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Impact of Vitamin D Elements and Osteoporosis Factors in Postmenopausal Iraqi Women with T2DM

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Abstract

Osteoporosis is a systematic bone disorder characterized by bone mass loss and bone tissue architectural degeneration. The incidence of osteoporosis in women increases with age, reflecting the considerable increase in bone loss rate in postmenopausal women once estrogen loses its protective influence. The purpose of this study was to determine the levels of Osteocalcin, Vitamin D, and other parameters that influence bone quality and increased bone fragility in postmenopausal Iraqi women with type 2 diabetes mellitus (T2DM). The levels of vitamin D receptor and vitamin D binding protein in serum are studied for the first time in our study. The present study included 89 postmenopausal women aged 50-70 years old, 62 T2DM patients, and 27 controls. Ten of the T2DM patients were considered osteoporotic, 28 were considered osteopenia, and 22 were normal; this classification is according to the WHO criterion. After matching for body mass index (BMI) and age for patients and controls, results show a significant difference in serum Osteocalcin, vitamin D, and vitamin D binding protein levels in patients compared to controls. In our study, diabetic patients appear with high levels of Osteocalcin, vit.D, and vitamin D binding protein compared with non-diabetic control. The processes underlying diabetes mellitus-induced skeletal problems are unknown. Anti-diabetic medications might have an adverse or favorable effect on bone metabolism. The study concludes that managing skeletal health in postmenopausal women entails screening fracture risk factors, lowering modifiable risk variables through dietary and lifestyle modifications, and using a pharmacologic treatment for individuals at high risk of osteoporosis or fracture. Women with osteoporosis must be managed for the rest of their lives.

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1. Introduction

Osteoporosis (OP) and diabetes mellitus (DM) are two of the most frequent diseases [1]. OP is the asymptomatic decrease of bone mass that is most common in postmenopausal women; over 40% of these women will have an osteoporosis-related fracture at some point in their lives[2]. Diabetes mellitus (DM) is a chronic metabolic

condition that mostly affects adults and is linked to a high rate of morbidity and death [3]. Type 2 diabetes is caused by insulin resistance, a disease in which cells fail to utilize insulin efficiently, and insufficient insulin production, often associated with absolute insulin deficiency [4-7]. Individuals with T2DM, particularly those on hypoglycemic drugs and those with comorbidities such as neuropathy and retinopathy, are more prone to fall, leaving them more vulnerable to fractures. An increased incidence of fractures is also linked to hyperparathyroidism and renal osteodystrophy. [8]. In addition to the standard method of bone densitometry, various biochemical bone metabolism biomarkers are utilized to adiagnosis of osteoporosis [9]. One of those markers is Osteocalcin, also known as bone Gla protein, a marker for bone development. It induces insulin synthesis and enhances energy consumption and insulin sensitivity in target organs as a hormone. Increased circulation levels of Osteocalcin, especially by exogenous protein consumption, have been demonstrated in animal experiments to prevent obesity and glucose intolerance. Some epidemiological studies back up osteocalcin's involvement in maintaining human glucose and energy balance [10]. Monitoring Osteocalcin levels might help determine how well patients respond to therapy for metabolic bone disorders or anticipate a bone loss in postmenopausal women [10, 11]. Vit D insufficiency and T2DM share many risk factors, including African Americans, Asians, and Latinos, increasing obesity, advanced age, and a lack of physical activity (which may lead to less outdoor time or exposure to sunlight), which may not be a coincidence [12]. Furthermore, Vitamin D promotes bone health and helps to avoid osteoporosis and fractures. Vit D deficiency and inadequate dietary vitamin D are frequent in older people and are linked to an increasingly higher risk of fractures [13]. For most people, the synthesis of Vit D in the skin after exposure to UV-B radiation [290-315nm] is the main source of Vit D, which converts 7-dehydrocholesterol to provitamin D3. The molecule is pushed into the extracellular space and drawn to the capillary bed, where it binds to Vit D binding protein (DBP) and is delivered to the liver due to these structural modifications [14], Then, vitamin D3 is converted into 25-hydroxycholecalciferol (25OHD) by 25-hydroxylase (CYP2R1). This process mainly occurs in the liver, The final stage of the formation of active 1,25-dihydroxycholecalciferol (1,25 (OH) 2 D) is mediated by the enzyme 25-hydroxyvitamin D1α-hydroxylase (CYP27B1), which is found in proximal tubular cells[15], the active form $1,25(OH)_2 D_3$ enter the cells and bind to Vit D receptor (VDR) and react with the Vit D response elements[16, 17]. Vit D binding protein (DBP) is a multifunctional protein with a molecular weight of(52–59) kDa that has been well preserved during the evolution of vertebrates and is mainly produced in the liver [18]. Vit D receptor is required for most of vitamin D's actions, with 1,25 (OH) 2D as the primary ligand. Vit D receptor is considered a transcription factor. Intestinal epithelial cells, osteoblasts, parathyroid cells, and distal renal tubules express VDR, which is important in calcium and phosphate balance [19, 20]. Some researchers have looked at the link between obesity and lifestyle behaviors and osteoporosis and osteopenia, finding that a lower BMI and smoking were positively linked with osteoporosis or osteopenia in the general population and the T2DM group [21]. Discoveries on the multiple functions of Vit D include the presence of VDR in pancreatic β-cells, and the expression of 1α -hydroxylase in pancreatic β-cells, which is responsible for the conversion of 25(OH)D to $(1,25(OH)_2D)$. Vit D response elements are found in the human insulin gene promoter, and VDR is found in skeletal muscle. Furthermore, $1,25(OH)_2D$ directly activates human insulin receptor gene transcription, activates the peroxisome activator of δ-receptor proliferators, increases insulin receptor expression, and enhances insulin-mediated glucose transport [22]. Vitamin D insufficiency is linked to insufficient bone mass or insufficient bone remodeling, which can lead to bone fragility and an increased risk of fractures, since vitamin D regulates the interaction between osteoblasts, osteoclasts, and osteocytes [23]. Bone density decreases by around 10% on average during the menopausal transition phase [24].

2. Subjects and Methods

The current case-control study included 89 postmenopausal women aged 50-70 old, 62 of them are T2DM patients and 27 control. The study was conducted during the period from November 2020 to March 2021. A total of 89 postmenopausal women aged 50-70 years old participated in the study. They were recruited from the medical City of Baghdad Teaching Hospital, educational laboratory, and Kadhimiya Teaching Hospital. Among the postmenopausal women, 62 were T2DM patients, and 27 were controls. According to the WHO criterion, ten of the T2DM patients were considered osteoporotic: T-score less than -2.5 below the normal adult mean based on the established reference databases. And 28 of the patients were considered osteopenia as their T-score was between -2.5 and -1. And 22 patients who were T-score more than -1 were normal. Exclusion criteria included subjects who received osteoporosis therapy and Vit D. By measuring bone mineral density (BMD) with dual-energy x-ray absorptiometry (DXA), patients were diagnosed with osteoporosis and controls were diagnosed as normal, according to World Health Organization (WHO) diagnostic guidelines: •A T-score of -1.0 or above is considered

"normal."•Osteopenia is defined as a T-score of -1.0 to -2.5. •Osteoporosis is defined as a T-score of -2.5 or below. Serum FBS and lipid profile measured by spectrophotometer, serum Osteocalcin, Vit D, VDR and DBP measured by enzyme-linked immune sorbent assay (ELISA) using kits manufactured by Sunlong /China. BMI was measured for patients and control. The mean and standard deviation were used to analyze the data. The significance of difference was determined using the Student-t-test for two independent means.

3. Results and Discussion

Sixty-eight T2DM patients (postmenopausal women) and 27 control were included in this study, the results showed in Tables 1-7 and Figures 1-8, the mean age for the patients and control groups was (61.58±5.08), (56.66±5.56) respectively. The results also showed a significant difference in FBS between patients and control. Our results showed a non-significant difference in m±sd of BMI between patients and control groups, but both groups were classified as obese.

Table 1: Age and body mass index of patients and control groups.

Marker	Control	Patients	P value (sig≤0.05)
Age	56.66±5.56	61.58±5.08	≤0.001
BMI	30.66±7.24	31.77±6.86	0.494

Table 2: Fasting blood sugar and vitamin D levels in patients and control groups.

Marker	Control	Patients	P value (sig≤0.05)
FBS (mg/dl)	92.29 ± 17.79	178.96±83.4	≤0.001
Vit D (ng/ml)	21.23±2.55	24.29±2.82	≤0.001

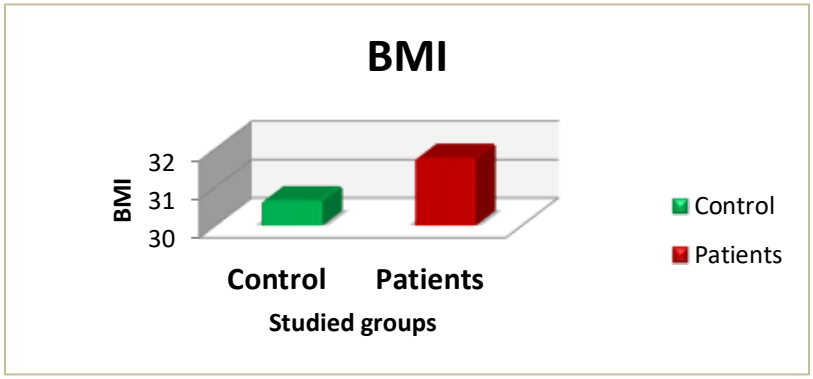

Figure 1: body mass index in patients and control groups.

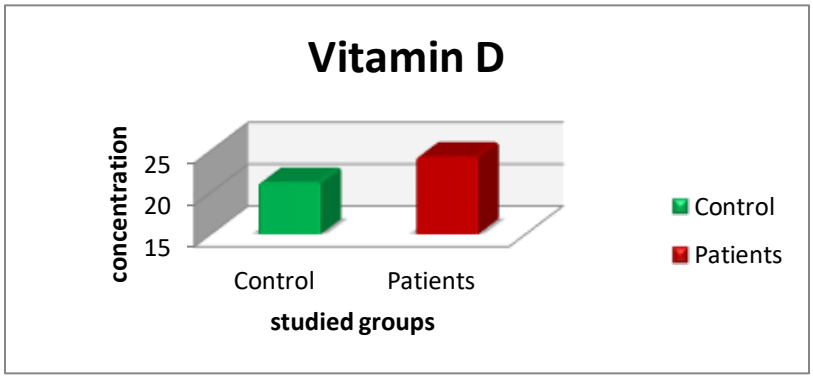

Figure 2: Levels of vitamin D in patients and control groups.

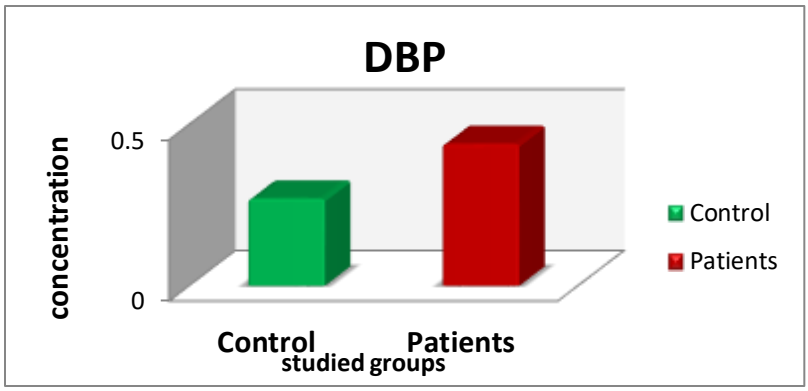

Figure 3: Levels of fasting blood sugar in patients and control group.

In this study, the difference in m±sd of Vit D was significant between patients and control, Vit D level was lower than the normal range (≥ 30 ng/dl) in both patients and control, but it was surprisingly lower in patients than control, these results agreed with Alhumaidi et al. who studied vitamin D deficiency in T2DM, and the results showed a low 25OHD level in patients and a lower level in control [25, 26]. Another cross-sectional study was done by Hidayat *et al.* [27], who evaluated the association between Vit D and t2dm in elderly subjects, the result revealed 78% Vit D deficiency in both patients and control, and explained this results by suggesting that variables like BMI, use of sun protection and sex had a substantial impact on the occurrence of Vit D deficiency. Women with menopause have thinner skin and a lower capacity for vitamin D production, and decreased vitamin D absorption in the intestine and hydroxylation of vitamin D in the liver and kidneys. These metabolic abnormalities will be accompanied by decrease outdoor activity and a lower vitamin D consumption in the diet [28, 29]. Some research has suggested that vitamin D may have a direct (through its function in pancreatic beta-cell activation and sensitive organs) or indirect (by calcium homeostasis control) favorable influence on insulin secretion and sensitivity [30]. Osteoporosis is a well-known fact among postmenopausal women, and several risk factors have been linked to this high prevalence, including vitamin, D deficiency [31]. The National, Health and Nutrition, Examination, Survey III (NHANES III) found a link between 25(OH) D and BMD in 13,432 participants, including whites, Hispanics, and blacks [32].

Table 3: Levels of vitamin D receptor and vitamin D binding protein.

Marker	Control	patients	P value (sig≤0.05)
VDR (ng/ml)	0.86±0.31	1.05±0.32	0.012
DBP (ug/ml)	0.27±0.12	0.44±0.13	≤0.001

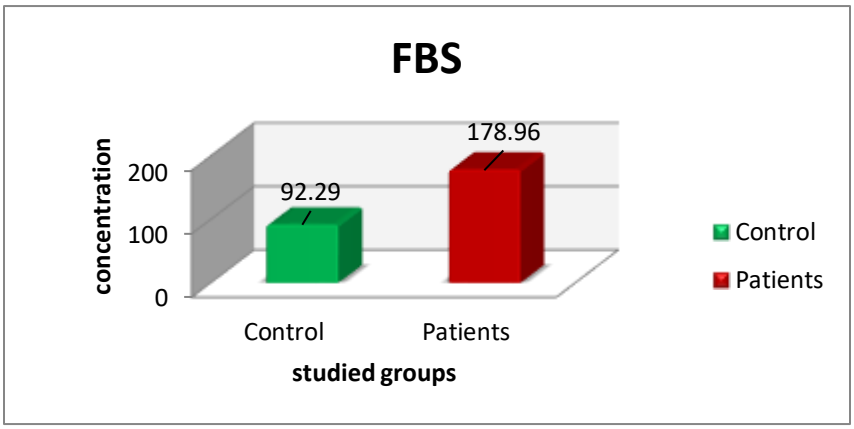

Figure 4: Vitamin D binding protein levels in patients and control groups.

The total 25(OH) D, which comprises DBP and albumin-bound 25(OH)D and the free form, is measured in the serum. Because the most of 25(OH) D is bound to DBP, the overall 25(OH) D concentration will be influenced by the serum DBP concentration. DBP levels remain relatively constant throughout life. However, they arise during pregnancy and estrogen supplementation. Low serum DBP levels can also be caused by protein loss in the urine

(as seen in some diabetic patients) [33]. This was consistent with our results which revealed a significant difference in Vit D and DBP between patients and control. The results of our study showed a non-significant difference in the serum level of VDR between patients and control groups. Vitamin D receptor is found in renal tubules, intestinal epithelial cells, parathyroid gland cells, skin (keratinocytes), pancreas (beta islet cells), pituitary gland, skeleton (osteoblasts and chondrocytes), mammary epithelial cells, germ tissues and immune system (monocytes, macrophages, and T-lymphocytes) [34].

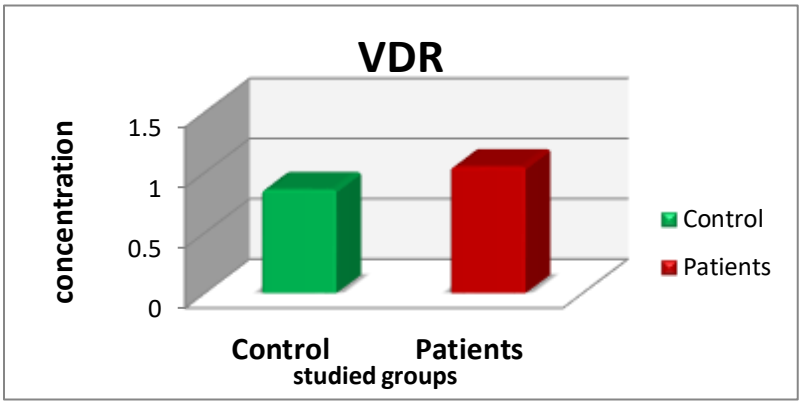

Figure 5: Levels of Vitamin D receptor in patients and control groups.

Vitamin D receptors may be playing an essential role in maintaining the maturity of β-cells which prevent them from undergoing dedifferentiation [35], The VDR's presence in bone cells shows that it directly affects bone. These receptors are found in osteoblasts as well as immature osteoclast precursor cells. It was previously thought that the active vitamin D metabolites' action on osteoclasts was indirect via osteoblasts [36]. Our study showed a non-significant difference in BMD between patients and control. These results agreed with Han et, al. [37] and Anaforoglu et, al. [38] ,while Raska et al. [39] disagree with these results they found that BMD in T2DM patients was higher than non-diabetic control.

Table 4: Measurements of spine bone mineral density and T.score.

Marker	Control	Patients	P value (sig≤0.05)
Spine T.score	-0.73±1.41	-1.0±1.31	0.39
Spine BMD g/cm2	0.97±0.15	0.94±0.18	0.46

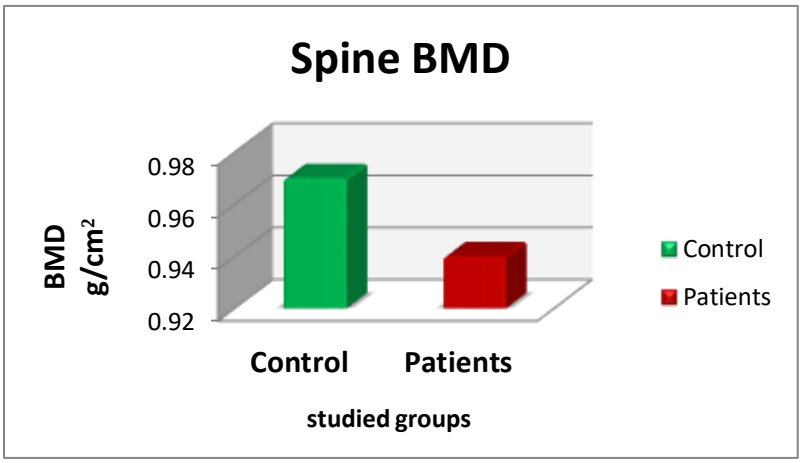

Figure 6: Spine bone mineral density for patients and control groups.

According to some data, type 2 DM patients may have normal or enhanced BMD [40-42]. Several studies have found additional benefits of metformin therapy, such as improved bone quality in diabetics and reduced fracture risk [43]. Metformin reduces hepatic glucose synthesis by blocking major gluconeogenesis enzymes and improves peripheral insulin sensitivity. Experiments have shown that it positively affects bone growth[44]. The Previous study has revealed that osteoclasts produced from the bone marrow enhance bone destruction in the

postmenopausal age when estrogen levels decline. Due to the mutually reinforcing nature of bone degradation and synthesis, an increase in bone breakdown also stimulates bone synthesis, resulting in a high rate of bone turnover[45]. Postmenopausal T2DM patients have the exact opposite problem. Unlike T1DM patients and osteoporotic postmenopausal women, T2DM patients experience slower bone loss. Data showing a reduction in bone turnover were discovered when biomarkers of bone turnover were investigated, particularly in well-controlled diabetes persons. This furthermore explains the existing phenomenon in T2DM patients of delayed bone loss [46, 47]. In a study of T2DM patients, Wakasugi and colleagues [48, 49] found that a substantial decline in BMD occurred only after the twentieth year of the illness, demonstrating the delayed bone loss pattern reported in T2DM patients.

Table 5: Level of serum osteocalcin in patients and control groups.

Marker	Control	Patients	P value (sig≤0.05)
Osteocalcin (ng/ml)	5.44±1.12	9.09±6.5	≤0.001

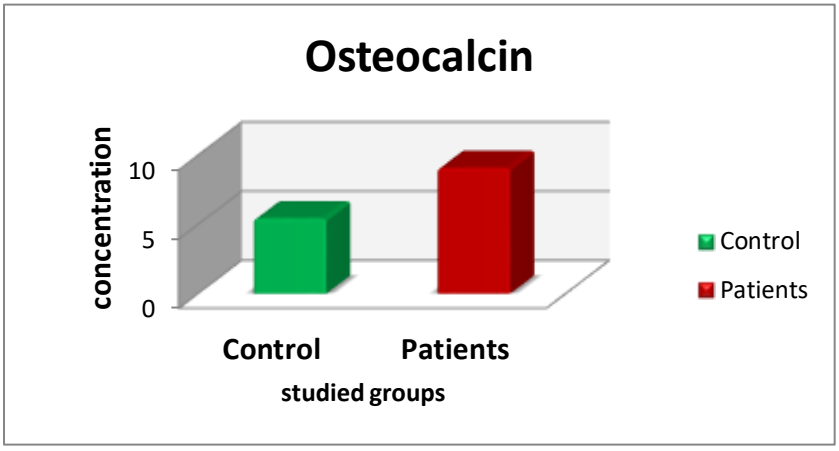

Figure 7: Levels of osteocalcin in patients and control groups.

Osteocalcin is produced during the formation of bones. It has a compact, calcium-dependent alpha-helical shape in which the Gamma Carboxyglutamic Acid (GLA) residues bind to hydroxyapatite in the bone matrix and facilitate absorption. Bone mineralization occurs in this manner. In osteoporotic women, calcium and phosphorus deficits reduce the development of hydroxyapatite crystals, allowing free Osteocalcin to circulate in the bloodstream. This could explain why Osteocalcin levels in the serum of osteoporotic postmenopausal women are higher [50]. This was agreed with the results of this study which revealed that serum Osteocalcin level in T2DM patients was significantly higher than in control.

Table 6: HDL, LDL, and VLDL levels in patients and control groups.

Marker	Control	Patients	P value (sig≤0.05)
HDL (mg/dl)	48.54±12.07	43.43±8.58	0.05
LDL (mg/dl)	114.19±13.25	113.76±40.46	0.94
VLDL (mg/dl)	39.51±13.34	30.96±9.14	0.004

Table 7: Total cholesterol and triglyceride levels in patients and control groups.

Marker	Control	Patients	P value (sig≤0.05)
TC (mg/dl)	186.34±40.02	179.95±51.08	0.57
TG (mg/dl)	157.93±95.62	163.61±92.23	0.79

In this study, there was a significant decrease in serum HDL-C in T2DM patients when compared with non-diabetic healthy control and this is in accordance with the study of Al-Tu'ma et al. [51]. Because androgen and estrogen receptors are present in visceral and subcutaneous adipocytes, endogenous sex hormones have altered the lipid profile in postmenopausal women. As a result, changes in endogenous sex hormone concentrations in middle-aged women's adipose tissues may disrupt lipid metabolism [52].

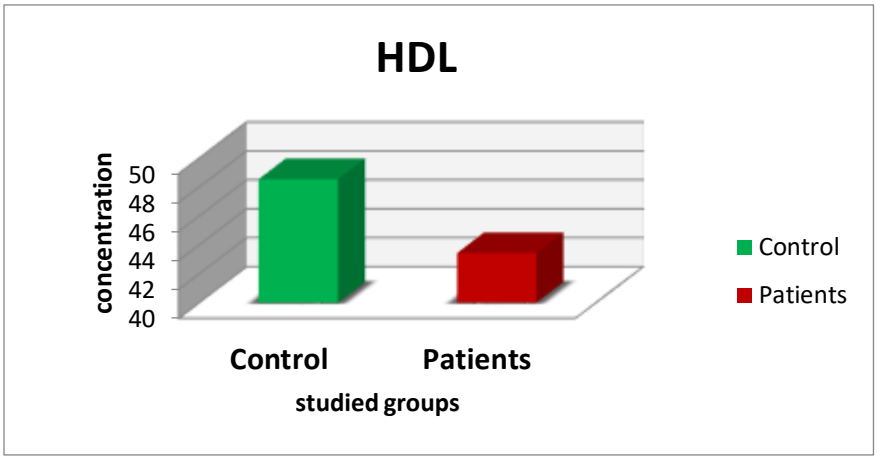

Figure 8: Levels of high-density lipoprotein in patients and control groups.

4. Conclusions

The current study's findings suggest that high LDL and low HDL levels may be linked to the development of osteoporosis in postmenopausal women. There is no significant difference in vitamin D levels between patients and control. In this study, bone mineral density was not affected by T2DM, and there are no differences in the prevalence of osteoporosis between diabetic and non-diabetic subjects.

Conflict of Interest

The authors declare that they have no conflict of interest.

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