Occurrence and risk factors of Vitamin D deficiency in Indian children living with HIV – A case–control study

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ABSTRACT

Background: Vitamin D deficiency (VDD) is highly prevalent in healthy individuals. Studies suggest that Vitamin D plays an important role in immune system. **Objective:** The objective of this study was to assess the frequency of VDD in Indian children living with HIV (CLHIV) and to find out the risk factors associated with it. **Materials and Methods:** It was a cross-sectional comparative study conducted in a tertiary care teaching hospital of North India. A total of 52 CLHIV were enrolled consecutively from the pediatric HIV center and an equal number of age- and sex-matched controls were enrolled from the pediatric outpatient department. Serum Vitamin D levels of cases and controls were assessed and compared. Various risk factors, both classical (age, sex, sunlight exposure, average dietary intake of calcium, and Vitamin D) and disease related (WHO and immunological stage, duration, and regimen of treatment), were evaluated for VDD in CLHIV. **Results:** The prevalence of VDD in cases and controls was 69.23% and 19.23%, respectively ($p<0.001$). The mean serum Vitamin D level of the cases (18.24±11.2 ng/dL) was significantly lower than that of controls (31.58±17.31 ng/dL) ($p<0.001$). The risk factor that predicted the occurrence VDD in CLHIV was a poor intake of Vitamin D. **Conclusion:** CLHIV are more prone to VDD; hence, there is a need to regularly evaluate, supplement, and monitor for Vitamin D status in these children.

Key words: *HIV, Hypovitaminosis D, India, Vitamin D deficiency*

Vitamin D plays a pivotal role in almost all the organ systems of the human body. Vitamin D deficiency (VDD) is considered as an epidemic in India. The prevalence of VDD is highly variable and is reported to range from 34% to 70–100% in different Indian studies [1]. It is known that the prevalence of VDD varies with several factors such as age, sex, season, geographic region, sunlight exposure, and dietary intake. With the decreasing number of new infections and expanding accessibility to antiretroviral therapy (ART), the life span of children living with HIV (CLHIV) is prolonged [2]. VDD in HIV patients has been associated with immunological hyper-reactivity, advanced clinical HIV infection, and increased mortality [3-5].

Globally, various studies have assessed the prevalence of VDD in HIV positive adults, but there are a limited number of studies in children [6-9]. Moreover, studies from the Indian subcontinent are sparse. The present study was planned in the pediatric HIV center of a tertiary care teaching hospital of Northern Indian to assess the prevalence of VDD in CLHIV and to compare it with age- and sex-matched healthy controls and to find out the associated risk factors.

MATERIALS AND METHODS

This cross-sectional study was carried out in a tertiary care teaching institution from November 2013 to March 2015 after obtaining Institutional Review Board clearance. A total of 52 CLHIV under the age of 18 years were consecutively enrolled from the pediatric HIV clinic and an equal number of age- and sex-matched healthy controls were enrolled from the outpatient department of the hospital. A written informed consent was obtained from the parents/guardians. Children who were on systemic steroids, antiepileptic drugs, Vitamin D, or calcium supplements or suffering from kidney (serum creatinine >2 mg/dL) or liver disease (ALT >100 IU/L) were excluded from the study.

The age, sex, height, weight, body mass index (BMI), age at diagnosis, the WHO stage, route of transmission, and treatment details were recorded for all the subjects enrolled. Anthropometry was interpreted using BMI Z-scores as per the WHO guidelines as obesity with more than +2SD, thinness as <−2SD, and normal between +2SD and −2SD. Daily calcium and Vitamin D intake were calculated taking the average of 3 days by the recall method using locally available charts depicting the calcium and Vitamin D content of commonly eaten North Indian food items. Daily sunlight exposure was calculated by asking the parent/guardian to recall the number of hours spent by the child in the open under sunlight in a whole day, considering the child's daily activities and the child's clothing pattern. The exposure was expressed as the surface area of body parts directly exposed to

sunlight multiplied by the number of units, for which these parts were exposed in the whole day (min*m^2/day).

Venous blood was analyzed for Vitamin D, parathormone, calcium, phosphorus, alkaline phosphatase level, liver and kidney function tests, serum electrolytes, and complete blood counts. Serum 25-OH Vitamin D levels were estimated by chemiluminescence immunoassay technique and graded as per the Endocrine Society Clinical Practice Guidelines published in 2011. These were divided into VDD (<20 ng/mL), insufficiency (VDI) (21–29 ng/mL), and sufficiency (VDS) (>30 ng/mL) [10]. The normal reference ranges for other laboratory parameters were serum parathyroid hormone (PTH) (<65 pg/mL), calcium (8.6–10.3 mg/dL), phosphorus (4.0–7.0 mg/dL), and alkaline phosphatase (100–390 μ/L for males and 100–320 μ/L for females).

The sample size was calculated as 52 in both the groups on the basis of a similar study done by Meyzer *et al*. to detect a mean difference in Vitamin D level of 3.8 ng/ml between controls (14.2 ng/ml) and cases (10.4 ng/ml), with a standard deviation of 6.9 ng/ml in the control group and 5 ng/ml in cases, considering two-sided alpha of 0.05 and power of 80% [11].

Statistical testing was conducted with the SPSS version 21.0. The comparison of continuous variables between the groups was performed using Student's *t*-test. Nominal categorical data were compared using Chi-square test or Fisher's exact test as appropriate. Non-normally distributed continuous variables were compared using Mann–Whitney U-test. Univariate analysis followed by multivariate logistic regression was performed for analyzing the risk factors for VDD in CLHIV. For all statistical tests, p<0.05 was considered as statistically significant.

RESULTS

This study compared 52 CLHIV (mean age = 10.19±3.35 years) and 52 healthy controls (mean age = 9.64±3.14 years). The male-to-female ratio was 1.89. Comparison of the BMI revealed a significantly lower proportion of nutritionally normal subjects in the HIV-infected group (32/52, 61.54%) in comparison to controls (48/52, 92.31%) (p=0.001). Other subjects were either thin (34.62% of cases and 7.69% of controls) or obese (3.85% of cases and 0% of controls).

Table 1 depicts the comparative profile of the two groups. Daily intake of Vitamin D and daily sunlight exposure in CLHIV were significantly lower in comparison to the controls. Similarly, serum Vitamin D and serum phosphorus levels were significantly lower in cases than controls, while the serum PTH levels were significantly higher for cases than controls.

Assessment of Vitamin D levels revealed that 69.23% of cases and 19.23% of controls had VDD; 17.31% of cases and 32.69% of controls had VDI; and 13.46% of cases and 48.08% of controls had VDS. The difference was statistically significant between the two study groups (p<0.001). Chi-square test was used to analyze the association between Vitamin D statuses of the HIV cases with different risk factors (Table 2). The factors that were significantly associated with Vitamin D status were WHO stage (p=0.04) and average Vitamin D intake (p<0.005). Correlation analysis did not reveal any significant correlation between Vitamin D level and CD4 count (r=0.023, 95% CI=−0.25–0.29, p=0.87).

Risk assessment was done for VDD in cases for both the classical and disease-specific risk factors, namely, age, sex, BMI, average intake of calcium and Vitamin D, sunlight exposure, immunological and WHO stage, duration of treatment, and regimen which were evaluated by logistic regression. The factors which significantly predicted VDD by univariate analysis were average intake of calcium, average intake of Vitamin D (p<0.001), and WHO stage (p=0.01). Multivariate analysis revealed that only a lower average intake of Vitamin D predicted VDD (p=0.002).

DISCUSSION

Vitamin D plays an important part in the immune system and its deficiency is also implicated in the progression of HIV [12,13]. The absence of routine Vitamin D supplementation can interfere with the peak bone mass acquisition and could lead to an increased risk of low bone mineral density in these vulnerable children [14]. The present study observed a high prevalence of VDD in CLHIV (69.23%) in comparison to age- and sex-matched healthy controls (19.23%). Few previous studies carried out in other parts of the world also assessed the prevalence of VDD in CLHIV [11,15-20]. A study carried out in perinatally infected Thai adolescents aged 12–20 years, on long-term ART, observed a comparatively lower prevalence of VDD (24.7%) [18].

Another study published by Meyzer *et al*. carried out in HIV-infected children (≤24 years) who reported the frequency of VDD to be 38.9% [11]. Rutstein *et al*. reported the prevalence of VDD as 36% in perinatally HIV-infected children [16]. The high frequency of VDD in the present study could be due to the different ethnic backgrounds,

Table 1: Comparison of different parameters among cases (CLHIV) and controls

Variable (unit)	Cases (Mean±SD)	Controls (Mean±SD)	p-value
Weight (kg)	26.18±6.60	30.06±11.05	0.03
Height (cm)	129.18±15.59	133.22±18.09	0.23
Average intake of calcium (g/day)	0.39±0.09	0.41±0.07	0.41
Average intake of Vitamin D (IU/day)	138.65±53.03	172.81±49.33	0.001
Sunlight exposure (Min*m^2/day)	4±0.86	6.14±1.58	<0.001
Serum calcium (mg/dl)	9.32±0.42	9.43±0.42	0.12
Serum phosphorus (mg/dl)	4.31±0.6	4.81±0.8	<0.001
Serum alkaline phosphatase (IU/l)	307.44±150.63	251.29±78.95	0.06
Serum Vitamin D (ng/dl)	18.24±11.2	31.58±17.31	<0.001
Serum PTH (pg/ml)	74.49±107.73	41.48±54.48	0.02

CLHIV: Children living with HIV, PTH: Parathyroid hormone

Table 2: Association of Vitamin D status with different risk factors among CLHIV

Variable	Groups	Vitamin D status (n [%])			p-value
		Deficiency	Insufficiency	Sufficiency	
Age	≤10 years	19 (67.85)	5 (17.85)	4 (14.28)	0.97
	>10 years	17 (70.83)	4 (16.66)	3 (12.51)	
Sex	Male	23 (67.64)	4 (11.76)	7 (20.60)	0.06
	Female	13 (72.22)	5 (27.78)	0 (0.00)	
Route of transmission	Vertical	34 (69.38)	9 (18.36)	6 (12.26)	0.48
	Transfusion	2 (66.66)	0 (0.00)	1 (33.34)	
Nutritional status	Normal	22 (68.75)	6 (18.75)	4 (12.5)	0.63
	Thinness	13 (72.22)	3 (16.66)	2 (11.12)	
	Obesity	1 (50)	0 (0.00)	1 (50)	
WHO stage	1	29 (78.37)	5 (13.51)	3 (8.12)	0.04
	2	4 (57.14)	2 (28.57)	1 (14.29)	
	3	3 (60)	0 (0.00)	2 (40)	
	4	0 (0.00)	2 (66.66)	1 (33.34)	
Duration of treatment	<5 years	13 (72.22)	2 (11.11)	3 (16.67)	0.43
	6–10 years	20 (74.07)	5 (18.51)	2 (7.42)	
	>10 years	3 (42.85)	2 (28.57)	2 (28.57)	
ART regimen	ZLN	35 (68.62)	9 (17.64)	7 (13.74)	0.80
	ZLE	1 (100)	0 (0.00)	0 (0.00)	
Average Vitamin D intake (IU/day)	60–100	17 (100)	0 (0.00)	0 (0.00)	<0.005
	101–200	19 (73.07)	5 (19.23)	2 (7.7)	
	>201	0 (0.00)	4 (44.44)	5 (55.56)	
Sunlight exposure (Min m^2/day)	<5	28 (75.67)	4 (10.81)	5 (13.52)	0.14
	≥5	8 (53.33)	5 (33.33)	2 (13.34)	

CLHIV: Children living with HIV, ART: Antiretroviral therapy

inadequate dietary intake, lack of dietary supplementation of Vitamin D, and dark skin color. However, a similar prevalence was observed in a study published in the Western world. Kakalia *et al*. reported only 15% of the subjects with sufficient Vitamin D levels (>75 nmol/L) and the authors attributed this to possible poor sunlight exposure, sunscreen use, and poor dietary intake [17]. One study that evaluated the nutritional status of CLHIV from Southern India reported the prevalence of VDD to be 51.9% [20].

This study also observed a significantly lower serum Vitamin D levels in CLHIV in comparison to controls. This might be due to the disparity observed in the nutritional status of the two groups. A statistically significant difference was observed between the two groups with regard to an average intake of Vitamin D and sunlight exposure. This could be due to the presence of commonly occurring comorbidities in CLHIV that hamper adequate dietary intake. Furthermore, CLHIV are more likely to be less physically active than controls, hence having lesser sunlight exposure. The study by Rutstein *et al*. too observed a significantly lower Vitamin D levels among cases in comparison to controls [16].

However, many studies failed to report any significant difference in Vitamin D levels between HIV-positive subjects and controls [15]. Meyzer *et al*. reported a significantly higher mean level of Vitamin D in HIV-infected children than controls [11]. These results were attributed to the higher rates of Vitamin D supplementation in the HIV-infected group. Our study did not observe any correlation between Vitamin D status and ART. This could be due to the majority of our subjects being on nevirapine which in comparison to efavirenz caused less induction of cytochrome enzyme [21].

There were few limitations of the study. It failed to find any correlation between Vitamin D and the CD4 count, as reported in other studies [18,19]. The correlation of Vitamin D levels with viral load could not be done in CLHIV as this facility due to a lack of availability and cost involved. Another limitation of this study is that the effects of season and skin color on Vitamin D levels were not assessed. It was observed that the poor intake of Vitamin D significantly predicted the presence of VDD. This observation reinforced the importance of dietary intake of Vitamin D for the maintenance of adequate serum levels.

CONCLUSION

VDD is an important and frequently ignored comorbidity seen in Indian CLHIV. As it may lead to potentially severe acute and long-term consequences on skeletal as well as extra-skeletal functions, there is an urgent need to develop guidelines for screening, supplementing, and monitoring for VDD in CLHIV more so for developing countries.

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