Role of Calcium Deficiency in Development of Nutritional Rickets in Indian Children: A Case Control Study

Varun Aggarwal, Anju Seth, Satinder Aneja, Bhawna Sharma, Pitamber Sonkar, Satveer Singh, and Raman K. Marwaha

Departments of Pediatrics (V.A., A.S., S.A.) and Radiodiagnosis (P.S.), Kalawati Saran Children's Hospital and Lady Hardinge Medical College, New Delhi 110001, India; and Department of Endocrinology (B.S., S.S., R.K.M.), Thyroid Research Center, Institute of Nuclear Medicine and Allied Science, New Delhi 110049, India

Context: Nutritional rickets is usually attributed to vitamin D deficiency. Studies from some tropical countries have postulated low dietary intake of calcium as the cause of nutritional rickets. Both vitamin D and dietary calcium deficiency are highly prevalent in India. Information on their relative contribution in the development of rickets in Indian children is limited.

Objective: The aim was to study the role of calcium and vitamin D deficiency in causation of nutritional rickets in young Indian children.

Design and Methods: In a case-control study, 67 children with nutritional rickets and 68 age- and sex-matched healthy controls were compared for demographic factors, nutritional status, sun exposure (UV score), dietary calcium and phytate intake (for subjects not breast-fed at presentation), and biochemical parameters [serum calcium, inorganic phosphate, alkaline phosphatase, 25-hydroxyvitamin D (25OHD), and PTH].

Results: Mean intake of calcium (204 $\pm$ 129 *vs.* 453 $\pm$ 234 mg/d; $P < 0.001$) and proportion of calcium from dairy sources (41.7 *vs.* 88.6%; $P < 0.001$) were significantly lower in cases *vs.* controls. The dietary intake of phytate was also significantly higher in cases ($P = 0.01$). Median serum 25OHD level (interquartile range) in both cases and controls was in the range of deficiency [13.7 (10; 17.9) and 19.4 (12.3; 24.6) ng/ml, respectively]. There was no significant difference in the serum 25OHD level ($P = 0.08$) or sun exposure as measured by UV score ($P = 0.39$) among the cases and controls. In cases with rickets, significant negative correlations were seen between dietary calcium intake and radiological score ($r = -0.28$; $P = 0.03$) and PTH ($r = -0.26$; $P = 0.02$). No correlation was found between serum 25OHD level and radiological score or biochemical parameters of rickets.

Conclusions: Rickets develops when low dietary calcium intake coexists with a low or borderline vitamin D nutrition status. **(*J Clin Endocrinol Metab* 97: 3461–3466, 2012)**

Rickets, a common nutritional disorder, is usually considered to be secondary to vitamin D deficiency. However, in the last few decades, studies from some tropical countries have shown that calcium deficiency may play a more important role in the causation of rickets (1–6). Dietary calcium intake has been reported to be low in children (7) and adults (8) in India. Indian diet is predominantly vegetarian, based on cereals and legumes, and is often deficient in milk and milk products (9). The low calcium content of the diet is further compromised by the high level of phytates in the vegetarian diet.

On the other hand, studies from many nations across the globe, including India, have also shown a widespread prevalence of vitamin D deficiency (10–13). A recent

ISSN Print 0021-972X ISSN Online 1945-7197
Printed in U.S.A.

doi: 10.1210/jc.2011-3120 Received November 14, 2011. Accepted June 26, 2012.
First Published Online August 14, 2012

Abbreviations: ALP, Alkaline phosphatase; 25OHD, 25-hydroxyvitamin D.

study by us (14) has shown a high prevalence of vitamin D deficiency in lactating mothers and their healthy infants in Delhi. Because both calcium and vitamin D deficiency are likely to be present in children in India, it is not clear what plays a more important role in the development of rickets. We conducted this study to elucidate the relative contribution of calcium and vitamin D in the development of nutritional rickets in young Indian children.

Subjects and Methods

Subjects

One hundred consecutive children aged 6 months to 5 yr with clinical (wrist widening, bow legs, double malleoli, Harrison's sulcus, rachitic rosary, frontal and parietal prominence) and radiological features of rickets were recruited from the outpatient department of a tertiary care teaching hospital in North India from December 2007 to January 2009. Subjects with features suggestive of nonnutritional etiology (renal or hepatic disease, malabsorption states, antiepileptic drug intake, or any chronic illness) were excluded. Relevant laboratory evaluation was carried out to exclude nonnutritional rickets where indicated. Cases presenting with hypocalcemic seizures or with a history of consuming calcium or vitamin D supplements in the preceding 6 months were also excluded from the study. Of the 100 children screened, 18 had taken calcium/vitamin D supplements in the preceding 6 months, five had a chronic medical disease, seven were outside the eligible age range, and three refused consent. Hence, a total of 67 cases were included for the study. These cases were later randomized to receive calcium, vitamin D, or a combination of the two. The results of this randomized controlled trial will be published separately.

Sixty-eight age- and sex-matched healthy children attending the immunization clinic, with no features of rickets and no disease likely to affect vitamin D or calcium metabolism, constituted the control group. Participation was voluntary, and informed written consent was taken from the parent/guardian of each child. The study protocol was approved by the institutional ethical committee.

Data and sample collection

The recruited subjects were evaluated for demographic parameters (age, sex, religion, birth order, and monthly family income). A detailed dietary evaluation including history of breastfeeding and complementary feeding was obtained. The diet was evaluated by 24-h dietary recall and food frequency questionnaire to assess the daily consumption of calcium from both dairy and nondairy sources, phosphate, oxalate, fiber, and phytate. Food products in India including milk (except formula milk) are not routinely fortified with vitamin D. None of the children with rickets or healthy controls were on formula milk or any other infant foods fortified with vitamin D. Therefore, the dietary intake of vitamin D was negligible in both cases and controls, the only source of vitamin D for these children being sunshine.

Amount of sun exposure was measured by calculating UV score, taking into account the total body surface area [using the Lund and Browder charts (15)] exposed to the sun while wearing routine clothes and the duration of exposure by finding the average time spent outdoors per day (in minutes) during the period of direct sunlight (0900 to 1500 h). UV score was calculated by multiplying these two variables (minutes per square meter per day).

For the cases, information was sought about the nature, onset, and duration of the presenting features. Height (length for children up to 2 yr) and weight were recorded for cases and controls, and the Z scores for weight for age and height for age were calculated using World Health Organization 2006 growth reference standards (16).

Biochemical measurements

For both cases and controls, serum calcium, phosphate, and alkaline phosphatase (ALP) were measured. Serum calcium was measured by calorimetric method (normal range for calcium (total) was 8.8–10.8 mg/dl with an analytical sensitivity of 0.2 mg/dl and calcium (ionic) was 4.4–5.4 mg/dl). Serum phosphate and ALP were determined by photometric analysis. Normal range for serum phosphate was 3.8–6.5 mg/dl (analytical sensitivity, 0.3 mg/dl). The upper limit of normal serum ALP was 420 IU/liter with analytical sensitivity of 5 IU/liter.

Serum 25-hydroxyvitamin D (25OHD) and PTH levels were measured by electrochemiluminescence immunoassay using Cobas kit. Based on widely accepted pediatric standards, a serum 25OHD level of less than 20 ng/ml was defined as vitamin D deficiency (17, 18). The analytical sensitivity of the assay was 4 ng/ml. The normal range for serum PTH was 15–65 pg/ml, with analytical sensitivity of 1.2 pg/ml.

Radiological evaluation

Radiographs of left wrist and knee were obtained for each patient with rickets. They were evaluated by two separate observers (blinded to the treatment protocol) using the method developed by Thacher *et al.* (19) and scored on a 0–10 point scale. The mean value of the two scores was used for the analysis. The interclass correlation of scores observed between observers was 0.90, and the correlation within observers was 0.91 or greater, indicating good reproducibility.

Statistical analysis

The primary variables for comparison between children with rickets and controls were serum 25OHD concentration and dietary intake of calcium. The data were analyzed using Windows SPSS software (version 10; SPSS Inc., Chicago, IL). Parametric data are reported as mean ± SD. Nonparametric data are reported as median with interquartile range. The parametric variables were compared using the two-tailed Student's *t* test and nonparametric variables by Mann-Whitney *U* test. Dichotomous variables were compared using χ^2 test. Pearson and Spearman correlation coefficients were used to find correlation between parametric and nonparametric variables, respectively. *P* value of <0.05 was considered statistically significant.

Results

Demographic data

The demographic characteristics of the cases and controls are shown in Table 1. The cases of rickets had significantly larger family size and higher birth order than the controls and belonged to families with lower per-capita income. They were also significantly shorter and lighter

TABLE 1. Comparison of characteristics in cases with rickets and controls

Characteristic	Controls (n = 68)	Cases (n = 67)	*P* value
Age (months)	12 (8 to 24)	14 (9 to 22)	0.80
Gender	M = 38, F = 30	M = 35, F = 32	0.67
Religion	H = 59, Mu = 9	H = 56, Mu = 11	0.80
Family size	4.4 ± 0.6	4.8 ± 1.1	**0.04**
Birth order	1.2 ± 0.4	2.0 ± 1.0	**0.02**
Per-capita family income (thousand Indian rupees)[a]	2.8 ± 1.6	1.5 ± 1.3	**<0.001**
Height (Z score)	0.3 (0.1 to 1.1)	−1.9 (−2.6 to −0.7)	**<0.001**
Weight (Z score)	0.2 (−0.2 to 0.6)	−1.7 (−2.3 to −0.6)	**<0.001**
UV score (min · m^2/d)	0.7 (0.0 to 2.8)	0.8 (0.0 to 2.6)	0.39
BF until date (n)	14	41	**0.005**
Exclusively BF until date (n)	8	30	**0.001**
Breast-feeding duration (months)	8.6 ± 2.7	17.8 ± 2.1	**0.01**
Total dietary calcium (mg/d)[b]	453 ± 234	204 ± 129	**<0.001**
Dairy calcium (mg/d)[b]	401 ± 300	85 ± 131	**<0.001**
Fiber (mg/d)[b]	0.67 ± 0.66	0.69 ± 0.59	0.90
Phosphate (mg/d)[b]	451.4 ± 199.0	366.8 ± 179.4	0.06
Phytates (mg/d)[b]	13.4 ± 16.3	24.8 ± 21.9	**0.01**
Oxalates (mg/d)[b]	7.3 ± 5.1	6.7 ± 4.0	0.54
Radiological score[c]		9 (5 to 10)	

P values in *bold* indicate significant values. Parametric data are reported as means ± SD. Non-normally distributed data are expressed as median (25th to 75th percentiles). M, Male; F, female; H, Hindu; Mu, Muslim; BF, breast-fed.

[a] To convert to U.S. dollars, divide by 50 (1 U.S. dollar = approximately 55 Indian rupees).

[b] Dietary analysis was done for 54 controls and 26 cases who were not breast fed at presentation.

[c] Radiological evaluation was done only for cases of rickets.

than the controls. Only five of the cases and none of the controls had height for age and weight for age Z scores less than −3. Amount of sun exposure (as determined by UV score) did not vary significantly among cases and controls.

Clinical presentation

Among the cases of rickets, 20 presented with complaints primarily ascribable to rickets (bow legs, 14; delayed walking, 14). In the remaining 47 children, rickets was picked as an incidental finding. In these children, the presenting illnesses were: lower respiratory tract infection, 37; upper respiratory tract infection, six; and acute gastroenteritis, two. Two cases were siblings of patients and were evaluated during subsequent visits.

Dietary analysis

Children with rickets were breast-fed for a longer duration compared with controls (17.8 ± 2.1 *vs.* 8.6 ± 2.7 months; $P = 0.01$). Among cases, 41 children were breast-fed at presentation (30 exclusively breast-fed). Among controls, 14 children were breast-fed at the time of presentation (eight exclusively breast-fed). Because it was not feasible to measure the milk output of their mothers, we evaluated the diet for various constituents only for those children (54 controls and 26 cases) who were not breast-fed at presentation.

The mean total calcium intake was significantly lower among the cases than the controls (204 ± 129 *vs.* 453 ± 234 mg/d; $P < 0.001$). Also, the proportion of calcium obtained from dairy sources consumed by the cases was significantly lower compared with controls (41.7 *vs.* 88.6%, respectively; $P < 0.001$). The intake of phytate was also higher in cases compared with controls. There was no difference in the dietary intake of phosphate, fiber, and oxalate among the two groups. In cases with rickets, regression analysis (Fig. 1) showed a significant negative correlation (r = −0.28) between dietary calcium intake and radiological score ($P = 0.03$). The correlation between dietary calcium intake and serum PTH was also

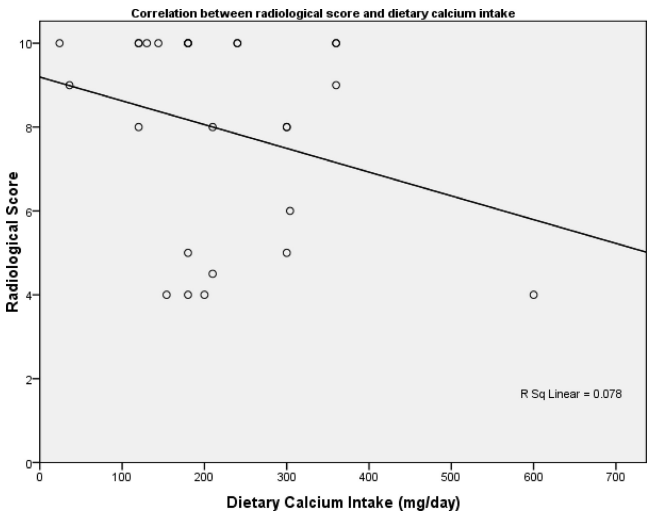

FIG. 1. Correlation between dietary calcium intake and radiological score of children with rickets.

TABLE 2. Biochemical parameters in cases with rickets (n = 67) and controls (n = 68)

Characteristic	Controls	Cases	*P* value	Normal range
Calcium (total) (mg/dl)	9.4 (8.9 to 10)	8.5 (8 to 9)	**<0.001**	8.8–10.8
Calcium (ionic) (mg/dl)	4.8 (4.6 to 4.9)	4 (3.6 to 4.3)	**<0.001**	4.4–5.4
Phosphate (mg/dl)	5 (4 to 5.4)	2.8 (2.4 to 3.4)	**<0.001**	3.8–6.5
ALP (IU/liter)	300 (200 to 361.2)	1257 (980 to 1452.2)	**<0.001**	<420
PTH (intact) (pg/ml)	47.9 (25.1 to 95.2)	130.7 (61.6 to 248.5)	**<0.001**	15–65
25OHD (ng/ml)	19.4 (12.3 to 24.6)	13.7 (10.0 to 17.9)	0.08	≥20

Data are expressed as median (25th to 75th percentiles). *P* values in *bold* indicate significant values.

significant (r = −0.26; $P = 0.02$). However, there was no correlation between dietary calcium intake and serum ALP and inorganic phosphate.

Biochemical assessment

Table 2 depicts the biochemical parameters among the cases and controls. Median (25th, 75th percentile) serum 25OHD levels in both cases and controls were in the range of vitamin D deficiency [13.7 (10.0, 17.9) and 19.4 (12.3, 24.6) ng/ml, respectively; $P = 0.08$]. Figure 2 shows the frequency distribution of serum vitamin D in cases and controls; 82.1% of cases and 54.4% of controls had serum vitamin D levels below 20 ng/ml, indicative of vitamin D deficiency ($P = 0.04$). There was no correlation between serum 25OHD level and radiological score or biochemical parameters of rickets. A significant correlation between serum ALP and radiological score (r = 0.32; $P = 0.009$) was seen among cases of rickets. Serum calcium (both total and ionic) levels were significantly lower among cases compared with healthy controls.

Comparison of profile of children with rickets who were breast-fed compared with those who were not

Children with rickets who were breast-fed at presentation were significantly younger [11 (7, 36) months; median (25, 75th percentiles)] than those who were not breast-fed [20 (14, 54) months; $P = 0.001$]. Serum 25OHD among breast-fed children with rickets [13.4 (8, 23.4) ng/ml] was not statistically different from those not breast-fed at presentation [14.1 (10.8, 53.6) ng/ml; $P = 0.18$]. The two groups also did not differ significantly in demographic (family size, birth order, per-capita income, height and weight Z scores), biochemical (serum calcium, phosphate, ALP, PTH), or radiological parameters.

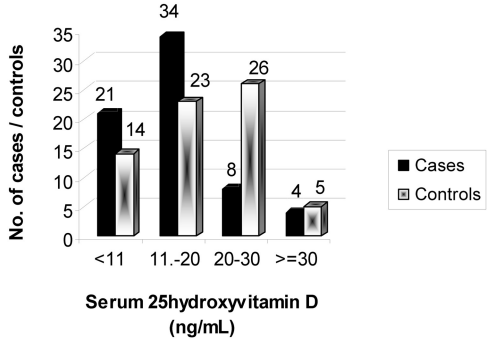

FIG. 2. Frequency distribution of serum 25OHD levels in cases and controls. To convert serum 25OH vitamin D levels to nanomoles per liter, multiply by 2.5.

Discussion

Nutritional rickets is usually thought to be caused by deficiency of vitamin D. However, studies from tropical countries [Nigeria (1) and South Africa (20)] have shown that low intake of calcium in the diet might be more important in causation of rickets. Dietary calcium intake of Indian population is very low (7, 8). Also, vitamin D deficiency is common in Indian children (13, 14). The present study was planned to investigate the relative contribution of vitamin D and calcium in the causation of nutritional rickets in Indian children. Our results show that children with rickets as well as healthy controls are vitamin D deficient, but cases of rickets have lower dietary calcium intake compared with controls.

The main presenting clinical features of rickets were delayed walking and bow legs (50% each). We had not included cases presenting with hypocalcemic seizures in the present study, a frequent presenting feature reported by other authors (13, 14). However, 91.04% of our cases of rickets had asymptomatic hypocalcemia at presentation; 62.6% of cases presented with another illness, and rickets was picked up as an incidental finding. The majority of these children had pneumonia (55.2%), a finding observed in other studies as well (21).

The cases of rickets belonged to lower socioeconomic status and had a higher family size compared with healthy controls. This explains the relatively lower intake of dairy products in cases. Because these families tend to stay in overcrowded areas, the exposure to sun is adversely affected. Maternal vitamin D deficiency due to similar environmental and dietary constraints can also adversely affect the vitamin D level of their infants, thereby increasing

their propensity toward rickets. Low 25OHD levels have been reported in mothers of infants with rickets (22).

The mean calcium intake among the cases of rickets was much lower than in the control group ($P < 0.001$), a finding previously reported by Balasubramanian *et al.* (6) from Lucknow, India, and Pettifor *et al.* (20) from South Africa. However, Thacher *et al.* (2) from Nigeria did not find any difference in calcium intake between cases and controls. The majority of cases with rickets consumed a cereal-based vegetarian diet. We found that the cases had a higher proportion of dietary calcium from nondairy sources and a higher phytate intake compared with controls. A high phytate content of diet is known to inhibit intestinal calcium absorption (23), especially the calcium from nondairy sources (24). A direct relationship between rickets and phytate intake has been reported by Robertson *et al.* (25). The amount of sun exposure did not vary significantly among the cases and controls. Previous studies from India (6) and Nigeria (2) have also reported similar sun exposure among the cases of rickets and controls.

We found a high prevalence of vitamin D deficiency in both cases and controls. Even in the apparently healthy control group, 54.4% of children had serum vitamin D levels below 20 ng/ml, indicative of vitamin D deficiency. In fact, the mean 25OHD level in both groups was in the range of deficiency, with no significant difference between the two. In an earlier study (14) from Delhi, we reported serum 25OHD below 10 ng/ml in 43.2% of healthy infants and 47.8% of their lactating mothers. In another study, Marwaha *et al.* (13) found 35.7% of adolescents from Delhi to have 25OHD levels below 9 ng/ml. These observations highlight the urgent need to consider strategies to combat vitamin D deficiency in these vulnerable sections of the population.

The median serum 25OHD levels in cases and controls in this study were 13.7 and 19.4 ng/ml, respectively. This difference did not reach statistical significance. Balasubramanian *et al.* (6) have reported mean 25OHD concentrations to be 20 ± 15.56 ng/ml among cases of rickets and 24.52 ± 14.36 ng/ml among controls. Similar findings have been reported by Fischer *et al.* (3) from Bangladesh and Thacher *et al.* (1) from Nigeria. These findings refute the earlier held belief that among cases of clinical rickets, the serum 25OHD levels are below 8 ng/ml (26). Despite similar 25OHD level, PTH levels were much higher in the cases, most likely due to associated dietary calcium deficiency as reflected in the dietary survey as well as the lower serum calcium levels.

Thus, the results of the study indicate that whereas vitamin D deficiency is prevalent in young children, mean 25OHD levels are not significantly different among cases of rickets and healthy controls. On the other hand, intake of dietary calcium, especially dairy calcium, is significantly lower among children with rickets. Thus, it seems that in our population, rickets develops when calcium deficiency is superimposed upon a low or borderline vitamin D status. Our findings support an observation by Pettifor (27) that intake of a diet low in calcium and high in phytate might account for rickets in communities where the vitamin D nutrition status is marginal. It is possible that the existing vitamin D deficiency gets exaggerated by hyperparathyroidism induced by calcium deficiency. This phenomenon has been reported by Clements *et al.* (28), who have shown in animal studies that the rate of inactivation of vitamin D in the liver is increased by calcium deprivation. The effect is mediated by high 1,25-dihydroxyvitamin D levels as a result of secondary hyperparathyroidism, thus promoting hepatic conversion of vitamin D to inactive polar metabolites. Low vitamin D status, in turn, impairs calcium absorption from the intestine, thereby exacerbating calcium deficiency. Thus, a combination of low dietary calcium and suboptimal vitamin D status will enhance both deficiencies and precipitate development of rickets in children.

It is important to realize that vitamin D deficiency does not cause rickets by directly affecting bone; instead, the effect is indirect by impairing intestinal calcium absorption. The main role of vitamin D is to ensure intestinal calcium delivery and not to act on the skeleton. Pure calcium deficiency has been reported to cause rickets, and studies including clinical trials have demonstrated healing with only calcium supplementation (1, 4, 5, 20). The critical importance of calcium in the treatment of rickets is also demonstrated by the fact that high-dose oral or intermittent iv calcium infusion is successfully used in the treatment of vitamin D-dependent rickets type 2 characterized by the absence of vitamin D receptor (29).

Our study has a few limitations that we would like to acknowledge. It is a case control study on a relatively small number of subjects. The method of assessing sun exposure by calculation of UV score is based on interviewing the parents for the duration of time spent outdoors and the amount of clothing. The method has its inherent limitations and can at best give a rough estimate of the sun exposure. Dietary evaluation was limited to those children who were not breast-fed. Thus, the crucial role played by calcium deficiency in causing rickets may not be valid for infants who are exclusively/predominantly breast-fed. These infants are unlikely to be calcium deficient, and thus vitamin D deficiency would play a relatively more important role in the etiology of rickets. We had excluded children presenting with hypocalcemic seizures who might have represented children more severely affected.

To conclude, our results indicate that in Indian children, rickets develops when low dietary calcium intake coexists with a low or borderline vitamin D nutritional status. An effective preventive strategy for rickets should be increasing dietary calcium intake and vitamin D supplementation in young children.

Acknowledgments

Address all correspondence and requests for reprints to: Brig. R. K. Marwaha, Flat no. 16, Gautam Apartments, Gautam Nagar, New Delhi 110049, India. E-mail: marwaha_ramank@hotmail.com.

Clinical Trials Registry-India registration no. CTRI/2010/091/000448.

Disclosure Summary: The authors have nothing to declare.

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