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## Original Research Article

## Risk factor analysis of vitamin d insufficiency in end-stage renal disease in CKD patients

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## ABSTRACT

Vitamin D insufficiency is prevalent in human populations and specifically, it affects the Chronic Kidney Disease (CKD) population due to the effects of Vitamin D on calcium homeostasis and bone health. Moreover, the association of Vitamin D deficiency with proteinuria is responsible for a major risk for End-Stage CKD patients. This work aimed to find a correlation (or relationship) between Vitamin D and CKD stages. The study included 70 Patients attending the Department of Medicine, GGSMCH Faridkot who was diagnosed with CKD. The population with Glomerular Filtration Rate (GFR) < 60 ml/min/1.73m<sup>2</sup> were observed in the present study. They were classified into several stages as per the criteria of CKD. The patients who were already on Vitamin D supplementation were excluded from this study. Accordingly, the routine investigations were performed on the considered group of patients. The Vitamin D levels of all the considered patients were estimated by ACCESS 2 Chemiluminescence. Patients with Vitamin D levels 10-30ng/ml and <10ng/ml were considered as insufficient and deficient, respectively. The Vitamin D levels (Mean  $\pm$  SD) in these patients were found to be lower concerning the severity of the kidney damage (Stage IIIa  $\Rightarrow$  37.83  $\pm$  7.13 ng/ml; IIIb  $\Rightarrow$  28.06  $\pm$  8.52 ng/ml; Stage IV  $\Rightarrow$  22.51  $\pm$  8.43 ng/ml; and Stage V  $\Rightarrow$  16.23  $\pm$  6.42 ng/ml) ( $p < 0.001$ ). The Vitamin D insufficiency prevalence was found as 58.6%, 64.72%, 75.4%, and 87.4% in all four stages. At the same time, the Vitamin D deficiency prevalence was found to be 1.8%, 2.5%, 11.8% & 12.6% in all four stages. Hence, this study highlights the role of insufficiency and deficiency in Vitamin D levels showing a significant association with the levels of kidney function in CKD Patients (specifically, those having advanced stage renal disease).

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## 1. Introduction

The key function of vitamin D includes the Maintenance of calcium and phosphorus homeostasis, thereby promoting bone mineralization. The two forms of Vitamin D exists, 1) vitamin D<sub>2</sub> (ergocalciferol), and 2) vitamin D<sub>3</sub> (cholecalciferol).<sup>1</sup> In the liver, Vitamin D (also known as steroid hormone), is hydroxylated to 25 hydroxylated vitamin D {25(OH)D}, which is the major articulating metabolite of vitamin D. In the kidney, the 25(OH)D is transformed to 1,25-dihydroxy vitamin D [1,25(OH)<sub>2</sub>D],

i.e. the active form by a 1-hydroxylase enzyme present in the kidney. This helps to maintain bone and muscle health through the regulation of calcium metabolism. Serum 25(OH)D concentrations are measured to clinically assess the status of vitamin D because it reflects both intakes as well as endogenous production.<sup>2</sup>

Vitamin D is associated with bone health, so it is acknowledged that vitamin D deficiency can lead to rickets (in children) as well as osteomalacia and osteoporosis (in adults). In recent years, various studies that vitamin D exhibits a broad array of metabolic and cell regulatory functions.<sup>3</sup> But, it is clear that the perfect operation of various organs and tissues all around the body requires

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sufficient levels of vitamin D. Especially, sufficient levels of vitamin D are very necessary for the Cardiovascular (CV) system, preventing tumor, proper functioning of the endocrine system and kidney functioning.<sup>4,5</sup> Vitamin D when binds to Vitamin D Receptor becomes the dominant player to inhibit cell proliferation (inducing cell apoptosis and differentiation), regulate immune function, protect organ function, and safeguard gene functions.<sup>6</sup> Vitamin D deficiency or insufficiency is nowadays a worldwide problem for children as well as adults.<sup>7</sup> Several studies provide the limit for vitamin D deficiency, i.e., 25(OH)D level of < 20 ng/ml (50 nmol/l) and vitamin D insufficiency, i.e., 21 to 29 ng/ml. The optimal concentration of 25(OH)D should at least be 30 ng/ml<sup>8</sup>. Various researchers have found that almost 40 to 100% of the U.S. community and European elderly community are having low levels of vitamin D. Also Vitamin D deficiency is more common in sunniest areas especially in Asian people. Moreover, in India and the Middle East, where maximum sunlight is there, 30 to 50% of the population had Vitamin D levels less than 50 mol/L.

In recent years, Chronic kidney disease (CKD) has evolved as a prominent disease affecting almost 15% of the adult population across the globe. This fact has been supported by the findings of a survey that highlighted the increase in the prevalence of CKD in the last 20 years.<sup>8</sup> People are not much aware of this disease and so they don't take it seriously when compared to other diseases like cardiovascular diseases and diabetes. Most of the patients are hospitalized only when they enter the last stages, which results in poor prognosis and huge disease burden.<sup>9</sup> Various existing researches also stressed the underlying risk factors about CKD incidence. Hence, in line with the above discussions, in the present study, we estimated the role of the variation in the levels of vitamin D with CKD Stages.

## 2. Aims and Objectives

1. The key objective of this study was to estimate Vitamin D levels with the advanced CKD stages.
2. The present study was done to find the association of hypovitaminosis D with the different stages of CKD.

## 3. Materials and Methods

The study was performed on 70 CKD Patients attending the Deptt of Medicine, Guru Gobind Singh Medical College, and Hospital, Faridkot, Punjab. A detailed medical history of CKD patients was also taken. The CKD subjects were divided into six categories according to the Clinical Practice Guidelines for Chronic Kidney Disease. The criteria concerning CKD were based on Glomerulation Filtration Rate calculated by using Creatinine clearance formula<sup>12</sup>. The various stages of CKD depending on GFR are as follows: (I  $\geq$  90, II : 60 – 89, III-a: 45-59, III-b: 30 –

44, IV: 15 - 29, and V: < 15 –all in ml/min/1.73m<sup>2</sup>). In this specific study, we have excluded two stages (I and II) as the value of GFR was > 60 ml/min/1.73m<sup>2</sup>. The patients already on Vitamin D supplementation were excluded from this study. Informed written permission (or consent) was taken from all the patients. Routine investigations were performed such as Serum Creatinine, Blood Urea, Albumin, Serum calcium, phosphorous, Alkaline Phosphatase on AU480, fully automated analyzer. The levels of Vitamin D for all patients were computed using Immunoassay analyzer ACCESS2. This was based on the competitive enzyme-mediated chemiluminescence method, in which one of the end products is light and this emitted light is measured in relative light units (RLU). Patients with Vitamin D levels 10-30ng/ml and <10ng/ml were considered insufficient and deficient respectively.

### 3.1. Statistical analysis

The descriptive data were calculated for all the variables. ANOVA technique was used to compare the statistical differences in the variables and the unpaired Student's t-test was performed to differentiate the normally distributed variables. The inter-group comparisons were examined using the chi-squared test. The association between vitamin D levels and CKD stages were observed and scrutinized through multivariate logistic regression analysis [the odds ratio considering 95% confidence intervals (CI)]. In the end, the statistical importance was accepted (or trusted) only if  $P < 0.05$ . All these statistical calculations were conducted using the SPSS software toolkit.

## 4. Results

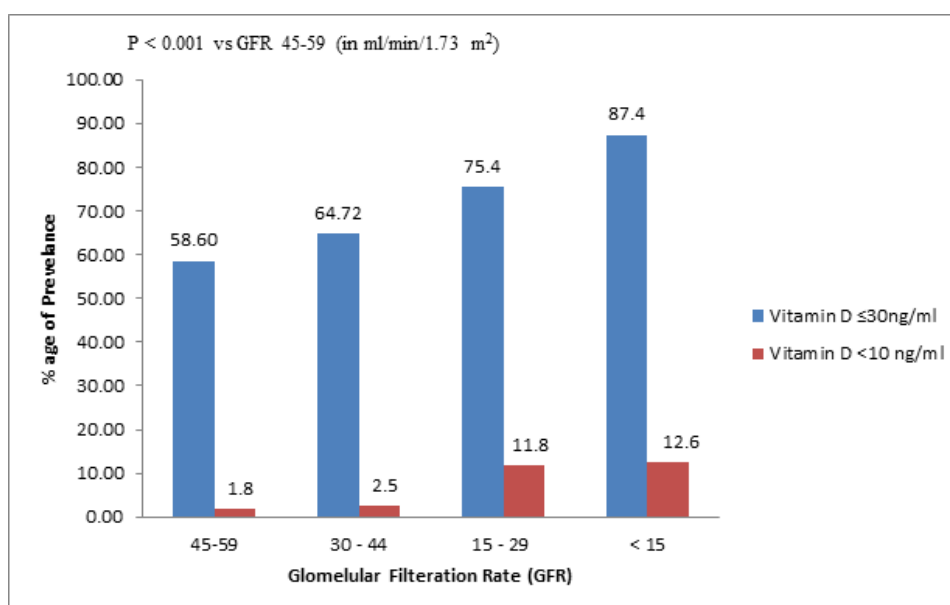
Patients with CKD and GFR less than 60ml/min/1.73m<sup>2</sup> were considered our study. Table 1 shows the baseline characteristics according to CKD status. Vitamin D levels (Mean  $\pm$  SD) were notably smaller according to gravity of renal impairment (CKD Stage IIIa=> 37.83  $\pm$  7.13 ng/ml; IIIb=> 28.06  $\pm$  8.52 ng/ml; Stage IV=> 22.51  $\pm$  8.43 ng/ml; and Stage V=> 16.23  $\pm$  6.42 ng/ml) ( $p < 0.001$ ).

Table 2 depicts the data of patients with Vitamin D level  $\leq$  30 ng/ml having a significant decrease in GFR, serum calcium levels and serum albumin levels. Moreover, there was an increase in BUN, Serum Creatinine, serum phosphorus and ALP ( $p < 0.001$ ). It was also evident that the prevalence of vitamin D deficiency increased with progression in CKD. The observed widespread prevalence of Vitamin D insufficiency and deficiency was 58.6%, 64.72%, 75.4%, and 87.4% and 1.8%, 2.5%, 11.8% & 12.6% in all the considered stages (Figure 1). In the analysis based on the multivariate logistic regression, a contrary association was noted between serum 25-hydroxyvitamin D  $\leq$  30 ng/mL and prevailing CKD stage 5 [calibrated odds

**Table 1:** Standard (or baseline) attributes of CKD Patients (Mean±SD)

Parameters	Stage III-aGFR—45 to 59 (N = 15)	Stage III-bGFR—30 to 44 (N = 10)	Stage IVGFR—15 to 29 (N = 25)	Stage VGFR<15 (N = 20)
GFR(ml/min/1.73m <sup>2</sup> )	51.04±5.03	37.87±4.76*	21.43±5.6*	7.26±3.2**
BUN (mg/dl)	20.67±4.37	29.78±7.4*	40.75±8.54**	55.4±6.3***
Serum Creatinine(mg/dl)	1.35±0.20	2.5±0.39*	2.53±1.23**	3.56±2.56**
Serum Albumin(g/dl)	4.27±0.32	4.15±0.28*	3.5±0.72*	3.28±0.97*
Serum Calcium(mg/dl)	9.77±1.56	9.68±1.3	8.58±0.6*	8.4±0.24*
Serum Phosphorus(mg/dl)	3.47±0.94	3.56±0.75	4.45±0.76*	4.7±1.38*
Alkaline Phosphatase (U/L)	72.5±40	80.4±64	92±48.2	160.4±50.98
Serum Vitamin D(ng/ml)	37.83 ± 7.13	28.06 ± 8.52 *	22.51 ± 8.43 *	16.23 ± 6.42**

\*\*\* p&lt;0.0001, \*\*p&lt;0.001, \*p&lt;0.05 versus Stage IIIa

**Fig. 1:** Visible prevalence (in %) for Vitamin D insufficiency/deficiency with respect to the CKD staging**Table 2:** Impact of Vitamin D insufficiency/deficiency concerning different variables

Parameters	Vitamin D levels (ng/ml)		p value
	>30 ng/ml	≤30 ng/ml	
GFR (ml/min/1.73m <sup>2</sup> )	43.14±12.05	23.48±18.08	<0.05 (S)
BUN (mg/dl)	22.4±8.7	34.7±15.46	<0.05 (S)
Serum Creatinine (mg/dl)	1.58±1.71	5.4±5.62	<0.05 (S)
Serum Albumin (g/dl)	4.2±0.56	3.37±0.48	<0.001 (HS)
Serum Calcium (mg/dl)	9.8±0.24	8.64±1.43	<0.05 (S)
Serum Phosphorus (mg/dl)	3.57±1.07	4.57±0.4	<0.001 (S)
Alkaline Phosphatase (U/L)	78.4±45.28	98.36±76.74	0.46 (NS)

p-value versus Vitamin D &gt; 30ng/ml in CKD patients; HS- Highly significant, S- Significant, NS- Non-significant.

ratio 2.19 (95% CI 1.07 to 4.48)] in the CKD population.

## 5. Discussion

In this study, we have found that vitamin D insufficiency/deficiency is very common among CKD patients. Regardless of the geographic location of India, 25(OH)D levels were less than 30 ng/mL in almost 59.8–84.5% of CKD patients (stage IIIa to Stage V). Moreover, despite being a tropical region, hypovitaminosis D has been reported for the Asian population with sufficiently normal kidney function.<sup>10</sup> Our results complement other observations that suggest any deficiency of Vitamin D is strongly related to the higher CKD stages. This may be because patients with CKD are on restricted protein and calorie intake which leads to low levels of Vitamin D.<sup>11</sup> Also, another reason may be the increasing modernization of Indian society because of which the number of hours spent indoors and use of sunscreens has increased which leads to Vitamin D deficiency.<sup>12</sup> Finally, a bigger concentration of urinary vitamin D metabolites loss is witnessed in patients with overt proteinuria.<sup>13</sup>

A study showed lower Vitamin D levels with the advancement of CKD stage when compared with the normal population ( $p < 0.001$ ).<sup>14</sup> During a long-term follow-up study, Melamed et al. observed that patients with Vitamin D levels  $< 15 \text{ ng/ml}$  had a higher risk of ESRD than those with higher levels of Vitamin D.<sup>15</sup> Moreover, a study done by La Clair et al. in 2005 have found vitamin D levels decreased in CKD group. Prevalence of Vitamin D insufficiency/deficiency for stages 4 and 5 was 29% and 17% respectively.<sup>16</sup>

Also, a negative (or inverse) relationship is witnessed between vitamin D levels and rennin angiotensin-aldosterone system (RAAS) abnormalities. RAAS is a core player in the regulation of blood pressure and developing kidney damage.<sup>17</sup> In animals, it was identified that by hindering 1,25(OH)(2)D synthesis there was an enhancement in renin production and also rennin suppression was there when 1,25 (OH)(2)D was injected.<sup>18</sup> Hence, 1,25 (OH)(2)D is one of the unique (or novel) negative (or inverse) endocrine regulators of the RAAS.<sup>19</sup> Not long ago, in a group study, it was established that serum 25-hydroxyvitamin D level was a self-sufficient (or independent) negative predictor of disease advancement and death in patients with stages 2–5 CKD.<sup>20</sup> Also there have been studies which have found a relationship between vitamin D with albuminuria in CKD and Type1 diabetes but failed to provide any evidence for the hypovitaminosis D in CKD.<sup>21,22</sup> Hereby our results are clearly presenting a marked association between Vitamin D and kidney damage risk in CKD prior to dialysis. The outcome of our study highlights a key public health concern that should be confirmed necessarily through extensive and group examinations in different inhabitants.

## 6. Conclusion

The study conducted in this work tries to analyze and demonstrates that any Vitamin D insufficiency, as well as deficiency, is strictly linked (or related) to the smooth functioning of kidneys in CKD Patients, specifically for the patients with advanced-stage renal disease.

## 7. Abbreviations

ESRD–End-Stage Renal Disease, CKD–Chronic Kidney Disease.

## 8. Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this article.

## 9. Source of Funding

None.

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