ORIGINAL ARTICLE

Vitamin D status in apparently healthy adults in Kashmir Valley of Indian subcontinent

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Background: The worldwide prevalence of vitamin D deficiency is reported to be high.
Objectives: To assess the vitamin D status in apparently healthy adults in Kashmir valley by measuring serum 25-hydroxyvitamin D (25 (OH) D).
Methods: 92 healthy natives (64 men and 28 non-pregnant/non-lactating women, aged 18–40 years), residing in Kashmir for at least last 5 years and not having any suggestion of systemic disease, were selected for this study. The samples were collected throughout the year in both summer and winter months. Vitamin D deficiency was defined as a serum 25 (OH) D concentration of <50 nmol/l and graded as mild (25–50 nmol/l), moderate (12.5–25 nmol/l) and severe (<12.5 nmol/l).
Results: Body mass index, total energy intake, and other nutritional parameters were comparable among subjects in different groups. Overall 76 (83%) of the subjects studied had vitamin D deficiency—25%, 33%, and 25% had mild, moderate, and severe deficiency, respectively. 49 of the 64 males and all but 1 of the 28 females were vitamin D deficient. The prevalence of vitamin D deficiency ranged from 69.6% in the employed group to 100% in the household group. Vitamin D deficiency was equally prevalent in subjects from rural and urban areas. Serum calcium and phosphorus values were comparable in subjects with and without vitamin D deficiency, while daily intake and urinary excretion of calcium were significantly lower in the former. Vitamin D deficient subjects had a significantly lower mean weekly exposure to sunlight.
Conclusions: In spite of abundant sunlight, healthy individuals in Kashmir valley are vitamin D deficient, particularly women. Serum 25 (OH) D concentrations are significantly related to sun exposure.

Serum 25-hydroxyvitamin D (25 (OH) D) is the major circulating metabolite of vitamin D and reflects the vitamin D inputs from cutaneous synthesis and dietary intake. The serum concentration of this metabolite of vitamin D is the most commonly used and the most sensitive index of vitamin D status.[1] There is mounting evidence that this fat soluble vitamin has beneficial effects on extraskeletal tissues and that amounts required for optimal health are probably higher than previously believed.[1] While overt vitamin D deficiency manifests as rickets and fractures in children and osteomalacia in adults, less severe deficiency has also been associated with detrimental skeletal consequences including secondary hyperparathyroidism, increased bone turnover, enhanced bone loss and fracture risk.[2 3] Numerous reports have shown that a relatively high proportion of people have vitamin D deficiency.[4] Vitamin D inadequacy does indeed constitute an epidemic in many populations across the world and has been reported in healthy children, young adults, and middle aged and elderly adults. Low 25 (OH) D concentrations (<20 ng/ml) have been reported in more than a third of otherwise healthy young adults aged 18–29 years, about a half of black women aged 15–49 years, 41% of outpatients aged 49–83 years, and up to 57% of general medicine outpatients in the USA. The picture is more dismal in Europe where a quarter to all of healthy adults and three quarters to all of hospitalised adults have such 25 (OH) D values.[5]

Low dietary vitamin D intake and poor exposure to sunlight are common causes of vitamin D deficiency in the general population. Sunlight is an important source of vitamin D. Skin pigmentation, clothing practices, latitude, and season are some of the factors that influence the cutaneous photochemical synthesis of vitamin D in healthy individuals.[6] Several studies have shown a high prevalence (50–97%) of vitamin D deficiency in tropical and subtropical regions of India and other South Asian countries, despite abundant sunlight.[7–11]

Kashmir valley is situated at an altitude of 1574–5425 feet above the sea level at latitudes 320 20′–340 50′ N and longitude 730 45′–750 35′ E in the Northern mountainous regions of India. However, the exposure to sunlight here is good and consumption of dairy products is acceptable. Though vitamin D deficiency has assumed pandemic proportions all over the world, there are no data on vitamin D status of this population and other similar populations in North India. Therefore, we carried this study to assess the prevalence of vitamin D deficiency in healthy volunteers, in Kashmir Valley.

PARTICIPANTS AND METHODS

In this study, 150 healthy volunteers, aged 18–40 years, were approached and screened for eligibility. The subjects had to be apparently healthy and residing in Kashmir valley for at least 5 years at the time of their recruitment into the study, without any suggestion of liver, kidney or gastrointestinal disease. Furthermore, pregnant and lactating women, and persons taking vitamin and mineral supplements or drugs that affect bone metabolism, were excluded from the study. Of the 150 individuals approached, only 92 fulfilled the criteria and are subjects of this study. The subjects were drawn from five distinct groups: medical professionals (23), employed (23), farmers (17), household (15), and students (14). The medical professionals included doctors and nurses from our institute. The demographic characteristics of excluded subjects were not significantly different from those of study subjects.

At initial evaluation of the subjects a detailed history was obtained, focusing on dietary habits, body area exposed to sun, and hours per week of exposure to sunlight. The subjects were interviewed for a 24 h diet recall and their nutritional status was assessed by estimating the composition of diet in terms of total energy, carbohydrate, protein, fat and calcium intake by using a semiquantitative food frequency questionnaire.[12] All the

Table 1 Demographic characteristics of study subjects

Characteristic	Farmer (n = 17)	Government employee (n = 23)	Household (n = 15)	Medical professional (n = 23)	Student (n = 14)	All groups (n = 92)
Gender (male:female)	17:0	20:3	0:15	16:7	11:3	64:28†
BMI (kg/m^2)	20.1 (1.8) (18.0–24.8)	21.1 (2.7) (16.5–27.6)	19.8 (2.5) (15.2–24.4)	20.3 (2.1) (17.7–24.8)	19.2 (1.6) (16.9–23.2)	20.2 (2.3) (15.2–27.6)
Season* (summer: winter)	8:9	10:13	7:8	17:6	6:8	48:44
Calcium (mmol/day)	9.0 (3.1) (1–13)	9.2 (2.5) (4–13)	7.2 (1.5) (5–11)	8.8 (2.2) (5–13)	8.2 (2.5) (5–13)	8.6 (2.5) (1–13)
Energy intake (kJ)	11676 (564) (10619–12923)	12019 (677) (10947–3407)	11899 (367) (10947–12677)	11778 (380) (11020–12386)	11641 (588) (10619–12698)	11818 (541) (10619–13407)
Carbohydrate intake (g/d)	574 (25) (528–614)	596 (35) (532–658)	590 (18) (542–614)	585 (19) (538–611)	584 (25) (540–627)	586 (26) (528–658)
Protein intake (g/day)	54.1 (5.2) (43–62)	55.5 (5.0) (50–65)	50.1 (7.4) (40–62)	53.6 (7.2) (40–65)	51.6 (7.3) (42–65)	53.3 (6.5) (40–65)
Fat intake (g/day)	36.5 (5.4) (25–47)	35.2 (4.2) (30–46)	36.2 (3.2) (30–42)	35.7 (4.4) (30–46)	36.8 (4.8) (30–47)	35.9 (4.4) (25–47)
Sun exposure (h/week)	25.1 (4.3) (14–28)	17.8 (7.2) (4–28)	14.7 (6.7) (4–28)	9.6 (7.4) (3–28)	16.9 (4.7) (6–21)	16.5 (8.1)† (3–28)
Area exposed (%)	19.0 (5.1) (10–30)	17.0 (4.9) (6–27)	11.4 (4.6) (5–21)	17.1 (5.0) (6–21)	16.9 (4.3) (6–21)	16.5) (5.3)† (5–30)

BMI, body mass index.
Values are mean (SD) and range, unless indicated otherwise.
*Season when sample was taken.
†p<0.001.

subjects had predominantly rice based diets, ate meat 2–3 times per week, and consumed fish and milk products only occasionally. Use of vitamin D fortified foods in Kashmir valley is negligible.

Sunlight exposure was determined from average daily duration of exposure to sun and percentage of body surface area exposed.[13] The average duration of cloud free sunshine during the year of study (2003) was 4.4 h/day in winter months (October to March) and 6.5 h/day in summer (April to September), according to the data provided by the Meteorology Department of Kashmir.

Examination included anthropometry and general physical and systemic examination; note was made of the colour of the skin in all subjects. Routine laboratory evaluation included kidney and liver function tests, and estimation of serum calcium, phosphorus and alkaline phosphatase, and daily urinary calcium excretion. Blood samples for 25 (OH) D were collected under basal conditions, taking precautions to avoid venostasis. The serum was refrigerated at −21℃ and stored until analysed; samples were collected throughout the year of study. After rapid extraction from serum with acetonitrile, 25 (OH) D was estimated by radioimmunoassay (RIA) procedure (catalogue No 68100, Dia Sorin, Stillwater, Minnesota USA). The intra- and interassay coefficients of variation ranged between 11.7–12.5% and 9.4–11.0%, respectively. Vitamin D deficiency was defined as serum 25 (OH) D concentration of <50 nmol/l and graded as mild (25–50 nmol/l), moderate (12.5–25 nmol/l), and severe (<12.5 nmol/l) as recommended by Lips.[3]

Table 2 Age and occupation in relation to vitamin D status

	Vitamin D deficient (n = 76) (%)	Vitamin D sufficient (n = 16) (%)	Total
Age group (years)			
20–24	15 (93.8)	1 (6.3)	16
25–29	36 (81.8)	8 (18.2)	44
30–34	17 (81.0)	4 (19.0)	21
35–40	8 (72.7)	3 (27.3)	11
Occupation			
Farmer	12 (70.6)	5 (29.4)	17
Government employee	16 (69.6)	7 (30.4)	23
Household	15 (100.0)	–	15
Medical professional	21 (91.3)	2 (8.7)	23
Student	12 (85.7)	2 (14.3)	14

Statistical analysis

Statistical Package for Social Sciences (SPSS) version 10.0 was used for statistical analysis of the data. A two-tailed p value was used for calculating statistical significance; a value of $p<0.05$ was taken to be significant. Logistic regression analysis was performed to compare factors affecting the prevalence of vitamin D deficiency.

RESULTS

Ninety-two subjects (64 males and 28 non-pregnant females) belonging to five different groups (farmer, employed, household, medical personnel, and students) were evaluated for vitamin D deficiency. The subjects studied had a mean (SD) age of 28.15 (4.9) years (range 18–40 years). The body mass index (BMI), total energy, carbohydrate, protein and fat intake of subjects in the five groups of subjects were comparable (table 1).

Overall, 76 (83%) of the subjects studied had vitamin D deficiency, defined as serum 25 (OH) D <50 nmol/l. More than three quarters (49 of 64; 76.6%) of the males and nearly all (27 of 28; 94.4%) of the females studied were vitamin D deficient. Mild, moderate, and severe deficiency of vitamin D was found in 25%, 33%, and 25% of the subjects studied, respectively. Between 70–100% subjects in different groups were documented to have vitamin D deficiency (table 2). The prevalence of vitamin D deficiency in subjects from rural and urban areas were comparable (80% of 50 subjects and 85.7% of 42 subjects, respectively); the mean (SD) vitamin D concentration was 28.35 (24.14) nmol/l in urban versus 32.10 (30.99) nmol/l in rural populations ($p>0.5$).

The mean (SD) daily calcium intake in subjects with vitamin D deficiency was significantly lower than that in subjects without vitamin D deficiency (306 (84.4) vs 444 (46.3) mg/day; $p<0.01$). Compared to subjects from other groups, farmers had a significantly higher mean exposure to sunlight in terms of hours per week and area exposed (table 1). Exposure to sunlight was related significantly to serum 25 (OH) D values.

Table 3 Demographic/biochemical characteristics and vitamin D status

	Vitamin D (nmol/l)				
	<12.5	12.5–25	25–50	⩾50	p Value
Women	14/23 (61%)	13/30 (40%)	0/23 (0%)	1/16 (6%)	<0.001
Calcium intake (mg/day)	6.21 (1.34)	7.69 (1.96)	10.0 (1.37)	11.52 (1.20)	<0.001
Sun exposure (h/week)	12.3 (7.1)	15.0 (8.7)	19.7 (7.0)	20.6 (6.5)	<0.001
BMI (kg/m^2)	20.1 (3.1)	20.0 (1.5)	19.6 (1.8)	21.5 (2.3)	>0.08
Serum calcium (mmol/l)	2.4 (0.1)	2.4 (0.1)	2.4 (0.1)	2.3 (0.1)	>0.1
Serum phosphorus (mmol/l)	1.29 (0.04)	1.28 (0.04)	1.31 (0.04)	1.30 (0.03)	<0.04
Alkaline phosphatase (U/l)	240 (35.5)	233 (35.40)	259 (115.7)	237 (28.0)	>0.5
24 urinary cal (mmol/day)	4.46 (0.95)	4.17 (0.80)	4.37 (1.35)	6.70 (1.70)	<0.003
Serum 25 (OH) vitamin D (nmol/l)	5.59 (3.18)	19.11 (3.82)	34.84 (6.38)	80.80 (26.64)	<0.001

BMI, body mass index.
Values are mean (SD) unless specified.

As a group, subjects with vitamin D deficiency had a significantly lower mean weekly exposure to sun compared to those who were vitamin D sufficient (15.6 (8.2) h vs 20.6 (6.5) h; p<0.03). Between subjects with and without vitamin D deficiency and among subjects with progressively severe vitamin D deficiency, there was significant difference in the mean exposure to sunlight measured in hours per week (table 3). There were no differences observed in the results of blood counts and lipid concentrations and the indices of liver and kidney functions in subjects with and without vitamin D deficiency. The serum concentrations of calcium, phosphorus, and alkaline phosphatase were comparable in subjects with and without vitamin D deficiency, but daily urinary excretion of calcium was significantly lower in the former.

All except one woman in our study were vitamin D deficient; probable factors for this deficient state were household occupation, decreased sun exposure, decreased body area exposure due to veiling, and decreased calcium intake (table 4). We did not find any consistent trend in the prevalence of vitamin D deficiency with age; similarly, there was no association between body mass index and serum 25 (OH) D concentrations in subjects without any and with variable severity of vitamin D deficiency (table 3).

DISCUSSION

We found a very high prevalence (83%) of vitamin D deficiency (defined as a serum 25 (OH) D concentration of <50 nmol/l) in apparently healthy adults from different walks of life. Despite abundant sunlight, a high prevalence of vitamin D deficiency has been reported from tropical and subtropical regions in India and other South Asian countries.[8–11] Arya and others demonstrated vitamin D deficiency (defined as 25 (OH) D concentration of <15 ng/ml) in two thirds of healthy urban North Indian hospital staff.[11] A recent study on school children from Delhi, India reported concentrations of 25 (OH) D <9 ng/ml in 35.7%; such low concentrations were far more frequent (42.3%) in children with low socioeconomic status than in those with high socio-economic status (27%).[14] Vitamin D inadequacy is particularly common among patients with osteoporosis. Globally, a quarter of postmenopausal women with osteoporosis were shown to have serum 25 (OH) D values <10 ng/ml, with the highest prevalence reported in central and southern Europe.[4] A study of Asian adults in the UK showed that 82% had 25 (OH) D values <12 ng/ml, with the proportion increasing to 94% during the winter months.[15]

Table 4 Demographic/biochemical characteristics of the study subjects: men vs women

	Men (n = 64)	Women (n = 28)	p Value
Age (years)	28.75 (4.90)	26.79 (4.71)	0.077
Occupation, n (%)			<0.001
Farmer	17 (100%)	0	
Government employee	20 (87%)	3 (13%)	
Household	0	15 (100%)	
Medical professional	16 (70%)	7 (30%)	
Student	11 (79%)	3 (21%)	
Sun exposure (h/week)	18.39 (7.86)	12.11 (7.04)	<0.001
Area exposed (%)	18.53 (4.02)	11.75 (4.77)	<0.001
Calcium intake (mmol/day)	9.20 (2.46)	7.11 (1.77)	<0.001
Haemoglobin (mmol/l)	8.64 (0.70)	7.09 (0.73)	<0.001
Serum albumin (g/l)	41.5 (8.1)	37.8 (4.6)	<0.03
25 (OH) vitamin D			
Mean (nmol/l)	37.66 (30.04)	13.77 (11.05)	<0.001
<5 nmol/l, n (%)	9 (39%)	14 (61%)	<0.001
5–10 nmol/l, n (%)	17 (57%)	13 (43%)	
10–20 nmol/l, n (%)	23 (100%)	0	
⩾20 nmol/l, n (%)	15 (94%)	1 (6%)	

*Values are mean (SD) unless specified.

Various factors have been found to affect the prevalence of vitamin D deficiency. Age (>80 years), race (non-white), BMI (>30 kg/m^2), use of medications known to affect vitamin D metabolism, lack of exercise, inadequate use of vitamin D supplementation (<400 IU/day), and education level were found to be significantly associated with vitamin D inadequacy.[16] The prevalence of vitamin D inadequacy ranged from 43% in subjects with one risk factor to 90% in those with five or more risk factors.[16] Regardless of the season, increasing age has been associated with lower 25 (OH) D values.[17] Contrary to the results of some other studies,[16 17] we did not find any effect of age on the prevalence of vitamin D deficiency. This is expected as the patients in our study belonged to a relatively narrow age range (18–40 years); also age was correlated with vitamin D deficiency or sufficiency only and not with the vitamin D values within these broad groups.

Adiposity has been reported to be strongly related inversely to serum 25 (OH) D and directly to parathyroid hormone (PTH) concentrations independent of age, sex, season, or smoking; the association has been found to be weaker if anthropometric measures only rather than precisely measured total body fat percentage is used, implying a specific role for adipose tissue.[18]

What this study adds

- Vitamin D deficiency is widely prevalent in the Kashmir valley of the Indian subcontinent.
- Despite abundant sunlight, vitamin D deficiency is seen in three quarters of healthy men and nearly all women.
- This study reaffirms the belief that vitamin D deficiency is a pandemic.

Implications of this study

- Implication of this study at the local/national level is the need to make physicians aware of the high prevalence of vitamin D deficiency in apparently healthy looking populations.
- Implication of this study at the global level is that urgent measures are needed for improving vitamin D status and eradicating the pandemic of vitamin D deficiency.

The lack of any consistent relationship between BMI and serum 25 (OH) D in our study needs to be seen in that perspective. In addition, none of the subjects in our study was actually obese (BMI >30 kg/m^2). Exposure to direct sunlight for as little as 5–10 min on the arms and legs between the hours of 10:00 and 15:00 during spring, summer and fall has been reported to be enough to prevent vitamin D inadequacy.[1 19] A large proportion of our study subjects with good amounts of exposure to sunlight were found to be vitamin D deficient. In particular, all but one of the women in our study were vitamin D deficient with significantly less sun exposure and decreased body area exposure than their male counterparts. This is attributable to the prevalent cultural practice of wearing clothing that covers most of the body and remaining indoors for household work, as has been reported from other areas with such cultural practice.[20 21]

The limitations of this study include a relatively small size, failure to study all subjects in both summer and winter, and lack of data on percentage of body fat of the subjects studied. Further, PTH levels were not available in our subjects; however, while some argue that the cut off level of 25 (OH) D in serum to define vitamin D deficiency should be linked to changes in PTH concentrations, at present there are no recommendations to measure PTH to supplement serum 25 (OH) D measurements for diagnosis of vitamin D deficiency.

We have demonstrated a very high prevalence of vitamin D inadequacy in apparently healthy, young, non-pregnant adults. It is likely to be even higher and more severe in children, the elderly, and in pregnant women. Given the importance of vitamin D in the regulation of calcium and phosphorous homeostasis and musculoskeletal health, its emerging role in extraskeletal health, and the magnitude of deficiency of this vitamin, fortification of certain commonly used food items may be required.

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