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"EVALUATE THE STATUS OF SERUM 25-HYDROXYVITAMIN D WITH URINARY ALBUMIN CREATININE RATIO AND TO SEE ITS ASSOCIATION WITH ECG CHANGES IN CHRONIC KIDNEY DISEASES

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ABSTRACT

Aims: Evaluate the status of serum 25-Hydroxyvitamin D with Urinary Albumin Creatinine Ratio and to see its association with ECG changes in chronic kidney diseases.

Study Design: Cross-sectional study.

Place and Duration of Study: Department of Biochemistry, Department of Medicine, Department of Nephrology Chirayu Medical College and Hospital, Bhopal, Madhya Pradesh between June 2019 to November 2019.

Methodology: 100 known or newly diagnosed CKD patients above 18 yrs of age were included. Patients on dialysis, Vitamin D therapy, obstructive nephropathy, gout, or having H/O IHD were excluded from the study. eGFR was calculated by CKD-EPI formula and patients were grouped into various stages of CKD based on KDOQI classification. Spot urinary albumin(immunoturbidimetric method) and urinary creatinine(buffered kinetic Jaffes reaction) were done on Cobas Integra 400 Plus and ACR was determined. Albuminuria (ACR) categories in CKD were listed as A1 : <30mg/g ; A2: 30-300mg/g and A3: >300mg/g Quantitative determination of total 25-hydroxyvitamin D in human serum was done on Cobas e411 based on electrochemiluminescence binding assay. 25-OH vitamin D deficiency , insufficiency and sufficiency were defined as levels <30 nmol/L , 30-50 nmol/L and > 50 nmol/L.12 lead ECG was done at the time of admission and interpretation done by a qualified physician based on accepted standard criteria.

Result: Out of 100 CKD patients 45 were in stage V, 36 in stage IV,14 in stage III and 5 were in stage II. 94% of patients were found to have vitamin D deficiency. 72% had vitamin D <30 nmol/L out of which 88.8% patients were in Stage IV and stage V CKD. Mean ACR was 344.9±163.5. A significant association was observed ($p<0.05$) between ACR and Vitamin D as well as severity of CKD and Vitamin D deficiency. ECG abnormalities were observed in 75% patients with 57% ECG abnormality present in CKD patients with vitamin D <30nmol/L. Significant association ($p<0.05$) was observed between Vitamin D status and ECG abnormalities.

Conclusion: 25-hydroxyVitamin D has been observed to have a renoprotective as well as cardioprotective role in CKD patients. This study can be of immense utility for alleviating progression and cardiovascular risk in CKD patients

KEYWORDS : Kidney Disease Outcome Quality Initiative (KDOQI); Chronic kidney disease(CKD) ; Electrocardiogram(ECG) ;Albumin Creatinine ratio(ACR)

INTRODUCTION

Chronic kidney disease (CKD) is one of the most commonly occurring non- communicable diseases in India with prevalence ranging from 1 to 13% [1,2] and is associated with significant morbidity, mortality and economic burden. Production of active vitamin D, 1,25- dihydroxycholecalciferol (calcitriol) requires the hydroxylation of the cholecalciferol molecule at both the 1 and the 25 position Hydroxylation at the 25 position occurs in the liver, whereas hydroxylation of the 1 position occurs in the kidney; this latter process is impaired in CKD with resulting deficiency in active vitamin D. 25-hydroxyvitamin D [25(OH)-VD] is the main circulating form of VD and its plasma levels are routinely measured as a marker of VD status. Expanding roles of the vitamin D endocrine system beyond calcemic regulation and its deficiency has not only been found to be widely associated with chronic kidney disease in humans, but to also accelerate the disease progression.[3] The virtual ubiquitous expression of the VDR in all nucleated cells, the presence of a functional 1 - hydroxylase in at least 10 different tissues apart from the kidney, and the very large number of genes that are under direct or indirect control of $1,25(OH)_2D$ all point toward a more universal role for the vitamin D endocrine system than just regulation of calcium/phosphate/bone metabolism.Urine albumin has been recommended as a sensitive marker for chronic kidney disease by US National Kidney Foundation Guidelines and is also a marker for generalized vascular endothelial dysfunction.

Recent observational evidence suggests strong links between low vitamin D levels and a range of cardiovascular conditions, including stroke, myocardial infarction, hypertension, and diabetes. Low Vitamin D levels can result in vascular smooth muscle cell proliferation, endothelial cell dysfunction, vascular and myocardial cell calcification, and increased inflammation.[4] Interventional studies are beginning to explore whether vitamin D supplementation can modify vascular health and prevent cardiovascular disease.

Greatly increased (16-fold) incidence of cardiovascular disease, particularly myocardial infarction, cardiac failure, sudden cardiac death and stroke has been observed in CKD patients. In CKD, arteriosclerosis becomes the dominant lesion. As a consequence, cardiac disease in patients with CKD can represent a spectrum from left ventricular hypertrophy associated with hypertension, to classical myocardial ischaemia, both irreversible (as in myocardial infarction) and reversible, and progressing to fibrotic myocardial disease with a high risk of sudden death, which is very common in patients with ESRD. The electrocardiogram (ECG) records the electrical activity of the heart at the skin surface and is important in detection of cardiac rhythm

abnormalities, cardiac conduction defects and detection of myocardial ischemia. ECG remains an essential tool for evaluation of cardiovascular disease and is important in detection of cardiac rhythm abnormalities, cardiac conduction defects and detection of myocardial ischemia. Resting ECG abnormalities are common in patients with CKD and they independently predict future cardiovascular events [5]

The aim of our study is to evaluate 25-OH vitamin D status with ACR and to assess its relation with cardiovascular events in patients presenting with CKD at Chirayu Medical College and Hospital.

MATERIALS AND METHODS:

The study design was cross-sectional in nature and was conducted by the Department of Biochemistry in collaboration with the Department of Medicine and Pathology at Chirayu Medical College and Hospital, Bhopal, Madhya Pradesh, over a period of 6 months from June 2019 to November 2019. Sample size of 100 was calculated with 95% confidence level and all patients above 18 yrs of age with known or newly diagnosed CKD were included. This work was approved by the Institute Ethics Committee and selection of cases was done from in-patient wards, ICU and from the Department of Medicine and Nephrology, Chirayu Medical College and Hospital ,Bhopal. The aim of the study and the protocol was explained to the patients and written informed consent was taken from the patients or nearest relatives before inclusion in the study. Patients on Vitamin D therapy, malignancies, obstructive nephropathy, gout, or having H/O IHD were excluded from the study. Patient was considered to have CKD if estimated GFR (eGFR) was less than <60 ml/min/1.73m^2 or if there was persistent proteinuria for 3 or more months. eGFR was calculated by CKD-EPI formula which has been found to be more accurate mainly due to a substantial decrease in systematic differences between mGFR and eGFR bias. Patients were further grouped into various stages of CKD based on KDOQI classification as defined by Nice guidelines 2012.[6-8] .

Albumin-to-creatinine ratio (ACR) is the first method of preference to detect elevated protein. Spot urinary albumin (immunoturbidimetric method) and urinary creatinine (buffered kinetic Jaffes reaction) were done on Cobas Integra 400 Plus analyzer ,Roche Diagnostics, GmbH, Mannheim, Germany using the cobas c pack reagent and ACR was determined. Albuminuria (ACR) categories in CKD were listed as A1 : <30mg/g ; A2: 30-300mg/g and A3: >300mg/g.

As serum 25(OH)D concentration is an excellent reflection of the vitamin D status due to rapid conversion of vitamin D into 25(OH)D and its long plasma half-life[9] quantitative determination of total 25-hydroxyvitamin D in human serum was done on Cobas e411,Roche-Diagnostics GmbH, Mannheim, Germany. Methodology was based on electrochemiluminescence binding assay. 25-OH vitamin D deficiency , insufficiency and sufficiency were defined as levels <30 nmol/L , 30-50 nmol/L and > 50 nmol/L[10]. 12 lead electrocardiograms (ECG) was done at the time of admission along with estimation of serum total 25-hydroxyvitamin D. Interpretation of ECG was done by a qualified physician based on accepted standard criteria[11] .ECG abnormalities were identified and mainly included ST-segment depression/elevation, T-wave inversion, ventricular conduction defects, AV block ,LVH.

Statistical analysis : Data analysis was done by SPSS version 23. For normality test Shapiro–Wilk test was used. Pearson chi square test was used for categorical variables to examine differences across 3 groups of 25- OH vitamin levels: group 1 >50 nmol/L, group 2 : 30–50 nmol/L; and group 3: ≤30 nmol/L. One way ANOVA was used to compare means among two or more groups.

Compliance with Ethical Standards: The study was approved by the institutional research ethics committee (Institutional ethics committee, CMCH,Bhopal) and certify that the study was performed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards. The aim of the study and the protocol was explained to the patients and written informed consent was taken from the patients or nearest relatives before inclusion in the study.

RESULTS:

Number of patients included in the study were 100 and entirely comprised of predialysis patients. Hypertensive patients with CKD were 70 and non -hypertensive patients with CKD were 30. Patients with frank diabetes were 50% , prediabetic 36% and non diabetic were 14 %. Mean age of patients was 44±14 years with 46% males and 54% females. 55% were in the age group of 18 – 45 yrs, 37% in age group of 46 – 65 yrs and 8 % were in age group of >65 yrs. The clinical and demographic characteristics of the patients with known or newly diagnosed CKD are presented in Table 1.

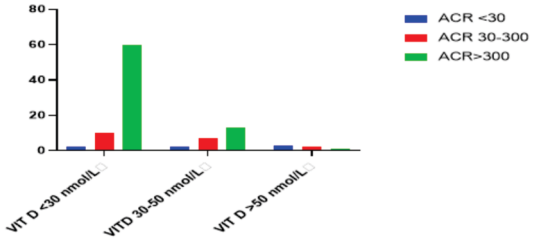
Table 1: Baseline characteristics across 25-OH Vitamin D categories

	Vitamin D Deficiency (<30nmol/L)	Vitamin D insufficiency (30-50nmol/L)	Vitamin D sufficiency (>50nmol/L)	P value
No.	72	22	6	
Age (Yrs)	55.6±12.5	54.2±10.2	32.4±14	<.001
Males	65.2% (30)	26 % (12)	8.6% (4)	0.32
Females	77.7 % (42)	18.5% (10)	3.7% (2)	
BMI (Kg/m2)	28.52±4.56	24.22±3.64	22.4±3.4	<0.001
Hypertension	77.8% (56)	18.6% (13)	1.4% (01)	<0.05
Non Hypertensive	22.2% (16)	30% (09)	16.7% (05)	
Diabetic	52.8% (38)	50 % (11)	16.6 % (1)	0.11
Pre diabetic	36.1% (26)	36.4 % (8)	33.3 % (2)	
Non diabetic	11.1% (08)	13.6 % (3)	50 % (3)	
Spot Urine ACR (mg/g)	390.7±158.7	230.4±161.9	185.6±110.7	<0.0001

Hypertensive and diabetic patients with CKD were found to have lower vitamin D as compared to non - hypertensive and prediabetic and non - diabetic CKD patients.

Mean eGFR of patients was 20.3 ml/min/1.73m^2 (calculated by CKD-EPI formula) and mean spot urinary ACR was 261.9 mg/g. 81% of CKD patients with urine ACR >300mg/g were found to have Vitamin D <30 nmol/L as shown in Fig 1.Hypertensive patients on ACE inhibitors were 28% out of which 10% had ACR >300 mg/g.

Figure 1 Vitamin D And Albumin Creatinine Ratio In CKD Patients



45% of patients were in stage V, 36% in stage IV,14 % in stage III and 5% in stage II based on KDIGO criteria .Mean duration of CKD was 3.6±4 months. 94% of patients were found to have vitamin D deficiency with 72% having vitamin D <30 nmol/L as shown in Table 2.

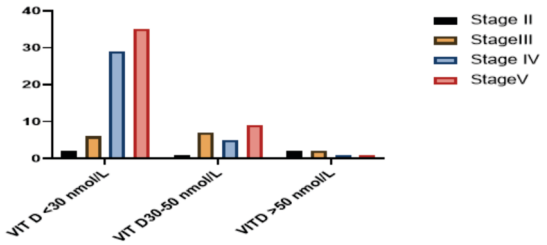
Table 2 Vitamin D Distribution In Ckd Patients

	Stages	VIT D (nmol/L)			Chi square	df	P value
		<30	30 -50	>50			
CKD Stages	II	02 (2.8%)	01 (4.5%)	02 (33.3%)	13.13	6	0.041
	III	06 (8.3%)	07 (31.8%)	01 (16.7%)			
	IV	29 (40.3%)	05 (22.7%)	02 (33.3%)			
	V	35 (48.6%)	09 (40.9%)	01 (16.7%)			

(@ significance at p <0.05)

77% of patients in Stage V and 80.5% in Stage IV CKD were found to have serum Vitamin D < 30nmol/L as shown in Fig.2.

Figure 2 : Distribution of Vitamin D in CKD Patients



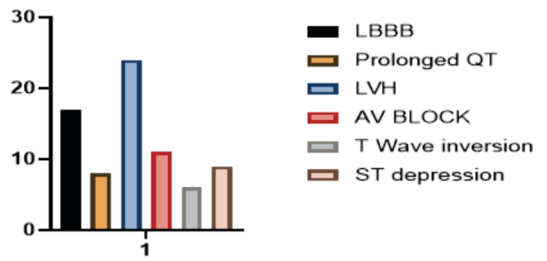
ECG abnormalities were observed in 75% patients with 57% ECG abnormality present in CKD patients with vitamin D <30nmol/L. Significant association (p<0.05) was observed between Vitamin D status and ECG abnormalities as shown in Table 3. Proportion of patients with abnormal ECG findings is shown in Fig.3. 32 % of the total ECG abnormalities detetected were LVH.

Table 3 Vitamin D Association With ECG

	Stages	VIT D (nmol/L)			Chi square	df	P value
		<30	30 -50	>50			
ECG	Normal	15 (20.8%)	6 (27.3%)	4 (66.7%)	6.283	2	0.043
	Abnormal	57 (79.2%)	16 (72.7%)	2 (33.3%)			

(@significance at p<0.05)

Figure 3 Proportion Of ECG Abnormalities



DISCUSSION

Significant association of ACR with 25-hydroxy Vitamin D deficiency in our study supported renoprotective action of vitamin D and could be due to withdrawl of inhibitory effect of Vitamin D on the Renin Angiotensin system (RAAS). Vitamin D has been shown to suppress the transcription of renin, inhibiting the RAAS and ultimately leading to a reduction in proteinuria through hemodynamic and non-hemodynamic pathways and has been observed to be an independent inverse predictor of disease progression and death in CKD patients [12].

Angiotensin II (AII) is the main effector of the RAAS and exerts its vasoconstrictor effect predominantly on the postglomerular arterioles, thereby increasing the glomerular hydraulic pressure and the ultrafiltration of plasma proteins. Previous clinical studies have shown an inverse relation between circulating Vitamin D and renin levels.[13]. ACE inhibitors have a proteinuria reducing effect which accounted for reduced ACR in hypertensive patients.

ECG abnormalities were found to be more in CKD patients with severe vitamin D deficiency. Higher incidence of ECG abnormalities in CKD patients with vitamin D <30 nmol/L in our study could be explained by a regulatory effect of vitamin D on cardiomyocytes and vascular smooth muscle cells. Our findings were consistent with other studies which showed Vitamin D to have beneficial effects on most cells of the vascular wall with an inverse association between $1,25(OH)_2D$ levels and blood pressure or plasma renin levels. [14,15]. Increased thrombogenicity and decreased fibrinolysis have been observed in *VDR*-null mice which further explains the cardioprotective role of Vitamin D. Antiinflammatory actions of Vitamin D have been reported to play an important role in atherogenesis and inhibition of certain matrix metalloproteinases (MMPs) that are important in plaque instability and are known to increase in MI, notablyMMP-9 andMMP-2. [16]. In CKD dysregulation of vitamin D metabolism with raised levels of circulating FGF-23(phosphaturic hormone) and renal 24-hydroxylase (CYP24A1), the catabolic enzyme involved in 25(OH)D and 1,25(OH)2D3 degradation, contributes to vitamin D-deficiency in kidney disease. Raised FGF 23 in CKD as a result of dysregulation of vitamin D metabolism might contribute to cardiotoxic effect and ECG abnormalities as observed in the present study[17].

Serum Vitamin D levels decline early in the course of kidney dysfunction and with progression of CKD severity of Vitamin D deficiency has been found to increase in the present study.

CONCLUSION:

Low vitamin D status in the present study has been found to be associated with micro albuminuria and greater ECG abnormalities suggesting reno protective and cardio protective effect of vitamin D in CKD patients.

Clinical Significance:

Cardiovascular disease is a major cause of morbidity and mortality in patients with chronic kidney disease. Screening of CKD patients for Vitamin D status can be done to attain the noncalcemic benefit on cardiovascular protection. However excess of vitamin D can also have deleterious effects hence proper prospective, large-scale randomized trial are needed before recommendation of routine supplementation of 25(OH)-VD to alleviate associated adverse outcomes and reducing the impact of CKD on healthcare resources.

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