Original Article

Interrelationships Between 1,25 Vitamin D, and iPTH, in Progressive CKD: A Cross-Sectional Study

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Abstract

Background: Chronic kidney disease (CKD) has become a growing global health concern affecting nearly 700 million individuals and accounting for 4.6% of all annual deaths worldwide. The condition is defined with decreased renal function quantified by glomerular filtration rate (GFR) and evidence of kidney injury evidenced by proteinuria determined by the urine albumin-to-creatinine ratio (ACR). **Objectives**: this study was to investigate the association of 1,25(OH)2 D with iPTH in patients with progressive CKD. **Materials and Methods:** This study was cross-sectional and conducted at Al-Imam Al-Sadiq Hospital, Babil, Iraq. This study enrolled 164 patients (84 men and 80 women) with CKD stages 2-5. Patients were grouped by CKD stage: 20 stage 2 patients and 36 stage 3a, 3b, 4, and 5 patients. Inclusion criteria were derived from established CKD markers, including albuminuria and decreased GFR. Exclusion criteria included diagnosis of primary hyperparathyroidism, prior parathyroidectomy, pregnancy and lactation. Serum levels of urea, creatinine, 25 (OH) D, 1,25 (OH)2 D and intact Parathyroid Hormone (iPTH) were measured on blood sample. With regard to loacl factors, samples of urine were collected to evaluate microalbuminuria. Vitamin D and PTH were measured via ELISA, while the other parameters were determined by standard biochemical methods. **Results:** The study revealed a strong positive correlation between estimated Glomerular Filtration Rate (eGFR) and 1,25(OH)2 D levels, with a correlation coefficient of 0.545 ($p < 0.01$), a strong negative correlation between eGFR and iPTH levels (correlation coefficient of -0.587, $p < 0.01$), and a moderate negative correlation between 1,25 (OH)D and iPTH levels (correlation coefficient of -0.422, $p < 0.01$). **Conclusion:** This study showed a strong relationship between rising iPTH levels and a decline in 1,25 OH D with decreased eGFR (deteriorating CKD).

Keyword: CKD, eGFR, ACR, 25 OH D, iPTH

Introduction

Chronic kidney disease (CKD) is a worldwide health problem, affecting an estimated 700 million people worldwide. It is linked to a higher risk of premature death, with impaired kidney function accounting for at least 4.6% of annual deaths [1]. CKD is characterized by reduced renal function, assessed using the glomerular filtration rate (GFR) derived from serum creatinine concentrations, and the presence of kidney damage, which can be identified via imaging or proteinuria, often measured using the albumin-to-creatinine ratio (ACR). Vitamin D deficiency is prevalent among patients with CKD [2], who may experience deficits in both the active and inactive forms of vitamin D, including calcitriol (1,25-dihydroxyvitamin D). These deficiencies can arise from inadequate skin synthesis or dietary restrictions that limit the availability of cholecalciferol, the precursor of 25-hydroxyvitamin D [3]. Furthermore, CKD impairs the function of CYP27B1, the enzyme responsible for activating vitamin D, leading to decreased levels of 1, 25-dihydroxyvitamin D in

uremia and proteinuria associated with CKD [4]. Secondary hyperparathyroidism (SHPT) is a common complication of CKD that is characterized by elevated PTH levels [5]. PTH production decreases when blood levels of 1,25(OH)2D3 and calcium are elevated; conversely, PTH secretion increases when serum phosphate levels increase [6]. SHPT in CKD results from abnormalities in several metabolic markers, including increases in serum phosphate due to decreased renal phosphate excretion [7] and reductions in 1,25-dihydroxyvitamin D levels stemming from low serum 25-hydroxyvitamin D concentrations, thereby providing less substrate for conversion, and declines in blood calcium levels as renal function deteriorates [5], [8]. This study aimed to explore the relationship between 1,25(OH)2 D and iPTH levels in patients with progressive CKD.

Materials and Methods

Subjects

This cross-sectional study was conducted at the Al-Imam Al-Sadiq Hospital in Babil, Iraq. The study included 164 patients diagnosed with CKD based on the criteria of CKD: 20 patients with stage 2 CKD, 36 with stage 3a CKD, 36 with stage 3b CKD, 36 with stage 4 CKD, and 36 with stage 5 CKD. Individuals with primary hyperparathyroidism, previous parathyroidectomy, pregnancy, or lactation were excluded. The study followed the University of Basra's guidelines and Clinical Research Ethics conducted at Al-Imam Al-Sadiq Hospital in Babylon, and informed consent was obtained from patients or their relatives before including potential subjects. The study protocol was approved by the College of Medicine and Clinical Research Ethics Committee.

Biochemical measurements

Blood samples were obtained from patients via vein puncture. Blood (5 ml) obtained from each subject was dispensed in gel tubes and subjected to a clotting process for 20 min at room temperature (RT), followed by centrifugation at 3000 rpm for 20 min to separate the serum. Part of the serum that was used for estimation of urea and creatinine (kits supplied by BIOLABO/MALZY-FRANCE), and other aliquots were aliquoted into parts using Eppendorf tubes (0.5 ml) and stored at -20°C for subsequent analysis of 1,25 di OH vit D, 25 OH vit D, and intact PTH levels. iPTH was determined by sandwich ELISA following the manufacturer's recommendations (SunLong Biotech, China), while 1,25 di OH vit D and 25 OH vit D levels were measured using the competitive ELISA protocol recommended by the manufacturer (BT LAB Bioassay Technology Laboratory, China).

In a disposable urine cap, fresh urine samples were collected from each subject in the morning while fasting, then transferred to a test tube and centrifuged (4–5 min) at 3000 rpm to remove any precipitate, and the supernatant was stored in a freezer to determine the urine albumin creatinine ratio (ACR). Urine creatinine was measured using a creatinine kit supplied by BIOLABO/MALZY-FRANCE and Albumin in urine was measured using Microalbumin Test cartridges supplied by AGAPPE DIAGNOSTIC, INDIA. CKD-EPI was used to estimate GFR [9].

Statistical analysis

Data were analyzed using MedCalc statistical software and GraphPad Prism 9. A detailed analysis of the kidney function, evaluated as the mean ± S.D., was conducted relative to the stage of CKD, checking for differences between the means with one-way ANOVA and differences of proportions with Chi-square tests at levels of significance 0.05 and 0.01. These findings highlight the dynamic range and evolution of kidney function impairment, and support the

potential role of these biomarkers in disease monitoring and management. All the figures presented within this study were illustrated using GraphPad Prism 9 with great care for visualization of data distribution and statistical results.

Ethical approval

This study was performed according to the ethical rules for medical research involving human participants of Al-Imam Al-Sadiq Hospital in Babil, Iraq (164). Before sampling, the approval of the patient or his companion was taken. The study protocol and the subject information and the consent form were reviewed and approved by Al-Imam Al- Sadiq Hospital - Hilla - Iraq according to document number (4) on (02/01/2024) to get this approval.

Results

Table 1 categorizes the staging distribution of 164 patients diagnosed with chronic kidney disease. It breaks down the patient count and corresponding percentages across different stages of the disease. Each stage from 2 to 5 has the following representation: Stage 2 includes 20 patients (12.4%), Stages 3a and 3b include 36 patients each (21.9%), and Stages 4 and 5 also include 36 patients each (21.9%). This uniform distribution among later stages highlights the prevalence and severity of disease progression within the studied cohort.

Table 1: Distribution of CKD Stages Among Patients.

Stage	Number Of Patients	Percentage
Stage 2	20	(12.4 %)
Stage 3a	36	(21.9 %)
Stage 3b	36	(21.9 %)
Stage 4	36	(21.9 %)
Stage 5	36	(21.9 %)

Table 2 extracted from the document provides data on the prevalence of albuminuria across various stages of chronic kidney disease, categorized by albuminuria classification: A1 (<30 mg/g), A2 (30-300 mg/g), and A3 (>300 mg/g). Each category is assessed in stages 2, 3a, 3b, 4, and 5 of CKD, with the indicated for each stage.

The data includes the Percentage of Patients: Each cell in stages 2 to 5 shows the number and percentage of patients falling into each albuminuria category. Statistical Inference: The table provides a Chi-square value of 78.6 for the overall distribution, indicating highly significant differences across groups with a p-value of <0.001.

Table 2: Prevalence of Albuminuria across CKD Stages.

Albuminuria (ACR)		Stage of chronic kidney disease.					*Statistical inference*
		Stage 2 (N. 20)	Stage 3a (N. 36)	Stage 3b (N. 36)	Stage 4 (N. 36)	Stage 5 (N. 36)	
A1 < 30	10 8.5%	8 (40 %)	0 (0 %)	2 (5%)	0 (0%)	0 (0%)	X2 = 78.6
A2 30-300	46 70.7%	12 (60%)	22 (61%)	12 (78%)	0 (0 %)	0 (0 %)	< 0.001 C ***
A2 > 300	108 20.7%	0 (0%)	14 (39%)	22 (17%)	36 (100 %)	36 (100 %)	Significant

Table 3 Demonstrates intricately details the statistical interrelationships between a series of critical biomarkers that play pivotal roles in diagnosing and managing CKD. The biomarkers included are diverse, ranging from vitamin D metrics (1,25VitD in pg /ml and 25 Vit D in ug/l), parathyroid hormone levels (1,25 Vit D /PTH ratio, iPTH in Pg/ml), renal function indicators (serum creatinine in mg/dl, eGFR in ml/min/1.73 m^2), to proteinuria markers ACR in mg/g, microalbuminuria in mg/dl, and urine creatinine in mg/dl). Each entry within the matrix represents the Pearson correlation coefficient between pairs of these biomarkers,

quantifying the strength and direction of their linear relationships. Positive values in the matrix suggest a direct correlation, meaning that as one biomarker increases, the other also tends to increase. Conversely, negative values indicate an inverse correlation. For instance, high correlation coefficients between renal function markers like serum creatinine and eGFR highlight the expected inverse correlation, as worsening renal function (increased creatinine) correlates with lower filtration rates (decreased eGFR). This detailed correlation matrix is not only foundational for clinical assessments and monitoring the progression of CKD but also serves as a critical tool for research, guiding further exploration into how these biomarkers influence each other and the overall pathophysiology of kidney disease.

Table 3: Correlation Matrix of Biomarkers in CKD Study.

	1,25VitD pg/ml	1,25 vit D/iPTH	ACR mg/g	microalb. mg/dl	serum cr mg/dl	iPTH pg/ml	B. urea mg/dl	25 Vit D ug/l	urine cr mg/dl	eGFR ml/min/ 1.73 m²
1,25VitD pg/ml	1	0.949 P<0.001	-0.147 P=0.186	-0.220 P=0.047	-0.325 P=0.002	-0.244 P=0.027	-0.344 P=0.001	0.101 P=0.368	0.231 P=0.037	0.421 P=0.001
1,25 vit D/iPTH	0.949 P<0.001	1	-0.175 P=0.115	-0.235 P=0.033	-0.352 P=0.001	-0.340 P=0.001	-0.361 P=0.009	0.115 P=0.303	0.240 P=0.029	0.470 P<0.001
ACR mg/g	-0.147 P=0.186	-0.175 P=0.115	1	0.682 P<0.001	0.490 P<0.001	0.385 P=0.003	0.322 P=0.003	-0.110 P=0.325	-0.378 P=0.005	-0.419 P=0.001
microalb. mg/dl	-0.220 P=0.047	-0.235 P=0.033	0.682 P<0.001	1	0.486 P<0.001	0.238 P=0.031	0.350 P=0.001	-0.222 P=0.044	-0.340 P=0.001	-0.516 P<0.001
serum cr mg/dl	-0.325 P=0.002	-0.352 P=0.001	0.490 P<0.001	0.486 P<0.001	1	0.669 P<0.001	0.767 P<0.001	-0.353 P=0.001	-0.479 P<0.001	-0.807 P<0.001
iPTH pg/ml	-0.244 P=0.027	-0.340 P=0.001	0.385 P=0.003	0.238 P=0.031	0.669 P<0.001	1	0.547 P<0.001	-0.236 P=0.032	-0.406 P=0.002	-0.637 P<0.001
urea mg/dl	-0.344 P=0.001	-0.361 P=0.009	0.322 P=0.003	0.350 P=0.001	0.767 P<0.001	0.547 P<0.001	1	-0.393 P=0.003	-0.390 P=0.003	-0.700 P<0.001
25 Vit D ug/l	0.101 P=0.368	0.115 P=0.309	-0.110 P=0.325	-0.222 P=0.044	-0.353 P=0.001	-0.236 P=0.032	-0.393 P=0.003	1	0.247 P=0.025	0.501 P<0.001
urine cr mg/dl	0.231 P=0.037	0.240 P=0.029	-0.378 P=0.005	-0.340 P=0.001	-0.479 P<0.001	-0.406 P=0.002	-0.390 P=0.003	0.247 P=0.025	1	0.680 P<0.001
eGFR ml/min/ 1.73 m²	0.421 P=0.001	0.470 P<0.001	-0.419 P=0.001	-0.516 P<0.001	-0.807 P<0.001	-0.637 P<0.001	-0.700 P<0.001	0.501 P<0.001	0.680 P<0.001	1

Discussion

Assessment of renal function in CKD has evolved to incorporate multiple biomarkers, with urine ACR emerging as a critical tool for evaluating kidney health. Our study results revealed highly significant differences in ACR across the groups, with a p-value <0.001 (Table 2). This finding aligns with growing evidence supporting ACR's crucial role of ACR in CKD assessment and management.

Matsushita *et al*. conducted a meta-analysis of 48 cohorts including 637,315 participants from diverse populations. They found that a higher ACR was consistently associated with an increased risk of kidney failure, acute kidney injury, and progressive CKD across all eGFR levels. Importantly, they demonstrated that the addition of ACR to eGFR significantly improved the risk prediction of these outcomes [10]. This large-scale study emphasizes the complementary roles of ACR and eGFR in assessing kidney function and predicting outcomes, aligning with our findings, and those of Flaherty *et al* [11] also emphasized the importance of assessing both estimated eGFR and albuminuria in diagnosing and staging CKD, particularly in older adults.

eGFR showed a strong positive correlation with 1,25VitD (pg/ml), with a correlation coefficient of 0.545 and $p < 0.01$. This indicates that as eGFR decreases, 1,25-dihydroxyvitamin D levels also tend to decrease. These results align with and extend the findings of Levin *et al*. (2007), who conducted a comprehensive study of 1,814 patients with CKD stages 2-5. Their research provided robust evidence for the relationship between declining eGFR and decreasing 1,25 (OH)2 D levels across the CKD spectrum [12].

EGFR correlated negatively with iPTH (Pg/ml) r =-0.587 p<0.01. That is, intact parathyroid hormone concentrations increased with

decreasing eGFR. The aforementioned results are consistent with the study by Herawati et al. evidence for such an inverse correlation between eGFR and iPTH in CKD, as shown by Yamada et al. (2021) ($r = -0.540$; $p = 0.000$) [13].

The 1,25VitD has a moderate negative correlation (-0.422, $p < 0.01$) with iPTH. This means a negative correlation that is to say when 1,25VitD decreases the iPTH tends to increase. These results are consistent with, and build on, the work of Kim et al. (2022), reporting a remarkable difference in levels of 1,25(OH)2D (the most biologically active metabolite of vitamin D) along CKD stages, values progressively dropping with the worsening of CKD. They demonstrated that levels of 1,25(OH)2D in CKD decreased in stage 3 to stage 5 ($p < 0.001$). At the same time, they noticed that patients with more advanced CKD stages exhibited higher serum intact PTH levels ($p < 0.001$) [14].

Conclusion

Research has shown a strong relationship between rising iPTH levels and a decline in 1,25 OH D with eGFR decreases (deteriorating CKD).

List of abbreviations

CKD, chronic kidney disease; ACR, albumin-to-creatinine ratio; 1, 25(OH)2D 1,25 (OH)2 vitamin D; 25(OH)D:25 (OH) vitamin D; SHPT:Secondary hyperparathyroidism; PTH: Parathyroid hormone ELISA: Enzyme-Linked Immunosorbent Assay ; iPTH, intact Parathyroid Hormone;ANOVA: Analysis of Variance ; RT: Room Temperature.

Conflicts of interest The authors declare there are no conflicts of interest.

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