Vitamin D status in pregnant Indian women across trimesters and different seasons and its correlation with neonatal serum 25-hydroxyvitamin D levels

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Abstract
The present cross-sectional study was conducted to determine the vitamin D status of pregnant Indian women and their breast-fed infants. Subjects were recruited from the Department of Obstetrics, Armed Forces Clinic and Army Hospital (Research and Referral), Delhi. A total of 541 apparently healthy women with uncomplicated, single, intra-uterine gestation reporting in any trimester were consecutively recruited. Of these 541 women, 299 (first trimester, ninety-seven; second trimester, 125; third trimester, seventy-seven) were recruited in summer (April–October) and 242 (first trimester, fifty-nine, second trimester, ninety-three; third trimester, ninety) were recruited in winter (November–March) to study seasonal variations in vitamin D status. Clinical, dietary, biochemical and hormonal evaluations for the Ca–vitamin D–parathormone axis were performed. A subset of 342 mother–infant pairs was re-evaluated 6 weeks postpartum. Mean serum 25-hydroxyvitamin D (25(OH)D) of pregnant women was 23·2 (SD 12·2) nmol/l. Hypovitaminosis D (25(OH)D, < 50 nmol/l) was observed in 96·3 % of the subjects. Serum 25(OH)D levels were significantly lower in winter in the second and third trimesters, while serum intact parathormone (iPTH) and alkaline phosphatase levels were significantly higher in winter in all three trimesters. A significant negative correlation was found between serum 25(OH)D and iPTH in mothers ($r^2 = 0·367, P = 0·0001$) and infants ($r^2 = 0·56, P = 0·0001$). A strong positive correlation was observed between 25(OH)D levels of mother–infant pairs ($r = 0·779, P = 0·0001$). A high prevalence of hypovitaminosis D was observed in pregnancy, lactation and infancy with no significant inter-trimester differences in serum 25(OH)D levels.

Key words: Pregnancy; Trimesters; Mother–infant pairs; Serum 25-hydroxyvitamin D; Parathormone

High prevalence of hypovitaminosis D has been established in all age groups across the world (1). The problem of hypovitaminosis D is likely to worsen during pregnancy because of the active transplacental transport of Ca to the developing fetus. Mother–offspring studies in Western populations have confirmed that optimal vitamin D supply not only influences the course of pregnancy, but is also required for fetal and neonatal Ca homeostasis, bone maturation and mineralisation (2–6). Breast-fed infants born to vitamin D-deficient mothers are at risk for developing vitamin D deficiency and its metabolic sequelae (7–12). Divergent data on the status of 25-hydroxyvitamin D (25(OH)D) levels in different trimesters of pregnancy are available, with different investigators reporting either a decline (13) or an increase (14) or absence of change with progression of pregnancy (15,16). Furthermore, most studies have evaluated mothers in the third trimester and correlated their serum vitamin D levels with the newborn’s cord blood 25(OH)D levels (17–19). In view of the aforementioned facts, we have (1) evaluated maternal 25(OH)D levels in different trimesters, (2) assessed the impact of seasonal variation on serum vitamin D status, and (3) correlated maternal and newborn vitamin D status by concurrent evaluation of serum 25(OH)D levels in mother–infant pairs at 6–8 weeks postpartum.

Methods
Setting
Subjects were recruited between April 2006 and October 2007, from the obstetrics outpatient department of the Armed Forces...
Clinic and Army Hospital (Research and Referral), Delhi, which is a primary care provider for families of armed forces personnel currently residing in Delhi. The present study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving human subjects/patients were approved by the Institutional Human Ethics Committee at Army Hospital (Research and Referral). Written informed consent was obtained from all subjects/patients.

Subjects

Healthy women (n 541) with uncomplicated, single, intrapartum gestation in any trimester were consecutively recruited, and anthropometric, nutritional, biochemical and hormonal investigations were carried out once at the time of first contact. All women who were approached agreed to participate in the study. These women, all of whom were housewives, belonged to lower–middle socio-economic strata, with 85 % having completed 12 years of schooling. Fasting blood samples were drawn without venostasis under basal conditions. Serum was separated in a cold centrifuge, and three aliquots were made, one of which was used immediately to measure ionised and total Ca, inorganic P and serum alkaline phosphatase (ALP), while the other two were stored at −80°C for assessing 25(OH)D and intact parathormone (iPTH). Women with any chronic hepatic or renal illness, malabsorption syndrome, medications (current and past) and vitamin supplements that can affect the Ca–vitamin D–parathyroid hormone axis were excluded from the study.

All women who completed their pregnancy were invited 6–8 weeks postpartum for clinical and biochemical evaluation of mother–infant pairs. Of the 541 recruited pregnant women, only 342 mother–infant pairs could be studied. The remaining mothers were unavailable for comment, as they had gone back to their native villages after delivery, which is a common local tradition.

Hormonal assays

Serum 25(OH)D was measured by RIA using a commercial kit (Diasorin, Stillwater, MN, USA). The normal range for 25(OH)D was 22·5–92·5 nmol/l (9–37 ng/ml), with analytical sensitivity being 3·75 nmol/l (1·5 ng/ml). Serum iPTH was measured by immunoradiometric assay with a commercial kit (Diasorin). The normal range for iPTH was 13–54 pg/ml, with analytical sensitivity being 0·7 pg/ml. Commercial kits (Roche Diagnostics GmbH, Mannheim, Germany) were used to measure serum Ca, P and ALP. Total Ca was estimated by the colorimetric method. The normal range for total Ca was 2·24–2·74 mmol/l (90–110 mg/ml) in infants (2–2 years old) and 2·09–2·54 mmol/l (84–102 mg/ml) in adults, with analytical sensitivity being 2·mg/l. Serum P and ALP were determined by photometric analysis. The normal range for P was 0·97–2·25 mmol/l (30–70 mg/ml) in infants and 0·87–1·45 mmol/l (27–45 mg/ml) in adults, with analytical sensitivity being 3 mU/l. The normal upper limit of ALP was 107·61U/l in infants and ≤240 U/l in non-pregnant women. The analytical sensitivity of ALP was 5IU/l. Serum ionised Ca was estimated by the ion exchange method and its normal range was 1·12–1·32 mmol/l (44·8–5·280 mg/l) in adults and 1·125 mmol/l (40–50 mg/l) in infants. Vitamin D deficiency was classified using Lips criteria based on 25(OH)D levels as mild (25–50 nmol/l (10–20 ng/ml)), moderate (12·5–25 nmol/l (5–10 ng/ml)) and severe (<12·5 nmol/l (5 ng/ml)) hypovitaminosis D.

Dietary analysis

Nutrient intake was calculated using the 24 h dietary recall method. During pre-testing, three separate 24 h dietary recalls were recorded from fifty subjects (two on weekdays and one on a weekend). Since no difference was found between weekday and weekend intakes, only one 24 h dietary recall was taken during the final study. Detailed descriptions of foods consumed along with their quantities, as estimated by standardised household measures, were noted. Raw weights were then calculated and used to estimate nutrient intake using the Nutritive Value of Indian foods (National Institute of Nutrition, 2001).

Statistical analysis

Data were analysed using STATA-9.0 (Stata Corp LP, College Station, TX, USA). Descriptive statistics are expressed as numbers (percentages) or means and standard deviations/medians (ranges) as appropriate. Seasonal differences in biochemical parameters were tested using Student's t test and Wilcoxon's rank-sum test for non-normal data. Spearman’s rank correlation coefficient was used to determine the strength of the relationship between variables, since data were non-normal. P<0·05 was considered significant.

Results

The basic characteristics of women are given in Table 1. The mean age of pregnant women was 24·6 (SD 2·8) (range 19–30) years. The mean age at marriage was 20·3 (SD 1·5) years. There were 219 (40·5 %) women with their first pregnancy.

Vitamin D status of pregnant women

A total of 521 women (96·3 %) were found to be vitamin D deficient (25(OH)D ≤ 50 nmol/l), with 36·8, 41·8 and 17·7 % falling into the mild (25–50 nmol/l), moderate (12·5–25 nmol/l) and severe (<12·5 nmol/l) hypovitaminosis D categories, respectively. Mean serum total Ca, ionised Ca, P, alkaline phosphate, 25(OH)D and iPTH were 2·33 (SD 0·31), 1·19 (SD 0·23), 1·22 (SD 0·25), 182·07 (SD 4·05), 2·33 (SD 0·31) pmol/l and 64·9 (SD 44·8) pmol/l, respectively. A highly significant negative correlation was observed between vitamin D and iPTH (r = −0·317, P<0·001) and between vitamin D and ALP (r = −0·232, P<0·0001). Seasonal differences observed in various biochemical and hormonal parameters during the three trimesters are shown in Table 2. In comparison with the values reported in summer, serum 25(OH)D levels were

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The mean vitamin D intake was similar in all trimesters (0.2 ± 0.3 (SD 0.4)) but did not reach statistical significance (0.3 (SD 0.3)).

### Parameters

<table>
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<th>Mean</th>
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<th>Range</th>
<th>Mean</th>
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<td>19–30</td>
<td>24·26</td>
<td>2·82</td>
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<td>1·62</td>
<td>18–26</td>
<td>20·35</td>
<td>1·54</td>
<td>18–26</td>
<td>20·05</td>
<td>1·46</td>
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<td>3·17</td>
<td>14·27–26·31</td>
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<td>3·16</td>
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<td>7959</td>
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<td>7829</td>
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<td>Hb (g%)</td>
<td>10·3</td>
<td>1·26</td>
<td>8·4–14·6</td>
<td>10·36</td>
<td>1·55</td>
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<td>7·86</td>
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<td>10–60</td>
<td>25</td>
<td>10–60</td>
<td>9</td>
<td>15·6</td>
<td>3–45</td>
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<td>n (%)</td>
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<td></td>
<td>n (%)</td>
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<td>Members in the family Frequency</td>
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<td>58–3</td>
<td>90</td>
<td>41–2</td>
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<td>82</td>
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<td>3</td>
<td>61</td>
<td>39–1</td>
<td>113</td>
<td>51–8</td>
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<td>74</td>
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<td>4</td>
<td>2–5</td>
<td>15</td>
<td>6–8</td>
<td></td>
<td>11</td>
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<td>74</td>
<td>47–4</td>
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<td>50</td>
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<td>&gt; 2</td>
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<td>20–5</td>
<td>57</td>
<td>26–1</td>
<td></td>
<td>38</td>
<td>22–1</td>
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</table>

### Vitamin D status in Indian pregnant women

The biochemical profile of lactating mothers is presented in Table 3. A total of 341 (99·7 %) lactating mothers had serum 25(OH)D levels < 50 nmol/l, with 19·3, 51·2 and 29·2 % suffering from mild (25–50 nmol/l), moderate (12·5–25 nmol/l) and severe (< 12·5 nmol/l) hypovitaminosis D, respectively. A highly significant negative correlation was found between 25(OH)D and iPTH (r = −0·310, P = 0·0001) and between 25(OH)D and ALP (r = −0·217, P = 0·0001), respectively, in lactating mothers.

### Vitamin D status of lactating mothers

The biochemical profile of lactating mothers is presented in Table 3. A total of 341 (99·7 %) lactating mothers had serum 25(OH)D levels < 50 nmol/l, with 19·3, 51·2 and 29·2 % suffering from mild (25–50 nmol/l), moderate (12·5–25 nmol/l) and severe (< 12·5 nmol/l) hypovitaminosis D, respectively. A highly significant negative correlation was found between 25(OH)D and iPTH (r = −0·310, P = 0·0001) and between 25(OH)D and ALP (r = −0·217, P = 0·0001), respectively, in lactating mothers.

### Vitamin D status of exclusively breast-fed infants

The biochemical profile of infants is shown in Table 3. No significant difference was observed in serum 25(OH)D levels in infants born in summer and winter (data not shown). A total of 338 infants (98·8 %) had serum 25(OH)D levels < 50 nmol/l, with 38·0, 44·5 and 16·3 % classified as mild (25–50 nmol/l), moderate (12·5–25 nmol/l) and severe (< 12·5 nmol/l) hypovitaminosis D, respectively. A highly significant negative correlation was also observed between 25(OH)D and iPTH levels of infants (r = −0·56, P = 0·0001; data not shown).

### Correlations between the vitamin D status of mother–infant pairs

As shown in Fig. 1, a strong positive correlation was found between 25(OH)D, (r = 0·779, P = 0·0001), ionised Ca (r = 0·166, 0·06).
We have reported vitamin D status of pregnant women hailing from lower–middle socio-economic strata. The nutritional, educational and obstetric data of these women were consistent with that described for this socio-economic class(23), thereby making the information generated generalisable for this group. In the present study, 96 % of pregnant women had hypovitaminosis D, which is the highest reported prevalence in the literature. Several other studies from developing and developed nations across the world have reported that the prevalence of hypovitaminosis D (25(OH)D, 25 nmol/l) in pregnancy ranged from 18 to 84 % (24–26,10,27–32). So South Asians, both in their country of origin and after migration to Europe or the UK, have been found to have lower serum 25(OH)D concentrations than white Caucasians (2 6,33–35 ) due to a range of factors including skin pigmentation, covered-up clothing (especially common in women), restricted outdoor physical activity and low dietary vitamin D intake(25,36).

The present findings have shown the status of 25(OH)D, Ca, ALP and iPTH during different trimesters of pregnancy. There was no significant difference in the prevalence of 25(OH)D deficiency (25(OH)D, 50 nmol/l)) among pregnant women in the three different trimesters, both in summer and winter. A study of pregnant Iranian women has shown that

Table 2. Seasonal differences between biochemical parameters in the three trimesters of pregnancy
(Mean values, standard deviations, medians, ranges and number of subjects)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Summer</th>
<th>Winter</th>
</tr>
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<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>Ca (mmol/l)*</td>
<td>2.37</td>
<td>0.09</td>
</tr>
<tr>
<td>PO4 (mmol/l)*</td>
<td>1.25</td>
<td>0.14</td>
</tr>
<tr>
<td>ALP (IU/l)*</td>
<td>177.45</td>
<td>43.78</td>
</tr>
<tr>
<td>Ionised Ca (mmol/l)*</td>
<td>1.190</td>
<td>0.045</td>
</tr>
<tr>
<td>25(OH)D (nmol/l)†</td>
<td>23.4</td>
<td>11.3</td>
</tr>
<tr>
<td>iPTH (pg/l)‡</td>
<td>687</td>
<td>502</td>
</tr>
<tr>
<td>Median</td>
<td>730</td>
<td>810</td>
</tr>
<tr>
<td>Range</td>
<td>71.5–3611</td>
<td>134.7–2200</td>
</tr>
</tbody>
</table>

First trimester

Second trimester

Third trimester

Table 3. Biochemical profile of mothers and their infants at 6 weeks postpartum
(Mean values, standard deviations, medians, and ranges, n=342)

<table>
<thead>
<tr>
<th>Parameters</th>
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<th>Infants</th>
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<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
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<tr>
<td>Ca (mmol/l)</td>
<td>2.37</td>
<td>0.09</td>
</tr>
<tr>
<td>PO4 (mmol/l)</td>
<td>1.25</td>
<td>0.13</td>
</tr>
<tr>
<td>ALP (IU/l)</td>
<td>213.20</td>
<td>42.41</td>
</tr>
<tr>
<td>Ionised Ca (mmol/l)</td>
<td>1.18</td>
<td>0.05</td>
</tr>
<tr>
<td>25(OH)D (nmol/l)†</td>
<td>19.6</td>
<td>8.3</td>
</tr>
<tr>
<td>iPTH (pg/l)‡</td>
<td>655</td>
<td>583</td>
</tr>
<tr>
<td>Median</td>
<td>102–1872</td>
<td>150–1863.2</td>
</tr>
<tr>
<td>Range</td>
<td>655</td>
<td>583</td>
</tr>
</tbody>
</table>

ALP, alkaline phosphatase; 25(OH)D, 25-hydroxyvitamin D; iPTH, intact parathormone.

* Mean values were significantly different as tested by the independent t test between summer and winter: P<0.001, P<0.01, P<0.05.
† Mean values were not significantly different between trimesters: P=0.81 (first v. second trimester), P=0.12 (second v. third trimester), P=0.07 (first v. third trimester).
‡ Mean values were significantly different as tested by Wilcoxon’s rank-sum test between summer and winter: P<0.001 (first trimester), P<0.01 (second trimester), P<0.05 (third trimester).

ALP, alkaline phosphatase; 25(OH)D, 25-hydroxyvitamin D; iPTH, intact parathormone.

P=0.0001) and iPTH (r=0.534, P=0.0001) levels of mothers and infants.

Discussion

We have reported vitamin D status of pregnant women hailing from lower–middle socio-economic strata. The nutritional, educational and obstetric data of these women were consistent with that described for this socio-economic class(23), thereby making the information generated generalisable for this group. In the present study, 96 % of pregnant women had hypovitaminosis D, which is the highest reported prevalence in the literature. Several other studies from developing and developed nations across the world have reported that the prevalence of hypovitaminosis D (25(OH)D < 25 mmol/l) in pregnancy ranged from 18 to 84 % (24–26,10,27–32). South Asians, both in their country of origin and after migration to Europe or the UK, have been found to have lower serum 25(OH)D concentrations than white Caucasians (2 6,33–35 ) due to a range of factors including skin pigmentation, covered-up clothing (especially common in women), restricted outdoor physical activity and low dietary vitamin D intake(25,36).

The present findings have shown the status of 25(OH)D, Ca, ALP and iPTH during different trimesters of pregnancy. There was no significant difference in the prevalence of 25(OH)D deficiency (25(OH)D < 50 nmol/l)) among pregnant women in the three different trimesters, both in summer and winter. A study of pregnant Iranian women has shown that
60% of the women in the first trimester, 48% in the second trimester and 47% in the third trimester had either severe or moderate vitamin D deficiency (15). In an earlier study conducted in Asian women residing in London, it has been found that 25(OH)D concentration was <25 nmol/l in 25% of subjects in the first trimester, which reduced in the third trimester (17).

Total serum Ca and ionised Ca values showed no variation across trimesters in the present study. Studies in the literature have shown either no change in serum Ca values (15,16,37) or an increase (17,36–40) or a decrease (41,42) with progression of pregnancy. The constancy of serum ionised Ca values has also been reported by other investigators (15,43,44). Similar to earlier reports (15,37,45,44), there was no change in serum P during the course of pregnancy both in summer and winter. Mean serum ALP showed a non-significant decline in the present study. This is in contrast with the findings of Ainy et al. (37) who attributed the increment in ALP, related to placental production, to lack of vitamin D supplementation and insufficient dietary intake. We observed no significant difference in mean serum 25(OH)D concentration in the three trimesters, both in summer and winter, which is in concordance with the reports from Reddy et al. (15) and Selly et al. (16). In contrast, Sanchez et al. (14) found that 25(OH)D concentration increased in the second and third trimesters, the increase being attributable to food and supplement intake and sun exposure. In a longitudinal study of pregnant women, Ardawi et al. (13) showed a moderate, but statistically significant, decrease towards the end of pregnancy both in summer and winter. The decrease was attributed to the particular dietary and cultural habits followed by the subjects. In India, there is no fortification of food products with vitamin D, and there is no clear guidelines recommending mandatory vitamin D supplementation during pregnancy. These factors could partly explain the absence of variation in serum 25(OH)D levels during the three trimesters of pregnancy.

Several reasons for change in serum 25(OH)D levels in pregnancy have been postulated. These include altered hepatic 25-hydroxylase activity, change in iPTH levels and increased fetal metabolic activity (14,43,45–47). Another study has suggested that rise in iPTH was responsible for the increased absorption of vitamin D in mothers (48).

There is no consistent pattern in the change in serum iPTH levels during the different trimesters of pregnancy. Most studies conducted in populations replete with Ca and vitamin D have reported a gradual decline in serum iPTH levels with evolution of pregnancy. In contrast, studies from the Gambia, Asia and other regions with low Ca and vitamin D intake often do not report any decline in iPTH levels during pregnancy (49,50). Other causes of varying results could include methodological differences in assays resulting in the measurement of multiple different immunoactive but biologically inactive fragments of parathormone (49). In addition, the contribution of placenta-derived parathormone-related peptide to the different aspects of bone mineral metabolism, including renal 1α hydroxylation of 25(OH)D, may also be partly responsible for the variation in iPTH values in the three trimesters reported in different studies (49,51–53).

Marked seasonal variation in serum 25(OH)D levels was observed in the present study. A progressive fall in 25(OH)D levels in pregnant women during winter months due to the reduced availability of sunshine has been described in European and US populations (54–57), as well as from India and other Asian countries (52,54,57,58,59). In addition, serum 25(OH)D concentration also depends on the extent of the body surface area exposed, which is likely to be reduced due to the style of dressing in winter (8,60).

High prevalence of vitamin D deficiency in apparently healthy lactating mothers (99.7%) and exclusively breast-fed infants (98.8%) observed in the present study only reiterates our earlier observation (12) as well as those of other workers (57,60–63). Mothers with suboptimal vitamin D status have offspring with reduced intra-uterine and postnatal skeletal development (7,10). The impact of maternal vitamin D status on the neonate’s serum 25(OH)D levels is apparent from the strong correlation reported by us in the present study. Although a similar correlation between 25(OH)D levels of mothers and newborns has been reported earlier, most investigators have measured cord blood 25(OH)D levels to establish the relationship (18,19,31).

Mother–offspring studies in Western populations have shown associations of maternal body build, diet, nutritional status, smoking and physical activity with bone mass in newborns and children (2–4,5,7,10). The importance of nutrition, mainly Ca, has been acknowledged with regard to pregnancy outcome (64). Greater maternal consumption of Ca and Ca-rich foods, especially milk and milk products, in mid- to late pregnancy has been associated with improved bone outcomes in children (9). In the present study, mothers had low Ca intakes, consistent with other low-income groups in India (65). The mean dietary Ca intake (408.11 ± 167.2 mg/d) of mothers was just 60% of the RDA given by the Indian Council of Medical Research (22), and the intake of other macronutrients was also far below the RDA. Also, vitamin D supplementation is not a part of antenatal care programmes in India, which worsens the situation further.
These data reinforce the need to provide greater emphasis on maternal nutrition to improve neonatal and childhood bone health.

**Conclusion**

We conclude that there is a high prevalence of hypovitaminosis D among pregnant women and their infants in India. Serum 25(OH)D levels were uniformly low across all three trimesters, with a tendency to decline in winter. There was a strong positive correlation between maternal and infant serum 25(OH)D levels. Further research, preferably by randomised controlled trials, is needed to establish the effects of vitamin D supplementation during pregnancy on the bone health of women and their children.

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