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Serum vitamin D status and bacterial vaginosis prevalence and incidence in Zimbabwean women

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Abstract

BACKGROUND—Bacterial vaginosis, a highly prevalent vaginal condition, is correlated with many adverse reproductive outcomes. In some studies, low vitamin D (measured as serum 25-hydroxy-vitamin D, 25(OH)D) has been associated with increased prevalence of bacterial vaginosis.

OBJECTIVES—We examined the cross-sectional association between vitamin D status and prevalence of bacterial vaginosis, separately for pregnant and non-pregnant women. Using prospectively-collected data, we also characterized the effect of time-varying vitamin D status on incident bacterial vaginosis.

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STUDY DESIGN—We quantified 25(OH)D in stored sera collected quarterly from 571 Zimbabwean women participating in the Hormonal Contraception and Risk of HIV Acquisition Study. The analysis was restricted to women not using hormonal contraception. We characterized associations between vitamin D insufficiency (defined as 25(OH)D ≤30 ng/mL vs. >30 ng/mL) and prevalence of bacterial vaginosis among non-pregnant women at the enrollment visit, and among pregnant women at the first follow-up visit that pregnancy was detected. Among women who were negative for bacterial vaginosis at enrollment (n=380), we also assessed the effect of time-varying vitamin D status on incident bacterial vaginosis. We used the Liaison 25 OH vitamin D total assay to measure 25(OH)D. Bacterial vaginosis was diagnosed via Nugent score.

RESULTS—At enrollment, prevalence of bacterial vaginosis was 31% and overall median 25(OH)D was 29.80 ng/mL (interquartile range: 24.70-34.30 ng/mL): 29.75 ng/mL (interquartile range: 25.15-33.95 ng/mL) among women with bacterial vaginosis and 29.90 ng/mL (interquartile range: 24.70-34.50 ng/mL) among women without bacterial vaginosis. Among pregnant women, the prevalence of bacterial vaginosis was 27% and overall median 25(OH)D was 29.90 ng/mL (interquartile range: 24.10-34.00 ng/mL): 30.80 ng/mL (interquartile range: 26.10-36.90 ng/mL) among women with bacterial vaginosis and 29.10 ng/mL (interquartile range: 23.80-33.45 ng/mL) among women without BV. Vitamin D levels ≤30 ng/mL were not associated with prevalence of bacterial vaginosis in non-pregnant women (adjusted prevalence ratio: 1.04; 95% confidence interval: 0.81-1.34) or pregnant women (adjusted prevalence ratio: 0.88, 95% confidence interval: 0.51-1.54). Vitamin D levels ≤30 ng/mL were similarly not associated with incident bacterial vaginosis (adjusted hazard ratio: 0.98, 95% confidence interval: 0.73-1.31). Our findings were robust to alternative specifications of vitamin D status including using a cutpoint for vitamin D deficiency of <20 ng/ml vs. ≥20 ng/mL, and modeling 25(OH)D as a continuous variable.

CONCLUSION—Among reproductive-age Zimbabwean women, insufficient vitamin D was not associated with increased BV prevalence or incidence. Given established associations between BV and poor reproductive outcomes, identification of factors leading to high BV prevalence is urgently needed.

Keywords

bacterial vaginosis (BV); incidence; prevalence; vitamin D; Zimbabwe

INTRODUCTION

Bacterial vaginosis (BV), a condition characterized by reduction in vaginal lactobacilli and overgrowth of largely anaerobic microorganisms, affects nearly 1 in 3 women worldwide.[1] BV is an extremely important determinant of women's health and is linked to spontaneous abortion, premature delivery, increased HIV transmission, and other reproductive morbidities.[1-5]

The causes of BV are not clear. This condition occurs more often in women with more sex partners and higher coital frequency,[6] new partners,[7] female partners,[8] unprotected sex,[9] herpes simplex virus type 2 (HSV-2) infection,[10] and some vaginal hygiene practices.[11-12] In the United States (US), a persistent association exists between race and BV, even after adjustment for other BV risk factors:[13] in nationally-representative data, 52% of Black

women vs. 23% of white women had prevalent BV.[14] The strong correlation between race and BV suggests that a factor more common in Black women is also a risk factor for BV. We hypothesized that vitamin D insufficiency could explain this racial disparity. Vitamin D stimulates mechanisms associated with pathogen elimination.[15] Like BV, vitamin D insufficiency is significantly more prevalent among Black women than white women.[16] Racial differences in vitamin D status are thought to stem from evolutionary adjustments to different levels of sun exposure, and reflect corresponding variations in vitamin D metabolism, vitamin D receptor polymorphisms and signaling.[17-18]

Evaluation of the association between vitamin D levels – measured as serum 25-hydroxy-vitamin D, or 25(OH)D – and BV prevalence has been limited. Some cross-sectional studies in pregnant women report significant links between low vitamin D and increased BV prevalence,[19-22] while others, largely in non-pregnant women, find no link.[20, 23-24] ("Low" varies by study, defined as 25(OH)D <30 ng/mL [19-20] or <20 ng/ml [21-23]). Prospective evaluations of the vitamin D-BV relationship are less common. Three randomized trials of vitamin D supplementation reported no difference in BV prevalence[25-26] or recurrence[27] between women who received, or did not receive, vitamin D supplementation. In contrast, a vitamin D supplementation trial of women with asymptomatic BV reported significantly improved BV cure rates among those receiving vitamin D.[28]

While a number of studies have reported on associations between vitamin D and BV among Black women in the US, none to date have focused on African women. Studies from the region report BV prevalence between 30% and 40%, and some higher than 50%,[29] confirming the high burden of disease among African women. To fill this gap in the existing literature, we present an ancillary study of the Hormonal Contraception and Risk of HIV Acquisition (HC-HIV) study. Among reproductive-age, urban-dwelling women in Zimbabwe, we characterized the association between a) vitamin D status and BV prevalence (separately for non-pregnant and pregnant women) and b) time-varying vitamin D status and BV incidence.

METHODS

Study design, setting and population

The HC-HIV Study was a prospective cohort study conducted from 1999-2004 in Zimbabwe, Uganda and Thailand, which assessed the effect of hormonal contraception on women's risk of HIV acquisition.[30] Women eligible for HC-HIV were recruited from family planning and general healthcare clinics, and were aged 18 to 35 years, HIV-negative, non-pregnant, and sexually active. HC-HIV participants were enrolled in three approximately equal-sized contraceptive groups: oral contraceptive pills, injectable depot medroxyprogesterone acetate (DMPA), and non-hormonal or no contraception. Contraceptive group was not randomized. Women returned quarterly for up to two years to assess time-varying contraceptive use and HIV status. In the present analysis, because hormonal contraception affects BV risk,[31] we only included women using non-hormonal or no contraception. For efficiency, we restricted the sample to Zimbabwean women only. This project quantified vitamin D levels in serum frozen approximately a decade earlier; levels of 25(OH)D have been shown to be stable in frozen sera.[32]

Data collection and assessment of laboratory outcomes

At all study visits, time-varying demographic and sexual behavior data were captured through interviews. Participants received examinations with specimen collection and testing for sexually transmitted and reproductive tract infections, including BV (by both Amsel criteria[33] and Nugent scoring[34]), yeast, HIV, chlamydia, gonorrhea, trichomoniasis, syphilis, and HSV-2.[30] Serum aliquots from each visit were frozen at −80°C. For this analysis, we quantified 25(OH)D in sera using the Liaison 25 OH vitamin D total assay (DiaSorin, Saluggia, Italy). According to current US guidelines, 25(OH)D <20 ng/ml is considered deficient, 20-30 ng/ml is insufficient, and >30 ng/ml is adequate.[35]

Ethical approval

Women in the HC-HIV study provided written informed consent; the form specified participants' approval to store sera for future research. HC-HIV was approved by ethics committees at collaborating institutions in the US and Zimbabwe. This ancillary study was approved by the Joint Research Ethics Committee at the University of Zimbabwe 15 July 2011 (JREC/192/11), by the Medical Research Council of Zimbabwe on 30 September 2011 (MRCZ/A/1635), and by the Ohio State University Institutional Review Board on 13 March 2012 (2011H0265).

Statistical analysis

Statistical analyses were performed using SAS (Version 9.3, Cary, NC). For our primary analyses, we constructed a binary variable dichotomizing vitamin D into deficient/insufficient (25(OH)D ≤30 ng/mL) vs. adequate (>30 ng/mL). The primary outcome was BV by Nugent score,[34] dichotomized as BV-negative (Nugent score 0-6) vs. BV-positive (Nugent score 7-10). Slides of vaginal material were created at the time of physical examination and stored; microscopists performing Nugent scoring were trained and had their readings validated before large-scale reading at the conclusion of the study. Batches of 25-100 Gram-stained slides were shipped to the University of California, San Francisco Chlamydia/Virology Research Laboratory regularly throughout the slide-reading period for external quality control. For the small number of visits where Nugent score was missing (3%) we used Amsel criteria to classify BV status. To be BV-positive by Amsel criteria, three of four criteria must be met: vaginal pH >4.5; characteristic vaginal discharge; "clue cells" on wet mount; and positive "whiff" test with addition of KOH to a swab of vaginal material.[33]

To determine whether vitamin D was associated with prevalent BV in non-pregnant women, we examined data from the HC-HIV enrollment visit. We constructed a log-binomial regression model with a generalized estimating approach to produce an unadjusted prevalence ratio (PR) for the association between deficient/insufficient vitamin D (25(OH)D ≤30 ng/mL) and BV prevalence in non-pregnant women. We selected log-binomial models because BV prevalence was above 10%, the cutoff value above which logistic regression is not recommended.[36] We obtained the adjusted prevalence ratio (aPR) using a model that controlled for demographics and previously-identified correlates of BV (age, education, parity, partner circumcision status, sexual frequency, vaginal hygiene practices, condom use, and HSV-2 serostatus).[10, 37-38]

We repeated this analysis to assess whether vitamin D was associated with prevalent BV in pregnant women. Pregnant women were not eligible to enroll in HC-HIV, but for each woman who became pregnant during follow-up, we measured vitamin D and BV at the first visit that pregnancy was detected. As above, we constructed log-binomial models to characterize the unadjusted and adjusted associations between deficient/insufficient vitamin D status and BV prevalence in pregnant women.

To examine the prospective effect of time-varying vitamin D status on BV incidence, we constructed an analysis dataset of women who were BV-negative at enrollment and had valid 25(OH)D and BV data during follow-up visits (n=380). Women were censored after the first visit when BV was detected, or the last visit in the study for women who remained BV-free throughout follow-up. We specified Cox proportional hazards models using continuous time (measured in days) and robust variance estimation[39] to estimate hazard ratios (HRs) comparing BV incidence among women with deficient/insufficient (25(OH)D ≤30 ng/mL) vs. adequate (>30 ng/mL) vitamin D status.

For all analyses, we used the serum vitamin D measure from the same visit when BV status was assessed. Because vitamin D levels change slowly over time in the absence of supplementation,[40] vitamin D status at a given visit is an acceptable representation of women's vitamin D status at the time that BV (if present) was developing.

Sensitivity analyses

We undertook several sensitivity analyses to evaluate the robustness of our findings. First, we repeated both the prevalence and incidence analyses using a lower cutpoint for vitamin D: 25(OH)D <20 ng/mL. Second, we repeated both analyses using 25(OH)D as a continuous variable. (We also examined the association between vitamin D and BV using splines, but observed no change in the outcomes or fit of the models, so present only the results from the continuous coding of vitamin D.) Third, for BV incidence analyses, we examined the effect of baseline Nugent score on the association between vitamin D and BV incidence. Whereas in the primary analysis women with normal vaginal flora at enrollment (Nugent score 0-3) and women with intermediate flora (Nugent 4-6) were both coded BV-negative, this sensitivity analysis examined the effect of time-varying vitamin D on BV incidence among these two groups separately.

RESULTS

Sample characteristics

Of the Zimbabwean HC-HIV participants (n=2296), 640 (28%) were using non-hormonal or no contraception at enrollment. When we further restricted the sample to women who had valid BV results from the enrollment visit and retrievable serum for vitamin D testing, 571 women (89% of eligible participants) were included in this ancillary analysis. Included women were similar to excluded women in all assessed characteristics, except that included women were less likely to have chlamydial (p<0.001) or gonococcal infection (p=0.004) at enrollment.

We observed no significant differences between women by vitamin D status at enrollment (Table 1). Median age was 26 years (interquartile range (IQR): 22-30 years). A third of women (31%) had BV. The prevalence of other infections at enrollment was low, except for HSV-2 (57% seropositivity). The median number of lifetime male partners was 1 (IQR: 1-2) and the median number of monthly sex acts was 12 (IQR: 8-21). Half of women (49%) had adequate vitamin D levels (>30 ng/mL) at enrollment. Median vitamin D level at enrollment was 29.80 ng/mL (IQR: 24.70-34.30 ng/mL): 29.75 ng/mL (IQR: 25.15-33.95 ng/mL) among women with BV and 29.90 ng/mL (IQR: 24.70-34.50 ng/mL) among women without BV.

We also examined characteristics of the subset of participants who became pregnant during the study, overall and by vitamin D status (Table 2). The general patterns observed in pregnant women were similar to the patterns seen in the overall population. BV prevalence was 27%, and overall median 25(OH)D was 29.90 ng/mL (IQR: 24.10-34.00 ng/mL): 30.80 ng/mL (IQR: 26.10-36.90 ng/mL) among women with BV and 29.10 ng/mL (IQR: 23.80-33.45 ng/mL) among women without BV. Neither gestational age nor pregnancy outcomes were captured in the HC-HIV data.

Vitamin D and BV prevalence

Among non-pregnant women (n=571), 25(OH)D ≤30 ng/mL was not associated with BV prevalence in unadjusted analyses (PR: 1.06, 95% CI: 0.83-1.35). After adjustment, the PR was essentially unchanged (aPR): 1.04, 95% CI: 0.81-1.34) (Table 3). Among pregnant women (n=141), we also found no association between vitamin D ≤30 ng/mL and BV prevalence in either unadjusted (PR: 0.84, 95% CI: 0.49-1.44) or adjusted analyses (aPR: 0.88, 95% CI: 0.51-1.54) (Table 3).

Vitamin D and BV incidence

Among women who did not have BV at enrollment (n=380), deficient/insufficient vitamin D was not associated with development of incident BV during follow-up in unadjusted (HR: 0.97, 95% CI: 0.72-1.30) or adjusted analyses (aHR: 0.98, 95% CI: 0.73-1.31) (Table 3).

Sensitivity analyses

Our primary findings were robust to alternative specifications of vitamin D (Table 4). When we modeled using a cutpoint of vitamin D deficiency (25(OH)D <20 ng/ml vs. ≥20 ng/mL), we still observed no association between vitamin D and prevalent or incident BV. When we analyzed vitamin D as a continuous variable, the change in BV prevalence and incidence per 1-ng/mL change in 25(OH)D again remained null. Finally, when stratifying by Nugent score at enrollment, deficient/insufficient vitamin D was not associated with BV incidence either among women with normal vaginal flora at enrollment nor among women with intermediate vaginal flora at enrollment (Table 4).

COMMENT

Among healthy, urban, Zimbabwean women not using hormonal contraception, serum vitamin D status was not associated with prevalence or incidence of BV. This lack of

association persisted for non-pregnant and pregnant women and after adjustment for confounding variables. BV incidence was not correlated with time-varying vitamin D status in women who started the study with normal vaginal flora or in those with intermediate flora at enrollment.

When we initiated this project, prior research on vitamin D and BV prevalence and incidence was mixed. Nearly all existing research focused on pregnant women. Given associations between BV and poorer pregnancy outcomes,[1] understanding how vitamin D impacts pregnancy is a public health priority.[41] At least four cross-sectional analyses among pregnant women in the US have reported that low vitamin D status is associated with prevalent BV, even after adjustment for, or stratification by race and other confounding variables.[19-22] Our finding of no association between vitamin D and BV among pregnant women in Zimbabwe differs from these cross-sectional studies, but agrees with two trials of vitamin D supplementation in pregnant women. Those trials examined BV as a secondary outcome and reported no significant effect of vitamin D supplementation on BV.[25-26, 42] Considering non-pregnant women, our finding of no association between vitamin D and BV prevalence agrees with published cross-sectional studies.[20, 23-24]

Approximately half (49%) of participants had adequate vitamin D levels (>30 ng/ml) at enrollment, and 9% had levels below 20 ng/ml. Some debate continues about the serum 25(OH)D level for optimal human health,[35, 43-44] and thresholds to define vitamin D deficiency, insufficiency and adequacy are not wholly consistent. In our sample, no participant had very low 25(OH)D (<10 ng/ml), so we could not examine the impact of severe vitamin D deficiency. However, our findings are robust across alternative specifications of vitamin D insufficiency, which suggests that in this population of African women living in a subtropical setting, vitamin D is not a meaningful factor in BV pathogenesis.

We used the DiaSorin Liaison 25 OH vitamin D total assay to measure 25(OH)D, whereas earlier studies used the Diasorin RIA assay[19, 21] or mass spectrometry.[23] Each assay quantifies 25(OH)D, but differences in quantification approaches could have led to somewhat different estimates of vitamin D insufficiency. However, assay sensitivity is not expected to be differential with regard to BV status, and so the differences between assays are unlikely to explain differences in the observed associations between vitamin D and BV across studies.

The BV incidence analysis presented here is the first to examine the association between time-varying vitamin D status and BV using cohort data. Although not directly comparable, two randomized, prospective trials that examined the effect of vitamin D supplementation on BV found opposite effects. Among 118 BV-positive women in the US, our team found no reduction in BV recurrence among women randomized to high-dose vitamin D supplements (50,000 IU/week for one month, then 50,000 IU/month for five additional months) compared to placebo (hazard ratio (HR): 1.11, 95% CI, 0.68-1.81).[27] Conversely, in a trial of 208 BV-positive Iranian women, those receiving low-dose vitamin D supplements (2000 IU/day) had significantly improved BV cure rates compared to placebo (odds ratio: 10.1, 95% CI: 4.8-21.3).[28] Both trials enrolled non-pregnant women and demonstrated marked increases in

25(OH)D in the intervention arm, but differences in design could explain the opposite findings. Our trial of US women enrolled participants with symptomatic BV, provided standard BV therapy together with vitamin D, used weekly high-dose supplements, and did not require documented vitamin D insufficiency to enroll (although only 10 participants (8%) had 25(OH)D ≥ 30 ng/mL).[27] Taheri *et al.* enrolled women with asymptomatic BV, did not provide BV therapy (consistent with treatment guidelines for asymptomatic BV), provided daily low-dose supplements, and vitamin D insufficiency (<30 ng/mL) was an eligibility criterion.[28] Future studies should assess whether any effect of vitamin D on the vaginal microbiome is modest compared to other host factors, and whether vitamin D is only impactful in asymptomatic BV where local inflammation may be less severe; a modest effect of vitamin D may not be detectable when inflammation is more profound, such as during symptomatic BV.

Individual-level changes in vitamin D over the study and across seasons were small (annual peak-trough seasonal difference in 25(OH)D over the full population was 3.5 ng/mL) compared to what is observed in supplement studies (19.1 ng/mL increase in the intervention arm in[28]). It is possible that more profound shifts in vitamin D could impact BV risk, but the lack of variability in vitamin D across our population was too modest to observe any consequence for BV prevalence or incidence.

As an ancillary study, our analysis has important limitations. Our findings only apply to women using non-hormonal or no contraception. We selected this analysis population because DMPA and contraceptive pills lead to reduced BV risk,[31] and with limited resources to quantify vitamin D from stored sera, we wanted to isolate any effect of vitamin D among the women expected to have higher BV burden. Vitamin D testing occurred in 2013, 10-13 years after sera was collected and frozen. As noted earlier, 25(OH)D has been shown to be stable in frozen sera.[32] However any degradation, if it occurred is likely to be non-differential with respect to BV status. Notably, measured levels of 25(OH)D in samples from the present study were higher than in samples from our vitamin D trial,[27] which were stored for only 1-2 years before 25(OH)D quantification. In that trial, vitamin D levels were in line with nationally-representative surveys for US women: median 25(OH)D at enrollment was 16 ng/ml.

Recent research reveals that racial differences in 25(OH)D status might be driven by evolutionary adjustments leading to variations by race in vitamin D catabolism, vitamin D receptor polymorphisms, vitamin D binding protein and other signaling molecules.[17-18] For example, while Black individuals in the US have significantly lower 25(OH)D than whites, because of correspondingly lower levels of vitamin D-binding protein, Blacks have similar levels of bioavailable vitamin D as their white counterparts.[45] Another recent study found that low vitamin D status was associated with increased coronary heart disease and bone mineral density in whites but not in Blacks.[17-18] All HC-HIV participants were Black, but this does not exclude the possibility of important genetic variations in the study population. Unfortunately, quantification of these determinants was outside the scope of this study.

This large, methodologically-strong study makes an important contribution to the BV literature. The population was comprised of participants at high risk of BV: sexually-active,

reproductive-aged women who were not using hormonal contraception. It is the first study to characterize associations between vitamin D and BV in a low-resource setting. Because of the primary purpose of the parent study, nearly all key variables related to BV risk were measured, permitting excellent confounder control. Many of these host factors associated with BV risk were unmeasured in previous studies of vitamin D and BV. For example, HSV-2 infection is associated with increased prevalence, incidence and persistence of BV, perhaps because host responses to intermittent reactivation and replication of virus create a local environment that is inhospitable to healthy vaginal flora.[10, 46] In addition, women partnered with circumcised men have lower BV risk than women with uncircumcised partners, likely because the absence of a foreskin leads to a reduction in the bacterial load to which women are exposed during sex.[38]

In Zimbabwe as in many parts of the world, BV prevalence is high and BV-associated morbidities are substantial. Because vitamin D insufficiency can be corrected with inexpensive supplements that have other health benefits,[43] documentation of a vitamin D-BV association could have led to supplementation interventions that could meaningfully impact women's health worldwide. However, our null findings reinforce current trends in the literature that suggest no significant association between vitamin D and BV prevalence and incidence. Given the established associations between BV and negative health outcomes, effective interventions to reduce BV's impact continue to be urgently needed.

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Table 1

Participant characteristics at enrollment, stratified by vitamin D status (n=571)[a]

Characteristic	All women (n=571)	Deficient/ Insufficient Vitamin D [b] (n=294)	Adequate Vitamin D [c] (n=277)	p-value[d]
	n (%)	n (%)	n (%)	
Marital Status				0.64
Single	18 (3.2)	11 (3.7)	7 (2.5)	
Married	499 (87.4)	257 (87.4)	242 (87.4)	
Divorced/Widowed	54 (9.5)	26 (8.8)	28 (10.1)	
Employed	278 (48.7)	154 (52.4)	124 (44.8)	0.07
Infections detected				
Bacterial vaginosis [e]	176 (30.8)	93 (31.6)	83 (30.0)	0.67
Normal flora (Nugent 0-3)	254 (44.5)	128 (43.5)	126 (46.5)	
Intermediate flora (Nugent 4-6)	128 (22.4)	66 (22.5)	62 (22.4)	
BV (Nugent 7-10)	171 (30.0)	89 (30.3)	82 (39.6)	0.94
Missing Nugent score	18 (3.2)	11 (3.7)	7 (2.5)	
Candidiasis	87 (15.2)	41 (14.0)	46 (16.6)	0.42
Chlamydia	2 (0.4)	1 (0.3)	1 (0.4)	1.00
Gonorrhea	13 (2.3)	3 (1.0)	10 (3.7)	0.05
Trichomoniasis	34 (6.0)	13 (4.4)	21 (7.6)	0.16
Syphilis	14 (2.5)	7 (2.4)	7 (2.5)	1.00
HSV-2 seroprevalence	324 (56.7)	161 (54.8)	163 (58.8)	0.33
Condom use [f]				0.77
Always	382 (67.3)	200 (68.3)	182 (66.2)	
Sometimes	84 (14.8)	38 (13.0)	46 (16.7)	
Never	102 (18.0)	55 (18.8)	47 (17.1)	
Ever engaged in commercial sex [g]	3 (0.5)	1 (0.3)	2 (0.7)	0.61
Any vaginal practice other than using water for cleaning [g]	137 (24.0)	70 (23.8)	67 (24.2)	0.92

	n (%)	n (%)	n (%)	
Male partner circumcision status				0.96
Circumcised	74 (13.0)	39 (13.4)	35 (12.6)	
Uncircumcised	437 (76.8)	223 (76.4)	214 (77.3)	
Do not know	58 (10.2)	30 (10.3)	28 (10.1)	
Primary partner risk [f, h]	219 (38.4)	118 (40.1)	101 (36.5)	0.37

	Median (IQR)	Median (IQR)	Median (IQR)	p-value [d]
Age (years)	26 (22-30)	26 (22-30)	26 (22-29)	0.62
Education (years)	10 (9-11)	10 (9-11)	10 (9-11)	1.00
Number of male sex partners, lifetime	1 (1-2)	1 (1-2)	1 (1-2)	0.47
Number of male sex partners [f]	1 (1-1)	1 (1-1)	1 (1-1)	0.46
Number of live births, lifetime	2 (1-3)	2 (1-3)	2 (1-3)	0.19
Number of monthly sex acts [f]	12 (8-21)	12 (8-23)	12 (8-20)	0.91
Vitamin D (25(OH)D)	29.8 (24.7-34.3)	24.9 (21.4-27.5)	34.5 (31.9-38.7)	<0.0001

[a] Pregnancy at enrollment was an exclusion criterion, so all women in Table 1 were non-pregnant.

[b] Deficient/insufficient vitamin D = 25(OH)D ≤30ng/mL

[c] Adequate vitamin D =25(OH)D >30ng/mL

[d] Chi-square, Fisher's exact or Cochran-Mantel-Haenszel tests for categorical variables, and Wilcoxon-Mann-Whitney test for continuous variables

[e] BV assessed primarily by Nugent score. For the 3% of visits where Nugent score was missing, BV was classified using Amsel criteria.

[f] In a typical month in the last 3 months

[g] In last 3 months

[h] 'Primary partner risk' is a composite variable that indicates that the participant's primary partner is HIV-positive, or has abnormal discharge from the penis, or weight loss, or spent nights away from the home, or that the partner has sex with other women

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Table 2

Characteristics of pregnant participants, stratified by vitamin D status (n=141), at the visit that pregnancy was detected.

Characteristic	All pregnant women (n=141)	Deficient/ Insufficient Vitamin D [a] (n=73)	Adequate Vitamin D [b] (n=68)	p-value[c]
	n (%)	n (%)	n (%)	
Marital Status				0.90
Single	3 (2.1)	1 (1.4)	2 (2.9)	
Married	128 (90.8)	67 (91.8)	61 (89.7)	
Divorced/Widowed	10 (7.1)	5 (6.9)	5 (7.4)	
Employed	65 (46.1)	30 (41.1)	35 (51.5)	0.22
Infections detected				
Normal flora (Nugent 0-3)	71 (53.0)	35 (51.5)	36 (54.6)	
Intermediate flora (Nugent 4-6)	25 (18.7)	15 (22.1)	10 (15.2)	0.52
BV (Nugent 7-10)	38 (27.0)	18 (24.7)	20 (29.4)	
Candidiasis	31 (22.0)	20 (27.4)	11 (16.2)	0.11
Chlamydia	1 (0.7)	0 (0.0)	1 (1.5)	0.48
Gonorrhea	3 (2.2)	2 (2.8)	1 (1.5)	1.00
Trichomoniasis	7 (5.0)	4 (5.5)	3 (4.5)	1.00
Syphilis	5 (7.1)	1 (2.9)	4 (11.1)	0.36
HSV-2 seroprevalence	92 (65.3)	47 (64.4)	45 (66.2)	0.82
Use of condoms [d]				0.12
Always	40 (28.8)	16 (21.9)	24 (36.4)	
Sometimes	59 (42.5)	32 (43.8)	27 (40.9)	
Never	40 (28.8)	25 (34.3)	15 (22.7)	
Commercial sex [e]	0 (0)	0 (0)	0 (0)	N/A
Any vaginal practice other than using water for cleaning [e]	46 (32.6)	24 (32.88)	22 (32.4)	0.95
Partner circumcision status				0.99
Circumcised	18 (13.1)	9 (12.9)	9 (13.4)	

Characteristic	All pregnant women (n=141)	Deficient/ Insufficient Vitamin D [a] (n=73)	Adequate Vitamin D [b] (n=68)	p-value [c]
	n (%)	n (%)	n (%)	
Uncircumcised	107 (78.1)	55 (78.6)	52 (77.6)	
Do not know	12 (8.8)	6 (8.6)	6 (9.0)	
Primary partner risk [d, f]	56 (39.7)	27 (37.0)	29 (42.7)	0.49
Inconsistent condom use [d]	99 (70.21)	57 (78.08)	42 (61.76)	0.03
	Median (IQR)	Median (IQR)	Median (IQR)	p-value [c]
Age (years)	25 (22-28)	25 (21-28)	26 (22-28.5)	0.28
Education (years)	10 (9-11)	10 (8-11)	10 (9-11)	0.91
Number of male sex partners, lifetime	1 (1-2)	1 (1-2)	1 (1-2)	0.93
Number of male sex partners [d]	1 (1-1)	1 (1-1)	1 (1-1)	0.31
Number of live birth, lifetime	2 (1-3)	2 (1-3)	2 (1-3)	0.54
Number of monthly sex acts [d]	14 (8-22)	15 (9-24)	12 (8-20)	0.26
Vitamin D (25(OH)D	29.7 (25.3-34.4)	25.5 (22.6-27.6)	34.7 (32.1-39.7)	<0.0001

[a] Deficient/insufficient vitamin D = 25(OH)D ≤30ng/mL

[b] Adequate vitamin D =25(OH)D >30ng/mL

[c] Chi-square, Fisher's exact or Cochran-Mantel-Haenszel test for categorical variables and Wilcoxon Mann Whitney test for continuous variables

[d] In a typical month in the last 3 months

[e] In last 3 months

[f] 'Primary partner risk' is a composite variable that indicates that the participant's primary partner is HIV-positive, or has abnormal discharge from the penis, or weight loss, or spent nights away from the home, or that the partner has sex with other women

Table 3

Unadjusted and adjusted associations between vitamin D status and the prevalence and incidence of BV among Zimbabwean women.

	Unadjusted		Adjusted [a]	
BV prevalence	**PR**	**(95% CI)**	**aPR**	**(95% CI)**
Non-pregnant women				
Deficient/insufficient vs. adequate vitamin D (≤30 ng/mL vs. >30 ng/mL)	1.06	(0.83, 1.35)	1.04	(0.81, 1.34)
Pregnant women				
Deficient/insufficient vs. adequate vitamin D (≤30 ng/mL vs. >30 ng/mL)	0.84	(0.49, 1.44)	0.88	(0.51, 1.54)

BV incidence	**HR**	**(95% CI)**	**aHR**	**(95% CI)**
Time-varying deficient/insufficient vs. adequate vitamin D (≤30 ng/mL vs. >30 ng/mL)	0.97	(0.72, 1.30)	0.98	(0.73, 1.31)

[a] Adjusted models control for age, education, parity, and several time-varying variables: HSV-2 status, circumcision status of primary male partner, and sexual behaviors measured as “in a typical month in the last three months”: any intravaginal hygiene practice other than cleansing with water, sexual frequency, condom use, and number of male sex partners.

Table 4

Sensitivity analyses examining the effect of alternative specifications of vitamin D status on the associations between vitamin D and BV prevalence and incidence

	Unadjusted		Adjusted [a]	
BV prevalence	**PR**	**(95% CI)**	**aPR**	**(95% CI)**
Non-pregnant women				
Deficient vs. insufficient/adequate vitamin D (<20 ng/mL vs. ≥ 20 ng/mL)	0.73	(0.44, 1.22)	0.75	(0.45, 1.27)
Per 1-unit increase in serum 25(OH)D (ng/mL, continuous measure)	1.00	(0.98, 1.01)	1.00	(0.98, 1.02)
Pregnant women				
Deficient vs. insufficient/adequate vitamin D (<20 ng/mL vs. ≥ 20 ng/mL)	N/A	-- [b]	N/A	-- [b]
Per 1-unit increase in serum 25(OH)D (ng/mL, continuous measure)	1.00	(0.97, 1.04)	1.00	(0.97, 1.04)
BV incidence	**HR**	**(95% CI)**	**aHR**	**(95% CI)**
Deficient vs. insufficient/adequate vitamin D (<20 ng/mL vs. ≥20 ng/mL)	1.03	(0.52, 2.01)	1.13	(0.56, 2.25)
Per 1-unit increase in 25(OH)D (ng/mL, continuous measure)	1.00	(0.98, 1.02)	1.00	(0.98, 1.02)
BV incidence stratified by baseline Nugent score	**HR**	**(95% CI)**	**aHR**	**(95% CI)**
Among women with normal flora at enrollment (Nugent 0-3)				
Deficient/insufficient vs. adequate vitamin D (≤30 ng/mL vs. >30 ng/mL)	1.11	(0.73, 1.69)	1.16	(0.76, 1.76)
Among women with intermediate flora at enrollment (Nugent 4-6)				
Deficient/insufficient vs. adequate vitamin D (≤30 ng/mL vs. >30 ng/mL)	0.75	(0.49, 1.17)	0.77	(0.49, 1.21)

[a] Adjusted models control for age, education, parity, and several time-varying variables: HSV-2 status, circumcision status of primary male partner, and sexual behaviors measured as “in a typical month in the last three months”: any intravaginal hygiene practice other than cleansing with water, sexual frequency, condom use, and number of male sex partners.

[b] This estimate could not be calculated because the analysis population contained no pregnant, BV-positive women with 25(OH)D <20 ng/mL.