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Original article

Vitamin D status and its relationship with bone mineral density in a healthy Iranian population

Patricia Khashayar[a,b,*], *Hamid Reza Aghaei Meybodi*[a], *Mohsen Rezai Hemami*[c], *Abbasali Keshtkar*[d,e], *Hans Peter Dimai*[f], *Bagher Larijani*[c]

[a] *Osteoporosis Research Center, Endocrinology and Metabolism Clinical Sciences Institute, Tehran University of Medical Sciences, Tehran, Iran*

[b] *Center for Microsystems Technology (CMST), Ghent University, Ghent, Belgium*

[c] *Endocrinology and Metabolism Research Center, Endocrinology and Metabolism Clinical Sciences Institute, Tehran University of Medical Sciences, Tehran, Iran*

[d] *Department of Health, The Ministry of Health and Medical Education (MOHME), Tehran, Iran*

[e] *Department of Nutrition, The Ministry of Health and Medical Education (MOHME), Tehran, Iran*

[f] *Department of Internal Medicine, Division of Endocrinology and Metabolism, Medical University of Graz, Graz, Austria*

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ABSTRACT

Objectives: Considering the controversial results regarding the relationship between vitamin D levels and bone mineral density in different populations, the present study was designed to evaluate this correlation in a healthy Iranian population.

Methods: Using a random cluster sample of apparently healthy men and women, this multicenter cross-sectional study was carried out among 4450 individuals living in urban areas of five major cities in Iran. Bone mineral density (BMD) values at different sites were analyzed along with the serum levels of 25(OH)D and PTH. Analysis of variance (ANOVA) was used to estimate the main effects, through comparing the mean values of these markers based on the bone mineral density status of the study group in each sex.

Results: 25(OH)D levels were inversely correlated with BMD values at total hip ($r = -0.062$ in men and $r = -0.057$ in women) and spine ($r = -0.076$ in men and $r = -0.107$ in women). After adjusting the data for age, the inverse correlation was no longer statistically significant.

Conclusion: Serum 25(OH)D levels are inversely correlated with bone mass values in both sexes.

Correlação entre os níveis de vitamina D e densidade mineral óssea em uma população iraniana saudável

RESUMO

Objetivos: Considerando os resultados controversos sobre a relação entre níveis de vitamina D e densidade mineral óssea em diferentes populações, o presente estudo foi desenhado para avaliar esta correlação em uma população iraniana saudável.

Palavras chave:
Densidade mineral óssea
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* *Corresponding author.*
E-mail: patricia.kh@gmail.com (P. Khashayar).

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Biomarcadores
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Métodos: Usando uma amostra aleatória de homens e mulheres aparentemente saudáveis, este estudo transversal multicêntrico considerou 4450 indivíduos que vivem em na região urbana de cinco grandes cidades no Irã. Os valores da densidade mineral óssea (DMO) foram analisados em conjunto com os níveis séricos de 25(OH)D e PTH. Análise da variação (ANOVA) foi utilizada para estimar os principais efeitos através da comparação entre os valores médios destes marcadores e a condição da densidade mineral óssea de cada gênero nesta amostra de estudo.

Resultados: Níveis de 25(OH)D foram inversamente proporcionais aos valores de DMO a nível do quadril (r = -0.062 em homens e r = -0.057 em mulheres) e coluna vertebral (r = -0.076 em homens e r = -0.107 em mulheres). Após ajuste dos dados para idade, a correlação negativa não foi mais estatisticamente significante.

Conclusão: Níveis séricos de 25(OH)D são inversamente correlacionados com os valores de massa óssea em ambos os gêneros.

Introduction

Osteoporosis is a systemic disease characterized by low bone mass and microarchitectural deterioration of bone tissue, resulting in an increased risk of fracture; if identified early in its course, however, many of the fractures can be prevented.[1]

Apart from modifiable lifestyle factors, bone mineral density (BMD) is influenced by several genetic, environmental, and hormonal factors.[2,3] While the influence of biochemical markers on the fracture risk has been well documented in previous studies, the association between serum 25(OH)D levels and BMD in different ethnicities residing in different geographic areas remains controversial. Many of them have reported no direct relationship between serum 25(OH)D levels and BMD was observed.[4]

The present study therefore was designed to evaluate the relationship between vitamin D status, bone mineral density and PTH in a healthy Iranian population.

Materials and methods

Subject selection

This study is part of a comprehensive survey (IMOS) assessing the prevalence of osteoporosis and related factors among healthy adults (age range: 20–70 Years), representative sample of Iranian population living in urban areas, in the urban areas of five major cities of Iran (Tehran, Tabriz, Mashhad, Shiraz and Booshehr) in late winter 2001 (February–March).

Details on the survey design and methods have been reported previously.[5] Briefly, the IMOS used a random cluster sampling design to draw five provincially representative, independent samples of healthy adults excluding those taking medications that could modify bone metabolism, those with hepatic or renal disorders, metabolic bone disease, hypercortisolism, malabsorption, sterility, oligomenorrhea, diabetes, malignancy, and immobility for more than 1 week as well as the pregnant and lactating women. The Research Ethics Committee of the Endocrine and Metabolism Research Center (EMRC) approved the protocol of this study. An informed consent was obtained from the subjects before they entered the study.

All subjects underwent a detailed medical examination, measurement of bone mineral density at different sites, and certain biochemical testing. Apart from demographic data, the subjects were asked about their menopausal status and the years passed since their menopause. Menopause, in this study, was defined as previous natural or surgical cessation of menstruation for more than 12 months.

Biochemical tests

A fasting blood sample (10 cm^3 of venous blood) was taken from all participants at their residence place. Sample centrifuge and serum extraction were done in the field. The samples were then frozen and sent to the EMRC laboratory for further analysis.

Serum Ca and P levels were analyzed by a calorimetric method using Chem. Enzyme Lab Kit; Iran. The normal laboratory range for serum Ca was 8.6–10.8 mg/dl and for serum P was 2.3 to 5 mg/dl. Serum levels of vitamin D (25 (OH) D) and PTH were measured with RIA (Radio-Immuno-Assay) method (IDS Ltd Kit; UK) and IRMA (Immuno-Radiometric) method (Diasorin Kit; USA), respectively. Normal range for serum 25(OH)D and PTH were 23–113 ng/ml and 13–54 pg/ml, correspondingly. The inter- and intra-assay variations for the markers were 8%/6.8% and 8.9%/6.1%, respectively.

Based on 25(OH)D values, subjects were classified as those suffering from vitamin D deficiency (≤20 ng/ml), – insufficiency (mild deficiency) (20–30 ng/ml) and – sufficiency (higher than 30 ng/ml). The complete method used to determine the 25(OH)D levels for classifying the participants is described in our previous studies.[6,7]

Bone mineral density

In each city, patients underwent an L1–L4 anteroposterior lumbar spine, hip and its sub-regions DXA study with a Lunar DPXMD densitometer (Lunar 7164, GE, Madison, WI) equipped with NHANES III dataset by a trained operator according to the manufacturer's instruction. Results were expressed as *T*- and

Z-scores. Quality control procedures were carried out in accordance with the manufacturer's recommendations. In each city, the instrument variation was determined regularly by a weekly calibration procedure using a phantom supplied by the manufacturer (the phantom equilibrium was sent from one city to another after each testing). The interdevice variance was checked several times during the study period. There was an irrelevant small difference between the reported measures which was negligible. Precision error for BMD measurements was 1–1.5% in the lumbar and 2–3% in the femoral regions.

Based on World Health Organization Study Group recommendation, BMD values were classified as normal, osteopenic and osteoporotic.[8] Since, there is no widely accepted definition of osteoporosis in males, the WHO criteria for testing osteoporosis in the female population was similarly used for this group.

Statistical analysis

Data were entered to Microsoft Access Databank. In view of the fact that a different instrument was used to assess the BMD in subjects who were recruited in Booshehr; data gathered in this city was assumed to be missing. All statistical analyses were performed with SPSS 13.0 for Windows (SPSS, Chicago, IL) based on a pair-wise approach, and *P* values lower than 0.05 were considered statistically significant.

All analyses were conducted separately for each gender, and then for the group overall. Means ± SD were used to express standard descriptive statistics. Categorical variables were expressed as percentages and compared using Chi-square. Differences among means were investigated by analysis of variance (ANOVA) with post hoc test. The two-sided Student's *t*-test was used to compare the mean biochemical values in different groups. The association between the outcome variable (BMD) and the biochemical variables was examined by bivariate analysis and then by adjusted stepwise multiple regression analyses.

Results

A total of 4450 individuals with the mean age of 42.6 ± 13.9 years were studied; from among them 1900 (42.7%) were male. Overally some 246 (6.5%) of the total studied subjects (4.8% of males and 7.7% of females) were reported to have osteoporosis. There was a significant difference between the frequencies of osteoporosis among the two genders (*P*-value < 0.001). Osteopenia, on the other hand, was reported among 848 (58.6%) of the studied females and some 600 (41.4%) of the studied men.

Serum levels of 25(OH)D was significantly higher among those suffering from osteoporosis (Table 1). The Pearson's correlation coefficients between the study variables revealed an inverse correlation between 25(OH)D levels and BMD values at total hip ($r = -0.062$ in men and $r = -0.057$ in women) and spine ($r = -0.076$ in men and $r = -0.107$ in women) (Table 2).

The correlation between PTH levels and BMD values at all sites failed to remain significant after adjusting data for gender. Each year increase in age was associated with 1.061 and 1.141 higher risk of developing osteoporosis in males and females, respectively. After adjusting the data for age, the inverse correlation between age, and 25(OH)D levels and BMD values at all sites was no longer statistically significant (data not shown).

Discussion

Hypovitaminosis D and osteoporosis is frequent in individuals living in countries with abundant sunshine such as Iran.[9–12] The reason behind this finding, however, remains unclear. Many believe the optimal level of 25(OH)D, which leads to maximum suppression of circulating iPTH levels, should be defined based on functional rather than an epidemiological definition.[13]

Considering the correlation between serum 25(OH)D levels and BMD values controversial results are found. While certain studies have failed to report any correlations between these two variables, others have revealed a positive association between serum 25(OH)D levels and BMD values.[4,14–18] Arya et al. reported a significant correlation between serum 25(OH)D levels and BMD values at proximal femur.[11] They concluded that subclinical 25(OH)D deficiency has an adverse effect on bone mass and therefore is linked with low BMD in these subjects. Villareal et al. similarly suggested that women with low serum 25(OH)D levels should be referred for osteoporosis screening, stressing that low BMD could be the only symptom in these women.[19]

On the contrary with these studies, the present research demonstrated a significant but negative relation between 25(OH)D levels and BMD values at all the studied sites.

Apparent discrepancies between our study and previous ones can be contributed to the fact that many of these population-based studies have recruited subjects with a relatively good health status, and therefore lower prevalence of severe vitamin D deficiency and osteoporosis.[14] The probable association between 25(OH)D and BMD may also vary based on the various sites used for densitometry measurement owing to different composition of trabecular and cortical bone tissue. Moreover, sampling from a population-based study, and comparatively lower prevalence of severe vitamin D deficiency might increase the generalizability of our results. In addition, the higher serum levels of 25(OH)D in osteoporotic patients could be secondary to the fact that osteoporosis is more common among the elderly and this age group are more frequently treated with Vitamin D supplements. Considering our exclusion criteria, individuals taking such supplements should not have been recruited in the present study; this comes while many people, particularly, older individuals, have received the supplements without being aware of their name.

The mean serum 25(OH)D concentration of the whole study population was 35.37 ± 30.3 ng/ml and some 67.2% of the studied subjects were reported to have moderate to severe vitamin D deficiency regardless of their gender. Except for trochanter, there was no difference in the correlation between these variables in the two genders. It should be noted that the higher vitamin D levels reported in osteoporotic cases in this study could be contributed to the lower number of studied osteoporotic cases compared to that of normal cases. Moreover, considering the subgroup analysis and the fact that the retired

Table 1 – The mean values of the studied biochemical markers based on bone mineral density categorization.

	Bone mineral density categorization (no. of cases)			P value
	Osteoporosis (246)	Osteopenia (1448)	Normal (2096)	
25(OH)D (ng/ml)	42.03 ± 34.59	35.97 ± 26.49	33.04 ± 23.78	<0.001
PTH (pg/ml)	28.12 ± 16.19	28.82 ± 16.18	28.73 ± 17.72	0.856
Ca (mg/dl)	9.44 ± 0.54	9.43 ± 0.56	9.46 ± 0.57	0.454
P (mg/dl)	3.22 ± 0.49	3.15 ± 0.48	3.19 ± 0.47	0.109

Data expressed as mean ± SD.

Table 2 – The partial correlation between biochemical markers and BMD values after controlling for gender (A – male and B – female).

	25(OH)D	PTH	Ca	P	BMD (femoral neck)	BMD (trochanter)	BMD (total hip)
A - Male:							
PTH	−0.101[a]						
Ca	0.053[b]	−0.032[a]					
P	0.077[a]	0.088[a]	0.213[a]				
BMD (femoral neck)	−0.073[a]	0.023	0.070[a]	0.137[a]			
BMD (Trochanter)	0.000	−0.008	0.044	0.074[b]	0.770[a]		
BMD (total Hip)	−0.062[b]	0.013	0.064[b]	0.090[a]	0.876[a]	0.934[a]	
BMD (spine)	−0.076[a]	0.038	0.022	0.083[a]	0.633[a]	0.616[a]	0.666[a]
B - Female:							
PTH	−0.175[a]						
Ca	0.079[a]	−0.038					
P	0.075[a]	−0.072[a]	0.245[a]				
BMD (femoral neck)	−0.095[a]	−0.024	0.004	0.004			
BMD (Trochanter)	−0.074[a]	−0.046	−0.037	−0.054[b]	0.806[a]		
BMD (total Hip)	−0.057[b]	−0.042	−0.021	−0.034	0.889[a]	0.926[a]	
BMD (spine)	−0.107[a]	−0.009	−0.030	−0.053[b]	0.699[a]	0.624[a]	0.689[a]

[a] Correlation is significant at the 0.01 level (2-tailed).
[b] Correlation is significant at the 0.05 level (2-tailed).

and elderly population in Iran spends more time outdoors, it could be concluded that they are exposed to more sun and therefore have higher 25(OH)D levels.

Certain studies have revealed an inverse correlation between serum PTH levels and BMD values, particularly at the femoral neck, pointing out the catabolic role of PTH on cortical bones.[15,20] These studies have also shown that decreased levels of serum calcium are associated with defects in mineralization and consequently low BMD.[13] In line with these studies, Hosseinpanah et al. reported a negative correlation between PTH levels and BMD values at femoral neck in the absence of similar correlations between serum 25(OH)D and BMD of other sites.[21] Sadat Ali et al.[22] showed that vitamin D levels significantly influence BMD reading among Saudi individuals, pointing out a significant positive correlation between 25OHD level and BMD and significant negative correlation with parathyroid hormone in the studied groups. The present study, on the contrary, reported PTH to be inversely correlated with BMD values at all sites rather than spine. The correlation, however, was reported to be significant only at the femoral trochanter.

The present study was conducted on healthy individuals based on their self-reported history; a potential bias of undiagnosed underlying diseases, therefore, is probable. Additionally, the cross-sectional nature of the present study is an important limitation of this study. Moreover, this study only measured five biochemical markers, namely PTH and 25(OH)D, while more recent studies have linked markers such as intact osteocalcin (OC) to BMD values and fracture risk.[23,24] In addition, 25(OH)D measurements were performed in winter, when its levels are believed to be at the lowest level compared to other months of the year. It should be noted that the present study was an observational study in which the effect of important factors such as population differences (gender, age, ethnic, sex, extent of sun exposure, and vitamin D intake) was not assessed. Large prospective studies, therefore, are needed to better evaluate the correlation between biochemical markers and BMD values in different populations.

Conflict of interest

Dr. Dimai reports receiving payments for board membership, consultancy, expert's testimony, travels, meetings and lectures for his work on osteoporosis (Amgen, Daiichi-Sankyo, Eli Lilly, Kyphon, Medtronic, Merck Sharp & Dohme, Novartis, Nycomed, Sanofi-Aventis, Servier). His institution, the Medical University of Graz, also reports receiving grants for Dr. Dimai's work on osteoporosis (Amgen, Daiichi-Sankyo, Eli Lilly, Kyphon, Medtronic, Merck Sharp & Dohme, Novartis, Nycomed, Sanofi-Aventis, Servier).

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