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BACTERIAL VAGINOSIS AND SEASON, A PROXY FOR VITAMIN D STATUS

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Abstract

BACKGROUND—Low serum vitamin D concentration has been associated with increased prevalence of bacterial vaginosis (BV) among pregnant women, but the few studies conducted in non-pregnant women have produced inconsistent results. Since serum vitamin D concentration is generally higher in the summer and fall than winter and spring, if vitamin D insufficiency causes BV then BV would be expected to be more common during seasons with lower vitamin D concentrations.

METHODS—The Longitudinal Study of Vaginal Flora followed women in Birmingham, Alabama (33.5° latitude) quarterly for up to one year. We employed a case-crossover design with conditional logistic regression among women who attended visits in each season, to assess the adjusted association between season and BV. We compared each woman's BV status in summer, fall, and spring to her own status in winter.

RESULTS—Among the 3620 women in the parent study, 2337 attended visits in each season; BV prevalence was 40% in winter, 38% in spring, and 41% in summer and fall. 1335 women had BV at some, but not all visits and were therefore included in the case-crossover analysis. Season was not associated with BV in women who were BV-negative at study entry (odds ratio versus winter were 1.0 for spring, 1.0 for summer and 0.9 for fall, p=0.81). Among women BV-positive at study entry, the corresponding odds ratios were 0.9, 1.4 and 1.4 (p<0.001).

CONCLUSION—These results do not support an association between vitamin D, measured through the proxy variable of season, and bacterial vaginosis.

Keywords

bacterial vaginosis; seasonality; vitamin D

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Bacterial vaginosis (BV) is a condition of unknown etiology in which the usual Lactobacillus predominant vaginal bacteria are replaced with overgrowth of *Gardnerella vaginalis* and mixed anaerobic organisms.[1] It is the most common cause of vaginal discharge in reproductive-age women, [2] and women with BV are at increased risk of preterm birth,[3] postoperative gynecologic infection[4] and acquisition of sexually transmitted diseases,[5] including HIV.[6] African-American women have consistently been demonstrated to have increased BV prevalence compared with white women; the increase is not explained by differences in demographic characteristics, or by differences sexual or hygienic behaviors.[7]

Recently, reduced serum concentration of 25 hydroxy-vitamin D, the standard marker of vitamin D status, has been associated with increased BV prevalence among pregnant women.[8–11] However, only two studies of vitamin D and BV have been conducted in non-pregnant women; one failed to find an association,[8] and the other found vitamin D deficiency to be associated with BV in HIV-positive, but not HIV-negative women.[12] As vitamin D is important for immune function, and deficiency has been associated with both immune disorders and chronic infection,[13,14] an association between vitamin D deficiency and BV is plausible. Synthesis of pre-vitamin D in skin exposed to ultraviolet-B light is the major source of vitamin D in humans,[15] and due primarily to darker skin color, African-American women have lower serum vitamin D concentrations than white women.[16] Therefore, insufficient or deficient vitamin D may account for some of the increased BV prevalence seen among African-American women. However, serum concentration of free vitamin D, the biologically available fraction, may not differ between African-Americans and whites,[17] calling into question the role of vitamin D in explaining the racial disparity in BV. Previous studies of the BV-vitamin D association have been cross-sectional, in which both BV and vitamin D were assessed at a single point in time. Since serum vitamin D concentration is usually higher in the summer than the winter, if low vitamin D were a cause of BV then BV ought to be more prevalent in the winter than the summer among women followed longitudinally. This report describes the seasonality of BV prevalence among non-pregnant women followed for one year.

METHODS

This report is a secondary analysis of the Longitudinal Study of Vaginal Flora.[18] Non-pregnant, 15–44 year old women were recruited from August 1999 to February 2002 upon presentation for a routine health visit to one of 12 clinics in the Birmingham, Alabama area. Exclusion criteria were significant medical or gynecological conditions, receiving chronic antibiotics (daily for at least 30 days), planning to leave the area in the next 12 months, and inability to provide informed consent. The study was approved by the IRBs of the University of Alabama at Birmingham, the Jefferson County Health Department and the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development. All participants provided written informed consent. This secondary analysis of previously collected data was determined not to constitute human subjects research by the Nationwide Children's Hospital IRB.

Women were seen at a research clinic for an initial visit, and then for quarterly visits for up to a year of follow-up. At each visit the woman was interviewed in a private office by a female interviewer. In addition to demographic factors, the interview included detailed questions on lifestyle, sexual and personal hygienic behaviors during the past 6 months for the initial interval and the past 3 months for subsequent interviews. Also at each visit the woman underwent a pelvic examination, at which time a cotton swab was obtained for vaginal Gram stain, which was evaluated according to the Nugent scoring system.[19] BV was defined as a Nugent score of 7–10; 10% of Gram stained slides were re-read in a different laboratory, with a high level of agreement for the presence of BV (kappa=0.81). Asymptomatic women who had BV by either Nugent [19] or Amsel [20] criteria were not routinely treated.

Data were analyzed with the case-crossover method.[21] Seasons were defined as winter (December – February), spring (March – May), summer (June – August) and fall (September – November), with winter considered the referent category. The case-crossover design compares each woman's BV status in spring, summer and fall with her own status in winter using conditional logistic regression. Because each woman serves as her own control, the method inherently controls for factors that do not vary over time, such as genes, skin type, and most demographic characteristics. Only women who are discordant on the outcome—those who had BV on at least one, but not all visits—contribute to the analysis. [21] Characteristics that may vary across the year, such as sexual, hygienic and health-related behavior, are not inherently controlled by this approach. Therefore the conditional logistic regression model included these confounding factors, in addition to season. Confounding factors were determined *a priori* and included vaginal and receptive oral sex frequencies, number of recent sex partners, having a recent new sex partner, current use of hormonal contraception, current douching frequency, type of menstrual protection (with amenorrhea as the referent category), preference for showering versus bathing, use of genital powder, towelettes and sprays, smoking, alcohol drinking, current weight and receipt of metronidazole since the last study visit (or in the past 2 weeks for the first study visit). We assessed whether these characteristics varied significantly by season using conditional logistic regression (for binary factors) or by fixed-effect linear regression (for continuous factors). We assessed whether the season-BV association varied by race, or by BV status at study entry, by including a product interaction term between season and race (African-American vs. all other races) and between season and initial BV status. All calculations were conducted using SAS 9.3 or STATA 12. A two-tailed p-value <0.05 was considered statistically significant, and no correction was made for multiple comparisons.[22]

RESULTS

The Longitudinal Study of Vaginal Flora enrolled 3620 women. This analysis was limited to women who were seen at least once in each season. There were 2337 such women: 714 never had BV at any visit, 288 had BV at all visits, and 1335 had BV at some but not all visits. Thus the final sample comprised the 1335 women (contributing 6392 visits) whose BV status varied during the study.

The cohort was predominantly African-American, low-income and overweight-to-obese (Table 1). The frequency of BV at all visits, for all women and separately by BV status at the initial visit, is presented in Table 2. Among women BV-negative at entry there was no difference in BV by season, but among women BV-positive at entry, BV was more common in the summer and fall than in winter or spring. The frequency of BV at the enrollment visit did not vary significantly by season of that visit (46.1% in winter, 46.1% in spring, 50.6% in summer, 48.0% in fall, p=0.62). Dietary intake data [23] were available at a single point in time from 909 of the 1335 women, and mean total vitamin D intake, from both diet and supplements, did not differ by season (188 IU in winter, 214 in spring, 192 in summer, 204 in fall, p=0.47).

Table 3 describes modest seasonal variation in confounding factors. Both vaginal sex frequency and number of recent sex partners were elevated in the summer. Women weighed more and reported more alcohol consumption at winter visits, but were less likely to have recently received metronidazole at summer visits. They were more likely to use powder on their genitals in the fall, and towlettes in the winter and spring. The prevalence of showering was highest in the summer. None of the other measured behaviors varied significantly by season.

Unadjusted and adjusted odds ratios for BV in each season are presented in Table 4. Among all women, compared to winter, the unadjusted odds ratios for BV were 0.9 (95% confidence interval 0.8–1.1) in the spring, 1.2 (1.01–1.4) in the summer, and 1.1 (0.97–1.3) in the fall. However, the seasonal effect on BV differed significantly (p<0.001) by whether the woman was BV-negative or positive at study entry. Among women BV-negative at entry, the odds ratios for BV, compared to winter, were 1.0 (0.8–1.2) in spring, 1.0 (0.8–1.2) in summer, and 0.9 (0.8–1.1) in fall; the overall association between season and BV was not significant (p=0.81). However, among women BV-positive at entry, the odds ratios were 0.9 (0.7–1.1) in spring, 1.4 (1.2–1.7) in summer, and 1.4 (1.1–1.7) in fall; the overall association between season and BV was highly significant among these women (p<0.001).

Adjustment for sexual, health and hygienic behaviors had little impact on the odds ratios. Among all women, BV remained most prevalent in the summer, least prevalent in spring, and intermediate in winter and fall. Among women who were BV-negative at entry, season remained unassociated with BV; among women who were BV-positive at entry, BV was most common in the summer and fall, least common in the spring and intermediate in winter. In none of the models did the association between season and BV differ between African-Americans and women of other races (all p-values for interaction between race and season were >0.4).

DISCUSSION

This study, which compared the same women across all four seasons, did not find that BV was more common in the winter or spring months, when serum vitamin D is expected to be lowest. Rather, season was unassociated with BV among women who were BV-negative at study entry, while among women who were BV-positive at entry, BV was statistically significantly more common in the summer and fall, when serum vitamin D is expected to be

highest. Adjustment for numerous sexual, hygienic and other factors that varied with season had minimal impact on the results. Our results suggest that vitamin D insufficiency is unlikely to explain increased BV risk.

BV has a high rate of relapse and remission.[24,25] It is plausible that women with BV at enrollment are more susceptible to future BV episodes. Residual or unmeasured confounding by sexual, hygienic or other behavioral factors (which appear to be somewhat more common during the same seasons when BV prevalence was highest), rather than vitamin D insufficiency, may be responsible for the variation in BV by season observed in these women. On the other hand, women who were BV-negative at enrollment may have lower susceptibility to BV, such that the variation in BV-associated behaviors by season was not profound enough to lead to differences in BV prevalence by season. Alternatively, seasonal differences in BV by baseline BV status may be explained by seasonal differences in factors associated with BV incidence vs. BV remission. Women who were BV-negative and then became positive experienced incident BV; in contrast, women who were BV-positive at enrollment and became negative during their follow-up experienced a remission. The etiology of BV is not well-understood, and it is possible that different factors are associated with the development of BV and with conversion to healthy vaginal flora. However, we believe that in neither cohort (BV-positive and BV-negative at enrollment) do our results support a strong role for vitamin D insufficiency in the pathogenesis of BV.

Previous studies of the association of vitamin D with BV have had inconsistent results. Reduced serum vitamin D has been associated with increased BV prevalence in pregnant women.[8–11] However, there have been few studies conducted in non-pregnant women. One did not find an association between vitamin D and BV,[8] and the other found an association between reduced serum vitamin D and increased BV prevalence in HIV-positive, but not HIV-negative women.[12]

Strengths of the study include a large number of women who were followed over all four seasons, systematic assessment of bacterial vaginosis, and collection of detailed data on individual behaviors that might confound a seasonal difference in BV. Use of the crossover design has the advantage of inherently controlling for many factors which are difficult to measure and could confound the association between season and BV, yet also brings the limitation that only women who changed BV status over the year were included in the analysis. The crossover design inherently excludes both women who had BV at every visit and women who never had BV at any visit; 43% of women who were seen in every season were not included in the analysis for this reason. Although not biasing *per se*, this limitation means that the associations in this report apply only to women who were intermittently BV-positive.

A second limitation is that season was used as a proxy for vitamin D status but no actual serum concentrations were measured. In temperate climates, serum vitamin D concentration is generally higher in the summer and fall than in the winter and early spring,[26–29] but seasonal fluctuations in serum vitamin D may be less among African-Americans than among whites.[26,28,29] However, the seasonal changes we observed in BV prevalence were similar among African-American and white women. Seasonal variation also may differ by

latitude. At 42 degrees latitude or higher, sun exposure in winter did not result in sufficient ultraviolet B radiation to produce previtamin D, but at 34 degrees latitude or less (which includes Birmingham), exposed skin can produce previtamin D in both winter and summer.[30] Therefore the seasonal fluctuation in vitamin D may be less in the southern than in the northern U.S.

Behavior may modify seasonal variation, particularly if people in hot climates limit their outdoor activities during summer days and increase them during winter days. Such behavior has been posited to explain a paradoxical seasonal variation in umbilical cord 25-OH-vitamin D concentration, a proxy for maternal third trimester concentration, in New Orleans.[31] However, a cross-sectional study of umbilical cord serum 25-OH-vitamin D concentration in Charleston, SC, which is at comparable latitude to Birmingham, found significantly higher values among both white and African-American women from April 1 to October 31 than from November 1 to March 31.[29] Dietary changes also can blunt or even reverse the seasonal variation in serum vitamin D,[32] although in our population dietary vitamin D intake did not vary by season. Therefore, we believe that season is a good proxy for serum vitamin D in these predominantly African-American women in Birmingham Alabama.

A final limitation is that although we controlled for numerous factors that could vary by season and also might cause BV, there may be relevant factors that were not measured. Furthermore, adjustment for hygienic behaviors would be inappropriate if these behaviors were undertaken to alleviate symptoms of BV. However, adjustment for hygienic behaviors had little impact on the results. Since visits took place throughout the entire season and the questionnaire asked only about events since the last visit, the timing of behaviors might not correspond to the season of interview, particularly for women interviewed early in the season.

In summary, our findings that BV is more common in the summer than the winter, particularly among women who were BV-positive at study entry, do not support an association between vitamin D, measured through the proxy variable of season, and BV. If vitamin D insufficiency is a risk factor for BV, our results suggest that its effect is slight or dwarfed by other, more proximal factors that vary by season. Future studies should assay serum vitamin D concentration in a group of women followed across multiple seasons, as well as investigate the role of other factors that vary by season, such as physical activity, temperature and daylight length. Answers to these questions may provide new insights into the enigmatic pathogenesis of BV.

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SUMMARY

In a longitudinal study, bacterial vaginosis was more common in summer and fall among women BV-positive at entry, but season was not associated with BV among women BV-negative at entry.

Table 1

Characteristics of the Study Population of Women Who Had Bacterial Vaginosis at Some, but not All Visits (n=1335)

Baseline Characteristic	
Race (n (percent), 1 missing)	
African-American	1170 (88%)
Other	164 (12%)
Years of education (n (percent), 3 missing)	
<12	500 (38%)
12	517 (39%)
>12	315 (24%)
Household income, $/month (n (percent), 102 missing)	
<500	278 (23%)
500–800	371 (30%)
801–3000	517 (42%)
3001+	67 (5%)
Age, years (mean (SD), 1 missing)	24.7 (6.9)
Weight, kg (mean (SD), 9 missing)	79.9 (23.9)
Height, cm (mean (SD), 9 missing)	163.7 (6.7)
Body Mass Index, kg/m^2 (mean (SD), 11 missing)	29.7 (8.4)

Table 2

Prevalence of Bacterial Vaginosis by Season

	Winter	Spring	Summer	Fall	P-value
All women (n=1335*)	48%	45%	51%	50%	0.007
BV-negative at entry (n=689)	43%	42%	42%	40%	0.81
BV-positive at entry (n=639)	53%	48%	60%	60%	<0.001

* BV status at first visit missing for 7 women

Table 3

Seasonal variation in confounding factors

Factor	Winter	Spring	Summer	Fall	P-value
Sexual Factors					
Mean vaginal sex frequency*	2.2/week	2.2/week	2.4/week	2.4/week	0.004
Mean oral sex frequency*	0.6/week	0.6/week	0.6/week	0.6/week	0.79
Mean number recent sex partners*	0.90	0.90	0.97	0.95	<0.001
New sex partner*	8%	8%	8%	8%	0.78
Hormonal contraception	47%	47%	49%	49%	0.79
Health-related Factors					
Weight (kg)	81.8	80.6	80.2	81.2	<0.001
Smoking	28%	29%	28%	30%	0.18
Alcohol drinking	54%	53%	51%	51%	0.009
Metronidazole since last visit**	15%	15%	13%	15%	0.04
Hygiene-related Factors					
Douching*	43%	43%	44%	45%	0.14
Powder on genitals*	23%	23%	21%	25%	0.03
Towelette use*	9%	8%	7%	7%	0.03
Feminine deodorant spray*	13%	12%	10%	12%	0.07
Showers only*	9%	9%	11%	9%	†
Baths only*	15%	16%	15%	15%	†
Baths and showers*	76%	75%	74%	77%	†

*In past 6 months at initial study visit, in the past 3 months for follow-up visits

**In past 2 weeks for first study visits, since previous visit for follow-up visits

†shower vs bath and shower, p=0.01; bath only vs. bath and shower, p=0.13; bath only versus shower only, p=0.07.

Table 4

Odds ratio for Bacterial Vaginosis by Season

	Winter	Spring	Summer	Fall	P-value
All Women					
Unadjusted	1.0	0.9 (0.8–1.1)	1.2 (1.01–1.4)	1.1 (0.97–1.3)	0.007
Adjusted for sexual factors	1.0	0.9 (0.8–1.1)	1.2 (1.00–1.3)	1.1 (0.96–1.3)	0.01
Adjusted for sexual and health-related factors	1.0	0.9 (0.8–1.1)	1.2 (0.99–1.3)	1.1 (0.96–1.3)	0.01
Adjusted for sexual, health-related and hygienic factors	1.0	0.9 (0.8–1.1)	1.2 (1.01–1.4)	1.1 (0.96–1.3)	0.01
Women BV-negative at Study Entry					
Unadjusted	1.0	1.0 (0.8–1.2)	1.0 (0.8–1.2)	0.9 (0.8–1.1)	0.81
Adjusted for sexual factors	1.0	1.0 (0.8–1.2)	1.0 (0.8–1.3)	0.9 (0.8–1.2)	0.83
Adjusted for sexual and health-related factors	1.0	1.0 (0.8–1.2)	1.1 (0.9–1.3)	0.9 (0.8–1.2)	0.75
Adjusted for sexual, health-related and hygienic factors	1.0	1.0 (0.8–1.2)	1.0 (0.8–1.3)	0.9 (0.8–1.2)	0.86
Women BV-positive at Study Entry					
Unadjusted	1.0	0.9 (0.7–1.1)	1.4 (1.2–1.7)	1.4 (1.1–1.7)	<0.001
Adjusted for sexual factors	1.0	0.9 (0.7–1.1)	1.4 (1.1–1.7)	1.3 (1.1–1.6)	<0.001
Adjusted for sexual and health-related factors	1.0	0.8 (0.7 -1.03)	1.3 (1.1–1.7)	1.3 (1.1–1.7)	<0.001
Adjusted for sexual, health-related and hygienic factors	1.0	0.8 (0.7–1.1)	1.4 (1.1–1.7)	1.4 (1.1–1.7)	<0.001

Adjusted by conditional logistic regression. Sexual factors- frequency of vaginal intercourse, frequency of receptive oral sex, number of recent sex partners, new partner since last study visit, current hormonal contraception use. Health factors- current weight, smoking, alcohol, receipt of metronidazole since last study visit. Hygienic factors- douching, power, towelette, genital deodorant spray, shower versus bath.