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Maternal Vitamin D Status and Child Morbidity, Anemia, and Growth in Human Immunodeficiency Virus-Exposed Children in Tanzania

Julia L. Finkelstein, MPH, SM, SCD[1,2,3], **Saurabh Mehta, MBBS, SCD**[1], **Christopher Duggan, MD, MPH**[2,4], **karim P. Manji, MD, MMED**[5], **Ferdinand M. Mugusi, MD, MMED**[6], **Said Aboud, MD, MMED**[7], **Donna Spiegelman, SCD**[3,8], **Gernard I. Msamanga, MD, SCD**[9], and **Wafaie W. Fawzi, MBBS, DRPH**[2,3,10]

[1]Division of Nutritional Sciences, Cornell University, Ithaca, NY

[2]Department of Nutrition, Harvard School of Public Health, Boston, MA

[3]Department of Epidemiology, Harvard School of Public Health, Boston, MA

[4]Division of GI/Nutrition, Children's Hospital, Boston, MA

[5]Department of Pediatrics, Muhimbili University of Health and Allied Sciences, Dar es Salaam, Tanzania

[6]Department of Internal Medicine, Muhimbili University of Health and Allied Sciences, Dar es Salaam, Tanzania

[7]Departments of Microbiology and Immunology, Muhimbili University of Health and Allied Sciences, Dar es Salaam, Tanzania

[8]Department of Biostatistics, Harvard School of Public Health, Boston, MA

[9]Department of Community Health, Muhimbili University of Health and Allied Sciences, Dar es Salaam, Tanzania

[10]Departments of Global Health and Population, Harvard School of Public Health, Boston, MA

Abstract

Background—Vitamin D may help prevent adverse pediatric outcomes, including infectious diseases and growth failure, based on its role in immune and metabolic functions. We examined the association of maternal vitamin D status and pediatric health outcomes in children born to HIV-infected women.

Methods—Vitamin D status was determined in 884 HIV-infected pregnant women at 12 to 27 weeks of gestation in a trial of vitamin supplementation (not including vitamin D) in Tanzania.

Corresponding Author: Julia L. Finkelstein, 218 Savage Hall, Division of Nutritional Sciences, Cornell University, Ithaca, NY, 14853, Phone: 607-355-9180, Fax: 607-255-1033, jlf288@cornell.edu.

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Information on child morbidities, anemia and hypochromic microcytosis, and anthropometry was recorded through monthly clinic visits. Generalized estimated equations and Cox proportional hazards models were used to assess the relationships of outcomes with maternal vitamin D status.

Results—A total of 39% of women had low vitamin D levels (<32 ng/mL). Children born to women with low vitamin D status were 1.11 times more likely to report cough during follow-up (RR: 1.11; 95% CI: 1.02-1.21). No significant associations were noted for other respiratory symptoms, diarrhea, or anemia outcomes. Low maternal vitamin D status was associated with significantly increased risk of stunting (height-for-age z-score <-2; RR: 1.29; 95% CI: 1.05-1.59) and underweight (weight-for-age z-score <-2; RR: 1.33; 95% CI: 1.03-1.71).

Conclusions—Maternal vitamin D status may be an important risk factor for respiratory infections, and ensuring optimal growth in HIV-exposed children.

Keywords

Vitamin D; Morbidity; Anemia; Growth; Children; HIV/AIDS

Introduction

Vitamin D is an immunomodulator (1) with an extensive role in both innate and adaptive immunity (2-4). Vitamin D may help prevent child morbidities, based on its involvement in the development of the fetal and infant immune system (5). Children with rickets, the classical vitamin D deficiency disease, experience an increased occurrence of infections, although the mechanisms involved are not fully understood (6, 7). Adequate vitamin D status has also been associated with a lower risk of infectious diseases during childhood, such as acute respiratory infections and pneumonia (8).

Vitamin D is required for fetal growth and may help regulate placental development and function (9); its role in regulation of calcium and phosphorus homeostasis may also be important in ensuring optimal pregnancy outcomes (10) and subsequent child growth (11). Maternal vitamin D intake or supplementation during pregnancy has been associated with increased infant birth weight, head and arm circumference, and skinfold thickness (12, 13).

There have been no studies assessing the relationship of maternal vitamin D status with health outcomes in HIV-exposed children, particularly from resource-limited settings. We conducted a prospective observational analysis of the association of maternal vitamin D status with pediatric outcomes, including morbidity, anemia and hypochromic microcytosis, and growth, in children born to HIV-infected women in Tanzania.

Methods and Materials

Study Design and Population

The Trial of Vitamins was a randomized placebo-controlled trial in Tanzania. This study was conducted to examine the effects of multivitamins (excluding vitamin D) on mother-to-child HIV transmission, disease progression, and adverse pregnancy outcomes, among 1,078 HIV-infected pregnant women and their children (14, 15). The design of this trial has been described previously (16). Briefly, women were assigned in a two-by-two factorial design to a daily oral dose of: 1) vitamin A (30 mg beta-carotene plus 5000 IU preformed vitamin A); 2) multivitamins excluding vitamin A (20 mg B1, 20 mg B2, 25 mg B6, 100 mg niacin, 50 μg B12, 500 mg C, 30 mg E, and 0.8 mg folic acid); multivitamins including vitamin A, or placebo. This regimen did not include vitamin D. All women received ferrous sulphate (400 mg, equivalent to 120 mg ferrous iron) and folate (5 mg) daily.

Ethics

Informed consent was obtained from all mothers. The research protocol was approved by the Research and Publications Committee of Muhimbili University College of Health Sciences, and the Institutional Review Board of the Harvard School of Public Health.

Assessment of Baseline Covariates

Structured interviews were conducted at the baseline clinic visit to collect information on demographic characteristics and obstetric history. Study physicians performed a complete medical examination and collected blood, urine, stool, and vaginal swab specimens to assess co-infections. Participants were counseled regarding the risks and benefits of infant feeding options for HIV-infected mothers, as per World Health Organization guidelines and standard of care in Tanzania at that time.

Assessment and Definitions of Exposure

Maternal vitamin D status was assessed at enrolment based on serum concentrations of 25-hydroxyvitamin D [25(OH)D]. Blood samples were obtained from participants at the enrollment visit, and stored at or below −70°C. In 2005, laboratory analyses were conducted on stored plasma specimens (≤ −70°C) at Children's Hospital Boston, to evaluate maternal serum 25(OH)D concentrations, using the Nichols fully automated chemiluminescence ADVANTAGE 25(OH)D assay system (Nicholas Institute Diagnostics, San Juan Capistrano, CA). Vitamin D status was categorized as insufficient (<32 ng/mL or <80 nmol/L) versus sufficient, based on requirements for optimal calcium homeostasis (17, 18) and previous studies (19), and in quintiles based on the distribution of vitamin D levels in this population. Results were similar when using quintiles, unless reported in the text below.

Follow-up Procedures

Clinical assessments were performed at monthly and interim visits to evaluate maternal and child health status, including morbidities and anthropometry. Children were followed for a median of 58 months (IQR: 13-69). Participants who missed a clinic visit or traveled outside of Dar es Salaam were followed-up *via* home visits, to establish maternal and infant vital status.

Laboratory Methods

Whole blood samples were collected from the children at birth (range 0-21 days), six weeks (range 21-49 days), and 3-monthly thereafter. Laboratory samples were tested in batch, and instruments were calibrated daily using standardized procedures.

Infant HIV status was determined from blood samples collected at birth, six weeks, and 3-monthly thereafter. The Amplicor HIV-1 detection kit (Roche Diagnostic System, Branchburg, NJ, US) was used to determine HIV status for infants less than 18 months of age. In children ≥ 18 months, HIV infection was defined by a positive ELISA, and confirmed by Western blot.

Hemoglobin concentrations were assessed using a CBC5 Coulter Counter (Coulter Corporation, Miami) or the cyanmethemoglobin method with a colorimeter (Corning Inc., Corning, NY).

Assessment and Definition of Outcomes

Morbidities—At each clinic visit, a study physician examined the children and noted the presence of clinical morbidities. Respiratory signs and symptoms were assessed, including: fever, cough, difficulty in breathing, chest retractions, and difficulty in eating, drinking, or

breastfeeding. Respiratory signs and symptoms were classified as: 1) cough alone; 2) cough with fever; 3) cough with rapid respiratory rate; or 4) cough plus at least one additional symptom: difficulty breathing; chest retractions; or refusal to breastfeed, eat, or drink. Infant respiratory rate was measured using a stopwatch on the day of the monthly visit. Rapid respiratory rate was defined as ≥50 breaths per minute for infants and ≥40 for children older than one year.

At each clinic visit, trained study nurses asked mothers to report episodes of child morbidity during the previous month. Diarrhea was defined as ≥3 watery stools in a 24-hour period in the previous month; mothers were asked if the stools contained blood or mucous. Diarrhea episodes were defined as acute (≥1 but <14 days) or persistent (≥14 days of diarrhea). Episodes of acute diarrhea were classified as dysenteric if mucus or blood was present; other episodes were categorized as watery diarrhea.

Anemia and Hypochromic Microcytosis—Anemia and severe anemia were defined as hemoglobin concentrations of less than 11.0 and 8.5 g/dL, respectively. Thin blood films with Leishman's stain were prepared and examined microscopically. We examined peripheral red blood cell morphology for hypochromasia and microcytosis. Hypochromic microcytic anemia was categorized as severe (hypochromasia ≥2+ and microcytic cells observed), moderate (hypochromasia ≥1+ and microcytic cells observed), or mild (hypochromasia ≥1+ with or without microcytosis). Participants diagnosed with severe anemia received clinical management as per standard of care, including iron supplementation.

Growth—Anthropometric measurements were obtained by trained research assistants using standardized procedures and calibrated instruments. As per World Health Organization guidelines, child height was measured as supine length up to 24 months of age, and standing height from 24 months onwards (20). Z scores were calculated using standard World Health Organization methods (20). Stunting and severe stunting were defined as height-for-age (HAZ) Z-score <-2 and HAZ <-3, respectively; underweight and severe underweight as weight-for-age (WAZ) Z-score <-2 and WAZ <-3; and wasting and severe wasting as weight-for-height (WHZ) Z-score <-2 and WHZ <-3.

Statistical Analyses

The mean number of episodes per child per year (±SD) was calculated for cough, other respiratory signs and symptoms, and diarrhea. Generalized estimating equations (GEE) with an exchangeable working covariance structure were used to examine the relationship between maternal vitamin D status and these outcomes (21). Follow-up data for each child were grouped into four half-year periods.

Cox proportional hazards models were used to assess the relationships of maternal vitamin D status with time to first episode of child anemia, hypochromic microcytosis, and growth outcomes (22). For children without the outcome of interest, follow-up ended on the date of the last visit or death.

We explored potential non-linearity of the relationships between continuous vitamin D levels and the risk of outcomes non-parametrically, using stepwise restricted cubic splines (23, 24). A partial likelihood ratio test for non-linearity compared the model with only the linear term to the model with the linear and the selected cubic spline terms. If non-linear associations are not reported, they were not significant.

The approach proposed by Rothman and Greenland was used to control for confounding (25); all known or suspected risk factors for outcomes were included in models (26). The

risk factors included in multivariate models were maternal age (years), education, occupation, WHO HIV disease stage, CD4 T-cell counts, short stature (height <150 cm), anemia (Hb<11.0 g/dL), mid-upper arm circumference (cm), and multivitamin regimen; and child's sex, gestational age (weeks), birth weight (< 2,500 g), mid-upper arm circumference (cm), breastfeeding status, and HIV status. Variables such as breastfeeding status and HIV status were allowed to vary with time. We also allowed covariates such as CD4 T-cell counts and MUAC to vary with time; however, results did not change on their inclusion. We examined effect modification of the relationship between low vitamin D status and outcomes by variables such as HIV status and low birth weight, by introducing an interaction term in the model; these terms were not statistically significant. The missing indicator method was used to account for missing covariate data (27).

Statistical analyses were performed using SAS software version 9.2 (SAS Institute Inc., Cary, NC, US).

Results

Of a total of 1,078 women enrolled in the parent trial (1995-1997), 884 women had vitamin D concentrations available. A total of 347 (39%) women had low vitamin D concentrations (<32 ng/mL) (Table 1). Follow-up information for pediatric morbidity, and anemia and hypochromic microcytosis, and anthropometric data, were available for 609, 884, and 732 children, respectively. Children were followed for a median duration of 58 months (IQR: 13-69).

Children born to the women included in this study, on average, experienced 1.4 episodes of diarrhea and 4.6 episodes of cough per year during follow-up (**See** Table, Supplemental Digital Content 1). Children born to women with low vitamin D levels were 1.11 times more likely to develop cough during follow-up (95% CI: 1.02-1.21) in multivariate models. No significant associations were observed for other respiratory signs and symptoms or diarrhea. Findings were similar in quintile analyses, and results did not significantly differ by child's HIV status.

A total of 60%, 37%, 10%, and 4% of the children born to women with low vitamin D had an episode of anemia, severe anemia, severe hypochromic microcytosis, and macrocytosis, respectively, compared to 68%, 45%, 12%, and 7% of the children born to women with adequate levels of vitamin D. No significant association was observed for anemia outcomes with vitamin D status (**See** Table, Supplemental Digital Content 2). Findings were similar in quintile analyses, and results did not significantly differ by child's HIV status.

A total of 58%, 44%, and 29% of the children born to women with low vitamin D became stunted, underweight, and wasted during follow-up respectively, compared to 56%, 40%, and 25% of the children born to women with adequate levels of vitamin D. Children born to women with low vitamin D status had a 29% increased risk of stunting (HAZ<-2) (RR=1.29, 95% CI: 1.05-1.59), and 33% increased risk of underweight (WAZ<-2) (RR=1.33, 95% CI: 1.03-1.71) in multivariate models (**See** Table, Supplemental Digital Content 3). Findings were similar in quintile analyses, and did not significantly differ by child's HIV status.

Discussion

In this study, low maternal vitamin D status was associated with significantly increased risk of cough among children during the follow-up period. No associations were observed between low maternal vitamin D status and adverse pediatric outcomes such as diarrhea, other respiratory signs and symptoms, or anemia and iron deficiency. A significantly

increased risk of stunting and underweight was observed in children born to women with low vitamin D status at baseline.

The role of maternal vitamin D status and morbidities of their children has not been previously examined among children born to HIV-infected women. However, the association between vitamin D status and reduced risk of infectious diseases in childhood has been found in other studies in HIV-uninfected populations. Vitamin D is a known immunomodulator (1), and has an extensive role in innate and adaptive immunity (2-4). It can enhance cell-mediated immunity (2), phagocytic activity of macrophages (3), and the number and cytolytic activity of natural killer cells (4). Additionally, toll-like receptor stimulation of human macrophages up-regulates the expression of vitamin D receptors and conversion to 1,25-dihydroxyvitamin D, the biologically active metabolite (28). In the presence of adequate 25-hydroxyvitamin D, the activation of the up-regulated vitamin D receptors leads to induction of cathelicidin, an antimicrobial peptide capable of killing pathogens such as *Mycobacterium tuberculosis* intracellularly. These effects on the immune system may explain the relationship of low vitamin D levels with increased risk of cough observed in this paper.

Vitamin D also contributes to the development of the fetal immune system (5). Children with stronger immune systems may be more resistant to infectious diseases; this may explain the decreased risk of cough observed in this study. Vitamin D has also been associated with decreased risk of a broad range of infectious diseases during childhood, such as acute respiratory infections and pneumonia (8).

In this study, analyses suggested no association between maternal vitamin D levels and risk of severe anemia and hypochromic microcytosis. The relationship between vitamin D and iron status has not previously been evaluated among children born to HIV-infected women. However, previous analyses in the parent study among HIV-infected mothers found that lower vitamin D status at baseline was associated with significantly higher risk of maternal anemia during the pregnancy and postpartum periods (29), and adequate vitamin D status was an important predictor of resolution of anemia and hypochromic microcytosis (Finkelstein, submitted). There are several plausible biological mechanisms by which vitamin D could modulate the risk of anemia and iron deficiency. For example, vitamin D deficiency could lead to anemia *via* increased inflammation or marrow myelofibrosis (30). An association between low vitamin D status and lower hemoglobin levels has been observed in earlier studies in individuals with renal disease in NHANES III (31) and in studies in children in minority communities in Britain (32, 33). However, there is limited evidence regarding biological mechanisms, and the relationship between vitamin D and iron status needs to be further explored in pregnant women and their children in resource-limited settings.

The association observed between vitamin D insufficiency and stunting and underweight is consistent with previous research in HIV-uninfected populations, and may be explained by its important role in somatic and bone growth. Vitamin D is required for fetal growth and may help regulate placental development and function (9); its role in regulation of calcium and phosphorus homeostasis may also be important in the etiology of pregnancy outcomes (10, 34, 35). For example, in a placebo-controlled trial among pregnant Indian women, participants were randomized to receive 600,000 IU of vitamin D or placebo, twice during the seventh and eight months of pregnancy (13). Infants born to women who received vitamin D supplements had increased intrauterine growth, birth weight, and head circumference, compared to placebo.

Vitamin D inadequacy is not generally associated with equatorial regions, due to year-round sunlight. Therefore, it was unexpected to observe a prevalence of almost 40% vitamin D insufficiency (<32 ng/mL) in this study, in a country just six degrees from the Equator. However, other studies have observed high rates of vitamin D insufficiency among African American pregnant women in the United States (19) and among presumably HIV-uninfected pregnant women in the Gambia (36). The participants in the current study resided in a primarily urban area in Dar es Salaam, and perhaps may have reduced exposure to direct sunlight. Further, HIV infection itself may contribute to the lower vitamin D concentrations observed in this study.

Our analysis is distinct from previous studies due to its extensive assessment of child morbidities, and prospective evaluation of incident health outcomes in a cohort of children born to HIV-infected women. Participants were also followed for a relatively long duration, with a median of 58 months.

Our study has a few limitations. One major limitation of this analysis is that we assessed only one measurement of maternal vitamin D levels at 12 to 27 weeks of gestation, and did not evaluate child vitamin D concentrations. However, data from other studies suggests that there is a moderately strong correlation between maternal baseline vitamin D levels and cord and infant blood vitamin D concentrations (19). Another limitation is that the vitamin D assay used does not accurately measure vitamin D_2, the form obtained through dietary supplements; however, the use of supplements was unlikely in this population.

The cut-off for adequate vitamin D status was selected to make results more clinically relevant and comparable to previous studies. However, similar results were obtained using vitamin D quintiles based on its distribution in this population. This analysis was also conducted among children born to HIV-infected women who were not on anti-retroviral therapy (ART). Therefore, findings may not be generalizable to HIV-infected pregnant women and children receiving ART. During the past decade, the advent and rapid scale-up of highly-active antiretroviral therapy (HAART) has dramatically altered the landscape of HIV standard care and treatment in resource-limited settings. HAART is today the single-best intervention for preventing HIV infection in children or slowing down HIV disease progression in adults. Emerging research shows that vitamin D deficiency/insufficiency is widespread all over the world and addressing it is important for both HIV and non HIV-infected or exposed mothers and children, respectively. We hypothesize that the association of vitamin D status with child morbidity, anemia, and anthropometric status is biologically plausible and independent of HIV status and needs to be carefully examined in randomized controlled trials.

In summary, our study provides support for a potentially beneficial role of adequate maternal vitamin D status on pediatric health outcomes, including cough and optimal growth. Findings need to be confirmed in the setting of a randomized controlled trial; if found to be effective, vitamin D supplementation may be a potential adjunct treatment to reduce the risk of morbidities and promote optimal growth in children born to HIV-infected women.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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