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The relation of bone mineral density with the serum levels of 25 hydroxy-vitamin D in premenopausal and postmenopausal individuals: vitamin D and bone health

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Abstract

The frequency of osteoporosis increases by aging. Although the role of vitamin D on bone metabolism is well-known, the results of the trials investigating the relation of vitamin D with the severity of osteoporosis and osteopenia are contradictory. Our aim is to analyze the relation of the serum level of vitamin D with densitometric T and Z scores in pre- and postmenopausal women. A total of 267 participants that are composed of 86 healthy (41 premenopausal, 45 postmenopausal), 90 osteopenic (40 premenopausal, 50 postmenopausal), 91 osteoporotic (35 premenopausal, 56 postmenopausal) individuals were included in the study. Patients under the age of eighteen and over eighty years, as well as those with medical data of additional comorbidities (congestive heart failure, hepatic insufficiency, thyroid disease and chronic kidney disease, primary and secondary hyperparathyroidism) were excluded from the study. Vitamin D, calcium, phosphorus, parathormone, alkaline phosphatase levels were recorded. Participants underwent to bone densitometry examination in Bagcilar Education and Research Hospital. Our study was conducted on women and they were divided into groups as osteoporotic, osteopenic and healthy individuals, and grouped as premenopausal and postmenopausal among themselves. The mean vitamin D level of postmenopausal healthy group was significantly higher than that of premenopausal healthy group. Premenopausal patients with osteoporosis had higher mean ALP levels than premenopausal osteopenic group. We failed to determine an association between vitamin D level and T and Z scores.

Administration of vitamin D replacement is excessively common among healthy postmenopausal women, independent from bone densitometry examination. Vitamin D level may not always reflect the exact status of bone densitometry.

KeyWords: Osteoporosis, osteopenia, vitamin D, bone densitometry

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Introduction

Osteoporosis is a systemic bone disorder which is defined as loss of bone mass and deterioration of bone microarchitecture, eventually leads to increased risk of fracture (l). As the mean lifespan globally increases, osteoporosis become more prevalent and turns into a public health burden. Osteoporosis has a silent period varying from months to years before trabecular bone loss become evident. The risk factors of osteoporosis are well defined and more frequent in female individuals. Although osteoporosis is more common among postmenopausal women, premenopausal individuals without an additional risk factor are under enhanced risk of bone disorders, compared to males (2,3).

Bone mineral densitometry (BMD) is an easy and reliable way of assessing bone mineral metabolism. BMD can noninvasively assess the density of bone from lumbar spine or femoral neck by using dual-energy X-ray absorptiometry (DXA) (4). BMD works as the quantitative calculation of the X-ray energy absorbed by bones which is turned to areal density and measured in g/cm^2 (5). Decreased density of bone mineral is the major determinant of osteoporosis and osteopenia. Female individuals reach their peak bone density in the fourth decade of life, which gradually decreases in the following decades. While T score is used for assessing the bone metabolism in postmenopausal women and males aged >50 years, Z score is used for determining osteoporosis and osteopenia in premenopausal women and males younger than 50 years (6).

Vitamin D which is produced in epidermal cells by the stimulation of ultraviolet-B rays, has important biological actions. The biologically active form of the vitamin D is 1-25 dihydroxycholecalciferol, also known as calcitriol. However, due to its short half-life, the level of 25-hydroxy (OH) vitamin D (with a half life of 3 weeks and 1000 times higher serum level), is a better way of assessing the activity of the vitamin D.(7) .

The impact of the serum 25-OH vitamin D on bone metabolism is well-studied. We aimed to analyze the correlation of the serum levels of 25-OH-D with densitometric T and Z score in the post and pre-menopausal women.

Methods

A total of 267 individuals that admitted to the Internal Medicine outpatient service of Bagcilar Education and Research Hospital, Istanbul between 1 April 2017 and 15 April 2020, and underwent to bone mineral densitometry were enrolled to the study. After examining the medical recordings of the participants, the groups were adjusted according to BMD values and classified into 3 groups; 86 participants with normal BMD (41 premenopausal, 45 postmenopausal) formed the healthy group, 90 participants were in osteopenic group (40 premenopausal, 50 postmenopausal), and 91 participants were in osteoporotic group (35 premenopausal,56 postmenopausal). The patients with chronic drug use, BMI< 18.5 kg/m^2, hypertension, ischemic heart disease, stroke, thyroidal dysfunction, autoimmune disorders, chronic liver disease, chronic kidney disease, primary or secondary hyperparathyroidism, Paget disease, collagen tissue disease and

smoking habit were excluded. The study was approved by Ethics committee of Bagcilar Education and Research Hospital (Ethics committee approval date and number are 29.05.2020 and 2020.05.2.11.067).

Written informed consent was acquired from all subjects participating in this study, according to the declaration of Helsinki.

Blood samples were collected from antecubital vein after 8-hour fasting period and centrifugated for 5 minutes at 2000 turn before analysis. Biochemical analysis including glucose, urea creatinine, uric acid, aspartate transaminase (AST), alanine transaminase (ALT), lactate dehydrogenase (LDH), calcium, sodium, potassium, total protein, albumin, total cholesterol, low density lipoprotein (LDL) cholesterol, high density lipoprotein (LDL) cholesterol and triglyceride measurements was performed by using photometric method in Siemens Advia 1800 device. MAPSS laser technique and LED Flow cell technology was used for hemogram analysis (Cell Dyn Sapphire Abbott Laboratories, Abbott Park, Illinois USA). Hemoglobin A1c (HbA1c) level was measured via Adams A1c HA-8180 analyzer by using High Performance Liquid Chromatography (HPLC) method.

Serum levels of 25-OH-vitamin D, thyroid stimulating hormone (TSH) and parathormone (PTH) were analyzed by using commercially available kits (Roche diagnostic test kits) in the Roche diagnostic company Cobas 8000 autoanalyzer (Basel, Switzerland). Eclia (ElectroChemiLuminescence) method was used for the analysis of hormonal parameters. Bone densitometry examination was performed in the Nuclear Medicine Department of Bagcilar Education and Research Hospital, Istanbul by using DMS (2016) stratos DR model Densitometry device (France). Osteopenia and osteoporosis was defined as BMD T score -1 to -2.5 and below -2.5, respectively (8).

Statistical methods

Data were analyzed using the NCSS (Number Cruncher Statistical System) 2007 Statistical Software (Utah, USA). All data were expressed as mean ± SD. Prior to the study, power analysis (G Power 3.1 programme) was performed to determine the minimum sample size, and reached a result of 93 participants. The differences between the study groups were analyzed by using Student's t-test. Quantitative data were analyzed by McNemar's test and chi-square test. Pearson correlation analysis and Spearman's rho correlation analysis were used to evaluate the correlation of variables. Dunn's multiple comparison test was used for subgroup comparison. The variables significant in univariate analysis were included in multivariate logistic regression analysis (only significant correlation coefficients are reported). A p-value of < 0.05 was considered to be statistically significant.

Results

Regarding to the levels of biochemical and hormonal variables of the premenopausal group; all participants had similar calcium, phosphorus, PTH and vitamin D levels with

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any BMD level. But osteoporotic group had significantly higher Alkaline Phosphatase (ALP) levels compared to other 2 groups (p:0.039) (Table-1).

Table-1: The comparison of biochemical, hormonal and bone densitometric parameters of the premenopausal individuals

Premenopausal	Normal BMD group n:41	Osteopenic group n:40	Osteoporotic group n:35	p
Age (years)	44,9±4,27	41,93±7,82	40,6±9,44	**0,035**†
Calcium (mg/dl)	9,65±0,53	9,59±0,43	9,68±0,73	0,789†
Phosphorus (mg/dl)	3,39±0,5	3,22±0,48	3,41±0,51	0,178†
Parathormon (pg/mL)	58,83±28,97	50,13±28,63	57,17±40,43	0,273‡
Vitamin D (ng/ml)	18,27±9,21	19,93±8,95	22,71±12,44	0,150‡
Alkaline Phosphatase (U/L)	74,83±30,99	69,15±20,59	84,34±25,68	**0,039**‡
T Score	-0,18±0,85	-1,83±0,38	-3,15±0,51	**0,0001**‡
Z Score	0,23±0,87	-1,49±0,48	-2,82±0,51	**0,0001**‡

†One-way variant analysis‡Kruskal Wallis test
BMD: Bone Mineral Density

The comparison of age, calcium, phosphorus, PTH, vitamin D and ALP levels were similar between osteoporotic, osteopenic and normal BMD subgroups in the postmenopausal group. As expected, T and Z scores of osteoporotic group was lower than those of other 2 groups (p:0.0001 and p: 0,0001; respectively) (Table-2).

Table-2: The comparison of biochemical, hormonal and bone densitometric parameters of the postmenopausal individuals

Postmenapozal	Normal BMD group n:45	Osteopenic group n:50	Osteoporotic group n:56	p
Age (years)	61,87±8,41	63,84±8,29	64,41±8,98	0,314†
Calcium (mg/dl)	9,75±0,44	9,64±0,41	9,73±0,54	0,453†
Phosphorus (mg/dl)	3,51±0,50	3,43±0,57	3,41±0,60	0,664†
Parathormon (pg/mL)	55,02±28,73	52,66±22,66	61,68±45,87	0,976‡
Vitamin D (ng/ml)	26,56±17,65	23,58±9,17	25,56±15,49	0,902‡
Alkalen Phosphatase (U/L)	81,04±26,12	72,5±16,56	76,59±26,94	0,380‡
T Score	0,09±0,98	-1,89±0,36	-3,18±0,57	**0,0001**‡
Z Score	1,63±1,22	-0,24±0,63	-1,52±0,62	**0,0001**‡

†One-way variant analysis‡Kruskal Wallis test
BMD: Bone Mineral Density

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In the correlation analysis, we failed to determine a significant relation between T and Z scores and serum levels of calcium, phosphorus, and PTH in both the postmenopausal and the premenopausal group. However vitamin D level was inversely correlated to T and Z score in premenopausal individuals (r:-0.227 and r:-0.209; respectively and p:0.014 and p:0.025; respectively). Similarly the correlation of T score and ALP was significant in the same group (r:-0.191 and p:0.04) (Table-3).

Table-3: Correlation of Densitometric parameters with biochemical and hormonal variables of the postmenopausal and premenopausal individuals

		Premenopausal		**Postmenopausal**	
		T Score	**Z Score**	**T Score**	**Z Score**
	R	**0,185**	**0,353**	-0,075	**0,203**
Age (years)	**p**	**0,046**	**0,0001**	0,358	**0,012**
	R	0,019	0,031	0,013	0,037
Calcium (mg/dl)	**p**	0,837	0,743	0,871	0,656
	R	-0,068	-0,023	0,06	0,046
Phosphorus (mg/dl)	**p**	0,467	0,807	0,462	0,574
	R	0,027	0,058	-0,121	-0,094
Parathormon (pg/mL)	**p**	0,772	0,538	0,139	0,251
	R	**-0,227**	**-0,209**	-0,025	-0,012
Vitamin D (ng/ml)	**p**	**0,014**	**0,025**	0,764	0,883
	R	**-0,191**	-0,159	0,031	0,017
Alkaline Phosphatase (U/L)	**p**	**0,04**	0,089	0,704	0,834

Discussion

The present study showed that healthy postmenopausal women had higher vitamin D levels than healthy premenopausal counterparts, which reflects unnecessary administration of vitamin D therapy. Additionally, serum vitamin D level does not always correlate with T and Z scores which may cause confusion among physicians.

In a multicentral study on postmenopausal women by Lips et al, the mean serum vitamin D level was 28.3 ng/ml in a group of patients receiving osteoporosis treatment and healthy controls (9). Similarly, it was 26.8 ng/ml in another study by Rizzoli et al. (10). The mean serum vitamin D level (25.6 ng/ml) in our study was in accordance with the literature. Rizzoli R et al defined deficiency of vitamin D as less than 30 ng/ml which was observed in the 64% of study population, while 31% of the participants had vitamin D level < 20 ng/ml. Authors determined that insufficiency of 25(OH) D was highest among individuals from Middle East Asia (%81.8) and other parts of Asia (%71.4) (10).

On the otherhand, the complex interaction of these variables still requires further explanation. For example, it is expected to observe higher frequency of osteoporosis among women in Britain that significantly have lower exposure to sunshine compared to a Nigerian women. On the otherhand, Nigerian women may suffer from inadequacy of having a balanced diet which also has crucial importance on the development of osteoporosis. Our country is far from both poles which may have impact on the inconsistency of our results.

Negative effect of low serum 25(OH) D level on preserving bone microrchitecture has been observed. Nevertheless, no consensus has been established on the optimal vitamin level. American Medical Institue (Institute of Medicine, IOM) has accepted the level of 25 (OH) D $\geq$ 20 ng/ml as adequate (11). Whereas, American Endocrine Society, National Osteoporosis Foundation, International Osteoporosis Foundation and American Geriatric Society concluded that 25 (OH) D > 30 ng/ml is necessary to decrease the risk of falls and fracture (12-15).

Lips et al determined that 400 IU of vitamin D was ineffective to reduce hip fracture risk in Dutch women in 1996 (16). In 1995, Dawson -Hughes et al failed to observe adequate protection against bone loss by 200 IU/day vitamin D supplementation in healthy postmenopausal women (17). As a result, it is reasonable to state that 400-800 IU/day vitamin D with adequate calcium intake is essential to preserve bone formation (18). On the contrary, osteoporotic group was receiving vitamin D therapy without calcium supplementation which is the possible explanation of our results. Moreover, some osteoporotic patients with high vitamin D levels (>30 ng/ml) were still receiving vitamin D therapy.

Similar to our results, White et al failed to determine a significant association between bone densitometry score and vitamin D levels in a South African population (19). On the contrary, Pekinnen et al observed a relation between vitamin D level and bone densitometry measurements in school-age children, and emphasized that vitamin D level is the major determinant of bone densitometry (20). Inconsistent with both studies, Zhou et al concluded that the effect of vitamin D is modified by dietary factors, especially by fat intake in a study on 424 Chinese individuals (21). Another study from Turkey which was conducted in postmenopausal individuals determined that low body mass index (BMI) is associated with low BMD (22).

There are some limitations of our study. Because of retrospective design of the study, we could not get the information about the frequency of sunshine exposure and dietary habits. Additionally, we do not exactly know body mass index values of the participants of our study which has a significant impact on bone metabolism. The number of cases in our

study was relatively low, depending on our strict exclusion criteria. Many patients were excluded from the study due to irregular follow-ups or comorbidities such as hypertension, heart failure, autoimmune disorder, chronic renal or liver disease, thyroidal dysfunction and prolactinoma. The study population was Turkish citizens from Bagcilar region of the European side of Istanbul with similar socioeconomic status and nutritional habits which may provide relative homogeneity. However, it may not be appropriate to generalize the results of our study for other populations since there may be effects of environmental factors such as diet or lifestyle as well as genetic factors. On the other hand, it is almost impossible to equalize the sun exposure of the study population. A prospective and randomized controlled study considering the dietary habits and physical activities of participants may help to produce more meaningful results.

Conclusion

In conclusion, screening the skeletal health of pre- and postmenopausal individuals is of vital importance to diminish morbidity and mortality rates. However, rather than replacing it for every postmenopausal women, vitamin D supplementation should be individualized for women at moderate or high risk of osteoporosis, and should be accompanied by calcium and phosphorus supplementation when necessary. Better understanding of the pathophysiology of the disorder may help to diminish the rate of complications. Further large scaled studies with longer follow-up are warranted to reach more precise conclusion.

Conflict of interest

The authors have no conflict of interest in this paper.

Financial disclosure

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