UNIVERSITY OF MEDICINE AND PHARMACY
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MEDICINE

DIAGNOSTIC AND THERAPEUTIC UTILITY OF VITAMIN D IN CHRONIC KIDNEY DISEASE-MINERAL AND BONE DISORDER

PhD THESIS SUMMARY

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Working hypothesis and general objectives

Vitamin D is essential for the human body, being involved, by exerting its endocrine actions, in the homeostasis of mineral and bone metabolism, but with effects also on other levels, by means of exerting its paracrine actions, such as the modulation of the immune system, cellular proliferation and differentiation and cardiovascular system (1). The present research focuses on vitamin D deficiency: its definition, implications and the correct therapeutic approach in patients with chronic kidney disease (CKD).

Chronic kidney disease associated mineral and bone disorder (CKD-MBD) is a category of complications that occurs invariably and relatively early during the progression of CKD. They encompass biochemical abnormalities, bone anomalies, and ectopic calcifications (2). CKD-MBD is considered to be a non-traditional cardiovascular (CV) risk factor with an impact on cardiovascular morbidity and mortality, and this relationship is mediated through increases in FGF23 levels, development of secondary hyperparathyroidism (SHPT), vitamin D deficiency, and vascular calcifications (3). At the same time, bone anomalies are associated with a higher incidence of fractures, which also increases the risk of death.

The introduction of the CKD-MBD concept has allowed progress in understanding its pathogenesis. For example, relationships between calcidiol levels and bone turnover markers have been suggested, although the type of bone lesion in CKD-MBD is still accurately determined only by bone biopsy. Moreover, supporting data concerning the utility of the calcidiol - bone turnover markers relationship are limited.

On the other hand, advances in CKD-MBD therapy have been much more limited. Among the components of CKD-MBD, the improvement of SHPT constitutes one of the central therapeutic strategies. For this purpose, guidelines propose a gradual therapeutic approach, starting with the correction of nutritional vitamin D deficiency with natural vitamin D compounds and continuing, in the absence of achieving correction, with vitamin D receptor activators (2). However, the effects of natural vitamin D compounds on SHPT have essentially different mechanisms compared to vitamin D receptor activators.

Native compounds provide calciferol, the substrate for successive hepatic and renal hydroxylation, which lead to the formation of calcitriol, the active endocrine form of vitamin D. Correcting calciferol nutritional deficiency is effective in correcting SHPT as long as there

is sufficient renal hydroxylase activity, that is, in most cases, as long as the eGFR is greater than 15 mL/min.

Vitamin D receptor activators do not require renal hydroxylation. Additionally, some may have selective activity, preferentially inhibiting the parathyroid gland. In other words, they have two advantages over natural compounds: (i) they can be active even when eGFR drops below 10-15 mL/min, and (ii) the risk of causing hypercalcemia and hyperphosphatemia is lower. However, the cost of therapy is much higher.

It is possible that extrarenal hydroxylation also contributes to the formation of active endocrine calcitriol, and the calcidiol produced as a result of nutritional supplementation with cholecalciferol may stimulate parathyroid vitamin D receptors, reducing PTH levels. Therefore, it is possible that supplementation with native vitamin D compounds may allow the correction of SHPT even when there is a renal hydroxylase deficiency in patients with eGFR lower than 10-15 mL/min, but studies on this effect are inconclusive.

The hypotheses of this work are:

1. In non-dialyzed patients with advanced CKD, there is a relationship between bone turnover markers and calcidiol levels.

2. Vitamin D deficiency may contribute to the development of arterial lesions (arteriosclerosis, atherosclerosis).

3. Correcting nutritional vitamin D deficiency may reduce PTH levels similarly to vitamin D receptor activators in non-dialyzed patients with advanced CKD and secondary hyperparathyroidism.

Consequently, the main objectives of this work were:

1. To evaluate the prevalence of vitamin D deficiency and its determinants, as well as the relationships between vitamin D deficiency, bone turnover markers, and subclinical markers of vascular calcifications.

2. To assess the therapeutic efficacy in controlling CKD-MBD parameters and the therapeutic safety of treatment with native vitamin D (cholecalciferol) compared to a vitamin D receptor activator (paricalcitol).

General methodology

To test the proposed hypotheses, two studies were conducted.

Study 1:

- Observational, multicentric
- 128 patients, divided based on serum 25(OH)D levels:
  - <15 ng/mL (n = 81);
  - ≥15 ng/mL (n = 47).
- Selection criteria:
  - Inclusion: CKD (<60 mL/min/1.73m^2 and/or urinary albumin/creatinin ratio >30 mg/g, both preexisting for at least 3 months prior to inclusion in the study)
- Exclusion: Anticipated need for renal replacement therapy within the next 12 months, malignancies, active infections, prior treatment with vitamin D compounds
- Main parameters
  - CKD-MBD associated: serum calcium, phosphate, bone alkaline phosphatase 25(OH)D, PTH;
  - Arterial lesions associated: IMT, Kaupilla score, CAVI.
- Statistical analysis (performed using Analyse-it Medical Edition and IBM SPSS Statistics 20):
  - Group comparisons: Student t, ANOVA, Kruskal-Wallis, Pearson's Chi2;
  - Relationships between parameters: Kendall τ; bivariate and multivariate regression models

Study 2.

- Interventional, randomized, multicentric.
- Subjects:
  - Control (colecalciferol, 1000 UI/day), n=23;
  - Intervention (paricalcitol 1or 2 mcg/day), n=24.
- Primary. Median changes between the end-of-study versus baseline levels for:

- Parathormone ($\Delta iPTH_{6-0}$), calcidiol [$\Delta 25(OH)D_{6-0}$] and total alkaline phosphatase (ΔALP_{6-0}) (i.e. variation of serum concentrations), for the biochemical parameters,
- CAVI ($\Delta CAVI_{6-0}$) and ABI (ΔABI_{6-0}), for the arterial parameters.

- Safety parameters
  - Incidence of hypercalcemia episodes (>10.5 mg/dL), hyperphosphatemia episodes (>5 mg/dL).
  - Change in eGFR at 6 months compared to baseline values (ΔeGFR6-0)
- Selection criteria:
  - Inclusion:
    - CKD (<60 mL/min/1.73m^2 and/or urinary albumin/creatinin ratio >30 mg/g, both preexisting for at least 3 months prior to inclusion in the study).
    - elevated iPTH above the upper normal reference value of the laboratory (>75 pg/mL)
  - Exclusion: Anticipated need for renal replacement therapy within the next 12 months, malignancies, active infections, prior treatment with vitamin D compounds
- Statistical analysis (performed using IBM SPSS Statistics 20):
  - Group comparisons (intergroup and intra-group): Student t, ANOVA, Kruskal-Wallis, Mann-Whitney, Pearson's Chi2.

Study 1: Vitamin D deficiency, bone turnover markers and arterial calcifications in non-dialysis chronic kidney disease patients

Introduction

Inadequate levels of vitamin D are a global public health concern, but the definitions of vitamin D insufficiency and deficiency remain controversial in the medical community (4–6). Data from the literature report high prevalence rates of vitamin D deficiency worldwide, with significant variability due to geographical, climatic factors, as well as population factors such as sex, age, ethnicity, or socio-economic context (7–11).

For patients with CKD, the prevalence of vitamin D deficiency is higher (12), reaching 20-30% in CKD stages G4-G5 (13), independent of other determinants. This difference is directly related to the presence of CKD and can be explained by several categories of causes, such as substrate (cholecalciferol) deficiency or mechanisms intrinsically related to CKD, such as CKD-MBD complications (14,15).

Studies in the general population suggest a relationship between vitamin D deficiency and cardiovascular (CV) risk, though this has not been confirmed in interventional studies (16). This relationship is even stronger in CKD, consecutive to CKD-MBD, and, in particular, due to vascular calcifications.

In Romania, to date, only one study has addressed the issue of vitamin D status in the general population (17). Regarding CV risk in CKD patients with vitamin D deficiency, only one Romanian study has evaluated the relationships between vitamin D status and subclinical markers of cardiovascular disease, identifying vitamin D deficiency as an independent predictor only for the reduction of the ankle-brachial index (ABI) among a series of evaluated subclinical cardiovascular damage parameters (18).

Materials and Methods

Objectives

1. To assess the prevalence of vitamin D deficiency and to identify its determinants in pre-dialysis CKD patients.
2. To assess the effects of vitamin D deficiency on bone turnover markers.
3. To assess the relationships between vitamin D deficiency and subclinical arterial calcification parameters at the intima and media levels.

Study design and assessments

This was a cross-sectional, prospective study that enrolled patients from two tertiary nephrology centers in the southern part of the country.

The enrollment period lasted 10 months (from August 2010 to June 2011). Following the signing of the participation consent and confirmation of eligibility, the study procedures were initiated, and visits in Clinics (V1 and V2) were scheduled for data collection as follows:

- **Visit 1 (V1):** General data, patient history, clinical and laboratory data.
- **Visit 2 (V2):** Paraclinical data involving measurements of vascular parameters.

A total of 187 patients were considered eligible, of whom 151 agreed to participate in the study. Thirteen did not attend V1 as scheduled, 6 did not attend V2, and 4 withdrew their consent to participate in the study.

The final study cohort consisted of 128 patients, divided based on serum 25(OH)D levels: <15 ng/mL - subjects with vitamin D deficiency, grouped in the deficiency group (n = 81), and ≥15 ng/mL, in the sufficient level group (n = 47) (**Figure 5.1**).

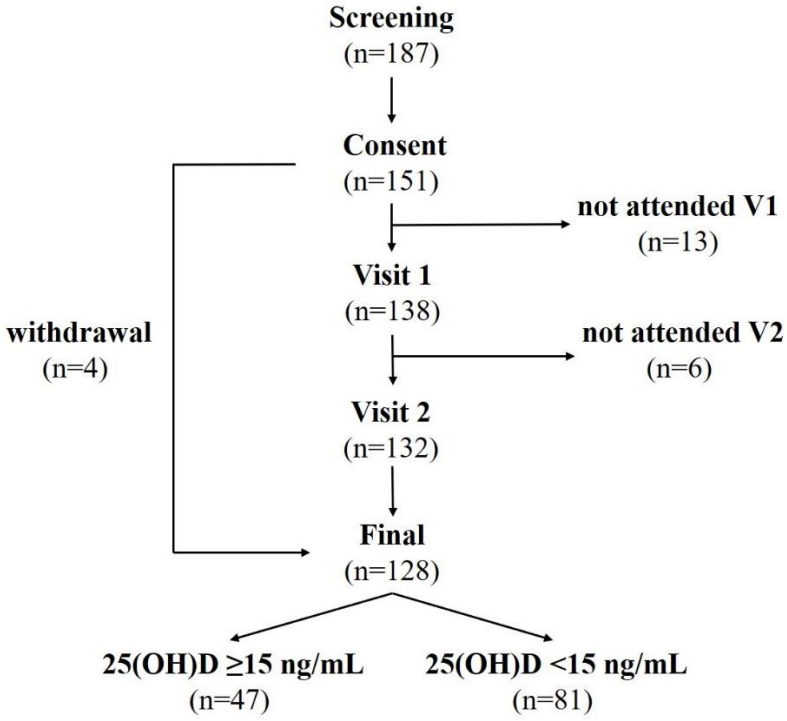

Figure 5.1. Diagram of Study 1

Selection Criteria are detailed in the General Methodology section.

Study Parameters

General parameters: Identification data (name, surname), demographic data (age, sex).

Medical History:

- Primary renal disease, CKD category (G3-G5) based on the CKD-EPI 2009 formula.
- Presence and duration of cardiovascular comorbidities: hypertension (HTN), diabetes mellitus (DM), and vascular disease in the coronary (CAD), cerebral (CVD), or peripheral (PAD) territories.
- Current medication.

Clinical parameters:

- Height, weight, and calculation of body mass index (BMI).
- Systolic and diastolic blood pressure (BP), with calculation of mean arterial pressure (MAP).

Paraclinical parameters:

- Blood: routine tests, mineral-bone metabolism tests, inflammation markers.
- Urinary: creatinine and urinary albumin (g/L), albuminuria (urine albumin-to-creatinine ratio, mg/g).
- Vascular parameters (arterial disease):
    - Atherosclerosis**:** Intima-media thickness (IMT). IMT values >0.08 cm were considered abnormally elevated.
    - Vascular Calcification: Aortic calcification score (Kauppila score). Values ≥1 were considered abnormally elevated.
    - Vascular Stiffness: Cardio-ankle vascular index (CAVI). A CAVI value >9 indicated arterial stiffness (arteriosclerosis), a value ≤8 indicated normal arterial elasticity, and values between 8 and 9 were considered borderline (19).

Results

Prevalence of vitamin D deficiency and its determinants

Vitamin D deficiency had a prevalence of 63%, with a median serum calcidiol level of 12.8 ng/mL across the entire study cohort

Table 5.2. Factors associated with 25(OH)D deficiency

	Bivariate	0		Multivariate†		
	Kendall's τ (95% CI)		Sig.	B±SE	Beta (95% CI)	Sig.
Age (years)	-0.17 (-0.28; -0.06)		0.005	-0.51±0.21	0.60 (0.40; 0.91)	0.02
Sex (F/M)*	2.18 (1.02; 4.65)		0.04	0.97±0.44	2.65 (1.11;6.30)	0.04
Diabetes mellitus (Yes/No)*	3.4 (1.3;8.8)		0.01	0.63±0.58	1.87 (0.60; 6.63)	0.06
eGFR (mL/min)	0.08 (-0.04; 0.21)		0.18			
ACR (mg/g)	-0.13 (-0.25; 0.00)		0.03			
Serum calcium (mg/dL)	-0.04 (-0.16; 0.08)		0.49			
Phosphate (mg/dL)	-0.08 (-0.20; 0.03)		0.18			
Parathormone (pg/mL)	-0.12 (-0.23; -0.01)		0.05	-0.004±0.00	1.00 (0.99; 1.00)	0.02
CRP (mg/L)	-0.21 (-0.33; -0.10)		0.001	-0.39±0.22	0.68 (0.44; 1.04)	0.07

*****Odds ratio
†Model of binary logistic regression 25(OH)D <15 vs. ≥15 ng/mL
Nagelkerke R^2 0.27; p=0.000; Hosmer and Lemeshow Test p=0.45
Variable entered on step 1: Age, Sex (M/F), Diabetes mellitus (Y/N), C-Reactive protein, Parathormone, Urinary albumin/creatinine ratio (ACR)

In the bivariate analysis model, advanced age, female sex, the presence of diabetes mellitus (DM), and higher C-reactive protein (CRP) values were correlated with serum calcidiol levels of less than 15 ng/mL. Among mineral parameters, parathyroid hormone (PTH) was the only factor that influenced vitamin D levels. Estimated glomerular filtration rate (eGFR) did not affect serum vitamin D levels, unlike albuminuria, which was negatively correlated with vitamin D deficiency (**Table 5.2**).

In the multivariate analysis, independent predictors of vitamin D deficiency were advanced age and female sex (among general factors) and higher PTH levels (among phosphorus-calcium metabolism factors) (**Table 5.2**).

Vitamin D deficiency and bone turnover markers

In the bivariate regression model, higher serum levels of alkaline phosphatase (ALP were correlated with younger age, higher phosphate and PTH levels, and lower calcidiol levels. In the multivariate regression model, only PTH remained an independent predictor of ALP (**Table 5.3**.).

Conversely, PTH was an independent predictor of vitamin D deficiency, being inversely correlated with serum vitamin D levels (**Table 5.2.**). Therefore, a higher ALP level was

independently associated with increased serum PTH levels and lower calcidiol levels, which may suggest increased bone turnover induced by vitamin D deficiency.

Table 5.3. Determinants of serum alkaline phosphatase

	Bivariate			***Multivariate**		
	Kendall's τ (95% CI)	0	Sig.	B±SE	Beta (95.0% CI)	Sig.
Age (years)	-0.19 (-0.3; -0.08)		0.00	-0.03±0.09	-0.03 (-0.21; 0.14)	0.70
Calcium (mg/dL)	-0.01 (-0.13; 0.11)		0.84			
Phosphate (mg/dL)	0.15 (0.02; 0.274)		0.02			
PTH (pg/mL)	0.14 (0.01; 0.27)		0.02	0.20±0.09	0.20 (0.03; 0.37)	0.02
25(OH)D (ng/mL)	-0.16 (-0.27; -0.05)		0.01	-0.15±0.09	-0.15 (-0.33; 0.02)	0.09

Linear regression model; R2=0.05; p=0.02
Dependent variable: serum alkaline phosphatase
Predictors: Age; Calcium; Phosphate; Parathormone (PTH); 25(OH)D
All variables were transformed for accuracy

Evaluation of the relationships between vitamin D deficiency and subclinical arterial calcification parameters

Each of the cardiovascular parameters, both clinical and paraclinical (except for CAVI), were significantly and negatively correlated with vitamin D deficiency (**Table 5.4.**).

Table 5.4. The relationships between 25(OH)D and the investigated cardiovascular parameters

	Kendall's τ 95% CI			**0**	**Sig.**
Antecedents of CVD	-0.20	0.30	-0.09		0.007
Intima-media thickness	-0.17	0.29	-0.04		0.01
Aortic calcifications score	-0.19	0.31	-0.08		0.005
Cardio-ankle vascular index	-0.08	0.19	0.04		0.24

Carotid intima-media thickness (IMT)

The median carotid intima-media thickness (IMT) was within normal limits across the entire cohort (0.08 cm), but it was higher in the vitamin D deficiency group (0.08 cm vs. 0.06 cm, p=0.03). In the bivariate analysis, IMT was directly correlated with age, BMI, presence of diabetes, and CRP levels, and inversely correlated with vitamin D levels. None of the other parameters with potential pathogenic roles in the formation and calcification of atheroma

plaques were associated with IMT values. After adjustment, age remained the only independent predictor for IMT value (**Table 5.6**).

Aortic calcification score (Kauppila score)

An abnormal aortic calcification score (Kauppila score) (≥1) was present in 57% of the study participants. A higher aortic calcification score was directly correlated with age, diabetes mellitus (DM), CRP levels, and inversely correlated with 25(OH)D levels, as determined by bivariate analysis.

In multivariate analysis, only advanced age and lower 25(OH)D levels remained independent predictors of a higher calcification score (**Table 5.6**). Therefore, it can be inferred that vitamin D deficiency appears to promote the development of focal intimal vascular calcifications within atheroma plaques.

Cardio-ankle vascular index (CAVI)

Approximately half of the study participants had pathological CAVI values. Although those in the vitamin D deficiency group had a higher CAVI than those without deficiency (10.5 vs. 9.9), the difference was not statistically significant (p=0.27). Bivariate analysis identified weak associations with older age and lower 25(OH)D levels. In logistic regression model, only older age kept its statistical significance (**Table 5.6**).

Testing for associations between the evaluated arterial disease parameters revealed a significant correlation of CAVI with the aortic calcification score (Kendall τ 0.28; $p<0.000$), suggesting a relationship between the degree of atheroma plaque calcification, dependent on vitamin D deficiency levels, and arterial stiffness.

Table 5.6. Determinants of carotid intima media thickness, aortic calcification score and cardio-ankle vascular index in CKD patients

			Sig.
Carotid Intima-Media Thickness	**B±SE**	**Exp(B) (95% CI)**	
Age (years)	0.03±0.01	0.52 (0.02; 0.04)	0.000
Body mass index (kg/m^2)	0.02±0.01	0.13 (0.00; 0.05)	0.10
Diabetes mellitus	0.22±0.18	0.10 (-0.13; 0.58)	0.22
C reactive protein (mg/dL)	0.01±0.00	0.11 (0.00; 0.01)	0.14
25(OH)D (ng/mL)	-0.01±0.01	-0.04 (-0.03; 0.02)	0.57
Constant	-2.55±0.46	(-3.47; -1.64)	0.000
Model of linear regression; adjusted R^2 0.36; p=0.000 Dependent variable Intima media thickness			
Aortic calcifications score	**Estimates (95% CI)**		
Age (years)	0.15 (0.10; 0.20)		<0.000
Diabetes mellitus	-0.16 (-2.0; 1.67)		0.86
C-reactive protein (mg/dL)	-0.01 (-0.01; 0.05)		0.58
25(OH)D (ng/mL)	-0.14 (-0.26; -0.03)		0.01
Constant	-3.32 (-6.85; 0.20)		0.06
Model of linear regression: adjusted R^2 0.30; p <0.0001 Dependent variable Aortic calcification score			
Cardio-ankle vascular index	**B±SE**	**Exp(B) (95% CI)**	
Age	0.09±0.02	1.10 (1.06; 1.14)	0.000
Body mass index (kg/m2)	-0.03±0.05	0.97 (0.88; 1.06)	0.47
25(OH)D (ng/mL)	-0.01±0.04	1.00 (0.92; 1.08)	0.99
Constant	-2.00±2.36	0.14	0.40
In each model were introduced variables which were significantly correlated with dependent variable (**Table IV**) Model of binominal logistic regression CAVI >9 vs. ≤9 Nagelkerke R^2 0.45; p=0.000; Hosmer and Lemeshow Test p=0.21			

Study 2: Nutritional or active vitamin D for the correction of mineral metabolism abnormalities in non-dialysis chronic kidney disease patients?

Introduction

The onset of CKD-MBD) depends on the level of renal function, as measured by eGFR. These abnormalities usually appear relatively early, with changes occurring as early as the eGFR drops below 60 mL/min (corresponding to CKD stage G2), being the case for FGF23 and Klotho (3). Regarding the biochemical component of CKD-MBD detectable by routine laboratory measurements, vitamin D deficiency (i.e. calcitriol deficiency) typically occurs concurrently with the increase of serum parathyroid hormone (PTH), usually when eGFR drops below 60-45 mL/min (corresponding to CKD stages G2-G3a) (3,20). Hypocalcemia, becomes apparent at eGFR below 30 mL/min, and hyperphosphatemia at eGFR below 30-20 mL/min, respectively (20,21). These changes appear initially as compensatory responses to declining renal function in order to maintain normal mineral homeostasis. Over time, however, they become maladaptive and tend to progressively worsen, alongside with the decline in renal function.

The importance of recognizing and treating CKD-MBD lies in its impact on cardiovascular morbidity and mortality in CKD patients. These complications are recognized by cardiologists and regarded as non-traditional cardiovascular risk factors (22).

In light of these findings, it becomes clear that nephrologists have a crucial responsibility to appropriately manage CKD-MBD, in order to delay the onset of these complications and mitigate their severity and clinical consequences, especially when it comes to those with a long-term impact.

The most recent KDIGO guideline recommends a step-by-step approach to correct mineral metabolism anomalies, with one of the most important aspects being the treatment of secondary hyperparathyroidism (SHPT) (2). The recommendation of the initiation of treatment is based on documenting an increasing trend in PTH. The proposed approach of treatment consists of firstly correcting modifiable factors influencing PTH synthesis and secretion: native vitamin D deficiency, hypocalcemia, hyperphosphatemia, or excessive dietary phosphate intake. For patients with CKD stages G3a-G5 in pre-dialysis, treatment with active vitamin D or vitamin D analogs is not recommended; these are reserved for CKD stages G4-

G5 with severe and progressive SHPT, due to the risk of hypercalcemic secondary effects and contested clinical benefits (2,23).

It is worth noting that the above recommendations have a level of evidence 2C, allowing the attending physician to choose the most appropriate therapeutic approach for a particular patient. The thresholds defining hyperparathyroidism, the biological values at which treatment is recommended and the choice of treatment remain, nevertheless, vague.

It is well-known that supplementation with native vitamin D compounds corrects nutritional deficiency, yet it remains controversial whether it also corrects other parameters of mineral metabolism, particularly SHPT. The rationale for using native vitamin D compounds to improve SHPT involves providing a substrate for extrarenal tissues, where 1α-hydroxylase is present and can therefore the active form of vitamin D could be synthesized. At the same time, the efficacy of active vitamin D compounds (VDRA) in correcting biochemical abnormalities (particularly SHPT and, to a lesser extent, calcitriol deficiency) is undisputed, though their non-endocrine benefits are supported by weak observational associations.

Therefore, the impact of treatment with native vitamin D or VDRA on CKD-MBD components remains unclear. In this context, the present study aimed to compare the effects of treatment with a native vitamin D compound versus a selective vitamin D receptor activator on biochemical abnormalities and arterial disease parameters in pre-dialysis CKD patients. The modifications in the aforementioned guideline deepen the issues related to vitamin D compound treatments, which only adds value to the results of this study. This was the first clinical study in Romania with these objectives.

Materials and methods

Objectives

1. Assesement of therapeutic efficacy on:
- biochemical components of CKD-MBD: parathormone (iPTH), calcidiol [25(OH)D], and total alkaline phosphatase (ALP),
- arterial function parameters: cardio-ankle vascular index (CAVI) and ankle-brachial index (ABI),
2. Assesement of treatment safety, by evaluating the effect on calcium, phosphate and glomerular filtration rate (eGFR).

To achieve these objectives, primary and secondary parameters of efficacy and safety were established, as detailed in the general methodology.

Study design and assessments

This was a prospective, interventional, open-label, randomized trial which enrolled patients from two tertiary nephrology centers in the southern part of the country.

Study duration was 6 months, with prior screening phase of 19 months (March 2010 - September 2011). The study was organized into two phases:

1. Screening - patients' inclusion and randomization 1:1, stratified by CKD G category, depending on the eGFR (G3-G5);

2. Intervention (6 months) - administration of one of the two compounds:

- Cholecalciferol, at a fixed dose of 1000 UI/day,
- Paricalcitol, at a dose of 1 or 2 mcg/day based on baseline iPTH levels: <500 pg/mL - 1 mcg/day and ≥500 pg/mL - 2 mcg/day, respectively.

After treatment allocation, subjects and parameters of interest were evaluated periodically at different frequencies according to the study protocol.

Target reductions for iPTH levels considered safe was a 30-60% decrease from baseline values. If this target was achieved, the dose of paricalcitol was maintained. Alternatively, the dose was adjusted based on the values of the safety parameters (calcium and phosphate levels) and the PTH levels respectively.

A total of 195 patients were initially evaluated. Of these, 157 met the criteria for CKD. Only 76 agreed to participate in the study. Among the 76, 48 had iPTH levels consistent with the definition of SHPT (i.e., iPTH >75 pg/mL) and were randomized 1:1 to receive either cholecalciferol (VitD group) or paricalcitol (VDRA group). During the study, one subject from the VitD group was excluded. The final statistical analysis included 23 subjects who received cholecalciferol and 24 subjects who received paricalcitol (**Figure 6.1**).

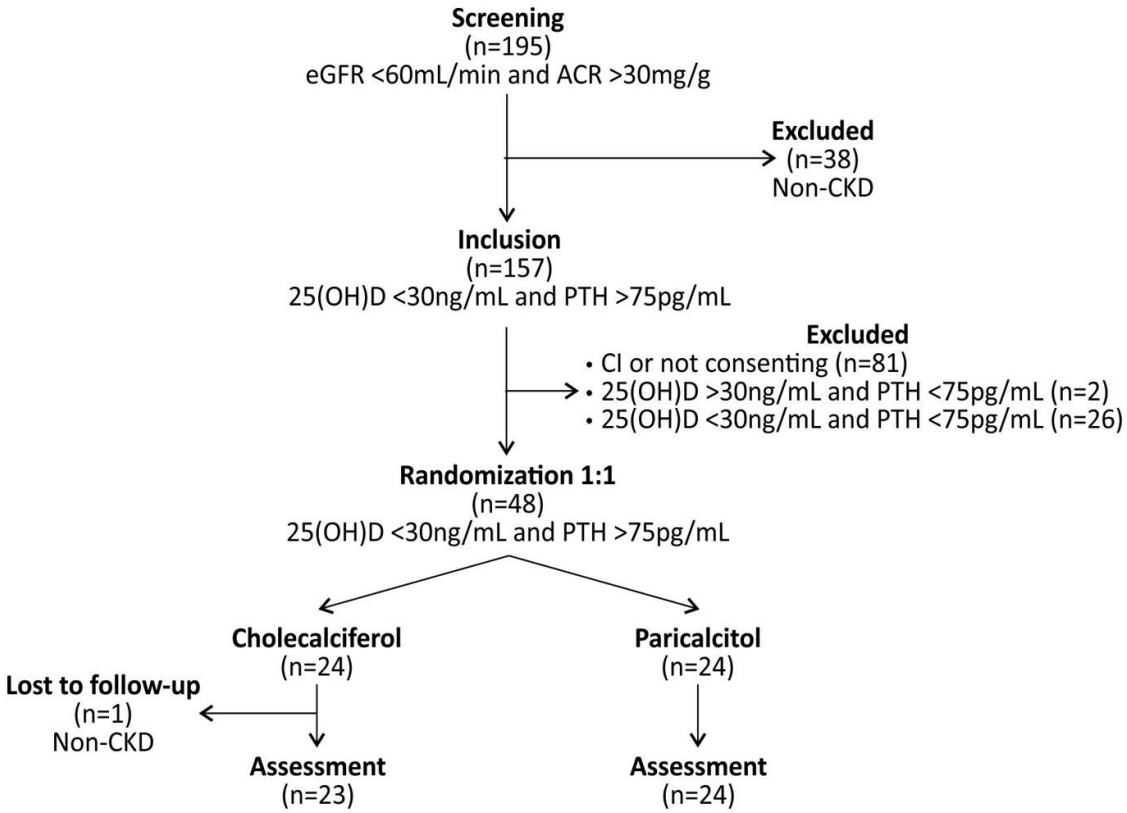

Figure 6.2. Diagram of Study 2

Selection Criteria are detailed in the General Methodology section.

Study Parameters

General parameters: Identification data (name, surname), demographic data (age, sex).

Medical History:

- Primary renal disease, CKD category (G3-G5) based on the MDRD4 formula;
- Presence and duration of cardiovascular comorbidities: hypertension (HTN), diabetes mellitus (DM), and atherosclerotic disease;
- Current medication, Smoking.

Clinical parameters:

- Height, weight, and calculation of body mass index (BMI);
- Systolic and diastolic blood pressure (BP), with calculation of mean arterial pressure (MAP).

Paraclinical parameters:

- Blood: routine tests, mineral-bone metabolism tests, inflammation markers.
- Urinary: creatinine and urinary albumin (g/L), albuminuria (urine albumin-to-creatinine ratio, mg/g), phophaturia, calciuria, urinary urea,
- Vascular parameters (arterial disease):
    - CAVI: a value >9 indicated the presence of arterial stiffness (arteriosclerosis). A value ≤8 indicated normal arterial elasticity. Values between 8 and 9 were considered borderline (19,24).
    - ABI: Normal ABI values are considered to be between 0.9 and 1.3. Values <0.9 were considered indicative of atherosclerosis (25). Values >1.3 were considered indicative of arteriosclerosis (26).

Results

Primary efficacy parameters

After the therapeutic intervention, the variation in iPTH in the cholecalciferol group was positive, reflecting an increase, while in the paricalcitol it was negative, reflecting a decrease in serum levels. The difference in iPTH variation between the two groups was significant (p=0.008) (**Table 6.2, Figure 6.1**). There was a trend towards a decrease in iPTH in the paricalcitol group, while the cholecalciferol group showed a trend towards an increase in iPTH at the end of the study. The difference between groups in iPTH variation became statistically significant by the end of the study.

These results suggest that there is an efficacy difference between the two preparations regarding SHPT in CKD, and that its correction may require a longer period of treatment (in this study, 6 months) (**Figure 6.1**).

The median increase in 25(OH)D levels compared to baseline was significantly higher in the cholecalciferol group at each visit (p<0.001) (**Table 6.2, Figure 6.2**). These results suggest superior efficacy of native vitamin D in improving vitamin D status compared to paricalcitol.

At the end of the study, those who received paricalcitol had a significant reduction in the median level ALP compared to those who received cholecalciferol. This is even more remarkable when taking into account that its median baseline level was significantly higher in the paricalcitol group (**Table 6.2, Figure 6.3**).

Tabel 6.2. The variation of the primary parameters of the study after the therapeutic intervention

Parameter	Group	Baseline (Bs)	End of study (EOS)	Difference EOS - Bs	p
iPTH (pg/mL)	VitD	80.8* (68.2; 95.4)	110.0 (64.5; 170)	13.3 (-8.1; 24.0)	0.008
	VDRA	161.0 (106.6; 294.3)	137.5 (85.4; 217)	-35.2 (-65.5; -12.6)	
25(OH)D (ng/mL)	VitD	11.6 (8.7; 15)	25.8* (22.9; 34.2)	15.5 (13.3; 17.1)	<0.001
	VDRA	15.7 (12.8; 18.2)	15.2 (9; 20.5)	0.4 (-5.4; 2.9)	
ALP (UI/L)	VitD	68* (61; 101)	59 (49; 75)	-16 (-23; -9)	0.02
	VDRA	95.5 (78; 126)	69 (47; 81)	-29 (-51; -14)	
CAVI	VitD	9.7 (9.4; 10.8)	9.7 (9.0; 10.3)	- 0.1 (-0.6; 0.2)	0.9
	VDRA	11.1 (10.2; 12.1)	10.3 (9.5; 12)	-0.15 (-0.3; 0)	
ABI	VitD	1.1 (1.1; 1.2)	1.1 (1.0; 1.2)	0.01 (-0.04; 0.05)	0.2
	VDRA	1.2 (1.1 1.2)	1.1 (1.0; 1.2)	-0.02 (-0.07; 0.01)	
eGFR (mL/min)	VitD	30 (25.4; 37.2)	29 (18; 41)	-2 (-5; 0)	0.3
	VDRA	25 (21.9; 33.9)	22 (14; 32)	-4 (-6; -2)	
ACR (mg/g)	VitD	47 (101; 582)*	38 (24; 198)	-5 (-39; 20)	0.6
	VDRA	286 (322; 1452)	207 (32; 550)	-19 (-202; 30)	

Data are presented as median and 95%CI; *significant difference ($p<0.05$) baseline-end of study, p: comparisons od median values (EOS - Bs) between groups
iPTH: intact parathormone; 25(OH)D: calcidiol; ALP: total alkaline phosphatase; CAVI: cardio-ankle vascular index; ABI: ankle-brachial index; eGFR: estimated glomerular filtration rate; ACR: albumin/creatinine ratio (mg/g); VDRA - Vitamin D receptor activator group (paricalcitol); VitD - cholecalciferol group

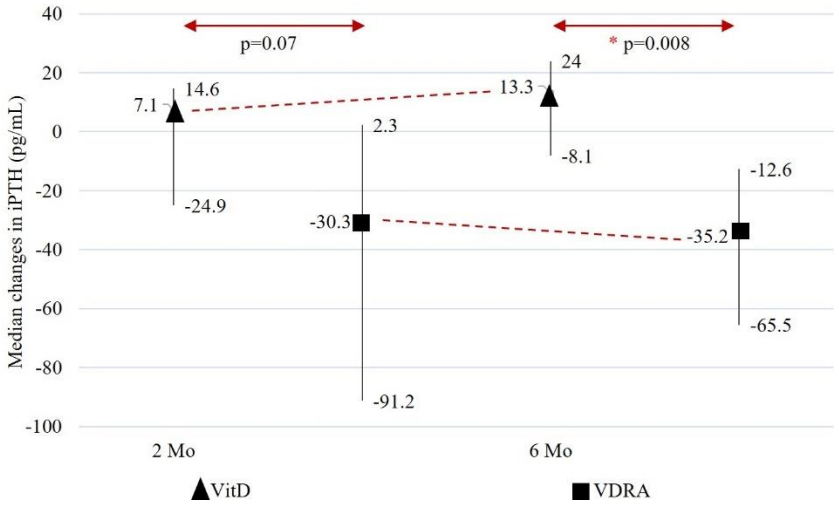

Figure 6.1. Median changes of PTH at 2 months and at EOS

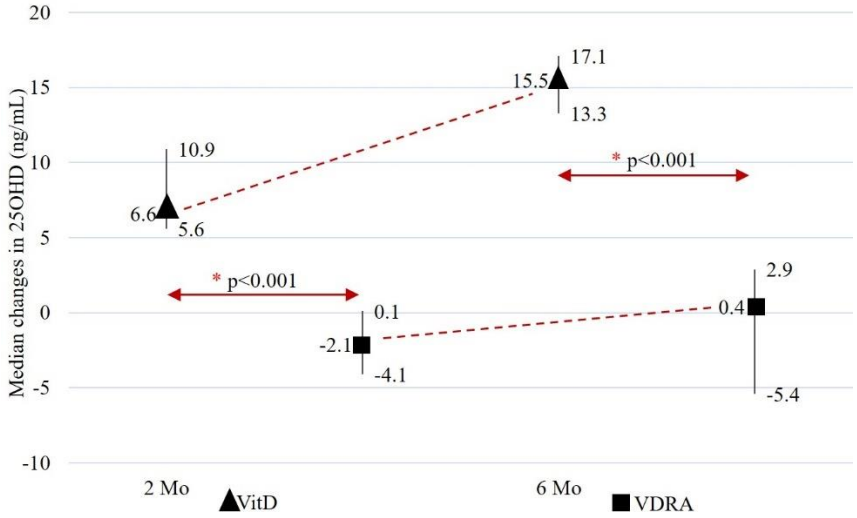

Figure 6.2. Median changes in calcidiol at 2 months and EOS

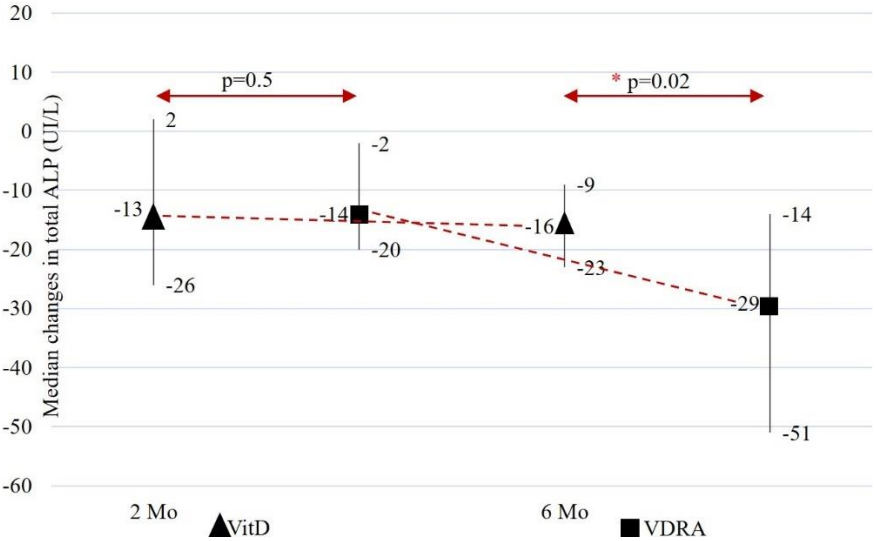

Figure 6.3. Median changes in total ALP at 2 months and EOS

There were no significant changes between groups at the end of the study versus baseline for none of the arterial parameters (**Table 6.2.**).

Secondary Efficiency Parameters

Effects on serum parathyroid hormone

The percentage of patients with a reduction of at least 30% in the median iPTH at the end of the study compared to baseline was almost double in the VDRA group (25% vs. 13%),

but the difference was not significant. However, the percentage of patients who had any degree of reduction in iPTH levels revealed a superior efficacy of paricalcitol treatment (**Table 6.3**).

The serum iPTH levels at intermediate visits in the cholecalciferol group remained relatively constant, except for the final visit, when it showed a tendency to increase. On the other hand, the paricalcitol group showed a marked reduction in iPTH levels between baseline and the first visit (2 months), despite significant higher initial values in this group. Subsequently, the levels tended to progressively increase, which cancelled the significant differences between groups at the end of the study (**Figure 6.4**).

Effects on serum calcidiol

The proportion of patients whose vitamin D status normalized was significantly higher in the cholecalciferol-treated group, while it remained unchanged in those treated with paricalcitol (**Table 6.2, Figure 6.5**). All patients in the cholecalciferol group showed an increase in serum calcidiol levels, in contrast to the paricalcitol group - just over half of the cases (100% vs. 54%, p<0.001) (**Table 6.2**). The increase in 25(OH)D was prompt, consistent, and progressive only in the cholecalciferol group (**Figure 6.6**).

These results argue for the undeniable utility of natural vitamin D compounds in correcting inadequate vitamin D status in pre-dialysis CKD patients. At the same time, they highlight the lack of benefit in this regard from VDRA.

Table 6.3. Effects of therapeutic interventions on secondary efficacy and primary safety parameters

Parameter	VitD (n=23)	VDRA (n=24)	Difference between groups (VitD-VDRA)*	p
Patients with iPTH >30% at EOS (%)	13	25	-12 (-17; 27)	0.3
Patients with any reduction of iPTH at EOS (%)	39	71	- 32 (-3; -56)	0.03
Patients with 25(OH)D levels ≥30 ng/mL (%)	39	8	31 (7; 53)	0.02
Patients with increased 25(OH)D levels at EOS (%)	100	54	46 (28; 65)	<0.001
Patients with CAVI reduction compared to baseline (%)	52	67	0.53 (0.30; 0.94)	0.3
Patients with CAVI reduction <9 (%)	4	8	0.49 (0.14; 1.68)	0.2
Hypercalcemia incidence (>10.5 mg/dL) (%)	0	4	2 (-3; 7)	0.5
Hyperphosphatemia incidence (>5 mg/dL) (%)	13	17	12 (-6; 27)	0.9

Expressed as median (95% CI);
VitD - cholecalciferol group; VDRA - Vitamin D receptor activator group (paricalcitol); iPTH: intact parathormone; 25(OH)D: seric calcidiol.

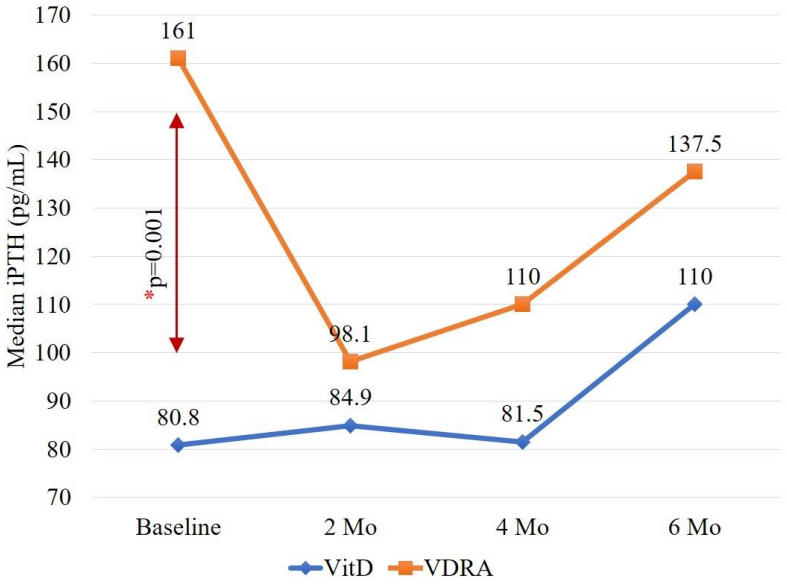

Figure 6.4. Serum iPTH levels at study moments in VitD and VDRA groups

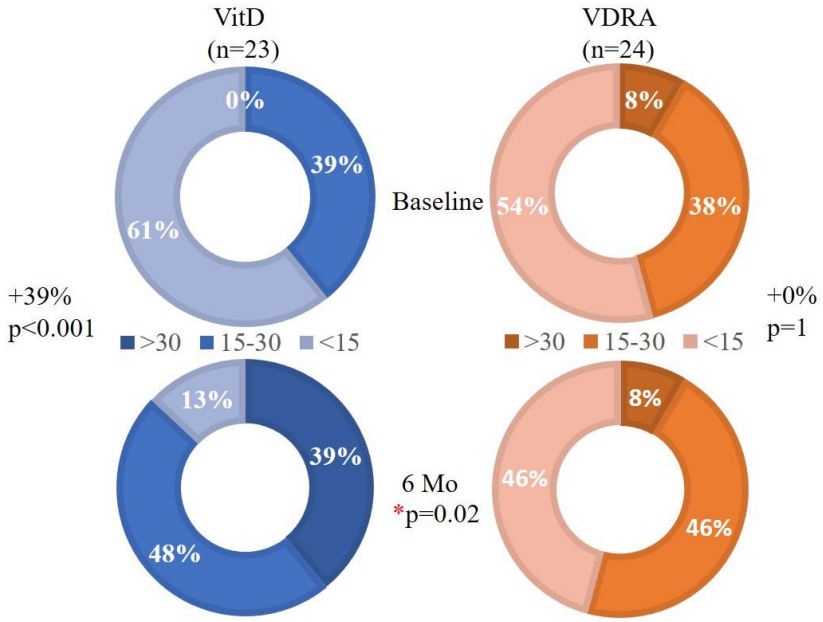

Figure 6.5. Percentage of patients with normalization of vitamin D status (calcidiol ≥30 ng/mL)

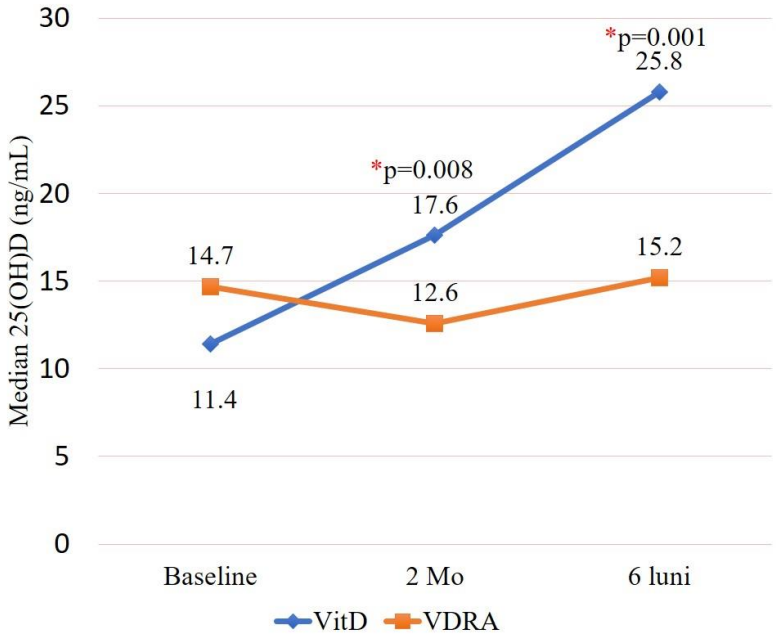

Figure 6.6. Serum calcidiol levels at study moments in VitD and VDRA groups

Effects on total alkaline phosphatase

The evolution of the median values of total alkaline phosphatase in each treatment group is shown in **Figure 6.7**.

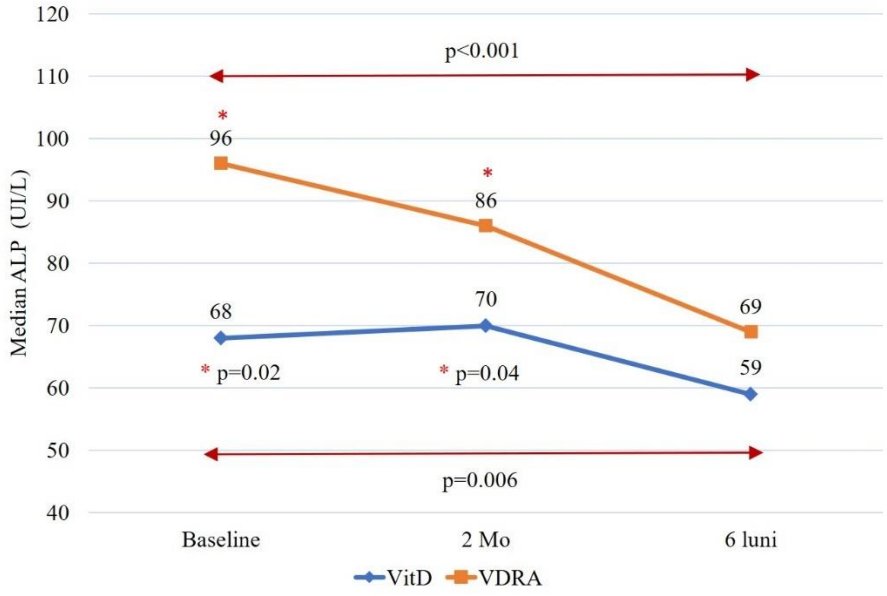

Figure 6.7. Serum ALP levels at study moments in VitD and VDRA groups

The patients treated with paricalcitol experienced a prompt, linear, and significant decrease in ALP compared to baseline, starting from the first intermediate evaluation. This effect was not observed in those treated with cholecalciferol. The reduction effect of paricalcitol on ALP values was strong enough to diminish and eventually cancel the significant difference that existed at baseline by the end of the study period (**Figure 6.7**.), similar to effects exerted on iPTH.

Effects on Arterial Parameters

None of the arterial parameters were influenced by either of treatments (**Table 6.3**). There were no significant differences in the median values of ABI and CAVI at the end of the study compared to baseline. These results suggest the absence of any effect on arterial function parameters by either type of vitamin D compounds, which could be partially explained by the relatively short duration of therapeutic intervention.

Primary Safety Parameters

The incidence of hypercalcemia (CaT >10.5 mg/dL) was low and similar between the groups. Hyperphosphatemia (Pi >5 mg/dL), though more frequent, had a similar incidence in both groups (**Table 6.3.**). The variation in renal function at the end of the study (ΔeGFR6-0), evaluated by eGFR did not differ between the two groups (p=0.3, **Table 6.2.**).

Secondary Safety Parameters

At the end of the study, renal function seemed to be influenced by paricalcitol treatment when compared to baseline within each group. In the group that received cholecalciferol, eGFR remained almost unchanged, while in the paricalcitol group, a slight but significant reduction (by 2 mL/min) was observed (p=0.01). Serum calcium and phosphate concentrations did not vary significantly during the study in either group.

Conclusions and personal contributions

Vitamin D deficiency is a global public health issue, characterized by high prevalence and association with increased cardiovascular risk, which is closely related to its pleiotropic effects. The prevalence of inadequate vitamin D status is higher, and its relationship with cardiovascular morbidity and mortality is more pronounced in patients with CKD. Additionally, the burden of CKD and its complications is increasing with the aging of the population. All of these justify the focus on the subject of vitamin D deficiency and its implications in patients with renal impairment. As such, pre-dialysis CKD patients from southern Romania were evaluated to assess vitamin D deficiency and the effects of the therapeutic intervention with vitamin D preparations on the CKD-MBD parameters.

As shown in the first study, the prevalence of vitamin D deficiency in non-dialysis CKD population is high, with more than a half of the subjects having serum calcidiol levels below 15 ng/mL. Compared to the general population in Romania, the prevalence of vitamin D deficiency in the pre-dialysis CKD patients in the study group was nearly two and a half times higher, and the average serum calcidiol level in the deficient population was half as much. This aligns with the ideas previously mentioned. When comparing the prevalence of vitamin D deficiency in CKD patients from southern Romania to those in Europe, the Romanian population seem to also have higher values - about 1.5 to 2.5 times higher, depending on the reported data.

The conclusion is that in Romania, the prevalence of inadequate vitamin D status in CKD patients is higher compared to non-CKD individuals and CKD patients from other countries. This emphasizes the need to focus the attention on systematic screening methods and on the supplementation strategies with native compounds for this population. Developing national medical protocols for treating vitamin D deficiency in CKD patients, independent of CKD-MBD onset, is of interest.

The at-risk population among CKD patients for vitamin D deficiency in the presented study were the elderly, women, and those with more severe SHPT, suggesting the need for more effective therapeutic intervention on SHPT, potentially through correcting vitamin D deficiency status. This lays the foundation for the second study. Furthermore, SHPT was associated with higher bone turnover, and vitamin D deficiency, being associated with SHPT,

seems to be negatively affecting bone turnover. Although the clinical effects are less dramatic, they are associated with musculoskeletal complications such as increased fracture risk, pain, and bone deformities, impacting quality of life and increasing morbidity and mortality. This highlights the importance of a correct and complete CKD-MBD treatment.

Vitamin D deficiency was associated with a higher prevalence of cardiovascular disease and was an independent predictor of arterial calcification, without being associated with other evaluated vascular disease markers. This aligns with the literature data: one of the pathogenic mechanisms through which vitamin D influences cardiovascular health is VDR-mediated modulation of arterial calcifications. Specifically, arterial calcifications in CKD are more likely to occur in the media, resulting in increased arterial stiffness. However, in the analyzed cohort, increased arterial stiffness was primarily due to calcification in atherosclerotic plaques.

This indicates that the etiopathogenesis of mineral metabolism disorders and arterial calcifications in CKD patients is complex and intertwined, with vitamin D deficiency playing a crucial role. Proper therapeutic management vitamin D deficiency has the potential to improve SHPT and cardiovascular prognosis. Therefore, the second study aimed to compare the efficacy of two different vitamin D derivatives, one native and one selective vitamin D receptor activator (VDRA).

The study group was similar to that in the first study: pre-dialysis CKD patients from southern Romania with vitamin D deficiency, who also had SHPT. The two compounds were tested following randomization, and the therapeutic intervention was of medium duration.

The main results revealed that native vitamin D treatment was effective only in improving vitamin D deficiency; neither the compound per se nor the supplementation of the substrate was sufficient to influence SHPT. However, the native compound doses were high enough to affect vitamin D status but likely insufficient to impact SHPT. On the other hand, the VDRA effect on improving SHPT and the lack of effect on correcting vitamin D deficiency became evident. The efficacy on vascular parameters was modest: neither compound influenced vascular stiffness markers, at least not in these doses and not after a medium-term administration. Both compounds were safe, at least in the used doses and in patients with moderate renal dysfunction who did not have pre-existing phosphate or calcium metabolism alterations.

The second study, the only one in the country with this design and objectives, had results similar to those in the literature. They reinforce the hypotheses about the role of various vitamin D compounds in treating CKD-MBD, highlighting, on one hand, the limitations of each compound and, on the other, the potential benefits of their combined use in medical practice to improve CKD-MBD, by simultaneously addressing its multiple components.

It became evident that simply correcting vitamin D deficiency was insufficient to improve SHPT and that only VDRA effectively improved SHPT and bone turnover in CKD patients. Additionally, the irreversible nature of vascular changes after vitamin D administration, regardless of the preparation type, became clear, allowing speculation on the superior role of prevention in these cases. The contributions of the second study clarify the effects of the two vitamin D compounds on various CKD-MBD parameters and highlight the importance of their sequential or concurrent use, given their different actions and broader therapeutic efficacy.

To conclude, these are potential new research directions in this field:

1. Since selective vitamin D receptor activators seem to mainly correct the endocrine effects of vitamin D deficiency, it is possible that the pleiotropic effects, including the cardiovascular effects, are to be better corrected with native vitamin D derivatives. Therefore, studies evaluating the efficacy and safety of combining native vitamin D with vitamin D receptor activators on various CKD-MBD components are needed.

2. Mineral and bone metabolism disorders begin in the early stages of CKD, with cumulative effects on the skeleton and blood vessels over time. Structural vascular changes are less reversible in advanced CKD stages. Hence, correcting nutritional vitamin D deficiency may be more effective if initiated earlier during CKD progression. This hypothesis should be tested in prospective controlled studies.

3. The results suggest that arterial stiffness is influenced by vitamin D deficiency, primarily through endothelial plaque calcification rather than media remodeling. This observation also requires further studies.

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