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Citation

Mehta, Saurabh, David J. Hunter, Ferdinand M. Mugusi, Donna Spiegelman, Karim P. Manji, Edward L. Giovannucci, Ellen Hertzmark, Gernard I. Msamanga, and Wafaie W. Fawzi. 2009. "Perinatal Outcomes, Including Mother#to#Child Transmission of HIV, and Child Mortality and Their Association with Maternal Vitamin D Status in Tanzania." *J INFECT DIS* 200 (7) (October): 1022–1030. doi:10.1086/605699.

Published Version

doi:10.1086/605699

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Published in final edited form as:

J Infect Dis. 2009 October 1; 200(7): 1022–1030. doi:10.1086/605699.

Perinatal Outcomes, Including Mother-to-Child Transmission of HIV, and Child Mortality and Their Association with Maternal Vitamin D Status in Tanzania

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Abstract

Background—Vitamin D is a strong immunomodulator and may protect against adverse pregnancy outcomes, mother-to-child transmission (MTCT) of human immunodeficiency virus (HIV), and child mortality.

Methods—A total of 884 HIV-infected pregnant women who were participating in a vitamin supplementation trial in Tanzania were monitored to assess pregnancy outcomes and child mortality. The association of these outcomes with maternal vitamin D status at enrollment was examined in an observational analysis.

Results—No association was observed between maternal vitamin D status and adverse pregnancy outcomes, including low birth weight and preterm birth. In multivariate models, a low maternal vitamin D level (<32 ng/mL) was associated with a 50% higher risk (95% confidence interval [CI], 2%–120%) of MTCT of HIV at 6 weeks, a 2-fold higher risk of MTCT of HIV through breast-feeding among children who were HIV uninfected at 6 weeks (95% CI, 1.08–3.82), and a 46% higher overall risk of HIV infection (95% CI, 11%–91%). Children born to women with a low vitamin D level had a 61% higher risk of dying during follow-up (95% CI, 25%–107%).

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Potential conflicts of interest: none reported.

Conclusions—If found to be efficacious in randomized trials, vitamin D supplementation could prove to be an inexpensive method of reducing the burden of HIV infection and death among children, particularly in resource-limited settings.

Since the first demonstration of its antirachitic properties by Elmer McCollum in 1922 [1], vitamin D has been shown to have a potential role in several health outcomes, including colorectal cancer [2] and infectious diseases, such as tuberculosis [3,4]. Vitamin D is a known immunomodulator [5]; it can improve cell-mediated immunity [6] and the phagocytic capacity of macrophages [7], and it can also increase the number and the cytolytic activity of natural killer cells [8]. Recent research has also highlighted the importance of vitamin D in the innate immune response, via the Toll-like receptor pathway [4]. Toll-like receptor stimulation of human macrophages up-regulates expression of the vitamin D receptor and induces the enzyme CYP27b1, which catalyzes the conversion of 25-hydroxyvitamin D₃ [25(OH)D] to 1,25-dihydroxyvitamin D₃ [1,25(OH)D], the biologically active vitamin D metabolite. In the presence of adequate 25(OH)D, activation of the up-regulated vitamin D receptors leads to induction of cathelicidin, an antimicrobial peptide capable of killing such pathogens as *Mycobacterium tuberculosis* intracellularly [4]. Cathelicidin also has several other important biological effects [9], such as neutralization of the effects of lipopolysaccharide [10], stimulation of angiogenesis [11], and chemotaxis of neutrophils, monocytes, and T cells [12].

Vitamin D may have a role in preventing adverse pregnancy outcomes, mother-to-child transmission (MTCT) of human immunodeficiency virus (HIV), and child mortality. The conventional role of vitamin D in calcium metabolism is important for fetal skeletal development [13]; approximately 25–30 g of calcium are transferred to the fetus, most of it during the last trimester [13]. The importance of vitamin D in this process may be indicated by the 50%–100% increase in 1,25(OH)D concentrations in the second and third trimesters [14]. In the last trimester, this increase is not accompanied by a similar increase in vitamin D-binding protein, leading to higher levels of free circulating 1,25(OH)D [15]. Recent research has suggested that vitamin D may also help regulate placental development and function [16], further contributing to fetal growth.

Vitamin D may also affect development of the fetal immune system [17], by virtue of its immunomodulatory properties. The potential ability to fight diseases such as tuberculosis, a leading cause of death in HIV-infected patients, could lead to decreased mortality [18].

A role for vitamin D in HIV infection has been postulated on the basis of a few laboratory and human studies [5]; however, there have been no published studies that have directly examined the association of vitamin D with pregnancy outcomes, MTCT of HIV, or death among children born to HIV-infected women. In the Trial of Vitamins [19,20], which was conducted in Tanzania, HIV-infected pregnant women received supplementation with a multivitamin regimen that did not include vitamin D and were monitored to observe pregnancy outcomes and disease progression, providing an opportunity to expand our knowledge about vitamin D status and health. This knowledge is of particular relevance to HIV infection and developing countries, because high rates of subclinical vitamin D deficiency have been identified in these countries [21].

METHODS

Study population

The study design has been described in detail elsewhere [22]. In brief, 1078 HIV-infected pregnant women of 12–27 weeks' gestation (hereafter referred to as "baseline") were randomized to receive daily doses of 1 of the following 4 regimens from enrollment until at least 18 months postpartum: (1) vitamin A alone (30 mg of β -carotene and 5000 IU of

performed vitamin A); (2) multivitamins, including vitamin A (30 mg of β -carotene and 5000 IU of preformed vitamin A, 20 mg of vitamin B₁, 20 mg of vitamin B₂, 25 mg of vitamin B₆, 100 mg of niacin, 50 μ g of vitamin B₁₂, 500 mg of vitamin C, 30 mg of vitamin E, and 0.8 mg of folic acid); (3) multivitamins, excluding vitamin A; or (4) placebo. In addition, all women received iron and folate tablets daily and chloroquine as malaria prophylaxis weekly, in accordance with national guidelines for antenatal care in Tanzania. The primary objectives of this trial were to examine the effect of multivitamin supplementation on pregnancy outcomes, maternal and child mortality, and MTCT of HIV among HIV-infected pregnant women.

Informed consent was obtained from all the participants, and the institutional review boards at the Muhimbili University College of Health Sciences, the National AIDS Control Program of the Tanzanian Ministry of Health, and The Harvard School of Public Health approved the study protocol.

Assessment of covariates at baseline

Structured interviews were conducted during the baseline visit, to collect information on demographic characteristics, including age and education and clinical and obstetric history. Gestational age was based on the women's recollection of the date of their last menstrual period. Study physicians performed a medical examination and collected blood, urine, and stool samples and vaginal swab specimens. The stage of HIV disease was classified in accordance with World Health Organization (WHO) guidelines [23]. Trained research assistants obtained anthropometric measurements, including weight and height, using standardized procedures.

Laboratory methods

Blood samples were obtained from participants at the enrollment visit, and plasma was stored at or below -70°C . Maternal vitamin D status was assessed using serum levels of 25(OH)D (hereafter referred to as "vitamin D levels"), which were measured by use of the fully automated chemiluminescence Advantage 25(OH)D assay system obtained from Nicholas Institute Diagnostics.

Total leukocyte counts were determined using a CBC5 Coulter Counter (Coulter), and differential white blood cell counts were determined manually. Absolute counts of CD4, CD8, and CD3 cells were measured using the FACSCount system (Becton Dickinson). HIV-1 serostatus was determined using an enzyme-linked immunosorbent assay (ELISA) (Wellcozyme; Murex Biotech), and positive results were confirmed using Western blot analysis (Bio-Rad).

Samples, such as serum and genital swab specimens, were used to diagnose candidiasis and sexually transmitted infections, including syphilis and gonorrhea. Malaria parasites were identified in Giemsa-stained thick-smear blood films. At the time of diagnosis, sexually transmitted infections, malaria, and intestinal parasitoses were treated according to standards of prenatal care established by the Tanzanian Ministry of Health.

Assessment of outcome and outcome definitions

Using a standard beam balance, a research midwife measured infant birth weight to the nearest 10 g immediately after birth. Low birth weight was defined as a birth weight of <2500 g, preterm birth was defined as delivery before 37 weeks' gestation, severe preterm birth was defined as delivery before 34 weeks' gestation, and small-for-gestational-age status was defined as a birth weight below the 10th percentile for gestational age [24]. Fetal death was defined as either miscarriage (occurring at <28 weeks' gestation) or stillbirth (occurring at ≥ 28 weeks' gestation). For live births, a composite adverse pregnancy end point was created that used information on small-for-gestational-age status, birth weight <2500 g, and preterm birth; all 3

events were weighted equally. This outcome was defined a priori and is consistent with previous publications from this trial [25,26].

Whole-blood samples were collected from the children at birth (range, 0–21 days), at 6 weeks after birth (range, 21–49 days), and at 3-month intervals thereafter. The Amplicor 1.5 HIV-1 detection kit (Roche Diagnostic System) was used to determine HIV status for infants <18 months of age. Infants ≥ 18 months of age had HIV infection diagnosed by ELISA, and the diagnosis was confirmed by Western blot analysis. If a blood sample obtained at the last visit tested negative for HIV DNA or a plasma specimen obtained from a child ≥ 18 months of age at that same last visit tested negative for HIV by ELISA, then the child was considered to be HIV uninfected.

Infants who were HIV infected at birth were most likely infected in utero, whereas those who were HIV uninfected at birth but were HIV infected at 6 weeks after birth probably acquired infection during the intrapartum period or as a result of breast-feeding in the first few weeks of life. Children who were first found to be HIV infected after 6 weeks of age were assumed to have acquired infection through breast-feeding. The vital status of the children was tracked by means of either regular clinic visits or home visits when a scheduled clinic visit was missed. The composite end point “HIV infected or dead at birth” was defined on the basis of whether an infant survived and remained free of HIV infection during the first 21 days of life, whereas the end point “HIV infected or dead by the end of follow-up” was used to denote whether a child survived follow-up and was free of HIV infection in the first 24 months of life. Both of these composite end points were defined a priori.

Statistical analyses

The cutoff value used to define vitamin D insufficiency was 32 ng/mL, which is considered to be optimal for calcium homeostasis [27,28] and has been used in similar studies [29]. We also defined quintiles of vitamin D on the basis of its distribution in the population. Risk ratios and 95% confidence intervals were estimated using binomial regression with the log-link function for all pregnancy outcomes and HIV transmission at birth and at 6 weeks [30]. When the log-binomial model failed to converge, log-Poisson models, which provide consistent but not fully efficient estimates of the risk ratio and its confidence intervals, were used [31,32]. For death and HIV transmission outcomes occurring after 6 weeks of age, Cox proportional hazards models were fit [33]. In addition, we investigated evidence of any nonlinear association between continuous vitamin D levels and the risk of major outcomes, including MTCT of HIV and child mortality, nonparametrically with stepwise-restricted cubic splines [34]. In these analyses, tests for nonlinearity used the likelihood ratio test, with the model using only the linear term compared with the model using the linear and the cubic spline terms. We included the following maternal variables at baseline in all multivariate models: age at enrollment, WHO HIV disease stage, CD4 cell count, and multivitamin regimen. The approach proposed by Rothman and Greenland [35] was used to control for confounding by additional variables in this analysis. According to this approach, all known or suspected measured risk factors for the outcome were included in the model, if they led to a >10% change in the estimate of the risk ratio or hazard ratio for vitamin D status [36]. Observations for which data for covariates were missing were retained in the analysis by use of the missing indicator method for variables missing >1% of the observations [37]. Statistical analyses were performed using SAS software (version 9.2; SAS Institute).

RESULTS

Vitamin D levels at baseline were known for 885 of the 1078 women enrolled in the trial. One woman was excluded because she had stage 4 HIV disease. The characteristics of these 884 women at baseline are presented in table 1. The average maternal age (\pm SD) at enrollment was

24.6 ± 5 years, and >70% of the women described their occupation as housewife. More than 80% of the women in this analysis had WHO stage 1 HIV disease, and >70% had a body mass index of 18.5–25 kg/m² at enrollment. Twin births (*n* = 24) were excluded from the analysis of pregnancy outcomes but were retained in the analysis of HIV infection and death as end points. The results obtained in the analysis with low versus adequate vitamin D levels were essentially similar to those obtained in the analysis with vitamin D quintiles; only the results from analyses of low versus adequate vitamin D levels are presented below (data not shown).

In this subset of the overall cohort, the risks of low birth weight, preterm birth, and small-for-gestational-age status were 12%, 27%, and 11%, respectively (table 2). For this subset of women with vitamin D measurements, these incidences, along with those for other outcomes presented subsequently, are comparable to those obtained for the entire cohort [19,38,39]. After multivariate adjustment for maternal age, HIV disease stage at baseline, CD4 cell counts, and multivitamin supplementation, low levels of vitamin D were not associated with the risk of low birth weight, preterm birth, small for gestational age, or the composite end point of an adverse pregnancy outcome (table 2).

Fetal loss was the outcome of 7.6% of all pregnancies, and 8.1% of infants were HIV infected at birth (table 3). Of the children who were known to be HIV uninfected at birth, 18.1% became infected by 6 weeks of age. There was no observed association between vitamin D status and fetal loss, HIV infection at birth, or HIV infection at 6 weeks of age, for those infants known to be HIV uninfected at birth, after multivariate adjustment (table 3). However, a significant association was observed between vitamin D status and the composite end point of HIV infection or death at delivery and HIV infection at 6 weeks. Children born to women with low vitamin D levels had a 49% greater risk of dying or being HIV infected at birth than did children born to women with sufficient vitamin D levels (95% CI, 7%–109%). A similar increase in the risk of HIV infection at 6 weeks of age was observed for children born to women with low vitamin D levels at baseline (risk ratio [RR], 1.50 [95% CI, 1.02–2.20]).

Over the first 24 months of follow-up, a total of 30.1% children were infected with HIV (table 4). In multivariate Cox regression models that assessed time to HIV infection as the outcome (table 4), children who were born to women with low vitamin D levels at baseline and who were known to be HIV uninfected at 6 weeks of age had a 2-fold higher incidence of acquiring HIV infection through breast-feeding than did children who were born to women with sufficient vitamin D levels (incidence rate ratio [IRR], 2.03 [95% CI, 1.08–3.82]). The overall rate of incidence of HIV infection by 24 months of age was 46% higher in the group with low vitamin D levels (95% CI, 11%–91%) than in the group with sufficient vitamin D levels. A nonlinear association was observed between continuous vitamin D levels and the risk of HIV transmission (*P* = .01) (figure 1); mothers with the lowest vitamin D levels had the highest risk of MTCT of HIV, and the risk decreased linearly as vitamin D levels increased. Although the risk of MTCT appeared to increase again at the highest levels of vitamin D, this risk was not significant.

During follow-up, 36.6% of the children died (fetal loss was included in this statistic); 46.0% of children died during follow-up or were HIV infected (table 4). A 61% increase in the rate of incidence of death during follow-up among live births was observed for infants born to women with low vitamin D levels at baseline (95% CI, 25%–107%). We examined effect modification of the association between a low vitamin D level and child mortality by HIV status, by introducing an interaction term in the model. This term was not statistically significant (*P* = .15). For children who remained HIV uninfected during follow-up, a similar increase in overall mortality was observed (IRR, 1.66 [95% CI, 1.23–2.23]); among children who became HIV infected, the increase in mortality was lower and not statistically significant (IRR, 1.36 [95% CI, 0.95–1.94]), suggesting independent and separate effects of vitamin D on

HIV infection and death. Furthermore, children born to mothers with the lowest vitamin D levels had the highest risk of dying ($P = .02$) (figure 1), and the risk decreased linearly as vitamin D levels increased. The risk of child mortality appeared to increase again at the highest vitamin D levels; however, this finding was not significant. Finally, for the composite end point of HIV infection or death by the end of follow-up, a 50% increase in risk (95% CI, 23%–63%) was observed for the group with low vitamin D levels.

DISCUSSION

In the present study, we examined the association of maternal vitamin D status at baseline (12–27 weeks' gestation) with adverse pregnancy outcomes, MTCT of HIV, and child mortality. No association was observed between a low maternal vitamin D level (<32 ng/mL) and such adverse pregnancy outcomes as low birth weight, preterm birth, and small-for-gestational-age status. An increased risk of being HIV infected or of dying at birth was observed for children born to women with a low vitamin D level at baseline; a low maternal vitamin D level was also associated with HIV transmission via breast-feeding and with higher infant mortality during follow-up.

The role of maternal vitamin D status in pregnancy outcomes of HIV-infected women has not been previously studied; however, there is some literature suggesting a benefit of vitamin D in pregnant women who are not HIV infected. For example, Marya et al [40] conducted a study of Asian-Indian women, randomizing them to receive either 600,000 IU of vitamin D twice (once each during the seventh and eighth months of pregnancy) or no vitamin D supplements. Infants of mothers who received vitamin D had greater intrauterine growth and a higher birth weight than did infants of women who did not receive vitamin D supplements. However, the beneficial effects of vitamin D have been observed with relatively large doses of vitamin D supplements, which may lead to serum vitamin D levels that are much higher than those observed in our cohort. This could be a potential explanation for the lack of any observed association with vitamin D levels and pregnancy outcomes in this cohort. Furthermore, the rates of adverse pregnancy outcomes appear to be higher in HIV-infected women than in HIV-uninfected women. For example, in another trial involving 8468 HIV-uninfected pregnant women in Tanzania, our group determined that the incidence of preterm birth was only 17%, compared with the 27% incidence noted in the current analysis [41]. Thus, it is possible that the adverse effect of HIV infection on perinatal outcomes may mask the beneficial effect of vitamin D on these outcomes, if any.

The association of maternal vitamin D status with HIV transmission and death among children has not been previously studied. However, our results are in accordance with some small studies of nonpregnant HIV-infected populations that have shown an association between low vitamin D levels and increased HIV disease progression and higher mortality [5]. For example, in one longitudinal study in Norway, HIV-infected patients with 1,25(OH)D levels of <25 ng/L at baseline ($n = 9$) had a significantly shorter survival time than did patients with levels in the range considered to be normal ($n = 44$), after adjustment for CD4 cell counts [42].

Recent research has highlighted the role of vitamin D in the regulation of the immune system, particularly innate immunity, which might explain this finding [43]. Vitamin D is also known to have other immunomodulatory effects, such as improving the phagocytic capacity of macrophages, increasing the number of natural killer cells, and boosting cell-mediated immunity [6–8]. Vitamin D is known to contribute to the development of the fetal immune system; a stronger immune system may be more resistant to HIV infection and may explain the decreased risk of MTCT observed in the present study. This finding would also likely correlate with fewer infections and opportunistic illnesses during follow-up and, consequently, with decreased mortality. In addition, there is increasing evidence supporting the role of

vitamin D in fighting tuberculosis; tuberculosis is one of the primary killers in HIV-infected populations [18].

Vitamin D may also decrease MTCT of HIV or child mortality by reducing inflammation; in this cohort, we observed an association between a low vitamin D level and a higher CD8 cell count and erythrocyte sedimentation rate (S.M., D.S., S. Aboud, E.L.G., G.I.M., E.H., F.M.M., D.J.H., and W.W.F., unpublished data). Although the conventional role of CD8 cells is to function as cytotoxic killer cells, they may also be the effector cells in inflammation [44]. The involvement of vitamin D in modulating CD8 cells is also indicated by the fact that, of the major immune cells (CD8, CD4, and B cells and macrophages), CD8 cells express the highest concentration of vitamin D receptor [45].

The present study includes important and informative findings, considering the lack of similar data in other studies reported to date, as well as implications for potential interventions for the prevention of MTCT of HIV and child mortality. However, we had limited power to examine the exact timing of MTCT of HIV. We also do not know whether the levels of vitamin D may be depressed as a result of HIV disease and thus may be a consequence of an advanced stage of HIV disease and/or accelerated viral replication and not the cause thereof. However, most women in our analysis did not have advanced HIV disease at baseline (>80% had stage 1 disease). The association observed in the present study may be different in other populations with a different underlying nutritional or immunologic status, as well as in populations with access to antiretroviral therapy, and therefore the findings may not be fully generalizable. One limitation of our analysis is that we had only one measurement of vitamin D levels at baseline; however, data from other studies suggest that there is a positive correlation between vitamin D levels in cord blood and vitamin D levels at baseline [29]. Therefore, it may be reasonable to assume that vitamin D levels at baseline are predictive of vitamin D levels in the postpartum period.

Another limitation of the present study is that the assay that we used to assess vitamin D status does not measure vitamin D₂ accurately; however, this form of vitamin D is obtained through supplements, the use of which was unlikely in this population. In addition, our choice of a cutoff level for vitamin D is to make the results clinically more relevant and comparable to those of other studies. However, estimates of a similar effect were obtained for such major outcomes as overall HIV transmission and overall child mortality, by use of quintiles of vitamin D, on the basis of its distribution in this population (data not shown).

Recent studies estimated that 1 billion people worldwide have vitamin D insufficiency [28]. Daily intake of at least 800–1000 IU of vitamin D may be needed in the absence of adequate sun exposure, to maintain a circulating level of 25(OH)D >32 ng/mL [28,46]. If demonstrated to be effective in intervention studies, vitamin D supplementation could prove to be a relatively simple and inexpensive method to lower mortality among children and to help prevent MTCT of HIV as an adjunct to antiretroviral therapy.

Acknowledgments

We thank the mothers and children, the field teams (including physicians, nurses, midwives, supervisors, and laboratory staff), and administrative staff, all of whom made the study possible. We also thank Muhimbili Medical Centre, Muhimbili University College of Health Sciences, and the National AIDS Control Program in Dar es Salaam, Tanzania, for their institutional support.

Financial support: National Institute of Child Health and Human Development (grant R01 32257), the Fogarty International Center (National Institutes of Health grant D43 TW00004), and the Harvard School of Public Health.

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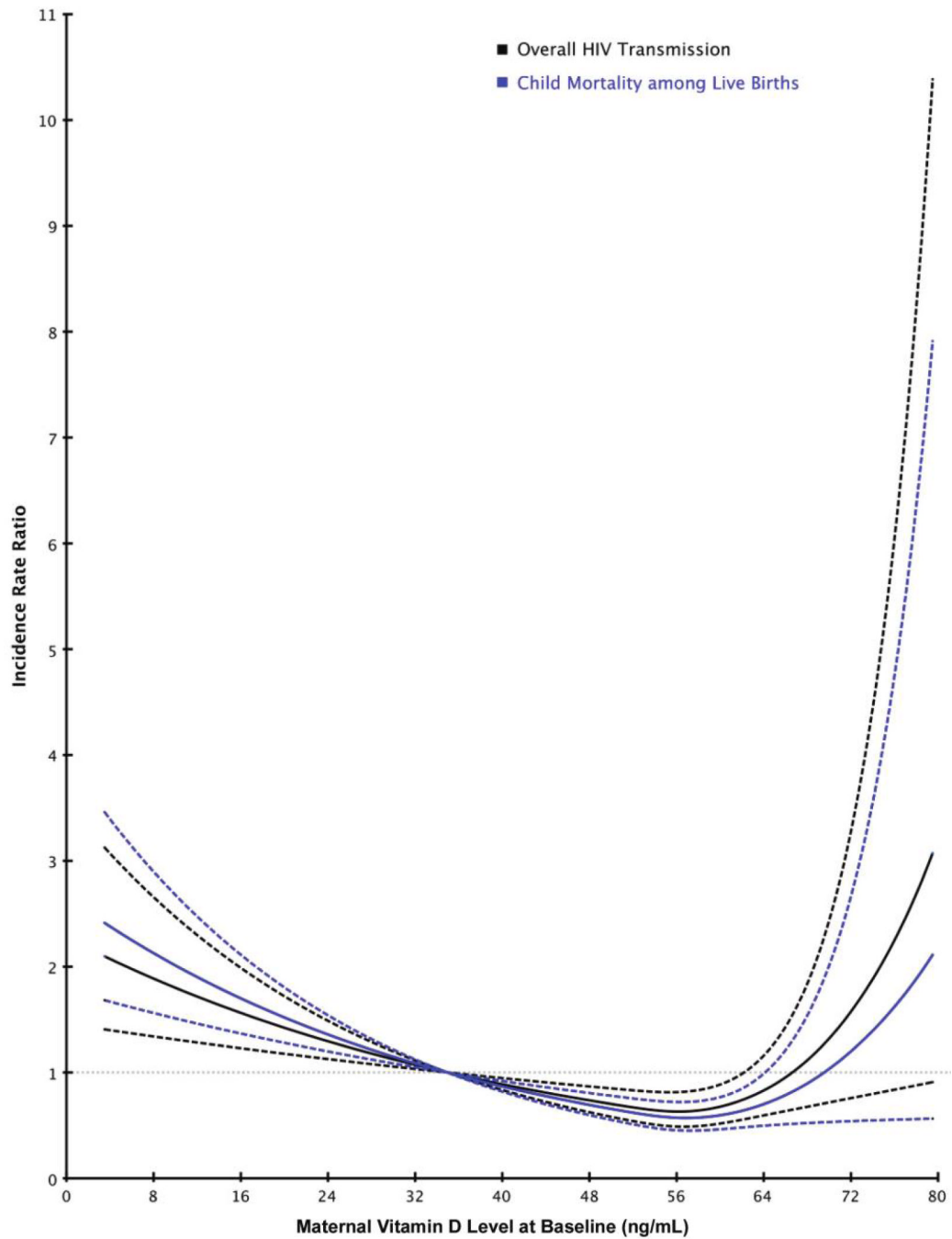


Figure 1. Association of maternal vitamin D level with the risk of mother-to-child transmission of human immunodeficiency virus (HIV) infection and child mortality among live births. Adjusted for maternal age at baseline, CD4 cell count, HIV disease stage, and multivitamin regimen received. Analyses of child mortality made additional adjustments for maternal death while the child was alive. Dotted lines denote 95% confidence intervals.

Table 1

Characteristics at Baseline of 884 Women Who Were Enrolled in the Trial and for Whom Data on Vitamin D Levels Were Available

Characteristic	Women with a low vitamin D level (<i>n</i> = 347)	Women with an adequate vitamin D level (<i>n</i> = 537)
Age, mean ± SD, years	24.6 ± 5	24.6 ± 5
≤500 shillings ^a spent on food per person per day	38.7	42.3
Occupation		
Housewife	71.5	74.1
Professional	3.2	2.6
Business	12.4	15.3
Public house	1.7	1.3
Employed	9.8	4.8
Other	1.4	1.9
WHO HIV disease stage		
1	80.1	85.1
2	17.3	14.0
3	2.6	0.9
Cell count, mean ± SD, cells/ μ L		
CD3	1283.0 ± 456.3	1209.6 ± 458
CD4	413.5 ± 202	428.7 ± 210
CD8	808.7 ± 354.1	722.0 ± 318
Vitamin D level, mean ± SD, ng/mL	24.2 ± 6	43.1 ± 9
Gestational age at randomization, mean ± SD, weeks	20.1 ± 4	20.5 ± 3
BMI, mean ± SD, kg/m ²	23.0 ± 3.2	23.4 ± 3.2

NOTE. Data are the percentage of women with the characteristic, unless otherwise indicated. BMI, body mass index; HIV, human immunodeficiency virus; SD, standard deviation; WHO, World Health Organization.

^aOne US dollar ≈ 500 Tanzanian shillings in 1995.

Table 2
 Low Maternal Vitamin D Level and Adverse Pregnancy Outcomes among Human Immunodeficiency Virus (HIV)–Infected Women in Tanzania

Outcome	Maternal vitamin D level, n/N (%)		Univariate analysis		Multivariate analysis ^a	
	Low	Adequate	RR (95% CI)	<i>P</i> ^b	RR (95% CI)	<i>P</i> ^b
Low birth weight ^c	28/261 (10.7)	52/414 (12.6)	0.85 (0.55–1.32)	.47	0.84 (0.55–1.28)	.42
Preterm birth ^d	69/289 (23.9)	135/469 (28.8)	0.83 (0.65–1.07)	.14	0.84 (0.65–1.07)	.15
Severe preterm birth ^e	26/289 (9.0)	44/469 (11.7)	0.77 (0.49–1.19)	.24	0.77 (0.50–1.18)	.23
Small for gestational age ^f	33/261 (12.6)	42/414 (10.1)	1.25 (0.81–1.91)	.31	1.25 (0.82–1.90)	.31
Adverse pregnancy outcome ^g	99/289 (34.3)	174/469 (37.1)	0.92 (0.76–1.13)	.43	0.92 (0.76–1.12)	.41

NOTE. Data are the no. of offspring with the outcome/no. of offspring assessed (% of offspring with the outcome). CI, confidence interval; RR, risk ratio.

^a All multivariate models were adjusted for multivitamin supplementation, maternal age at baseline, CD4 cell counts at baseline, and HIV disease stage at baseline.

^b *P* values are derived from log-binomial regression models.

^c Defined as <2500 g.

^d Defined as delivery at <37 weeks' gestation.

^e Defined as delivery at <34 weeks' gestation.

^f Birth weight below the 10th percentile for gestational age.

^g Composite of low birth weight, preterm birth, and small-for-gestational-age status.

Table 3
Human Immunodeficiency Virus (HIV) Infection and Mortality Outcomes up to 6 Weeks Postpartum among Offspring of HIV-Infected Women and the Association of Such Outcomes with a Low Maternal Vitamin D Level

Outcome	Maternal vitamin D level, n/N (%)		Univariate analysis		Multivariate analysis ^a	
	Low	Adequate	RR (95% CI)	P ^b	RR (95% CI)	P ^b
Fetal loss	32/336 (9.5)	33/519 (6.4)	1.50 (0.94–2.39)	.09	1.05 (0.63–1.74) ³	.86
Infant status at birth						
HIV infected	25/233 (10.7)	24/370 (6.5)	1.65 (0.97–2.83)	.07	1.54 (0.90–2.64)	.11
HIV infected or dead	57/265 (21.5)	56/400 (14.0)	1.54 (1.10–2.15)	.01	1.49 (1.07–2.09)	.02
Infant status at 6 weeks of age						
HIV infected	33/120 (27.5)	46/239 (19.3)	1.43 (0.97–2.11)	.07	1.50 (1.02–2.20)	.04
HIV infected after having been HIV uninfected at birth	21/97 (21.7)	32/196 (16.3)	1.33 (0.81–2.17)	.26	1.40 (0.86–2.28)	.17

NOTE. Data are the no. of offspring with the outcome/no. of offspring assessed (% of offspring with the outcome). CI, confidence interval; RR, risk ratio.

^c Adjusted for continuous CD8 cell counts at baseline and primiparity in addition to the covariates in footnote *a*.

^a All multivariate models adjusted for multivitamin supplementation, maternal age at baseline, CD4 cell counts at baseline, and HIV disease stage at baseline.

^b *P* values are derived from binomial regression models.

Table 4
Human Immunodeficiency Virus (HIV) Infection and Mortality Outcomes up to 24 Months Postpartum for Offspring of HIV-Infected Women and the Association of Such Outcomes with Low Maternal Vitamin D Levels

Outcome	Maternal vitamin D level, n/N (%)		Univariate analysis		Multivariate analysis ^d	
	Low	Adequate	RR (95% CI)	P ^b	RR (95% CI)	P ^b
HIV transmission through breast-feeding						
Among children known to be HIV uninfected at 6 weeks of age	18/87 (20.7)	24/193 (12.4)	1.89 (1.03–3.49)	.04	2.03 (1.08–3.82)	.03
Among children not known to be HIV infected at 6 weeks of age	47/222 (21.2)	62/392 (15.8)	1.55 (1.06–2.26)	.02	1.55 (1.06–2.27)	.02
Infant status at the end of follow-up						
HIV infected at 24 months of age ^c	95/270 (35.2)	122/452 (27.0)	1.49 (1.14–1.95)	<.01	1.46 (1.11–1.91)	<.01
Death among live births	118/304 (38.8)	130/486 (26.8)	1.64 (1.28–2.11)	<.01	1.61 (1.25–2.07) ^d	<.01
Overall mortality ^e	150/336 (44.6)	163/519 (31.4)	1.61 (1.29–2.01)	<.01	1.58 (1.26–1.97) ^d	<.01
Overall HIV infection or mortality ^e	181/336 (53.9)	212/519 (40.9)	1.54 (1.26–1.87)	<.01	1.50 (1.23–1.83) ^d	<.01

NOTE. Data are the no. of offspring with the outcome/no. of offspring assessed (% of offspring with the outcome). CI, confidence interval; RR, risk ratio.

^a All multivariate models adjusted for maternal age at baseline, HIV disease stage at baseline, CD4 cell counts at baseline, and regimen received.

^b P values are derived from Cox (proportional hazards) regression models.

^c Overall HIV infection.

^d Additionally adjusted for maternal death while child was alive.

^e Including fetal deaths.