The impact of atmospheric pollution on vitamin D status of infants and toddlers in Delhi, India

K S Agarwal, M Z Mughal, P Upadhyay, J L Berry, E B Mawer, J M Puliyel

Aims: To compare the vitamin D status of 34 children, 9–24 months old, living in an area of Delhi renowned for high levels of atmospheric pollution (Mori Gate), with a comparable age matched group of children from a less polluted (Gurgaon) area of the city.

Methods: Serum concentrations of calcium, alkaline phosphatase (ALP), parathyroid hormone (PTH), 25-hydroxyvitamin D (25(OH)D), and 1,25-dihydroxyvitamin D (1,25(OH)2D) were measured. Haze scores, regarded as a surrogate marker of solar UVB radiation reaching ground level, were measured in both areas.

Results: Mean 25(OH)D of children in the Mori Gate area was 12.4 (7) ng/ml, compared with 27.1 (7) ng/ml in children living in the Gurgaon area (p < 0.001). The median ALP (p < 0.05) and mean PTH (p < 0.001) concentrations were higher in children living in the Mori Gate area than in the Gurgaon area. The mean haze score in the Mori Gate area [2.1 (0.5)] was significantly lower (p < 0.05) than in the Gurgaon area [2.7 (0.4)], indicating less solar UVB reaching the ground in Mori Gate.

Conclusion: We suggest that children living in areas of high atmospheric pollution are at risk of developing vitamin D deficiency rickets and should be offered vitamin D supplements.

In humans the main source of vitamin D is that formed in the skin by conversion of 7-dehydrocholesterol to cholecalciferol (vitamin D3) on exposure to the sun's ultraviolet B (UVB) radiation. The importance of sunlight in the prevention and cure of rickets was observed over a century ago, by Palm, who found rickets to be most prevalent in cities where people were exposed to low levels of sunlight. Vitamin D is essential for skeletal health and its deficiency results in development of rickets in growing children and osteomalacia in adults. There is concern that increasing atmospheric pollution related haze from industrial and vehicular sources might lead to absorption of UVB photons, thereby reducing the cutaneous vitamin D synthesis.

Delhi (latitude 28.35°N) is one of the most polluted cities in the world; the vehicle population, a major contributor to the atmospheric pollution burden, has grown by over 12% annually for the past two decades. In this cross sectional study we assessed the vitamin D status of infants and toddlers living in a downtown area of Delhi, renowned for high levels of atmospheric pollution, with a comparable group of children from a relatively less polluted area on the outskirts of the city. We hypothesised that serum total 25-hydroxycholecalciferol (25(OH)D), a reliable measure of an individual's vitamin D status, of children living in the area with high levels of atmospheric pollution would be lower than in those living in the less polluted area of the city.

METHODS
All eligible children aged 9–24 months from a downtown area of Delhi with high levels of visible atmospheric pollution (Mori Gate) took part in this cross sectional study. Thirty four infants and toddlers were recruited from this area. A similar number of age matched subjects were recruited from the Gurgaon area, a less polluted area on the outskirts of the metropolitan boundary of the city. Families in both study areas received community healthcare from the Community Outreach Department of St Stephen's Hospital, Delhi. The study was approved by St Stephen's Hospital research ethics committee. Witnessed, verbal consent was obtained, as the majority of the parents were illiterate. Housing and the socioeconomic status of families in the two areas were similar. The infants in both areas spent most of the time indoors while the older toddlers played outside their dwellings. The families in both areas lived in single storey, one room houses with a small open space/communal footpath at the front of the house. The lack of designated safe play areas and the age of the subjects meant that they mainly spent most of the time indoors. The approximate average monthly income of families in both the areas was 1500–2000 Indian Rupees ($35–40). All the families studied were Hindu vegetarians. Children in both areas were breast fed and the rest of their diet consisted of boiled cereals, vegetables, and fresh cow or buffalo milk. None of the children was receiving vitamin or dietary supplements.

Haze scores at ground level were measured in both areas between 12 and 26 February 2000, at 0900, 1200, and 1600 hours, using a haze sensor, which had been modified by placing a filter that only allowed UVB radiation (285–310 nm) to be detected by the sensor's light detecting diode. An average daily haze score was used as a surrogate measure of solar UVB light reaching the ground level. Four ml of blood was collected from each child between 29 March 2000 and 10 April 2000. Blood in vacutainers, containing a clot activator (Greiner Labotechnic, Kremsmunster, Austria), was centrifuged for 10 minutes at 3000 rpm and the serum separated and stored at −24°C, pending biochemical analysis. Sufficient blood for biochemical tests was obtained from 26 children in the Mori Gate area and 31 children in the Gurgaon area. Serum calcium (Ca) concentration and alkaline phosphatase (ALP) activity were analysed using an autoanalyser (BPC Electra, Italy).

Vitamin D metabolites were measured by in-house assays as described in detail previously. Briefly, samples were extracted using acetonitrile and applied to C18 Silica Sep-paks. Separation of metabolites was by straight phase HPLC (Waters Associates, Milford, MA) using a Hewlett-Packard Zorbax-Sil
Table 1  Age, gender, haze score, and biochemical parameters of subjects from the Mori Gate and Gurgaon areas

<table>
<thead>
<tr>
<th></th>
<th>Mori Gate High pollution area</th>
<th>Gurgaon Low pollution area</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (months)</td>
<td>16 (4.1)</td>
<td>15.9 (3.8)</td>
</tr>
<tr>
<td>Haze score</td>
<td>2.1 (0.5)</td>
<td>2.7 (0.4)*</td>
</tr>
<tr>
<td>Gender</td>
<td>15 males, 11 females</td>
<td>15 males, 16 females</td>
</tr>
<tr>
<td>Ca (mg %)</td>
<td>9.7 (0.8)</td>
<td>9.6 (0.8)</td>
</tr>
<tr>
<td>ALP (IU/L), median (range)</td>
<td>498 (116–3739)</td>
<td>398* (196–780)</td>
</tr>
<tr>
<td>25(OH)D2 (ng/ml)</td>
<td>11.7 (7)</td>
<td>21.7 (7)**</td>
</tr>
<tr>
<td>25(OH)D3 (ng/ml)</td>
<td>2.4 (0.6) (n=5)</td>
<td>0</td>
</tr>
<tr>
<td>Total 25(OH)D (ng/ml)</td>
<td>12.4 (7)</td>
<td>21.7 (7)**</td>
</tr>
<tr>
<td>1,25(OH)2D (pg/ml)</td>
<td>73.7 (30)</td>
<td>55 (19)</td>
</tr>
<tr>
<td>PTH (pg/ml), median (range)</td>
<td>25 (5–284)</td>
<td>13.1** (1.6–37)</td>
</tr>
</tbody>
</table>

Except for ALP and PTH, all data are presented as mean (1 SD). Serum 25(OH)D2, was only detected in five children from the Mori Gate area and none from the Gurgaon area; it is presented as range only for the subjects from the Mori Gate area.

Thirty four subjects aged 9–24 months were recruited from each of the study areas; however, data are not provided for eight subjects from the Mori Gate area and three subjects from the Gurgaon area due to failed venepunctures.

*p<0.05, **p< 0.01, ***p<0.001.

RESULTS

As table 1 shows, mean age of children in the Mori Gate and Gurgaon areas was not different. In both areas 15 subjects were boys. In both areas 10 subjects were 9–12 months old, 15 were 13–18 months old, and nine were 19–24 months old. Table 1 shows the results of serum Ca, ALP, 25(OH)D2, 25(OH)D3, total 25(OH)D, 1,25(OH)2D, and PTH. The mean total serum 25(OH)D concentration of children in the Mori Gate area was significantly lower than of those living in the Gurgaon area. In contrast, the mean serum concentrations of 1,25(OH)2D was quantified by radioimmunoassay as described in detail elsewhere." The adult reference range is 20–50 pg/ml, sensitivity 1.25 pg/assay tube, and intra- and inter-assay CV 7.8% and 10.5% respectively. A

Serum intact parathyroid hormone (PTH) was measured using an immunoradiometric assay (Nichols Institute Diagnostics, San Juan, Capistrano, USA); adult reference range 10–60 pg/ml, sensitivity 1 pg/ml, intra- and inter-assay CV 3% and 6% respectively.

SPSS, version 9 for Microsoft Windows was used for statistical analysis. The normally distributed data are presented as mean (1 SD); for the non-normally distributed variables, the data are presented as median and range. The normally distributed data between the two groups were analysed using the unpaired Student’s t test; those not normally distributed were analysed using the Mann–Whitney t test. All tests were two tailed, and p values less than 0.05 were considered statistically significant.

DISCUSSION

We assessed the vitamin D status of infants and toddlers who lived in similar types of housing in two areas of the city with different levels of atmospheric pollution. We found that subjects living in the Mori Gate, an area with high levels of atmospheric pollution, had significantly lower mean serum total 25(OH)D concentrations compared to those living in the less polluted area (Gurgoan). Twelve children living in the Mori Gate were vitamin D deficient (total 25(OH)D <12 ng/ml) and three had severe vitamin D deficiency (total 25(OH)D <5 ng/ml). As expected, the mean PTH and the median ALP levels were significantly higher in subjects from the Mori Gate than in those from Gurgoan. One infant had biochemical features of vitamin D deficiency rickets, with very low serum total 25(OH)D and high PTH and ALP concentrations. In contrast, none of the children in the Gurgoan were vitamin D deficient.

The most important source of vitamin is that produced in the skin after exposure to UVB sunlight. Serum 25(OH)D concentrations in infants show a seasonal variation, reflecting the importance of cutaneous synthesis as the main source of this vitamin, even at this age. In this study, the vitamin D status was measured in late March and early April, and therefore reflects the vitamin D status of the subjects at the end of the winter season. We used the modified haze sensor to measure indirectly the effect of atmospheric pollution on reducing solar radiation in the UVB range reaching ground level, in the two areas. This inexpensive, but validated instrument relies on conversion of UVB light into a voltage by the light emitting diode. The higher the amount of atmospheric pollution, the lower the amount of UVB light reaching ground level and thus the lower the haze score. We found that the haze score was significantly lower in the more polluted Mori Gate area than in the less polluted Gurgoan area.

Dietary sources of vitamin D are limited mainly to oily fish, eggs, and fortified foods, which were not consumed by the...
infants studied. None of the children were receiving supplementary vitamins, although five children from the Mori Gate area had small but measurable amounts of 25(OH)D3 (range 1.8–3.2 ng/ml) but none from the Gurgaon area. Vitamin D3 is of plant origin and small amounts may be obtained from diet; it can also be supplied as supplements, but this was not the case in this study. Therefore, the differences in the vitamin D status of the children in two areas cannot be explained by the differences in their diets. Results of this study suggest that the haze caused by aeropollutants, such as ozone, reduced UVB sunlight reaching ground level; this is likely to be responsible for reduced cutaneous vitamin D synthesis in children living in the Mori Gate area.

There are several limitations to our study, which include its cross sectional design and lack of clinical and radiological assessment for features of rickets in the subjects. Dietary intake of vitamin D was not formally assessed but this would have only contributed a very small proportion to the body pool. We did not collect data on the duration of time spent outdoors by the subjects, and ideally we should have measured the UVB exposure of the individual child during the study period. The modified haze scores should have been measured during the summer months, a period of maximal cutaneous vitamin D synthesis; however, we believe that measurements in February reflect the trends in solar UVB reaching the ground level in the two areas of the city during summer months. Nevertheless, our data clearly indicate that infants and toddlers living in a more polluted area of Delhi had lower serum levels of vitamin D than those living in a less polluted area of the city.

From the results of this study, we conclude that children living in areas of high atmospheric pollution are at risk of developing vitamin D deficiency rickets. The findings of Palm in 1890 are still relevant today. The importance of atmospheric pollution on reducing effective UVB levels, even in traditionally sunny countries at low latitudes. Children living in such areas should be offered vitamin D supplements. A double blind, randomised, placebo controlled trial, involving oral administration of a single large dose vitamin D supplementation at the onset of winter is currently underway. We suggest that atmospheric pollution control measures need to be urgently implemented in order to safeguard general and skeletal health of children growing up in cities such as Delhi.

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