High prevalence of vitamin D deficiency among pregnant women and their newborns in northern India[1,2]

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ABSTRACT

Background: Vitamin D deficiency is prevalent in India, a finding that is unexpected in a tropical country with abundant sunshine. Vitamin D deficiency during pregnancy has important implications for the newborn and infant. There are few data from India about the prevalence of hypovitaminosis D in pregnancy and in the newborn.

Objective: Our aim was to determine the prevalence of osteomalacia and hypovitaminosis D in pregnancy and in cord blood and to correlate maternal 25-hydroxyvitamin D [25(OH)D] status with sun exposure, daily calcium intake (dietary plus supplemental), and intact parathyroid hormone (PTH) concentrations.

Design: Serum calcium, inorganic phosphorus, 25(OH)D, heat-labile alkaline phosphatase, and PTH were studied in 207 urban and rural pregnant subjects at term. Alkaline phosphatase and 25(OH)D were measured in the cord blood of 117 newborns.

Results: Mean maternal serum 25(OH)D was 14 ± 9.3 ng/mL, and cord blood 25(OH)D was 8.4 ± 5.7 ng/mL. PTH rose above the normal range when 25(OH)D was <22.5 ng/mL. Eighty-four percent of women (84.3% of urban and 83.6% of rural women) had 25(OH)D values below that cutoff. Fourteen percent of the subjects had elevated alkaline phosphatase (17% of urban and 7% of rural subjects). Calcium intake was uniformly low, although higher in urban (842 ± 459 mg/d) than in rural (549 ± 404 mg/d) subjects ($P < 0.001$). Maternal serum 25(OH)D correlated positively with cord blood 25(OH)D ($r = 0.79$, $P < 0.001$) and negatively with PTH ($r = -0.35$, $P < 0.001$).

Conclusion: We observed a high prevalence of physiologically significant hypovitaminosis D among pregnant women and their newborns, the magnitude of which warrants public health intervention. *Am J Clin Nutr* 2005;81:1060–4.

KEY WORDS Vitamin D, pregnancy, osteomalacia, parathyroid hormone, newborn, sunlight, dietary calcium

INTRODUCTION

Vitamin D deficiency is unexpected in a tropical country such as India, where there is abundant overhead sun for most or all of the year. Nevertheless, hypovitaminosis D, resulting in severe osteomalacia, has been observed in adolescents in India (1). This paradox may be partly explained by the many prevalent social and cultural practices in India that preclude adequate exposure of adolescent girls and young women to sunshine. Revealing clothing is frowned on in traditional Indian households, both rural and urban. Newly married females are expected to cover themselves even more and are discouraged from outdoor activity. Increasing urbanization that results in poor outdoor activity and greater pollution, coupled with skin pigment, may further compound this problem (2).

Furthermore, milk, the primary source of calcium, is an expensive food in India. Deficient calcium intake has been shown to be the cause in a large proportion of childhood rickets in India (3) and other tropical countries (4, 5) and to contribute to adolescent osteomalacia (1, 3). Dietary calcium replenishment produced healing of rickets independent of vitamin D in those rickets patients with normal serum 25-hydroxyvitamin D [25(OH)D] concentrations (3, 4). Experimental studies in a rat model showed that dietary calcium deficiency caused secondary vitamin D deficiency and that calcium replenishment improved serum 25(OH)D concentrations (6). It is possible that the same mechanism may be active in human calcium-deficiency rickets or osteomalacia.

In a population that already has a high prevalence of vitamin D deficiency and poor dietary calcium intake, the problem is likely to worsen during pregnancy because of the active transplacental transport of calcium to the developing fetus. Hypovitaminosis D during pregnancy has important consequences for the newborn, including fetal hypovitaminosis D, neonatal rickets and tetany, and infantile rickets (7, 8). Rickets during infancy has been associated with higher prevalence of lower respiratory tract infections (9), the largest cause of infant mortality in India.

There are few data on serum 25(OH)D concentration and the prevalence of osteomalacia among pregnant women from India (10, 11). This study was undertaken to determine the prevalence of clinical or biochemical osteomalacia and maternal and fetal hypovitaminosis D among urban and rural northern Indian women and to study the correlation of those prevalences with calcium intake, sun exposure, serum 25(OH)D, and plasma intact parathyroid hormone (PTH).

SUBJECTS AND METHODS

Subjects

Pregnant women were recruited from Queen Mary's Hospital, King George Medical University, Lucknow (lat 26.8°N), which

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caters to predominantly low and middle socioeconomic groups. The hospital serves both women who have never had antenatal care, who are predominantly from the villages surrounding the city of Lucknow, and women who have had regular antenatal care at the hospital itself, who are predominantly urban women. We attempted to recruit all women with a full-term live pregnancy who presented to the hospital in a 3-mo period from September to November 2002 and their infants. Exclusion criteria were chronic liver disease, renal disease, or treatment with antitubercular or antiepileptic drugs in the previous 3 mo. Because of a shortage of labor room staff, we were able to register only 207 of the 572 women who had term live newborns during the 3-mo study period. One hundred fifty-seven subjects were Hindu, and 50 were Muslim. Of the Muslim subjects, 29 women from both the urban and rural areas practiced purdah, in which the veil covers the whole body except hands and face.

Detailed history and examination were performed with special regard to current and past pregnancies and labor, socioeconomic status, and clinical features suggestive of osteomalacia (eg, proximal muscle weakness, bone pain, tenderness, or fractures) or past rickets. Daily intake of dietary calcium and vitamin D was calculated from a food-frequency questionnaire. The food-frequency questionnaire was validated for calcium in a sample of 30 subjects against a 5-d diet record (D Pandey, unpublished observations, 2004). A strong correlation of dietary calcium estimation was observed between the food-frequency questionnaire and the diet record ($r = 0.653$, $P < 0.001$). Any supplemental calcium intake in the current pregnancy was also noted. Daily sun exposure was assessed by taking a detailed history of the daily routine separately during summer and winter seasons and of the type of clothing worn. Sunshine exposure was calculated as hours of exposure/d × percentage of body surface area (BSA) exposed. Birth weight, crown-heel length, largest diameter of anterior fontanelle, and head circumference of newborns were examined.

Oral informed consent was obtained from all subjects. Approval from the institutional ethics committee was obtained.

Biochemical analysis

Maternal blood was collected in the nonfasting condition before labor and immediately transported on ice to the Sanjay Gandhi Institute for assay within 24 h for serum alkaline phosphatase (AP), calcium, albumin, and phosphorus. Sera were stored at −70 °C for future analysis of serum 25(OH)D and PTH. Cord blood samples ($n = 117$) were similarly processed for AP activity and serum 25(OH)D.

Serum total calcium, albumin, and inorganic phosphorus were analyzed spectrophotometrically (Sigma Diagnostics, St Louis, MO). Serum calcium was corrected for serum albumin. Serum AP was measured spectrophotometrically (Boehringer Mannheim, Mannheim, Germany). To exclude placental isoenzyme (stable after heating for 15 min at 56 °C), heat-labile AP (HLAP) was analyzed (12). The normal upper limit for maternal HLAP was taken as that for total AP in our laboratory for an adult population (125 U/L), and the normal upper limit for cord blood AP was taken as 165 U/L (13). Serum 25(OH)D was assayed by using a commercial radioimmunoassay kit (Diasorin, Stillwater, MN). The sensitivity of this assay is 1.5 ng/mL, and the total imprecision CV is 8.2% at 22.7 ng/mL. Although the reference range given by the manufacturer of the assay is 9–38 ng/mL, those values represent a small number of subjects living in temperate latitudes and do not necessarily represent a true normal range for 25(OH)D. On the basis of physiologic correlates such as PTH, that range is more likely to be 20–80 ng/mL (14–17). The normal range of cord blood 25(OH)D was similarly taken as 20–80 ng/mL. Plasma PTH assay was performed by using a commercial immunoradiometric assay kit (normal range: 9–55 pg/mL; Diagnostic Systems Laboratories, Webster, TX). The sensitivity of this assay is 6 pg/mL, and the interassay CV is 10.5%.

TABLE 1
Clinical characteristics of registered and not registered subjects

	Registered ($n = 207$)	Not registered ($n = 365$)
Age (y)	24.0 ± 4.1[1]	24.7 ± 5.1
Weight at term (kg)	55.1 ± 6.5	52.5 ± 4.3
Parity	1.1 ± 1.2	1.8 ± 1.1[2]
Birth weight (kg)	2.9 ± 1.7	2.7 ± 2.0[2]
Hindu/Muslim	157/50	267/98

[1] $\bar{x}$ ± SD (all such values).
[2] Significantly different from registered subjects, $P < 0.001$ (Student's t test).

Statistical analysis

Data are presented as mean (±SD). Statistical analysis was conducted by using SPSS FOR WINDOWS software (version 9.0; SPSS, Chicago, IL). Proportions were compared by using the chi-square test. Group means were compared by using Student's t test. Nonparametric data were log transformed and compared by using Student's t test. Correlations were studied by using Spearman's correlation coefficient. To ascertain the 25(OH)D concentration below which PTH rose above the normal range, a linear regression analysis was performed. All complete pairs of values were used to derive a cutoff of 25(OH)D. Significance at $P \leq 0.05$ was taken for two-sided tests.

RESULTS

No difference between the subjects registered and those not registered was observed in age, weight at term, or religion (**Table 1**). However, the registered subjects had significantly lower parity and their newborns had significantly higher birth weight than did the nonregistered subjects and their newborns. None of the subjects had clinical evidence of osteomalacia, as defined by proximal muscle weakness and bony pains or tenderness. Biochemical osteomalacia (HLAP >125 U/L) was present in 29 subjects (14%). Subjects with biochemical osteomalacia had lower serum inorganic phosphorus and higher PTH than did women with normal HLAP (**Table 2**). However, maternal serum 25(OH)D, dietary calcium intake, and cord blood 25(OH)D did not differ significantly between the groups.

Maternal serum 25(OH)D <10 ng/mL was found in 88 women (42.5%), whereas 138 women (66.7%) had values <15 ng/mL. Plasma PTH was significantly higher (125 ± 153 and 51 ± 39 pg/mL, respectively; $P < 0.001$) and cord blood 25(OH)D was significantly lower (5.2 ± 3.0 and 11.8 ± 5.9 ng/mL, respectively; $P < 0.001$) in mothers with 25(OH)D concentrations <10 ng/mL than in mothers with 25(OH)D concentrations >10 ng/

TABLE 2
Differences in biochemical indexes and daily calcium intake among subjects with and without biochemical osteomalacia[1]

	Subjects with osteomalacia (n = 29)	Subjects without osteomalacia (n = 178)
Corrected serum calcium (mg/dL)	9.4 ± 0.6[2]	9.4 ± 0.7
Serum phosphorus (mg/dL)	3.6 ± 1.2	4.2 ± 1.7[3]
Serum 25(OH)D (ng/mL)[4]	12.1 ± 8.0	14.3 ± 9.5
Maternal hypovitaminosis D [n (%)][5]	26 (89.7)	148 (83.1)
Serum PTH (pg/mL)[6]	127 ± 180	74 ± 89[7]
Cord blood AP (U/L)[8]	172 ± 200	114 ± 61
Cord blood 25(OH)D (ng/mL)[4]	8.1 ± 7.4	8.5 ± 5.4
Daily calcium intake (mg/d)[9]	813 ± 435	737 ± 466
Daily calcium intake <RDA [n (%)][5,10]	22 (75.8)	138 (77.5)
Sun exposure score over past 3 mo (h/d × % BSA exposed)	4.9 ± 4.5	6.2 ± 6.0

[1] Biochemical osteomalacia = heat-labile alkaline phosphatase >125 U/L. 25(OH)D, 25-hydroxyvitamin D; PTH, parathyroid hormone; AP, alkaline phosphatase; RDA, recommended dietary allowance; BSA, body surface area.

[2] $\bar{x}$ ± SD (all such values).

[3,7] Significantly different from subjects with osteomalacia (Student's t test after log transformation of data and of proportions with chi-square test): [3]$P < 0.05$, [7]$P < 0.005$.

[4] Normal = 20–80 ng/mL.

[5] Hypovitaminosis D = < 22.5 ng 25(OH)D/mL.

[6] n = 157 (with osteomalacia, n = 24; without osteomalacia, n = 133). Normal = 9–55 pg/mL.

[8] Normal = < 165 U/L.

[9] Dietary plus supplemental calcium intake.

[10] RDA = 1200 mg/d.

mL. Maternal serum 25(OH)D showed a strong positive correlation with cord blood 25(OH)D (r = 0.79, $P < 0.001$) and a moderate negative correlation with maternal plasma PTH (r = −0.35, $P < 0.001$) (**Figure 1**). The regression equation between serum 25(OH)D and plasma PTH yielded a 25(OH)D value of 22.5 ng/mL, below which PTH rose beyond the upper limit of normal. Eighty-four percent of women had 25(OH)D concentrations <22.5 ng/mL. A weak correlation also existed between maternal HLAP and cord blood AP (r = 0.19, $P < 0.05$). Maternal serum 25(OH)D did not correlate with HLAP, sun exposure, or daily calcium intake.

A comparison of women of urban and rural backgrounds is shown in **Table 3**. Sun exposure was significantly lower in urban subjects than in rural subjects in the last trimester of pregnancy (urban: 4.1 ± 3.2 h/d × %BSA exposed; rural: 9.7 ± 8.1 h/d × %BSA exposed; $P < 0.001$) as well as over the previous year (urban: 7.5 ± 5.6 h/d ×%BSA exposed; rural: 11.6 ± 8.4 h/d × %BSA exposed; P = 0.005). Despite this finding, the mean serum 25(OH)D concentration in urban women did not differ significantly from that in rural women (urban: 14.0 ± 9.5 ng/mL; rural: 14.1 ± 8.9 ng/mL; NS). In contrast, the dietary calcium intake was significantly lower in rural than in urban women (549 ± 404 and 842 ± 459 mg/d, respectively; $P < 0.001$). Mean maternal PTH and HLAP were significantly higher in urban women. Total daily calcium intake, mean HLAP, 25(OH)D, and PTH did not differ significantly between women practicing purdah and women not practicing purdah.

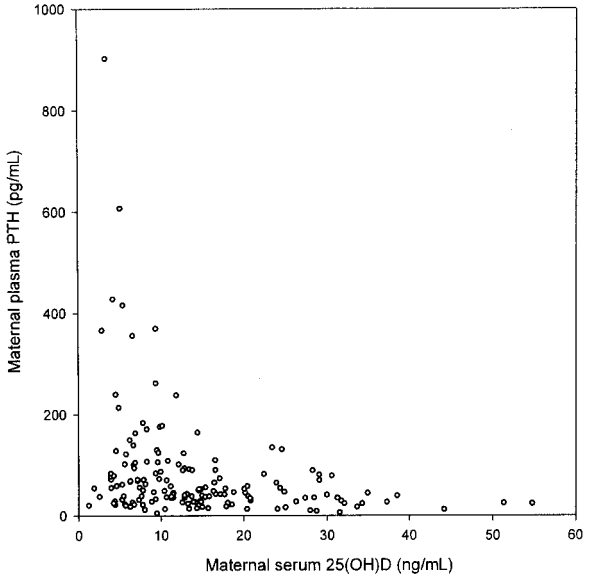

FIGURE 1. Scatter plot showing relation of intact parathyroid hormone (PTH) and serum 25-hydroxyvitamin D [25(OH)D] in mothers' blood (n = 157). Regression equation (by linear regression analysis): PTH = [−3.32 × 25(OH)D] + 129.76.

The mean cord blood 25(OH)D in neonates was low (8.4 ± 5.7 ng/mL). A large proportion of neonates (95.7%) had hypovitaminosis D [serum 25(OH)D <20 ng/mL]. Mean AP was 131

TABLE 3
Clinical characteristics and biochemical indexes of urban and rural women[1]

	Urban women (n = 140)	Rural women (n = 67)
Sun exposure score over past 3 mo (h/d × % BSA exposed)	4.1 ± 3.2[2]	9.7 ± 8.1[3]
Sun exposure score over past 1 y (h/d × % BSA exposed)	7.5 ± 5.6	11.6 ± 8.4[4]
Daily calcium intake (mg/d)[5]	842 ± 459	549 ± 40[3]
Daily calcium intake <RDA [n (%)][5,6]	101 (72)	59 (88)[7]
Daily vitamin D intake (IU/d)	16.4 ± 7.4	16.5 ± 7.7
HLAP (U/L)[8]	87 ± 60	73 ± 31
Elevated HLAP [n (%)][9]	24 (17)	5 (7)
Serum 25(OH)D (ng/mL)[10]	14.0 ± 9.5	14.1 ± 8.9
Maternal hypovitaminosis D [n (%)][11]	118 (84)	56 (84)
Maternal PTH (pg/mL)[12]	94 ± 127	57 ± 49

[1] BSA, body surface area; RDA, recommended dietary allowance; HLAP, heat-labile alkaline phosphatase; 25(OH)D, 25-hydroxyvitamin D; PTH, parathyroid hormone.

[2] $\bar{x}$ ± SD (all such values).

[3,4,7] Significantly different from urban women (Student's t test after log transformation of data and of proportions with chi-square test): [3]$P < 0.001$, [4]P = 0.005, [7]$P < 0.05$.

[5] Dietary plus supplemental calcium intake.

[6] RDA = 1200 mg/d.

[8] Normal = 30–125 U/L.

[9] Elevated HLAP = > 125 U/L.

[10] Normal = 20–80 ng/mL.

[11] Hypovitaminosis D = < 22.5 ng 25 (OH)D/mL.

[12] Normal = 9–55 pg/mL.

U/L, and 20% of neonates had elevated AP. Neonates born to mothers who were vitamin D deficient or sufficient did not differ in anthropometry or AP (data not shown). Similarly, neonates with normal or low 25(OH)D did not differ in these variables (data not shown).

DISCUSSION

Our study presents the first large body of data on serum 25(OH)D and PTH in pregnancy from a population not observing purdah in a tropical country. The most important finding in our study is the unexpectedly high prevalence of hypovitaminosis D among pregnant women. The physiologic relevance of the finding is substantiated by the negative correlation with PTH and the positive correlation with cord blood 25(OH)D. Hypovitaminosis D and osteomalacia among pregnant South Asian women have been widely reported (10, 11, 18–25). However, all studies but a few (ie, 10, 11, 22, 23) were from temperate regions such as the United Kingdom (18–21, 24) and Norway (25), where the already low availability of overhead sun is compounded for Asian women by poor outdoor activity, pigmented skin, and excessive clothing. Vitamin D deficiency has also been noted in pregnant women in tropical countries, but all studies were in Muslim populations, in whom the practice of purdah might have played an important role (22, 23, 26–29). The only study to comment on serum 25(OH)D concentrations in pregnant non-Muslim women living in the tropics is from New Delhi (11), where the mean concentration in summer in 25 women was 21.9 ± 10.7 nmol 25(OH)D/L (8.6 ± 4.28 ng/mL).

We expected to find a higher serum 25(OH)D concentration in the rural women in our study than in their urban counterparts, who had distinctly poorer sun exposure. However, the results were contrary to expectation, with urban and rural women having equally low mean serum concentrations and equally high prevalence of the deficiency. The explanation could lie in the prolonged deficiency of dietary calcium intake among poorer parts of India (where most of the rural women in our study lived), because of the expensive nature of milk and milk products. Dietary calcium deficiency has been shown to lead to secondary vitamin D deficiency in rats (6). Similar findings are also suggested in studies on humans (3, 4). Our own studies among children with rickets and adolescent girls with rickets or osteomalacia who were from a lower socioeconomic population showed the average daily dietary calcium intake in these 2 groups to be 282 mg and 305 mg, respectively (1, 3). The higher intake of dietary calcium in the women in our study is likely to have been short-lived and attributable to the social custom of providing extra milk to pregnant and lactating women. Further studies are needed to document direct evidence of improvement in serum 25(OH)D with calcium supplementation in large numbers of subjects in our region.

Exactly how much sun exposure is needed for healthy people to maintain normal serum 25(OH)D is not clear. It would, of course, depend on latitude, season, skin pigment, and age. On the basis of his own studies, Holick (30) recommended that suberythemal exposure of face, arms, and hands (ie, ≈22% BSA) ≈3 times a week is probably sufficient for elderly people living in a temperate climate to maintain serum 25(OH)D at 20 ng/mL. It would be expected, then, that a similar amount of vitamin D should form in the skin of the women in our study, who were younger and lived in a more tropical latitude, and who exposed ≈11% of their BSA to sun for 1 h/d. In addition to the possible contribution of darker skin pigment and prolonged low intake of dietary calcium, the high amount of atmospheric pollution extant in Indian cities, including Lucknow, could be an important factor (2, 31).

The cutoff of 10 ng 25(OH)D/mL, which we used a priori for defining hypovitaminosis D, is conservative. The availability of simultaneous PTH and serum 25(OH)D allowed us to examine the relation between these 2 hormones. Most investigators now suggest higher values of 25(OH)D, eg, 15–30 ng/mL, as the cutoff below which PTH starts to rise sharply (14–16, 32). Investigators who used other surrogate markers such as intestinal calcium absorption and bone mineral density suggested 25(OH)D concentrations as high as 98 nmol/L (39.2 ng/mL) to define normalcy (33, 34). In our study also, the corresponding 25 (OH)D value was 22.5 ng/mL. Accordingly, 84% of the women in our study would be declared vitamin D–deficient.

Cord blood 25(OH)D strongly correlated with maternal values, which is in keeping with reports in the literature (19, 35–37). The cutoff for hypovitaminosis D in neonates is still being debated. No evidence suggests that neonatal 25(OH)D concentrations are different from those in adults. Zeghoud et al (36) found neonatal 25(OH)D concentrations <30 nmol/L (12 ng/mL) to be associated with elevated PTH, and they proposed that concentration as the cutoff for diagnosing hypovitaminosis D in the newborn. We were unable to study the status of neonatal calcium and PTH. However, on the basis of what is known in the literature, we can conclude that a large proportion of our newborns have 25(OH)D concentrations that will predispose them to neonatal hypocalcemia and infantile rickets and to the attendant morbidity (8, 38, 39).

In the current study, 14% of the mothers had elevated HLAP (which indicated biochemical osteomalacia), as did 14% of the newborns. Although none of these women had clinical features suggestive of osteomalacia, the biochemical profile (ie, low serum phosphorus and elevated PTH) is that typically seen in osteomalacia. Brooke et al (19) reported elevation of HLAP in 20% of Asian subjects from the United Kingdom with serum 25(OH)D concentrations <25 nmol/L (10 ng/mL), whereas only 2% of those who had serum 25(OH)D concentrations >25 nmol/L had elevated HLAP. Rab and Baseer (22) from Pakistan reported elevated total AP in 26% of pregnant women. Daily vitamin D intake was low (88 ± 14 IU/d) in their subjects, but serum 25(OH)D was not measured. Marya et al (10) from India reported elevated HLAP in 13% and hypocalcemia in 44% of their pregnant subjects who were not receiving vitamin D supplementation, whereas none of the subjects supplemented with vitamin D (600 000 IU twice in the 7th and 8th mo of gestation) had elevated HLAP. That study also did not comment on serum 25(OH)D.

At present, vitamin D supplementation is not a part of antenatal care programs in India. The US National Academy of Sciences mentions 400 IU as the dietary reference intake for vitamin D during pregnancy. However, several investigators worldwide are arguing for revised higher guidelines for vitamin D allowance during pregnancy and lactation (40). So far, the concern expressed by those investigators is mainly for women in temperate climates, especially those with greater skin pigmentation, and for women living in tropical regions but observing purdah, such as those in the Middle East. On the basis of our results, we conclude

that such recommendations perhaps are also warranted for pregnant Indian women not practicing purdah, so that they may remain healthy and provide adequate vitamin D to their fetuses. The exact cause of or factors contributing to the occurrence of hypovitaminosis D in rural women in a tropical country remain to be elucidated in future studies.

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