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Research article

# SERUM 25(OH) VITAMIN D AND ITS ROLE IN SECONDARY HYPERPARATHYROIDISM IN PATIENTS WITH CHRONIC KIDNEY DISEASE

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## ABSTRACT

**Background & Objective:** Chronic Kidney Disease (CKD) is emerging as an important public health problem across the world. In severe cases, the disease progresses towards end stage renal disease (ESRD) which is associated with several complications. In recent years, 25 (OH) Vitamin D has been identified as a risk factor for ESRD. Vitamin D plays a vital role in regulating parathyroid hormone (PTH) synthesis and release. Decreased Vitamin D levels are identified as a risk factor for secondary hyperparathyroidism (SHPT) which is one of the major complications of CKD and is associated with renal osteodystrophy. The present study was planned to assess the status of 25 (OH) Vitamin D and its role in secondary hyperparathyroidism in patients with CKD. **Materials and Methods:** The study was conducted on 50 diagnosed cases of CKD (stage 4 and 5), age ≤ 60 years. Age and sex matched healthy subjects (n = 50) constituted the control group. Serum 25(OH) Vitamin D and iPTH were estimated for all the enrolled subjects. BMI and eGFR were also calculated. All variables were compared among the control and diseased group. **Results:** Vitamin D levels were significantly low in the CKD group as compared to the control group ($p<0.001$). 72% of the total CKD patients were deficient in Vitamin D levels. Serum iPTH was significantly higher in the CKD patients ($p = 0.000$). A significant negative correlation was observed between iPTH and Vitamin D ($r = -0.614$).**Conclusion:** The study suggests that patients of CKD are at high risk of Vitamin D deficiency. Vitamin D deficiency has a strong association with the pathogenesis of SHPT.

**KEYWORDS:** Chronic Kidney Disease, Vitamin D, Parathyroid hormone, Secondary Hyperparathyroidism, End Stage Renal Disease.

## INTRODUCTION

In the last few decades, chronic kidney disease (CKD) has emerged as a global health problem of epidemic proportions. It has a major effect on healthcare costs and world productivity, particularly in developing countries where the young people are the most afflicted population. Although in many persons CKD remains an asymptomatic pathologic condition that progresses slowly. For many others, CKD represents a progressive irreversible process that ultimately requires renal replacement therapy. The burden of CKD includes not only progression to end-stage renal disease (ESRD), but also complications related to renal impairment and increased risk of cardiovascular diseases[1]. Cardiovascular disease events are the leading cause of morbidity and mortality in patients with CKD. Left ventricular hypertrophy and vascular calcification are more prevalent among patients with CKD. Additionally, hyperparathyroidism and hypovitaminosis D are independent risk factors for vascular calcification[2, 3].

With the growing global epidemic of traditional risk factors like hypertension and diabetes in particular, the prevalence of CKD and kidney failure is rising continuously[4, 5]. In the past ten years, the incidence and prevalence of end-stage renal disease have doubled and expected to continue to rise steadily in the future. In addition to the well-known risk factors; several "*nontraditional*" risk factors may contribute to the progression as well as higher risk of death in patients with CKD compared to the general population [1, 6-8]. The increased morbidity and mortality related to CKD may be reduced with early detection and effective management of risk factors to prevent or delay further progression of renal dysfunction and its associated complications.

In recent years, vitamin D deficiency has been recognized as a prominent feature of CKD. Growing evidences suggest that progression of CKD and many of the secondary complications like disturbance in bone mineral metabolism, inflammation, cardiovascular disease, anemia and neuropathy may be linked to vitamin D [1, 3, 6-8]. In-vitro studies indicate that vitamin D besides maintaining bone mineral homeostasis, involved in a wide range of physiological functions, including regulation of

cytokines, inflammatory and fibrotic pathways, the rennin-angiotensin system, vascular and cardiac cell function, immune response modulation, cell growth and differentiation[9-12].

The data from experimental and clinical studies suggest that vitamin D protects kidney by targeting two major pathways: the local RAS and the NF-kB pathways that promote renal damage and progression of kidney disease[13, 14].

Deficiency of vitamin D has been identified as a risk factor for end-stage renal disease and co-existing cardiovascular disease, and overall mortality in patients with CKD[15, 16]. Additionally, vitamin D deficiency causes an increase in parathyroid hormone, which increases insulin resistance and is associated with diabetes, hypertension, inflammation, and cardiovascular risk[17].

Secondary hyperparathyroidism is a common and major cause for concern as high PTH is associated with the development of renal osteodystrophy and cardiovascular complications[3]. In a recent study serum iPTH level ≥ 400 pg/ml is identified as an independent risk factor for high radial artery intimal thickness[2]. Epidemiological studies have also shown that damage of large arteries is a major contributing factor to morbidity and mortality in patients with CKD and in those with ESRD[18].

Therefore, the present study was planned to investigate the association of 25(OH) vitamin D with secondary hyperparathyroidism in patients with chronic kidney disease (CKD).

## MATERIAL & METHOD

**Study design:** Case control analytical study

**Ethics approval**: The study was conducted after seeking approval from the institutional ethics committee and informed consent was on obtained from the participants

**Sampling method:** Consecutive sampling method

**Sample size:** In each group 50 participants

**Locus of study:** Department of Nephrology, Mahatma Gandhi Medical College and Hospital, Jaipur

**Time frame:** during May to November 2015.

**Inclusion criteria:** Fifty diagnosed cases of CKD (stage 4 and 5) as per the guidelines of National Kidney Foundation [19], age up to 60 years. Fifty age and sex matched healthy subjects constituted the control group.

**Exclusion criteria:** Patients with acute renal failure, Primary hyperparathyroidism, thyroid or parathyroid surgeries and on Vitamin D supplementation were excluded from the study.

**Grouping:** Group 1: CKD patients, Group 2: Control

**Methodology:** All participants underwent physical examinations including anthropometric assessments and biochemical assessments. Patient's medical records were also reviewed for history of hypertension, diabetes and other associated co-morbidities. Blood samples were collected using standard aseptic technique and analyzed for Serum Urea, Creatinine, Uric acid by dry chemistry on VITROS 4600 and 25(OH) Vitamin D and iPTH by CLIA[20] on VITROS ECI. eGFR was calculated using Cockeroft and Gault formula.GFR is the best measure of the kidneys functioning level. It is calculated based on blood creatinine level, age, race, gender and other contributing factors.

**Statistical Analysis:** Results obtained were analyzed using a statistical package program (SPSS 17 Inc; Chicago II, USA) for social science. Data were presented as mean ± SD and subjected to statistical analysis. P-value $\leq$ 0.05 was considered as statistically significant. All variables were presented in the two groups as mean $\pm$ SD and compared by applying student's't' test (Table 1).

## RESULTS

Mean age of CKD patients (38.980 ± 10.32 years) was comparable with healthy subjects. Body mass index in CKD group (20.90 kg/m$^2$) was comparable with healthy subjects (21.36kg/m$^2$). As anticipated, mean eGFR was significantly lower (14.31± 4.19 ml/min) in CKD group (p<0.000). The mean values of renal profile parameters were further compared in the CKD and control group. All parameters viz. urea, creatinine and uric acid were significantly higher in CKD patients as compared to healthy subjects. The mean serum 25-hydroxyvitamain D level was also observed to be significantly lower (p<0.001) in CKD patients (19.29± 0.77 ng/ml) as compared to healthy subjects (39.37±10.801 ng/ml).

The CKD patient group was further subdivided on the basis of concentration of 25-hydroxy vitamin D levels (Table 2). It was observed that 72% of the subjects were in the "**deficient**" group (< 20 ng/ml). The "**insufficient**" group (20-<30 ng/ml) included 12% of the total patients while only 16 % were having **"normal"** (>30 ng/ml) Vitamin D levels.

Mean serum iPTH levels were significantly higher in CKD population (301.77 ± 218.17 pg/ml) as compared to age-matched healthy subjects with normal kidney function (41.19 ± 16.21 pg/ml). To evaluate the association between Serum iPTH levels and Vitamin D levels, Pearson's correlation was applied. A significant negative correlation (r=-0.614; p=0.000) was observed between the two variables. This confirms that Vitamin D deficiency plays a major role in the development of secondary hyperparathyroidism (SHPT).

**Table 1: Distribution of variables between CKD patients and Control group**

| Variables | CKD patients | Control | tvalue | P-value |
|---|---|---|---|---|
| Age (years) | 38.98± 10.32 | 38.28± 13.72 | 0.29 | NS |
| BMI (kg/m$^2$) | 20.90± 2.52 | 21.36± 3.39 | 0.77 | NS |
| eGFR (ml/min) | 14.3± 4.2 | 106.37± 13.0 | - 48 | 0.000 |
| S. Urea (mg/dl) | 109.3± 48.3 | 26.32± 5.17 | 12.1 | 0.001 |
| S.Creatinine (mg/dl) | 8.56± 2.9 | 0.72 ± 0.13 | 19.1 | 0.000 |
| S.Uric acid (mg/dl) | 7.87 ± 2.57 | 4.22 ± 1.47 | 8.71 | 0.000 |
| 25(OH)VitaminD (ng/ml) | 19.29± 10.77 | 39.37 ± 10.8 | - 9.3 | 0.000 |
| S. iPTH (pg/ml) | 301.7± 218.2 | 41.19± 16.2 | 8.44 | 0.000 |

**Table 2: Distribution of CKD patients on the basis of 25 (OH) Vitamin D levels**

| 25(OH) Vitamin D (ng/ml) | CKD Patients | |
|---|---|---|
| | No. of cases(n) | % |
| Deficiency <20 ng/ml | 36 | 72 |
| Insufficiency 20-<30 ng/ml | 6 | 12 |
| Normal 30-100 ng/ml | 8 | 16 |
| Total | 50 | 100 |

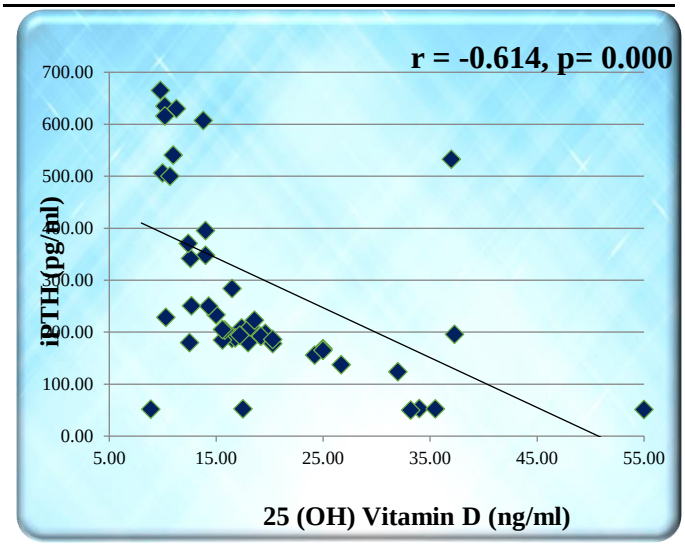


**Figure 1: Correlation of serum 25(OH) vitamin D with iPTH levels**

## DISCUSSION

Serum creatinine is the most commonly used marker for assessing kidney functions in patients with chronic kidney disease. GFR uses the creatinine level and additional factors to provide a better estimate of kidney function. The use of serum urea is recommended by the Kidney Disease Outcome Quality Improvement clinical practice guideline to assess dialysis clearance. Recently, uric acid has also been resurrected as a potential contributory risk factor in the development and progression of CKD [21]. Pilot studies also suggest that lowering plasma uric acid concentrations may slow the progression of renal disease in subjects with CKD[22-24]. For the present study, the renal function variables including eGFR were significantly higher in the CKD patient group.

Findings of the study suggest that prevalence of vitamin D deficiency or insufficiency is high among patients with CKD, which is in accordance with previous studies[25, 26]. Several factors are involved in determining the vitamin D status of an individual. In patients with CKD, severely impaired renal function, proteinuria/ albuminuria and uremia increase the risk of low serum 25(OH) Vitamin D[25]. Gonzalez EA *et al* 2004[27] reported 86% prevalence of 25(OH) D deficiency/ insufficiency in pre-dialysis patients with normal glomerular filtration rate (eGFR) and 97% in patients on dialysis. In addition to a high prevalence of 25 (OH) D deficiency or insufficiency, patients with CKD also demonstrate profound reductions in 1,25$(OH)_2$ D levels, especially those reaching ESRD. Decreased renal 1-α hydroxylase activity is the primary cause, and this activity is not only affected by the reduction of functional renal mass, it is also suppressed by hyperuricemia, metabolic-acidosis and uremic toxins disorders commonly seen in advanced CKD[26, 28]. Under normal circumstances, the synthesis of 1,25$(OH)_2$D is not substrate (25[OH]D) dependent; however, in CKD patients, renal 1α-hydroxylase becomes substrate (25[OH]D) dependent, and a higher concentration of precursor 25(OH)D is likely needed to reach adequate 1,25(OH)2D levels [29]. The data from experimental and clinical studies suggest that vitamin D protect the kidney by targeting two major pathways that promote renal damage and progression of kidney disease: the local RAS and the NF-kB pathway[13].

The study observed a significant negative correlation between Vitamin D and iPTH levels in the CKD patients. This observation is in accordance with previous studies conducted on CKD patients[3, 16, 30, 31]. PTH secreted from parathyroid glands maintain the correct balance of calcium and phosphorous in the body. PTH is involved in the homeostasis of bone metabolism by regulating the level of calcium in the blood, release of calcium from bone, absorption of calcium from the intestine, and excretion of calcium in the urine. Consequently, the levels of calcium and other minerals involved in bone metabolism, such as phosphorus and vitamin D, affect the secretion of PTH by the parathyroid gland. The findings of current study indicate that patients with long standing CKD are at high risk of vitamin D deficiency which is further a major contributor in the pathogenesis of SHPT.

Secondary hyperparathyroidism develops universally in patients with CKD, especially those on long-term dialysis therapy[32]. It is characterized by excessive secretion of parathyroid hormone (PTH) and parathyroid hyperplasia, resulting in bone disorder, soft tissue calcification and significantly increased risk of morbidity and mortality. It is well accepted that development of parathyroid hyperplasia is associated with down-regulation of the vitamin D receptor (VDR) and the calcium-sensing receptor (CaSR)[33]. As kidney disease progresses, parathyroid VDR and CaSR levels decrease in parallel with the severity of parathyroid hyperplasia[34]. Recently, enhanced parathyroid expression of the potent growth promoter transforming growth factor alpha (TGF-α) and its receptor, the epidermal growth factor receptor (EGFR), has been identified as one of the main causes of parathyroid hyperplasia and the reduction of VDR in CKD[35].

1,25 $(OH)_2$ D deficiency promotes parathyroid gland growth (hyperplasia) and increased PTH synthesis through loss of the ability to up regulate vitamin D receptor expression within parathyroid cells[36]. The end result is elevated serum PTH and abnormal calcium (Ca) and phosphorus (P) balance. The combination of persistently high PTH and low 1,25- $(OH)_2$ D is associated with bone loss, cardiovascular disease, immune suppression and increased mortality in patients with end-stage kidney failure.

Elevated PTH and hyperphosphatemia were recently identified as risk factors for mortality in dialysis patients[37]. Clinical data indicates that vitamin D treatment is an important

factor that may mitigate the effects of SHPT and hyperphosphatemia on cardiovascular mortality. Deficiency of vitamin D is not only limited to the active hormone, calcitriol but calcidiol (25- hydroxycholecalciferol) is also deficient in most patients with chronic kidney disease (CKD), independent of their underlying renal function. Decrease in calcitriol occurs relatively early in the progression of kidney disease and may predate the increase in PTH. These changes in calcitriol and PTH contribute to the maintenance of relatively normal serum calcium concentrations until the GFR decreases to <20–25%; however, the result is the potential development of bone and vascular disease.

The findings of the above study suggest that patients of CKD are at risk of developing hypovitaminosis D due to several interrelated complex processes. The hydroxylation of Vitamin D to Vitamin D3 (1,25-dihydroxy Vitamin D) occurs in renal tissue, therefore deficiency of D3 becomes obvious in CKD patients. However, a reduction in the levels of 25(OH) Vitamin D is a matter of concern for clinicians as well as research personnel. The present study reported a highly significant negative correlation between Vitamin D and iPTH levels. This indicates that with fall of Vitamin D levels, the risk of developing SHPT increases which itself is a risk factor for ESRD. The study therefore, recommends further research on the effect of renal dysfunction and hyperparathyroidism on other markers of bone development and mineralization viz. Calcium, Phosphorus, Alkaline phosphatase, Calcitonin etc.

The balance of calcium, phosphorus, vitamin D, and iPTH is complex and interrelated. So patients must adhere to dietary restrictions, therapies, and complicated medication regimens. These factors create barriers to achieving and maintaining control of SHPT.

## CONCLUSION

The findings of the above study suggest that patients of CKD are at risk of developing hypovitaminosis D. There was highly significant negative correlation between Vitamin D and iPTH levels. The study recommends routine screening for Vitamin D levels in patients of CKD. Identification of patients with low Vitamin D levels will be helpful in timely management of the patients as well as minimizing the risk of associated complications.

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**Conflict of interest:** Nil

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