Original article:

Study of Vitamin D levels in patients with Chronic Kidney Disease

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Abstract:

Introduction: Apart from classical functions, vitamin D has autocrine function. Autocrine function of vitamin D has a potential impact on the morbidity and mortality in the Chronic Kidney Disease patients. This study is undertaken to observe probable modality, if present, between post Hemodialysis (HD) decrease in vitamin D levels and clinical deterioration in CKD patients. ***Method & Material:*** 63 patients (32 males and 31 females) of Stage – 5 CKD were studied. There vitamin D was estimated before and after HD on first and subsequent maintenance HDs all patients with or without comorbidities. ***Results:*** Pre HD total vitamin D levels were 'deficient' (58.73%) and 'sufficient' in 38.1%, almost equal in patients of both sexes despite intake of supplement containing Calcium and Calcitriol. Post HD vitamin D levels were detected to be markedly low, seems to be 'washed out' during HD, in 85.71% of patients (29 males and 27). Vitamin D 'wash out' effect was also observed in subsequent maintenance HDs. Females and elderly patients were more prone to vitamin D 'wash out'. Conventional therapy with vitamin D supplements can replenish but could not prevent its post HD 'wash out'. ***Conclusion:*** It seems, in CKD the deleterious effects of post HD vitamin D deficiency are due to appreciable autocrine dysfunctions resulting in cardio vascular diseases (CVDs) and comorbidities especially diabetes mellitus add to worsening of CVDs, which are the main causes of high morbidity and mortality in these patients.

Keywords: Chronic Kidney Disease; Vitamin D; vitamin D Receptor; Hemodialysis

Introduction:

Vitamin D has emerged as a vital compound with newly ascribed autocrine functions vastly different from its classical function in mineral homeostasis. To ignore the significance of vitamin D and its potential impact on morbidity and mortality in the Chronic Kidney Disease (CKD) patients is no longer appropriate.Now it is established that role of vitamin D is no longer restricted to calcium and phosphate homeostasis[1,2] but also important in cell differentiating and antiproliferative[1,3]; acts in different tissues i.e. renal, cardiovascular, and immune systems[2,4];regulates rennin – angiotensin system (RAS)[5]and the nuclear factor (NF) κ B pathway[4]. It was thought till now that the vitamin D activation 1,25Dihydroxy vitamin D [1,25 (OH)2 D]takes place primarily in the kidney and only regulates bone and mineral metabolism[1].The wide distribution of the 1-α-hydroxylase and vitamin D receptors (VDRs) in non-renal tissues such as the skin, vascular smooth muscle cells, pancreas, kidney, heart, immune system[3,7] has shown 25 hydroxy vitamin D [25 (OH) D]activation to 1,25 (OH)2 D takes placein these tissues*in situ*[2,6].

The kidney appears to be a major target organ for both the classical [activation of 25 (OH) vitamin D to

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1,25 (OH)2 D] and non-classical (autocrine) actions of vitamin D asVDRs are highly expressed in kidneys (4). The non-classical effects of vitamin D may play a relevant role in the mortality and morbidity of CKD patients, specifically affecting the possible progression of their renal disease and coexisting cardiovascular disease (CVD), hypertension (HTN), diabetes mellitus (DM) and progression of CKD, which is the major cause of death in this population[4,5]. The autocrine role of vitamin D in the CKD is to regulateRAS with activation of angiotensin II. That is, in the absence of vitamin D autocrine activity in CKD patientslikely to have deleterious effects on the vasculature, renal parenchymal and blood pressure. With vitamin D supplements, these deleterious effects are reported to get reversed and beneficial effects noticed in blood pressure and cardiovascular state (8).We have observedin some CKD patients sudden clinical deterioration after hemodialysis (HD). They were not having fluid overload, left ventricular failure (LVF) or serious systemic infection. This study is undertaken to observe probable modality, if present,between post HD decrease in vitamin D levels and clinical deterioration in CKD patients.

Study design: This was about two years, single centre,retrospective and prospective, randomized, non intervention and blind study carried out in Tertiary Referral Hospital in Himachal Pardesh (India).

StudyParticipants:

Inclusion criteria: Retrospective and prospective patients of Stage - 5 CKD (Glomerular Filtration Rate ≤ 15 ml / min per 1.73 m²), due to any cause, requiring maintenance HD.

Exclusion criteria: Patients of Chronic Heart Failure (CHF), terminally ill,Hypercortisolism, thyroid dysfunction, acromegaly/gigantism, on steroids, chronic infectious diseases; chronic intestinal diseases.

Total patients: 69 patients (35 males and 34 females, male: female 1:1).

Duration of study: from Jun 2014 to Oct 2016.

Study design: Kidney Disease Outcomes Quality Initiative (KDOQI)2002, and subsequently adopted with minor modifications by Kidney Disease Improving Global Outcomes (KDIGO) in 2004formed the basis of classifying CKD. Estimated Glomerular Filtration Rate (eGFR) was calculated by the abbreviated MDRD equation: 186 x (Creat / 88.4)$^{-1.154}$ x (Age)$^{-0.203}$ x (0.742 if female) x (1.210 if black). Stage – 5 CKDpatients, who consented for the study, were enrolled. They were evaluated for associated co-morbidities, previous HD, post - HD complications, supportive treatment for CKD and associated co-morbidities.History was taken to inquire into thevitamin D supplements consumption as prescribed for CKD and also to rule out any steroid intake, chronic infections or intestinal diseases, endocrinopathies except DM. Physical examination included: recording of blood pressureand other relevant vital parameters; presence of anemia, edema, cardiovascular state and systemic examination relevant to CKD, associated co – morbidities.

*Biochemical measurements:*Pre HD, total vitamin D was measured by using *DIAsource 25OH Vitamin D Total ELISA 90KAP1971/F1*kit manufactured by DIAsource ImmunoAssays SA (Rue du Bosquet, 2 B-1348 Louvain – la Neuve, Belgium). Levels of vitamin D, as per 'kit', as shown in Table – 1, were accepted for result interpretation. Apart from blood urea, serum creatinine, serum electrolytes, serum calcium & phosphate, enrolled patients were also subjected to relevant laboratory, radiological and ECG tests depending on associated co – morbidities for management during subsequent follow up. Total Vitamin D was estimated again on maintenance post HD. Followupincluded clinical and laboratory evaluation relevant for pre and post dialysis.

Results:

69 patients (35 males and 34 females, male: female 1:1) of stage – 5 CKD consented for study. During study 6 patients (3 males and 3 females) left due to change of station. Age and sex-wise and comorbidities-wise distribution of patients is shown in Table – 2 and Table – 3.

(Table – 1) (Accepted Vitamin D values)

Test Parameter	Interpretation				Unit
	Deficiency	Insufficiency	Sufficiency	Toxicity	
Vitamin D	≤ 10	10 – 29	30 – 100	≥ 100	Ng/Ml

(Note: Above values as per DIAsource 25OH Vitamin D Total ELISA 90 kit standard)

(Table – 2) (Age and sex wise distribution of patients)

Participants	< 31 year	31 – 50	51 – 70	>70
Males	01	06	22	01
Females	02	13	16	02
Total	03	19	38	03

(Table – 3) (Associated Co morbidities wise distribution of patients)

Age distribution	HTN M	F	DM M F	HTN & DM M F	Others• F	
< 31 Years	-	01	- 01	-	0	-	-
31 – 50	04	11	02 03	-	-	-	-
51 – 70	13	10	05 03	060	01	-
>71	01	-	-01	01 01	-	-
Total	17	22	0708	07 01	01	-

(HTN= Hypertension, DM = Diabetes mellitus, M = males, F = females.

•= one patient of post pyelonephritis CKD)

Pre hemodialysis total vitamin D levels were 'deficient', equally in both sexes, in 58.73% of CKD patients (18 males and 19 females), 'sufficient' vitamin D was in 38.1% (13 males and 11 females), one male and one female were in 'toxicity' range. Details revealed they were consuming supplement containing Calcium and Calcitriol in heavy dosages. Post hemodialysis vitamin D levels were detected to be markedly low, seems to be 'washed out' during HD, in 85.71% of patients(29 males and 27 females) and 'sufficient' in only 14.29% (5 males and 4 females). No patient was in 'toxicity' range. Pre and post dialysis is shown in figure - 1

(Vitamin D Levels Pre and Post Hemodialysis)
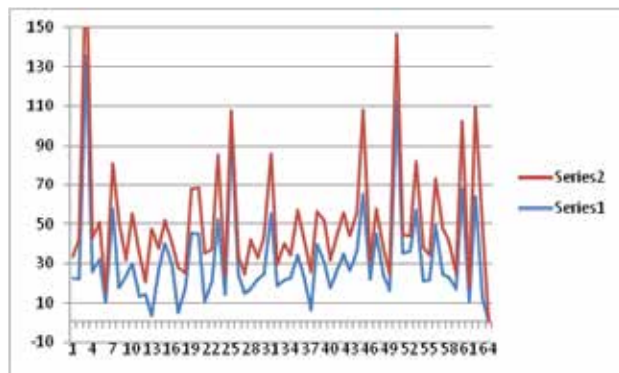
(Figure – 1: it depicts Predialysis vitamin D levels in RED and Post Hemodialysis vitamin D levels in BLUE)

Same vitamin D 'wash out' effect was also observed in subsequent maintenance HDs as depicted in figure – 2. This phenomenon was observed despite administration of appropriate calcium and vitamin D supplements along with other conventional treatment of CKD.

(Vitamin D Levels Pre and Post Hemodialysis)
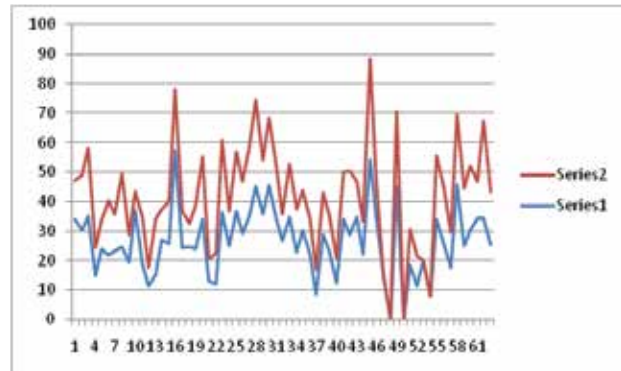
(Figure – 2: it depicts Predialysis vitamin D levels in RED and Post Hemodialysis vitamin D levels in BLUE)

Post HD low vitamin D levels were found in 53 out of 63 patients[(84.13%; males = 26 (41.27%) and females = 28 (42.86%)] patients as depicted in (Table – 4).Females HTN with CKD signalled the worst outcome in post HD. In females vitamin D levels were low in 20 (19 HTN +1 HTN & DM) out of 23 (86.96%). Whereas in males, vitamin D levels were lessin 19 patients (13 HTN + 6 HTN & DM) out of 24(79.17%). In DM arm there was insignificant difference between both sexes: males 13 [7 DM + 6 HTN & DM (92.86%)] and females 8 [7 DM + 1 HTN & DM (88.89%)].Average values depicted in (Table – 5)about the outcome of pre- HD and post – HD effects on vitamin D revealed lower vitamin D levels were found in females as compared to males in subsequent HDs. Sex related vitamin D 'wash out' was observed in CKD associated with any co-morbidity, that is, females continued to have lower vitamin D levels post HD irrespective of any associated co-morbidity. During conventional therapy of CKD with vitamin D supplements did not prevent the post HD vitamin D 'wash out'.

(Table – 4) Post Dialysis Vitamin D levels

Patients	HTN		HTN& DM		DM		Post HD CKD Patients	
Vitamin D level	Total	Post HD	Total	Post HD	Total	Post HD	Total	Post HD
Males	17	13(76.47%)	7	6(85.71%)	7	7 (100%)	31	26 (41.27%)
Females	22	19(86.36%)	11	11 (100%)	8	7(100%)	31	27 (42.86%)

(Note: HTN: Hypertension, HTN & DM: Hypertension and Diabetes Mellitus, DM: Diabetes Mellitus, Post HD: Post Hemodialysis)

(Table – 5) (Average Vitamin D levels Pre - and Post – HD)

CKD & Co-morbidities	Pre Hemodialysis				Post Hemodialysis			
	First		Subsequent		First		Subsequent	
	M	F	M	F	M	F	M	F
All CKD patients	32.38	32.05	29.40	23.34	19.94	18.57	16.03	14.81
HTN	33.74	38.78	27.82	25.22	22.09	15.50	16.05	14.36
DM	29.21	29.09	26.93	24.88	15.12	19.49	12.72	17.59
HTN & DM	27.18	16.73	32.64	45.19	17.55	29.02	13.37	9 25

Discussion:

Before discussing the results of this study, it is imperative to recapitulate the new established roles of vitamin D i.e. autocrinepathway (*non – classical pathway)* in peripheral tissues and CKD.

New established roles of vitamin D: Lots of evidence has shown involvement of vitamin D in numerous regulatory processes in the body and, as previously maintained, the role of vitamin D is no longer solely restricted to its *classicalpathway* of maintaining calcium and phosphate homeostasis[1, 3]. Vitamin D seems to be involved in cell differentiating and antiproliferative factor, action in different tissues i.e. renal, cardiovascular, and immune systems[1-4,9]. Vitamin D, through *non – classical pathways* (autocrinepathway) regulates RAS[3] and nuclear factor (NF) κB pathway[3].These emerging roles of vitamin D establish a new paradigm intreatment approach to address both the classical and non-classical effects of vitamin D in patients of vitamin D deficiency, particularly those with CKD, where hypovitaminosis D is disproportionately high as compared to non - CKD patients.

Autocrine(Non –Classical)Pathway of vitamin D: It appears that most of 25 (OH) Dmetabolised via the peripheral autocrine pathway. In peripheral tissues 25 (OH) D attaches to intracellular VDRsand synthesises 1,25(OH)2 D*in situ* and participates in the intracellular signalling cascades bridging external signals to the gene transcription. Therefore, intracellularly 1,25(OH)2Dregulates cellular proliferation and differentiation, inflammation, the immune system and the endocrine system, including RAS, insulin resistance and lipid metabolism[1, 6].

*Autocine pathway of Vitamin D in CKD patients:*Both the classical and non-classical pathways for vitamin D actions do exist in kidneys as VDRs are widely expressed in this organ[4]. It is now documented disproportionately high incidence of hypovitaminosis D in CKDpatients[1, 4-5]. Because of marked VDRs deficiency and ineffective vitamin D autocrine functionin CKD, RAS appear to be dysregulated.In diabetic nephropathy, both hyperglycemia and hypovitaminosis D also appear to activate RAS as shown by enormous increase in angiotensin II concentration in renal tissues, thisactivated angiotensin-II is likely to have deleterious effect on blood pressure, vascular smooth muscles, cardiac muscles and renal parenchyma resulting in hypertension, cardiac hypertrophy and water retention[8, 10]. Because of these pathophysiological changes, CVDs seem to be the main cause of mortality in CKD patients. Evidences have also shownan inverse relationship between vitamin D levels and degree of albuminuria, which is an important diagnostic hallmark of CKD (11).Vitamin D repletion, both in humans and animals, positively alters this dysfunction and may

be significant in affecting premature mortality in CKD[5,8].

Vitamin D regulates NF-κB pathway through autocine action. In CKD patients withvitamin D deficiency, unregulated and activated NF-κB cascade has role in progression of renal disease and diabetic nephropathy through the production of cytokines, chemokines and other inflammatory factors.Vitamin D has been shown to inhibit the activation of NF-κB and there is an inverse relationship between serum vitamin D levels and the degree of tissue inflammation present in various kidney diseases[4, 12-13].

*Present Study:*Stage – 5 CKD patients, due to any cause, participated in this study. Majority of the patients were in age group 51 - 70 (60.32%), males 22(34.92%) and females 16 (25.4%). Patients in all age groups (Males 18 and females 19), irrespective of any associated co-morbidity (ies),were found vitamin D deficient / insufficient(58.73%). As prescribed dose of vitamin D supplement varies from institution to institution and health providers, these results are at variance with other study where deficiency / insufficiency were found to be 88.9% and 6.7%, respectively[14]. It could be due to difference in doses / amount ofvitamin Dconsumption, population studied and number of HDs a patient has undergone.

On first Pre dialysis average vitamin D levels in over all CKD with HTN patients were sufficient (>30ng/ml), but deficient (<30ng/ml) in CKD patients with co –morbidities like DM and HTN & DM. Post HDs vitamin D levelsremained uniformly low (<30n/mi) in all patients irrespective of morbidities (15). It is also substantiated by other studies[15,16]. It is difficult to explain in patients with comorbidity (HTN & DM) that ininitial post HD thatvitamin D levels found to be in sufficient range (> 30ng/ml). However, in subsequent post HDs, there was significant reduction in vitamin D levels in both sexes. Our findings differ from other studies where comorbidities like HTN, HTN & DM were reported to be independent of vitamin D levels[17, 18] but these comorbidities worsen with low vitamin D levels[15], especially, in post HD period. In post HDs, elder patients and females in all age groups had significantly lower levels of vitamin D than malesirrespective of any associated comorbidity, findings consistent with other studies[15,19,28-29].

Studies have suggested that 25 (OH) Ddeficiency is an unrecognized contributor to development of CVDs and mortality. Deficient vitamin D, especially < 17.8ng/ml, was associated with a 26% increased rate of all-cause mortality[15]. However, major contribution in this study was by female and elderly CKD patients.Majority of our patients were from low socio economic background (inadequate vitamin D supplements), had less exposure to sunlight particularly in winters(high hilly terrain where sun rises late and sets early) resulting in appreciable low 25 (OH) D levels. These findings were consistent with other study[15].

Possible mechanisms of hypovitaminosis D in CKD: Many mechanisms seem to be responsible in hypovitaminosis D in CKD: loss of functional renal mass leading to decrease in production of 1 α (OH)ase[20]; suppression of 1 α (OH)ase activity by metabolic acidosis[21]; hyperphosphatemia[22]; uremic toxins[23]; elevation of fibroblast growth factor 23 (FGF 23) upregulating 24 (OH)ase and downregulation of conversion of 25 (OH) D to 1,25 (OH)2D [24]; age related increase in 24 (OH)ase gene expression and hence increase in clearance of 1,25 (OH)2D[25]and probably estrogen deficiency with decrease production of 1,25 (OH)2D, decrease suppression of 24,25 (OH)2D resulting in net vitamin D deficiency [26] andvitamin D-deficiency may further accelerate CKDprogression[27].

Post HD Vitamin D 'wash – out': Apart from above factors operating in CKD and factors related to thesocio – economic and geographical background of participants, dialysis patients are at a greater risk of vitamin D deficiency and many of the diseases thought to be associated with it[16]. How does vitamin D, being fat soluble, get 'washed out' during HD? Fat soluble vitamin D needs lipid medium only during digestive processes. After absorption from gut or after synthesis in skin from7 – dehydrocholesterol by Ultra Violet (UV B) radiations, it getsactivated into 25(OH)D in liver. 25(OH)D circulates in blood after binding with Vitamin D – binding protein (VDBP).VDBP also carries vitamin D metabolites. VDBP, also known as *gc– globulin,* a multifunctional protein belongs to albumin gene family (GC-gene) and is found in plasma, ascitic fluid, cerebrospinal fluid and on surface of many cells.VDBP electrophoresisidentified two different phenotypes VDBP-2 and VDBP-2 2.There was an increased proportion of the DBP 2 allele in HD patients. The median serum DBP concentration was lowest in the DBP 2-2 group. The need for oral vitamin D differed significantly between DBP phenotypes, and was greatest in DBP 2-2 [30].

Various studies have demonstrated the loss of VDBP in urine and dialysate (in peritoneal effluent more than in HD effluent) and contribute tocirculating

low 25(OH)D levels in chronic peritoneal dialysis [PD] patients[31 - 33]. But these studies were conducted in pediatric age groups. In our study, pre HD total vitamin D level deficiency was equal in both sexes i.e. in 58.73% of CKD patients (18 males and 19 females) almost similar to as reported by other (34-35), 'sufficient' vitamin D was in 38.1% (13 males and 11 females), these results are at variance with other observations[31 – 33], because in post HD vitamin D levels were found to be appreciably low in 85.71% of our patients (29 males and 27 females) whereas 'sufficient' in only 14.29% (5 males and 4 females). It seems that during HD there was significant 'wash out' of VDBP bound 25(OH)D, hence exacerbating their already existing vitamin D deficiency / insufficiency. Irrespective of associated comorbidity (ies), females has shown greater loss of 25(OH)D than males. This 'wash out' effect has been observed also in subsequent maintenance HDs, despite adequate vitamin D supplements.

Possible etiopathogenesis of deterioration in clinical condition in CKD patients:

Numerous evidences, both in animals and humans, have documented the involvement of vitamin D, through autocrine effects, in numerous regulatory processes in the body i.e. cell differentiating and antiproliferative factor, action in different tissues i.e. renal, cardiovascular, and immune systems, apart from classical pathway of calcium and mineral homeostasis[1-4,9]. Vitamin D also regulates RAS and nuclear factor (NF) κB pathway[3].

Hypovitaminosis D is documented to be disproportionately high in CKD patients[1, 4-5]. Because of renal tissue damage VDRs are marked deficient resulting in ineffective vitamin D autocrine effects. RAS appears to be dysregulated and with angiotensin-II activation. In diabetic nephropathy, hyperglycemia and hypovitaminosis D also dysregulate RAS resulting in excessive angiotensin II activation[10]. Activated angiotensin II seems to have deleterious effect on blood pressure, vascular smooth muscles, cardiac muscles and renal parenchyma resulting in hypertension, cardiac hypertrophy and water retention[8]. It is observed that with further decrease in 25 (OH) D levels, mean systolic and diastolic pressure, body mass index (BMI) and percentage of DM have increased and mean serum albumin levels decreased[15]. Therefore, HTN and DM may likely to get exacerbated, insulin resistance appear and hypoalbuminemia develops resulting in extravasation of intravascular fluid into extravascular tissues and spaces complicating clinical picture further. The non-classical effects of vitamin D may play a relevant role in the mortality and morbidity of patients with CKD, specifically affecting the possible progression of their renal disease and coexisting CVD, HTN, DM and progression of CKD, which is the major cause of death in this population[4,5].

In CKD patients, hypovitaminosis D unregulate and activate NF- κB cascade resulting in progression of renal disease and diabetic nephropathy through the production of cytokines, chemokines and other inflammatory factors. Various studies have observed an inverse relationship between serum vitamin D levels and the degree of tissue inflammation present in various types of kidney disease [4, 12-13]. This effect may be because vitamin D supplements have shown to inhibit the activation of NF-κB.

Aim of this study was to observe, why clinical condition deteriorates after HD in CKD patients. 25(OH)D deficiency is an silent contributor to the development of CVD, development of multiple risk factors for CVD, cancer and mortality[36]. Low 25(OH)D levels in HD patients have been shown to be associated with all-cause-mortality[37]. Because of 25(OH)D deficiency, functioning of non-classical pathway functions inefficiently, thereby, inadequately regulating various cell functions including RAS, Insulin resistance and lipid metabolism[1,6]. Therefore, CKD patients having 25 (OH)D levels in the lowest quartile were associated with 70% and 78% higher risk of CVD mortality and all-cause- mortality respectively. However, this association was not significant statically in the fully adjusted model (38). Major cause of CVD mortality was 76% atherosclerotic CVD, 19% cerebrovascular and 5% congestive heart failure in one follow up study of 8.7 years. Females having both low (<20ng/ml) and high (>50ng/ml) vitamin D levels were associated with increased rate of mortality (38).

Treatment paradigm for vitamin D in CKD – a new shift:

As the new role of vitamin D unfolded, the treatment paradigm for vitamin D in CKD has now shifted to ensure requirements of both the classical and non-classical pathways. As vitamin D deficiency is disproportionately high as compared to non - CKD patients, significance and benefits of vitamin D therapy and its potential impact on morbidity and mortality cannot be ignored. It is noted that use of activated vitamin D in CKD patients is associated with decreased mortality[39] and inhibits rennin-angiotensin system[40]. In CKD, 25-(OH)-vitamin D

supplementation is recommended at the inception of the disease, with the addition of calcitriol replacement beginning in Stage 3[1]. Because of non – classical effects of vitamin D, it is suggested to revised recommendations for the daily intakes both in normal and CKD patients. Oral vitamin D supplementation seems to be cost-effective, have better control of mineral metabolism, attenuation of inflammation, reduction in erythropoietin dose and possibly improvement of cardiac dysfunction without evident toxicity[41]. Recent studies have recommended daily requirement in normal population to be as high as 4,000 international

units (IU) to maintain optimum levelsand it is extrapolated that higher doses may be required in CKD patients to overcome the more profound deficits[1]. For safe and adequate replenishment of vitamin D, one should consider that for each 100 IU of vitamin D administered, the serum level of 25-(OH)D will rise by 1 ng/mL, although there is wide individual variation in the response to vitamin D dose.One should also take into account that vitamin D requirement is greatest for the CKD patients with VDBP phenotype 2-2.Therefore, vitamin D treatment needs monitoring among VDBP 2-2 phenotype group with CKD[30].

Limitations of the Study:

1.Small size of the study.

2. In this study only total serum vitamin D is estimated and not the fractions i.e. 25 (OH) D and 1,25 (OH)2 D separately. Because concentration of circulating 25 (OH) D is 1000 time more than 1, 25 (OH)2 D (32,42). It is 25(OH)D which is important for non-classical effects of vitamin D. In CKD, especially after HD, plasma levels of 25(OH)D determine the CVD mortality and all-cause-mortality, possible progression of their renal disease and coexisting CVD, HTN, DM and progression of CKD (4,5). Activated 1,25 (OH)2 D circulates in plasma in small fraction which seems to be insignificant in the present context.

3. Only one parameter i.e. vitamin D is considered in this study. In CKD there is vast biochemical derangement – ranging from serum electrolytes, serum calcium, phosphate, hormones, plasma albumin and products transported by it, RAS components and metabolites. It was not possible to establish correlation between post HD vitamin D changesand all of these altered parameters.

4. Long term effect of 25 (OH)D deficiencycould not be ascertained because of small duration of this study. It may be possible that in post HD period, rapid clinical deterioration occurs due to sudden dysregulation of intracellular pathophysiology at molecular level. It needs molecular level study which is not possible in this institution.

Conclusion: Hemodialysis exacerbates vitamin D deficiency, especially 25(OH) D, in CKD patients. Increase in CVD mortality and all- cause-mortality with acceleration of multiple risk factors for CVD and progression of renal disease are attributed to decrease in 25(OH) D. In this study, we tried to establish that 25(OH) D 'wash – out' during HD may be responsible for rapid deterioration of the clinical condition of CKD patients.It seems after HD, due to sudden decrease in 25(OH) D, there is sudden intracellular environment changes at molecular level. Probably, these changes may be responsible for rapid clinical deterioration. However, it is recommended to undertake further study to unfold intracellular dysregulation at molecular level as the cause of sudden deterioration in clinical condition in CKD patients in post HD period.

Conflict of Interest: No conflict of interest to disclose.

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