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Effect of 25-hydroxy Vitamin D and Hemodialysis on Vitamin D Axis in Sudanese Renal Failure Patients

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Authors' contributions

This work was carried out in collaboration between both authors. Author MEAM designed the study, managed the literature search, performed the statistical analysis and supervised the work. Author NAS participated in the study design and performed the practical work. Both authors read and approved the final manuscript.

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Short Research Article

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ABSTRACT

Objective: The aim of this study is to investigate the effect of hemodialysis on vitamin D axis through measuring the blood level of25-hydroxyvitamin D, parathyroid hormone (PTH), calcium and phosphate in renal failure patients before and after dialysis compared to their level in healthy subjects.
Materials and Methods: 16 Sudanese patients with renal failure under hemodialysis and vitamin D dose and 16 healthy subjects were involved in this study after informed consent.
Results: The mean of the 25-hydroxyvitamin D concentration in the control, pre dialysis and post dialysis were 0.75, 1.31, and 0.84 µmol/l sequentially. There was no significant difference between the control and post dialysis samples (p-value = 0.44) and there was significant difference between the means of the control and pre dialysis samples (p< 0.000) and between the pre dialysis and post dialysis samples (p< 0.000). The means of phosphate concentration in the three samples were 3.31,

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6.26 and 3.19 mg/dl respectively. Regarding the results of the parathyroid hormone (PTH) and calcium; the means of the PTH in the control, pre dialysis and post dialysis samples were 18.08, 18.01 and 21.96 pg/ml. Concerning the means comparisons, there was no significant difference between all the means. The means of calcium of the three groups were 10.58, 9.96 and 10.14 mg/dl. However, there was no significant variation between the means.
Conclusion: 25-hydroxyvitamin D treatment caused significant increase in the blood concentration of 25-hydroxyvitamin D and phosphate and an insignificant decrease in PTH and calcium.

Keywords: Renal failure; hemodialysis; vitamin D axis.

1. INTRODUCTION

Kidney or renal failure means that the kidneys stop removing the waste from the blood. Stage 5 kidney failure is, also, known, as End- Stage Renal Disease (ESRD) and it is treated by dialysis or a kidney transplant [1].

During the year 2014, in Sudan, renal failure was the fifth cause of death with 4.3% of the total death cases [2]. The major risk factors of chronic renal failure in Sudan are chronic glomerulonephritis, obstructive nephropathy (renal stones), hypertension and diabetes mellitus [3].

Vitamin D axis involves 25-hydroxyvitamin D, calcitriol, Para Thyroid Hormone (PTH), calcitonin, Fibroblast Growth Factor- 23, calcium and phosphate. The relation between the different vitamin D axis members is complex but the major mechanism that controls most of them is the feedback mechanism [4].

Vitamin D is one of the fast soluble vitamins. It is hydroxylated at position 25 in the liver and at position one in the kidney to be converted to calcitriol; the active form of vitamin D. However, some tissues has the capability of forming calcitriol from 25-hydroxyvitamin D like the prostate, breast, colon, β cells and skin. Normally vitamin D increases the absorption of calcium and phosphate from the intestine, maintains bone mineralization through affecting osteogenesis and osteoclastogenesis processes and it affects the kidneys through regulating it own homeostasis and increasing the re absorption of calcium and phosphate [5].

Calcitriol is regulated negatively by calcitriol through feedback inhibition, inversely by calcium and positively by PTH. Vitamin D and phosphate are negatively regulated by Fibroblast Growth Factor-23 (FGF-23). However, the regulation of calcitriol is majorly through the regulation of the 1-α hydroxylase synthesis and activity [6].

Para Thyroid Hormone (PTH) is a peptide hormone with 84 amino acids. It is secreted from the parathyroid gland in response to hypocalcaemia, low calcitriol and hyperphosphatemia. It stimulates the osteoclasts leading to hypercalcemia and hyperphosphatemia and it enhances production of calcitroil through activating the 1-α hydroxylase. The PTH increases the reabsorption of calcium from the renal tubules and it decreases the reabsorption of phosphate. However, high PTH level leads to hypercalcemia and hypophosphatemia [7,8].

Phosphate plays many functional roles in our bodies including mineralization of bones, regulation of enzyme activity and involvement in hormone action and energy metabolism. Blood phosphate level is regulated by three factors; PTH, vitamin D and FGF-23. The phosphate activates the PTH synthesis and the PTH reduces the blood level of phosphate .i.e. there is feedback inhibition mechanism. Vitamin D increases the blood phosphate level through increasing its absorption from the intestine and increased phosphate level leads to the inhibition of calcitriol synthesis. The FGF-23 is a protein synthesized by the osteoclasts and osteoblasts it affects the blood phosphate level through inhibiting the renal re absorption and through reducing the rate of calcitriol synthesis in the kidney by ceasing the rate 1-α hydroxylase. By the two mechanisms FGF-23 reduces the blood level of phosphate. Regarding the effect of phosphate on the FGF-23, it is registered that oral phosphate administration increases the FGF-23 levels in animals and humans [4].

Calcium plays different functions including bone mineralization, working as coenzyme, regulation of enzyme activity and muscle contraction. Blood calcium concentration is regulated within narrow range by three hormones; calcitriol, PTH and calcitonin. Calcitriol increases the blood concentration of calcium through increasing its absorption from the gut and reabsorption from

the renal tubules and it regulates bone resorption and formation. Calcium is the potent stimulus of PTH and high concentration of calcium reduces the PTH blood level. Calcitonin is a peptide hormone synthesized in the thyroid gland. Calcitonin is secreted in response to hypercalcemia and gastro intestinal tract hormones like gastrin. Decreased calcium concentration leads to reduced calcitonin secretion. Calcitonin inhibits osteoclastic bone resorption [9].

Many studies stated that vitamin D axis is affected in patients with renal diseases like Chronic Kidney Disease (CKD) and End Stage Renal Disease (ESRD) [10-12].

The objective of this article is to investigate the effect of 25-hydroxy vitamin D and hemodialysis on vitamin D axis in the blood of Sudanese renal failure patients. The members of vitamin D axis measured were 25-hydroxyvitamin D, phosphate, PTH and calcium.

2. MATERIALS AND METHODS

2.1 Research Design

This work is classified as a pilot, quantitative, descriptive and case control research design.

2.2 Study Subjects and Ethical Issues

This study involved sixteen renal failure patients under hemodialysis and 25 hydroxyvitamin D dose (0.25 µg/day) from Kosti and Eldwium cities in the White Nile state- Sudan. Sixteen age range (≥ 20, 21- 44, 45- 64, 65≤) and sex matched healthy subjects were involved in this study as control subjects. All the study subjects joined this study after informed consent. This study was implemented after academic and ethical approval from the Sudan Academy of Sciences.

2.3 Hemodialysis

The duration of the hemodialysis was very variable ranging from one to nine years**.** Every dialysis session was 4 hours and was done three times a week. The dialysate was containing calcium with a concentration of 1.5 mmol\L.

2.4 Sampling

5 mls of intravenous blood samples before and after hemodialysis were obtained from each hemodialysis patient. From each control subject 5 mls of intravenous blood sample was taken once. The samples were taken in EDTA tubes and the plasma was separated and stored according to each analysis parameter instructions.

2.5 Analysis Procedure

25-hydroxyvitamin D was measured in the blood samples using ELISA technique. The procedure of the analysis was according to the manufacturer instructions (EUROMIN 25-OH vitamin D ELISA kit, ORDER NO: EQ 6411-9601).

Phosphate concentration in the blood of the study subjects was evaluated using the spectrophotometric method of BioSystem company- Spain. The kit title is Phosphorus and its code number is 11508 170 ml. The analysis was done according to the kit leaflet instructions.

PTH concentration was measured using the automated ELISA procedure of TOSOH biosciences- Japan. The research followed step by step the analysis procedure of the kit entitled: ST AIA-PACK Intact PTH, Cat No. 0025213.

The concentration of calcium in the blood was determined using the colorimetrical method of Centronic GmbH- Germany. The analysis steps of the manufacturer was followed strictly (Centronic GmbH Calcium-Arsenazo fluid Monoreagent, order No. CF06000100).

3. RESULTS AND DISCUSSION

3.1 Results

The patients and the healthy subjects were composed of 11 males and 5 females each. The age range of the patients was (28- 70) and their mean age was 47.2 while the age range and the mean of the control subjects were (21- 53) and 32.4 respectively.

25- hydroxyvitamin D ranges in the blood samples of the control subjects, pre-dialysis and post-dialysis patients were (0.39- 1.18 µmol/l), (0.79- 1.89 µmol/l) and (0.09- 1.92 µmol/l) sequentially. The mean values of 25-hydroxyvitamin D concentration in the three samples sequentially were 0.75, 1.31 and 0.84 µmol/l (Table 1 and Fig. 1). However, nine of the pre-dialysis patients were with high 25-hydroxyvitamin D concentration and 7 (6 in the upper limit and 1 in the mid of the normal range)

were within the normal range compared to the control subjects. Regarding the post-dialysis 25-hydroxyvitamin D concentrations, 3 were above the normal range, two were below the normal range and 11 were within the normal range (range of the control subjects).

The comparison of the three means using One Way ANOVA test showed the significant increase in 25-hydroxyvitamin D in the pre-dialysis sample compared to the control sample (p-value < 0.000) and insignificant increase in the post-dialysis sample (p-value= 0.44). There was significant decrease in 25-hydroxyvitamin D concentration when the pre-dialysis mean was compared to the post-dialysis mean (p-value< 0.000). The statistical analysis results showed that the renal failure patients were characterized by high concentration of 25-hydroxyvitamin D and the hemodialysis significantly decreased the blood concentration of 25-hydroxyvitamin D.

The ranges and the mean values of phosphate in the three groups were as follows; control subjects (2.7- 4.1, 3.31mg/dl), pre-dialysis (3.4-9.6, 6.26 mg/dl) and post-dialysis (1.2- 7.5, 3.19 mg/dl) (Table 1 and Fig. 1). In the pre-dialysis group samples, 13 were above the control group range and three were within the control group range. Regarding the post-dialysis group samples, 7 samples were below the normal range, 5 samples were mildly higher than the upper limit of the range and 4 samples were within the normal range. The one way ANOVA test results highlighted the significant increase of phosphate concentration in the blood of renal failure patients under hemodialysis (p-value< 0.000) and that the hemodialysis significantly decreased the concentration of phosphate (p-value < 0.000). However, there was no significant decrease in the phosphate after hemodialysis compared to the phosphate concentration in the control subjects (p- value= 0.814). As 25-hydroxyvitamin D, renal failure patients under hemodialysis were characterized by high phosphate level.

Unlike the 25-hydroxyvitamin D and phosphate, PTH and Calcium in the blood of renal failure patients under hemodialysis were insignificantly decreased compared to their means in the control subjects (PTH p- value= 0.98, Calcium p-value= 0.25). Also, PTH and Calcium were insignificantly increased in the blood of the patients after dialysis compared to their level before dialysis (PTH p- value = 0.42, Calcium p- value = 0.75). The ranges and means of PTH and Calcium in the three study samples were presented in Table 1 and Fig. 1.

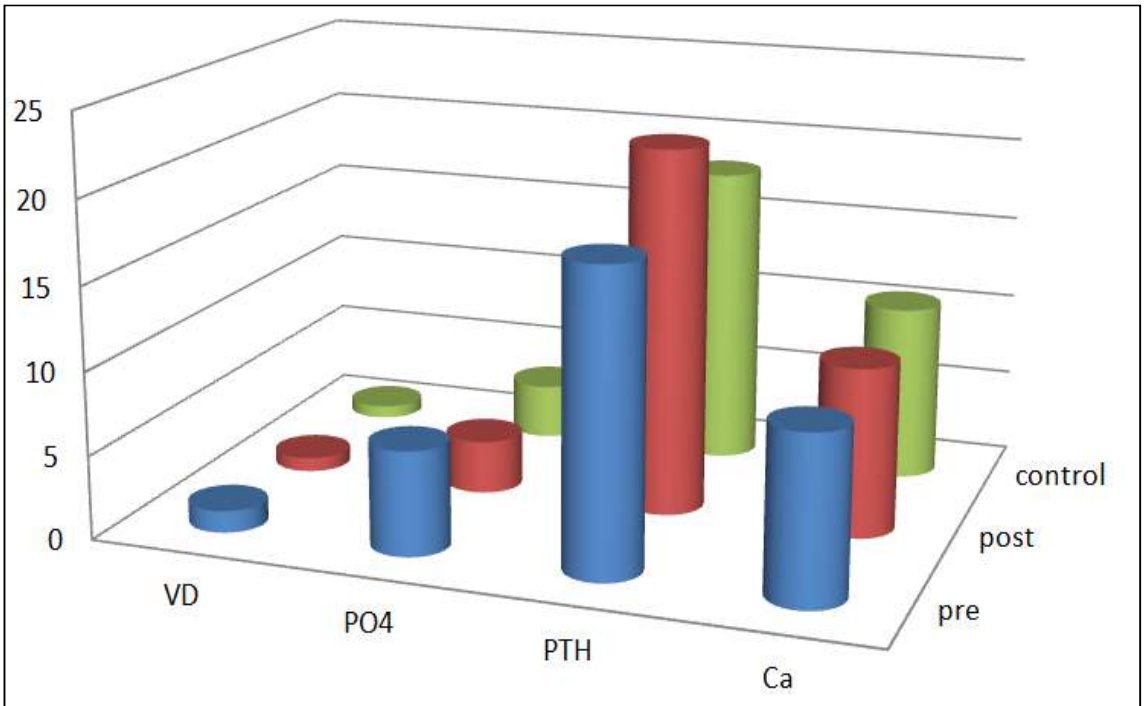
The results of this study can be summarized as follows: The 25-hydroxy vitamin D therapy for the renal failure patients leaded to significantly increased phosphate and insignificant decrease in PTH and Calcium. The hemodialysis significantly decreased the concentration of vitamin D and phosphate while the PTH and the calcium insignificantly increased. The results reflected the directly proportional relationship between vitamin D and phosphate and the inverse relationship between vitamin D and PTH and calcium.

3.2 Discussion

This study registered a significant positive effect of 25-hydroxyvitamin D therapy on the blood phosphate level and an insignificant negative effect on the blood level of PTH and calcium.

Table 1. Ranges, mean values and standard deviation (STD) of analytes within the different study groups

Analyte	Group	Range	Mean	STD
25- hydroxyl vitamin D	Control	0.39-1.18	0.75	0.19
µmol/l	Pre-dialysis	0.79-1.89	1.31	0.29
	Post-dialysis	0.09-1.92	0.84	0.47
Phosphate mg/dl	Control	2.7-4.1	3.31	0.43
	Pre-dialysis	3.4-9.6	6.26	1.84
	Post-dialysis	1.2-7.5	3.19	1.76
PTH pg/ml	Control	15.6-21.5	18.08	1.49
	Pre-dialysis	0.1-55.7	18.01	16.68
	Post-dialysis	7- 54	21.96	16.96
Calcium mg/dl	Control	9.5-12	10.58	0.78
	Pre-dialysis	7.9-13.3	9.96	1.38
	Post-dialysis	6.5-12.8	10.14	2.01



	VD	PO4	PTH	Ca
pre	1.31	6.26	18.01	9.96
post	0.84	3.19	21.96	10.14
control	0.75	3.31	18.08	10.58

Fig. 1. Means of the analytes in the three samples

The figure shows the mean concentration of 25 hydroxyvitamin D (µmol/l), phosphate (mg/dl), PTH (pg/ml) and calcium (mg/dl) in the control group and hemodialysis patients (before and after hemodialysis)

Spectra laboratories stated that high dose of 25-hydroxyvitamin D leaded to hyperphosphatemia, hypo parathyroidism and hypercalcemia [13]. It is obvious that our results are similar to the report of spectra in the case of phosphate and parathyroid hormone and against our finding since 25-hydroxyvitamin D caused low concentration of calcium.

Similar to our findings Sprague SM and his colleagues in 2001 [14] found that doses of paricalcitriol caused hypoparathyroidism and hyperphosphatemia, however, this study used 25- hydroxyvitamin D rather than paricalcitriol (19-nor-1,25 dihydroxvitamin D2).

PTH is elevated in the blood of hemodialysis patients with high and low 25-hydroxyvitamin D [15], this result is similar to our results since we registered low 25-hydroxyvitamin D accompanied by high PTH in the samples after dialysis. It is against our findings in the pre-dialysis sample since we registered high 25-hydroxyvitamin D accompanied with decreased level of PTH.

We have found an inverse relation between 25-hydroxyvitamin D and PTH since vitamin D decreased after dialysis while PTH increased, similarly, Mucsi concluded that there was a negative relation between 25-hydroxyvitamin D and the PTH level in hemodialysis patients [16].

Blair D and his research group in 2008 conducted a research which aimed to investigate the effect of ergocalciferol supplementation on stage 5 of chronic kidney disease and they found

that ergocalciferol increased the level of 25-hydroxyvitamin D, decreased the calcium, phosphate and PTH [17]. However, we have obtained decreased PTH and calcium but increased phosphate level with increased 25-hydroxyvitamin D.

Another study mentioned that 25-hydroxyvitamin D decreased the PTH and increased the phosphate and calcium which is similar to our findings except for calcium [18].

Unlike our findings, Angel L M DE Francisco and his colleagues in 1998 [19] suggested that high serum phosphate level in hemodialysis patients caused high PTH and high to normal calcium level since we registered that high phosphate level caused low PTH and calcium.

From the above literature, it is clear that the relations between vitamin D, phosphate, PTH and calcium are very complicated with variable relations. However, some studies obtained some results similar to our findings, e.g. inverse relationship between 25-hydroxyvitamin D and PTH and the positive effect of vitamin D on the phosphate level (directly proportional relationship). Depending on our literature survey we did not find any previous study, which registered the negative effect of 25-hydroxyvitamin D on calcium level, which is removed by the decrease of vitamin D (when 25-hydroxy vitamin D decreased after dialysis, calcium increased). One study concluded that hemodialysis solutions contain calcium [20], which may be the reason of increased calcium after hemodialysis.

Regarding the effect hemodialysis on the levels of the study parameters, this study found that hemodialysis leaded to significant decrease in the blood level of 25-hydroxyvitamin D and phosphate while it insignificantly increased the level of the PTH and calcium. After searching the literature our results can be compared as follows:

With regard to the effect of hemodialysis on the concentration of 25-hydroxyvitamin D, Yousefzadeh and his colleagues in 2014 [21] stated that the concentration of total 25-hydroxyvitamin D was low after hemodialysis in black patients compared to white ones without affecting the concentration of the free form the vitamin. This finding is similar to our finding since Sudanese may be considered as black African/ Arab. The decreased level of the total 25-hydroxyvitamin D may be due to its removal by the dialysis process or may be due to the insignificant increase in the PTH which leads to the conversion of the 25-hydroxyvitamin D to 1, 25-hydroxyvitamin D through increasing the expression of 1α- hydroxylase enzyme.

Hemodialysis is well known to decrease the level of phosphate [22,23], this finding is similar to our finding concerning the phosphate concentration.

Regarding the effect of hemodialysis on calcium concentration, Skrabal and his group in 1974 expressed that the major factor affecting the concentration of calcium after dialysis is the concentration of the calcium the dialysate solution which is 6mg/100 ml dialysate [24].

Similar to our finding Felix Ciovicescu stated that calcium and bicarbonate were significantly increased after hemodialysis [25].

Francisco Maduell registered decrease of phosphate and PTH and increase of Calcium concentration after hemodialysis with two different dialysates [26], this result is comparable to our findings except for PTH.

Anneke Bech measured plasma ionized calcium and PTH before and after hemodialysis and he found that plasma ionized calcium was reduced while PTH was increased, the dialysate was containing 1.25 mmol/l calcium [27]. We found increased PTH and calcium after hemodialysis which may be due to the calcium concentration in the dialysate.

4. CONCLUSION AND RECOMMENDA-TIONS

4.1 Conclusions

The conclusions of this study are:

1- Treatment of Sudanese renal failure patients with 25 hydroxyvitamin D caused a significant increase in Phosphate concentration and an insignificant decrease in PTH and calcium concentration.
2- Hemodialysis leaded to significant decrease in 25 hydroxyvitamin D and phosphate and to insignificant increase in PTH and Calcium concentration in the blood of Sudanese hemodialysis patients.

4.2 Recommendations

1- Large scale study is highly recommended.
2- The dose of the 25 hydroxyvitamin D should be included in the study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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