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Original Research

Effect of weekly high-dose vitamin D3 supplementation on the association between circulatory FGF-23 and A1c levels in people with vitamin D deficiency: A randomized controlled 10-week follow-up trial

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Abstract

Purpose: This randomized clinical trial (RCT) was designed to assess the effect of vitamin D supplementation (VD3) on the relationship between the levels of fibroblast growth factor-23 (FGF-23) and osteocalcin factor (OSC) with glycated hemoglobin (A1c) values in the blood in a sample of Jordanian males and female adults with vitamin D deficiency. **Subjects and methods:** This RCT was designed to randomly split (78) eligible participants into two groups: experimental (Group A) and interventional (Group B). Group B was supplemented with a weekly dose of 50,000 IU VD3 for eight weeks. Fasting baseline and follow-up (10 weeks; 8 weeks supplementation plus washout two weeks) of serum 25 hydroxyvitamin D (25OHD), fibroblast growth factor-23, (FGF-23), glycosylated hemoglobin (A1c), parathyroid hormone (PTH), calcium, and phosphorus were assayed. A paired T-test was used to assess the changes in fibroblast growth factor-23, A1c, and OSC levels over the follow-up period. **RESULTS:** Significant increase in follow-up level of 25OHD (40.04 ± 11.61 vs. 15.35 ± 5.41, PC < 0.001), FGF-23 (114.04 ± 103.8 vs. 87.40 ± 82.21, PA = 0.02) and A1c (5.63 ± 0.33 vs. 5.38 ± 0.32, PA = 0.01). **Conclusion:** High doses of vitamin D supplementation (50,000 IU/week) may have potentially negative effects on glycemic control, which might be related to changes in serum osteocalcin than in FGF23 levels.

Keywords: vitamin D deficiency; vitamin D3; fibroblast growth factor-23; A1c; OSC

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INTRODUCTION

Vitamin D is made of ergocalciferol (Vitamin D2) and cholecalciferol (Vitamin D3); its deficiency (VDD) is a significant health issue with a high morbidity rate.[1] Vitamin D supplements have been recommended to prevent cancer, diabetes, and cardiovascular disease.[2] Recent research has indicated that VDD results in negative health effects associated with significant morbidity in Jordan and other Mediterranean countries.[2-5] This is particularly concerning given the abnormally high prevalence of type 2 diabetes mellitus (T2DM) in Jordan.[6,7] Since VDD is linked to poor glucose metabolism,[8-10] it is possible that taking vitamin D supplements could help lower fasting blood glucose (FBG). [11] Furthermore, central obesity increases the risk of developing insulin resistance (IR) and type 2 diabetes; this is an important area of research because there is a high prevalence of both overweight and obesity in Jordan; VDD is a serious health risk that requires to be treated.[12,13]

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Moreover, several research studies have established that the osteoblast-derived hormone osteocalcin (OSC) regulates muscular function, male fertility, and insulin production by attaching to the G protein-coupled receptor family C (GPRC6A) receptor. In contrast, the G protein-coupled receptor family c (GPR158) receptor mediates cognitive functioning.[14] OSC correlates to fat mass, insulin sensitivity and secretion, glucose metabolism, and glycemic fluctuation.[15] The direct effects of 1-alpha and 25 dihydroxy-vitamin D3 (1α,25D3) on osteoblasts may be demonstrated by isolated VD receptors in osteoblasts.[16] VD modulates mineralization, osteoblast proliferation, and differentiation [17]. Chronic kidney disease (CKD) in the form of diabetic nephropathy might adversely affect the management of diabetes mellitus.[18] Over time, the millions of tiny filtering units in each kidney are damaged by elevated blood sugar levels resulting in renal dysfunction as a long-term complication of diabetes mellitus. Improvements in serum levels of both fibroblast growth factor-23 (FGF-23) and soluble alpha-Klo (s-KL) are related to this change.[19] In addition, phosphorus levels are controlled by an endocrine hormone, FGF-23, created and secreted by osteocytes, which influence renal tubules and are involved in VD metabolism.[20]

On the other hand, many RCTs utilizing different VD3 treatment protocols have failed to demonstrate that VD3 can reduce A1c levels in T2DM patients.[21,22] However, while low to moderate VD3 dosages (1600 to 4000 IU) were shown to have no significant effect on A1c in previous studie,[21,22] some RCTs (Barham 2021; Nada and Shaheen (2017) have found a link between high VD_3 supplementation and a significant reduction of A1c and FBG. However, based on available data, no randomized clinical trials (RCTs) have investigated the effects of high doses of VD3 on the association between FGF-23 or OSC and A1c levels in people with VDD. Therefore, this RCT was designed to assess the effect of VD3 on the relationship between the levels of FGF-23 and OSC with A1c values in the blood in a sample of Jordanian adults with VDD.

METHODS

Study design and participants

This RCT was approved by the Institutional Review Board (IRB) panel of the Applied Science Private University (ASU) (protocol number. 2020-PHA-23) and was conducted between December 2020 and March 2021 during the winter season. It was executed following the Helsinki Declaration and its tenets.

Participants were Jordanian males and females from the ASU campus, as well as their friends and relatives, with an average age at the start of the study of 37.88 ± 9.53 years (range 22 to 55). Enrolled participants consented in writing to complete this clinical trial.

Internal medicine physicians at Ibn Al-Haytham Hospital and Laboratory in Amman, Jordan, provided a confirmed diagnosis of VDD for all eligible participants. Due to the association between extended treatment of VD3 and kidney stone formation, participants having a history of chronic illnesses, such as kidney disorders, were excluded from the RCT.[23] Participants with chronic health conditions such as osteoporosis, thalassemia, cancer, and endocrine disorders or a documented history of immune reactions to VD supplements were also excluded from the trial.

Participants who fulfilled the eligibility criteria were notified by the research team and invited to attend the baseline meeting, where their age, body mass index (BMI; kg/m^2), alanine aminotransferase (ALT; U/L), A1c (percent), calcium (mg/dL), phosphorus (mg/dL), parathyroid hormone (PTH) (pg/mL), 25-hydroxyvitamin D (25OHD) (ng/mL), and urea (mg/dL) were collected.

Intervention

The baseline and follow-up measurements of the anthropometric and clinical variables were recorded before and after VD3 supplementation. At the end of the interventional protocol, which is 8 weeks, the participants entered a washout period of 2 weeks. Then, follow-up measurements were collected for all the participants.

Before and after vitamin D_3 administration, baseline and follow-up assessments of both anthropometric and clinical variables were obtained. After the 8-week interventional protocol, the subjects entered a 2-week washout period, followed by a final follow-up measurement.

An independent statistician selected the study groups utilizing a computer-generated randomization program, where the eligible participants (n =78) were divided into two cohorts, as depicted in the companion chart (Figure 1): Participants in Group A (Experimental group) received no treatment and participated as the control group. Participants in Group B (Intervention group) received 50,000 IU of VD3 in a Hi Dee soft gelatin capsule once weekly (United Pharmaceuticals Company, Amman, Jordan). The vitamin D_3 therapy protocol was applied in conformity with the Endocrine Society's Clinical Guidelines for the treatment of VDD in adults.[24] As demonstrated, VD_3 was administered to humans for one year without producing any toxicity.[25]

Anthropometric measurements

This RCT was conducted at Pharmacy school laboratories/ ASU throughout the winter of 2020 since the timing of blood sampling is essential for minimizing seasonal variations in total VD blood levels.[26] In addition, anthropometric data, including height in meters (Ht), body weight (BW) in kilograms, body mass index (BMI) in kilograms/meter squared, waist (W) circumference in meters, hip (H) circumference in meters, and waist to hip ratio (WHR) were recorded at the inception and the conclusion of the trial.

Clinical parameters assays

In the clinical laboratories department at Ibn Al-Haytham Hospital in Amman, Jordan, qualified technicians collected baseline venous blood samples from fasting participants using labeled Eppendorf® tubes for serum measurement of the clinical variables.

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Vitamin D_3

The LIAISON® 25-hydroxyvitamin D Assay (DiaSorin), the chemiluminescence immunoassay method, was utilized to determine the total serum levels of vitamin D (25OHD). The assay has a detection sensitivity of approximately 4 ng/mL, a 100 % cross-reactivity with both 25OHD metabolites, $25OHD_2$ and $25OHD_3$, and assesses the total serum 25OHD concentration.

FGF-23

Using an enzyme Immunoassay kit, serum concentrations of FGF-23 were quantified. The sensitivity of this method was 0.08 p-mol/l (= 0.6 pg/ml).

Parathyroid Hormone

An enzyme Immunoassay kit assayed parathyroid hormone (PTH) levels in the serum (PTH Intact EIA -3645, DRG Diagnostics, Marburg, Germany). The analytical sensitivity was 1.57 pg/mL for this assay.

Calcium and Phosphorus

Calcium and phosphorus (PO4) levels in serum were measured utilising spectrophotometry (Clinical Chemistry RAL Analyzers Clima Plus, Spain) and kits (CALCIUM-ARSENAZO kit (M11570i-15) and Phosphorus Phosphomolybdate/Uv Kit) (M11508i-18, BioSystems, Spain).

Glucose and Triglycerides

Both fasting blood glucose (FBG) and triglyceride (TG) serum levels were assayed using an enzymatic colorimetric technique on a Roche Cobas C501 analyzer (GLUC3 application, Roche, Mannheim, Germany). TG-BioSystems kits were used to assay serum TG levels (M11528i-20, BioSystems, Barcelona, Spain).

Statistical Analysis

The statistical analysis was conducted using version 27.0 of the Statistical Package for the Social Sciences (SPSS) for Windows (Chicago, IL, USA). A paired T-test was carried out to determine any significant differences between both study groups before and after vitamin D_3 supplement administration. Using an Independent T-test, any significant differences in the mean values of each parameter between the two study groups were analyzed for statistical significance. Multiple linear regressions with the step-wise method were performed to study the associations and potential factors influencing dependent variables (DVs) at baseline and the study's conclusion. The Kolmogorov – Smirnov test was applied to assess the normality of distribution for measured values.

RESULTS

The consort diagram (Figure 1) indicates that seventy-eight out of one hundred twenty-seven eligible participants completed all trial stages. Fifty-three percent (n = 41) of the participants were females. Participants stated that 17 % of their fathers and 24 % mothers had diabetes mellitus. Additionally, 47.4% of the participants reported sun exposure on a daily basis.

Baseline characteristics of participants

The baseline mean age of the trial participants was 37.88

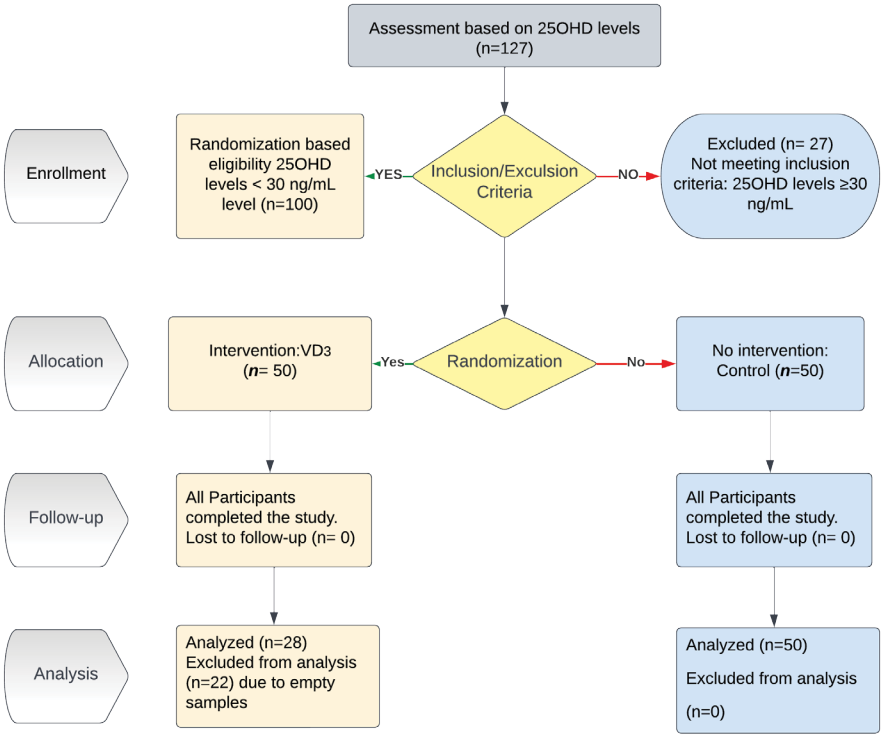

Figure 1. The study design companion flowchart illustrating the number of participants screened, recruited, and randomly assigned to the intervention group.

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± 9.53 years, as seen in Table 1. The BMI for the study cohort was 28.70 ± 5.79 kg/m^2, indicating that most participants were overweight. All participants in the trial had an average 25OHD concentration of 16.14 ± 5.81 ng/ml, indicating a deficiency in vitamin D. Subjects having vitamin levels of 30 ng/mL or higher were excluded from this RCT. Table 2 presents a descriptive analysis of clinical parameter mean values. Each test result was within acceptable limits.

Table 1. Anthropometric Baseline descriptive characteristics (*n* = 78)

Parameter	MMean ± SD
Age (years)	37.88 ± 9.5
Weight (kg)	79.85 ± 17.3
Height (cm)	167.64 ± 7.5
BMI (kg.m^{-2})	28.70 ± 5.8
Waist (cm)	95.22 ± 15.2
Hip (cm)	109.35 ± 12.4

BMI: body mass index; SD: Standard deviation.

Table 2. Baseline descriptive clinical parameters (*n* = 78)

Parameter	Mean ± SD	Normal Range
25OHD (ng/ml)	16.14 ± 5.81	30-50
FGF-23 (pg/ml)	50.64 ± 11.58	N/A
FBG (mg/dl)	78.28 ± 10.65	70-110
A1c (%)	5.20 ± 0.32	< 6.5%
OSC (ng/ml)	0.74 ± 0.33	N/A
PTH (pg/ml)	37.01 ± 8.81	9-90
Calcium (mg/dl)	9.61 ± 1.53	8.6-10.3
PO4 (mg/dl)	4.06 ± 0.23	2.5-4.5
TG (mg/dl)	121.61 ± 58.87	Up to 150

Note: 25OHD: 25-hydroxy vitamin D; FBG: fasting blood glucose; A1c: glycated hemoglobin; OSC: osteocalcin; PTH: parathyroid hormone; PO4: phosphate; FGF-23: fibroblast growth factor-23; TG: triglycerides; TC: total cholesterol; HDL: high-density lipoprotein; LDL: low-density lipoprotein; TC/HDL: total cholesterol/high-density lipoprotein ratio; SD: standard deviation. * NA, not applicable.

Results at the conclusion of the trial

25OHD levels

At the conclusion of the trial, a paired t-test showed the mean serum values of 25OHD were increased with a significant change of about-24.5ng/ml (40.04 ± 11.61vs 15.54 ± 5.02, P^A < .001) in the B group, as shown in Table 3. The independent t-test showed a statistically significant difference in the mean serum levels of 25OHD between the B and A groups (40.04 ± 11.61 vs. 15.35 ± 5.41, P^C < .001).

A1c levels

There was a significant change between the mean A1c of baseline and follow-up among those in the B group (5.63 ± 0.33 vs. 5.38 ± 0.32, P^A = .01). However, according to the independent t-test analysis, groups B and A had no significant differences in their mean A1c levels at the inception and conclusion of the trial, as shown in Table 3.

FGF-23 levels

At a 10-week follow-up, the mean FGF-23 levels significantly increased with a change of about -27 ng/ml (114.04 ± 103.8 vs. 87.40 ± 82.21, P^A = .02) in the B group, as shown in Table 3. The FGF-23 levels were significantly higher in group B than group A (114.04 ± 103.8 and 52.67 ± 8.28), respectively, with a P-value of P^C < .001.

Serum OSC

Follow-up osteocalcin levels in the B group were statistically insignificant and higher than baseline levels (0.84 ± 0.75 vs. 1.08 0.93 ng/ml, P = .206). The independent t-test showed that the mean levels of OSC were insignificantly higher in the B group than in the A group, which was 0.84 ± 0.75 and 1.01 ± 0.47, respectively, with a P^C = .217 as shown in Table 3.

Multivariate step-wise regression analysis

The multivariate step-wise regression analysis was conducted to indicate significant mediating factors (IDVs) (Table 4) on the circulatory levels of selected variables indicative of glycemic control at 10-week follow-up supplementation of VD3 at 50,000 IU once a week.

FGF-23 levels were only mediated by cis-gender factor (R = 0.329, R2=0.108, F-test = 5.819, B = 0.329, P = 0.020). Changes in A1c values observed in the VD3 interventional group were significantly mediated by TC levels (R = .340, R2 = .116, F-test = 6.270, B = .340, P-value = .016). TC levels were positively associated with A1c at the conclusion of the trial, and it seems to be involved in the positive relationship between elevated 25OHD levels and A1c values observed at the end of this trial (R = .340, R2 = .116, F-test = 6.270, B = .340, P-value = .016).

DISCUSSION

This RCT is the first clinical investigation to examine the effect of large doses of vitamin D_3 (50,000 IU VD_3/week) on serum FGF-23 levels in participants with VDD. The primary outcome of this trial was that VD_3 significantly increased serum concentrations of FGF-23 and PTH.

At the beginning of the study, there were 78 Jordanian participants. The average age of eligible subjects was 37.88 ± 9.53 years, and their mean BMI was 28.70 ± 5.79, indicating that the majority of trial participants were overweight as indicated in Table 1.

Numerous international and regional research has demonstrated a negative connection between BMI and the concentration of serum 25OHD. This trial's VDD screening data for the ASU society revealed a mean serum 25OHD concentration of approximately (16.14 ± 5.81 ng/mL), indicating that they had VDD. These findings were consistent with prior local research showing a high prevalence of VDD among Jordanians.[3,6]

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Table 3. Baseline and follow-up results of study variables for Experimental and Interventional groups

Variable	Group	A (Control) *n = 50*	B (Treatment) *n = 28*	*P*-value
25OHD	Baseline	17.20 ± 6.97	15.54 ± 5.02	P^B = 0.22
	Follow-up	15.35 ± 5.41	40.04 ± 11.61	**P^C < .001***
	Difference	1.85	-24.5	
	P^A	**< .05***	**< .001***	
A1c	Baseline	5.15 ± 0.53	5.38 ± 0.32	P^B = 0.07
	Follow-up	5.38 ± 1.13	5.63 ± 0.33	P^C = 0.14
	Difference	-0.23	-0.25	
	Follow up CV%	21	5.86	
	P^A	**0.11**	**< .05***	
FGF-23	Baseline	49.46 ± 12.00	87.40 ± 82.21	P^B = 0.24
	Follow-up	59.36 ± 11.43	114.04 ± 103.8	**P^C < .05***
	Difference	-9.9	-26.64	
	Follow up CV%	19.26	91.02	
	P^A	0.09	**< .02***	
OSC	Baseline	0.71 ± 0.37	1.01 ± 0.47	P^B = 0.239
	Follow-up	1.08 ± 0.93	0.84 ± 0.75	P^C = 0.217
	Difference	-0.37	0.17	
	Follow up CV%	86.11	8.97	
	P^A	0.08	**< .02***	

Note: P^A: P-value for paired t-test between baseline and 10 weeks follow-up of the study for both groups; P^B**:** P-value for independent T-test between baseline means of B and A groups, P^C**:** P-value for independent T-test between 10 weeks follow-up means of B and A. * Indicates a P value of < .05. **Abbreviations:** A**:** Experimental group (Control); B**:** Intervention group (Treatment).

Table 4. Multiple step-wise linear regression analysis to investigate the predictor variables at follow-up for the (B) group

Dependent variable	Univariate effect estimates	Coefficient			
		B	F	R	R^2
FGF-23	Gender	0.329	5.819	0.329	0.108
A1c	TC-post	0.340	6.270	0.340	0.116

Abbreviation: **B:** Intervention group; **A1c:** glycated hemoglobin; **R:** correlation coefficient; **TC:** total cholesterol; **DM:** diabetes mellitus;

After 8 weeks of 50,000 IU VD_3, a statistically significant positive change in serum 25OHD level was detected, with a mean difference of -24.50 ($p < 0.05$) at the conclusion of the study. Several RCTs had shown comparable results.[27-29]

Some RCT studies found that VD_3 significantly affects A1c levels, contradicting our findings.[30-32] Unfortunately, these results were inconsistent and vague.[33]

Regarding FBG, our results are consistent with most published research.[31,34,35] A study conducted by Lemieux et al. (2019) on 96 newly diagnosed T2DM participants who had VDD and were supplemented with a VD3 dose (5000 IU/Day) reported no difference in FBG levels six months later.[31] However, the VD3 dose used by Lemieux was slightly less than the dose conducted in the current trial. In addition, Mitchell et al. (2015) failed to demonstrate any effect of the high VD_3 dose (50,000 IU/week) for three months on the FBG levels in healthy VDD patients.[35] Furthermore, neither the sensitivity of the insulin receptor nor insulin secretion was affected. However, $A1_C$ remains the most accurate predictor of glycemic control, and it is essential for diabetes diagnosis and monitoring.[36] A weekly dose of 50,000 IU elevated A1c levels considerably in those with VDD. These results were found after 10 weeks of follow-up (after a 2 week washout period). The results were consistent with those of earlier RCTs conducted on Jordanians with VDD using the same study methodologies.[37,38] Along with the results of this trial, Lips et al. (2019) indicated that the mean A1c levels were lower in those with 25OHD ≥30 ng/ml as opposed to those with suboptimal 25OHD levels.[10]

In addition, Rezagholizadeh's observations, which supported VD_3 for glycemic management, lacked follow-up data from the interventional group. While there is no agreement regarding the potential efficacy of the treatment of VD3 in the management of diabetes, the hypothesis appears to be closer to a U-shaped curve. As a result, some studies reported a strong negative correlation between serum 25OHD levels and A1c; lower levels of 25OHD were associated with insulin resistance (IR).[39] Furthermore, another RCT conducted on 96 obese adolescents (BMI = 31.5 ± 4.2) aged 11 to 13 years revealed that 2000 IU/Day of VD_3 for three months did not impact $A1_C$ levels or IR.[40]

Moreover, no significant reduction in mean A1c values was observed at the conclusion of clinical studies, including 125 patients with T2DM.[41] In contrast, high doses of VD_3 were evaluated to determine their effect on A1c levels. For instance, Nada and Shaheen (2017) concluded a correlation between VD_3 and a significant decrease in A1c (7.9 ± 1.7 vs. 7.4 % ± 1.2 %, P < .01) and FBG (9.1 ± 4.3 vs. 7.4 ± 2.4 mmol/L, P = .034) following a VD3 treatment plan of 45,000 IU/week for 8 weeks and then after a dose of 22,500 IU/week for 16 weeks, followed by calcium tablet supplementation for 6 months. The percentage reduction in A1c was 0.54%, whereas the decrease in FBG was 1.22 mmol/L.[41]

FGF-23 levels were considerably elevated by weekly dosing of 50,000 IU in individuals with VDD. These findings were observed at 10 weeks of follow-up (after 2 weeks of washing out), as seen in Table 3. In a study published by Burnett-Bowie et al. (2012), 18 to 45-year-old participants (n = 90) treated with 50,000 IU of VD_2 (ergocalciferol) each week for three months in subjects with 25 OHD ≤ 20 ng/mL increased level of FGF-23 relative to placebo.[42]

Trummer et al. (2018) found that VD3 did not affect FGF-23 levels in hypertensive patients with 25 OHD levels below 30 ng/mL. A systematic review and meta-analysis of RCTs from multiple databases comprised 21 papers, with 23 studies included in the final analysis.[43] The selected studies included 1925 people who were followed for 8–156 weeks. Results were unaffected by the study duration (p = 0.14), age class (p = 0.09), or assay provider (p = 0.11). Overall, the meta-analysis of RCTs revealed that VD_3 administration of >2000 IU/d VD or activated VD significantly increased FGF-23 concentrations, particularly in patients with end-stage kidney or heart failure.[43]

In Uzum et al. (2010) study, the population consisted of women with VDD who were randomly assigned to one of three groups: healthy women with VDD (n = 18, mean age 29.1 ± 9.9 years), healthy women with VD insufficiency (control group; n = 19, mean age 28.5 ± 5.2 years), and women patients with genetically determined hypophosphatemic rachitis (n = 13, mean age 26.1 ± 14.9 years).[36] These patients received the standard therapy regimen of VD3 150,000 IU once, followed by D3 880 IU and calcium carbonate 1000 mg daily for six weeks. Serum FGF-23, 1,25 $(OH)_2$ D, calcium, phosphate, bone turnover indicators, intact PTH, and urine calcium and phosphate excretion were compared between the groups. After a standard treatment protocol, the VDD group women were reviewed. Significantly, lower serum FGF-23 concentrations were found in women with VDD compared to those with VD insufficiency and hypophosphatemic rachitis. Upon replacement of VD, serum FGF-23 and phosphate concentrations significantly decreased (p < 0.05). Before VD replacement, a significant negative correlation existed between FGF-23 and PTH in patients (r = -.469, p < .05). According to the result of Uzum et al. 2010 study, decreasing FGF-23 concentrations, which decline further during VD replacement therapy, may promote bone mineralization by exerting a counter-regulatory influence on phosphate homeostasis. Lower baseline 1,25 $(OH)_2$ D concentrations and hypophosphatemia during treatment may have dominant effects on FGF-23 concentrations in VDD, resulting in lower FGF-23 concentrations at baseline and during replacement therapy.[36]

CONCLUSION

This trial provides vital insight into the effects of VD_3 on healthy Jordanian participants diagnosed with VDD. Eight weeks of therapy increase 25OHD, FGF-23 by VD_3 supplement.

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CONFLICTS OF INTEREST

The authors report no conflicts of interest.

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