INDIAN JOURNAL OF ORTHOPAEDICS
January 2006
Volume 40 : Number 1 : P. 41-45

Relationship between vitamin D insufficiency in osteoporosis and blood bone biochemistry

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Background: Vitamin D insufficiency is associated with increased PTH secretion, which in turn leads to bone resorption.

Method: In the present study primary involution osteoporosis and vitamin D_3 levels were studied in 62 subjects including thirty one controls. The biochemical analysis of serum calcium, phosphorus, ALP, albumin and vitamin D3 [1,25 $(OIH)_2D_3$] levels was done and 1,25 $(OIH)_2D_3$ levels were significantly decreased in osteoporotic patients when compared to non-osteoporotic control group.

Results: There was a significant correlation of magnitude of sun exposure and vitamin D intake in the diet with 1,25 $(OH)_2D_3$ levels. However, no correlation could be obtained between vitamin D3 levels with increasing age. Our results significantly demonstrated that assays for routinely used bone biochemistry parameters including serum calcium, phosphate, parathormone and alkaline phosphatase are not representative of hypovitaminosis D even in those whose serum PTH is elevated and only reliable way to confirm this is to do vitamin D levels.

Conclusion: The diagnosis of hypovitaminosis D should be made on the basis of clinical suspicion, arising from an awareness of risk factors, leading to direct measurements of serum vitamin D.

Key-words: Osteoporosis, 1,25 $(OH)_2D_3$ levels, Bone biochemistry

Introduction

Osteoporosis is a skeletal disorder characterized by low bone mass and microarchitectural deterioration of bone tissue with a consequent increase in bone fragility and susceptibility to fracture risk. Vitamin D is needed to maintain calcium homeostasis, skeletal integrity and muscle strength[1]. Vitamin D insufficiency is associated with an increased risk of fracture due to both proximal weakness leading to increased body sway with a propensity to fall[2] and to skeletal fragility from secondary hyperparathyroidism (increased bone turnover and decreased bone density), or from the development of osteomalacia[3-5].

Aging is associated with decrease sun exposure, oral intake and skin activation of Vitamin D, and Vitamin D absorption. All of these factors may contribute to Vitamin D insufficiency, which is required for calcium absorption and bone mineralization. The low serum Vitamin D is associated with increased PTH secretion, which in turn leads to bone resorption, and increased renal calcium excretion[3, 6].

It has been presumed that Indians are vitamin D sufficient as the Indian subcontinent situated between 8.4N and 37.6 N latitude, has adequate sunshine and UVb rays (290-315 nm) reaching the earth's surface throughout the year. However, a recent study has suggested a high prevalence of subnormal 25(OH) D concentration amongst healthy Indians[7].

It is estimated that by the year 2020, a world population of more than 1000 million people will be aged 60 years or above, with more than 700 million of them in developing countries[8]. More than 142 million of this group will be in India[8]. By the year 2020, it is projected that 75% of all deaths in developing countries would be age related[8]. One in 4 women and one in 8 men older than 50 years are believed to have osteoporosis[9, 10].

In the present study, it was planned to evaluate Serum 1,25-dihydroxycholecalciferol levels in patients with clinical features suggestive of reduced bone mass with evidence of osteoporosis to confirm vitamin D deficiency in patients in comparison to age and sex matched controls and to investigate whether routine bone biochemistry can be used to predict vitamin D deficiency.

Material and methods

In a prospective randomized study 31 (16 males and 15 females) patients of primary osteoporosis in the age group 40-80(mean 67 years) years were selected who had clinical features suggestive of reduced bone mass viz low backache

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Table I. Relationship of vitamin D levels with sun exposure

Sun Exposure (hrs/week)	Male		Female		Total	
	P	C	P	C	P	C
Low(<4hrs)	18.86±3.82	19.00±2.12	15.82±2.13	13.30±3.26	17.04±3.21	16.15±3.98
Moderate(4-8 hours)	26.34±5.38	29.04±5.54	19.33±2.52	19.48±2.35	24.43±5.68	25.46±6.56

or generalized weakness or malaise or chronic fatigue syndrome or a hip fracture and radiological evidence of osteoporosis at one or more sites (including lumbosacral spine, neck of femur, distal radius). An equal number of age and sex matched healthy controls were included in the study. The patients admitted in the wards with osteoporotic hip fractures constituted a majority of the patients.

Patients taking HRT and anticonvulsants, having a chronic debilitating illness (cancer, AIDS, CHF, COPD), renal disease (serum creatinine >1.5mg/dl), liver disease (serum bilirubin >2.0mg/dl), malabsorption syndrome, IBD, chronic pancreatitis, or small bowel resection, and patients taking Vitamin D were excluded.

A detailed questionnaire was given to the patients with regard to diabetes mellitus/ambulatory status—whether working outdoors/house bound/bed ridden/history of sun exposure in a week (little <4 hours; moderate – between 4 to 8 hours; substantial > 8 hours)/diet recall which included a questionnaire with regard to daily intake of foods containing vit-D. Detailed clinical examination including general physical examination, systemic examination and local examination was done. X-rays as relevant for the patients viz. X-ray pelvis, spine and clinical interest were done and patients classed into based on hip[11] and spine[12] osteoporosis indices. Laboratory investigations including an admission screen haemogram with ESR; renal and liver function tests; serum calcium (corrected to albumin levels); phosphate; and alkaline phosphatase levels were measured by automated standard methodology and Vitros slides.

Serum 1,25-dihydroxycholecalciferol were measured by radioimmunoassay using Biosource1, 25 $(OH)_2$- Vit D, RIA – CT Kit and intact PTH by two–site immuno-radiometric assay**.** Serum samples were treated with extraction solvent and applied on cartridges to separate 1,25 $(OH)_2D_3$ from other vitamin D metabolites. The sample, standard and control were incubated with the tracer solution in label coated tubes. A fixed amount of I^{125} labelled 1,25 $(OH)_2$ Vit D competes with 1,25 $(OH)_2$ Vit D from either standard or extracted samples for a fixed amount of specific antibody coated on the inner surface of incubation tubes. After an overnight incubation, an aspiration step followed by a washing step stops the competition reaction. The tubes are counted in a gamma counter for 60 seconds.

Calculations of results: The bound radioactivity is calculated as a percentage of the binding determined at zero standard point (a) according to the following formula.

$$B/B_0 \times 100 = \frac{\text{Counts (standard or sample)}}{\text{Counts (zero standard)}} \times 100$$

The amount of 1,25 $(OH)_2$ D_3 was calculated from the standard curve in picogram/ml. The relations between PTH and other variables were examined by one-way analysis of variance and linear regression giving the most significant difference in bone markers and 1,25 $(OH)_2$ D_3 levels. Results with a value of $p<0.10$ were considered statistically significant.

Results

After applying the exclusion criteria, 31 patients (16 males and 15 females) with primary osteoporosis in the age group 40-80(mean 67 years) years were selected. Instead of using basal serum 1,25 $(OH)_2$ D_3 levels as determined from young populations, we used a control age and sex matched group who closely resembled the patient group with respect to sun exposure, dietary intake and other variables. We derived baseline reference levels of 1,25 $(OH)_2$ D_3 from the control group and compared them to the patient group.

The vitamin D_3 levels were found to be lower than one SD below the mean reference value of controls (21.35±4.74»16.61) in 47% of female patients and 56% of male patients (29.5 ± 7.41 » 22.09) with the levels being 22.6±5.75 in male patients with osteoporosis and 16.5±2.47 in female patients with osteoporosis (Table I).

Overall irrespective of sex, 42% of the patients had vitamin D_3 levels below one SD of the mean reference value of the controls (25.55 ± 7.47 » 18.07). The levels of 1,25-(OH)2 D3 were significantly higher in male subjects as compared to females in both patients and controls ($p< 0.05$).

Among the patients with hip fractures who constituted majority (78%) of patients, 57% patients had vitamin D3 levels below the lower reference value for controls (18.07pcg/ml).

Table II. Serum-calcium level (mg/dl)

Serum Calcium level (mg/dl)	Male		Female		Total	
	P	C	P	C	P	C
Low (<8.6)	8(50)	6(37.50)	8(53.33)	9(60)	16(51.61)	15(48.39)
Normal (8.6-10.2)	7(43.75)	10(62.50)	7(46.67)	6(40)	14(45.16)	16(51.61)
High (>10.2)	1(6.25)	-	-	-	1(3.23)	-
Mean Ca.level	8.48±0.89	8.65±0.56	8.28±0.39	8.69±0.65	8.39±0.69	8.37±1.66
p-value	>.10		<. 05		>.10	

Table III. ALP levels (IU/L)

ALP Level (IU/L)	Male		Female		Total	
	P	C	P	C	P	C
<50	2(12.50)	-	-	1(6.67)	2(6.45)	1(3.23)
50-100	4(25)	6(37.5)	1(6.67)	5(33.33)	5(16.13)	11(35.48)
100-150	7(43.75)	7(43.75)	13(86.66)	4(26.67)	20(64.52)	11(35.48)
>150	3(18.75)	3(18.75)	1(6.67)	5(33.33)	4(12.90)	8(25.81
Mean ALPLevel	110.99±41.60	141.18±93.70	127.17±21.77	133.56±68.42	118.82±33.97	130.94±83.86
p-Value	>.10		>.10		>. 10	

Table IV. Correlation of 1,25 Dihydroxycholecalciferol levels with other variables.

Variable	Male		Female		Total	
	P	C	P	C	P	C
1. Age	.030	.237	.285	.617***	.102	.272
2. vitamn-D Intake	.248	.672***	.694**	.846***	.433**	.401**
3. Sun Exp	.246***	.627**	.631**	.842**	.378***	.402**
3. S.Ca	.206	.519**	.074	.664***	.217	.454**
5. ALP	.168	.067	.541**	.052	.322	.026
6.S.Phosphate	.149	.137	.631	.407**	.385	.239
7.S.PTH	.188	.087**	.451	.055	.412	.033

*** p<0.01 NS - Not significant(p>0.10)
** p<0.05
* p<0.1

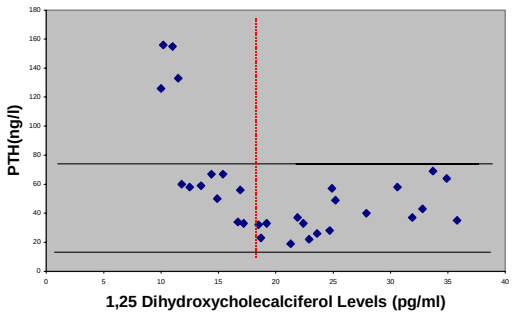

Fig 1. Relationship between 1,25 Dihydroxychole-calciferol levels (pg/ml) and PTH

The mean PTH levels were 56 pg/ml (range, 14-178 pg/ml). Compared with the published reference range of 12-72ng/l[13], only 13% of the osteoporotic patients had elevated PTH levels compared to deficient Vitamin D_3 levels in 42% of osteoporotic patients. The remaining 87% patients had PTH levels within the hospital standard laboratory reference. The mean serum calcium levels were 8.9 mg/dl in the patients with osteoporosis (Table II). It was not found to be a significant univariate predictor of low vitamin D_3 levels in both male and female patient group. But calcium levels were positively related to $1,25(OH)_2 D_3$ levels in controls in both sexes. Overall irrespective of sex, the control group had significantly high ($p<0.05$) vitamin D_3 levels and proportionately high serum calcium levels. The mean Alkaline phosphatase levels was 89IU/L and phosphate was 3.1mg/dl and overall none of them had a correlation with low $1,25(OH)_2 D_3$ levels (Tables III, IV). No significant relationship could be established between age

of the subjects and any of the bone biochemistry levels or low vitamin D_3.We found a significant correlation of magnitude of sun exposure and vitamin D intake with vitamin D_3 levels across all respondent groups and especially in patient group ($P<0.01$).

Discussion

This study confirms the high prevalence of hypovitaminosis D in the osteoporotic patients of both sexes in the Indian population as compared to controls. This was alarmingly high to the tune of 57% in the patients presenting with hip fractures. Our results significantly demonstrated that assays for routinely used bone biochemistry parameters including plasma calcium, phosphate, parathormone and alkaline phosphatase are not representative of hypovitamininosis D and only reliable way to confirm this is to do vitamin D levels. Clinical suspicion based upon history and an awareness of risk factors should remain the gold standard for requesting vitamin D measurements. But these may still well be needed for assessment and monitoring the need for vitamin D and calcium supplementation.

We used an age and sex matched population of non-osteoporotic controls compared to previous studies where patients undergoing elective hip replacement have been used as controls but this group may not replicate co-morbid pathology, nutritional and sun exposure characteristics of the patient group. Clinical suspicion of hypovitamininosis D should be based on an awareness and assessment of risk factors. Thomas et al [14] found that in addition to insufficient sun exposure and being housebound, inadequate dietary vitamin D intake is an independent predictor of vitamin D insufficiency in a study of 164 patients in a general medical ward in Massachusetts, USA. Furthermore occlusive dressing or veiling also poses a significant risk for vitamin D deficiency, irrespective of latitude or average hours of daily sunshine, as illustrated in a study from Lebanon where clothing is completely occlusive and 60% of women had serum vitamin D levels less than 10 ng/L[15].

As persistent vitamin D deficiency leads to secondary hyperparathyroidism, it has been suggested that an elevation in PTH is a sensitive pointer to significant hypovitaminosis D[16,17]. Haden et al observed that serum vitamin D as high as 62.4 nmol/L was associated with a compensatory increase in PTH[18], but in our study only 13% of the osteoporotic patients had elevated PTH levels compared to deficient Vitamin D_3 levels in 42% of osteoporotic patients. A review of earlier studies of vitamin D status of both young and old subjects, where individual values of both are given, shows that this blunted PTH response in the presence of hypovitaminosis D may be a common but unrecognized occurance[19,21]. Similar findings were given by Serhan et al [22] where among an Indo Asian population with a high prevalence of hypovitaminosis D (58%), only 30% of the subjects had secondary hyperparathyroidism. Sahota[23] has reported that 50% of patients with hypovitamininosis D (<30 nmol/L) fail to develop hyperparathyroidism, and as a result have lower 1,25 Dihydroxyvitamin D, and hence a lower serum calcium as a result of less calcium absorption.

Although it has been shown that different degrees of hypovitaminosis D are associated with a rise in ALP and PTH, the absolute values still remain within the reference ranges[22], and it is unlikely that these subtle changes would alert a clinician to the diagnosis.

This is very important as in this population the other hip needs to be protected and supplementation started as this certainly can help generate preventive strategies to contain the growing epidemic of osteoporosis, for the entire population as well as for those at highest risk. This is further supported by the fact that 57% of patients with hip fractures in this study had low vitamin D_3 levels, which is quite significant as one of the causative factors for their proneness to osteoporosis and consequent fractures.

A meta-analysis of previous controlled trials suggests that vitamin D and calcium treatment reduces the incidence of fractures among frail elderly population[24]. It can be safely postulated that patients who are being treated for hip fractures may benefit from calcium and vitamin D_3 supplementation particularly to enhance healing as they will tend to remain indoors and will not ordinarily be exposed to sunlight till their fractures heal.

In summary this study demonstrates that the abnormalities of routine markers of bone profile, beyond laboratory reference ranges, are unable to discriminate patients with vitamin D insufficiency, even in those whose serum PTH is elevated. Therefore the finding of normal ALP, calcium, phosphate, or PTH should not be interpreted as implying normal vitamin D status. The diagnosis of hypovitaminosis D should be made on the basis of clinical suspicion, arising from an awareness of risk factors, leading to direct measurements of serum vitamin D.

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