Original article

Vitamin D deficiency among children aged 10-18 years in Sri Lanka

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Abstract

Background Vitamin D deficiency (VDD) and insufficiency (VDI) are public health problems in many countries, and limited data is available on the prevalence of VDD/VDI in Sri Lanka.

Objectives To determine the prevalence and associated factors of VDD in children aged 10-18 years.

Methods This was a cross-sectional study among school children aged 10-18 years at national level. A representative sample of 2525 children were recruited from July to November 2017. Serum 25(OH)D concentration and the patterns of vitamin D rich food consumption were assessed. VDD and VDI cut offs were set at serum 25(OH)D concentrations of <12 ng/mL and 12-20 ng/mL, respectively as defined by global consensus in 2016.

Results The mean serum 25(OH)D level was 19.3±7.4 ng/mL. The prevalence of VDD and VDI were 13.2% (95% CI: 11.9%-14.5%) and 45.6% (95% CI: 43.7%-47.5%), respectively. The prevalence of VDD was highest in the central province (32.2%) and highest prevalence of VDI was in the Sabaragamuwa province (58.9%). VDD and VDI were lowest in North Central province (0.7% and 34.7%, respectively). Significantly higher serum 25(OH)D levels were observed with male gender (p=0.000), BMI for age <-2SD (p=0.000), daily milk consumption (p=0.000) and residing in dry zone (p=0.000).

Conclusions Though Sri Lanka is a tropical country, VDD is prevalent among school children aged 10-18 years. It is important to develop a VDD preventive strategy, especially for high risk groups.

Introduction

During the past decade, interest in Vitamin D (VD) has increased due to the associations of VD with immune function, cardiovascular health, and even cancer occurrence as observed in multiple studies [1]. The clinical role of VD and its metabolites is expressive due to its interrelationship with calcium homeostasis and bone metabolism [2]. Vitamin D deficiency (VDD) presents as a pyramid with its severe most presentations being rickets in children and osteomalacia in adults at the top of the pyramid. These clinical presentations are rarely seen now a days but subclinical VDD, as detected by low serum 25-hydroxyvitamin D(25(OH)D), is prevalent, VDD in children is an obstacle for them reaching their genetically programmed height and peak bone mass [3].

There is a disagreement about the best threshold of 25(OH) D levels to define VDD and VDI as the association between clinical presentations such as rickets or osteomalacia and levels of 25(OH) D has been inconsistent. This inconsistency is also seen between 25(OH)D level and biochemical markers such as elevated alkaline phosphatase. Thus, studies have adopted different methods to define cut-off values to determine VD sufficiency, insufficiency and deficiency [4].

There is a high prevalence of VDD worldwide when 25(OH)D threshold of 30 ng/mL is considered, whereas level below 10 ng/mL is common in South Asia and the Middle East [5]. A study revealed that more than 90% of patients aged 10-65 years were VDD when they presented with nonspecific muscle aches, bone aches and pain [6]. Identification and treatment of VDD is important for optimum musculoskeletal health [1]. VDD in children has been reported increasingly since the mid-1980s despite plentiful sunshine in some countries [7]. In Indian male and female adolescents the prevalence of severe VDD

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(<9.5 ng/mL) was 27% and 47%, respectively [8]. VDD (<20 ng/mL) prevails in the Middle East and North Africa, with rates varying from 30-90% [7]. Female gender, winter season, lower vitamin D consumption and poor sunlight exposure due to cultural dress codes and pigmented skin were the risk factors of VDD identified in this study [7].

Sri Lanka is an island located near the equator between latitudes 5055' and 9051' N and longitudes 79041' and 81053'E. It has a maximum land length and width of 432 km and 224 km, respectively. Sri Lanka is a tropical country, with monthly average temperature varying between 26°C and 33°C in the lowlands and 7°C and 21.6°C in the central highlands. The country is divided according to the amount, seasonality and variability of rainfall into three zones (wet, intermediate and dry zones) [9]. Only a few studies on vitamin D have been conducted in Sri Lanka and these are either restricted to one region or adult populations [10,11].

A study conducted in Southern Sri Lanka found severe (25(OH)D <5.2 ng/mL), moderate (25(OH)D 5.2-10.0 ng/mL), mild (25 (OH) D 10.1-14.0 ng/mL) and overall VDD (25(OH)D ≤14 ng/mL) among community-dwelling healthy women to be 21.4%, 19.1%, 15.7% and 56.2%, respectively [10]. The age and sex adjusted prevalence of VDD (25(OH)D <20 ng/mL) and VD insufficiency (25(OH)D 20-30 ng/mL) were 57.2% and 31%, respectively in an adult Sri Lankan urban population [11].

Considering the high prevalence reported in these studies and limited data particularly in children, this study was conducted under the following objective: to determine the prevalence and associated factors of VDD in children aged 10-18 years in Sri Lanka at national and provincial levels.

Methods

Data sources

Data from the Sri Lankan national nutrition and micronutrient survey conducted among children aged 10-18 years was used for analysis (12). The study design was a cross-sectional school-based survey and included 2700 adolescents aged 10-18 years. This study used a cluster random-sampling technique and clusters were defined as all government schools excluding primary schools. The government school system retains about 80% of children between 10-17 years in Sri Lanka [13]. From each province 15 schools, selected randomly, were studied (a total of 135 schools). In each selected class, 20 adolescents (10 male and 10 female) were randomly selected using attendance registers. The survey was conducted from July to November 2017. Children answered a food frequency questionnaire, which was validated through similar previous surveys conducted by the Medical Research Institute (MRI) to determine the daily consumption of vitamin D rich food items. Among the food consumed by the children, it was found that only milk and biscuit contain

a considerable quantity of vitamin D. Therefore, average vitamin D content of milk and biscuits was obtained by conducting a market survey and scanning food labels. The Indian food composition tables were used for all other food items [14]. Weight and height were measured by trained MRI staff using UNICEF UNISCALES and stadiometer, while adhering to the WHO standardized techniques in recording the measurements to the nearest 0.1 kg and 0.1 cm, respectively [15]. The parents of children provided written informed consent before their enrolment in the study. Ethical clearance was obtained from the MRI ethics committee.

Laboratory methods

Blood samples were collected from 2525 children (93.5% of the sample) by venepuncture to red-top tubes by qualified nurses at designated centres. Blood was allowed to clot at room temperature and then centrifuged to separate serum within 2 hours of collection by the medical laboratory technologists. Serum samples were stored at -20°C for maximum of 5 days until transported with ice packs to the central laboratory every week and then stored at -80°C until the analysis. Serum 25(OH)D was quantified by chemiluminescence immunoassays (DiaSorin, Italy) with sensitivity of ≤4.0 ng 25(OH)D/mL. The reference standard was run on the analyser to check for correct calibration and fraction before the samples were analysed. Internal commercially prepared quality control samples (LIASON level 1 and 2) were included in every batch to determine between-batch precision of the assays. Samples of 25 (OH) D concentrations below 12 ng/mL and above 40 ng/mL were analysed in duplicates. The inter assay coefficient variance (CV) was 4.9%. Intra-assay level 1 and intra-assay level 2 CV were 7.5% (mean 16.8±1.3) and 5.9% (mean 53.6±3.2), respectively.

Data analysis

IBM Statistical Package for Social Sciences® (SPSS) version 23.0 was used for statistical analyses. Serum 25(OH)D levels was expressed as mean ±SD. Chi-square test, Mann Whitney U test and F test were used to determine the factors associated with serum 25(OH)D level. The $p<0.05$ was considered as statistically significant. In the original dataset, age was calculated with birthdays extracted from the class attendance register that was available for all the children. Weight and height were analysed using the WHO Anthro-Plus 2009 software [16]. Child nutritional status was classified according to BMI-for-age-sex as thin (<-2SD), adequate (between -2SD to +1SD), overweight (between +1SD to +2SD) or obese (>+2SD) and linear growth was classified according to height-for-age as stunted (<-2SD), or adequate (≥-2SD) as defined by the WHO [15]. We categorized vitamin D status as sufficient, insufficient and deficient when 25(OH)D concentrations were >20-100 ng/mL, 12-20 ng/mL and <12 ng/mL, respectively according to the global

guidelines [17]. Parathyroid hormonal level was performed in a 300-sub sample to reconfirm the cut-off level (details are not provided in this paper).

Results

A total of 2525 children (53.3% female) were included in the study. Mean age of the sample was 14.0 ± 2.0 years. In the study sample, mean BMI was 17.8 ± 3.5 kg/m^2 and it ranged from 10.7 to 41.0 kg/m^2. Percentages of stunting, thinness, overweight and obesity in the study sample were 13.4, 27.0, 8.5 and 2.2, respectively (Table 1). Among the study subjects, the prevalence of VDD, VDI, VDS were 13.2% (95% CI: 11.9%-14.5%), 45.6% (95% CI: 43.7%-47.5%) and 41.2% (95% CI: 39.3%-43.1%), respectively (Table 2). There was a higher percentage of VDD in females than in males (18.9 Vs 6.7; p=<0.001). Overweight children had a higher prevalence of VDD compared to thin children (16.4 Vs 11.0; p=<0.001). There was a wide variation in VDD prevalence between provinces. The highest prevalence was found in the Central province and the lowest in the North Central province (32.2% [95% CI:26.8%-37.6%] Vs 0.7% [95% CI: -0.3%-1.7%]; P=<0.001). There was no significant difference in VDD within age groups and also between stunted vs non-stunted. Mean serum 25(OH)D level in the study sample was 19.3 ± 7.4 ng/mL and ranged from 4.0 to 70.0 ng/mL. It was observed that, among boys of all age groups, mean serum 25(OH)D concentrations (20.6-23.8 ng/mL) were above the cut-off level of VDS. Among girls of all age groups, mean 25(OH)D levels were below the cut-off for VDI (16.7-18.1 ng/mL). Mean serum 25(OH)D level in thin children was significantly higher compared to obese children (20.9 ± 8.0 Vs 16.4 ± 4.9 ng/mL; p<0.001). Mean 25(OH)D level significantly varied between provinces (14.6-24.1 ng/mL; p<0.001). The highest mean 25(OH)D levels was observed in the North Central province (24.1 ± 8.3 ng/mL) while the lowest in the Sabaragamuwa province (14.6 ± 4.9 ng/mL). Commonly consumed vitamin D rich daily food items were milk and biscuits. Though fish contains the highest amount of vitamin D, 70.3% of children consumed fish, only 2-3 times per week. Second highest food source of vitamin D was milk. One serving of milk (20g) contained 3.6 ± 2.5 µg of vitamin D due to fortification. It was observed that 21.2%, 26.4% and 29.1% of children had never consumed margarine, butter or cheese, respectively (Table 4). Table 5 shows, the multiple regression analysis, the variables account for 22% of the variance in 25(OH)D levels. Female gender, living in Sabaragamuwa or Central province, being obese or overweight showed significant negative correlations with serum 25(OH)D level. Living in North Central or North Western provinces, being thin and consuming milk daily showed significant positive correlations with serum 25(OH)D level.

Table 1. Baseline information of study sample

Child and household characteristics	Mean ± SD / No (%) / % (CI)
Child's sex	
Boys (n)	1178 (46.7)
Girls (n)	1347 (53.3)
Age (years)	
Mean ± SD	14.0 ± 2.0
Nutritional status of children	
Mean HAZ score	-1.0 ± 0.9
Mean BMI Z score	-1.1 ± 1.4
Mean BMI ± SD (kg/h^2)	17.8 ± 3.5
BMI range (kg/h^2)	10.7 - 41.0
Stunting (height-for-age < -2SD)	13.4 (12.1-14.7)
Thin (BMI-for-age-sex < -2SD)	27.0 (25.3-28.7)
Overweight (BMI-for-age-sex > + 1SD)	8.5 (7.4-9.6)
Obesity (BMI-for-age-sex > + 2SD)	2.2 (1.6-2.8)
Total	2525 (100.0)

HAZ – height for age Z score; BMI Z score – Body Mass Index Z score

Table 2. Prevalence/percentage of VDD, VDI, VDS in children aged 10-18 years by child characteristics and place of residence

Child characteristics and place of residence	No of subjects	25 (OH) D level (% and confidence interval)		
		<12 VDD	12-20 VDI	20-100 VDS
Sex*				
Girls	1347	18.9	50.3	30.8
Boys	1178	6.7	40.2	53.1
Age in years				
10	91	8.8	48.4	42.9
11	292	12.3	43.2	44.5
12	266	9.8	51.9	38.3
13	303	11.6	46.2	42.2
14	422	15.9	46.2	37.9
15	534	13.7	45.3	41.0
16	405	12.3	43.2	44.4
17-18	212	17.9	42.9	39.2
Height group				
Stunted	339	13.3	44.5	42.2
Not stunted	2186	13.2	45.7	41.1
BMI group*				
Thin	681	11.0	37.2	51.8
Adequate	1600	13.7	48.4	37.9
Overweight	189	16.4	47.1	36.5
Obese	55	14.5	61.8	23.6
Province*				
North Central	271	0.7 (-0.3-1.7)	34.7 (29.0-40.4)	64.6 (58.9-70.3)
North Western	270	1.9 (0.3-3.5)	37.0 (31.2-42.8)	67.1 (61.5-72.7)
Northern	287	8.7 (5.4-12.0)	44.9 (39.2-50.7)	46.3 (40.5-52.1)
Western	280	8.9 (5.6-12.2)	45.7 (39.9-51.5)	45.4 (39.6-51.2)
Eastern	288	11.1 (7.5-14.7)	51.4 (45.6-57.2)	37.5 (31.9-43.1)
Southern	277	13.0 (9.1-16.9)	45.8 (40.0-51.6)	41.2 (35.5-46.9)
Uva	296	13.5 (9.6-17.5)	46.3 (40.5-52.1)	40.2 (34.5-45.9)
Sabaragamuwa	280	28.2 (23.0-33.4)	58.9 (53.2-64.6)	12.9 (9.0-16.8)
Central	276	32.2 (26.8-37.6)	44.6 (38.9-50.4)	23.2 (18.3-28.1)
Total (95% CI)	2525	13.2 (11.9-14.5)	45.6 (43.7-47.5)	41.2 (39.3-43.1)

*P=<0.001.

Table 3. Mean serum 25OHD levels in children aged 10-18 years by age, sex and province

Age and sex		Mean serum 25OHD levels	
Sex*	Age in years	No of subjects	NG/ML
Female	10	46	18.1 (6.2)
	11	148	17.4 (6.2)
	12	132	16.8 (5.9)
	13	168	16.9 (6.0)
	14	234	16.7 (6.8)
	15	264	18.0 (6.5)
	16	220	18.0 (7.1)
	17-18	135	17.5 (6.6)
	Total	1347	17.4 (6.5)
Male	10	45	23.8 (9.5)
	11	144	21.5 (6.3)
	12	134	21.2 (6.8)
	13	135	22.2 (8.4)
	14	188	21.7 (8.2)
	15	270	20.6 (7.9)
	16	185	21.7 (7.9)
	17-18	77	21.0 (7.5)
	Total	1178	21.5(7.8)
Both sex	10	91	20.9 (8.5)
	11	292	19.4 (6.6)
	12	266	19.0 (6.8)
	13	303	19.2 (7.6)
	14	422	18.9 (7.8)
	15	534	19.3 (7.4)
	16	405	19.7 (7.7)
	17-18	212	18.8 (7.2)
Province*			
North Central		271	24.1 (8.3)
North Western		270	22.4 (6.7)
Northern		287	19.8 (6.5)
Western		280	19.5 (6.2)
Uva		296	19.5 (8.2)
Eastern		288	19.3 (7.0)
Southern		277	19.0 (6.9)
Central		276	15.5 (6.8)
Sabaragamuwa		280	14.6 (4.9)
BMI categories*			
Thin		681	20.9 (8.0)
Adequate		1600	18.9 (7.2)
Overweight		189	18.0 (6.3)
Obese		55	16.4 (4.9)
Total		2525	19.3 (7.4)

P=<0.001; Range of 25OHD level = 4.0-70.0 ng/mL

Table 4. Daily consumption of vitamin D rich food items among children aged 10-18 years and vitamin D content of typical serving size

Vitamin D rich food items	Serving size of food	Vitamin D (µG) in a serving mean±SD	Consumption frequency (%)				
			More than once /daily	Daily	2-3 times per week	Seldom	Never
Fish (small and big, shell, dry fish)	30g	5.4±8.0[2]	0.2	3.9	70.3	23.5	**2.1**
Milk powder / liquid	20g /100ml	3.6±2.5[1]	17.8	35.4	13.0	30.7	**3.1**
Margarine	10g	1.6±0.1[1]	0.0	0.2	7.0	71.6	**21.2**
Biscuits	30g	0.6±0.5[1]	1.6	17.9	49.5	30.5	**0.6**
Yogurt	80g	0.7[2]	0.0	0.9	21.8	73.0	**4.3**
Cheese	1 slice	0.2[2]	0.0	0.2	3.6	67.0	**29.1**
Egg	1 egg (60g)	1.0[2]	0.0	1.1	44.4	50.5	**3.9**
Butter	**10G**	**0.02[2]**	**0.0**	**0.2**	**5.3**	**68.0**	**26.4**

[1]Values obtained through the market survey and averaged;

[2]Values were obtained from Indian food composition table

Table 5. Child characteristics in relation to serum 25OHD levels, VDD, VDI and VS children years aged 10-18 of as identified by multiple regression analysis

Child and household characteristics	B	SE	P	Confidence interval
Serum 25OHD levels ng/ml[•]				
Living in north central province vs other provinces	4.6	0.4	.000	**3.7 – 5.4**
Living in north western province vs other	2.9	0.4	.000	**2.1 – 3.8**
Children consumed milk daily vs other	1.4	0.3	.000	**0.8 – 2.0**
Thin children vs other	1.2	0.3	.000	**0.6 – 1.8**
Children consumed milk 2-3 times per day vs other	1.1	0.4	.003	**0.4 – 1.8**
Living in Sabaragamuma province vs other provinces	-4.8	0.4	.000	**-5.6 – -4.0**
Living in central province vs other provinces	-4.0	0.4	.000	**-4.8 – -3.1**
Female vs male	-3.7	0.3	.000	**-4.2 – -3.1**
Obesity children vs other	-3.2	0.9	.000	**-4.9 – -1.4**
Overweight children vs other	-1.2	0.5	.017	**-2.2 – -0.2**

[•]$F_{(10,2514)} = 73.7$, $P<0.000$, $R^2 = 0.22$

Discussion

This is the first national study which assessed VDD in children aged 10-18 years in Sri Lanka. Although Sri Lanka is a tropical country with sunlight throughout the year with ample opportunity to obtain sun exposure, we found 13.2% and 45.6% of children aged 10-18 years to have VDD and VDI, respectively according to the cut-off provided in the recent global consensus [17].

We measured serum 25(OH)D concentration to assess the vitamin D status, which is the gold standard. It reflects the amount of vitamin D taken from the diet and that produced in the skin in response to sunlight (UVB) exposure [5]. The use of diverse cut-offs for VDD and laboratory techniques to detect 25(OH)D in previous studies made it difficult to make a comparison between studies [18-24]. The criterion standard method of testing 25(OH)D is liquid chromatography mass spectrometry (LC/MS) and in our study immunoassay was used. There is minimum variability between results of immunoassays and LC/MS at low 25(OH)D concentrations [25-26]. Hence using immunoassays in our study may not have affected the validity of test results due to high prevalence of low 25(OH)D concentrations in our sample.

The mean serum 25(OH)D concentrations of our study subjects was 19.3±7.4 ng/mL, which is lower than the cut off defined by global consensus (≥20 ng/mL) [17]. These results are consistent with studies involving children in other countries. Vitamin D insufficiency (<20ng/mL) varied from 5-57% in children of other tropical countries and also among other age groups used for studies already conducted in Sri Lanka [10-11, 26-32].

Our results suggest that the vitamin D status of children in our study group was positively associated with male gender, low BMI for age-sex, dry zone lowlands and consuming milk daily. Vitamin D status was negatively associated with living in wet zone highlands and obesity. It was not associated with stunting and age.

Similar to results reported in other studies, male had a lower prevalence of VDD compared to females [26-32] and this pattern was noted in all age groups. This could partly be due to males having more sun exposure because of the tendency of males to engage in outdoor sports more than females.

Considering BMI for age-sex, 13.4%, 27.0%, 8.5% and 2.2% of children in our study sample were stunted, thin, overweight and obese, respectively. We found no association between stunting and VDD. The highest VDD was found in obese children and the lowest in thin children. Other studies also found a similar association between VDD and BMI [31-32].

There was a marked difference in mean 25(OH)D concentrations between provinces highlighting the need for further studies. We found a higher prevalence of VDD in Sabaragamuwa and Central provinces and these provinces are situated in the wet zone highlands of the country. There is frequent rainfall and a colder climate (average 16°C) when compared with other parts of the country. Conversely lower prevalence of VDD was reported from the North Western and North Central provinces, which are situated in the dry zone lowlands.

The primary determinant of vitamin D status in any population is the exposure to ultraviolet-B (UV-B) rays. VDD is associated with limited outdoor activity during the midday due to hot climate or scorching sun, dark skin pigmentation and covering up of the body due to the cold climate or cultural reasons etc. [5]. Our study group was comprised of school children and schools function from 7.30 A.M. to 1.30 P.M. providing an opportunity for the children to get exposed to UV-B at peak hours: from 10 A.M. to 3 P.M. [5]. Typical Sri Lankan school uniforms allow for 20-50% body skin exposure and with 35-40 minutes of peak UV-B exposure, this is sufficient to obtain the daily vitamin D requirement [34]. The results of our study indicate that the average sun exposure during their daily activities was insufficient for almost half of this study group to synthesize the daily requirement of vitamin D. Therefore, we need in-depth studies on sunlight exposure and vitamin D status in Sri Lankan children to decide on the conditions of VD production and safety issue. More than 35-40 minutes of high UV index sun exposure may not be safe due to the increased risk of eye damage and damage to the immune system [35, 36].

Daily consumption of food rich in vitamin D was low in the study sample but of all the vitamin D rich foods, milk was a part of the daily diet in 50% of this population. Our study showed, most of the milk powder in the market was fortified with vitamin D, however this fortification may be inadequate for this population and adequately fortified milk powder could be used as an alternative source of vitamin D.

This study has a few limitations. First, data were collected from school children and children out of school were not included. However, 80% of children between 10-18 years are school going in Sri Lanka. Second, blood samples were collected during the period of June to November, which are the monsoon months meaning there is more rainfall to the wetland areas and colder climate in the highlands but also signifies drought season for the dry zone areas. As a result, the data presented most likely represents the worst-case scenario for wet zone highland areas and best-case scenario for dry zone lowlands. Third, all risk factors associated with VDD were not analysed (eg. season, latitude, colour of the skin, cultural dress habits, estimated sunlight exposure, calcium levels etc.) in this study.

Conclusion

Though Sri Lanka is a tropical country, vitamin D deficiency is an emerging problem in children aged 10-18 years. It highlights the need to adopt strategies to prevent

VDD in this population. We need to raise awareness and promote active outdoor activity to get safe sun exposure to obtain the optimum daily vitamin D requirements. It is also important to promote the consumption of vitamin D fortified food in this population.

Author contributions

Renuka Jayatissa and Sarath Lekamwasam conceptualised, designed and wrote the paper. Ranbanda Jayawardana, Samantha Ranasinghe, Amila Perera, and KH De Silva conducted the national survey, analysed the data set and assist to write the paper. All authors read the manuscript, made a substantial contribution to the revision and approved the final manuscript.

Conflicts of interest

The opinions expressed are those of the authors do not necessarily reflect the views of the institutions that they are affiliated with. The authors declare that there are no conflicts of interest.

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