Prevalence of Abnormal Serum 25-HydroxyVitamin D and Its Association with Hemoglobin Level in Pre-Dialysis Chronic Kidney Disease (CKD) Patients

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Abstract

Background: Chronic kidney disease (CKD) has been a growing health burden worldwide.

Aim: To evaluate 25 (OH) vit D levels in non-dialysis CKD patients and investigate if this deficiency independently correlates with lower hemoglobin levels.

Subjects and methods: This was a cross-sectional study performed on 90 subjects at outpatient clinic of Beni-Suef University hospital & outpatient clinic at EL Galaa military hospital, from August 2020 till the patients were collected.

Results: The prevalence of abnormal vitamin D (< 30 ng/mL) was 92.9%in group A with insufficiency 78.6% and deficiency (14.3%) while prevalence of abnormal vitamin D (< 30 ng/mL) was 100% in group B with insufficiency 15.4% and deficiency (84.6%). The prevalence of abnormal vitamin D was 100% in group C with insufficiency 0% and deficiency (100%) while prevalence of abnormal vitamin D was 83.3% ingroup D with insufficiency 38.9% and deficiency (44.4%). There was a significant difference between the four groups regarding prevalence of abnormal vitamin D ($p<0.001$). The mean hemoglobin was 10.28± 0.83 g/dl, 9.01± 0.65 g/dl, 8.36±0.69 g/dl and 13.23± 1.35 g/dl in group A, group B, group C and group D respectively. Control group showed significant increase in Hb. compared to group A, group B, group C and group D.

Conclusion: Abnormal 25OHD metabolite prevalence in pre-dialysis CKD patients is linked to lower hemoglobin and higher PTH levels, potentially causing anemia and hyperparathyroidism.

Keywords: CKD; Vitamin D; Hemoglobin.

Introduction

Worldwide, the burden of chronic kidney disease (CKD) is rising **(1)**.

Numbers of prevalent CKD patients will continue to climb reflecting the expanding senior population and increasing numbers of patients with diabetes and hypertension **(2)**.

Numerous consequences, including as electrolyte imbalance, fluid overload, problems with bone and mineral metabolism, and anemia, continue to plague patients with chronic kidney disease (CKD) **(3)**.

Chronic kidney disease (CKD)-related anemia is a complicated consequence that frequently remains untreated, increasing risk of morbidity, death, and medical expenses **(4)**.

The aim of this study was to determine whether there was an independent relationship between decreased hemoglobin levels and 25 (OH) vitamin D insufficiency in patients with chronic kidney disease (CKD) who were not receiving dialysis.

Patients and methods

This was a cross-sectional study performed on 90 subjects at outpatient clinic of Beni-Suef university hospital & outpatient clinic at EL Galaa military hospital, from August 2020 till the patients were collected. They were divided into 72 pre-dialysis CKD patients and 18 healthy individuals as (control group), 72 pre-dialysis divided into: Group (A): includes 14 CKD stage 2 patients with GFR 60 - 90 ml / min per1.73 m2, group (B): includes 26 CKD stage 3 patients with GFR 30 - 60 ml / min per 1.73 m2, group (C): includes 32 CKD stage 4 patients with GFR 15 - 30 ml / min per 1.73 m2 and group (D): includes 18 healthy individuals as control group.

Inclusion criteria: Adult patients above the age of 45 years and pre-dialysis patients with GFR < 60 ml\min. Stage 3 A moderate CKD (GFR = 45-59 ml\min). Stage 3 B moderate CKD (GFR 30-44 ml\min). Stage 4 severs CKD (GFR 15-29 ml\min). Stage 5 end stage CKD (GFR < 15 ml\min) not on dialysis.

Exclusion criteria: Patients with renal replacement therapy, patients with prolonged use of vit D therapy within 12 months of screening, macrocytic hyperchromic anemia and microcytic hypochromic anemia.

Methods

All patients were subjected to Full medical history, full medical examination, D-Imaging: Pelvic, abdominal ultrasound and laboratory investigations: Renal functions, complete blood count reticulocytic count, iron profile and 25 (OH) vit D levels done by Stat fax 2100 ELISA reader, made in USA. Vit D levels were determined using an enzyme immuno assay called Vit D Quanti microlisa. The test was based on delayed competitive ELISA, where samples were incubated with a diluent to release vitamin D from the binding protein in the sample. The sample competed with the biotin conjugated for binding anti-vit D antibodies, increasing sensitivity for low concentration samples. The enzyme conjugated detected the binding of 25OH Vit D – Biotin, and the color reaction was started and stopped after a defined time. The test's principle was that the color intensity was inversely proportional to the concentration of vitamin D in the sample. Sample preparation involved using only human serum, removing the serum from the clot to avoid hemolysis, and avoiding heat inactivated, icteric, hyperlipemic, and hemolysed samples. Samples were tested using Architect plus i1000 SR and Vitros 4600.

Sample Size: A previous study [(5)] was used to calculate the sample size. sample size was calculated by formula as 1.962(0.05)2/.012. So, it can be relied upon in this study, based on this assumption, sample size was calculated according to these values produced a minimal samples size of 68 cases were enough to find such a difference. Assuming a drop-out ratio of 5%, the sample size will be 72 patients.

Ethical consideration

All subjects participated in the study were asked to sign a consent before inclusion in the study in addition to approval of the ethical committee for the study on 1/9/2020.

Statistical analysis

Microsoft Excel 2016 for Windows, a part of the Microsoft Office suite, was used to gather, code, and enter data into a spread sheet. Microsoft Excel 2016 was produced by Microsoft Corporation in the United States. The IBM Statistical Package for Social Sciences (SPSS) software was utilized to analyze the data (IBM SPSS Statistics for Windows, Version 26.0. Armonk, NY: IBM Corp). The distribution's normality was confirmed using the Kolmogorov-Smirnov test. For continuous data, mean ± standard deviation was used, whereas numbers and percentages were used for categorical data. A statistical significance threshold of less than 0.05 was applied. The chi-square test, analysis of variance (ANOVA or F test), Kruskal-Wallis test, post Hoc testing, and correlation analysis (using spearman's approach) were the tests that were employed.

Results

Table (1): Demographic characteristics among the studied groups

		Group (A)(n.= 14)		Group (B)(n.= 26)		Group (C)(n.= 32)		Group (D)(n.= 18)		Test value	P-value
		No.	%	No.	%	No.	%	No.	%		
Gender	**Male**	5	35.7%	0	0.0%	0	0.0%	0	0.0%	X^2= 1.867	0.393
	Female	9	64.3%	26	100.0%	32	100.0%	18	100.0%		
Age (years)	**Mean± SD**	60.14± 8.47		55.58± 8.69		62.63± 7.97		40.22± 2.82		KW= 10.17	**0.017**
	Median	61.5		50.5		65.0		40.0			
	Range	47.0- 72.0		45.0- 73.0		47.0- 75.0		35.0- 45.0			

p≤0.05 is considered statistically significant, p≤0.01 is considered highly statistically significant, SD: standard deviation, analysis done by X^2: Chi-Square Test& KW: Kruskal-Wallis Test.

This cross-sectional study was conducted on 72 pre-dialysis CKD patients and 18 healthy individuals as control group. The study was conducted at outpatient clinic of Beni-Suef University hospital & outpatient clinic at EL Galaa military hospital. The studied cases were divided into 4 groups: Group (A): includes 14 CKD stage 2 patients with GFR 60 - 90 ml / min per1.73 m2. Group (B): includes 26 CKD stage 3 patients with GFR 30 - 60 ml / min per1.73 m2. Group (C): includes 32 CKD stage 4 patients with GFR 15 - 30 ml / min per1.73 m2. In addition, group (D): includes 18 healthy individuals as control group, Among the studied groups, females predominated, comprising 64.3% in group A and 100% in groups B, C, and D, with no statistically significant gender difference (p > 0.05). The mean ages were 60.14±8.47 years (group A), 55.58±8.69 years (group B), 62.63±7.97 years (group C), and 40.22±2.82 years (group D). A significant age difference was observed (p = 0.017), with group C patients being notably older than group B patients. (Table 1).

Table (2): GFR, hemoglobin, vitamin D and PTH in the studied groups

		Group (A) (n.= 14)	Group (B) (n.= 26)	Group (C) (n.= 32)	Group (D) (n.= 18)	KW/F	
						Test	P-value
	Mean± SD	71.09± 8.33	35.20± 5.10	23.64± 4.08	107.34± 17.47		

GFR (ml / min per 1.73 m2)	**Median**	67.70	34.15	24.70	102.40	KW= 81.82	**<0.001**
	Range	61.20- 87.50	30.0- 55.70	15.0- 29.90	90.50- 137.40		
Hemoglobin (g/dl)	**Mean± SD**	10.28± 0.83	9.01± 0.65	8.36± 0.69	13.23± 1.35	KW= 65.42	**<0.001**
	Median	10.15	9.0	8.15	13.40		
	Range	9.0- 12.0	6.60- 10.0	7.20- 10.0	10.80- 16.0		
Vitamin D (ng/ml)	**Mean± SD**	20.60± 9.11	13.67± 5.64	10.68± 4.19	23.52± 7.18	F= 20.46	**<0.001**
	Median	22.0	14.50	10.20	21.10		
	Range	0.5.0- 34.10	3.50- 22.30	2.10- 17.70	14.70- 37.10		
PTH (pg/dl)	**Mean± SD**	64.50± 5.52	83.08± 7.14	113.06± 14.49	23.52± 7.18	KW= 79.48	**<0.001**
	Median	65.5	84.5	114.5	21.10		
	Range	53.0- 71.0	72.0- 95.0	85.0- 141.0	14.70- 37.10		

p≤0.05 is considered statistically significant, p≤0.01 is considered highly statistically significant, SD: standard deviation, analysis done by F: One-Way ANOVA Test.& KW:Kruskal-Wallis Test.

Group A, B, C, and D had mean GFRs of 71.09± 8.33, 35.20± 5.10, 23.64± 4.08, and 107.34±17.47, respectively. Between groups A and C, there was a statistically significant drop in mean GFR. The GFR of the control group was significantly higher than that of groups A, B, and C. In group A, group B, group C, and group D, the mean hemoglobin was 10.28± 0.83 g/dl, 9.01± 0.65 g/dl, 8.36±0.69 g/dl, and 13.23± 1.35 g/dl, respectively. As CKD worsened, the mean hemoglobin progressively dropped. Compared to groups A, B, C, and D, the control group's Hb level increased significantly. In group A, the mean vitamin D level was 20.60± 9.11 ng/ml; in group B, it was 13.67±5.64 ng/ml; in group C, it was 10.68± 4.19 ng/ml; and in group D, it was 23.52± 7.18 ng/ml. When the vitamin D levels of groups A, B, C, and D were compared, it was found that there had been a statistically significant ($p < 0.01$) drop in levels. Despite the fact that there was no statistically significant difference in the drop in vitamin D levels between groups A and D and B and C. As the stage of CKD advanced, there was a considerable rise in the mean serum PTH levels. Group A had the highest mean serum PTH value at 64.50±5.52, group B at 83.08±7.14, group D at 113.06±14.49, and group D at 23.52±7.18 pg/ml. Statistical analysis revealed that group A's mean PTH level increased significantly more than group C's (Table 2).

Table (3): Prevalence of abnormal vitamin D among the studiedgroups

		Group (A) (n.= 14)		*Group (B) (n.= 26)*		*Group (C) (n.= 32)*		*Group (D) (n.= 18)*		*Chi-Square Test*	
		No.	*%*	*No.*	*%*	*No.*	*%*	*No.*	*%*	**Test value**	**P-value**
Vitamin D	**Normal**	1	7.1%	0	0.0%	0	0.0%	3	16.7%	48.45	**<0.001**
	Vitamin D insufficiency	11	78.6%	4	15.4%	0	0.0%	7	38.9%		
	Vitamin D deficiency	2	14.3%	22	84.6%	32	100.0%	8	44.4%		

p≤0.05 is considered statistically significant, p≤0.01 is considered highly statisticallysignificant,
analysis done by X^2: Chi-Square Test

The prevalence of abnormal vitamin D (< 30 ng/mL) was 92.9% in group A with insufficiency 78.6% and deficiency (14.3%) while prevalence of abnormal vitamin D (< 30 ng/mL) was 100% in group B with insufficiency 15.4% and deficiency (84.6%). The prevalence of abnormal vitamin D was 100% in group C with insufficiency 0% and deficiency (100%) while prevalence of abnormal vitamin D was 83.3% in group D with insufficiency 38.9% and deficiency (44.4%). There was a significant difference between the four groups regarding prevalence of abnormal vitamin D (p<0.001) as vitamin D deficiency was significantly increased steadily as CKD progressed (Table 3).

Table (4): Correlation between Vitamin D level and differentvariables in group A

	Vitamin D level	
	r	**p- value**
Age	-.205-	.483
S. Creatinine	.548	**.042**
S. Urea	-.110-	.707

GFR	-.385-	.175
Hb	.389	.169
MCV	.210	.472
S.iron	.270	.350
Ca	.453	.104
Po4	-.547-	**.043**
PTH	-.368-	.195

p≤0.05 is considered statistically significant, p≤0.01 is considered highly statisticallysignificant,
r: Spearman rho

Table 4 shows that there was a significant negative connection (r=-0.547, p=0.043) between the vitamin D level and PO4, and a significant positive correlation (r=0.548, p=0.042) between the vitamin D level and s. creatinine in group A.

Table (5): Correlation between Vitamin D level and differentvariables in group B

	Vitamin D level	
	r	**p- value**
Age	.240	.238
S. Creatinine	-.086-	.676
S. Urea	-.237-	.243
GFR	-.046-	.823
Hb	.193	.345
MCV	.089	.664
S.iron	-.082-	.690
Ca	.289	.153
Po4	.088	.670
PTH	-.307-	.127

p≤0.05 is considered statistically significant, p≤0.01 is considered highly statisticallysignificant,
r: Spearman rho

Table 5 shows that there was no significant association (p>0.05) between the vitamin D level in group B and age, creatinine, urea, GFR, Hb, MCV, iron, Ca, PO4, and PO4.

Table (6): Correlation between Vitamin D level and differentvariables in group C

	Vitamin D level	
	r	**p- value**
Age	.261	.149
S. Creatinine	-.194-	.288
S. Urea	-.198-	.277
GFR	.022	.906
Hb	-.170-	.352
MCV	-.039-	.833
S.iron	.119	.516
Ca	.425	**.015**
Po4	-.132-	.470
PTH	.201	.271

p≤0.05 is considered statistically significant, p≤0.01 is considered highly statisticallysignificant,
r: Spearman rho

Table 6 shows a substantial positive connection (r=0.425, p=0.015) between the vitamin D level and s. Ca in group C.

Table (7): Correlation between Vitamin D level and differentvariables in group D

	Vitamin D level	
	r	**p- value**
Age	-.171-	.498
S. Creatinine	-.011-	.965
S. Urea	-.006-	.982

GFR	.232	.355
Hb	.241	.335
MCV	-.279-	.262
S.iron	.336	.172
Ca	.079	.754
Po4	.313	.206
PTH	.078	.760

p≤0.05 is considered statistically significant, p≤0.01 is considered highly statisticallysignificant,
r: Spearman rho

Table 7 shows that there was no significant connection (p>0.05) between the vitamin D level in group D and age, creatinine, urea, GFR, Hb, MCV, iron, Ca, PO4, and PO4.

Discussion

Chronic kidney disease (CKD) has been a growing health burden worldwide **(6,7),** and the patients with CKD continue to suffer from a wide range of complication including electrolyte imbalance, fluid overload, bone and mineral metabolism disorder to anemia **(8).**

In our study showed that as regard demographic characteristics among the studied groups. There was predominance of female gender in the four groups as there were 64.3%females in group A, 100% females in groups B, C and D. There was no statistically significant difference between the four studied groups regarding gender (p-value was >0.05). The mean age was 60.14± 8.47 years in group A, 55.58± 8.69 years in group B, 62.63± 7.97 years in group C and 40.22± 2.82 years in group D. Significant difference was found between the four studied groups regarding age (p-value was 0.017) and pairwise comparison showed that patients in group C were significantly older than patients in group B.

According to the current investigation, **Sah & Adhikary, (5)** set out to find out how often aberrant 25(OH) D is in patients with non-dialyzed CKD and to further look into how it relates to Hb level. In this cross-sectional investigation, 172 clinically stable individuals who were not receiving dialysis and had an eGFR at CKD stages 2-4 were investigated. Patients were divided into three groups according to their 25(OH) D levels: group 1 (< 20 ng/mL), group 2 (20–30 ng/mL), and group 3 (> 30 ng/mL). The mean ages of group 2 were substantially higher (< 0.05) than those of groups 1 and 3. Likewise, there were significant differences in the mean Hb levels across the groups, and these differences were shown to rise over time as the 25(OH) levels rose (P < 0.05). There were no variations based on gender.

In our study showed that regarding GFR, hemoglobin, vitamin D and PTH in the studied groups, we found that the mean GFR 71.09± 8.33, 35.20± 5.10, 23.64± 4.08 and 107.34± 17.47 in group A, group B, group C and group D respectively. There was a statistically significant decrease in mean GFR from group A to group C. Control group showed significant increase in GFR compared to group A, group B and group C. The mean hemoglobin was 10.28± 0.83 g/dl, 9.01± 0.65 g/dl, 8.36± 0.69 g/dl and 13.23± 1.35 g/dl in group A, group B, group C and group D respectively. The mean hemoglobin decreased steadily as CKD progressed. Control group showed significant increase in Hb. compared to group A, group B, group C and group D. The mean vitamin D level in group A was 20.60± 9.11ng/ml, 13.67±5.64 ng/ml in group B, 10.68± 4.19 ng/ml in group C and 23.52± 7.18 ng/ml in group D. Comparison of levels of vitamin D between group (A vs. B), (A vs. C), (B vs. D) and (C vs. D) showed that the decline in levels was statistically significant ($p < 0.01$). Although the decline in levels of vitamin D between group A vs. D and group B vs. C was not statistically significant. The mean serum PTH levels was significantly increased as the stage of CKD progressed. The mean serum PTH values in group A, B, C and D were 64.50± 5.52, 83.08± 7.14, 113.06± 14.49 and 23.52± 7.18 pg/ml, respectively, and there was a statistically significant increase in mean PTH level from group A to group C.

In agreement with our results **Aggarwal et al. (9)** reported there was significant difference between different stages of CKD as regard eGFR, PTH and vitamin D that however disagreement with our results the Hb level was non-significantly differed between different stages of CKD.

In agreement with our results **Shastry & Belurkar, (10)** reported that Hb level was significantly associated with the advance in the stage of CKD.

In our study showed that the prevalence of abnormal vitamin D (< 30 ng/mL) was 92.9% in group A with insufficiency 78.6% and deficiency (14.3%) whereas prevalence of abnormal vitamin D (< 30 ng/mL) was 100% in group B with insufficiency 15.4% and deficiency (84.6%). Group C had a 100% frequency of aberrant vitamin D with 0% insufficiency and 100% deficiency, whereas group D had an 83.3% prevalence with 38.9% insufficiency and 44.4% deficiency. The incidence of aberrant vitamin D varied considerably ($p<0.001$) between the four groups, and the degree of vitamin D insufficiency increased significantly with the progression of CKD.

This is consistent with the findings of **Tapper et al. (11),** who showed that blood 25-OH-vitamin D levels were gradually reduced from stage 2 to 5. Of those, 31 (22.1%) had vitamin D insufficiency and 31 (22.1%) had deficiency.

The link between vitamin D level and several factors in our investigation revealed the following outcomes: Vitamin D level and s. creatinine showed a significant positive association ($r=0.548$, $p=0.042$) in group A, although there was a substantial negative correlation ($r=-0.547$, $p=0.043$) between vitamin D level and PO4.The vitamin D level in group B did not significantly correlate with age, creatinine, urea, GFR, Hb, MCV, iron, Ca, PO4, or PO4 ($p>0.05$).The vitamin D level and s. Ca in group C showed a strong positive connection ($r=0.425$, $p=0.015$).The levels of vitamin D in group D did not significantly correlate with age, creatinine, urea, GFR, Hb, MCV, iron, Ca, PO4, or PO4 ($p>0.05$).

Regression analysis, however, revealed that serum 25(OH) D was inversely correlated with age ($P = 0.006$) and I PTH ($P = 0.025$), but positively correlated with male individuals ($P = 0.02$), serum albumin ($P = 0.002$), and eGFR ($P = 0.042$). Both univariate and multivariate analysis revealed a positive correlation ($P < 0.05$) between the concentration of Hb and 25(OH) D **(5).**

Additionally, **Sharaf El Din et al.(12)** reported that there was a significant negative correlation between serum 25(OH)D and serum P ($r = −0.46$), serum PTH ($r = −0.69$), serum UA ($r = −0.73$),

and urine albumin/creatinine ratio (ACR) (r = −0.8), as well as a statistically significant positive correlation (two-tailed P <0.001 if r ≥0.082) between serum 25(OH)D and serum Ca (r = 0.3). Serum 25(OH)D did not significantly correlate with age, BMI, or eGFR.

Conclusion

A high prevalence of abnormal 25 OH D metabolite was observed in pre dialysis CKD patients. Our study showed that lower level of Vit D is associated with lower level of hemoglobin and higher level of PTH and could play a role in development of anemia and hyperparathyroidism in these patients. Further studies with larger sample size and longer follow up are needed to confirm our results and to identify risk factors of adverse events.

Recommendations

Further studies with larger sample size are needed to confirm the current results. Further multicenter studies are needed to confirm the current results. We recommend using Serum 25-Hydroxy Vitamin D in Pre-Dialysis Chronic Kidney Disease (CKD) Patients for follow up of patients. We recommend the combination between Serum 25-Hydroxy Vitamin D and other diagnostic methods for better accuracy. Regular monitoring of Vit D and hemoglobin level in pre dialysis patients in addition to early and propre treatment of hypovitaminosis D and anemia to prevent more advanced complications.

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