P < .05$). The same finding was noticed for serum 25(OH)D with a mean level of 23.5 ± 16 vs. 22.2 ± 11.5 in female compared with male patients ($P < .05$, Table 2). Serum level of 25(OH)D was normal in 28%, insufficient in 23%, and deficient in 49% of cases. Level of 1-25$(OH)_2$D was normal in 32%, low (< 48 pmol/L) in 60.9% and toxic (> 108 pmol/L) in 7.1%. Eleven patients with toxic levels of 1-25$(OH)_2$D had never received high dose calcitriol (arbitrarily defined as more than 0.25 µg/d). Serum level of 1-25$(OH)_2$D was zero in 3 patients and more than 2 times of upper limit normal i.e., > 350 pmol/L in 3 other patients. Of the 3 patients with 1-25$(OH)_2$D level of zero, 1 patient had no history of the current calcitriol intake and had used a cumulative dose of 30 pearls of calcitriol (each 0.25 µg) since the onset of dialysis. But the other two were under treatment with 1.5 to 2 calcitriol pearls with the cumulative dose of 492 and 4067 pearls based on the duration of ESKD (3 and 7 years, respectively). Of the 3 patients with 1-25$(OH)_2$D serum levels > 350 pmol/L, one had no history of recent or current calcitriol supplementation, and the other two patients had used a cumulative dose of 216 and 1145 calcitriol pearls with the current daily dose of 1 and 5 pearls, respectively.

Pearson correlation test results showed no significant relationship between 1-25$(OH)_2$D and 25(OH)D serum levels ($P > .05$, r = 0.12). By categorizing patients into two groups with and without 25(OH)D deficiency, the mean values of 1-25$(OH)_2$D was 49.6 ± 40.8 and 58.96 ± 6 in these

Table 1. Clinical and Medical Characteristics of Patients

Variables	
Age (Mean ± SD)	56.5 ± 16.2 (21 to 76)
Gender (n (%))	
Male	104 (66.03)
Female	52 (33.97)
Transplantation History (n)	24
Smoking (n)	19
Addiction (n)	0
Cause of ESKD (%)	
Unknown	37.2
DM	20.5
ADPKD	9
HTN	8.3
GN	5.7
Multiple Myeloma	5.1
Reflux Nephropathy	3.2

Abbreviations: ESKD, end-stage kidney disease; DM, diabetes mellitus; ADPKD, autosomal dominant polycystic kidney disease; HTN, hypertension; GN, glomerulonephritis.

Table 2. Mean, Standard Deviation and the Range of 25(OH) vitD, 1,25$(OH)_2$D, iPTH, and FGF23 Levels

Variables	Mean (± SD)	Maximum	Minimum
25 (OH)D, pmol/L	22.66 ± 13.18	73.50	3.00
1-25 $(OH)_2$D, ng/mL	54.31 ± 61.10	459.30	0
iPTH, pg/mL	509.33 ± 370.73	1855.00	33.70
FGF23, pg/mL	580.41 ± 255.56	1235.00	155.00

Abbreviations: iPTH, intact parathyroid hormone; FGF23, fibroblast growth factor 23; 25(OH)D, 25-hydroxyvitamin D; 1-25$(OH)_2$D, 1-25 dihydroxyvitamin D.

groups, respectively, with no significant differences ($P > .05$). Similarly, by the evaluation of correlation between serum levels of 1-25$(OH)_2$D and 25(OH)D with cumulative and current doses of vitamin D and calcitriol, only a significant direct and positive relationship was observed between the current dose of vitamin D, and 25(OH)D and 1-25$(OH)_2$D levels ([r = 0.33, $P < .001$] and [r = 0.32, $P < .001$], respectively) (Table 3).

By categorizing the subjects based on 1-25$(OH)_2$D serum levels into two groups of with and without deficiency, no significant difference was observed in the levels of inflammatory markers including ESR, CRP, and ferritin between the 2 groups. However serum albumin level was higher in patients with 1-25$(OH)_2$D deficiency (3.86 ± 0.8 vs. 3.6 ± 0.3, $P < .05$). Regarding the same analysis for the 25(OH)D, levels of serum albumin and other inflammatory markers were not different between those cases with and without vitamin D deficiency.

Correlations between current and cumulative consumption of calcidiol and calcitriol, FGF23 and iPTH levels were studied and only the cumulative calcitriol consumption was significantly correlated with iPTH level (r = 0.25, $P < .001$; Table 4). Furthermore the correlation between ESKD duration and iPTH, FGF23 and ALP levels was studied and only a significant and positive correlation was found between ESKD duration and iPTH level (r = 0.202, $P < .05$; Table 5).

The mean 1-25$(OH)_2$D / 25(OH) D ratio was 0.9 pg/ng in our study.

Table 3. Correlations Among 25$(OH)D_3$, 1,25$(OH)_2$D, and Clinical and Laboratory Variables

Variables	25 (OH)D (pmol/L)		1-25 $(OH)_2$D (ng/mL)	
	P	r	*P*	r
Age	> .05	0.01	> .05	0.03
ESKD Duration	< .05*	0.19	< .001*	0.25
BMI	> .05	0.13	> .05	0.05
Calcidiol (current)	< .001*	0.33	< .001*	0.32
Calcidiol (cumulative)	> .05	0.41	> .05	0.03
Calcitriol (current)	> .05	0.10	> .05	0.35
Calcitriol (cumulative)	> .05	0.08	> .05	0.10
FGF23, pg/mL	> .05	0.06	< .001*	0.36
iPTH, pg/mL	> .05	0.03	> .05	0.02

Abbreviations: iPTH, intact parathyroid hormone; FGF23, fibroblast growth factor 23; 25(OH)D, 25-hydroxyvitamin D; 1-25$(OH)_2$D, 1-25 dihydroxyvitamin D; ESKD, end-stage kidney disease; BMI, body mass index.
*Correlation is significant at the .05 level.

Table 4. Correlations Between Current and Cumulative Consumption of Calcidiol and Calcitriol and FGF23 or iPTH

Variables	FGF23	iPTH
Calcidiol (current)	*P* > .05 r = 0.08	*P* > .05 r = 0.04
Calcidiol (cumulative)	*P* > .05 r = 0.05	*P* > .05 r = 0.14
Calcitriol (current)	*P* > .05 r = 0.02	*P* > .05 r = 0.06
Calcitriol (cumulative)	*P* > .05 r = 0.00	*P* < .001* r = 0.25

Abbreviations: iPTH, intact parathyroid hormone; FGF23, fibroblast growth factor 23.
*Correlation is significant at the .05 level.

Table 5. Correlations Between iPTH, FGF23, ALP, and ESKD Duration

iPTH vs. FGF23	*P* > .05 r = -0.03
iPTH vs. ALP	*P* < .001 r = 0.49
iPTH vs. ESKD Duration	*P* < .05* r = 0.20
FGF23 vs. ALP	*P* > .05 r = -0.08
FGF23 vs. ESKD Duration	*P* > .05 r = 0.11
ALP vs. ESKD Duration	*P* > .05 r = 0.13

Abbreviations: iPTH, intact parathyroid hormone; FGF23, fibroblast growth factor 23; ALP, alkaline phsophatase; ESKD, end-stage kidney disease.
*Correlation is significant at the .05 level.

DISCUSSION

Vitamin D deficiency is prevalent in patients with CKD and maintenance hemodialysis patients, however the results of various studies are controversial and a prevalence from less than 10% to over 80% has been reported in different studies.[13-19] In the current study, the prevalence of vitamin D deficiency and insufficiency were 49% and 24%, respectively (totally 72.9%).

In our study, 95.5% of the patients had FGF23 level higher than normal values, however the highest level was 1235 pg/mL and values higher than 1000 times reported in different studies were not seen in our patients. The normal range of FGF23 is not well defined.[20] However, cross sectional studies showed that in ESKD patients undergoing hemodialysis, FGF23 level increases over time and often exceeds 1000 times more than normal range; the highest FGF23 levels were reported in patients with ESKD, which is attributed to its increased synthesis in bone and reduced degradation,

although its clearance by kidneys or dialysis also seems to be ineffective.[20] A direct and significant relationship was found between the levels of FGF23 and 1-25(OH)2D as in the review of literature.[21,22] This result probably shows the positive feedback of 1-25(OH)2D on FGF23 in order to suppress 1-alpha hydroxylase enzyme.

There was no significant correlation between the serum levels of 25(OH)D and 1-25$(OH)_2$D in our study. In a previous study on vitamin D status in sepsis, a strong correlation was reported between 25(OH)D and 1-25$(OH)_2$D serum levels.[23] However other studies have shown a positive correlation between these two metabolites of vitamin D only in patients with 25$(OH)_2$D deficiency.[8,24-26] In these patients 1-25$(OH)_2$D synthesis may be reduced due to lack of substrate, i.e. 25(OH)D.[8] Based on this, we compared the mean values of 1-25$(OH)_2$D in patients with and without 25(OH)D deficiency and no difference was observed. This issue needs more study in CKD patients with different levels of 1-alpha-hydroxylase. In a study by Lips and colleagues, it was noted that in cases of severe 25(OH)D deficiency, treatment with vitamin D may increase the serum level of 1,25-dihydroxy vitamin D.[8]

In the current study we also examined the correlation between current and cumulative dosages of vitamin D and calcitriol supplements consumed by the patients and the serum levels of the relative vitamins. We found only a direct and significant relationship between the current vitamin D consumption and 1-25$(OH)_2$D and 25(OH)D serum levels, which is quite expectable, considering 25(OH)D as the substrate of 1-25$(OH)_2$D. However ingestion of calcitriol for PTH suppression was not associated with high or toxic levels of 1-25$(OH)_2$D, which was our main concern. We could not find any studies regarding the correlation between consumption of vitamin D and/or calcitriol and 1-25$(OH)_2$D serum levels and we suggest more studies on this important issue especially at cellular level. In the study of the correlation between inflammatory markers such as ferritin, CRP, ESR, and albumin with 1-25$(OH)_2$D and 25(OH)D levels, only a significant and direct relationship was found between albumin and 25(OH)D level, which may indicate higher vitamin D levels in patients with better nutritional status. In a study on 118 healthy female subjects in 2016, no significant correlation was found between the levels of vitamin D and inflammatory markers.[27] In a recent in depth review on the correlation of inflammation and vitamin D metabolism, the authors believed that vitamin D deficiency is not the cause, but the effect of inflammation, and by referring to studies showing low levels of 25(OH)D in spite of higher levels of 1-25$(OH)_2$D under inflammatory conditions, attributed this effect to impairments in the endogenous metabolism of vitamin D under inflammatory conditions.[28] This review emphasizes that measuring 25(OH)D levels alone cannot reflect the endogenous metabolism of vitamin D and the status of vitamin D in the body, and its metabolite (1-25$(OH)_2$D) should also be checked.[28] Based on this, and as many patients on maintenance dialysis can be assumed to be in chronic inflammatory state, we tried to find a correlation between vitamin D deficiency and inflammatory markers, but no significant difference in the level of inflammatory markers was found between patients with and without vitamin D deficiency. There was a significant and direct correlation between the duration of ESKD and the 25(OH)D and 1-25$(OH)_2$D serum levels. This may have been due to vitamin D supplementation over time, as we showed the correlation between cumulative dose of D supplement used and both 25(OH)D and 1-25$(OH)_2$D serum levels. We could not find a similar finding in literature review.

Considering the risk factors of vitamin D deficiency including age, obesity and diabetes mellitus, we examined the relationship between these risk factors and the level of vitamin D, and, contrary to other studies no significant relationship was found.[18,29,30] In terms of gender the mean serum level was even higher in female subjects. Better supplementation in females or other unknown causes may be responsible for this finding. Previously Pasquali *et al.* had specifically examined the serum levels of 1-25$(OH)_2$D and 25(OH)D in a variety of populations including the early stages chronic kidney disease, kidney transplantation, hemodialysis and primary hyperparathyroidism, and defined the mean ratio of 1,25-dihydroxyvitaminD to 25(OH)D as the index of adequacy for vitamin D hydroxylation.[3] Pasquali found the lowest ratio of 1.1 in hemodialysis patients, and an increasing trend in patients with

lesser degrees of kidney failure (1.77 pg/ng in subjects with CKD without dialysis and 4.11 pg/ng in the ones without kidney disease).[3] We determined this ratio, which theoretically could show how much 1-25$(OH)_2$D (in picograms) exists per nanogram of 25(OH)D in our patients. The ratio was 0.9 pg/ng and close to the ratio found by Pasquali *et al* (expected value in non-CKD patients CKD > 2.2 pg/ng).[3] This ratio may be used to determine the degree of dysregulation of vitamin D metabolism and the need for compensation of 1,25$(OH)_2$D.

CONCLUSION

Concerns about over-treatment with calcitriol were the main reason for the conduction of the current study. The result of our study did not show any significant relationship between the amount current or cumulative calcitriol intake and the serum level of 1-25$(OH)_2$D. A strong and direct correlation between the serum levels of 25(OH)D and 1-25$(OH)_2$D with current consumption of vitamin D supplements, confirms the findings of other studies on the importance of extra-renal 1-alpha-hydroxylase activity, which is recently noticed in patients with kidney failure more than ever. However due to heterogenic pattern of consumption of the abovementioned supplements before and during the study, the results of statistical tests should be interpreted with caution.

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