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Comparison of Levels of Vitamin D, Vitamin D Receptor and Vitamin D Binding Protein in Women with Osteoporosis and Osteopenia

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ABSTRACT

Aims Osteopenia describes a decrease in bone mineral density below normal reference values, yet not low enough to meet the diagnostic criteria for osteoporosis. The aim of this study was to compare the levels of vitamin D, vitamin D receptor and vitamin D binding protein in women with osteoporosis and osteopenia.

Instruments & Methods This descriptive study was conducted on 110 postmenopausal women aged 52 to 73 years who referred to Baghdad Medical City Hospital from January 2021 to October 2021. Subjects were divided into three groups based on T-score results: control, osteopenia, and osteoporosis. Blood samples were collected from each participant to assess biochemical tests and dual energy X-ray absorptiometry used to diagnose osteoporosis.

Findings There was no significant difference in vitamin D and vitamin D receptor levels between the groups ($p>0.05$). Vitamin D binding protein level in healthy group was significantly higher than osteoporosis group ($p=0.005$), and DBP level in osteopenia was significantly higher than osteoporosis ($p=0.001$), but there was no significant difference between healthy and osteopenia groups ($p=0.53$). Differences between groups were significant in terms of bone mineral density ($p<0.001$).

Conclusion Comparison of levels of vitamin D, vitamin D receptor, and vitamin D-binding protein among women with osteoporosis and osteopenia, despite differences between the two groups in bone mineral density, does not show a significant difference.

Keywords Osteoporosis; Osteopenia; Vitamin D; Vitamin D Binding Protein; Vitamin D Receptor

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Introduction

Osteoporosis is a systemic bone disease characterized by a decrease in bone mass and deterioration of the microstructure of bone tissue. The World Health Organization (WHO) defines osteoporosis as a decrease in bone mass (50%) and bone quality (50%) [1]. It is a "quiet illness" that may go unnoticed until a fall occurs**.** The bones that make up the entire skeleton have multiple functions. They are active objects in themselves. They are constantly destroyed and regenerated during the remodeling process and become fragile with age. At the age of 30, maximum bone mass is reached. After that, it begins to decline and decreases significantly in postmenopausal women. Osteoporosis occurs when bone resorption exceeds bone formation [2].

Many risk factors affect the development of osteoporosis, including a sedentary lifestyle or risk of falling, weight loss, smoking, alcohol drinking, age, gender, being white, vitamin D deficiency, and diabetes mellitus. The prevalence of osteoporosis in women increases with age from 2% at 50 to more than 25% at 80 years old, which reflects the significant increase in bone loss rate in postmenopausal women after losing the protective effect of estrogen. Mechanical forces that do not normally cause a fracture cause these fractures. Fractures linked with poor Bone Mineral Density (BMD) are known as osteoporotic fractures [3].

The incidence of both osteopenia and osteoporosis increases with age. Osteopenia which refers to low bone mass, is not considered pathological, and usually has no clinical symptoms; however, it can be regarded as a precursor of osteoporosis, which is clinically indicated. Anabolic or anti-absorption drugs are used for treatment [4].

Vitamin D Receptor (VDR) is a transcriptional regulator protein and a member of the nuclear receptor superfamily. Binding to the active form of vitamin D (1,25-dihydroxy vitamin D) is important for the biological functions of vitamin D, which is expressed in the nuclear membrane of different cells [5]. Vitamin D has many functions, including promoting the absorption of minerals and calcium in the intestines and regulating the balance of calcium and phosphorus in the blood. In addition, it plays an important role in preventing osteoporosis and fighting cancer cells. Furthermore, vitamin D may prevent excessive loss of minerals in the kidneys and protect the elderly from the impact of Alzheimer's disease. It can also activate the body's immune system. The most important role of vitamin D is to maintain bone health by activating osteoclasts, and hence bone calcification and muscular strength [6].

For most people, the main source of vitamin D is skin synthesis after exposure to Ultraviolet B (UV-B) radiation (290-315 nm). UVB radiation acts on the upper epidermis of the skin to convert 7-dehydrocholesterol to pro-vitamin D3 through photolysis of the B-ring structure and subsequent isomerization. These structural changes cause the molecule to no longer fit into the plasma membrane and are pushed into the extracellular space and attracted into the capillary bed, where it binds to DBP (Vitamin D Binding Protein) and is transported to the liver. Maximum production is reached after 10-15 minutes of sun exposure in summer, depending on skin pigmentation [7]. 25 (OH) 2D acts as the primary ligand. VDR is considered a transcription factor. Intestinal epithelial cells, osteoblasts, parathyroid cells, and distal renal tubules all express VDR, which is important in calcium and phosphate balance. VDR is a member of the family of steroid hormone receptors. VDR was first discovered in the small intestine, but it now detected in almost all tissues where it has appeared. Vitamin D, not unexpectedly, affects a variety of cellular functions through VDR [8].

The vitamin D receptor acts as a heterodimer in the nucleus and regulates vitamin D target genes along with the Retinoic Acid X Receptor (RXR). The heterodimer compound interacts with the Vitamin D Respond Elements (VDRE) in DNA, thereby regulating the transcription of many vitamins D target genes, including bone-related genes. The key function of 1,25 Vit D3 / VDR is to regulate mineral and bone homeostasis [9].

The human VDR gene is located on chromosomes 12 and 15. Human and mouse genes consist of 8 coding exons. Two non-coding exons were found in mouse genes, and at least six non-coding exons were found in human genes [10].

According to the World Health Organization (WHO) guidelines, osteoporosis is diagnosed by measuring BMD with Dual-energy X-ray Absorptiometry (DXA). DXA results are presented in the form of a T-score. Young women aged 20 to 29 years are expressed as the Standard Deviation (SD) of this indicator (males use the same estimate because there is no reason to believe that bone formation and loss are different for different sexes). T-score compares BMD with people of the same age and race. This is not a measure of bone strength, but low reading indicates that other factors (such as other diseases or medication) affect bone density [11].

This study aimed to compare the levels of vitamin D, BMD, DBP, VDR, and other variables related to bone-building in women with osteoporosis and osteopenia and healthy women.

Instruments and Methods

This descriptive study was conducted on 110 postmenopausal women aged 52 to 73 years who were referred to Baghdad Medical City Hospital and Baghdad Teaching Hospital in Baghdad, Iraq, from January 2021 to October 2021.

Subjects were selected by random sampling method and divided into three groups based on T-score results, including control (n=45), osteopenia (n=46),

and osteoporosis (n=19) groups.

Blood samples were collected intravenously with a volume of 5 ml from each participant. The sample was placed in a gel tube. A centrifuge was used at 4000 rpm for 10 minutes to separate the blood and the samples were stored at 20°C for biochemical tests related to the study.

The physical characteristics were assessed using anthropometric measurements and Body Mass Index (BMI) was calculated by the following equation:

$$BMI\ (kg/m^2) = \text{Weight in kg} / (\text{Height in meter})^2$$

In the lumbar spine (first to fourth lumbar vertebrae), the BMD was measured by a Dual-energy X-ray Absorptiometry (DXA) at Baghdad Medical City Hospital. All women in the study measured their DXA for the first time and had no previous history of low bone density, osteoporosis, or osteoporosis treatment. All BMD values were expressed in g/cm^2.

According to diagnostic classification criteria published by a WHO study group in 1994, the normal category has a BMD within 1.0 SD of the young adult female reference mean (T-score greater than or equal to -1.0 SD); osteopenia has a BMD more than 1.0 but less than 2.5 SD below the young adult female reference mean (T-score less than -1 and greater than -2.5 SD), and osteoporosis has a BMD 2.5 or more SD below the young adult female reference mean (T-score less than or equal to -2.5 SD).

The Enzyme-Linked Immunosorbent Assay (ELISA) kit used in this study employs the Sandwich ELISA method, which is a specific antibody (Vitamin D3, DBP, and VDR) that is pre-coated on a Microelisastriplate in this kit. A specific antibody combines with the standards or samples in Microelisastriplate wells. This is followed by the treatment of each Microelisastripplate with a Horseradish peroxidase (HRP-conjugated) (Vit D3, DBP, and VDR) antibody. Each unnecessary part is removed and each well is filled with Tetramethylpiperidine (TMB) substrate solution. When the stop solution is added, only wells containing (Vit D3, DBP, and VDR) and HRP conjugated (Vit D3, DBP, and VDR) antibodies will turn blue before yellowing. Optical Density (OD) was determined using spectrophotometry.

Data were analyzed by one-way Analysis of Variance (ANOVA) and Tukey's post hoc test using SPSS 16 software and Microsoft Office Excel 2013. Data were presented as mean±standard deviation (SD).

Findings

The prevalence of osteoporosis was 17.3%, osteopenia 41.8%, and healthy 40.9%. The mean of the studied variables in different groups is shown in Table 1.

There was no significant difference between the healthy, osteopenia, and osteoporosis groups in terms of mean age ($p>0.05$).

Significant differences were observed between different groups in terms of BMI so BMI in the healthy group was significantly higher than the osteopenia ($p=0.002$) and osteoporosis ($p=0.007$) groups, but no significant difference was observed between osteopenia and osteoporosis groups ($p=0.80$).

There was no significant difference in vitamin D and VDR levels between the study groups ($p>0.05$).

DBP levels showed a significant difference between groups; DBP level in the healthy group was significantly higher than osteoporosis group ($p=0.005$), as well as DBP level in osteopenia was significantly higher than in osteoporosis ($p=0.001$), but there was no significant difference between healthy and osteopenia groups ($p=0.53$).

Spin BMD in each group of osteopenia and osteoporosis showed a significant decrease compared to the healthy group ($p<0.001$). Also, the osteopenia group showed a very significant increase compared to the osteoporosis group ($p<0.001$).

In addition, the differences in T-scores between the healthy groups, osteopenia, and osteoporosis groups were very significant in comparison with each other ($p<0.001$).

Table 1) The mean of the studied variables in different groups

Variables	Healthy	Osteopenia	Osteoporosis
Age (years)	59.72 5.62	59.81±6.26	61.56±4.30
BMI (kg/m^2)	34.40±7.59	29.45 ±5.75	28.95 ±5.49
Vitamin D (ng/ml)	24.23±3.05	22.94±3.20	22.31±2.39
DBP (µg/ml)	0.41±0.03	0.43±0.15	0.28±0.06
VDR (ng/ml)	1.00±0.37	1.03±0.30	0.87±0.25
Spin BMD (g/cm^2)	1.08±0.10	0.89±0.10	0.76±0.10
Spin T. score	0.32±0.73	-1.32±0.58	-2.88±0.52

BMI: Body Mass Index, BMD: Bone Mineral Density, DBP: Vitamin D Binding Protein, VDR: Vitamin D Receptor

Discussion

This study aimed to compare the levels of vitamin D, BMD, DBP, VDR, and other variables related to bone-building in women with osteoporosis and osteopenia and healthy women.

There was no significant difference between the healthy, osteopenia, and osteoporosis groups in terms of mean age. Age is considered a risk factor for osteoporosis, elderly people are at higher risk for developing osteoporosis especially women [12]. Tanaka *et al*. found in their study that age is significantly higher in patients with osteoporosis and this disease is more prevalent in women [13]. Zanker *et al*. reported that the contentious reduction in the bone mass with age is because of alterations in cell allocation. Bone formation and bone resorption processes are performed simultaneously and are well coordinated under the age of thirteen to reach the beak bone mass, after which bone mass begins to decline by an average of 0.5% per year [14].

The results show that subjects with high BMI have a lower risk of developing osteoporosis than those with low BMI, which is consistent with some previous studies [15]. This can be explained by the favorable effects of greater mechanical loading on bone and increased estradiol levels due to enhanced conversion of androgen precursors to estrogen in a larger volume of adipose tissue, which is thought to be due to higher BMD in overweight/obese patients. Xu *et al*. reported that a higher BMI may stimulate new bone formation and limit bone loss, this is considered an effective measure to capture the weight-bearing element of a bigger load on the skeleton. On the other hand, being overweight can alter BMD by affecting the release of hormones from adipose tissue, and estrogen is involved in bone formation [16].

There was no significant difference in vitamin D between the study groups. Interestingly, the results of the present study are consistent with Kuchuk *et al*. who studied vitamin D in patients with osteoporosis; their results showed a high prevalence of low serum 25(OH)D in women with postmenopausal osteoporosis [17]. Osteoporosis is a well-known fact among postmenopausal women, and several risk factors, including vitamin D deficiency, are associated with this high prevalence. Holick *et al*. reported that more than 60% of postmenopausal women have a level of 25(OH)D deficiency, including the population of sunny countries [18]. Cauley *et al.* conducted a 7.1-year case-control study in which blood 25(OH)D levels were assessed in fractured patients and compared with controls. They discovered that serum 25(OH)D was lower in patients and also found that vitamin D deficiency was associated with a higher risk of fractures. This increased risk of fracture was not only independent of the frequency of falls, but also independent of physical function, frailty, renal function, and secondary steroid hormone levels, and was partially mediated by increased bone resorption. As a result, serum 25(OH)D levels of 20 ng/ml are associated with an increased risk of hip fracture [19].

DBP level in the healthy group was significantly higher than osteoporosis group, as well as DBP level in osteopenia was significantly higher than osteoporosis, but there was no significant difference between the healthy and osteopenia groups.

Additional actions attributed to DBP include potential direct actions on bone resorption [20]. Early data points to associations between differences in DBP levels and bone density [18]. Jorde *et al*. reported that the total 25(OH)D measured in serum is comprised of DBP and albumin-bound to 25(OH)D as well as the free form. The overall concentration of 25(OH)D is affected by the serum concentration of DBP because the majority of 25(OH)D is bound to DBP. Vitamin D binding protein levels remain relatively constant throughout life, however, increase during pregnancy and estrogen supplementation. Low serum DBP levels can also be caused by a loss of protein in the urine (as seen in some diabetics) [21]. In a study conducted by Martínez-Aguilar *et al*. about using serum DBP as a new biomarker to diagnose osteoporosis and fracture risk in postmenopausal women, they showed that DBP levels in patients with osteoporosis were significantly lower than in normal individuals, which agrees with the results of the present study. They also concluded that DBP could be used as a diagnostic biomarker for osteoporosis [22].

There was no significant difference in VDR levels between the study groups. The presence of VDR in bone cells shows that it has direct effects on bone. These receptors are found in osteoblasts as well as immature osteoclast precursor cells. It has previously been thought that the effect of active metabolites of vitamin D on osteoclasts is indirect through osteoblasts [23]. Furthermore, Lim *et al*. mentioned that VDR is essential for bone turnover, BMD, and calcium homeostasis [24]. Ebeling *et al*. reported that VDR concentration in the intestine decreases with age, leading to intestinal resistance to 1,25 (OH) 2D, which leads to decreased calcium absorption and increased bone resorption and bone loss [23].

The results of this study showed that the differences between groups were very significant in terms of spin BMD and T-score.

The National Health and Nutrition Examination, Survey III (NHANES III) found a link between BMD and 25(OH)D in 13,432 participants, including whites, Hispanics, and blacks [25]. Another worldwide investigation on the link between vitamin D level and BMD in postmenopausal women with osteoporosis found a significant positive relationship between BMD and 25(OH)D with a threshold of 50 nmol/l. Chapuy *et al*. reported that vitamin D has an essential and complementary function in musculoskeletal health, therefore appropriate levels of vitamin D are considered the first step in osteoporosis treatment, also vitamin D supplementation may help prevent falls and fractures [26, 27]. In their study on the influence of vitamin D on osteoporosis, Palmieri *et al*. reported that vitamin D plays a major role in the stimulation of bone matrix synthesis and bone maturation, but may also increase the activity of osteoclasts and affect bone cell differentiation [28]. However, as a result, vitamin D and its metabolites are implicated in the process of osteoporosis. As reported by Sunyecz *et al*., vitamin D deficiency reduces the absorption of calcium in the intestine and stimulates osteoclasts to break down bone tissue and release calcium and other minerals into the bloodstream which increases the risk of fractures and reduces BMD [29]. Ji & Yu *et al*. reported that the average decline in BMD during the menopausal transition

period is roughly 10%. About half of women lose bone faster, perhaps as much as 10%-20% in the 5-6 years leading up to menopause. Fast bone losers account for about 25% of postmenopausal women and can be identified by measuring bone loss and resorption indicators [30].

The prevalence of osteoporosis was 17.3%, osteopenia 41.8%, and healthy 40.9%. These results are supported by another study in turkey by Demir *et al.*, which showed that the prevalence of osteoporosis in postmenopausal women is 16% [31]. A study reported that the prevalence of osteoporosis was 37% and osteopenia was 44% [32]. In Saudi Arabia, the study by Sadat *et al*. showed that the prevalence was 44% [32]. The most common osteoporotic fractures due to the high concentration of trabecular bone structure affect the thoracic and lumbar vertebrae, pelvis, and wrist. These fractures are significantly more common in women than men. This disease is one of the primary causes of morbidity and complications among the elderly. Early detection and prevention of osteoporosis should be given the highest priority when considering the high costs of osteoporosis treatment and its consequences [33].

Conclusion

Comparison of levels of vitamin D, vitamin D Receptor (VDR), and vitamin D-Binding Protein (DBP) among women with osteoporosis and osteopenia, despite differences between the two groups in Bone Mineral Density (BMD), does not show a significant difference.

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