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Vitamin D status and associated factors among HIV-infected children and adolescents on antiretroviral therapy in Kampala, Uganda

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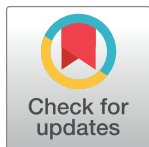
Abstract

Background

A high prevalence of suboptimal serum vitamin D has been reported among HIV infected children even in countries with high sunshine abundance throughout the year. Vitamin D is a potent immune modulator of innate and adaptive immune responses. Vitamin D regulates immune responses through the vitamin D receptor on CD4 cells. We aimed to determine the vitamin D status of HIV infected children and factors associated with suboptimal vitamin D.

Methods

This was a cross sectional study. We enrolled children aged between 6 months and 12 years attending an outpatient paediatric HIV clinic. Serum 25-hydroxyvitamin D (25(OH)D) was measured using the electrochemoluminisence method. Suboptimal vitamin D was defined as 25(OH)D <30 ng/ml, vitamin D insufficiency and deficiency were 21–29 ng/ml and <20 ng/ml respectively. Anthropometry, physical exam and medical history were documented. Logistic regression was performed.

Results

We enrolled 376 children with mean age (sd) 8.05 years (3.03), a median (IQR) duration of ART of 5.9 years (3.2–8.4). Majority of the children (64%) had been exposed to non nucleoside reverse transcriptase inhibitors (NNRTIs). A third were severely immunosuppressed (CD4% ≤15%) at ART initiation. At the time of the study, the majority (89%) were virologically suppressed (VL <1000 copies/ml). Prevalence of 25(OH)D <30 ng/ml was 49 (13%) of 375 participants and 11 (3%) had 25(OH)D <20 ng/ml. Lopinavir/ritonavir regimen was independently associated with 25(OH)D <30 ng/ml; OR 0.27 CI (0.13–0.57), p value-0.002. Serum 25(OH)D <20 ng/ml was associated with CD4 count ≤15% at ART initiation OR 6.55 (1.30–32.9), p value—0.023 and use of NNRTIs; OR 10.9(1.22–96.2), p value—0.03.

Conclusion

We found a low prevalence of suboptimal vitamin D compared to earlier reports. Severe immunosuppression at ART initiation and use of NNRTIs increases odds of deficiency. Vitamin D supplementation should be considered in severely immunosuppressed children initiating ART.

Introduction

Human Immunodeficiency Virus (HIV) remains a burden in low-income countries despite the many interventions currently in place. In 2016, 2.1 million children under 15 years of age were living with HIV/AIDS globally, with two-thirds living in Sub Saharan Africa (SSA) [1]. Although Antiretroviral Therapy (ART) prolongs life and improves quality of life among HIV infected children [2], there is evidence of persistent inflammation and immune dysregulation in HIV infected individuals even with effective ART [3]. This inflammation coupled with cumulative drug toxicities predispose them to metabolic complications and bone diseases [3].

Vitamin D is a prohormone that has antinflammatory effects [4]. Vitamin D deficiency (VDD) is associated with greater inflammation by upregulation of inflammatory markers like, IL-6, TNF-α, activated monocyte phenotypes (CX3CR1+ and CCR2+) in HIV-infected patients [5], which have been related to tissue dysfunction, comorbidity development, AIDS progression, and death in HIV-infected individuals [6]. Severe VDD is associated with low CD4 counts and increased markers of inflammation in ART-naïve HIV-infected patients [7]. Additionally, high vitamin D levels have been associated with protection against the development of IRIS events [8] and decreased incidence of pulmonary tuberculosis and mortality among HIV-infected patients [9].

The main source of vitamin D is sunshine exposure. Children infected with HIV may be predisposed to VDD due to limited sun exposure as a result of ill health caused by opportunistic infections, concomitant treatment with ART and medications for opportunistic infections like ketoconazole which impair vitamin D metabolism [10] and also reduced dietary intake of vitamin D [11]. ART especially efavirenz may impair vitamin D metabolic pathways [12–14]. Long duration of ART is associated with vitamin D insufficiency (VDI) while a low CD4 count <200/µl, advanced stages of disease and current efavirenz use were independently associated with severe VDD [12]. Protease inhibitors (PI) use has shown no association with VDD [15]. VDD is also associated with opportunistic infections like tuberculosis and oral candidiasis as shown among HIV infected adults in a Tanzanian study [9].

The role of vitamin D as a potent immune modulator of innate and adaptive immune responses has been described [16–19]. Vitamin D regulates the immune responses through the vitamin D receptor (VDR) on CD4 cells. Vitamin D inhibits excessive production and action of T–helper 1 (innate immune system) thus prevents cellular inflammation [20, 21]. Furthermore, it plays a role in the innate immunity through activation of Toll-like receptors (TLRs) that leads to induction of the antimicrobial peptide cathelicidin and killing of intracellular bacteria [22]. Therefore VDD may affect the innate and adaptive immune response thus leading to disease progression [23].

The Endocrine Society defines sufficient vitamin D as a level of serum vitamin D (25(OH) D) $\geq$ 30–100 ng/ml while levels below 30ng/ml are suboptimal. The suboptimal level is further categorized into VDD and VDI [10]. VDD is defined as 25(OH)D <20 ng/ml,usually it manifests as bone disease [10]. VDI defines serum 25(OH)D between 20–29 ng/ml which are levels

that may be associated with other disease outcomes [10]. A median serum level of 25(OH)D of 26 ng/ml is associated with reduced overall risk of cardiovascular mortality while in adolescents a level of 25(OH)D >26 ng/ml is associated with lower odds of elevated blood pressure and other diseases like T1DM [24]. High rates of suboptimal serum vitamin D in HIV-infected children have been reported in the range of 29–90% depending on the season, latitude, and patient ethnicity[25–27]. Observational studies suggest that vitamin D status may impact HIV disease severity [28–30]. A study in South Africa showed that a higher CD4 count had decreased odds of VDD among HIV infected children [31].

VDI is increasingly being reported even among healthy children in Uganda despite being located at the equator with an abundance of the sun [32–34]. This is possibly due to change in lifestyle amongst the general population. From our observation, children living within and around the capital city where majority of these studies have been conducted are increasingly being kept out of the sun because of more indoor activities at school, changes in housing with limited space for outdoor play and increased screen time due to urbanisation. Screening and supplementation for vitamin D are not routinely done in HIV infected children and adolescents in low-income settings despite the recommendation by the Endocrine Society that calls for screening for vitamin D status in HIV infected patients due to high risk of VDD [10]. However, there is no evidence for supplementation in an environment which is rich in sunshine throughout the year. This study was designed to assist in developing hypotheses for bigger studies to understand the role of vitamin D in HIV infection in a setting with an abundance of sunshine and a high burden of infectious diseases. Therefore our aim was to determine the vitamin D status among HIV infected children in Uganda and the factors associated with suboptimal vitamin D. The factors measured included clinical, immunological and laboratory markers.

Methods

Study design and participants

This was a cross-sectional study carried out between April 2019 and September 2019 at the Baylor HIV Paediatric Clinic, located at the Mulago National Referral Hospital Complex in Kampala the capital city of Uganda. Uganda is located at the Equator, Latitude 0° N with an average temperature of 26°C in Kampala. The study was carried out over 6 months' duration consisting both the wet rainy season of April to June and the dry hot season from July to September. The sun is present throughout the year; however, in the rainy season majority of the children especially those younger children < 5 years remain indoors, over swaddled and rarely play in the sun. We enrolled HIV infected children attending the clinic aged between 6 months and 12 years, who were taking ART, and those whose parents or guardians provided written informed consent and assent for children ages ≥ 10years.

We excluded those who were ill (fever > 38.5 degrees Celsius, difficulty in breathing, alterd level of consciousness and / or in shock) on the day of enrollment, those who were already on vitamin D supplementation other than the multivitamin supplements given at the clinic and children who were currently on steroids for at least 2 weeks because of an independent association of chronic steroid use and low serum 25(OH)D [35]. Those with known chronic kidney disease, liver disease and heart disease were also excluded.

Procedures

Systematic sampling was used as the children came into the clinic for triage. The study nurse approached every 10th child in the queue to assess eligibility.

A structured questionnaire was used to collect clinical data related to HIV illness from the eligible participants' clinic chart. Clinical data collected included WHO clinical staging of HIV/AIDS [36], CD4 cell percentage and absolute count at the time of ART initiation. Additionally, we captured the current viral load on ART, the current ART regimen and duration on that ART as well as any other current medications. We also captured the previous number of admissions and any previous severe infections since ART initiation from the patient and the history of sun exposure.

Anthropometric measurements were taken, including the weight in kilograms and height/length in centimetres of the children. Height-for-age and weight-for-age Z-scores were calculated using the WHO reference charts [37]. Weight-for-age Z-score and height-for-age Z-score < -2 SD was considered as underweight and stunted respectively. Weight-for- height Z-score <-2 SD for children aged less than 5 years and BMI for age Z-score < -2 SD for those aged 5 years and above was considered as wasted. Physical examination was done to assess for overt features of rickets.

Laboratory testing: A venous blood sample of 5 mL was drawn into a plain (red top) blood collection tube from each child. Blood was centrifuged, and serum was collected and stored at -80˚C. Serum vitamin D level was determined by electrochemiluminescence immunoassay using a technique called Elecsys vitamin D assay. This is an immunoassay supplied by Roche Diagnostics, Germany, which measures the vitamin D concentrations in the range of 4–100 ng/ml. Suboptimal 25(OH)D was defined as a level < 30ng/ml. VDI was defined as serum levels of 25(OH)D between 21–29 ng/ml and VDD was defined as serum levels of 25(OH)D < 20 ng/ml [10]. The parathyroid hormone (PTH) was measured using electrochemiluminescence immunoassay. Serum PTH level > 65 pg/ml was considered as hyperparathyroidism.

Serum calcium, phosphorus, alkaline phosphatase (ALP) were analyzed using a fully automated COBAS 6000 (Roche Diagnostics GmbH, Germany) machine. The calcium level of 8-10mg/dl (2–2.5 mmol) was considered normal, phosphorus: normal range infancy; 4.5–8.3 mg/dl (1.45–2.68 mmol), childhood; 3.7–5.6 mg/dl (1.19–1.8 mmol), alkaline phosphatase levels; was considered raised level at a value > 400 IU/dl. Viral suppression was considered at viral copies < 1000/ml as defined in the Uganda National HIV treatment guidelines [38].

Ethics

Written informed consent was obtained from parents or guardians of all study participants. Written assent was obtained from all children aged 10 years and above. Ethical approval was granted by the institutional review board for human studies at Makerere University School of Biomedial Sciences (#SBS HDREC-618), Uganda National council of science and Technology (#HS294ES), Baylor College of Medicine, Houston, Texas (# H-45391) and University of Minnesota, Minneapolis, Minnesota (#STUDY00005173).

Statistical considerations

Data entry and cleaning was done using Epidata version 3.1. Data was analyzed using statistical package, STATA Version 15.0. Patient characteristics were reported as frequency and percentage for categorical variables, mean and standard deviation (SD) for the normally distributed continuous variables with outliers and median and inter-quartile range (IQR) for continuous variables without outliers.

To determine the vitamin D status of the children; we got the proportion of the children with serum 25(OH)D <30 ng/ml from the total enrolled sample as suboptimal vitamin D level, we then further determined the proportion with categories of serum 25(OH)D <20 ng/ml, 21–29 ng/ml and ≥30 ng/ml as VDD, VDI and sufficient D level respectively.

To determine the factors associated with suboptimal vitamin D; our major outcome was defined as a dichotomized variable based on 25(OH)D < 30 ng/ml and > 30 ng/dl. Socio-demographic, clinical variables and biochemical variables were compared against the outcome using chi-square test or fisher's exact when cell sizes were small (< 5 children). Multivariable analysis for factors independently associated with serum 25(OH)D <30 ng/ml were performed using a logistic regression model. Further analysis was done to determine variables that were associated with vitamin D level <20 ng/ml. A p-value <0.05 was considered statistically significant. The sample size of 376 was calculated using an assumed prevalence of vitamin D deficiency among HIV infected children of 57% from a Tanzanian study [39], with a margin of error of 5% and a standard normal value at confidence interval of 95% ($Z_{\alpha2}$ = 1.96).

Results

We enrolled 376 particpants between April 2019 and September 2019.

The study Profile is shown in Fig 1 below.

Baseline characteristics

The mean age (SD) of the study participants was 8.05 years (3.03) with a median (IQR) duration of ART of 5.9 years (3.2–8.4). The median age (IQR) of ART initiation was 1.58 years (0.75–3.25). Majority of the children 64% (242 of 376 participants) had been exposed to non nucleoside reverse transcriptase inhibitors (NNRTIs); efavirenz and nevirapine. About 65 participants (17%) were underweight (weight for age zscore < -2) and more than a quarter (28.7%) of the participants were stunted (Table 1). Only 17 of the 376 participants (4.5%) had suffered from tuberculosis. Almost 99% of the study participants reported daily sun exposure.

About 2% (9 of 376 participants) had typical clinical signs of severe vitamin D deficiency including bone deformities such as bow legs, 'knock- knees' or rachitic rosary. Three participants had bow legs, 5 had 'knock- knees', 1 had a rachitic rosary. However, only one child with bow legs had 25(OH)D < 30ng/ml, all the rest of the 8 children had sufficient vitamin D level. All these children were on ART and were virologically suppressed. All the children with bow legs and rachitic rosary were stunted while only one child with 'knock-knees' was stunted. None of the children with bow legs was wasted while the one with rachitic rosary and two with 'knock-knees' were wasted.

The mean (SD) viral load (VL) of the participants at ART initiation was 531,226.1 (1542359) as compared to the current mean (SD) VL 15,871.1 (164030.6). Majority, 321 of 360 participants (89%) were virologically suppressed (VL <1000copies/ml) at the time of the study. Normocalcemia was detected in 305 participants (80%), none was hypocalcemic and 70 participants (18.7%) were hypercalcemic. Most of the participants 345 (92%), had normal parathyroid hormone while 282 participants (75%) had normal serum phosphorus and a quarter of participants (92) had low phosphorus.

Vitamin D status of HIV infected children

Sufficient serum 25(OH)D ≥ 30 ng/ml was demonstrated in 326 participants (87%) while we found suboptimal level of serum 25(OH)D < 30 ng/ml in 49 participants (13%). Of those with suboptimal vitamin D; 11 of the 49 participants (22%) had 25(OH)D <20 ngml (VDD) while 38 participants (78%) had serum levels in range of 20–29 ng/ml (VDI). The median (IQR) 25 (OH)D was 49.64 ng/ml (7.29–89.75).
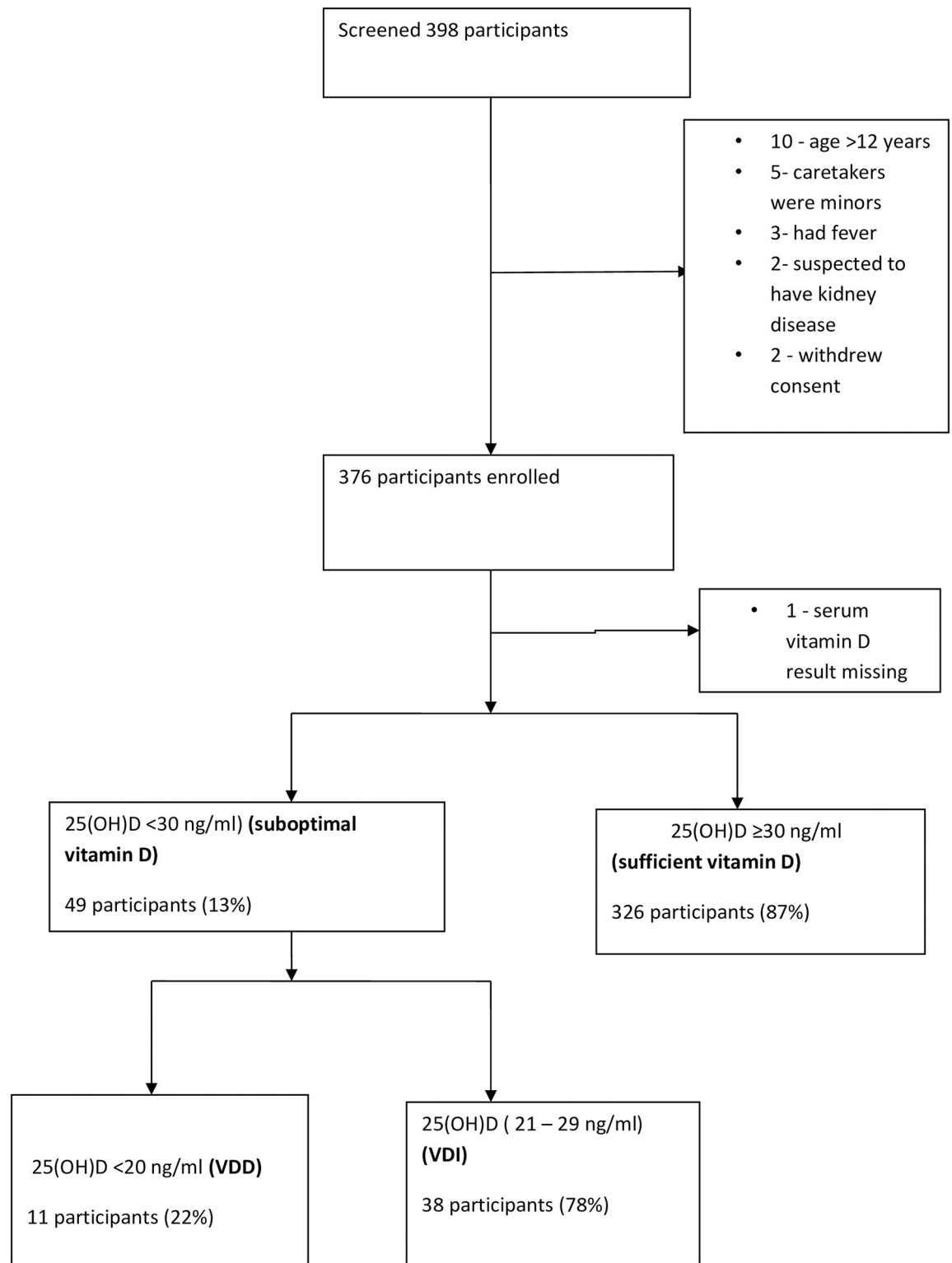
Fig 1. The study profile.

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Table 1. Sociodemographics and clinical characteristics.

Characteristic	25(OH)D ≥30 ng/ml, n (%)	25(OH)D <30 ng/ml, n (%)	P value
Age (n = 375)			
<5	64(92.8)	5(7.2)	
5–12 yrs	262(85.6)	44 (14.4)	0.120
Gender (n = 375)			
Male	150(84.7)	27(15.3)	
Female	176(88.9)	22(11.1)	0.236
CD4%at initiation (n = 338)			
> 15	194(87.8)	27(12.2)	
≤15	100(85.5)	17(14.5)	0.548
Current Viral load (copies/ml) (n = 359)			
≥ 1000	36(92.3)	3(7.7)	
<1000	274(85.6)	46(14.4)	0.260
Height for age (n = 370)			
≥-2 z score	231(88.5)	33(11.5)	
< -2 z score	91(85.8)	15(14.2)	0.669
Weight for age (n = 374)			
≥ -2 z score	269(82.8)	40(81.6)	
< -2 z score	56(17.2)	9(18.4)	0.845
Weight for ht/ BMI for age Z-score (n = 370)			
≥ -2 z-score	309(87.3)	45(12.7)	
< -2 z-score	13(81.3)	3(18.7)	0.486
WHO stage at ART initiation (n = 375)			
I& II	78(87.6)	11(12.4)	
III &IV	248(86.7)	38(13.3)	0.821
Previous admissions (n = 371)			
None	268(85.6)	45(14.4)	
Once	31(93.9)	2(6.1)	0.200
2–5 times	24(96)	1(4)	0.177
First ART regimen(n = 370)			
No	140(86.4)	22(13.6)	
Yes	183(88.0)	25(12.0)	0.655
*ART Regimen currently(n = 375)			
AZT/3TC/ EFV OR ABC/3TC/EFV OR TDF/3TC/	69(78.4)	19(21.6)	
AZT/3TC/ NVP OR ABC/3TC/NVP OR TDF/3TC/	38(90.5)	4(9.5)	0.101
AZT/3TC/ LPV/r OR ABC/3TC/LPV/r OR AZT	193(95.5)	9(4.5)	0.001
Dolutegravir/Others	31(72.0)	12(28.0)	0.426
Duration for taking ART(n = 369)			
< 6 years	168(88.9)	21(11.1)	
≥ 6 years	153(85.0)	27(15.0)	0.073
Alkaline phosphatase (n = 371)			
< = 400 iu	244(87.1)	36(12.9)	
>400 iu	78(85.7)	13(14.3)	0.727

AZT—zidovudine, 3TC—lamivudine, EFV- efavirenz, ABC-abacavir, NVP- nevirapine, TDF- Tenofovir, LPV/r-lopinavir/ritonavir.

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Factors associated with suboptimal vitamin D

Children on lopinavir/ritonavir regimen were less likely to have serum 25(OH)D <30 ng/ml; OR 0.27 CI (0.13–0.57), p value—0.001 (Table 2).

However, it was noted that children with serum 25(OH)D <20 ng/ml (VDD) had low CD4 count (< 15%) at ART initiation OR 6.55(1.30–32.9), p value—0.023 as shown in Fig 2. They were also more likely to be on NNRTIs containing regimens with efavirenz and nevirapine OR 10.9(1.22–96.2), p value—0.03 as shown in Table 3. No biochemical markers like calcium, phosphorus, parathyroid hormone or alkaline phosphatase were independently associated with VDD.

Discussion

Our study found a low prevalence (13%) of suboptimal vitamin D (25(OH)D <30 ng/ml) as compared to previously reported studies among the HIV-infected children; with prevalence between 29–90% [25, 28, 31], and also among 135 healthy children aged <6 months the prevalence of 25(OH)D <20 ng/dl was 84% [40] while it was 48% among 95 healthy Ugandan children aged <7 years [32]. The major source of vitamin D is exposure to sunlight. Sunlight is abundant even in the rainy seasons in Uganda. Our study enrolments run across both the rainy season and dry season over 6 months. During the rainy season many children in our setting are kept indoors and are over swaddled especially for those aged <5 years. However, three quarters of our participants were enrolled in the dry months of June to September. We are uncertain whether the prevalence would be the same if this study was conducted in a purely rainy season and if the study population were of younger children aged <5 years.

Vitamin D deficiency has been neglected in many sunny regions despite increasing global reports of deficiency in these areas [41]. Therefore, there are few reports to compare our results with in the region regarding vitamin D status and HIV infected children. A study done in Tanzania found a high prevalence of insufficiency of 80% in HIV infected children [39], however, this study was conducted among infants thus making it difficult to compare with our study population with a mean age of 8 years. Also, the studies done among healthy children in Uganda were conducted among children of a young age group aged less than 8 years [33]. We believe the risk factors for vitamin D deficiency among the younger children aged < 5 years are plausibly different from those older [42, 43]. During a child's infancy and preschool years, children tend to spend more time taking part in outdoor activities. As they grow, sedentary activities increase and adolescents tend to limit their outdoor activities because they prefer activities such as watching television or playing computer games. Additionally, their time outdoors is decreased because of prolonged study at school with outdoor physical activity usually limited to physical education at school. Also, dietary intake changes with age as the ingestion of unbalanced diets like fast and highly processed foods increases with age while the consumption of other nutritious foods containing vitamin D decreases [44].

This current study population comprised of older HIV infected children (mean age = 8years) and majority were healthy with virological suppression thus the children were more likely to be active and with good sun exposure thus the low prevalence.

Further still, the numbers of healthy children studied in previous reports in Uganda were too few to provide generalizable data to the population [33, 34]. Additionally, the lower prevalence may be accounted for by the different assay methods used for evaluating serum vitamin D by the various studies. This study used electrochemiluniscence assay while Cusick et al. [33] used chemiluminescent immunoassay and the high-performance liquid chromatography tandem Mass spectrometry was used by Sudfeld et al. in the Tanzanian study [39]. Multiple

Table 2. Factors associated with suboptimal vitamin D (serum 25(OH)D <30 ng/ml) among HIV infected children.

Characteristic	Unadjusted Odds ratio, 95% CI	P value	Adjusted Odds ratio,95% CI	P value
Age				
<5	Ref		Ref	
5–12 yrs	2.14(0.82–5.64)	0.120	1.16(0.33–4.04)	0.814
Gender				
Male	Ref			
Female	0.69(0.38–1.27)	0.236		
CD4%at initiation categorized				
> 15	Ref		Ref	
≤15	1.22(0.64–2.35)	0.548	1.12(0.56–2.21)	0.748
Current Viral load (copies/ml)				
≥ 1000	Ref			
<1000	2.01(0.59–6.81)	0.260		
Height for age				
≥-2 z-score	Ref			
< -2 z-score	1.15(0.59–2.23)	0.669		
Weight for age				
≥ -2 z-score	Ref			
< -2 z-score	1.08(0.49–2.35)	0.845		
Weight for ht/ BMI for age Z-score				
≥ -2 z-score	Ref			
< -2 z-score	1.58(0.43–5.78)	0.486		
WHO stage				
I& II	Ref		Ref	
III &IV	1.09(0.53–2.22)	0.821	1.65(0.71–3.84)	0.243
Previous admissions				
None	Ref			
Once	0.38(0.09–1.67)	0.200		
2–5 times	0.25(0.03–1.88)	0.177		
First ART regimen				
No	Ref			
Yes	0.87(0.47–1.61)	0.655		
*ART Regimen currently				
AZT/3TC/ EFV OR ABC/3TC/EFV OR TDF/3TC/	Ref		Ref	
AZT/3TC/ NVP OR ABC/3TC/NVP OR TDF/3TC/	0.38(0.12–1.21)	0.101	0.35(0.10–1.18)	0.092
AZT/3TC/ LPV/r OR ABC/3TC/LPV/r	0.27(0.13–0.57)	0.001	0.25(0.11–0.59)	0.002
Dolutegravir/Others	1.41(0.61–3.25)	0.426	1.49(0.61–3.68)	0.381
Duration for taking ART				
< 6 years	Ref		Ref	
≥ 6 years	1.83(0.94–3.53)	0.073	1.21(0.57–2.56)	0.624
Alkaline phosphatase				
< = 400 iu	Ref			
>400 iu	1.12(0.57–2.23)	0.727		

AZT—zidovudine, 3TC—lamivudine, EFV- efavirenz, ABC-abacavir, NVP- nevirapine, TDF- Tenofovir, LPV/r- lopinavir/ritonavir.

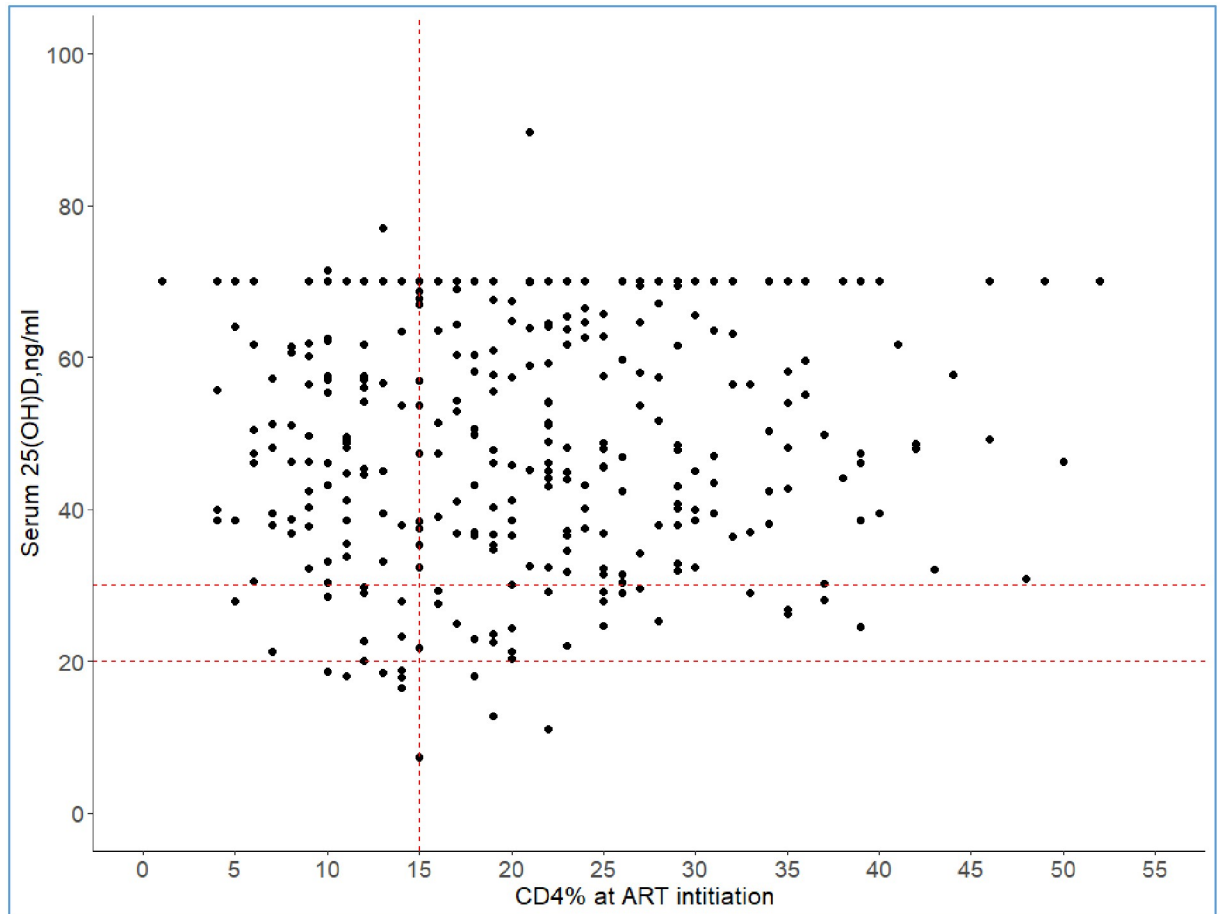https://doi.org/10.1371/journal.pone.0253689.t002

Fig 2. A graph of serum 25(OH)D against CD4% at ART initiation. The horizontal dotted lines show the 25(OH)D level at 20 ng/ml and at 30 ng/ml. The vertical doted line denotes the CD4% at 15% level. P value was calculated using chi square test comparing CD4% ≤15 and >15 with 25(OH)D <20 ng/ml and above.

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methodologies for 25(OH)D measurement exist but these are subject to variability due to inter assay differences in performance thus may explain the difference in results [45].

In this study we also found an association between severe immunosuppression at ART initiation and VDD. Other studies by Rutstein et al. [28] also found that decreased CD4 count was correlated to VDD and Mirza et al. [31] also found that a low CD4 count had an increased odds of vitamin D deficiency, although Kim et al. [46] found no association. Given the evidence demonstrating the role of vitamin D in innate and adaptive immunity, the association between low CD4 and VDD could be explained by the role played by the vitamin D and its receptor in activation and T-cell receptor signaling in naive T-cells [5], and it agrees well with data showing that VDD is associated with clinical progression [9] and lower CD4 counts even before initiation of ART [7], and with a poorer CD4 restoration on treatment [17]. However, we were unable to determine the association with the current CD4 count because the current treatment policy uses the viral load for ART monitoring and not the CD4 counts as previously [38].

The current HIV treatment guidelines in Uganda recommend initiation of ART at the earliest opportunity in all people with confirmed HIV infection, regardless of clinical stage or CD4 cell count [38]. This reduces the risk of delaying treatment and thus less likely to have many

Table 3. Factors associated with serum 25(OH)D <20 ng/ml (VDD) among HIV infected children.

Characteristic	25(OH)D > 20 ng/ml, N (%)	25(OH)D < 20 ng/ml, N (%)	Unadjusted Odds ratio, (95% CI)	P value	Adjusted odds ratio	P value
Age						
0–4 years	68 (18.7)	1(9.1)	Ref			
5–12 years	296 (81.3)	10(90.9)	2.3(0.29–18.3)	0.432		
Gender						
Male	169(46.4)	8(72.7)	Ref			
Female	195(53.6)	3(27.3)	0.33(0.08–1.24)	0.101		
CD4%at initiation categorized						
≤15	218(66.7)	3(27.3)	Ref		Ref	
>15	109(33.3)	8(72.7)	5.33(1.39–20.5)	0.015	6.55(1.30–32.9)	0.023
Weight for age Z-score						
≥ -2	301(82.9)	8(72.7)	Ref			
< -2	62(17.1)	3(27.3)	1.82(0.47–7.06)	0.386		
BMI for age Z-score						
≥-2	346 (96.1)	8(80.0)	Ref		Ref	
< -2	14(3.9)	2(20.0)	6.18(1.19–31.8)	0.029	4.91(0.64–37.7)	0.126
WHO stage						
I& II	88(24.2)	1(9.1)	Ref		Ref	
III &IV	276(75.8)	10(90.9)	3.19(0.40–25.3)	0.272	3.23(0.35–29.6)	0.298
First ART regimen						
No	161(44.7)	1(10.0)	Ref		Ref	
Yes	199(55.3)	9(90.0)	7.28(0.91–58.1)	0.061	4.98(0.56–44.1)	0.149
Duration on ART						
<6 years	182(50.8)	7(63.6)	Ref		Ref	
≥6 years	176(49.2)	4(36.4)	0.59(0.17–2.05)	0.408	0.41(0.08–2.00)	0.269
Current Viral load (copies/ml)						
≥1000	39(11.2)	0(0.0)	Ref			
<1000	309 (88.8)	11(100.0)	Cannot be calculated			
Current ART Regimen						
*LPV/r or PI based regimen	199 (54.7)	3 (27.3)	Ref		Ref	
NVP/EFV based regimen	165(45.3)	8(72.7)	3.22(0.84–12.3)	0.088	10.9(1.22–96.2)	0.032
Alkaline phosphatase (n = 371)						
< = 400 iu	276(76.7)	4(36.4)	Ref		Ref	
>400iu	84(23.3)	7(63.6)	5.75(1.64–20.12)	0.006	3.86(0.82–18.0)	0.085
Serum calcium						
2–2.5 mmol	295(81.3)	9(81.8)	Ref			
Above 2.5mmol	68(18.7)	2(18.2)	0.96(0.20–4.56)	0.963		

* AZT—zidovudine, 3TC—lamivudine, EFV- efavirenz, ABC-abacavir, NVP- nevirapine, TDF- Tenofovir LPV/r- lopinavir/ritonavir.

children starting ART with severe immunosuppression as compared to 7–10 years ago. This plausibly may explain the lower prevalence of VDD in this study, only 30% had started ART with CD4% <15. Therefore with this finding, it would be important to consider supplementation of all HIV infected children with severe immunosuppression with vitamin D because it's an easy and cost effective intervention but also re-emphasize early initiation of ART for better outcomes.

We found no association between viral load and serum vitamin D although other studies have found that higher serum 25(OH)D is also associated with lower RNA viral load [46]. Majority (89%) of our study population were virologically suppressed with normal serum 25 (OH)D. Vitamin D has been shown to increase autophagy, a process utilized by immune cells to kill intracellular pathogens [47]. Autophagy induced by physiological concentrations of 1,25 (OH) vitamin D, leads to inhibition of HIV replication in HIV infected macrophages as demonstrated in vitro studies [48]. The children in our study had been receiving ART for a median duration of six years and many were healthy, therefore, the immunologic, HIV stage and other HIV related factors may have been normalized and could have compensated for the effects of the vitamin D status. We believe this could have been different in children who may have had advanced disease and may have just been initiated on ART.

Our study found an association between efavirenz use and VDD/VDI. Several studies have documented reduced vitamin D level in people with HIV treated with efavirenz [13, 27, 49]. Longitudinal studies demonstrate a 5 ng/mL decrease in 25(OH)D within 6–12 months after initiation of an efavirenz-based regimen [49] and an improvement on switching to darunavir based regimen which is a PI regimen [50]. The effect of efavirenz is hypothesized through the induction of cytochrome P450 enzyme, 24 hydroxylase which inactivates 25(OH)D and 1,25 $(OH)_2D$ [49, 51].

We also found that lopinavir/ritonavir regimen was associated with lower odds of sub optimal vitamin D. This is consistent with other studies which report that ritonavir is associated with lower odds of low vitamin D [52], although some studies report no association [15]. In vitro, protease inhibitors have been shown to inhibit 25-hydroxylase and 1- α hydroxylase in a dose-dependent and reversible manner [53], resulting in decreased production of 1,25(OH)2D. Therefore it's imperative to study the effect of various PIs on the vitamin D status.

Limitations of this study

Our study had some limitations; we did not objectively assess the dietary and environmental effect (sun exposure) on the vitamin D status of our study participants. Additionally, we were unable to assess the effects of vitamin D deficiency/ insufficiency on bone due to financial constraints. We did not carry out any radiological tests for bone changes.

Conclusions

In conclusion, we found a low prevalence of suboptimal vitamin D among stable virologically suppressed HIV infected children in Uganda. The use of lopinavir/ ritonavir was associated with reduced odds of suboptimal vitamin D, while efavirenz was associated with deficiency. We recommend that HIV infected children who are severely immunosuppressed or with advanced disease at ART initiation be supplemented with vitamin D.

Supporting information

S1 Appendix. Study questionnaire stamped.
(DOCX)

S1 Dataset. Vitamin D study complete data April 2020.
(XLS)

Acknowledgments

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References

1. Joint United Nations Programme on HIV/AIDS. UNAIDS data 2017. Geneva: Accessed on;https://www.unaids.org/en/resources/documents/2017/2017_data_book.

2. Violari A, Cotton MF, Gibb DM, Babiker AG, Steyn J, Madhi SA, et al. Early antiretroviral therapy and mortality among HIV-infected infants. New England Journal of Medicine. 2008; 359(21):2233–44. https://doi.org/10.1056/NEJMoa0800971 PMID: 19020325

3. Deeks SG, Lewin SR, Havlir DV. The end of AIDS: HIV infection as a chronic disease. Lancet (London, England). 2013; 382(9903):1525–33. https://doi.org/10.1016/S0140-6736(13)61809-7 PMID: 24152939

4. Wei R, Christakos S. Mechanisms underlying the regulation of innate and adaptive immunity by vitamin D. Nutrients. 2015; 7(10):8251–60. https://doi.org/10.3390/nu7105392 PMID: 26404359

5. Lucas RM, Gorman S, Geldenhuys S, Hart PH. Vitamin D and immunity. F1000Prime Rep. 2014; 6:118-. https://doi.org/10.12703/P6-118 PMID: 25580272

6. Jiménez-Sousa MÁ, Martínez I, Medrano LM, Fernández-Rodríguez A, Resino S. Vitamin D in Human Immunodeficiency Virus Infection: Influence on Immunity and Disease. Front Immunol. 2018; 9:458-. https://doi.org/10.3389/fimmu.2018.00458 PMID: 29593721

7. Legeai C, Vigouroux C, Souberbielle J-C, Bouchaud O, Boufassa F, Bastard J-P, et al. Associations between 25-Hydroxyvitamin D and Immunologic, Metabolic, Inflammatory Markers in Treatment-Naive HIV-Infected Persons: The ANRS CO9 «COPANA» Cohort Study. PLOS ONE. 2013; 8(9):e74868. https://doi.org/10.1371/journal.pone.0074868 PMID: 24058636

8. Musselwhite LW, Andrade BB, Ellenberg SS, Tierney A, Belaunzaran-Zamudio PF, Rupert A, et al. Vitamin D, D-dimer, interferon γ, and sCD14 levels are independently associated with immune

reconstitution inflammatory syndrome: a prospective, international study. EBioMedicine. 2016; 4:115–23. https://doi.org/10.1016/j.ebiom.2016.01.016 PMID: 26981576

9. Sudfeld CR, Giovannucci EL, Isanaka S, Aboud S, Mugusi FM, Wang M, et al. Vitamin D status and incidence of pulmonary tuberculosis, opportunistic infections, and wasting among HIV-infected Tanzanian adults initiating antiretroviral therapy. The Journal of infectious diseases. 2013; 207(3):378–85. https://doi.org/10.1093/infdis/jis693 PMID: 23162137

10. Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, Heaney RP, et al. Evaluation, Treatment, and Prevention of Vitamin D Deficiency: an Endocrine Society Clinical Practice Guideline. The Journal of Clinical Endocrinology & Metabolism. 2011; 96(7):1911–30.

11. Rodriguez M, Daniels B, Gunawardene S, Robbins G. High frequency of vitamin D deficiency in ambulatory HIV-positive patients. AIDS research and human retroviruses. 2009; 25(1):9–14. https://doi.org/10.1089/aid.2008.0183 PMID: 19108690

12. Theodorou M, Serste T, Van Gossum M, Dewit S. Factors associated with vitamin D deficiency in a population of 2044 HIV-infected patients. Clinical nutrition (Edinburgh, Scotland). 2014; 33(2):274–9.

13. Welz T, Childs K, Ibrahim F, Poulton M, Taylor CB, Moniz CF, et al. Efavirenz is associated with severe vitamin D deficiency and increased alkaline phosphatase. AIDS (London, England). 2010; 24 (12):1923–8. https://doi.org/10.1097/QAD.0b013e32833c3281 PMID: 20588161

14. Wohl DA, Orkin C, Doroana M, Pilotto JH, Sungkanuparph S, Yeni P, et al. Change in vitamin D levels and risk of severe vitamin D deficiency over 48 weeks among HIV-1-infected, treatment-naive adults receiving rilpivirine or efavirenz in a Phase III trial (ECHO). Antivir Ther. 2014; 19(2):191–200. https://doi.org/10.3851/IMP2721 PMID: 24430534

15. Orkin C, Wohl DA, Williams A, Deckx H. Vitamin D deficiency in HIV: a shadow on long-term management? AIDS reviews. 2014; 16(2):59–74. PMID: 24759452

16. Connor RI, Rigby WF. 1α, 25-Dihydroxyvitamin D 3 inhibits productive infection of human monocytes by HIV-1. Biochemical and biophysical research communications. 1991; 176(2):852–9. https://doi.org/10.1016/s0006-291x(05)80264-5 PMID: 1673842

17. Aziz M, Livak B, Burke-Miller J, French AL, Glesby MJ, Sharma A, et al. Vitamin D insufficiency may impair CD4 recovery among Women's Interagency HIV Study participants with advanced disease on HAART. AIDS (London, England). 2013; 27(4):573.

18. Mehta S, Giovannucci E, Mugusi FM, Spiegelman D, Aboud S, Hertzmark E, et al. Vitamin D status of HIV-infected women and its association with HIV disease progression, anemia, and mortality. PloS one. 2010; 5(1):e8770. https://doi.org/10.1371/journal.pone.0008770 PMID: 20098738

19. Kamen DL, Tangpricha V. Vitamin D and molecular actions on the immune system: modulation of innate and autoimmunity. Journal of molecular medicine. 2010; 88(5):441–50. https://doi.org/10.1007/s00109-010-0590-9 PMID: 20119827

20. Lemire JM. Immunomodulatory actions of 1, 25-dihydroxyvitamin D3. The Journal of steroid biochemistry and molecular biology. 1995; 53(1–6):599–602. https://doi.org/10.1016/0960-0760(95)00106-a PMID: 7626516

21. Baeke F, Korf H, Overbergh L, van Etten E, Verstuyf A, Gysemans C, et al. Human T lymphocytes are direct targets of 1, 25-dihydroxyvitamin D3 in the immune system. The Journal of steroid biochemistry and molecular biology. 2010; 121(1–2):221–7. https://doi.org/10.1016/j.jsbmb.2010.03.037 PMID: 20302932

22. Liu PT, Stenger S, Li H, Wenzel L, Tan BH, Krutzik SR, et al. Toll-like receptor triggering of a vitamin D-mediated human antimicrobial response. Science. 2006; 311(5768):1770–3. https://doi.org/10.1126/science.1123933 PMID: 16497887

23. Viard J-P, Souberbielle J-C, Kirk O, Reekie J, Knysz B, Losso M, et al. Vitamin D and clinical disease progression in HIV infection: results from the EuroSIDA study. AIDS (London, England). 2011; 25 (10):1305–15. https://doi.org/10.1097/QAD.0b013e328347f6f7 PMID: 21522006

24. Thacher TD, Clarke BL. Vitamin D insufficiency. Mayo Clin Proc. 2011; 86(1):50–60. https://doi.org/10.4065/mcp.2010.0567 PMID: 21193656

25. Eckard AR, Tangpricha V, Seydafkan S, O'Riordan MA, Storer N, Labbato D, et al. The relationship between vitamin D status and HIV-related complications in HIV-infected children and young adults. The Pediatric infectious disease journal. 2013; 32(11):1224–9. https://doi.org/10.1097/INF.0b013e318286c793 PMID: 23360833

26. Meyzer C, Frange P, Chappuy H, Desse B, Veber F, Le Clesiau H, et al. Vitamin D deficiency and insufficiency in HIV-infected children and young adults. The Pediatric infectious disease journal. 2013; 32 (11):1240–4. https://doi.org/10.1097/INF.0b013e3182a735ed PMID: 23907260

27. Dao CN, Patel P, Overton ET, Rhame F, Pals SL, Johnson C, et al. Low vitamin D among HIV-infected adults: prevalence of and risk factors for low vitamin D Levels in a cohort of HIV-infected adults and

comparison to prevalence among adults in the US general population. Clinical Infectious Diseases. 2011; 52(3):396–405. https://doi.org/10.1093/cid/ciq158 PMID: 21217186

28. Rutstein R, Downes A, Zemel B, Schall J, Stallings V. Vitamin D status in children and young adults with perinatally acquired HIV infection. Clinical nutrition. 2011; 30(5):624–8. https://doi.org/10.1016/j.clnu.2011.02.005 PMID: 21641097

29. Teichmann J, Stephan E, Discher T, Lange U, Federlin K, Stracke H, et al. Changes in calciotropic hormones and biochemical markers of bone metabolism in patients with human immunodeficiency virus infection. Metabolism. 2000; 49(9):1134–9. https://doi.org/10.1053/meta.2000.8609 PMID: 11016893

30. Giacomet V, Vigano A, Manfredini V, Cerini C, Bedogni G, Mora S, et al. Cholecalciferol supplementation in HIV-infected youth with vitamin D insufficiency: effects on vitamin D status and T-cell phenotype: a randomized controlled trial. HIV clinical trials. 2013; 14(2):51–60. https://doi.org/10.1310/hct1402-51 PMID: 23611825

31. Mirza A, Wells S, Gayton T, Smotherman C, Rathore A, Kraemer D, et al. Vitamin D Deficiency in HIV-Infected Children. Southern medical journal. 2016; 109(11):683–7. https://doi.org/10.14423/SMJ.0000000000000556 PMID: 27812706

32. Hamdoun E. Current Definition of Vitamin D Status Misclassifies Maladapted Children of First Generation African Immigrants to the Northern US. Thesis. University of Minnesota. https://conservancyumnedu/handle/11299/190599. 2017.

33. Cusick SE, Opoka RO, Lund TC, John CC, Polgreen LE. Vitamin D insufficiency is common in Ugandan children and is associated with severe malaria. PloS one. 2014; 9(12):e113185. https://doi.org/10.1371/journal.pone.0113185 PMID: 25470777

34. Nabeta HW, Kasolo J, Kiggundu RK, Kiragga AN, Kiguli S. Serum vitamin D status in children with protein-energy malnutrition admitted to a national referral hospital in Uganda. BMC research notes. 2015; 8(1):418. https://doi.org/10.1186/s13104-015-1395-2 PMID: 26346815

35. Skversky AL, Kumar J, Abramowitz MK, Kaskel FJ, Melamed ML. Association of glucocorticoid use and low 25-hydroxyvitamin D levels: results from the National Health and Nutrition Examination Survey (NHANES): 2001–2006. The Journal of Clinical Endocrinology & Metabolism. 2011; 96(12):3838–45. https://doi.org/10.1210/jc.2011-1600 PMID: 21956424

36. World Health Organization. Interim WHO clinical staging of HVI/AIDS and HIV/AIDS case definitions for surveillance: African Region. World Health Organization, 2005.

37. World Health Organization. WHO anthro survey analyser and other tools. 2017. Accessed on: https://www.who.int/tools/child-growth-standards/software.

38. Ministry of Health U. CONSOLIDATED GUIDELINES FOR PREVENTION AND TREATMENT OF HIV IN UGANDA. June 2018. Accessed on: http://libraryhealthgoug/publications/hivaids/consolidated-guidelines-prevention-and-treatment-hiv-uganda-1.

39. Sudfeld CR, Duggan C, Aboud S, Kupka R, Manji KP, Kisenge R, et al. Vitamin D status is associated with mortality, morbidity, and growth failure among a prospective cohort of HIV-infected and HIV-exposed Tanzanian infants. The Journal of nutrition. 2015; 145(1):121–7. https://doi.org/10.3945/jn.114.201566 PMID: 25527666

40. Cissy N, Paul L, Edison M, Fox L. Survey of Vitamin D Status in Breastfeeding Infants Under the Age of 6 Months Attending the Growth Monitoring Clinic at Rubaga Hospital. ESPE Abstracts. 2014; 82.

41. Prentice A. Vitamin D deficiency: a global perspective. Nutrition reviews. 2008; 66(suppl 2):S153–S64. https://doi.org/10.1111/j.1753-4887.2008.00100.x PMID: 18844843

42. Celep G, Durmaz ZH, Çamurdan AD. Vitamin D Status in a Population of Well Children: Amasya Sample. Puerto Rico health sciences journal. 2019; 38(4).

43. Sahin ON, Serdar M, Serteser M, Unsal I, Ozpinar A. Vitamin D levels and parathyroid hormone variations of children living in a subtropical climate: a data mining study. Italian journal of pediatrics. 2018; 44(1):1–7.

44. Antonucci R, Locci C, Clemente MG, Chicconi E, Antonucci L. Vitamin D deficiency in childhood: old lessons and current challenges. Journal of Pediatric Endocrinology and Metabolism. 2018; 31(3):247–60. https://doi.org/10.1515/jpem-2017-0391 PMID: 29397388

45. Snellman G, Melhus H, Gedeborg R, Byberg L, Berglund L, Wernroth L, et al. Determining vitamin D status: a comparison between commercially available assays. PloS one. 2010; 5(7):e11555–e. https://doi.org/10.1371/journal.pone.0011555 PMID: 20644628

46. Kim JH, Gandhi V, Psevdos G Jr, Espinoza F, Park J, Sharp V. Evaluation of vitamin D levels among HIV-infected patients in New York City. AIDS research and human retroviruses. 2012; 28(3):235–41. https://doi.org/10.1089/AID.2011.0040 PMID: 21644847

47. Yuk J-M, Shin D-M, Lee H-M, Yang C-S, Jin HS, Kim K-K, et al. Vitamin D3 induces autophagy in human monocytes/macrophages via cathelicidin. Cell host & microbe. 2009; 6(3):231–43. https://doi.org/10.1016/j.chom.2009.08.004 PMID: 19748465

48. Campbell GR, Spector SA. Hormonally active vitamin D3 (1α, 25-dihydroxycholecalciferol) triggers autophagy in human macrophages that inhibits HIV-1 infection. Journal of Biological Chemistry. 2011; 286(21):18890–902. https://doi.org/10.1074/jbc.M110.206110 PMID: 21454634

49. Brown TT, McComsey GA. Short communications-association between initiation of antiretroviral therapy with efavirenz and decreases in 25-hydroxyvitamin D. Antivir Ther. 2010; 15:425.

50. Julie Fox BP, Manyu Prakash, Jose Arribas, Andrew Hill, and Christiane Moecklinghoff. Improvement in Vitamin D Deficiency Following Antiretroviral Regime Change: Results from the MONET Trial. AIDS research and human retroviruses. 2011; 27(1):29–34. https://doi.org/10.1089/aid.2010.0081 PMID: 20854196

51. Pascussi JM, Robert A, Nguyen M, Walrant-Debray O, Garabedian M, Martin P, et al. Possible involvement of pregnane X receptor–enhanced CYP24 expression in drug-induced osteomalacia. The Journal of clinical investigation. 2005; 115(1):177–86. https://doi.org/10.1172/JCI21867 PMID: 15630458

52. Yin M, Stein E. The Effect of Antiretrovirals on Vitamin D. Clinical Infectious Diseases. 2011; 52(3):406–8. https://doi.org/10.1093/cid/ciq169 PMID: 21217187

53. Cozzolino M, Vidal M, Arcidiacono MV, Tebas P, Yarasheski KE, Dusso AS. HIV-protease inhibitors impair vitamin D bioactivation to 1, 25-dihydroxyvitamin D. AIDS (London, England). 2003; 17(4):513–20. https://doi.org/10.1097/00002030-200303070-00006 PMID: 12598771