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Normative Values of Vitamin D Among Iranian Population: A Population Based Study

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Abstract: There is no agreement about normal level of vitamin D and its deficiency stages. For finding normative value of Vitamin D and evaluating the state of vitamin D level in Iranian population we revised the data that was collected in Iranian national Multi-center Osteoporosis Study (IMOS). We chose 5 cities with different climates; individuals were selected by random cluster sampling. Healthy people aged 20-69 were entered into the study and serum vitamin D and PTH levels were measured. We stratified subjects based on their vitamin D levels in 7 groups and compared mean PTH levels of adjacent groups. We evaluated 5329 blood samples for vitamin D and PTH and found three steps of PTH elevation with decreasing vitamin D levels for women (40, 25 and 12 nmol L^{-1}) and two (35 and 25 nmol L^{-1}) for men. We use these values as cutoff levels for definition of normal, mild, moderate and severe vitamin D deficiency states. Based on these cutoffs, prevalence of all stages of vitamin D deficiency was unexpectedly high in all cities. Vitamin D deficiency state was seen in 75.1% of women and 72.1% of men. The high prevalence of vitamin D deficiency in Iran is similar to the results of other studies in Middle East area and indicates a need for a careful search for a determination of cause and need for regular fortification program.

Key words: Iran, normal serum vitamin D level, PTH, vitamin D deficiency, IMOS

INTRODUCTION

Vitamin D is an essential factor for normal metabolism of bone and bone minerals; it is further required in several non-bone related metabolic processes that encompass either Vitamin D Receptor (VDR) mediated or non VDR-mediated mechanisms (Brown *et al*., 1999). Vitamin D is supplied to the human body by foods as well as through the photosynthesis of vitamin D by Ultraviolet-B irradiation of skin (Holick *et al*., 1981; MacLaughlin *et al*., 1982; Holick and Vitamin, 2002).

Despite the high UV exposure in tropical countries, some evidence suggests a high prevalence of vitamin D deficiency in specific age and sex groups in these areas (Sedrani, 1984; Sedrani *et al*., 1983; Dawodu *et al*., 2003). Specifically, several reports from the Middle East indicate that vitamin D deficiency is highly prevalent in this area (Sedrani *et al*., 1983; Sedrani, 1984; Taha *et al*., 1984;

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Azizi *et al*., 2000; Alagol *et al*., 2000; Goswami *et al*., 2000; Dawodu *et al*., 2003). Therefore, elucidation of vitamin D status at the national level is of crucial importance for making any decisions about food fortification or screening programs.

Normal level of serum vitamin D is also a matter of debate (Lips, 2004). There are multiple levels suggested for normal vitamin D, mild moderate and severe vitamin D deficiency (Lips *et al*., 1988; Lips, 2004; Himmelstein *et al*., 1990; Chapuy *et al*., 1997; Thomas *et al*., 1998; Ooms *et al*., 1995; Chapuy *et al*., 1992). In this study, we chose a method for a definition of normal vitamin D and mild, moderate and severe vitamin D deficiency.

This study is a report of the Iranian national Multi-center Osteoporosis Study (IMOS) designed for investigation of bone mineral density and influencing factors on it in the normal Iranian population. The IMOS was performed cross-sectional in five cities of different climates and latitudes in Iran (i.e., Bushehr, Mashhad, Shiraz, Tabriz and Tehran).

Generally, the main aim of this study was defining normative values of Vitamin D level among apparently healthy Iranian population and then, based on these values finding the prevalence of Vitamin D deficiency states in this community.

MATERIALS AND METHODS

In this study, we chose five cities in the country with different climates and latitudes (i.e., Bushehr, Mashhad, Shiraz, Tabriz and Tehran). Individuals were selected using random cluster sampling by dividing cities into multiple foci based on distribution of population. Trained operators met citizens at their home in each focus. Blood samples of eligible persons, after filling an informed consent, were drawn and centrifuged within 30 min in their place of residence. Serum samples were transferred to the local participant laboratories and frozen and then sent to Endocrinology and Metabolism Research Center (EMRC) Laboratory, Tehran by mobile freezers. The sampling was performed in late winter (February and March 2001).

The study protocol was approved by the research ethics committee of EMRC, Medical ethics research center and ethics committee of the Iran ministry of health and medical education. All participants were also invited to have a Bone Mineral Density measurement in the local university of medicine in the same city. Information was collected in the Iranian Multi-center Osteoporosis Study database.

Subjects

Healthy men and women aged 20-69 years were eligible for enrolment. Exclusion criteria were chronic disease such as known hepatic or renal disease, metabolic bone disease, malabsorption, sterility, oligomenorrhea, type I diabetes, hypercortisolism, known malignancy, immobility for more than one-week, pregnancy, lactation, medications influencing bone metabolism, smoking more than 10 cigarettes per day, alcoholism; using medication affecting vitamin D and calcium metabolism and recent vitamin D use (oral in preceding 2 weeks and injection in preceding 6 month).

Measurements

Serum concentrations of 25-hydroxy-vitamin D (25(OH) D) were measured by Radioimmunoassay method (Biosource Europes.A, Ò). Serum intact PTH measurement was also done using RIA (Diasorin, Ò). Intra-assay and inter-assay coefficients of variation were 5.3 and 8.2%, respectively, for 25-OH-D and 2 and 4.6%, respectively for PTH.

Statistical Analysis

Based on the biologic relationship of vitamin D to PTH, we used a method for determination of health based normative values of serum vitamin D for our community. We know there are multiple adverse effects of chronically elevated levels of PTH on general and bone health. Based on the negative relationship between vitamin D and PTH, we assumed; in the normal population, that sufficient levels of vitamin D are associated with serum PTH levels within the normal range and with decreasing vitamin D, PTH level rises. We stratified serum levels of vitamin D to seven groups (<12.5, 12.5-24.9, 25-29.9, 30-34.9, 35-39.9, 40-44.9 and >45 nmole L^{-1}). PTH levels were analyzed as continuous quantitative variable. For analysis of data, we used one way ANOVA test. After assessment of normality of PTH distribution in each group, we transformed data for reaching normal distribution by square root extraction of PTH levels. Then by Scheffe method, we compared mean PTH levels in adjacent groups. Adjacent groups with no significant difference in PTH levels were pooled together and regarded homogeneous. Then PTH levels were compared between new homogeneous groups using linear contrast method. In decreasing fashion, the first significant difference between mean PTH levels was regarded as cutoff levels of mild vitamin D deficiency and second and third as cutoff levels of moderate and severe vitamin D deficiency, respectively.

In next step, based on these cutoff levels, we evaluated prevalence of mild, moderate and severe vitamin D deficiency in males and females of each city. For prevention of probable effect of different age distributions in subjects of each city, we entered weight of age groups of community in our modeling according to the last census of Iranian Statistical Center.

Extracted data were analyzed by SPSS for windows 11.5 software and STATA version 8/SE and significance level was 0.05 in all tests.

RESULTS

Finally, we evaluated 5329 blood samples for vitamin D and PTH levels. Table 1 is demonstrating mean levels of PTH in the initial vitamin D groups for each sex.

Figure 1-3 show mean value of PTH levels in different vitamin D subgroups in men, women and both sexes, respectively, homogeneous groups are presented with ellipses and cutoff levels are viewed by curved arrows. As shown in Fig. 1-3, we have found stepwise elevations in PTH levels with decreasing Serum vitamin D levels. There was 2 steps for PTH levels in male subjects at Vitamin D levels of 25 and 35 nmol L^{-1} and three steps of PTH elevation in female population at 12.5, 25 and 40 nmol L^{-1}. Median and inter quartile range of vitamin D were 26 and 24 in female and 30 and 20.1 in male population, respectively. These values for PTH were 24 and 18 in female and 23 and 16 in male population, respectively.

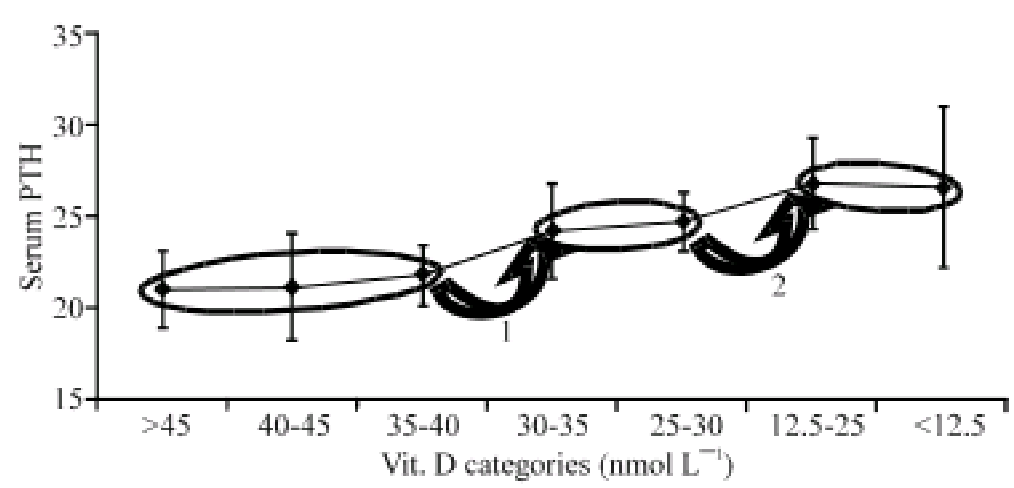

Fig. 1: Mean PTH levels in different vitamin D levels among Iranian men Ellipses: Homogeneous groups, Arrows: Significant differences, 1: Contrast value = 0.33, p<0.0001 (cutoff for mild vitamin D deficiency) 2: Contrast value = 0.22, p = 0.05 (cutoff for moderate or severe vitamin D deficiency)

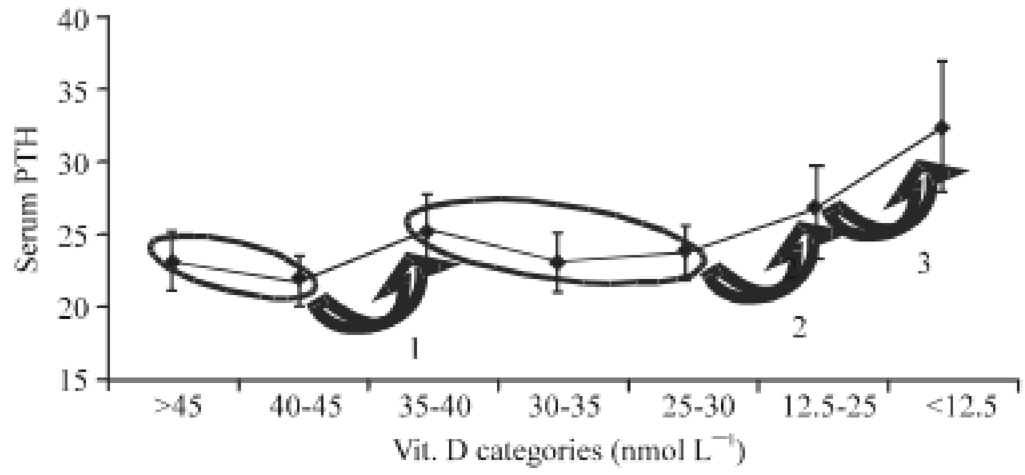

Fig. 2: Mean PTH levels in different vitamin D levels among Iranian women, Ellipses: Homogeneous groups, Arrows: Significant differences, 1: Contrast value = 0.17, p<0.05 (cutoff for mild vitamin D deficiency) 2: Contrast value = 0.26, p = 0.001 (cutoff for moderate vitamin D deficiency) 3: Contrast value = 0.50, p = 0.001 (cutoff for severe vitamin D deficiency)

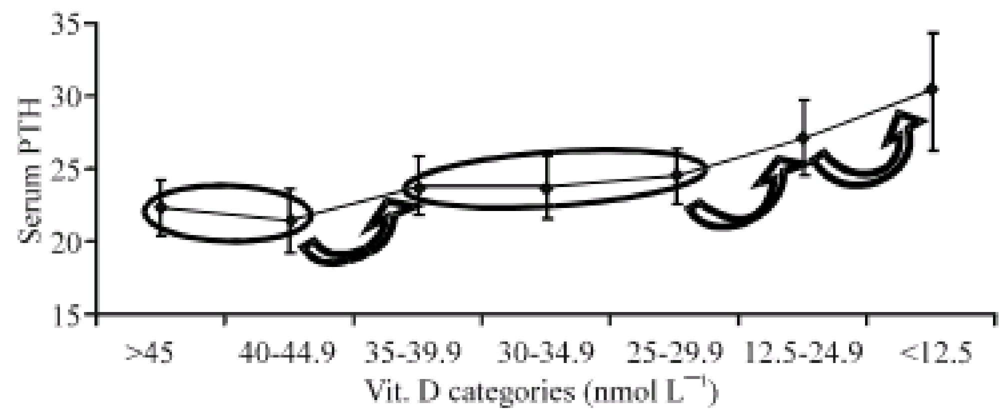

Fig. 3: Mean PTH levels in different vitamin D levels among all subjects, Ellipses: Homogeneous groups, Arrows: Significant differences, 1: Contrast value = 0.23, p<0.0001 (cutoff for mild vitamin D deficiency) 2: Contrast value = 0.31, p<0.0001 (cutoff for moderate vitamin D deficiency) 3: Contrast value = 0.30, p = 0.007 (cutoff for severe vitamin D deficiency)

Table 1: Mean and standard deviation of serum PTH levels in various vitamin D subgroups*

Vit. D categories (nmol L^{-1})	Sex Male	n	Female	n	Total	n
>= 45	20.99 (1.43)†	454	22.89 (1.40)	601	22.25 (1.39)	1055
40-44.9	21.10 (1.72)	145	21.60 (1.29)	109	21.42 (1.50)	254
35-39.9	21.77 (1.29)	195	25.08 (1.52)	166	23.80 (1.43)	361
30-34.9	24.15 (1.58)	238	22.88 (1.41)	214	23.66 (1.48)	452
25-29.9	24.68 (1.27)	294	23.52 (1.33)	299	24.52 (1.39)	593
12.5-24.9	26.75 (1.57)	667	26.46 (1.63)	1038	27.10 (1.61)	1705
<12.5	26.50 (2.08)	55	31.82 (2.11)	114	30.31 (2.00)	169

*: Distributions of PTH levels were not normal, so data were transformed (by extraction of square root of values); mean and standard deviation of PTH levels after retransformation is presented in this table, †: Numbers inside parentheses are the standard deviations

Using values of stepwise elevation of PTH as cutoff values for definition of vitamin D deficiency states (normal, mild, moderate and severe vitamin D deficiency) the prevalence of vitamin D deficiency was 75.1% in female population (mild: 27.2%, moderate: 42.8% and severe:5.1%) and 72.1 in males (mild: 41.1%, moderate and severe: 35.8%). Prevalence of Vitamin D deficiency states are shown in Table 2 and 3. The prevalence of vitamin D deficiency was compared between two sexes in every city. There was no significant deference in prevalence of mild vitamin D deficiency between two sexes in Tehran and Bushehr [(p = 0.212) and (p = 0.15), respectively]. In other cities the prevalence of mild

Table 2: Prevalence of vitamin D deficiency in females in each city

City	Mild Vit. D def. 25-39.9 nmol L^{-1} (CI 95%)	Moderate Vit. D def. 12.5-24.9 nmol L^{-1} (CI 95%)	Severe Vit. D def. <12.5 nmol L^{-1} (CI 95%)
Tehran	15.8 (12.2-19.4)	54.9 (49.9-59.9)	10.0 (6.9-13.1)
Tabriz	21.3 (17.6-25)	45.3 (40.8-49.8)	9.4 (6.7-12)
Mashhad	32.6 (28.7-36.6)	35.2 (31.1-39.2)	0.2 (0-0.6)
Shiraz	25.9 (21.3-30.4)	51.0 (45.9-56.2)	5.2 (2.8-7.6)
Bushehr	42.5 (37.2-47.9)	28.4 (23.5-33.2)	0.1 (0-0.4)
Total¶	27.2 (25.3-29.1)	42.8 (40.7-44.9)	5.1 (4.1-6)

¶: The prevalence rates are age-adjusted

Table 3: Prevalence of vitamin D deficiency in males in each city

City	Mild Vit. D def. 25-34.9 nmol L^{-1} (CI 95%)	Moderate/Severe Vit. D deficiency <25 nmol L^{-1} (CI 95%)
Tehran	20.1 (14.7-25.4)	62.0 (55.6-68.4)
Tabriz	39.4 (35-43.8)	36.2 (32-40.4)
Mashhad	41.1 (35.6-46.6)	35.8 (30.5-41.1)
Shiraz	40.5 (35.7-45.3)	29.2 (24.8-33.6)
Bushehr	37.7 (31.2-44.2)	11.1 (6.8-15.3)
Total¶	37.3 (34.9-39.6)	34.8 (32.5-37)

¶: The prevalence rates are age-adjusted

vitamin D deficiency was significantly higher in men, (Tabriz: $p = 0.0212$ Mashhad: $p = 0.007$ and Shiraz: $p<0.0001$). Moderate or severe vitamin D deficiency was significantly more prevalent in female sex in Tehran, Tabriz, Shiraz and Bushehr ($p = 0.041$, $p<0.0001$, $p<0.0001$ and $p<0.0001$, respectively). In Mashhad, moderate or severe vitamin D deficiency had a trend to higher prevalence in male sex ($p = 0.92$).

There was no significant difference between mean PTH levels of pre and post menopausal females in each vitamin D group and PTH rise with vitamin D decrease was uniform in pre and postmenopausal subjects.

DISCUSSION

There were several methods suggested for determination of normal levels of vitamin D. These methods were historically based on antirrhachitic levels of vitamin D but gradually they have become more physiologic so at present, normal levels of vitamin D are based on the negative relationship between vitamin D and PTH, positive effect of vitamin D supplementation on $1,25(OH)_2D$, or effect of vitamin D on BMD or fractures (Lips, 2004).

Because this study was part of a large scale, cross sectional study, we consider the negative correlation between PTH and vitamin D for the basis of our study. In this method PTH after transformation showed stepwise elevations, two steps in men and three steps in women were found. So, we defined mild vitamin D deficiency in women, between 25 and 39.9 nmol L^{-1}, moderate 12.5-24.9 and severe deficiency below 12.5 nmol L^{-1}. Mild vitamin D deficiency in men was defined between 25 and 34.9 nmol L^{-1} and moderate/severe deficiency state below 25 nmol L^{-1}. The different results might be due to the effect of different levels of sun exposure, different levels of daily calcium consumption which diminishes the need for elevated levels of vitamin D for suppression of PTH, or possible innate difference in calcium and vitamin D regulatory mechanism in two sexes. Presence of two steps in men despite three steps in women also might be because of the effect of very low prevalence of vitamin D deficiency states which can induce a third rise of PTH among men (severe vitamin D deficiency). Although the presence of two or three steps in PTH rise may only means the exponential elevation of PTH with decreasing vitamin D levels, in clinical viewpoint we can accept these cutoffs because chronically elevated PTH can cause adverse effects.

Our cutoff levels for vitamin D sufficiency state are similar to levels suggested in other studies. There are two vitamin D supplementation studies for determination of normal vitamin D level based on the positive effect of vitamin D on serum level of 1,25$(OH)_2$D in the Netherlands (Lips *et al*., 1988) and the United states (Himmelstein *et al*., 1990) in these two studies. The serum level of 1,25$(OH)_2$D did not change significantly after supplementation of persons with baseline 25(OH)D levels more than 30 and 40 nmol L^{-1}, respectively. In French, postmenopausal women who were followed in the Suvimax study, a negative relationship between serum PTH and serum 25(OH)D below 78 nmol L^{-1} was shown (Chapuy *et al*., 1997). Another study performed on inpatients in the United States revealed similar results to the Suvimax study (Thomas *et al*., 1998). In Amsterdam Vitamin D Study, at baseline a negative correlation was detected between serum PTH and serum 25(OH)D only when serum 25(OH)D was lower than 25 nmol L^{-1} (Ooms *et al*., 1995). Analysis of vitamin D status in the MORE study (a study for assessment of raloxifen on osteoporosis) revealed levels of more than 50, 50-25, below 25, normal vitamin D level, mild and severe vitamin D deficiency, respectively (Chapuy *et al*., 1992).

Studies for assessment of prevalence of vitamin D deficiency were mostly limited to a small sample size or specific age and sex group. In countries with regular vitamin D fortification of foods (USA and some European countries), prevalence of vitamin D deficiency is between 1.6-14.8% in different age groups (Omdahl *et al*., 1982; Kinyamu *et al*., 1997; Burnard *et al*., 1990). In European countries with no regular vitamin D fortification program, the deficiency state is more prevalent (Vander *et al*., 1995).

Studies which assessed middle-aged and elderly people showed 14 to 59.6% vitamin D deficiency (Chapuy *et al*., 1997; Burnard *et al*., 1990). Vitamin D deficiency prevalence is much higher in Asian countries (Du *et al*., 2001). Studies in Middle East revealed vitamin D deficiency even in countries with high sun light (Sedrani *et al*., 1983; Sedrani, 1984; Taha *et al*., 1984; Azizi *et al*., 2000; Alagol *et al*., 2000; Goswami *et al*., 2000; Dawodu *et al*., 2003).

Results of the present study indicate a high prevalence of vitamin D deficiency in various cities of the country even in sea side and lower latitude. These results are similar to other studies in nearby countries and may indicate a need to food fortification by vitamin D in national level.

High prevalence of vitamin D deficiencies in all assessed cities may be due to different causes such as low exposure of skin to UVB despite abundant sun shine in these areas (life style factor), higher skin pigmentation of residents compared to western ethnic groups and low oral intake of vitamin D. Other factors such as VDR polymorphism in Iranian populations and low daily calcium intake which increase vitamin D levels needed for PTH suppression may contribute to this high prevalence of increased PTH. Low calcium intake also can increase vitamin D catabolism. There is evidence of increased activity of vitamin D 24-hydroxylase in Indian immigrants to United States compared to other Americans (Awumey *et al*., 1998). It is possible that Iranian population have similar genetic characteristics.

In this study, we did not assess daily calcium and vitamin D consumption in all cities, but in Tehran (in a part of present study) there was no significant difference between daily calcium intake of persons with normal and low vitamin D levels (Hashemipour *et al*., 2004).

Present study was performed in urban area but a decision about the need for a national fortification program should also include data from rural areas.

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