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Association of subclinical vitamin D deficiency with severe acute lower respiratory infection in indian children under 5 years

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Objectives: To determine whether subclinical vitamin D deficiency in Indian children under 5 years of age is a risk factor for severe acute lower respiratory infection (ALRI).
Design: A hospital-based case-control study.
Setting: Sanjeevani Paediatrics Hospital, a private hospital in Indapur, India.
Participants: A total of 150 children including 80 cases and 70 controls, aged 2–60 months, were enrolled. The case definition of severe ALRI as given by the World Health Organization was used for cases. Controls were healthy children attending outpatients' service for immunization.
Main outcome measure: Association of serum 25-hydroxyvitamin D3 (25OHD3) with severe ALRI, controlling for demographic and other potential risk factors.
Results: Serum 25OHD3 increased with age. Factors significantly associated with decreased risk of severe ALRI in univariate analysis were: exclusive breastfeeding in the first 4 months (cases 35/78 (45%), controls 41/64 (64%); $P=0.02$); introduction of dietary liquids other than milk only after 6 months (cases 46/70 (66%), controls 31/66 (47%); $P=0.03$); use of liquid petroleum cooking fuel (cases 32/80 (40%), controls 40/70 (57%); $P=0.04$); infant not covered in swaddling clothes when exposed to sunlight before crawling (cases 11/52 (21%), controls 25/54 (46%); $P=0.006$); and serum 25OHD3 > 22.5 nmol/l (cases 16/80 (20%), controls 48/70 (69%); $P<0.001$). In multivariate analysis, factors associated with significantly lower odds ratio for having severe ALRI were: serum 25OHD3 > 22.5 nmol/l (OR: 0.09; 95% CI 0.03–0.24; $P<0.001$) and exclusive breastfeeding in the first 4 months of life (OR 0.42; 95% CI 0.18–0.99; $P=0.046$), with age and height/age as significant covariates.
Conclusion: Subclinical vitamin D deficiency and nonexclusive breastfeeding in the first 4 months of life were significant risk factors for severe ALRI in Indian children.
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Introduction

Acute lower respiratory infection (ALRI), primarily pneumonia, is a common cause of morbidity and mortality in children younger than 5 y of age, particularly in developing countries (USAID, 2002). Consequently, considerable research has aimed at finding effective interventions against ALRI such as immunisation (Monto & Lehmann, 1998) and case management involving antibiotics (WHO, 1995). Micronutrient supplementation is another potential intervention. Many trials have investigated the benefits of vitamin A supplements, but these do not significantly decrease morbidity and mortality from ALRI, other than that due to measles, in spite of decreasing overall under-5 mortality (Vitamin A and Pneumonia Working Group, 1995). Zinc supplementation lowered ALRI incidence by 41% (Zinc Investigators' Collaborative Group, 1999).

Vitamin D deficiency is known to cause rickets and retard skeletal growth, but recently noncalcaemic roles of the vitamin have been also investigated. *In vitro* studies have shown the importance of 1,25-dihydroxyvitamin D3 for promoting and regulating immune responses (Rockett *et al*, 1998; Cantorna, 2000; Pichler *et al*, 2002), and these are also

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supported by studies in humans. Clinical vitamin D deficiency (rickets) was associated with a 13-fold increased risk of pneumonia in Ethiopian children less than 5 y of age (Muhe *et al*, 1997). In Yemen, 50% of children admitted to hospital for ALRI had vitamin D deficiency rickets (Banajeh *et al*, 1997), and in Kuwait 43% of children with vitamin D deficiency rickets had pneumonia (Lubani *et al*, 1989). Subclinical vitamin D deficiency, as indicated by low serum concentration of 25-hydroxyvitamin D3 (25OHD3), has been associated with increased risk of tuberculosis in adults, which was modified by polymorphisms in the vitamin D receptor (Wilkinson *et al*, 2000). However, we are unaware of studies that have investigated the role of subclinical vitamin D deficiency in ALRI caused by pathogens common among infants and young children in low-income countries. Such research is important given the increasing evidence that subclinical vitamin D deficiency is common even in countries at low latitude and with plentiful sunshine, including India (Atiq *et al*, 1998; Goswami *et al*, 2000; Andran *et al*, 2002).

Subjects and methods

A hospital-based case–control study was conducted in Sanjeevani Paediatrics Private Hospital, Indapur, India, in May–June 2002. The cases were children younger than 5 y of age admitted as in-patients or seen in the outpatient department with severe ALRI. A case definition of severe ALRI as given by the World Health Organization (1995) was used for the cases: the presence of lower chest indrawing with respiratory rate more than 60 min in infants less than 2 months, more than 50 min in infants 3–12 months and more than 40 min in children 13–60 months. The controls were healthy children younger than 5 y of age living in the same area as the cases and attending the clinic for vaccination during the study period. Controls were not matched with cases.

A structured questionnaire was used to obtain information concerning age of the child; education of parents, recorded as illiterate, primary education, secondary, graduate or postgraduate; type of housing; details of cooking fuel used in the household; smoking by any member in the family; and history of lower respiratory tract infection in family members. The child's immunisation history was elicited from health records brought by parents.

Breastfeeding history and the age of introduction of supplementary foods were recorded. The duration of breastfeeding was categorised as <4 months, 4–6 months, or >6 months, or as 'other' for infants who were aged less than 4 months and still breastfeeding. Parents were explained the meaning of 'exclusive breastfeeding' (breast milk and nothing else) and asked if their child was exclusively breastfed during its first 4 months of life.

Information regarding the practice of exposure of the infant to sunlight was collected, including whether the infant was first exposed to outdoor sunlight before she/he learned to crawl, the frequency and approximate duration of the infant's sun exposure and how the infant was clothed when taken out into the sun. Clothing was coded as swaddled or uncovered, although in this community tight swaddling clothes were used only for very young infants, less than about 4 months, and older infants were similarly well covered but in looser wraps.

Weight was measured using an electronic scale accurate to 5 g (Bench Weighing Scales, Universal Weighing Systems, Poona, India). Length of the child was measured to the nearest centimetre using an infantometer until the age of 2 years and thereafter height was measured using a stadiometer. Weight for age and height for age *Z* scores were with reference to the NCHS growth statistics.

Venous blood specimens were collected from both cases and controls. A commercial radioimmunoassay kit (^{125}I RIA Kit, Diasorin, UK) was used to measure 25OHD3 in serum for assessing vitamin D status. The lower detection limit of the kit was 12.5 nmol/l. For some analyses, in order to categorise 25OHD3 as normal or deficient, we chose the cutoff of 22.5 nmol/l recommended by the kit manufacturers or the possibly more appropriate higher cutoff of 50 nmol/L (Holick, 2002).

Data were analysed using SPSS (Version 11.0; SPSS Inc. Chicago). When used as a continuous variable, serum 25OHD3 was natural log transformed to normalise its distribution. Standard statistical tests were used including *t*-test, odds ratios and multiple logistic regression. For calculation of odds ratios, continuous variables were dichotomised: weight for age and height for age at Z score $=-2$, haemoglobin at 110 g/L and age of introduction of dietary liquids or solids at the group median values. $P<0.05$ was considered to be statistically significant.

Results

In total, 80 (51 boys, 29 girls) cases and 70 (38 boys, 32 girls) controls were enrolled. The mean age of all the children was 23.9 (sd 15.3) months; 15 children were 2–6 months, 25 were 6–12 months, 51 were 12–24 months, 22 were 24–36 months, 22 were 36–48 months and 15 were 48–60 months; there was no significant difference between cases and controls in age mean or distribution. The mean weight/age *Z*-score of the case children was −0.01 (sd 1.20) and of the control children was −0.10 (SD 0.97); $P=0.09$. The mean height/age of case children was −0.48 (SD 1.70) and of control children was −1.07 (SD 1.61); $P=0.03$. The mean blood haemoglobin for case children was 97 g/l (s.d. 17 g/l) and for control children was 100 g/l (s.d. 10 g/L); $P=0.18$. There were no differences between case and control groups in proportions of children with low weight/age, height/age or haemoglobin (Table 1).

Most families were in the middle-income ranges according to Indian government categories (Kumar, 1993); 63% of fathers and 52% of mothers had at least secondary education. There were no significant differences between cases and

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Table 1 Univariate associations between severe ALR1 in children, nutritional status and practices related to breastfeeding and sunlight exposure in infancy

	Case (n = 80)	*Control (n = 70)*	*Odds ratio (95% CI)*	P
Weight/age *Z* score > −2	76 (95%)	67 (96%)	0.85 (0.18–3.94)	0.84
Height/age Z score > −2	65 (81%)	49 (70%)	1.86 (0.87–3.97)	0.11
Haemoglobin > 110 g/l	16 (20%)	13 (19%)	1.10 (0.49–2.48)	0.83
Plasma 25OHD3 > 22.5 nmol/l	16 (20%)	48 (69%)	0.12 (0.05–0.24)	< 0.001
Plasma 25OHD3 > 50 nmol/l	4 (5%)	27 (39%)	0.08 (0.03–0.26)	< 0.001
Ever breastfed	78 (98%)	64 (91%)	3.66 (0.71–18.7)	0.10
Exclusively breastfed in first 4 months[a]	35/78 (45%)	41/64 (64%)	0.46 (0.23–0.90)	0.02
Introduction of liquids other than milk at ⩾6 months[b]	46/70 (66%)	31/66 (47%)	2.16 (1.09–4.32)	0.03
Introduction of solids at ⩾8 months[b]	47/63 (75%)	38/58 (66%)	1.55 (0.71–3.39)	0.28
Exposed to outdoor sun before learning to crawl	52 (65%)	54 (77%)	0.55 (0.27–1.13)	0.10
Not swaddled when exposed to outdoor sun[c]	11/52 (21%)	25/54 (46%)	0.31 (0.13–0.73)	0.006
Liquid petroleum cooking fuel	32 (40%)	40 (57%)	0.05 (0.26–0.96)	0.04

[a]Missing data for two cases and six controls.
[b]Cutoff = median value for the total group; missing diet results for several children.
[c]Includes only those exposed to sunlight before crawling.

controls in mothers' and fathers' education, number of siblings, family income, smoking history of family and type of house. Fewer households with case children (32/80, 40%) than with control children (40/70, 57%) used liquid petroleum as a cooking fuel (Table 1); the rest used biomass.

Breastfeeding was nearly universal but many stopped early. Of children at least 4 months old and for whom data were available ($n = 132$), 28 (21%) stopped before 4 months and 40 (30%) stopped between 4 and 6 months; there was no significant difference ($P = 0.58$) between cases and controls in the duration of breastfeeding. Only 54% of children overall were exclusively breastfed during their first 4 months of life and exclusive breastfeeding was significantly more common in control children than case children (Table 1). Control children received supplementary liquids other than milk at a slightly younger age than the case children, as was reflected in the proportion first receiving liquids before the median age of 6 months. There was no difference between groups in the age of introduction of solids (median = 8 months).

More controls than cases were exposed to outdoor sunlight before the age of learning to crawl, although the difference did not quite reach significance. Of those exposed to sunlight early, more case than control children were exposed while swaddled (Table 1). There were no differences in the duration or frequency of sunlight exposure among those exposed in early infancy (data not shown).

Serum 25OHD3 increased significantly with age. The correlation between natural log 25OHD3 and age in months appeared stronger for control ($r = 0.72$, $P < 0.001$) than for case children ($r = 0.31$, $P = 0.05$). The geometric mean serum 25OHD3 was significantly lower (*t* test; $P < 0.001$) in cases (geometric mean 22.8 nmol/l; 95% confidence interval (CI) 21.0–24.7) than controls (geometric mean 38.4 nmol/l; 95% CI 34.2–43.1). Serum 25OHD3 > 22.5 nmol/l was significantly less common in cases than controls (Table 1). Similar

Table 2 Serum 25-hydroxyvitamin D3 in young children according to sunlight exposure in infancy[a]

	Yes	*No*	P[b]
Exposed to outdoor sun before learning to crawl	30.9 (28.1–33.9) 106	25.1 (21.6–29.1) 44	0.02
Swaddled when exposed to outdoor sun in infancy	26.5 (23.9–29.4) 70	42.4 (36.5–49.3) 36	< 0.001

[a]Geometric mean nmol/l (95% confidence interval) *n*.
[b]Significance by *t*-test.

results were obtained when a 50 nmol/l cutoff for normal 25OHD3 was used.

Children who were exposed to sunlight as young infants had higher serum 25OHD3 than those who were not (Table 2). Of those who were sun-exposed in infancy, those exposed uncovered had higher serum 25OHD3 than those who were swaddled when taken outdoors. Blood haemoglobin, but not weight for age or height for age, correlated significantly ($P < 0.001$) with plasma 25OHD3.

Variables showing statistically significant associations with severe ALRI in univariate analyses were entered into a multiple logistic regression. The age of introduction of dietary liquids was not included since it was highly associated with exclusive breastfeeding and the missing data would reduce sample size. Age and haemoglobin were included as continuous covariates in the multivariate analysis, although they were not independently associated with ALRI, since they correlated with 25OHD3. Since information on swaddling was available only for the subset exposed to sunlight before crawling, we categorised sunlight exposure as either (1) exposed unswaddled or (2) not exposed or exposed swaddled. The 22.5 nmol/L cutoff was used for 25OHD3 because very few case children had levels

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Table 3 Factors associated with severe ALRI in multivariate analyses[a]

Factor (no. in group, no. in reference group)	*Odds ratio (95% CI)*	P
Plasma 25OHD3 $>$22.5 nmol/l (58, 84)	0.09 (0.03–0.24)	$<$0.001
Exclusively breastfed 4 months (76, 66)	0.42 (0.18–0.99)	0.046
Exposed to sun unswaddled before learning to crawl (34, 108)	0.63 (0.23–1.74)	0.37
Liquid petroleum cooking fuel (67, 75)	0.72 (0.31–1.65)	0.44
Continuous variables		
Age (months)	1.04 (1.01–1.08)	0.02
Height/age *Z*-score	1.38 (1.06–1.81)	0.02
Haemoglobin (g/l)	0.99 (0.96–1.02)	0.44

[a]Complete data were available for only 142 children.

greater than 50 nmol/L. Two-way interaction terms between the four categorical variables—exclusive breastfeeding, 25OHD3, sunlight exposure and cooking fuel—proved not to be significant and were removed from the analysis presented (Table 3). Exclusive breastfeeding in the first 4 months of life and 25OHD3 $>$ 22.5 nmol/l were associated with a significantly lower risk of severe ALRI. Age and height/age *Z* score were significantly positively associated with an increased risk of respiratory infection.

Discussion

In the multivariate analysis, adequate serum 25OHD3 and early exclusive breastfeeding were significantly associated with severe ALRI. Additional significant protective factors in the univariate analyses were the use of liquid petroleum rather than biomass cooking fuel and early exposure of the unswaddled infant to outdoor sunlight. Lack of breastfeeding and use of fuel other than liquid petroleum were risk factors for pneumonia in young children in a study similar to ours in northern India (Broor *et al*, 2001). The small but significant positive associations of height/age and age with respiratory infection may be chance findings resulting from the lack of matching of cases and controls by age and the fact that controls were shorter than cases.

The protective effect of breastfeeding against severe ALRI is well recognised (WHO collaborative Study Team on the Role
Q1 of Breastfeeding in the Prevention of Infant Mortality 2000). As for vitamin D status, although it is difficult to assume causality in a case-control study, it seems more likely that poor vitamin D status was a causal factor that increased
Q2 severe ALRI than that ALRI induced low serum 25OHD3. Firstly, *in vitro* studies (Rockett *et al*, 1998; Cantorna, 2000; Pichler *et al*, 2002) and clinical studies of rickets (Lubani *et al*, 1989; Banajeh *et al*, 1997; Muhe *et al*, 1997) support a role for vitamin D in protecting against infectious diseases. Secondly, although 1,25-dihydroxyvitamin D3 metabolism is altered in both the acute-phase response, including fever, to catch-scratch disease (*Bartonella henselae*; Bosch, 1998) and chronically in adults with HIV infection (Haug *et al*, 1998), neither of these infections induced changes in serum 25OHD3, the accepted measure of vitamin D status.

Although India has abundant sunlight, low serum 25OHD3 was common. Breast milk may not provide enough vitamin D for infants (WHO, 1998), especially if the mothers are also vitamin D-deficient (Atiq *et al*, 1998; Goswami *et al*, 2000; Andran *et al*, 2002). Therefore, additional vitamin D is needed from sunlight or other foods. The complementary or replacement foods in the diets of Indian infants and children are primarily cereals, cheese and yoghurts that are not fortified with vitamin D (National Institute of Nutrition,
India, 1996). Children may not be exposed to sunlight for Q3
lifestyle or cultural reasons. In addition, pollution may decrease the ultra-violet rays reaching the children (Agarwal *et al*, 2002).

The results of this study have important public health implications since, irrespective of any effect of vitamin D on respiratory illness, vitamin D deficiency is clearly a problem in this community. Messages on the importance of sunlight exposure of young children should be given to mothers and the general community. Cheap vitamin D-rich supplements such as cod liver oil could be advocated. Breastfeeding messages in this population need to be reinforced since only half of the children were exclusively breastfed for at least 4 months and many had completely stopped breastfeeding by 6 months of age. Messages supporting exclusive and longer breastfeeding could be given through the Baby Friendly Hospital Initiative to ensure that all children obtain the benefit of breast milk for reducing the burden of respiratory tract infection.

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