



Published in final edited form as:

Pediatr Infect Dis J. 2014 November ; 33(11): 1141–1147. doi:10.1097/INF.0000000000000428.

Vitamin D Insufficiency in HIV-Infected Pregnant Women Receiving Antiretroviral Therapy is not associated with Morbidity, Mortality or Growth Impairment in Their Uninfected Infants in Botswana

Kathleen Powis, MD, MPH^{1,2,3}, Shahin Lockman, MD, MSc^{2,3,4}, Laura Smeaton, MS⁵, Michael D. Hughes, PhD⁵, Wafaie Fawzi, MB BS, DrPH⁶, Anthony Ogwu, MB, BS³, Sikhulile Moyo, MSc, MPH³, Erik van Widenfelt³, Julia von Oettingen, MD⁷, Joseph Makhema, MB, ChB, MRCP³, Max Essex, DVM, PhD^{2,3}, and Roger L. Shapiro, MD, MPH^{2,3,8}

¹Departments of Internal Medicine and Pediatrics, Massachusetts General Hospital, Boston, MA United States

²Harvard School of Public Health, Department of Immunology and Infectious Diseases, Boston, United States

³Botswana Harvard AIDS Institute Partnership, Gaborone, Botswana

⁴Brigham and Women's Hospital, Infectious Disease Division, Boston, United States

⁵Center for Biostatistics in AIDS Research, Harvard School of Public Health, Boston, MA, United States

⁶Harvard School of Public Health, Department of Global Health and Population, Boston

⁷Boston Children's Hospital, Division of Endocrinology, Boston, MA, United States

⁸Division of Infectious Diseases, Beth Israel Deaconess Medical Center, Boston, MA, United States

Abstract

Background—Low maternal 25(OH)D (vitamin D) values have been associated with higher mortality and impaired growth among HIV-exposed uninfected (HEU) infants of antiretroviral (ART)-naïve women. These associations have not been studied among HEU infants of women receiving ART.

Methods—We performed a nested case-control study in the Botswana Mma Bana Study, a study providing ART to women during pregnancy and breastfeeding. Median maternal vitamin D values, and the proportion with maternal vitamin D insufficiency, were compared between women whose HEU infants experienced morbidity/mortality during 24 months of follow-up and women

Corresponding author: Kathleen Powis, 100 Cambridge St, 15th floor, Boston, MA 02114 (617)643-2054; Fax (617)643-9105; kpowis@partners.org.

Presented in Part: Powis KM, Fawzi W, Smeaton L, Hughes M, Lockman S, Ogwu A, Moyo S, Makhema J, Essex M, Shapiro R.L., Vitamin D insufficiency in HIV-infected women on HAART is not associated with morbidity, mortality or growth impairment of uninfected infants in Botswana. 20th Conference on Retroviruses and Opportunistic Infections, Atlanta, United States. March 3th to March 6th, 2013 [Abstract #920].

with non-hospitalized HEU infants. Growth faltering was assessed for never hospitalized infants attending the 24-month-of-life visit. Multivariate logistic regression models determined associations between maternal vitamin D insufficiency and infant morbidity/mortality and growth faltering.

Results—Delivery plasma was available and vitamin D levels assayable from 119 (86%) of 139 cases and 233 (84%) of 278 controls, and did not differ significantly between cases and controls (median 36.7 ng/mL; IQR 29.1- 44.7 vs. 37.1 ng/mL; IQR 30.0 - 47.2; $p = 0.32$). Vitamin D insufficiency (< 32 ng/mL) was recorded among 112 (31.8%) of 352 women at delivery and occurred most frequently among women delivering in winter. Multivariate logistic regression models adjusted for maternal HIV disease progression did not show associations between maternal vitamin D insufficiency at delivery and child morbidity/mortality, or 24-month-of-life growth faltering.

Conclusions—Vitamin D insufficiency was common among ART treated pregnant women in Botswana, but was not associated with morbidity, mortality or growth impairment in their HIV-uninfected children.

Keywords

HIV-exposed uninfected infants; maternal vitamin D; morbidity; mortality; growth

Introduction

In developing countries, antiretroviral treatment (ART) initiated prior to conception or during pregnancy and continued throughout breastfeeding has proven to be highly efficacious in preventing mother-to-child HIV transmission (PMTCT).¹⁻⁴ However, children exposed to HIV *in utero* who remain uninfected experience significantly higher morbidity and mortality compared with HIV-unexposed infants,⁵⁻⁷ and some studies have found growth impairment of HIV-exposed uninfected (HEU) infants.^{8,9} Understanding the reason for these disparities is an important scientific priority for childhood survival programs in HIV-affected areas.

Vitamin D is an immune regulatory hormone. *In vivo*, it has been demonstrated to affect CD4+ Th1-Th2 balance, inhibiting Th1 and augmenting Th2 cell development¹⁰ and Th2 enhanced cytokine expression¹¹. Vitamin D has also been found to mediate macrophage chemotaxis in response to inflammatory stimulus¹², to be associated with higher quantities and increased cytolytic activity of natural killer cells¹³, and to up-regulate innate immune system antimicrobial peptides¹⁴. In a pre-ART era study in Tanzania, maternal vitamin D insufficiency, defined as a vitamin D level < 32 ng/mL, was significantly associated with mortality of HEU infants.¹⁵ This cut-off was chosen by the Tanzania research team, as it was a level above which optimal calcium homeostasis is achieved.^{16,17} Infants born to the women enrolled in the Tanzania cohort were also found to have a significantly higher risk of stunting or being underweight if maternal vitamin D insufficiency was noted during the antenatal period.¹⁸ Other studies have demonstrated an association between the use of efavirenz-based ART and vitamin D deficiency.^{19,20} Scale-up of the World Health Organization's first line efavirenz-based ART regimen in pregnancy²¹ may therefore result

in a higher prevalence of infant hypovitaminosis D, as the fetus is entirely dependent on maternal stores of vitamin D,²² and this deficiency may be associated with higher infant mortality and/or poorer growth outcomes.

We therefore sought to define the relationship between maternal vitamin D insufficiency and infant outcomes within a cohort of women receiving ART in Botswana. Using a nested case-control design, we evaluated the association between maternal vitamin D insufficiency at delivery and infant morbidity/mortality. In a secondary analysis we evaluated the association between maternal vitamin D insufficiency at delivery and growth outcomes for those infants in the morbidity/mortality analysis who attended the 24-month-of-life study visit and were never hospitalized over the 24-month-period of the study.

Materials and Methods

Study Population

The Mma Bana Study, which enrolled 730 HIV-1 infected ART-naive pregnant women, has been described in detail previously.¹ Five hundred sixty participants who had CD4+ cell counts ≥ 200 cells/mm³ and no AIDS defining illnesses at enrollment were randomized between 26-34 weeks gestation to initiate either abacavir/zidovudine/lamivudine co-formulated as Trizivir (GlaxoSmithKline, Greenford, United Kingdom) (TZV) twice daily or zidovudine/ lamivudine co-formulated as Combivir (CBV) (GlaxoSmithKline) with lopinavir/ritonavir co-formulated as Kaletra (Abbott Laboratories, Abbott Park, IL) (CBV-KAL) twice daily. Another 170 women, who had a screening or previously documented CD4+ cell count of < 200 cells/mm³, were enrolled in an observational arm of the Mma Bana study between 18 weeks and 34 weeks gestation and monitored as they initiated Combivir and Nevirapine (CBV-NVP), the ART regimen specified at the time in Botswana's national HIV treatment guidelines. Mma Bana participants remained on ART during a period of breastfeeding, up to six months postpartum and continued ART indefinitely if they qualified for ART under national treatment guidelines. Gestational age was ascertained from an algorithm that relied upon maternal reported last menstrual period (LMP) and ultrasound performed prior to randomization. All study physicians received standardized ultrasound training. Study physicians relied upon ultrasound-identified fetal femur length and biparietal diameter to estimate ultrasound gestational age. Where the estimated gestational age of a first trimester ultrasound differed by more than one week from the woman's LMP, a second trimester ultrasound differed by more than two weeks, or a third trimester ultrasound differed by more than three weeks, the ultrasound dating was utilized as the gestational age.

Trained study nurses and physicians conducted clinical assessments at birth, monthly through seven months of life and then once every three months through 24 months of life starting at 9 months of life to evaluate maternal and child health status, including morbidity, mortality and anthropometric measures. During the visits, the child was weighed and recumbent length was obtained using a length measuring mat. Health records were reviewed and mothers were interviewed to determine if the child required hospitalization since the last scheduled study visit. If a child failed to attend a scheduled visit, attempts were made to contact the mother by phone or in person, to determine the infant's vital status.

Infant HIV DNA PCR testing was completed at birth, one, six and twelve months of life, and an HIV ELISA was completed at 18 months. Eight infants in the parent study tested positive for HIV, with all positive tests occurring within the first 6 months of life. These infants were excluded from the current analysis.

The primary analysis assessed the association between maternal vitamin D levels at delivery and HEU infant morbidity/mortality employing a retrospective nested case-control study design of women with HEU infants in the Mma Bana study. HEU Mma Bana study infants experiencing hospitalization, where hospitalizations were used as a surrogate for morbidity, or death by 24-months-of-life were defined as potential cases. Infants who had no reported hospitalizations while on study and were reported to be alive at last study contact were eligible to be selected as controls. Controls were randomly selected from the available possible controls to provide a 2:1 ratio for controls to cases from all remaining Mma Bana women with a live delivery. A sub-analysis was performed to ascertain whether maternal vitamin D insufficiency was specifically associated with infant respiratory or diarrheal disease resulting in hospitalization or death, as diagnosed by the health care providers in the public sector who treated or interfaced with the infant at the time of their hospitalization/death.

A secondary analysis to assess for any association between maternal vitamin D insufficiency at delivery and infant growth faltering among a subset of control infants at 24-months-of-life was performed. Only those women whose infants did not require hospitalization throughout the study period, attended the 24-month-of-life study visit, and had documented growth measurements at this visit were included in this secondary analysis. Growth faltering was defined as having a weight-for-age, length-for-age or weight-for-length z-score at the 24-month visit more than 2 standard deviations (SD) below the median of the reference population using World Health Organization (WHO) Child Growth Standards.²³

The Botswana Health Research Development Committee and the Harvard School of Public Health Human Subjects Committee approved the collection of blood and its utilization for research purposes in the Mma Bana study. Women who enrolled in the Mma Bana study provided written informed consent to participate in the Mma Bana study and human subjects research guidelines of Harvard School of Public Health and Botswana's Health Research Development Committee were followed in the conduct of this clinical research.

Sample Collection and Preparation

Samples of maternal peripheral blood were obtained from all HIV-infected women at delivery. The samples were processed and plasma was stored at -70°C. Samples selected for this sub-study were shipped frozen and kept below -60°C until time of the vitamin D assay.

Measurement of Maternal Vitamin D Levels

Plasma levels of 25-hydroxyvitamin D [25-OH vitamin D] were measured using a chemiluminescent microparticle immunoassay (Architect, Abbott Diagnostics, Abbott Park, IL). Assays were performed by laboratory personnel blinded to the study design and outcomes. The limit of detection is 4 ng/ mL [lowest concentration distinguishable from zero] and the limit of quantitation is 8.0 ng/mL [lowest concentration at which the observed

imprecision (CV) was less than 10%]. The Architect 25-OH vitamin D assay has an imprecision of 10% total CV. Using an absolute deviation from linearity of 10% the linear range is 13.1 ng/mL to 96.2 ng/mL. This assay has a 100%, and 82% cross-reactivity with 25 OH vitamins D3 and D2, respectively. Cross reactivity with 3-epi 25-OH vitamin D3 at a concentration of 100 ng/mL was less than 3%.

Statistical Analyses

Maternal characteristics involving continuous and ordinal variables were compared using Wilcoxon's Rank Sum test. Categorical variables were compared using Fisher's exact or Chi Square tests. Seasonality of maternal vitamin D insufficiency was assessed using a Fisher's exact test. Wilcoxon's Rank Sum test was used to compare median maternal vitamin D levels at enrollment between mothers of cases and controls in the morbidity/mortality analysis.

Infant Morbidity/Mortality—The proportion of women with vitamin D insufficiency, defined as a vitamin D level < 32 ng/mL, was determined and a Fisher's exact test was used to test for differences between cases and controls. A multivariate logistic regression model was used to estimate the contribution of maternal vitamin D insufficiency to infant morbidity/mortality, adjusting for factors found to have a p-value of 0.10 in univariate analyses. The multivariate model was also adjusted for potential confounders including CD4+ cell count (< 350 versus > 350 cells/mm³), pretreatment HIV RNA level (HIV-1 RNA 100,000 vs <100,000 copies/mL), and season of birth. Since breast milk is a poor source of vitamin D and the Mma Bana protocol called for 6 months of exclusive breastfeeding, a sub-analysis was performed to ascertain whether maternal vitamin D insufficiency was more prevalent among women whose infants experienced early morbidity or mortality, where “early” was defined as mortality/mortality events occurring within first six months of life. Sensitivity analysis was also performed to assess association between maternal vitamin D deficiency for levels < 20 ng/ml per the Endocrine Society Clinical Practice Guidelines and infant morbidity/mortality.²⁴

Infant Growth Faltering—A multivariate logistic regression model estimated the association between maternal vitamin D insufficiency and infant growth faltering at 24-months-of-life, and included factors found to have a p-value of 0.10 in univariate analyses. The multivariate model was also adjusted for potential confounders, including maternal CD4+ cell count (< 350 cells/mm³ versus > 350 cells/mm³), pretreatment HIV RNA level (HIV-1 RNA 100,000 vs <100,000 copies/mL), maternal body mass index (BMI) 1-month postpartum, preterm delivery, defined as delivery prior to 37.0 weeks gestational age, and presence or absence of growth faltering at birth, defined as a sex adjusted weight-for-age, length-for-age or weight-for-length z-score more than 2 standard deviations below the median value of the reference population using WHO Child Growth Standards.²³ While WHO Child Growth standards were developed for use with infants born at term, birth anthropometric z-scores were calculated for all children included in this secondary analysis, regardless of gestational age at birth, in order to derive the covariate of growth faltering at birth. Sensitivity analyses included women with hospitalized infants in the multivariate model.

All testing used a two-sided significance level of 0.05 with no correction for multiple testing. Statistical analyses were performed using SAS, version 9.3 (SAS Institute, Cary, North Carolina, USA).

Results

Maternal plasma specimens were available from 29 (97%) of 30 women who experienced the death of their child and 110 (96%) of 114 women who had a hospitalized child at least once during the first 24-months-of-life, yielding a total of 139 cases with available specimens. Maternal plasma specimens were available from 538 (98%) of 547 women who were identified as controls. Two hundred seventy eight maternal specimens were randomly selected from the available control specimens to match cases at a 2:1 ratio. Of these, maternal vitamin D levels at delivery were assayable from 119 (86%) of the 139 cases and from 233 (84%) of the 278 selected controls.

Overall maternal vitamin D results

The median vitamin D level at delivery among all 352 HIV-infected women included in this analysis was 37.1 ng/mL (interquartile range (IQR) 29.7 ng/mL, 46.0 ng/mL); 31.8% (112) of these women had vitamin D insufficiency (vitamin D level < 32 ng/mL). Of the 112 women with vitamin D levels below 32 ng/ml, 21 (18.8%) had a level < 20 ng/ml. Maternal vitamin D insufficiency at delivery varied significantly by season irrespective of infant outcome (Table 1). Deliveries occurring in the winter season were associated with the highest proportion of maternal vitamin D insufficiency, and those in the summer season, with the lowest proportion of vitamin D insufficiency. There was no significant association between overall infant hospitalizations or death and season of birth ($p = 0.19$).

Baseline characteristics among cases and controls for morbidity/mortality analysis

Overall, there were no significant differences in age, parity, enrollment CD4+ cell count, or pretreatment viral load between women who had a child experiencing morbidity/mortality (cases) in the first 24-months and those who gave birth to a child who did not (controls) (see Table. SDC 1). The median time on ART prior to delivery among women who gave birth to a child experiencing morbidity/mortality was 10.7 weeks compared with 11.6 weeks for women who gave birth to a child in the control group ($p=0.06$). A significantly higher proportion of children experiencing morbidity/mortality were male compared with children in the control group (58.8% versus 46.8%; $p= 0.04$) and had a birth weight below 2500 grams (23.5% versus 12.4%; $p=0.008$).

Maternal vitamin D levels at delivery for morbidity/mortality analysis

Maternal median vitamin D level at delivery and proportions of maternal vitamin D insufficiency did not differ significantly between cases and controls. The median vitamin D level at delivery among cases was 36.7 ng/mL (IQR 29.1 ng/mL, 44.7 ng/mL) compared with 37.1 ng/mL (IQR 30.0 ng/mL, 47.2 ng/mL) among controls ($p=0.32$). The proportion of vitamin D insufficiency at delivery among cases was 32.8%, compared with 31.3% among controls ($p=0.80$).

In univariate logistic regression, maternal vitamin D insufficiency at delivery was not associated with a significant risk of child morbidity (hospitalization) or mortality (OR 1.07; 95% CI 0.67 – 1.71; p=0.78). The same lack of effect was found in multivariate logistic regression analysis after adjustment for maternal CD4+ cell count (< 350 cells/mm³ versus > 350 cells/mm³), pretreatment HIV-1 RNA level (HIV-1 RNA ≥ 100,000 vs <100,000 copies/mL), self-reported household income, infant sex, infant birth weight (< 2500 grams versus ≥ 2500 grams), and season of birth (aOR 1.17; 95% CI 0.70, 1.98; p=0.55) (Table 2). There was no significant difference in maternal median vitamin D levels between cases/controls when analyses were restricted to infant morbidity/mortality events that occurred in the first 6 months of life (p=0.14). We also performed sensitivity analyses evaluating associations between vitamin D deficiency²⁴ (defined as < 20 ng/ml) and morbidity/mortality, and we found no statistically significant associations (data not shown).

Of the 119 children who were hospitalized or died, 47 children were diagnosed with diarrheal disease and 30 were diagnosed with a respiratory illness. Maternal vitamin D insufficiency was not associated with diarrheal disease, with 38.3% (18) of mothers with children hospitalized or who died from diarrheal disease experiencing vitamin D insufficiency compared with 31.3% (73) of women whose children did not have a diarrheal illness serious enough to require hospitalization or result in death (p=0.39). Prevalence of vitamin D insufficiency was lower among women of children who required hospitalization or experience death due to a respiratory illness (13.3% (4) versus 31.3% (73); p=0.05).

Maternal vitamin D insufficiency at delivery and child growth faltering at 24-months-of-life

A total of 184 (80%) children, out of 233 who had never been hospitalized, attended the 24-months-of-life study visit and had documentation of weight and length measurements. Thirty seven (20%) of the 184 children had growth faltering at 24-months-of-life. Comparing maternal and child characteristics between children with growth faltering at 24-months-of-life with children with normal growth, there were no significant differences between the two groups, including no significant differences in maternal median age, CD4+ cell count and HIV RNA level at enrollment, education level, marital status, length of infant *in utero* exposure to maternal ART's or infant gender (data not shown). In multivariate logistic regression analysis, maternal vitamin D adequacy at delivery was not significantly associated with a growth at 24-months-of-life (aOR 0.99; 95% CI 0.40 – 2.47; p =0.99), after adjusting for maternal reported income, maternal enrollment CD4+ cell count and HIV RNA level, maternal BMI 1-month postpartum, presence of infant growth faltering at birth, and delivery prior to 37.0 weeks gestational age (Table 3). High maternal income and absence of growth faltering at birth were associated with reduced odds of growth faltering at 24-months-of-life. A sensitivity analysis that included mothers with hospitalized infants evaluating the combined growth faltering endpoint at 24-months-of-life also found no associations between maternal vitamin D levels and growth outcomes (data not shown).

Discussion

This is the first study to evaluate the relationship between maternal vitamin D levels at delivery and hospitalizations, mortality and growth among HIV-exposed uninfected

breastfed infants in the setting of maternal ART in pregnancy. We noted a high prevalence of maternal vitamin D insufficiency at delivery of nearly 32%, and an expected seasonal variation. The prevalence of maternal vitamin D insufficiency in our study was comparable to the prevalence of 39% reported among HIV-infected pregnant women not yet on ART participating in Trial of Vitamins study in the Tanzania, where the same definition of vitamin D insufficiency was utilized.¹⁸ We found no association between maternal vitamin D insufficiency at delivery and infant morbidity, mortality, or growth outcomes at 24-months-of-life.

In the Tanzania Trial of Vitamins study, Mehta and colleagues found an association between maternal vitamin D insufficiency in pregnancy and HEU infant mortality through 24-months-of-life, reporting a mortality incident rate ratio of 1.66 (95% CI 0.95 – 1.94) for HIV-exposed uninfected born to mothers with vitamin D insufficiency.¹⁵ While our study demonstrated a negative finding of the association between maternal vitamin D insufficiency at delivery and the combined endpoint of HEU infant morbidity (hospitalizations) and mortality, the 95% confidence interval of the odds ratio for this association in our study ranged from 0.70 to 1.98, including the value found in the Tanzanian study. Therefore, the negative finding in our study may merely represent inadequate power to detect a statistically significant association, or it may represent the fact that maternal vitamin D insufficiency in pregnancy or at delivery is only associated with subsequent mortality of HEU infants, but not morbidity. Amukele and colleagues failed to find a significant association between either maternal or infant vitamin D levels and subsequent child morbidity through 24-months-of-life in a small cohort of HEU infants in Malawi.²⁵ However, their study in Malawi defined morbidity more broadly than our study, and included the presence of malnutrition by anthropometric measures or clinical documentation of illness.²⁵ Assuming that the illness in the Malawi study included outpatient visits and was not restricted to more severe illnesses warranting hospitalization, the lack of association between both maternal and infant vitamin D levels and morbidity in the Malawi study may only reflect the fact vitamin D may not influence or be associated with the occurrence of common childhood illnesses. In our study, we opted to evaluate the combined end point of morbidity and mortality, as there was not adequate power to study associations solely with the endpoint of mortality, as we only had 30 deaths among 709 live born infants.

A notable difference between the Tanzania study and our study was the use of ART during pregnancy. Unfortunately, we are unable to quantify the contribution of maternal ART during pregnancy on the health and survival of their HEU infants. It is possible that the use of maternal ART in pregnancy in Botswana, and subsequent maternal immune system reconstitution and/or improved maternal health, accounted for the different study outcomes. Other potential contributors to differences between our study and that in Tanzania include better availability of health care services and infrastructure and differences in environments. It is also possible that the frequently scheduled study visits in the Mma Bana study mitigated the impact of maternal vitamin D insufficiency; during Mma Bana study visits, infants were examined for signs/symptoms of illness and treated or referred to government health facilities, and infant growth and feeding practices were assessed with the provision of caregiver counseling.

Respiratory and diarrheal diseases are the predominant causes of death among HEU infants in resource-constrained settings in the first two years of life.^{26, 27} Some studies of HIV-unexposed infants have found an association between infant vitamin D levels and respiratory disease.^{28, 29} In a sensitivity analysis of our cohort, maternal vitamin D insufficiency was found to be significantly more prevalent among mothers of children who did not have a documented respiratory illness severe enough to warrant hospitalization or result in death. There was no significant difference in the prevalence of maternal vitamin D insufficiency comparing mothers of children who experienced diarrheal disease severe enough to require hospitalization or result in death and mothers of children without this level of severe diarrheal disease. In our cohort of mother-infant pairs, we did not have infant specimens available to assay vitamin D levels of our HEU children. Therefore, we cannot preclude that actual infant hypovitaminosis D may be associated with the two most frequent causes of death among HEU infants in resource-constrained settings.

We did not detect an association between maternal vitamin D insufficiency at delivery and growth faltering of HEU children at 24-months-of-life, either in the analysis of healthy infants alive at 24-months, or in the sensitivity analysis that also included an oversampling of ill (hospitalized) infants from the morbidity/mortality case-control study. In these analyses, the 24-month prevalence of underweight or stunted children was below Botswana's national prevalence for children under the age of 5 (11.9% and 31.2%, respectively in 2007) (Nnyepi et al. Child Nutrition Situation in Botswana: Observations from the 2000 and 2007 household surveys. Draft report commissioned by UNICEF. November 2011). This may reflect the higher level of care that infants in our cohort received during study visits, other biases inherent in the conduct of a clinical trial, or the earlier timing of our data at 24-months versus 5-years-of-life.

The primary aim of this study was to assess for any association between low maternal vitamin D levels at delivery in a cohort of HIV-1 infected women initiating ART in pregnancy and adverse outcomes among their HEU infants. While maternal vitamin D was not associated with increased infant morbidity/mortality or growth faltering, our logistic regression models did find low maternal income and low anthropometric measures at birth to be associated with increased infant morbidity/mortality, as well as with growth faltering at 24-months-of-life. Other studies conducted in resource limited settings have demonstrated low maternal income and infant underweight or stunting at birth to be significantly associated with growth faltering for non-HIV exposed children under the age of 5.³⁰⁻³² Additionally, the presence of either stunting and underweight has been shown to increase the risk of mortality from infectious disease in childhood³³⁻³⁵, while longitudinal studies have found stunting in infancy and early childhood to be associated with poorer cognitive and educational outcomes through the adolescent period^{36, 37}. Although our findings are not novel, they highlight the need for targeted health care and social programs for HEU infants at highest risk for poor health outcomes and their mothers.

In conclusion, in our cohort, maternal vitamin D insufficiency was highly prevalent among HIV-infected women at delivery, but was not associated with increased morbidity, mortality or growth impairment in HIV-exposed uninfected infants. Maternal ART or other factors in our study may have improved overall infant health and survival, mitigating the effect of

maternal vitamin D insufficiency. As global advances are made toward the WHO goal of virtually eliminating mother-to-child HIV transmission, an ever larger proportion of children born in settings with generalized HIV-epidemics will be born HIV-exposed uninfected. Further research is urgently needed to identify maternal and infant modifiable risk factors associated with the higher morbidity and mortality reported among HIV-exposed uninfected infants.

Acknowledgments

We are indebted to the women and infants who participated in the Mma Bana study, the Mma Bana study team staff, as well as the administration and staff at Scottish Livingstone, Deborah Retief Memorial, Athlone and Princess Marina Hospitals and the staff at the referring health clinics. We are grateful to Abbott Pharmaceuticals, GlaxoSmithKline, and the government of Botswana for the provision of study drugs.

Conflicts of Interests and Sources of Funding: MH has served as a paid Data Safety and Monitoring Board member for Boehringer Ingelheim, Pfizer, Tibotec and Medicines Development. LS served as a paid Data Monitoring Committee member for Pfizer.

Mma Bana study was supported by a grant (U01-AI066454) from the National Institute of Allergy and Infectious Diseases. Funding support from Brigham and Women's Global Women's Health Fellowship supported KP's salary during the Mma Bana study. KP received salary support from a Harvard University Center for AIDS Research grant (P30 AI060354) and a National Institute of Child Health and Human Development Grant (1K23HD070774-01A1) for this project. The Fogarty AITRP grant (D43 TW000004) provided funding for AO and SM. Mma Bana study drugs were provided by Abbott Pharmaceuticals, GlaxoSmithKline, and the government of Botswana.

References

1. Shapiro RL, Hughes M, Ogwu A, Kitch D, et al. Antiretroviral Regimens in Pregnancy and Breast-Feeding in Botswana. *N Engl J Med*. 2010; 362:2282–2294. [PubMed: 20554983]
2. Marazzi MC, Nielsen-Saines K, Buonomo E, et al. Increased Infant Human Immunodeficiency Virus-Type One Free Survival at One Year of Age in Sub-Saharan Africa with Maternal Use of Highly Active Antiretroviral Therapy During Breast-Feeding. *Pediatr Infect Dis J*. 2009; 28:483–487. [PubMed: 19483516]
3. De Vincenzi, I. Kesho Bora Study Group. Triple-antiretroviral (ARV) prophylaxis during pregnancy and breastfeeding compared to short-ARV prophylaxis to prevent mother-to-child transmission of HIV-1 (MTCT): The Kesho Bora randomized controlled clinical trial in five sites in Burkina Faso, Kenya and South Africa. Abstract # LBPECO1 In 5th IAS Conference on HIV Pathogenesis and Treatment; Cape Town, South Africa. 2009.
4. Chasela CS, Hudgens MG, Jamieson DJ, et al. Maternal or Infant Antiretroviral Drugs to Reduce HIV-1 Transmission. *N Engl J Med*. 2010; 362:2271–2281. [PubMed: 20554982]
5. Brahmabhatt H, Kigozi G, Wabwire-Mangen F, et al. Mortality in HIV-infected and uninfected children of HIV-infected and uninfected mothers in rural Uganda. *Journal of Acquired Immune Deficiency Syndromes*. 2006; 41:504–508. [PubMed: 16652060]
6. Marinda E, Humphrey JH, Iliff PJ, et al. Child mortality according to maternal and infant HIV status in Zimbabwe. *Pediatric Infectious Disease Journal*. 2007; 26:519–526. [PubMed: 17529870]
7. Shapiro RL, Lockman S, Kim S, et al. Infant morbidity, mortality, and breast milk immunologic profiles among breastfeeding HIV-infected and HIV-uninfected women in Botswana. *Journal of Infectious Diseases*. 2007; 196:562–569. [PubMed: 17624842]
8. Muhangi L, Lule SA, Mpairwe H, et al. Maternal HIV infection and other factors associated with growth outcomes in HIV-uninfected infants in Entebbe, Uganda. *Public Health Nutrition*. 2013; 16(9):1548–1557. [PubMed: 23507372]
9. Moraleda C, de Deus N, Serna-Bolea C, et al. Impact of HIV-exposure on health outcomes in HIV-negative infants born to HIV-positive mothers in Sub-Saharan Africa. *JAIDS*. 2013 In Press. 10.1097.

10. Cantorna MT, Zhu Y, Froicu M, Wittke A. Vitamin D status, 1,25-dihydroxyvitamin D₃, and the immune system. *Am J Clin Nutr.* 2004; 80(supl):1717S–20S. [PubMed: 15585793]
11. Boonstra A, Barrat FJ, Crain C, et al. 1 α ,25-Dihydroxyvitamin D Has a Direct Effect on Naïve CD4⁺ T Cells to Enhance the Development of Th2 Cells. *J Immunol.* 2001; 167:4974–4980. [PubMed: 11673504]
12. Bar-Shavit Z, Noff D, Edelstein S, et al. 1,25-dihydroxyvitamin D₃ and the regulation of macrophage function. *Calcif Tissue Int.* 1981; 33:673–676. [PubMed: 6275970]
13. Miriani E, Ravaglia G, Forti P, et al. Vitamin D, thyroid hormones and muscle mass influence natural killer (NK) innate immunity in healthy nonagenarians and centenarians. *Clin Exp Immunol.* 1999; 116:19–27. [PubMed: 10209500]
14. Liu PT, Stenger S, Li H, et al. Toll-like receptor trigger of a vitamin D-mediated human antimicrobial response. *Science.* 2006; 311:1770–1773. [PubMed: 16497887]
15. Mehta S, Hunter DJ, Mugusi FM, et al. Perinatal Outcomes Including Mother-to-Child Transmission of HIV, and Child Mortality and Their Association with Maternal Vitamin D Status in Tanzania. *JID.* 2009; 200:1022–1030. [PubMed: 19673647]
16. Bodnar LM, Simhan HN, Powers RW, et al. High Prevalence of Vitamin D Insufficiency in Black and White Pregnant Women Residing in the Northern United States and Their Neonates. *Journal of Nutrition.* 2007; 137(2):447–452. [PubMed: 17237325]
17. Holick MF. Vitamin D Deficiency. *New England Journal of Medicine.* 2007; 357:266–281. [PubMed: 17634462]
18. Finkelstein JL, Mehta S, Duggan C, et al. Maternal Vitamin D Status and Child Morbidity, Anemia, and Growth in Human Immunodeficiency Virus-exposed Children in Tanzania. *Pediatr Infect Dis J.* 2012; 31:171–175. [PubMed: 22252204]
19. Allavena C, Delpierre C, Cuzin L, et al. High frequency of vitamin D deficiency in HIV-infected patients: effects of HIV-related factors and antiretroviral drugs. *J Antimicrob Chemother.* 2012; 67:2222–2230. [PubMed: 22589455]
20. Cervero M, Agud JL, Gracia-Lacalle C, et al. Prevalence of Vitamin D Deficiency and Its Related Risk Factors in a Spanish Cohort of Adult HIV-Infected Patients: Effects of Antiretroviral Therapy. *AIDS Research and Human Retroviruses.* 2012; 28(9):963–971. [PubMed: 22242918]
21. World Health Organization. [accessed 10-Mar-14] Consolidated guidelines on general HIV care and the use of antiretroviral drugs for treating and preventing HIV infection: recommendations for a public health approach. <http://www.who.int/hiv/pub/guidelines/arv2013/download/en/>
22. Salle BL, Delvin EE, Lapillonne A, et al. Perinatal metabolism of vitamin D. *American Journal of Clinical Nutrition.* 2000; 71(Suppl):S1317–1324.
23. World Health Organization. [Accessed December 18, 2009] Child Growth Standards. Available at: http://www.who.int/childgrowth/1_what.pdf
24. Holick MF, Binkley NC, Bischoff-Ferrari HA, et al. Evaluation, Treatment, and Prevention of Vitamin D Deficiency: an Endocrine Society Clinical Practice Guideline. *J Clin Endocrinol Metab.* 2011; 96(7):1911–1930. [PubMed: 21646368]
25. Amukele TK, Soko D, Katundu P, et al. Vitamin D levels in Malawian infant from birth to 24 months. *Arch Dis Child.* 2013; 98:180–183. [PubMed: 23220204]
26. Lockman, S.; Smeaton, L.; Shapiro, R., et al. Morbidity and mortality among infants born to HIV-infected mothers and randomized to breastfeeding versus formula-feeding in Botswana (Mashi Study); XVI International AIDS Conference; Toronto Canada. 13-18 August 2006; Abstract # TUPE0357
27. McNally LM, Jeena PM, Gajee K, et al. Effect of age, polymicrobial disease, and maternal HIV status on treatment response and cause of severe pneumonia in South African children: A prospective descriptive study. *Lancet.* 2007; 369:1440–1451. [PubMed: 17467514]
28. Maxwell CS, Carbone ET, Wood RJ. Better newborn vitamin D status lowers RSV-associated bronchiolitis in infants. *Nutrition Reviews.* 2012; 70(9):548–552.
29. Mohamed WA, Al-Shehri MA. Cord Blood 25-Hydroxyvitamin D Levels and Risk of Acute Lower Respiratory Tract Infection in Early Childhood. *J Trop Pediatr.* 2013; 59(1):29–35. [PubMed: 23022743]

30. Black RE, Victora CG, Walker SP, et al. Materna and Child Nutrition 1: Maternal and child undernutrition and overweight in low-income and middle income countries. *Lancet*. 2013; 382:427–51. [PubMed: 23746772]
31. Gordon NH, Halileh S. An Analysis of Cross Sectional Survey Data of Stunting Among Palestinian Children Less Than Five Years of Age. *Matern Child Health J*. 2013; 17:1288–1296. [PubMed: 22948964]
32. Ikeda N, Irie Y, Shibuya K. Determinants of reduced stunting in Cambodia: analysis of pooled data from Demographic Health Surveys. *Bull World Health Organ*. 2013; 91:341–349. [PubMed: 23678197]
33. Pelletier DL, Frongillo EA Jr, Schroeder DG, et al. The effects of malnutrition on child mortality in developing countries. *Bull World Health Organ*. 1995; 73:443–48. [PubMed: 7554015]
34. Caulfield LE, de Onis M, Blossner M, et al. Undernutrition is an underlying cause of child deaths associated with diarrhea, pneumonia, malaria and measles. *Am J Clin Nutr*. 2004; 80:193–98. [PubMed: 15213048]
35. Olofin I, McDonald CM, Ezzati M, et al. Associations of suboptimal growth with all-cause and cause-specific mortality in children under five years: pooled analysis of ten prospective studies. *PLoS One*. 8(5):e64636.10.1371/journal.pone.0064636 [PubMed: 23734210]
36. Walker SP, Wachs TD, Grantham-McGregor S, et al. Inequality in early childhood: risk and protective factors for early child development. *Lancet*. 2011; 378:1325–38. [PubMed: 21944375]
37. Grantham-Mcgregor S, Cheung YB, Cueto S, et al. Developmental potential in the first 5 years for children in developing countries. *Lancet*. 2007; 369:60–70. [PubMed: 17208643]

Table 1
Proportion of Low Maternal Vitamin D Levels by Season

	Maternal Vitamin D Level < 32 ng/mL	Maternal Vitamin D Level ≥ 32 ng/mL	p-value
Season			0.004¹
Jan-Feb-Mar (Summer)	16 (18.4%)	71 (81.6%)	
Apr-May-Jun (Fall)	34 (34.0%)	66 (66.0%)	
Jul-Aug- Sep (Winter)	34 (44.2%)	43 (55.8%)	
Oct-Nov-Dec (Spring)	28 (31.8%)	60 (68.2%)	

¹Fisher's exact test

Table 2
Multivariate Logistic Regression to Analyze Risk Factors Associated with Infant Morbidity/Mortality

Analyzed Risk Factors	Infants with Morbidity/Mortality (n= 119)	Healthy Infants (n = 233)	Odds Ratio (CI)	p-value
Maternal Income				0.09
None	57 (31.5%)	124 (68.5%)	Ref	
1 – 500 pula/year	39 (43.8%)	50 (56.2%)	1.51 (0.87 – 2.63)	0.14
501– 1000 pula/year	16 (35.6%)	29 (64.4%)	1.21 (0.58 – 2.49)	0.61
>1000 pula/year	7 (18.9%)	30 (81.1%)	0.46 (0.19 – 1.16)	0.10
Maternal Enrollment CD4+ Cell Count				0.19
350 cells/mm ³	56 (31.1%)	124 (68.9%)	0.72 (0.45 – 1.17)	
> 350 cells/mm ³	63 (36.6%)	109 (63.4%)	Ref	
Maternal Enrollment HIV RNA Level				0.09
< 100,000 cells/mL	88 (31.7%)	190 (68.3%)	Ref	
100,000 cells/mL	30 (41.1%)	43 (58.9%)	1.64 (0.93 – 2.91)	
Maternal Vitamin D Insufficiency				0.55
Yes	39 (34.8%)	73 (65.2%)	1.17 (0.70 – 1.98)	
No	80 (33.3%)	160 (66.7%)	Ref	
Quarter of Birth				0.34
Winter	29 (37.7%)	48 (62.3%)	Ref	
Spring	35 (39.8%)	53 (60.2%)	0.69 (0.35 – 1.35)	0.27
Summer	29 (33.3%)	58 (66.7%)	0.90 (0.45 – 1.78)	0.75
Fall	26 (26.0%)	74 (74.0%)	1.24 (0.64 – 2.42)	0.51
Infant Gender				0.09
Male	70 (39.1%)	109 (60.9%)	Ref	
Female	49 (28.3%)	124 (71.7%)	0.66 (0.41 – 1.06)	
Birth Weight Category				0.01*
Low birth weight (< 2500 grams)	28 (49.1%)	29 (50.9%)	2.14 (1.17 – 3.89)	
2500 grams	91 (30.9%)	204 (69.1%)	Ref	

* Statistically significant p-value

LEGEND: CI =Confidence Interval

Table 3
Multivariate Logistic Regression to Analyze Risk Factors Associated with Growth Faltering¹ at 24 months-of-life among Non-Hospitalized Children

Analyzed Risk Factors	Children with Growth Faltering ¹ at 24-months of life (n= 37)	Children without Growth Faltering at 24 months-of-life (n=147)	Odds Ratio (CI)	p-value
Maternal Income				0.01
None	29 (27.4%)	77 (72.6%)	Ref	
1 – 500 pula/year	5 (12.8%)	34 (87.2%)	0.27 (0.09 – 0.83)	
> 500 pula/year	2 (7.7%)	36 (92.3%)	0.20 (0.05 – 0.77)	
Maternal Enrollment CD4+ Cell Count				0.46
350 cells/mm ³	21 (21.4%)	77 (78.6%)	1.36 (0.60 – 3.12)	
> 350 cells/mm ³	16 (18.6%)	70 (81.4%)	Ref	
Maternal Enrollment HIV RNA Level				0.70
< 100,000 cells/mL	30 (19.6%)	100 (80.4%)	Ref	
100,000 cells/mL	7 (22.6%)	47(77.4%)	0.81 (0.28 – 2.35)	
Maternal Vitamin D Insufficiency		47 (83.9%)		0.99
Yes	9 (16.1%)	100 (78.1%)	0.99 (0.40 – 2.47)	
No	28 (21.9%)		Ref	
Maternal BMI 1-month Postpartum				0.85
< 20 kg/m ²	7 (28.0%)	18 (72.0%)	Ref	
20 kg/m ² - < 25 kg/m ²	18 (21.4%)	66 (78.6%)	1.10 (0.36 – 3.35)	
25 kg/m ²	10 (17.5%)	47 (82.5%)	0.85 (0.26 – 2.79)	
Birth Growth Faltering¹				0.01
Yes	19 (27.5%)	50 (72.5%)	Ref	
No	18 (15.8%)	96 (84.2%)	0.34 (0.15 – 0.80)	
Preterm Birth²				0.25
Yes	5 (16.7%)	25 (83.3%)	0.50 (0.16 – 1.61)	
No	32 (20.8%)	122 (79.2%)	Ref	

¹Using 2006 World Health Organization's (WHO) Child Growth Standards, growth faltering represents a weight-for-age (underweight), length-for-age (stunted) or weight-for-length (wasted) z-score more than 2 standard deviations below the median value of the reference population

²Preterm birth defined as birth before 37.0 weeks gestational age

LEGEND: CI = Confidence Interval