Original Article

Vitamin D Status in Healthy Indians Aged 50 Years and Above

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Abstract

Introduction: There is widespread prevalence of vitamin D deficiency from new-born to infancy, childhood and adult male and females (non-pregnant, pregnant and lactating). However, there is limited information of the vitamin D status in elderly Indians.

Material and Methods: The study was carried in 1346 healthy subjects more than 50 years of age residing in Delhi, India. These subjects, who were divided in two groups: Group-1 (50 - <65 years) and Group-2 (≥65 years), underwent anthropometric, biochemical and hormonal evaluation for vitamin D status Bone mineral density was measured by dual X-ray absorptiometry.

Results: There were 643 males and 703 females, with a mean age of 58.0 ± 9.5 years (range 50-84 years). Vitamin D deficiency [VDD, serum 25(OH)D levels < 20 ng/ml) was present in 1228 (91.2%) and Vitamin D insufficiency [VDI, serum 25(OH)D levels 20-<30 ng/ml] in 92(6.8%). There was no significant difference in prevalence of either VDD or VDI between two age groups and sexes. Serum 25(OH)D levels were negatively correlated with PTH levels (r -0.027, p <0.00001) and BMI (r -0.128, p 0.05). Prevalence of secondary hyperparathyroidism increased from 14.1% to 43.1% from VDI to severe VDD. PTH levels started rising at vitamin D level < 30 ng/ml. However, more than 50% of subjects with severe VDD had PTH levels within normal range. High prevalence of osteopenia (50.2%) and osteoporosis (31.2%) was observed in this population.

Conclusion: Hypovitaminosis D is universal above the age of 50 years in north India. Absence of a PTH response was observed in more than 50% of individuals with VDD, the cause of which merits further evaluation. Normal bone mass was observed in only 18.6% of study subjects.

Introduction

Vitamin D deficiency (VDD) has been documented across all age groups and both sexes from India and different parts of world.[1-4] However there is paucity of data on vitamin D status in population more than 50 years of age.[5] Vitamin D deficiency is associated with low bone mass, muscle weakness, and increases the risk of fracture. It has also been linked to infection, cardiovascular disease, malignancy, and autoimmunity which are commonly seen in the elderly, as are fragility fractures.[1] Hence this population study was under taken to assess the vitamin D status and its impact on bone mass in individuals above 50 years of age.

Material and Methods

The study was carried in population more than 50 years of age in Delhi, India (latitude 28.35º). A total of 1346 individuals were recruited from resident welfare associations and senior citizen associations from different locations in Delhi. Subjects with hepatic, renal, dermatological disorders, alcoholism, and receiving medication likely to adversely affect vitamin D status, were excluded from the study. Demographic, anthropometric and clinical data were ascertained and a detailed physical examination conducted. Individuals taking calcium (minimum 500 mg/day) and Vitamin D (200-400 IU) for >6 months were considered as taking supplements. Fasting blood samples were drawn for the estimation of serum 25(OH)D, intact parathyroid hormone, total and ionic calcium, inorganic phosphorus, and alkaline phosphatase. The study was approved by the ethics committee of the Institute of Nuclear Medicine and Allied Sciences and all subjects gave written informed consent.

Biochemical estimations were carried out using automated analyser (Hitachi 902; Roche, Manheim, Germany) and commercial kits (Roche). The normal range for serum total calcium, (8.8-10.2 mg/dl), ionic calcium. (1.12-1.32 mmol/L), inorganic phosphorus (2.7-4.5 mg/dl), and alkaline phosphatase were (females: <240 U/L; males: <270 U/L). The serum concentrations of 25(OH)D (reference range: 9.0-37.6 ng/ml) and PTH (reference range: 10-65 pg/ml) were measured by RIA (Diasorin, Stillwater, MN) and electrochemiluminescence assay (Roche diagnostics, GMDM-Manheim, Germany) respectively.. Serum 25(OH)D level of 20.0 - <30.0 ng/ml was classified as vitamin D insufficiency[1] (VDI), and levels < 20 ng/ml were classified as vitamin D deficiency (VDD). VDD was further categorized based on Lips classification as mild (10.0 – <20.0 ng/ml), moderate (5.0-<10.0 ng/ml) and severe (< 5.0 ng/ml).[2] Secondary hyperparathyroidism was defined by serum PTH level of >65pg/ml.

Bone mineral density (BMD) at anteroposterior (AP) lumbar spine (L1–L4), femur (femoral neck, Ward's triangle, and trochanter) and forearm (total, ultra distal and 33% radius) was measured using the Prodigy Oracle (GE Lunar Corp., Madison, WI) according to standard protocol. Quality control procedures were carried out in accordance with the manufacturer's recommendations. Instrument variation was determined regularly using a phantom supplied by the manufacturer and mean coefficient of variation was <0.5%. For in vivo measurements, mean coefficients of variation for all sites were <1%. The WHO classification was used to define osteopenia (T score between -1 and -2.5) and osteoporosis (T score < -2.5).[6] DXA was also used to measure percentage body fat.

Statistical analysis was carried out using STATA 9.0 (College Station, TX). Data were presented as mean ± SD or number (%) unless specified. All unpaired parametric data were analysed by student's t-test and non parametric data by chi-square test.

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Table 1 : Anthropometric, Biochemical and Vitamin D Status (All Subjects)

Variable/Age Group (1346)	50-65 (995)	>65(351)	P – Value
Anthropometric Parameters			
Height (cm)	159.2±8.5	160.3±9.3	0.037
Weight (kg)	70.3±12.7	66.6±12.0	<0.00001
BMI (kg/m^2)	27.8±4.9	25.9±4.1	<0.00001
Total Fat (%)	41.5±9.2	38.6±9.0	<0.00001
Biochemical Parameters			
S. Calcium (mg/dl)	9.6±0.4	9.8±0.4	<0.00001
Ionic Calcium (mmol/L)	1.16±0.05	1.15±0.05	0.306
S. Phosphorus (mg/dl)	3.6±0.4	3.5±0.5	<0.00001
ALP (IU/L)	224±69	211±61	0.0021
PTH (ng/ml)			
Mean±SD	59.5±39.8	54.7±34.9	
Median (Range)	52.7 (1.2-497.4)	49.8 (6.5-448.1)	0.052
Vitamin D Status			
Mean±SD	9.72±7.75	9.99±7.2	0.32
Median (Range)	8.21 (0.18-100.0)	8.12 (0.94-53.74)	
Vitamin D Deficiency	908 (91.3%)	320 (91.2%)	0.75
Vitamin D Insufficiency	69 (6.9%)	23 (6.6%)	0.90

Table 2 : Serum 25(OH)D categories

Severity	All (1346)	Male	Female
25(OH)D Levels (ng/dl)	9.79±7.61	9.81±6.79	9.78±8.30
Severe (<5 ng/ml)	376 (27.9%)	166 (25.8%)	210 (29.9%)
Moderate (5-<10 ng/ml)	457 (34.0%)	220 (34.2%)	237 (33.7%)
Mild (10-<20 ng/ml)	395 (29.4%)	201 (31.3%)	194 (27.6%)
VDI (20-<30 ng/ml)	92 (6.8%)	47 (7.3%)	45 (6.4%)

Pearson's correlation was calculated to assess the strength of relationship between 25(OH)D levels and other parameters. A p value of < 0.05 was considered statistically significant.

Results

In this cross sectional study 1346 subjects were studied. Mean age of subjects were 58.0±9.5 years (median 50.1 years, range 50-84 years). There were 643 males and 703 females. These subjects were divided in two groups: Group-1 - those 50 - <65 years (as all females were postmenopausal in this group) and Group-2 - those with 65 years or above (as senile osteoporosis sets in after 65 years of age).

Anthropometric and Biochemical Data

Weight, BMI and percentage of total body fat significantly decreased with age (Table 1). Height decreased significantly in females with age (153.7±5.6 vs. 152.6±6.3, p 0.037) but not in males (165.9±6.2 vs. 166.3±6.4, p 0.48). Serum phosphorus decreased significantly in males with age (3.5±0.4 vs. 3.3±0.5, p <0.00001) compared to females (3.8±0.4 vs. 3.8±0.4, p 0.68). There was no difference in intact PTH level between age groups and sexes.

Vitamin D Status

VDD was present in 1228 (91.2%) and VDI in 92(6.8%) among all (Table 1). There was no significant difference in prevalence of either VDD or VDI between two age groups (Table 1). There was no difference in prevalence of VDD (91.3% vs. 91.2%, p = 0.58) and VDI (7.3% vs. 6.4%, p = 0.26) between sexes (Table 2). There were almost equal distributions of severity of VDD among all and both sexes (Table 2). The only statistically significant correlation of serum 25(OH)D levels on multivariate analysis was a negative correlation with PTH levels, ALP and BMI (Table 3).

Table 3 : Correlation of Vitamin D Level with Anthropometric, and Biochemical Data (Multivariate Analysis)

Variables	Coefficient (r value)	P Value
Age	0.035	0.86
BMI	-0.058	0.03
Total Fat	-0.043	0.117
Calcium	0.039	0.149
Phosphorus	0.004	0.887
ALP	-0.075	0.006
PTH	-0.157	<0.00001

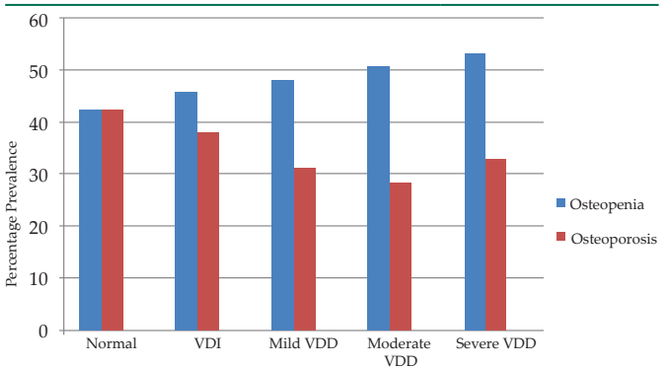

Fig. 1 : Prevalence of osteopenia and osteoporosis according to 25(OH)D Categories

There were 374 (27.8%) subjects with history of fracture [Male 179 (27.8%), and Female 195 (27.7%)]. Serum 25(OH)D levels were not significantly different between those with history of fracture (9.95±8.73 ng/ml) and those without (9.74±7.14 ng/ml; p = 0.88). Prevalence of VDD and VDI also did not differ among those with and without fracture (91.4% vs. 91.2%, p 0.88 and 7.2% vs. 6.7%, p 0.82). There was no statistically significant correlation of serum 25(OH)D levels with BMD at any site.

Seven hundred forty nine (55.6%) subjects were taking vitamin D and calcium supplements [Male 352 (54.7%), and Female 397 (56.5%)]. Serum 25(OH)D levels were not significantly different among those who were taking supplements (9.85±6.89 ng/ml) and those who were not (9.75±8.14 ng/ml; p = 0.793).

Overall prevalence of osteopenia and osteoporosis was 50.2% and 31.2% respectively. Serum 25(OH)D levels were not significantly different between those subjects with osteopenia (9.42±7.15 ng/ml) and osteoporosis (10.22±8.76 ng/ml, p =0.096). However prevalence of osteopenia progressively increased from 42.3% in those with normal vitamin D levels to 53.2% among those with severe VDD (Fig. 1).

PTH level and Secondary Hyperparathyroidism (SHPT)

Intact PTH levels were negatively correlated with serum 25(OH)D levels (r -0.157, p <0.0001) (Table 3). Compared to serum PTH levels (43.9 ± 12.4 pg/ml) of subjects with normal serum 25(OH)D, serum PTH in subjects with VDI, mild VDD, moderate VDD and severe VDD were higher by 10%, 21.5%, 31.7% and 53.3% respectively (Table 4). Prevalence of secondary hyperparathyroidism also increased from 14.1% to 43.1% from VDI to severe VDD. However, more than 50% subjects with severe VDD had PTH levels within normal range (Table 5).

Discussion

Wide spread VDD has been recognised in Indians of all age groups and both sexes.[3-5,7-9] The present study extended the assessment of vitamin D status in older age groups where there was limited data and showed that 91.2% subjects more than 50 years of age had VDD and an additional 6.8% subjects had VDI. Varying prevalence of VDD in the elderly has been reported

Table 4 : PTH Levels According to Vitamin D Status

Vitamin D Deficiency	PTH Levels (Median)	PTH % Increase	P Value
Severe (<5 ng/ml)	67.28±51.57 (59.6)	53.3%	<0.00001 (Chi-square for trend)
Moderate (5-<10 ng/ml)	57.8±31.3 (52.69)	31.7%	
Mild (10-<20 ng/ml)	53.35±33.34 (48.9)	21.5%	
Vitamin D Insufficiency (20-<30 ng/ml)	48.29±27.78 (44.85)	10.0%	
Normal	43.89±12.43 (42.95)		

globally. One of the highest prevalence among postmenopausal women >50 years reported in recent times have been from Croatia[10] and France,[11] where 92.5% and 89.9% had VDI. A high prevalence of VDI and VDD has also been reported from United States (Blacks, Hispanics, and Asians) UK, and Saudi Arabia where approximately 90% people above the age of 65 years had vitamin D level < 30ng/ml. [12-14]

Our study, from North India, confirms the high prevalence of VDD in both men and women in older age groups. The mean 25(OH)D level observed by us in this population of older subjects was 9.79±7.61 ng/ml. We and other investigators have earlier reported similarly low levels in different age of healthy individuals in north India, mean 25(OH)D level ranging from 4.5 ng/ml[15] – 20.85 ng/ml.[3] In contrast, higher serum 25(OH)D levels, 25.3±7.4 ng/ml in females in summer and 18.4±5.3 ng/ml in males in winter have been observed in paramilitary personnel who as a consequence of their professional duties have greater sunlight exposure.[16]

Studies from South India, while showing a high prevalence of VDD, have consistently reported higher mean serum 25(OH) D levels than those observed in north India. Harinarayan et al reported vitamin D level of 14.6±7 and 20.85±8.63 ng/ml in postmenopausal women in the age group of 50-67 years.[3,5] The present study was carried out at a latitude of 28.35° whereas study from south was done at latitude of 12.55° and 13.4°,[3] which may explain the difference in serum 25(OH)D levels at these two sites. An additional contributor to the low serum 25(OH)D levels in Delhi is the atmospheric pollution. In an earlier study, Agarwal et al[17] have shown a significantly higher level of serum 25(OH) D levels in infants from an area of lower atmospheric pollution compared to their peers from Delhi.

Serum 25(OH)D level was negatively correlated with PTH levels, ALP and BMI in multivariate analysis. Serum PTH levels have strong negative correlation with vitamin D levels[2] and a negative correlation between ALP and vitamin D levels has been reported[5]. A negative correlation between 25(OH)D and PTH has been also observed in elderly population[18] on admission to nursing home with fracture. Serum PTH levels plateau at 25(OH) D level of 20 ng/dl in this population. However SUVIMAX study observed rising trends in PTH at 25(OH)D value <30ng/dl[19] in young adult, whereas such threshold was observed at 10 ng/dl in population >70 year of age.[2] This can be interpreted as with increasing age there is decrease in threshold for rise in PTH in relation to 25(OH)D level.

VDD and VDI has been reported to be associated with increased risk of fracture,[1] however there are conflicting reports in the literature[2]. Serum 25(OH)D were not significantly different among those with or without history of fracture in both age groups and sexes. However age of occurrence of fracture, and the degree of trauma associated with the fracture were not ascertained in this study, limiting the interpretation of this information. In a study from the UK, patients with hip fracture were shown to have lower serum 25(OH)D levels compared with controls. This difference was not observed in case of fractures at other sites.[20] In our study, there was no difference in prevalence

Table 5 : Secondary Hyperparathyroidism According to Vitamin D Status

Vitamin D Status	Secondary PHPT		P Value
	Present	Absent	
VDD Severe (<5ng/ml)	162 (43.1%)	214 (56.9%)	<0.00001 (Chi-square for trend)
VDD Moderate (5-<10ng/ml)	153 (33.5%)	304 (66.5%)	
VDD Mild (10-<20ng/ml)	93 (23.5%)	302 (76.5%)	
VDI (20-<30ng/ml)	13 (14.1%)	79 (85.9%)	
Normal	0	26 (100.0%)	

of osteopenia or osteoporosis among subjects with VDD or VDI. However, there was a trend of increasing prevalence of osteopenia progressively as one progressed from mild to severe degree of vitamin D deficiency. Similar observation has been made by us in previous study among healthy young paramilitary personnel, where vitamin D and PTH levels were not statistically different between those with osteopenia or osteoporosis.[16]

There was no correlation of BMD at different sites with serum 25(OH)D levels in the present study. No consistent relationship has been reported between 25(OH)D levels and BMD in cross-sectional studies. A recent meta-analysis confirms the lack of a consistent association between 25(OH)D and BMD, with a consistent observable effect only present in older age groups.[21] A correlation has been reported between 25-OHD levels and hip BMD in subjects of South Asian descent.[21-23] However in other studies involving subjects of South Asian ethnicity, including those conducted by us in healthy young subjects and school girls, there was no correlation between 25(OH)D levels and BMD at any site.[16,24-26]

More than half of subjects in this study were taking calcium and vitamin D supplements, but there was no difference in serum 25(OH)D levels between those who took and did not take supplements. A lack of difference in serum 25(OHD) levels between those receiving and not receiving vitamin D supplements was also reported in an audit from Belfast.[20] Most of the subjects were taking between 200-400 IU of vitamin D3 (cholecalciferol), which is insufficient to normalize serum 25(OH) D levels in a vitamin D deficient population. One study from North India[15] reported requirement of 60,000-120,000 IU per month to achieve vitamin D level > 30 ng/ml. In another study[27] reported correction of vitamin D level to normal after 8 weeks supplementation with weekly supplementation of 60,000 IU. Both these studies highlight the need of regular supplementation of at least 2000 IU/day vitamin D supplementation to maintain normal vitamin D levels.

Serum PTH levels progressively increased from VDI to varying severity of VDD. Serum PTH levels were within normal range among subjects with serum 25(OH)D levels ≥30ng/ml. However, PTH levels above the upper limit of normal (secondary hyperparathyroidism, SHPT) were present in less than half of subjects with severe vitamin D deficiency (<5 ng/ml). The possible explanations for this phenomenon include probable adaptation to vitamin D deficiency or other associated genetic, nutritional or environmental factors which preclude an elevation of serum PTH levels. A study of vitamin D receptor polymorphism from this geographical region did not reveal any abnormality or increase expression explaining adaptability to VDD.[28] However decreased expression of calcium sensing receptor has been observed with vitamin D deficiency in rat and human parathyroid glands.[29,30] This may limit the ability of calcium and calcitriol to regulate PTH secretion in individuals with long standing VDD.[31] Variation in calcium intake and other dietary factor can lead to variation in effect of VDD on PTH.[2,32] Elderly subjects who are not physically active can have increased bone turnover due to immobility which causes suppression of

serum PTH.[2] However, all the subjects recruited for this study were mobile and had physical activity commensurate with their age. Nonetheless, the absence of an anticipated PTH response to low serum 25(OH)D levels is an observation, which merits further study.

Conclusion

Vitamin D deficiency is nearly universal above the age of 50 years in northern part of India, with no difference among those with and without a history of fractures or with and without intake of vitamin D supplements. The study highlights the inadequacy of a daily vitamin D intake of 200-400 IU in normalizing serum 25(OH)D levels in this population, which is consistent with recent observations which indicate that a daily intake of 2000 IU cholecalciferol would be required for this. The absence of PTH elevation despite severe vitamin D deficiency is an area for future research.

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