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ORIGINAL ARTICLE

EVALUATION OF SERUM 25-HYDROXYVITAMIN D IN CHILDREN WITH CHRONIC KIDNEY DISEASE ON DIALYSIS AT ZAGAZIG UNIVERSITY CHILDREN HOSPITALS

Ali Mohammed Abo Zaid [1], Seham Fathy Azab[1], Asmaa Mohammed Osh[2], Aya Ahmed Ragaey Kamar[1]

[1]Pediatrics Department, Faculty of Medicine, Zagazig University, Zagazig, Egypt

[2] Clinical Pathology Department, Faculty of Medicine, Zagazig University, Zagazig, Egypt

Corresponding Author:
Aya Ahmed Ragaey Kamar , Pediatrics Department,Faculty of Medicine, Zagazig University, Zagazig, Egypt
aya7osamaser@yahoo.com

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ABSTRACT

Background: Vitamin D deficiency and insufficiency in chronic kidney disease (CKD) patients on dialysis is associated with undesirable outcomes as rapid progression to later stages of renal insufficiency, and greater risk of mortality. At the mean time data is limited regarding if vitamin D deficiency and insufficiency is more present in children with chronic kidney disease on dialysis or not.

Subjects and Methods: this cross sectional study included 42 pediatric patients aged from 3-16 years with CKD on dialysis were enrolled, we evaluated serum 25-hydroxyvitamin D (25(OH)D) levels by enzyme-linked immunosorbent assay (ELISA).

Results: Out of 42 pediatric patients, 71.4% had 25(OH) D deficiency, 26.2% had vitamin D insufficiency and only 2.4% were vitamin D sufficient. 25(OH) D insufficiency and deficiency is more common in more severe cases of CKD and also more prevalent in children with longer duration of dialysis.

Conclusion: CKD patients on dialysis therapy are more likely to have 25(OH) D deficiency or insufficiency, vitamin D deficiency and insufficiency increase by increasing the severity of CKD and increasing the duration of dialysis.

Keywords: Vitamin D, chronic kidney disease, dialysis.

INTRODUCTION

Vitamin D has many important functions including skeletal development, prevention of hypertension, cardiovascular disease, diabetes mellitus, and malignancy. Vitamin D is present in diet or synthesized in the skin by ultraviolet B from the sun, in the liver it is converted in to 25(OH)D, then in the kidney to the biologically active 1,25(OH)2 vitamin D, parathyroid hormone (PTH) controls the hole process of vitamin D metabolism [1] . 25(OH) D is used as a parameter that shows vitamin d status as it is the major circulating form [2].

The decrease in serum Vitamin D level is a result of decrease exposure to the sun and decrease dietary supplementation, and it is common all over the world in all age groups [3] . The major risk factors of vitamin D deficiency and insufficiency in children is winter season, decrease exposure to the sun, dark skin races, older age, puberty, obesity, female, and low socioeconomic status [4] . Vitamin D deficiency in children presents many barriers against bone composition resulting in bone ache, deformities, growth impairment and fractures [5].

Patients with CKD can't prepare enough amounts of 1, 25(OH) 2D. These patients may also have low nutritional intake due to uremia-induced decrease of appetite, also dietary limitation of specific nutrients, as phosphorus,

resulting in decreased substrate for conversion to 1,25(OH)2D [6]. CKD causes cardiovascular, metabolic, and infectious complications, also vitamin D deficiency may be an important risk factor in this high-risk population. Recent researches in CKD connected vitamin D deficiency with diseases other than skeletal comorbidities, including diabetes mellitus, anemia, and inflammation. In studies of adults with CKD, vitamin D deficiency was an independent indicator of CKD progression and mortality. Vitamin D deficiency may also lead to bone disease in children with CKD [7].

The National Kidney Foundation clinical practice guidelines for bone and mineral metabolism focus on the use of vitamin D to decrease the high level of parathyroid hormone (PTH) in CKD. It is known that with progression of CKD there is high decrease in 1α-hydroxylase activity, the enzyme important for the metabolism of 25-hydroxyvitamin D2 to 1, 25-dihydroxyvitamin D3 (calcitriol). This leads to worsening of secondary hyperparathyroidism [8]. Dialysis patients are at higher risk of deficiency because of decreased photo production of vitamin D and low levels of vitamin D binding protein [9].

In this cross-sectional study we aim to evaluate 25(OH) vitamin D status in children with CKD on dialysis.

SUBJECTS AND METHODS

A written informed consent was obtained from all guardians of all participants and the study was approved by the research ethical committee of Faculty of Medicine, Zagazig University. The work has been carried out in accordance with The Code of Ethics of The World Medical Association (Declaration of Helsinki) for studies involving humans.

Study Design and Population: This cross-sectional study was conducted at Pediatric Nephrology Unit, Faculty of medicine, Zagazig University Hospitals during the period from January 2019 to July 2019. A total sample of 42 cases with CKD on dialysis therapy was included in the present study.

Inclusion criteria: Children diagnosed with chronic renal failure on dialysis, both sexes and all ages till 16 years old.

Exclusion criteria: CKD patients not undergoing dialysis treatment, patients who are receiving treatment that could influence vitamin D status like anticonvulsant and patients taking vitamin D supplementation.

Steps of performance: Stage of CKD was determined based on K/DOQI definitions [10]. 25(OH) Vitamin D levels <20 ng/ml (50 nmol/L) was 25(OH) vitamin D deficiency, from 20–29 ng/ml (50–72 nmol/L) was 25(OH) vitamin D insufficiency and ≥30 ng/ml (75 nmol/L) was adequate [11].

All cases has been assessed by full history taking including, careful clinical examination, laboratory tests as follow, complete blood count (CBC), serum Creatinine, calcium level, potassium level, phosphorus level, blood Urea Nitrogen and serum 25-OH D concentrations. Detailed information has been taken including, name, age, weight and time at which patient started dialysis

Laboratory Procedures: Sampling 2 ml of venous blood was obtained from children with CKD on dialysis before giving them heparin then blood was centrifuged at 3000 rpm for 10 minutes and separated into serum in sterile tubes and stored at – 20 degree C until time of analysis.

Test Principle: Quantities determination of 25 hydroxyvitamin D was performed using a double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) to determine the level of human 25-dihydroxy vitamin D in the collected samples.

The assay consists of adding 25-OH-D to monoclonal antibody Enzyme which is pre-coated with human 25-OH-D monoclonal antibody, incubated for 60 minutes at 37 degree C, dilute 30 times with distilled water as stand by, then drain the liquid, shake away the remaining water.

And then add chromogen solution a 50µl, then chromogen solution B 50µl to each well. Gently mixed, incubate for 10 min at 37 degree in a dark place, then add stop solution 50µl into

each well to stop the reaction (the blue changes into yellow immediately)

The chrome of color and the concentration of the human substance 25-OH-D of sample were positively matched.

The optical density (OD) was measured under 450 nm wavelengths which should be done within 15min after adding the stop solution.

The concentration of 25-OH-D in the samples was then determined by comparing the OD of the samples to the standard curve.

STATISTICAL ANALYSIS

Data were checked, entered and analyzed using SPSS version 23 for data processing. The following statistical methods were used for analysis of results of the present study.

Data were expressed as number and percentage for qualitative variables and mean $\pm$ standard deviation (SD) for quantitative one.

RESULTS

Vitamin D was deficient in (71.4%) of the study group, (26.2%) had insufficient Vitamin D and (2.4%) had sufficient Vitamin D as presented in table (1).

Both of the demographic and laboratory data of the study group were presented in table (2). No statistically significant differences were present as regards mean age, Albumin level, PTH level, RBCs counts, Hb level, WBCs counts, Calcium level, K level and Ph level.

Significant differences were observed regarding mean values of Duration of dialysis (p=0.01), urea level (p=0.001), creatinine level (p=0.002) and platelets counts (p=0.03).

there was statistically significant difference between patients with and without bone diseases in Vitamin D level with decreased Vitamin D level in patients with bone diseases than without with mean of (11.2 VS 15.4 respectively) as shown in table (3).

There was statistically significant negative correlation between VIT D level with duration of dialysis and creatinine (increased duration of dialysis and creatinine level was associated with decreased VIT D level) D level). Otherwise, there was no statistically significant correlation with other variables as shown in table (4) and figure (1) and figure (2).

Table (1): VIT D status in the study group:-

VIT D	**No(42)**	**Percent (%)**
Deficient <20 (ng/ml)	30	71.4%
Insufficient 21-29 (ng/ml)	11	26.2%
Sufficient >30 (ng/ml)	1	2.4%

Table (2): Relation between Vitamin D level with demographic and laboratory data in the study group:-

Variable	Deficient Vit D (30) mean ± SD (Range) Median	Insufficient Vit D (11) mean ± SD (Range) median	p-value
Age (months)	**129.6±42.9** **(60-192)**	**138±51.2** **(42-192)**	**0.5** **(NS)**
Duration of dialysis (months)	**89.4±45.6** **(48-192)**	**47.2±34.1** **(1-120)**	**0.01*** **(S)**
Urea (mg/dL)	81.4±119.5 (36-709) 84	61.6±10.2 (49-78) 53	**0.001**** **(HS)**
Creatinine (mg/dL)	7.7±2.3 (4-11.4) 7.5	6.6±1.6 (2.7-9.6) 5.4	**0.002*** **(S)**
Albumin (g/dL)	4.2±0.5 (2.5-4.9) 3.4	4.1±0.3 (3.2-4.7) 3.2	0.5 (NS)
PTH (pg/mL)	430.4±535.5 (6.9-1740) 440	402.1±384.2 (13-1427) 396	0.5 (NS)
RBCs (10^6/µL)	4.6±0.4 (3.9-5.5)	4.7±0.5 (3.8-5.3)	0.3 (NS)
Hb level (g/dL)	9.5±1.03 (7.7-12.5)	9.4±1.18 (7.1-10.9)	0.6 (NS)
WBCs (10^3 /µL)	6.6±1.7 (2.4-16)	7.26±1.8 (4.3-13.5)	0.7 (NS)
Platelets (10^3 /µL)	264.1±30.2 (110-608)	323.3±104 (132-633)	**0.03*** **(S)**
Calcium (mg/dL)	8.7±1.8 (1.1-11.9)	9.2±1.6 (7.5-13.4)	0.7 (NS)
K (mg/dL)	0.96±0.17 (4-8.2)	0.95±0.28 (5-7.9)	0.8 (NS)
Ph (mg/dL)	5.6±1.2 (2.6-7.9)	5.7±1.4 (3.2-7.9)	0.1 (NS)

**** Statistically significant difference (P ≤ 0.05)***

Table (3): Relation between VIT D level and presence of bone diseases in the study group:-

Variable	Bone diseased (22) mean ± SD (Range) Median		No bone disease (20) mean ± SD (Range) Median		Mann-Witenny Test	p-value
VIT D level (ng/ml)	11.2±9.1 (1.9-30.2) 10.7		15.4±8.3 (2.6-26.5) 16.5		3.4	**0.002* (S)**
VIT D level	**Bone diseased No.(22)**	**%**	**No bone disease No.(20)**	**%**	**χ^2**	**p-value**
Deficient <20 (ng/ml) (30)	17	56.7%	13	43.3%	5.6	**0.002* (S)**
Insufficient 21-29 (ng/ml) (11)	5	45.4%	6	54.6%		
Sufficient >30 (ng/ml) (1)	0.0	0.00%	1	100.0%		

* Statistically significant difference ($P \leq 0.05$)

Table (4): Correlation between VIT D level with patient investigations and patient characteristics in the studied group:

Variable	*VIT D level*			
	r	**^**	**p**	**SIG**
Age	**0.03**	**> 0.05**	**NS**	
Duration of dialysis	**-0.6**	**0.001****	**HS**	
Albumin	**0.06**	**> 0.05**	**NS**	
Urea	**0.1**	**> 0.05**	**NS**	
Creatinine	**-0.5**	**0.001****	**HS**	
PTH	**0.03**	**> 0.05**	**NS**	
Ca	**0.1**	**> 0.05**	**NS**	
K	**0.09**	**> 0.05**	**NS**	
Ph	**0.04**	**> 0.05**	**NS**	
RBCs	**0.2**	**> 0.05**	**NS**	
WBC	**0.09**	**> 0.05**	**NS**	
HB	**0.1**	**> 0.05**	**NS**	
Platelets	**-0.1**	**> 0.05**	**NS**	

**** Statistically significant difference ($P \leq 0.05$), S=significant, HS= highly significant, NS= Non-significant. ^Spearman correlation**

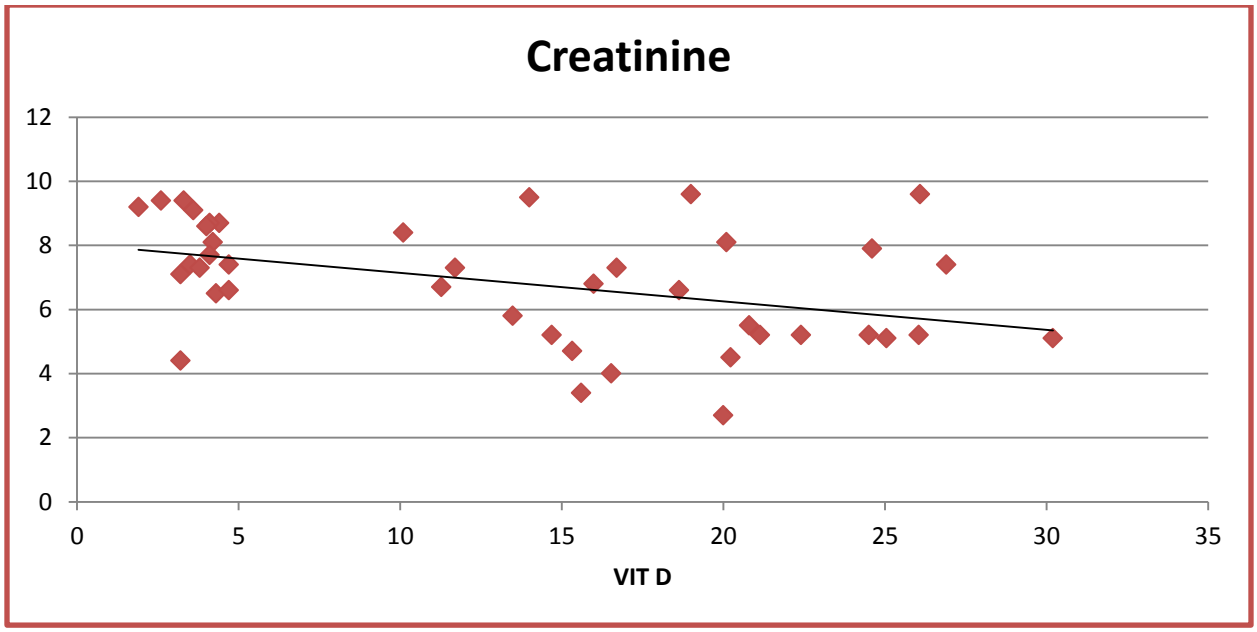

Fig (1): Scatter plot with line chart for negative correlation between creatinine level and Vit D level

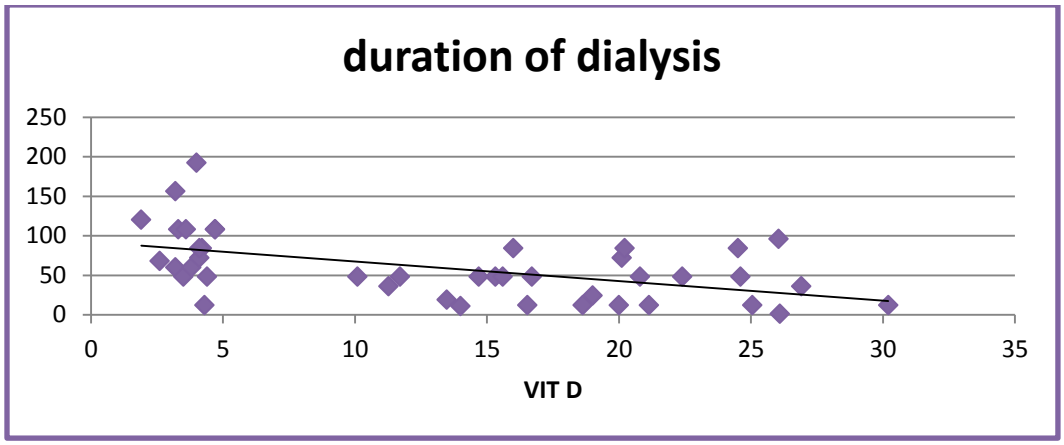

Fig (2): Scatter plot with line chart for negative correlation between duration of dialysis and Vit D level

DISCUSSION

An enhanced understanding of the role of vitamin D status in patients with renal insufficiency has the potential to change supplementation practices and the clinical care of these patients [6].

The aim of our study was to assess the relationship between serum 25(OH) D level and chronic kidney disease and dialysis therapy in pediatric patients. The data obtained show that Vitamin D level in the study group ranged from (1.9 to 30.2) ng/mL with a mean of (13.3±8.7) ng/mL, (71.4%) of the study group had 25(OH)D deficiency, (26.2%) had vitamin D insufficiency and only (2.4%) was vitamin D sufficient. That was very close to the American study done by Gonzalez et al. [12] who reported mean concentration of 25-OH-D of (10.7±6.8) ng/mL, 80% had 25-OH-D deficiency, 17% had insufficiency and only 3% had sufficient 25-OH-D. Also it was near to the American study done by Kalkwarf *et al.* [7] who observed 25-OH-D concentration in stage 5 patients on dialysis with a mean of (15.6±9.9) ng/mL and

40 patient out of 54 (74%) were 25-OH-D deficient.

In the current study, patients with 25(OH)D deficiency had been on dialysis for a period ranging from (48-192) months (mean 89.4 ± 45.6)month in comparison to patients with 25(OH)D insufficiency with range (1-120) months (mean 47.2 ± 34.1)month with statistically significant difference between vitamin D deficiency and insufficiency and dialysis duration, as with increased the duration of dialysis 25(OH)D deficiency increase. This result was similar to the study done by Ambrus et al. [13] who reported a statistically significant difference between duration of ESRD and in turn duration of dialysis and 25(OH)D deficiency (P = 0.001).

Our study showed that there was statistically significant difference between patients with deficient and insufficient Vitamin D level and urea and creatinine as with increased urea and creatinine level 25(OH)D deficiency increase with (P=0.001) for urea which is highly significant and (P=0.002) for creatinine which is significant that matched with the study done by Valencia and Arango [8] and the Chinese study done by Feng et al. [14] who found A negative relationship between creatinine level and vitamin d deficiency.

The current study conducted that there was statistically significant difference between patients with and without bone diseases in Vitamin D level with decreased Vitamin D level in patients with bone diseases than without with a mean of (11.2 VS 15.4 respectively), out of 42 patients included in our study 30 of them had vitamin D deficiency 17 (56.7%) of those deficient patients had bone disease while 13 (43.3%) had no bone disease, 11patients of the study group had vitamin D insufficiency 5 (45.4%) of them had bone disease and 6 (54.6%) didn't had bone disease, finally only one patient was vitamin D sufficient and had no bone disease (100%). That matched Ambrus et al. [13] who found in their study that was done on 130 patients who had been on maintenance hemodialysis for more than 6 months that 25(OH)D3 was significantly lower in patients with bone fractures history.

Our study found a negative correlation between VIT D level with duration of dialysis (increased duration of dialysis was associated with decreased VIT D level), this agrees with Ambrus et al. [13] who also found a negative correlation between VIT D level with duration of dialysis.

The current study also found a negative correlation between vitamin D level and creatinine level (increased creatinine level was associated with decreased VIT D level) this agrees with Valencia and Arango [8], who also found a negative correlation between vitamin D level and creatinine level.

The present study has a few limitations. There was no data about other behavioral or dietary factors such as daily activity, sun exposure, sunscreen use, or milk consumption. Our study included a relatively small number of patients in a single center, and there might be a limitation that our patients might not be representative of all pediatric patients with CKD on dialysis.

CONCLUSION

The present study revealed that Prevalence of 25(OH)D insufficiency and deficiency are very common in children with CKD on dialysis, it is more common in more severe cases of CKD and on longer duration of dialysis, also Bone disease in those patients may be a result of 25(OH)D deficiency and insufficiency.

RECOMMENDATIONS

From the perspective of this study we recommend, routine measurement of vitamin D in patients with CKD on dialysis, vitamin D should be supplemented to all patients with CKD on dialysis with appropriate doses according to their serum vitamin D level to prevent bone disease and complications in these patients, we also recommend further studies and using a larger number of patients in the future for better demonstration of the correlations in our study.

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