Clinical Endocrinology (2009) **70**, 680–684 doi: 10.1111/j.1365-2265.2008.03360.x

ORIGINAL ARTICLE

Vitamin D deficiency in rural girls and pregnant women despite abundant sunshine in northern India

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Summary

Context Vitamin D deficiency is common in urban Indians despite living in the tropics and its public health consequences are enormous. However, 70% of India is rural, and data from rural subjects, who are expected to have good sun exposure, are scant.

Objectives To determine the population prevalence of vitamin D deficiency in rural pregnant women and adolescent girls, compare serum 25-hydroxyvitamin D (25OHD) status in adolescent boys from the same families, and determine seasonal differences in serum 25OHD.

Design A cross-sectional study conducted over 18 months.

Subjects A random selection of 121 adolescent girls from a survey of a population of 8270 in a rural low socioeconomic community; 139 pregnant women in the second trimester; and a subset of 28 adolescent girls compared with 34 brothers.

Measurements Serum 25OHD, serum alkaline phosphatase (AP), sun exposure, and dietary calcium intake.

Results The age-adjusted community prevalence of vitamin D deficiency (25OHD < 50 nmol/l) in adolescent girls was 88·6%. Seventy-four per cent of pregnant women had vitamin D deficiency. Mean ± SD 25OHD in girls and women in summer was 55·5 ± 19·8 nmol/l compared to 27·3 ± 12·3 nmol/l in winter ($P < 0{\cdot}001$). Winter serum 25OHD in boys (67·5 ± 29·0 nmol/l) was higher than that in their sisters (31·3 ± 13·5 nmol/l, $P < 0{\cdot}001$).

Conclusion We report a high prevalence of vitamin D deficiency among pregnant women and adolescent girls from a rural Indian community. Boys are relatively protected. Seasonal variation in serum 25OHD is significant at latitude 26° N.

(Received 8 January 2008; returned for revision 13 February 2008; finally revised 1 May 2008; accepted 21 July 2008)

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Introduction

Despite abundant sunshine, vitamin D deficiency is highly prevalent in urban India.[1–3] This has been documented in children from New Delhi (latitude 28·3° N), pregnant women and newborns in Lucknow (26·8° N), and men and women from Tirupathi (13·4° N).[1–3] The paradox may be explained by insufficient outdoor activity in urban areas,[4] dark skin colour, modest clothing precluding adequate sun exposure,[5] poor dietary calcium causing secondary vitamin D deficiency,[5,6] and environmental pollution.[7]

In contrast to urban subjects, the rural population is expected to have excellent sun exposure. Studies on vitamin D status in rural Indians are few, and were not designed to give a population prevalence.[1,3] Seventy per cent of India's population is rural, and the consequences of vitamin D deficiency are serious. Neonates, infants and pregnant women are most vulnerable. Vitamin D deficiency in pregnancy leads to neonatal hypocalcaemia, infantile rickets and predisposition to lower respiratory tract infections, the main cause of infant mortality.[8,9] Adolescent girls are vulnerable because of early marriage and child bearing. Girls are discriminated against, with men and boys receiving the major share of nutrition in poor Indian households.

We undertook this study to determine the prevalence of vitamin D deficiency in adolescent girls and pregnant women in a rural community. In addition, we compared serum 25-hydroxyvitamin D (25OHD), dietary calcium intake and sun exposure in adolescent girls with that in adolescent boys from the same families. We also looked for seasonal differences in the level of serum 25OHD.

Subjects and methods

Subjects

The study was conducted in 2005 and 2006 in Barabanki district, 32 km from Lucknow (latitude 26·8° N). The majority of the families in this economically undeveloped community did not own land and worked as tenant farmers. Six villages having a combined population of 8270 were surveyed. Thirteen per cent of the population was Muslim and the rest Hindu. Demographic listing of every household yielded 671 girls in the age group 10–20 years. Based on a prevalence of vitamin D deficiency of 85% obtained in a previous study from our region,[1] a sample size of 152 was calculated to be sufficient to estimate the prevalence within 5% of the true value, with 95% confidence. Of the selection by computer-generated random numbers, 121 gave consent for the study. Thirty-four brothers (age 10–20 years) of 28 girls recruited during a single month in the winter season (December) were also studied in the same month. One hundred and fifty consecutive pregnant women registered with the local community female health worker were invited during the second trimester to participate in the study. One hundred and thirty-nine gave their consent. Responders and nonresponders did not differ in age, religion and family size. Among pregnant women, the two groups also did not differ in parity.

History was obtained for bone pain and weakness, and examination performed for proximal muscle weakness (difficulty in climbing stairs, inability to rise from squatting position), bone tenderness and deformities. Written informed consent was obtained from pregnant women and parents of the adolescent subjects, as well as assent of the latter. Ethics committee approval for conducting the study was obtained from both institutions.

Diet and sunlight exposure

A food frequency questionnaire was used for calculation of dietary calcium intake. The dietary pattern of rural, poor socioeconomic group Indians is remarkably monotonous from meal to meal and day to day, the only variety being provided by seasonal availability of vegetables and fruit.[10,11] The staple cereals in this region are rice and wheat.[10,11] Milk and milk products are prohibitively expensive; even if the farmers own cattle, they prefer to sell milk and are unable to use it for consumption by the family. Vitamin D intake was not calculated because a preliminary survey revealed a very low consumption of foods containing reasonable amounts of vitamin D (fatty fish such as salmon, herring and sardine, fish liver oil or eggs).

History was sought regarding the clothes worn in summer and winter, and the pattern of daily activities (for example, time spent walking to and from school, number of outdoor classes, time spent in the field, feeding cattle, etc.). These were also observed by field staff and recorded on a proforma. Sun exposure between 1000 and 1600 h was calculated separately for summer and winter months. The percentage of body surface area exposed to sunlight was calculated according to Wallace's rule of nine. Women, older girls and older boys exposed the face, forearms and hands during summer, and only face and hands during winter. Younger boys and girls also exposed their legs. Our calculation was not able to account for the different obliquity of incident rays of the sun on different body parts such as the face *vs.* the limbs.

Biochemical analysis

Blood samples were transported on ice to our laboratory. Biochemical analysis was performed within 24 h and serum was stored at –20 °C for future analysis of 25OHD. Serum samples of pregnant women were heated at 65 °C to exclude heat-stable placental alkaline phosphatase (AP). The normal upper limit of AP for adolescents was taken as three times the upper limit for adult women.

Serum 25OHD was measured by radioimmunoassay kit (Diasorin, Stillwater, MN). The sensitivity of this assay is 3·8 nmol/l. The interassay coefficient of variation (CV) is 9·4% at 21·5 nmol/l and 11·0% at 122·5 nmol/l. Intra-assay CV is 11·7% at 21·5 nmol/l and 12·5% at 122·5 nmol/l. Vitamin D deficiency was defined as serum 25OHD < 50 nmol/l. As there is still debate in the literature as to the definition of normal values in pregnancy, the same value was taken to define normalcy in pregnancy as for the adolescent group, for ease of comparison.

Statistical analysis

Data are presented as mean ± SD or median and interquartile range. Statistical analysis was performed using SPSS software (version 10·0). Body mass index (BMI) of adolescent girls was converted to standard deviation or *z*-scores, using a reference base of well-nourished Indian children and adolescents.[12] The Kruskall–Wallis test was used for comparison of means of multiple groups, the Mann–Whitney *U*-test and the χ^2-test for intergroup comparisons and Spearman's rank test for correlation. To test for the association of different variables with serum 25OHD, a stepwise multiple linear regression analysis was performed separately for pregnant women and adolescent girls. In the case of variables that were not normally distributed, log transformation was performed prior to analysis.

For the comparison of girls with their brothers, a generalized linear mixed effects model (using SAS version 9·1; SAS Institute, Cary, NC), taking into consideration family clustering, was used to examine differences of age, pubertal stage, calcium intake, sun exposure and serum 25OHD. The same model was used to examine for association between serum 25OHD and age, pubertal status, summer sun exposure, winter sun exposure, and daily calcium intake, separately in boys and girls and in the combined group.

A two tailed *P*-value < 0·05 was taken as significant.

Results

The clinical and biochemical features of adolescent and pregnant subjects are shown in Table 1. The age-adjusted population prevalence of vitamin D deficiency (serum 25OHD < 50 nmol/l) in 10–20-year-old girls according to the 2001 census figures of Barabanki district was 88·6%. Thirty-four per cent had 25OHD < 25 nmol/l. Serum 25OHD was 33·3 ± 16·0 nmol/l. Fifty-six adolescent girls (46%) complained of bone pain, but none had proximal muscle weakness or bone deformities. There was no difference in mean 25OHD between girls with BMI *z*-score > –1·0 or ≤ –1·0 (32·3 ± 16·5 nmol/l, *vs.* 35·0 ± 14·8 nmol/l, respectively, $P = 0{\cdot}3$). Thirty-two per cent of pregnant women had 25OHD < 25 nmol/l and mean 25OHD was 37·8 ± 19·8 nmol/l. Biochemical osteomalacia was present in 43%. None had clinical osteomalacia. Calcium intake was uniformly low in both groups. On univariate analysis, summer and winter sunshine had significant correlations with serum 25OHD, both in girls ($r = 0{\cdot}34$, $P < 0{\cdot}001$, for summer and $r = 0{\cdot}33$, $P < 0{\cdot}001$ for winter sun) and in pregnant women ($r = 0{\cdot}28$, $P < 0{\cdot}001$, for summer and $r = 0{\cdot}36$, $P < 0{\cdot}001$ for winter sun). Serum AP had a weak negative correlation with 25OHD in adolescent girls ($r = -0{\cdot}18$, $P = 0{\cdot}054$)

Table 1. Clinical and biochemical features in adolescent and pregnant women

	Adolescent girls (n = 121)	Pregnant women (n = 139)
Age (years)	14·3 ± 2·7	26·7 ± 4·1
Calcium intake (mg/day)	211 ± 158	214 ± 150
Summer sun exposure (h/day × % BSA*)	32·4 ± 21·9	35·4 ± 15·9
Winter sun exposure (h/day × % BSA)	29·9 ± 19·8	26·8 ± 8·1
Serum calcium (mmol/l)†	2·17 ± 0·22	2·29 ± 0·22
Serum inorganic phosphorus (mmol/l)‡	1·46 ± 0·3	1·2 ± 0·28
Serum alkaline phosphatase (U/l)§	524 ± 287	246 ± 126
Serum 25OHD (nmol/l)¶	33·3 ± 16·0**	37·8 ± 19·8
Vitamin D deficiency††	107 (88)	103 (74)
Biochemical osteomalacia‡‡	33 (27)	59 (43)

Data expressed as mean ± SD and n (%).
*BSA, body surface area; †normal serum calcium 2·0–2·5 mmol/l; ‡normal serum phosphorus 0·9–1·5 mmol/l; §normal serum alkaline phosphatase (AP) > 720 U/l in adolescent girls and heat-labile AP > 240 U/l in pregnant women; ¶normal serum 25-hydroxyvitamin D (25OHD) 50–375 nmol/l; **25OHD median (interquartile range) for adolescent girls 29·5 (23–43) nmol/l and for pregnant women 32·3 (22·7–50·1) nmol/l; ††25OHD < 50 nmol/l; ‡‡serum AP > 720 U/l in adolescent girls and heat-labile AP > 240 U/l in pregnant women.

Table 2. Clinical and biochemical characteristics of boys compared to sisters from same family, all examined in a single month (December)

	Boys (n = 34)	Girls (n = 28)	P-value
Age (years)	14·0 ± 3·0	14·4 ± 2·7	0·67
Pubertal staging	3·0 ± 1·5	3·7 ± 1·2	0·06
Calcium intake (mg/day)	384 ± 600	198 ± 159	0·11
Summer sun exposure (h/day × % BSA*)	49·1 ± 15·7	30·5 ± 20·7	< 0·001
Winter sun exposure (h/day × % BSA)	32·8 ± 6·1	31·4 ± 22·3	0·79
Serum 25OHD (nmol/l)	67·5 ± 29·0†	31·3 ± 13·5	< 0·001
Vitamin D deficiency‡	9 (27)	25 (89)	< 0·001
Biochemical osteomalacia	11 (32)	7 (25)	0·6

Data expressed as mean ± SD and n (%). Comparison of means by generalized linear mixed effects model. *BSA, body surface area; †median (interquartile range) 59 (48·3–84·8) nmol/l for boys and 29 (21·3–42·3) nmol/l for girls; ‡25OHD < 50 nmol/l.

and BMI with 25OHD in pregnant women (r = –0·17, P = 0·044). No correlation was found between 25OHD and calcium intake in either group and between 25OHD and BMI z-scores in adolescent girls. In a stepwise multiple linear regression model including age, BMI, parity, calcium intake and summer and winter sun exposure in pregnant women, summer sun exposure was the only independent parameter associated with serum 25OHD level (R^2 = 0·13, P = 0·000, coefficient B = 0·52, 95% confidence limits for B = 0·25–0·78). Similarly in girls, in a model including age, BMI z-score, pubertal staging, calcium intake and summer and winter sun exposure, summer sun exposure was the only independent parameter associated with serum 25OHD (R^2 = 0·197, P = 0·000, coefficient B = 0·3, 95% confidence limits for B = 0·19–0·42).

In the families of 28 girls recruited during December, there were 34 brothers between 10 and 20 years of age. These 28 girls were compared with the 34 boys. Calcium intake was higher in the boys, although the difference did not reach statistical significance (Table 2). Summer sun exposure was significantly greater in boys than in their sisters. Vitamin D insufficiency and severe deficiency were less common in boys. Serum 25OHD < 25 nmol/l was present in 3% boys *vs.* 36% girls (P < 0·001). In a generalized linear mixed effects model (which takes into consideration family clustering), including gender, age, pubertal staging, calcium intake and summer and winter sun exposure, gender remained the only significant predictor of serum 25OHD (Table 3).

Table 3. Generalized linear mixed effects model for association of various parameters with serum 25OHD in girls and boys of the same families

Variable	F-value	P-value
Gender	24·86	< 0·0001
Age	0·37	0·547
Pubertal stage	1·61	0·207
Calcium intake	1·72	0·203
Summer sun exposure	0·05	0·818
Winter sun exposure	0·21	0·653

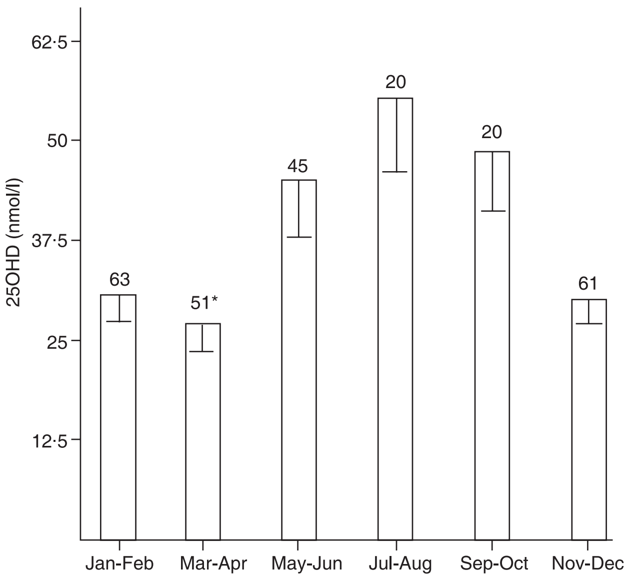

Fig. 1 Bar diagram depicting mean ± SD serum 25OHD of adolescent girls and pregnant women ($n = 260$) during various months of the year. The number of subjects sampled in each pair of months is depicted above each bar. *$P < 0·001$ *vs.* mean 25OHD in July–August.

Serum 25OHD varied significantly between summer and winter. The peak occurred during May to October and a trough during November to April (55·5 ± 19·8 nmol/l *vs.* 27·3 ± 12·3 nmol/l, $P < 0·001$) (Fig. 1). The prevalence of vitamin D deficiency was also significantly different during summer and winter (54% and 93%, respectively, $P < 0·001$).

Discussion

Our study provides the first Indian rural community prevalence data on vitamin D deficiency in adolescent girls and pregnant women, and reveals a high prevalence in both groups. The only other large study from India on the prevalence of vitamin D deficiency in adolescents was in urban subjects from New Delhi.[2] Serum 25OHD was < 50 nmol/l in about 90% of school-going adolescents and < 22·5 nmol/l in 36%. This study did not comment on the season in which the estimations were carried out. The study from Tirupathi revealed a large burden of vitamin D deficiency in adults from both urban and rural areas.[3] The sample size of subjects studied here was large. However, the urban sample of 943 subjects was a convenience sample, and for the rural sample of 205 subjects, there were no details of the size of the population from which this sample was drawn or the sampling method used.

A new finding from the present study was a marked seasonal variation in serum 25OHD. The only other study from a similar latitude commenting on a winter trough of 25OHD was from New Delhi, which reported a small group of 19 subjects to have significantly lower 25OHD during winter.[4] The peak daily energy from UV-B radiation falling on Delhi (on a similar latitude to Lucknow) is reported to be three times weaker in winter than in summer and the monthly dose about 8–10 times lower.[13] Apart from the temperate countries of northern Europe, where a strong seasonal variation in vitamin D would be expected, a few reports from Middle Eastern countries (Teheran, latitude 35° N and Beirut 33·5° N)[14,15] and Athens, Greece, 38° N have appeared in recent years,[16] commenting on seasonal variation in serum 25OHD. Our results bear relevance to the results of studies on vitamin D from regions around the tropics.[17]

Our findings lead to the question: how much UV exposure is necessary for Indians to form sufficient vitamin D? Previous experiments suggest that Indians require about three times the UV exposure as white Caucasians to produce the same amount of vitamin D.[18] Matsuoka *et al.*,[19] working with Oriental, Indian, Caucasian and Black volunteers of Fitzpatrick skin types II to VI, have also shown Caucasian skin to produce approximately 2·5 times the amount of circulating vitamin D as Indian skin exposed to the same amount of UV energy. Furthermore, it has been shown that whole-body exposure of Caucasian skin to one minimal erythemal dose of UV energy produces an amount of circulating vitamin D equivalent to an oral dose of 10 000 units of vitamin D.[20] Exposure to the noon sun in summer at Boston (latitude 42° N) produces minimal erythema after 15 min in Caucasian skin.[20,21] Thus, our women should require about 45 min to 1 h of whole-body sun exposure to produce the same amount of vitamin D, and are likely to produce 800 units per day when exposing only about 8% skin surface. Holick *et al.*[18] have also shown that the time to maximum previtamin D formation is 0·75–1·5 h in darker (type V) skin, in contrast to 0·25–0·5 h in fairer (type III A) skin, during exposure to equatorial amounts of UV energy. As previtamin D is formed in the skin, isomerization to inactive products also takes place simultaneously, resulting in removal of vitamin D during prolonged sun exposure. Furthermore, all the exposure (of 2–4 h per day) was not occurring in our subjects at noon, the peak time of UV energy. For all of these reasons, the formation may have been even less than 800 units per day. Such an amount may be unlikely to maintain adequate circulating levels of 25OHD.[22]

Another explanation for vitamin D deficiency in rural subjects could be their low dietary calcium intake. Dietary calcium deficiency leading to secondary vitamin D deficiency has been shown in experimental studies.[6] Small reports on humans with calcium deficiency rickets have also shown improvement in serum 25OHD with calcium replenishment.[5,23] Rural girls from Beijing (latitude 40° N), despite higher UV exposure than urban girls, had significantly lower 25OHD.[24] Similarly, lower socioeconomic group children from Delhi had significantly lower serum 25OHD than higher socioeconomic group children.[2] Both investigators attributed this finding to the lower calcium intake in rural and lower socioeconomic group children, respectively.

Serum 25OHD in boys was significantly higher than that in their sisters. Similar results have been published from urban India,[2] Lebanon[15] and Iran.[25] The confounding factors in these studies of season of sampling[2] or the use of burqas[15,25] were not present in our study. In rural India, boys are allowed greater freedom to be outdoors and preference in terms of diet. These social factors could impact upon vitamin D status.

The present study has certain limitations. Only 80% of the sample size calculated to give a true estimate of the prevalence of vitamin D deficiency among adolescent subjects consented for the study. The food frequency questionnaire and the activity questionnaire had not been previously validated. However, the dietary pattern of low socioeconomic group people in rural India remains remarkably similar from day to day, the only variety being brought about by seasonal changes in availability of foods. The record of sun exposure was not detailed enough to differentiate between exposure at noon (the period of strongest UV energy) *vs.* that at other times of the day. The seasonality report is from cross-sectional data, not a comparison of paired samples in summer and winter. PTH values were not recorded because of operational constraints. Nevertheless, intact PTH was reported by us in a previous study from the same region to increase at 25OHD < 50 nmol/l. An inverse correlation between maternal 25OHD and PTH ($r = -0{\cdot}35$, $P < 0{\cdot}001$) was also shown.[1]

In conclusion, we report a high prevalence of vitamin D deficiency among pregnant women and adolescent girls in an Indian rural community, which calls for public health attention. For the first time from India, a large study shows marked seasonal variation in circulating 25OHD, which has implications not only for therapy but also for the interpretation of future studies from similar latitudes.

Acknowledgements

Grant support BT/PR 3552/SPD/11/349/2002 (to V.B.) was received from the Department of Biotechnology, Government of India. We gratefully acknowledge the help of Canlan Sun, Division of Population Sciences, City of Hope National Medical Center, Duarte, CA, USA, for performing the generalized mixed effects model linear regression analysis; the expert technical assistance of P. K. Awasthi and Intsar Ahmed for laboratory analyses; and the assistance of Arjun Singh for studies in the community.

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