

# Analysis of Adequacy of 25-Hydroxi vitamin D3 Supplementation in Patients on Hemodialysis and Parathormone, Calcium and Phosphorus Level in the Blood of These Patients

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## ABSTRACT

**Introduction:** Appropriate vitamin D turnover is essential for many physiological function. Knowledge of it's function was improved in last two decades with enlargement of scientific confirmation and understanding of overall importance. In addition to classical (skeletal) roles of vitamin D, many other function (no classical), out of bone and calcium-phosphate metabolism, are well defined today. **Aim:** To analyze vitamin D level in the blood in dialysis and pre dialysis patients and evaluate efficacy of supplementation therapy with vitamin D supplements. **Methods:** Vitamin D3 level in form of 25-hydroxivitamin D3 was measured in dialysis and pre dialysis patients, using combination of enzyme immunoassay competition method with final fluorescent detection (ELFA). Parathormone was measured by ELISA method. Other parameters were measured by colorimetric methods. Statistical analysis was done by nonparametric methods, because of dispersion of results of Vitamin D and parathormone. **Results:** In group of dialysis patients 38 were analyzed. Among them 35 (92%) presented vitamin D deficiency, whether they took supplementation or not. In only 3 patients vitamin D deficiency was not so severe. Vitamin D form were evaluated in 42 pre dialysis patients. Out of all 19 patients (45 %) have satisfied level, more than 30 ng/ml. Moderate deficiency have 16 patients (38%), 5 of all (12%) have severe deficiency, and two patients (5%) have very severe deficiency, less than 5 ng/ml. Parathormone was within normal range (9.5-75 pg/mL) in 13 patients (34 %), below normal range (2 %) in one subject, and in above normal range in 24 (63 %). **Conclusion:** Vitamin D3 deficiency was registered in most hemodialysis patients; nevertheless supplemental therapy was given regularly or not. It is to be considered more appropriate supplementation of Vitamin D3 for dialyzed patients as well as for pre dialysis ones. In pre dialysis patient moderate deficiency is shown in half of patients but sever in only two.

**Key words:** Vitamin D, hemodialysis, parathormone, calcium, phosphorus.

## 1. INTRODUCTION

Appropriate concentration of 25-hydroxivitamin D3 in serum of hemodialysis patients is very important for two cornerstone purposes: the first one is to participate in bone metabolism and second to ensure phagocytosis of immune cells, neutrophils, monocytes, macrophages, neuroglia, etc. Supplementation of vitamin D3 is recommended in all guidelines for treatment of dialyzed patients. Essentially it is recommended for patients with any stage of chronic kidney disease (CKD). Even if hyperparathyroidism is present or not – impaired vitamin D3 and bone metabolism is present in many patients on hemodialysis. Analysis of difficulties with supplementation of vitamin D3 on

dialyzed patient is the aim of this study.

Not only for CKD and bone metabolism (calcium and phosphate homeostasis) the appropriate vitamin D plasma level (1) of great importance. Many of cardiovascular complications in patients with CKD may be linked to vitamin D deficiency. It's well known that CKD patients have a reduced ability to convert 25-(OH)-vitamin D3 into active form – 1.25 dihydroxy vitamin D. The most vital cellular uses of vitamin D include: Cell proliferating and anti proliferative factor in variety of tissue; Involved in regulatory immune system mostly for effective phagocytosis; Regulatory function in autoimmune diseases; Infection diseases; Cardiovascular diseases; Regulatory role

in renin-angiotensin system, diabetes including regulation of insulin resistance (2,3). Because of many new discovered roles of vitamin D all of these roles were divided in two groups: Classical (bone and calcium-phosphate homeostasis, skeletal health); No classical (no skeletal health) – the newly listed ones described above (4,5). Metabolic activation is mostly performed in kidney, but at the same time in many other tissues. Activation of Vitamin D in different places in the body is considered an autocrine pathway. Utilization of Vitamin D is very high in the whole body, with immediate local degradation. So, circulating component of vitamin D is minimal. Vitamin D binds to intracellular vitamin D receptor (VDR) and has one crucial role in transferring a signaling cascade that bridges external stimuli to gene transcription (1).

The Classical role of Vitamin D is its overall participation in general metabolism and calcium phosphate balance (6-8). The involvement of the kidneys in vitamin D metabolism is mainly considered as part of classical (skeletal) role. Vitamin D in kidneys is analyzed as a separate factor important in physiological kidney function and disease (9, 10). Hallmark of kidney diseases – proteinuria is analyzed many times to evaluate comprehensive role of vitamin D, separately. According to the Third National Health and Nutrition Examination Survey (NHANES III) (9) proteinuria is present twice the norm, in vitamin D deficient patients, in comparison with to subjects with normal vitamin D in plasma. Classical and no-classical pathways in vitamin D metabolism overlap in the kidneys. Anti-proteinuric effect of vitamin D is likely related to RAS-angiotensin II-mediated mechanism (7).

**2. AIM**

The aim of this study was to analyze vitamin D level in the blood in dialysis and pre dialysis patients and evaluate efficacy of supplementation therapy with vitamin D supplements.

**3. METHODS**

Vitamin D3 level in form of 25-hydroxivitamin D3 was measured using combination of enzyme immunoassay competition method with final fluorescent detection (ELFA). Analyzes were done by VIDAS laboratory machine using regencies produced by Biomerieux. The blood samples are mixed with pre-treatment reagent to separate vitamin D from its binding protein. The pretreated sample is then collected and transformed into the well that contains an alkaline phosphates labeled anti-vitamin D antibody (conjugate). The vitamin D antigen present in the sample and the vitamin D coating the inferior of the solid phase receptacle compete for binding sites on the anti-vitamin D antibody alkaline phosphate conjugate. In final detection step the substrate (4-methyl-umbelliferyl phosphate) is cycled and in and out of solid phase receptacle. The conjugate enzyme catalyzes the hydrolysis of this substrate into a fluorescent product (4-Methyl-umbelliferone). Fluorescence was measured at 450 nm. The intensity of the fluorescence is inversely proportional to the concentration of vitamin D antigen present in the sample.

Parathormone was measured by immunoassay technique. Concentration of calcium and phosphorus (as phosphate ion) were measured using standard colorimetric methods. Gathered data were calculated by standard statistical methods of descriptive statistic using statistical package Statistica for windows. Nonparametric method of linear regression was used because of high dispersion of result of vitamin D measurement.

**4. RESULTS**

On a group of 38 hemodialysis patients, a complete laboratory examination was performed. Among them, 20 females and 18 males. Of the 38 patients, 35 (92%) presented 25-hydroxivitamin D3 deficiency whether they took Vitamin D3 supplementation or not. No patient with normal plasma concentration of 25-hydroxivitamin D3 was present. In three cases (8 %) the concentration of vitamin D3 was borderline (Figure 1).

Vitamin D as 25-hydroxivitamin D3 form were evaluated in 42 pre dialysis patients) 22 female and 20 male). Among them as much as one half have vitamin D deficiency. Out of all 19 patients (45%) have satisfied level, more than 30 ng/ml. Moderate deficiency have 16 patients (38%), 5 of all (12%) have severe deficiency, and two patients (5%) have very severe deficiency, less than 5 ng/ml (Figure 2). In comparison of vitamin D level in dialysis and pre dialysis group, by linear regression and using Spearman rank correlation, statistical significance was shown (p=0.0016).

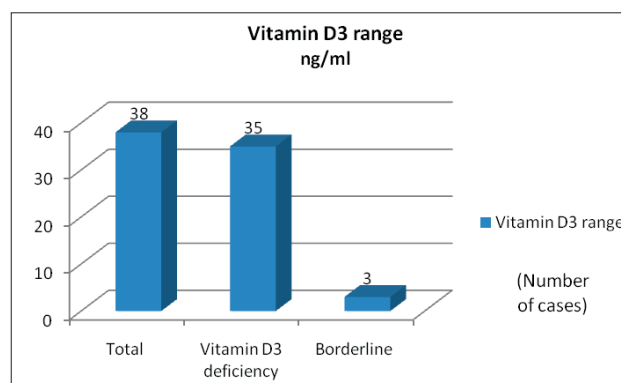


Figure 1. Number of cases with different Vit. D3 range in plasma. As severe Vit. D3 deficiency concentration is lower than 5 ng/ml

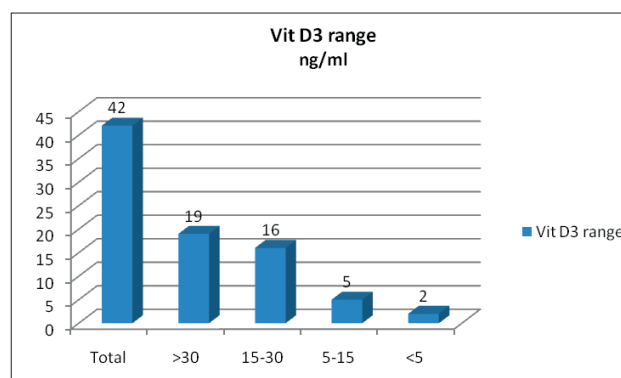


Figure 2. Number of cases with different Vit. D3 range in plasma. As severe Vit. D3 deficiency concentration is lower than 5 ng/ml. (predialysis)

In 27 patients (72%) calcium level in plasma were in normal range (2.15-2.60 mmol/L), in 10 patients (26%) calcium level were above normal range, and in only one patient (2%) the plasma calcium level was below normal range (Figure 3). Serum phosphorus was within normal range in 10 patients (26%), above normal range in 27 patients (71%), and in only one patient below normal range (2%) (Figure 4). Parathormone was within normal range (9.5-75 pg/mL) in 13 patients (34%), below normal range (2%) in one subject, and in above normal range in 24 (63%) of the 38 patients (Figure 5). Concentration of parathormone was significantly higher in eight patients (more than 500 pg/ml). The highest level was in one female with bilateral nephrectomy

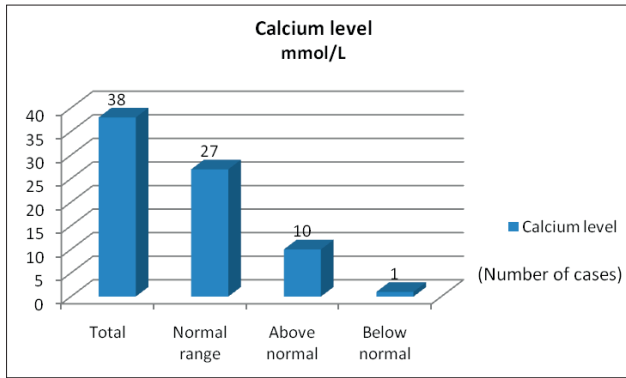


Figure 3. Number of cases with different calcium level range in plasma. Normal concentration is between 2.10-2.65 mmol/L.

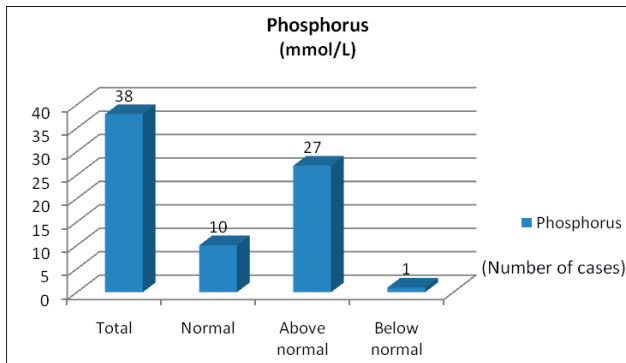


Figure 4. Number of cases with different level of phosphorus in plasma. Normal concentration is between 1.0-1.4 mmol/L.

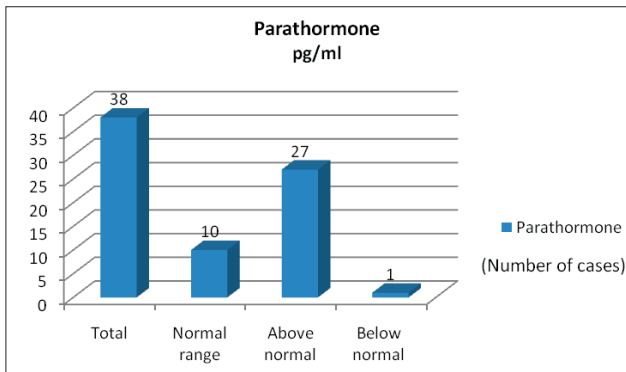


Figure 5. Number of cases with different level of parathormone in plasma. Normal concentration is between 9.5-75 pg/ml.

(2630 pg/ml). All patients were on vitamin D3 and calcium therapy, either with oral or intravenous supplementations. A few patients were being treated for hyper phosphatemia, but not continuously. Itching, as minor problem, was noted to be consistent in 8 (21%) out of 38 patients. Some patients had metastatic calcification occurring in the muscular tendons. These results were in concordance with others published in recent literature. It is important to note that most of the patients had low level of vitamin D3 despite of regular supplementation therapy.

**5. DISCUSSION**

Very large number of people worldwide suffers of vitamin D3 deficiency. It's considered as much as 20 percent of people worldwide were estimated (3). Vitamin D deficiency is most common in elderly people, postmenopausal women in patient with bad general metabolic status. It's considered that up to 70-

Serum 25(OH) D (mg/mL) [nmol/L]	Definition	Ergocalciferol Dose (Vitamin D <sub>2</sub> )	Duration (months)
(< 5) [ 12 ]	Severe vitamin D deficiency	50,000 IU/wk orally x 12 wks, then monthly	6 months
		500,000 IU as single I.M. dose	6 months
(5-15) [12-37]	Mild vitamin D deficiency	50,000 IU/wk x 4 weeks, then 50.000 IU/month orally	6 months
(16-30) [40-75]	Vitamin D insufficiency	50,000 IU/month orally	6 months

Table 1. Recommendation for therapy of Vitamin D with appropriate doses and duration.

80% of patients with CKD has vitamin D deficiency.

Multiple studies have shown an association between the use of vitamin D therapy in patients on dialysis and with CKD and improve survival. (11, 12). Active vitamin D therapy has been associated with slower progression of end stage renal disease (ESRD) (13).

Is it possible to improve overall outcome of vitamin D supplementation by special nutritional diet, rich vitamin D? Some studies have been done, but no benefit with diet supplementation (14). Only active vitamin D supplementation should be done, oral or intravenous (15, 16).

According to recommendation of KDOQI guidelines supplementation of vitamin D is recommended for dialysis patients as well as for those with stage 3 and 4 of CKD, if basal level of 25(OH)D is less than 30 ng/ml. Recommended doses in various level of vitamin D deficiency were shown in Table 1 (17). These guidelines recommended vitamin D supplementation any time if parathormone was above normal range nevertheless level if vitamin D in blood is satisfied or not (17).

Many observational studies have been done to evaluate calcium and phosphate levels during vitamin D supplementation therapy. In very large Meta-analysis of observational studies concerning vitamin D supplementation no changes of calcium and phosphate levels were shown (18).

Kidney are the primary sites for hydroxylation of vitamin D, and his process is as much inhibited as the global kidney function become worse. But it's known so, there are much more functions of vitamin D except of bone metabolism and calcium and phosphorus turnover (19).

Like in recent literature data (20,21), this study shows importance of vitamin D supplementation for dialysis patients and pre dialysis ones, as well. Our patients show vitamin D deficiency despite active vitamin D supplementation therapy. Some changes in supplementation protocols should be done. Moreover measurement of plasma levels of vitamin D should be scheduled for surveillance of dialysis and pre dialysis patients. In our study as much as one half of pre dialysis patients have vitamin D deficiency. Supplementation therapy of these patients is just sporadic. Some patients take combination of calcium and vitamin D in one tablet. This recommendation should be established as soon as possible, the best in primary care level.

**6. CONCLUSION**

Vitamin D3 deficiency, measured as 25-hydroxivitamin D3, was registered in most hemodialysis patients; nevertheless supplemental therapy was given regularly or not. It is to be considered more appropriate supplementation of Vitamin

D3 for dialyzed patients as well as for pre dialysis ones. In pre dialysis patient moderate deficiency is shown in half of patients but sever in only two.

CONFLICT OF INTEREST: NONE DECLARED.

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