Research Letter

Prevalence and Risk Factors for Vitamin D Deficiency Among Tanzanian HIV-Exposed Uninfected Infants

Summary

Vitamin D is essential for bone development and may also play an integral role in control of intracellular pathogens. Serum 25-hydroxyvitamin D levels were assessed at 6 months of age for 191 HIV-exposed uninfected infants enrolled in a trial of multivitamins (not including vitamin D) in Tanzania. A total of 66 infants (34.6%) were classified as vitamin D deficient (<20 ng/ml), 93 (48.7%) as vitamin D insufficient (20–30 ng/ml) and 32 (16.8%) as vitamin D sufficient (≥30 ng/ml). Independent risk factors for vitamin D deficiency were sampling during the rainy season and infant wasting. Infant breastfeeding, maternal CD4 T-cell count, maternal wasting status and maternal receipt of antiretroviral therapy were not associated with vitamin D deficiency. Low levels of vitamin D were highly prevalent among HIV-exposed uninfected infants in Tanzania, and longitudinal studies and clinical trials of supplementation are needed to assess the impact on child health.

Key words: HIV, vitamin D, vitamin D deficiency, infant, risk factors.

Vitamin D is a potent immunomodulator with effects on both adaptive and innate immune responses [1]. Maintaining adequate levels of vitamin D appears to be particularly important for control of intracellular pathogens through enhancement of cell-mediated immunity, production of antimicrobial peptides and phagocytic activity of macrophages [2, 3]. As a result, vitamin D–deficient infants may be at high risk for infectious diseases, particularly acute respiratory infections [4].

Vitamin D levels in breastfeeding infants are dependent on the vitamin D status of the mother [5]. Consequently, breastfed HIV-exposed uninfected infants may be at high risk for vitamin D deficiency because low levels of vitamin D are common in HIV-infected adult women owing to HIV-related factors and antiretroviral drugs [6, 7]. Infants who are not being breastfed to prevent mother-to-child transmission may also be at high risk for deficiency, if they are not consuming foods or infant formulas that contain adequate levels of vitamin D [8]. To our knowledge, no studies have assessed the prevalence and risk factors for vitamin D deficiency among HIV-exposed uninfected infants in any setting.

We conducted a cross-sectional study of vitamin D among 191 HIV-exposed uninfected 6-month-old infants enrolled in a randomized, double-blind, placebo-controlled trial assessing the effect of multiple micronutrients (vitamins B, C and E) on mortality and infectious disease (NCT00197730) [9]. The trial enrolled singleton infants between 5 and 7 weeks of age born to women ≥18 years old who tested HIV-positive at the 32nd week of gestation or earlier at eight antenatal clinics in Dar es Salaam, Tanzania, during August 2004 to November 2007. Infants were randomized to receive a daily oral supplement of multivitamins or placebo. All children were tested for HIV infection at 6 weeks by polymerase chain reaction using the Amplicor HIV-1 DNA assay version 1.5 (Roche Molecular Systems, Branchburg, NJ, USA). Infants who were HIV-infected at 6 weeks were excluded from this analysis, owing to possible effect modification by HIV status. Nevirapine prophylaxis was provided to mothers at the onset of labor and to the child within 72 h of birth. During the trial, highly active antiretroviral therapy (ART) availability increased dramatically for adults, and mothers in the study were treated according to Tanzanian Ministry of Health guidelines. The standard first-line regimen included stavudine, lamivudine and nevirapine.

Mothers and infants were followed for 24 months after enrollment at monthly clinic visits, during which research nurses preformed standardized interviews. Study nurses asked mothers about infant feeding in the past 7 days. Exclusive breastfeeding was defined as breast-milk-only feeding with no additional foods, whereas mixed breastfed infants were given artificial foods (cereal, milk, etc.) in addition to breast milk [10]. Anthropometric measurements were also obtained by study nurses. Weight-for-length z-scores for infants were calculated using World Health Organization Child Growth Standards, with moderate to severe wasting defined as a z-score <-2 and mild wasting as a z-score of -2.0 to -1.0 [11]. Mid-upper arm circumference (MUAC) was also obtained from the mother, and wasting was defined using a cutoff of <22 cm [12]. Blood samples from the mother were collected for an assessment of absolute CD4 T-cell count (FACSCalibur system, Becton Dickinson, San Jose, CA).

In this study, 191 HIV-exposed uninfected infants were randomly selected to have 25-hydroxyvitamin D (25[OH]D) quantified from serum samples obtained at the 6-month clinic visit (range: 5.5–6.5 months). Quantification of 25(OH)D was done by high performance liquid chromatography tandem mass spectrometry (HPLC-MS/MS) using an API-5000 (AB Sciex, Foster City, CA) at Children's Hospital

Boston [13]. Vitamin D deficiency was defined as 25(OH)D concentrations <20 ng/ml, insufficiency as 20–30 ng/ml and sufficiency as >30 ng/ml [14].

We investigated risk factors for vitamin D deficiency using binomial regression models to obtain risk ratio estimates [15]. In a few instances, the models did not converge, and log-Poisson models, which provide consistent, but not fully efficient, estimates of the relative risk and its confidence intervals (CIs), were used [16]. Variables to be included in the risk factor analysis were identified from the literature and included: season, infant sex, wasting (weight for length), breastfeeding method, randomized treatment regimen, mother's age, MUAC and receipt of ART. Missing data for covariates were retained in analyses using the missing-indicator method. All *p*-values were two-sided, and $p < 0.05$ was considered statistically significant. Statistical analyses were performed using SAS v 9.2 (SAS Institute Inc., Cary, NC, USA).

The mean 25(OH)D concentration for 191 HIV-exposed uninfected Tanzanian infants at 6 months of age was 23.7 (standard deviation: 8.1) ng/ml. A total of 66 infants (34.6%) were classified as vitamin D deficient (<20 ng/ml), 93 (48.7%) as insufficient (20–30 ng/ml) and 32 (16.8%) as vitamin D sufficient (≥30 ng/ml).

Univariate and multivariate analyses of risk factors for vitamin D deficiency are presented in Table 1. After multivariate adjustment, infants who were moderately to severely wasted had 2.42 (95% CI 1.29–4.55; $p = 0.008$) times higher risk of vitamin D deficiency as compared with infants with normal weight-for-length *z*-scores. Season was also significantly associated with vitamin D deficiency, with

TABLE 1
Characteristics of HIV-exposed uninfected infants at 6 months of age and risk factors for vitamin D deficiency (<20 ng/ml)

Characteristic	Vitamin D deficient (<20 ng/ml) Frequency (%) ($n = 66$)	25(OH)D ≥20 ng/ml Frequency (%) ($n = 125$)	Univariate Relative risk (95% CI)	*p*-value	Multivariate Relative risk (95% CI)	*p*-value
Infant sex						
Female	26 (39.4)	57 (45.6)	1.0	–	1.0	–
Male	40 (60.6)	68 (54.4)	1.18 (0.79–1.77)	0.415	1.43 (0.97–2.14)	0.071
Infant weight-for-length *z*-score						
Normal ($z > -1.0$)	8 (12.1)	23 (18.4)	1.0	–	1.0	–
Mild wasting ($-1 \leq z \geq -2$)	46 (32.6)	95 (76.0)	1.29 (0.67–2.40)	0.474	1.31 (0.74–2.31)	0.357
Moderate/severe wasting ($z < -2$)	12 (18.2)	7 (5.6)	2.45 (1.23–4.87)*	0.011*	2.42 (1.29–4.55)*	0.006*
Breastfeeding						
None	52 (78.8)	99 (79.2)	1.0	–	1.0	–
Mixed	7 (10.6)	10 (8.0)	1.20 (0.65–2.20)	0.565	1.14 (0.59–2.20)	0.686
Exclusive	7 (10.6)	16 (12.8)	0.88 (0.46–1.70)	0.712	0.86 (0.44–1.70)	0.672
Season						
Long rain (December to March)	26 (39.4)	36 (28.8)	1.0	–	1.0	–
Harvest (April to May)	6 (9.1)	18 (14.4)	0.60 (0.28–1.26)	0.178	0.57 (0.26–1.17)	0.119
Postharvest (June to August)	7 (10.6)	48 (38.4)	0.30 (0.14–0.64)*	0.001*	0.30 (0.15–0.62)*	0.001*
Short rain (September to November)	27 (40.9)	23 (18.4)	1.29 (0.87–1.90)	0.203	1.32 (0.89–1.96)	0.672
Randomized regimen						
Placebo	38 (57.6)	57 (45.6)	1.0	–	1.0	–
Multivitamin	28 (42.4)	68 (54.4)	0.73 (0.49–1.08)	0.119	0.71 (0.49–1.03)	0.068
Maternal age						
<30 years	44 (66.7)	74 (59.2)	1.24 (0.81–1.88)	0.321	1.06 (0.72–1.58)	0.761
≥30 years	22 (33.3)	51 (40.8)	1.0	–	1.0	–
Maternal MUAC						
Normal (≥22 cm)	57 (86.4)	117 (93.6)	1.0	–	1.0	–
Wasting (<22 cm)	9 (13.6)	8 (6.4)	1.62 (0.98–2.65)	0.058	1.41 (0.78–2.53)	0.256
Maternal CD4 T-cell count						
<350 cells/μl	18 (30.5)	39 (32.2)	1.0	–	1.0	–
≥350 cells/μl	41 (69.5)	82 (67.8)	0.93 (0.59–1.48)	0.762	0.83 (0.50–1.40)	0.497
Mother receiving ART						
No	40 (60.6)	78 (62.4)	1.0	–	1.0	–
Yes	26 (39.4)	47 (37.6)	1.05 (0.71–1.56)	0.808	0.92 (0.59–1.43)	0.703

* *p*-value <0.05

low risk during the harvest and postharvest period (April to August) and high risk during the long and short rains (September to March). Maternal wasting was borderline significant in the univariate analysis ($p = 0.058$), and randomization to multivitamins as compared with placebo was borderline significant in the multivariate analysis ($p = 0.068$).

In conclusion, the prevalence of low vitamin D levels (<30 ng/ml) is high among HIV-exposed uninfected infants in Tanzania, and the greatest risk for vitamin deficiency occurs during the rainy season. We also found infant wasting was associated with vitamin D deficiency; however, from this cross-sectional study, we are unable to assess whether malnutrition, which may be a surrogate for low intake of foods containing vitamin D, results in vitamin D deficiency or whether low vitamin D levels increase the risk or severity of infections that induce weight loss. The impact of breastfeeding on vitamin D status was not clear, and larger studies among HIV-infected mothers are needed. Longitudinal studies are also warranted to assess the association of vitamin D with incidence of infections among HIV-exposed infants as well as HIV disease progression for infected infants.

Ethics Statement

Written informed consent was obtained from all participants included in the parent trial. The trial protocol was approved by the institutional review boards of the Harvard School of Public Health, Muhimbili University of Health and Allied Sciences, Tanzania Food and Drugs Authority and National Institute of Medical Research.

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