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Vitamin D status of children in Kerala, southern India

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Abstract

Objective: To study plasma 25-hydroxyvitamin D (25(OH)D) status of children in Kerala, southern India, and its relationship with sociodemographic variables.
Design: Cross-sectional observational study.
Setting: Tertiary government hospital.
Participants: Children (*n* 296) with trivial acute illness were enrolled. Sun exposure and Ca and vitamin D intakes (7 d dietary recall) were documented. Serum Ca, P, alkaline phosphatase, plasma 25(OH)D and parathyroid hormone (PTH) were measured.
Results: Prevalence of vitamin D deficiency (plasma 25(OH)D < 30 nmol/l) was 11·1 % (median, interquartile range (IQR): 52·6, 38·4–65·6 nmol/l). Children who ate fish daily had significantly higher plasma 25(OH)D than those who did not (median, IQR: 52·5, 40·8–68·9 *v.* 49·1, 36·2–60·7 nmol/l; $P = 0·02$). Those investigated in the months of March–May showed highest 25(OH)D *v.* those enrolled during other times (median, IQR: 58·7, 45·6–81·4 *v.* 45·5, 35·6–57·4 nmol/l; $P < 0·001$). Plasma 25(OH)D correlated positively with serum P ($r = 0·24$, $P < 0·001$) and Ca intake ($r = 0·16$, P 0·03), negatively with age ($r = -0·13$, P 0·03) and PTH ($r = -0·22$, $P < 0·001$.). On linear regression, summer season (March–May), lower age, daily fish intake and higher Ca intake were independently associated with plasma 25(OH)D.
Conclusions: Prevalence of vitamin D deficiency is low in Kerala. The natural fish diet of coastal Kerala and the latitude may be protective. Public health policy in India should take account of this geographical diversity.

Keywords
25-Hydroxyvitamin D
Rickets
Fish
Latitude
Sun

India is situated in a tropical and subtropical region where there is abundance of sunlight. However, vitamin D deficiency has been documented commonly in both urban and rural populations of Indian neonates, infants, adolescents (especially girls) and pregnant women[1–4]. In urban Indians, indoor lifestyle and skin pigment are risk factors. In India, food is not fortified with vitamin D or Ca. Overt rickets, with softening and bending of bones, is a common problem encountered in the paediatric clinics in our country. However, only children with the most severe vitamin D and Ca deficiencies manifest overt rickets, with a larger pool of children with moderate deficiency at the bottom of the iceberg[5].

India is a large country, with a long coastline in the south and high mountains in the north. Geography and climatic conditions are diverse in this subcontinent, as are food habits. The literature documenting high prevalence of vitamin D deficiency in India is largely from inland cities or rural areas north of the Tropic of Cancer[1–4]. Particularly, literature is sparse in the toddler and pre-adolescent age group. The southern state of Kerala lies between latitudes 8°N and 12°N, entirely situated in the tropics, with abundant sunlight. There is heavy cloud cover during the South-West monsoon season from June to August, and some cloud cover also during the North-East monsoon in September and October, but the weather is warm throughout the year[6]. Being a coastal area, fatty fish like sardine are available in all seasons and are relatively cheap. The majority of Keralites are fish eaters. The present study aimed to find out whether these factors protect children in Kerala from developing vitamin D deficiency.

Methods

Participants

Consecutive children, between 6 months and 12 years of age, attending the paediatric outpatient department of one unit with trivial illness like upper respiratory tract infection, first episode of urinary infection and conjunctivitis were enrolled for the study after getting informed written

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consent from parents. Children suffering from severe acute illness requiring hospitalization and children having any chronic illness like bronchial asthma, heart disease and renal disease were excluded from the study. Children with clinical rickets and those who had received any vitamin D supplementation, antitubercular or anticonvulsant therapy in the past year were also excluded. The study protocol was approved by the ethics committee of the Government Medical College, Kozhikode. Parents of children gave written informed consent.

Data collection: diet and sunlight exposure

A 7 d diet recall method was used for calculating dietary Ca and vitamin D intakes. The staple diet in this region is rice and fish. Ragi, a cereal rich in both Ca and Fe, is a complementary weaning food[7]. History of clothing pattern in different seasons and activity pattern in different months was enquired about. Sun exposure (in minutes per day) from 10.00 to 16.00 hours was calculated based on typical activity pattern and clothing habits.

Blood samples and laboratory analyses

Serum Ca, inorganic phosphate and alkaline phosphatase were analysed on the same day (Erba EM 360 auto-analyser; Transasia Bio-Medicals Limited, Mumbai, Inda) and plasma was stored at −40°C until transportation on dry ice and assay for parathyroid hormone (PTH) and 25-hydroxyvitamin D (25(OH)D) by immunoradiometric assay and RIA, respectively (DiaSorin, Inc., Stillwater, MN, USA). The sensitivity of the assay for 25(OH)D was 3·75 nmol/l; the inter-assay CV in our laboratory was 14·1 % and the intra-assay CV was 8·6 % at 31 nmol/l. The sensitivity of the PTH assay was 0·64 pmol/l, with inter-assay CV of 10·5 %.

Statistical analysis

Analysis was done using the statistical software package PASW Statistics version 18. Sample size of 380 was estimated for obtaining a true prevalence of vitamin D insufficiency within 95 % confidence limits, with 80 % power, and was calculated presuming a prevalence of 50 %, lower than that reported (approximately 75 %) from northern parts of the country. The Kruskal–Wallis test was for used comparison of medians of multiple groups, the Mann–Whitney *U* test for comparison of medians between pairs of groups, the Spearman correlation coefficient for correlation, and linear regression was done to estimate the adjusted association of each individual factor with plasma 25(OH)D. Variables that were correlated with plasma 25(OH)D at $P < 0{\cdot}1$, or were clinically relevant factors, or were significantly different on comparison of means were included in the linear regression models. Collinearity between the variables in the regression models were checked using the variance inflation factor. Since variance inflation factors for all variables were less than 4, and models excluding any of the collinear variables like fish, vitamin D and Ca intakes did not change the outcome, we used a model with all the relevant variables. $P < 0{\cdot}05$ was considered statistically significant.

Results

A total of 296 consecutive children with trivial illness presenting to the outpatient department, and who gave consent, were enrolled in the study. Baseline characteristics, including median plasma 25(OH)D in the total population and in different subgroups, are shown in Table 1. Plasma 25(OH)D was in the sufficient range (>50 nmol/l) in 160 children (54·1 %), while 103 (34·8 %) had insufficient levels (30–50 nmol/l) and thirty-three children (11·1 %) were deficient (<30 nmol/l). Gender and egg and milk consumption patterns were not associated with plasma 25(OH)D. Although median plasma 25(OH)D was not different between toddler, mid-childhood and adolescent age groups, age was significantly negatively correlated with plasma 25(OH)D (Table 2). Significantly higher plasma 25(OH)D was observed in those consuming fish daily in comparison with those who consumed it less often than daily or never. Plasma 25(OH)D was significantly higher (median 58·7 nmol/l) among children whose blood sample was collected during the dry hot summer months (March–May) and was similar in all other seasons (median 46·6 nmol/l in December–February (dry season), 45·6 nmol/l in

Table 1 Baseline characteristics of the study group of children aged 6 months–12 years (*n* 296), Kerala, southern India, October 2015–March 2017

			25(OH)D (nmol/l)		
Characteristic	*n*	%	Median	IQR	*P* value
Total study group	296	100·0	52·6	38·4–65·6	–
Age					
< 5 years	138	46·6	54·3	39·6–70·9	0·15
5–10 years	100	33·8	50·5	38·5–62·1	
> 10 years	58	19·6	50·0	33·9–61·5	
Gender					
Male	156	52·7	54·0	39·7–68·2	0·08
Female	140	47·3	49·3	36·8–62·6	
Fish intake					
Every day	153	51·7	52·5	40·8–68·9	0·02
Not every day/never	143	48·3	49·1	36·2–60·7	
Milk consumption					
Nil	100	33·8	48·4	34·7–66·3	0·25
< 200 ml/d	143	48·3	55·6	41·2–67·5	
> 200 ml/d	53	17·9	51·9	38·8–60·4	
Egg intake					
Every day	59	19·9	54·25	41·0–68·25	0·34
Not every day/never	237	80·1	51·75	38·25–65·25	
Season of blood collection					
March–May	136	45·9	58·7	45·6–81·4	<0·001
Other seasons	160	54·1	45·5	35·6–57·4	

25(OH)D, 25-hydroxyvitamin D; IQR, interquartile range.

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Table 2 Summary of quantitative measures and their correlation with plasma 25-hydroxyvitamin D (25(OH)D) among children aged 6 months–12 years (*n* 296), Kerala, southern India, October 2015–March 2017

			Correlation of quantitative variable with plasma 25(OH)D	
Variable	Mean	SD	*r*	*P* value
Age (years)	5·9	3·05	−0·13	0·03
Plasma PTH (pmol/l)	49	49	−0·22	<0·001
Serum alkaline phosphatase (U/l)	281		−0·10	0·09
Serum Ca (mmol/l)	2·3	0·30	−0·06	0·33
Serum P (mmol/l)	1·6	0·29	0·24	<0·001
Sun exposure (min/d)	55	14	0·12	0·04
Ca intake (mg/d)	166	147	0·16	0·03
Vitamin D from food (μg/d)	3·8	2·57	0·14	0·02

PTH, parathyroid hormone.

Table 3 Linear regression results for the adjusted association of study variables with plasma 25-hydroxyvitamin D among children aged 6 months–12 years (*n* 296), Kerala, southern India, October 2015–March 2017

Variable	*β*	*P* value
Male gender	2·423	0·12
Daily fish intake	3·212	0·04
March–May season of sampling	8·332	<0·001
Age	−0·854	0·001
Ca intake	0·014	0·007
Sun exposure	0·013	0·82
Vitamin D from food	−0·02	0·07

Model $P<0·001$, model adjusted $R^2=0·15$.

June–August (monsoon season) and 38·4 nmol/l in September–November (retreating monsoon season)).

Mean daily Ca intake was 166 (SD 147) mg and mean daily vitamin D intake was 3·8 (SD 2·5) μg (153 (SD 103) IU). Of the study children, 251 (84·7 %) were exposed to sunlight for at least 60 min daily, with head, forearms, hands and legs (below knee) exposed to sunlight in the majority. Elevated alkaline phosphatase (>450 IU/l) was noted in fifteen (5·1 %) children. As shown in Table 2, plasma 25(OH)D showed a significant positive correlation with Ca intake, vitamin D intake, serum P and duration of sun exposure, and a negative correlation with PTH. Ca and vitamin D intakes were strongly correlated with each other ($r=0·73$, $P<0·001$), an expected result because the commonly eaten fish (sardine, mackerel, pomphret) are rich in vitamin D as well as Ca content. A linear regression model which included season of blood sampling, fish intake, gender, age, Ca intake, vitamin D intake and minutes of sun exposure indicated that summer season (March–May), lower age, eating fish daily and higher Ca intake were independently associated with plasma 25(OH)D (Table 3).

Discussion

Our data show that the mean plasma 25(OH)D of children in north Kerala is in the normal range (>50 nmol/l) and 11 % have plasma 25(OH)D in the deficient range (<30 nmol/l). These values indicate better vitamin D status than found in most Indian studies conducted in various geographical regions (Table 4).

Although the majority of these studies are from northern and central India, studies from regions closer to the equator, such as Tirupathi and nearby villages[8] and Chennai[9], have also reported severe vitamin D deficiency to be a relevant public health and clinical problem. Despite the populations studied by Harinarayan *et al.*[8] being mainly adult, it was a large study with detailed description about the dietary pattern. The authors reported mean serum 25(OH)D of 38·9 nmol/l in urban boys and 42·4 nmol/l in rural boys. Vitamin D intake in their population was very poor. The study from Chennai reported vitamin D deficiency to be the commonest single cause of hypocalcaemic seizures in infancy, reflecting poor maternal vitamin D nutrition[9]. Chennai is a coastal city at latitude 13·1°N. However, pregnant women in India, and their infants, are among the most vulnerable to vitamin D deficiency due to modest clothing pattern and increased physiological demand[1,2]. The authors did not comment on the fish intake of the mothers of the infants in their study.

International studies from geographical regions situated close to the equator like Kerala also report vitamin D 'deficiency'. A study from Sri Lanka of 340 pre-school children aged 2–5 years showed mean serum 25(OH)D to be 57·5 nmol/l, with 5 % of children having serum 25(OH)D < 25 nmol/l[10]. With regard to the cut-off for the definition of vitamin D deficiency, a recent global consensus statement has recommended that, for children, deficiency be defined as serum 25(OH)D < 30 nmol/l and insufficiency as serum 25(OH)D between 30 and 50 nmol/l[5]. It is in this context that recent studies in children from equatorial countries should be viewed. The four-nation SEANUTS (South East Asian Nutrition Survey) reports also mention widespread vitamin D 'deficiency', with about 40 % of children aged between 6 months and 12 years having serum 25(OH)D < 50 nmol/l[11–14]. However, mean serum 25(OH)D was above 50 nmol/l in all four countries. Like

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Table 4 Vitamin D status in children: comparison of studies in Indian literature

Study	Place	Study population	Age	Percentage deficiency (25(OH)D < 30 nmol/l)	25(OH)D (nmol/l) Mean	SD
Agarwal *et al.*[18]	Delhi: Mori Gate	Infants and toddlers	9–24 months	46	31	
	Delhi: Gurgaon	Infants and toddlers	9–24 months	0	67·75	
Tiwari and Puliyel[19]	Delhi (slums)	Sunder Nagar (Jan)	9–30 months	2*	9	25·0
		Rajiv Colony (Feb)	9–30 months	82·0	23·8	27
		Rajiv Colony (Aug)	9–30 months	84	17·8	22·0
		Gurgaon (Aug)	9–30 months	82	19	20
Sahu *et al.*[3]	Lucknow	Rural girls	10–20 years	34†	33·3	16
Harinarayanan *et al.*[20]	Tirupathi	Urban boys		81·5‡	38·9	3
		Rural boys		78·5	42·5	3·25
		Urban girls		62·8	46·25	4
		Rural girls		73·2	47·5	4
Marwaha *et al.*[4]	Delhi	Low SES	10–18 years	50·7§	26·0	N/A
		Upper SES		30·4	34·3	N/A
Khadilkar *et al.*[21]	Pune	Adolescent girls	12–16 years	70	N/A	N/A
Basu *et al.*[22]	Kolkata	Children	Total	19·7	47·5‖	N/A
			1–5 years		57·5	
			6–11 years		42·5	
			12–16 years		25·0	

SES, socio-economic status; N/A, data not available.
Tiwari and Puliyel used an ELISA (ImmunoDiagnostik), Basu *et al.* used a chemiluminescence assay (Roche Diagnostic) and the other studies used the DiaSorin RIA. For conversion, 1 ng/ml = 2·5 nmol/l.
*Definition of vitamin D deficiency used by Tiwari and Puliyel is 25(OH)D < 35 nmol/l.
†Definition of severe vitamin D deficiency used by Sahu *et al.* is 25(OH)D < 25 nmol/l.
‡Definition of vitamin D deficiency used by Harinarayanan *et al.* is 25(OH)D < 20·0 ng/ml (50·0 nmol/l)
§Definition of moderate deficiency used by Marwaha *et al.* is serum 25(OH)D < 25·0 nmol/l.
‖Values are medians.

us, all studies noted highest serum 25(OH)D in the youngest age groups. The prevalence of serum 25(OH)D < 30 nmol/l and the availability of serum PTH would have added value to the interpretation of these studies.

Vitamin D intake would be expected to be generous in all these countries, fish and seafood forming an important part of their diet. Intake was calculated only in the Malaysian SEANUTS study[14]. Diet contributed about 5–7 μg vitamin D daily, variably in different age groups. This finding is similar to ours. Fatty fish like sardine (*mathi*) and mackerel (*aila*) are rich sources of vitamin D[7] and are part of the staple diet in Kerala. Furthermore, fish is also a rich source of dietary Ca in this region.

Even as close to the equator as 8°N to 12°N, there is a significant seasonal variation in serum 25(OH)D. This is possibly due to cloud cover during the monsoon season, which blocks UV rays from reaching the earth's surface. Over Kozhikode, the India meteorological department describes the UV index to be 11–12 in January to March and 6–7 in June and July[15]. This is an important observation which should be kept in mind while planning studies or supplementation based on vitamin D levels. Indeed, season was the strongest predictor of serum 25(OH)D in our regression analysis. Recent studies from countries close to the equator such as Cambodia[16] or the nine-nation Mesoamerican study have commented on the low prevalence of vitamin D deficiency[17]. Documenting serum 25(OH)D < 30 nmol/l only in 2·9 % of 781 Cambodian children, the authors attributed the good status to outdoor activity and sun exposure rather than to dietary intake. In the latter study, although by no means representative of the population due to small sample sizes, mean serum 25(OH)D in 287 children was as high as 79·5 nmol/l, with just one child having serum 25(OH)D < 30 nmol/l and 3·6 % with serum 25(OH)D <50 nmol/l[17]. Although fortification of milk is prevalent in some of these countries, thereby providing dietary vitamin D to children, the adult population with the greatest outdoor activity in the same study had the highest serum 25(OH)D.

We have compared vitamin D status of children from various studies in India[3,4,8–22] (Table 4). Other Indian studies have reported a steep increase in the prevalence of vitamin D deficiency during adolescence, noticeably in girls, with increased physiological demands compounding poor outdoor activity and modest clothing. In studies from Pune in central India and Barabanki in northern India, 70 % of adolescent girls were found to have vitamin D deficiency[3,18]. We had only a small number of adolescents in our study and could not examine this issue.

Our study has some limitations. First, it is a hospital-based study and even though the children enrolled had only trivial illness, in the true sense they are not healthy subjects. Second, the same children were not sampled in all seasons of the year. Third, we did not meet our original sample size estimation; however, with a sample size of 296, we obtained a prevalence of vitamin D insufficiency of 46 %, with a relatively narrow precision of 5·6 %. Finally, the assay used for serum 25(OH)D estimation was not the gold-standard liquid chromatography–mass spectrometry technique, which could have introduced the confounding

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factor of assay drift. We tried to minimize this problem by performing all assays within a short period of time using a single lot number. Notwithstanding these limitations, we believe we can conclude that vitamin D deficiency in the pre-adolescent age is not as common or severe a public health problem in Kerala as it is in most other reports from the northern inland regions of India. There is significant seasonal variation in plasma 25(OH)D. Our study demonstrates that advantage can be taken of the rich natural resource of sunshine availability in some seasons of the year, in some parts of our country, to improve vitamin D nutritional status, before resorting to pharmacological supplements. Our results may also have implications for policy makers considering food fortification, drawing attention to the geographical diversity in our large country.

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