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Bacterial Vaginosis and Risk for *Trichomonas Vaginalis* Infection: A Longitudinal Analysis

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Abstract

Background—Bacterial vaginosis (BV) and *Trichomonas vaginalis* (TV), have been estimated to affect one-quarter to one-third of sexually active women worldwide, and are often found concurrently. Few studies have examined this relationship longitudinally to better understand the direction and temporality of this association.

Methods—Between 2005 and 2006, a cohort of 853 young, sexually active women was followed in Mysore, India; participants were interviewed and tested for BV and TV at baseline, and at three- and six-month visits. Generalized estimating equations were used to estimate how changes in vaginal flora between consecutive visits – as defined by Nugent diagnostic criteria for BV - were related to the risk of TV infection at the latter visit, adjusted for sociodemographic and behavioral covariates. Treatment was offered to women with TV and/or symptomatic BV.

Results—After adjustment for covariates, participants with abnormal flora at two consecutive visits had nine times higher risk of TV (95% CI 4.1, 20.0) at the latter visit, relative to those with persistently normal flora. An increased risk of TV was also observed for participants whose flora status changed from normal to abnormal (aRR 7.11, 95% CI 2.8, 18.2) and from abnormal to normal (aRR 4.50, 95% CI 1.7, 11.8).

Conclusions—Women experiencing abnormal flora during a three-month span appear to have significantly increased risk of acquiring TV infection. Reproductive-age women in low-resource settings found to have abnormal vaginal flora should be assessed for TV.

Keywords

Bacterial vaginosis; *Trichomonas vaginalis*; India; women; longitudinal analysis

INTRODUCTION

Bacterial vaginosis (BV) and *Trichomonas vaginalis* (TV) infection have been estimated to affect as many as one-quarter to one-third of sexually active females worldwide(1,2), and

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are often found concurrently(3). Both BV and TV have been associated with adverse birth outcomes such as prematurity and low birth weight(4,5), pelvic inflammatory disease(6,7), infertility(8,9), and acquisition of HIV and Herpes Simplex Virus type 2 (HSV-2) infections(1,10–12). Surprisingly, given the well-described association between BV and TV, few data that would help clarify the temporality of the relationship between BV and TV infection are available.

Understanding the relationship between BV and TV has been problematic because most studies have been cross-sectional in nature(13–17). Nevertheless, results of a number of studies suggest that TV colonization is increased in the presence of BV-defining phenomena such as elevated amine production, loss of facultative lactobacilli, and increased pH(18–22). The present study describes the results of a secondary analysis of data originally collected in a prospective cohort study examining the relationship of abnormal vaginal flora and incident HSV-2 infections among young reproductive age women in Mysore, India.

MATERIALS AND METHODS

Participant recruitment and laboratory methods have been described in detail elsewhere(23–25). In brief, 15–30 year old, non-pregnant, sexually active women were recruited in 2005–2006, through health education camps offered in the rural and peri-urban communities around Mysore city, in south India. At baseline, and three- and six-month visits, participants underwent an interviewer-administered questionnaire and a physical examination, during which biological specimens, including vaginal, high cervical swabs and venous blood, were collected. Of the 898 women who completed the baseline visit, 853 provided data from at least 2 consecutive study visits. These 853 participants comprise the study sample for this analysis.

TV infection was diagnosed based on a positive result from either wet-mount microscopy for detection of motile Trichomonads and/or culture (InPouch Culture Kit, BioMed Diagnostics, White City, OR, USA). All women had specimens collected for both TV tests. Women with TV infection were treated with a single dose of two grams of oral metronidazole, and the same treatment was given to participants to give to their sex partners. BV was diagnosed clinically using Amsel's criteria and treated with 400 mg oral metronidazole twice daily for one week(26). For analytic purposes, BV was diagnosed by Nugent scoring of gram stained vaginal smears(27). ELISA testing was done for HSV-2 IgG antibodies (Focus Technologies, Cypress, CA, USA). Institutional review boards at the University of California, Berkeley, and the Asha Kirana Hospital in Mysore, India, approved the study protocol. Informed consent was obtained from all study participants.

Statistical analysis

In a cross-sectional analysis of baseline data, increased prevalence of TV was found among those testing positive for BV (Nugent score 7–10) and among those with Intermediate scores (Nugent score 4–6), both of which indicate the presence of abnormal vaginal flora(24). Thus, for this analysis Nugent scores were categorized into a dichotomous measure of vaginal flora status, with 0–3 considered 'Normal' and 4–10 'Abnormal'(27). To estimate the relationship between changing vaginal flora status and TV infection risk, log-linear generalized estimating equations (GEE) with an exchangeable covariance structure, and bootstrapped results with 5000 repetitions for robust standard errors, were used(28). Four exposure patterns were defined based on participant vaginal flora status on a given visit (Abnormal or Normal) and status at the previous visit (Abnormal or Normal). The four patterns represent different exposure periods of abnormal vaginal flora between visits. With three study visits, participants could contribute two exposure patterns (for 0 to 3 months, and 3 to 6 months) to the GEE model, to estimate the risk of TV at 3 and 6 months, respectively.

The GEE model provides covariate-adjusted estimates of risk ratios (aRR) for TV by different vaginal flora exposure patterns between visits, compared to those whose Nugent tests scores were Normal at both visits (Figure 1). Eight hundred and twenty participants contributed at least one observation to the GEE model; unreadable vaginal smears accounted for all but three of the 33 participants not included in the analysis.

The selection of possible confounders and cut points for categorization of continuous variables were based on findings from the baseline cross-sectional analyses for BV and TV infections(12,24) and previously published literature. For the multivariable GEE model, variables with small strata (<10% of sample) were not controlled for. Strata from ordinal categorical measures were combined if the baseline TV analysis showed similar prevalences of TV across adjacent groups. For the religious identification measure, Hindu and Christian women were combined into a single 'Non-Muslim' category. In three observations where a participant's marital status was not recorded, her marital status at the previous visit was used, as there was minimal change in marital status over time in this cohort. For HSV-2 testing, 21 missing results were recoded to negative, as HSV-2 incidence was low in this cohort. A socioeconomic (SES) index was created using the first factor generated from a principal components analysis of assets owned, financial instruments used, and dummy-coded cooking stove type(29). SES score was made into a dichotomous categorical measure to create balanced categories of Low and High SES. Two participants with missing asset data were coded into the Low SES category.

Two additional GEE models were run to examine the sensitivity of results due to the clinical definition of abnormal flora. In the first case, abnormal flora was defined by a Nugent score of seven or higher. In the second case, abnormal flora was defined by an Amsel score of three or higher. A third GEE model was run using our original definition of abnormal flora (Nugent score of four or higher), but using only incident TV infections. All three additional GEE models used the same covariate and bootstrapping specifications as the original model. Data were analyzed using Stata 11.1 (StataCorp, College Station, TX, USA).

RESULTS

A description of the full cohort with baseline TV prevalence has previously been reported elsewhere (12); additional baseline measures are described here for the analyzed cohort. A majority of women had been with their partners for 7–19 years (78.1%), and these women were at higher risk of a TV diagnosis (9.4% vs. 6.5% for women who had partners for less than seven years). Women were equally divided across SES categories, with a higher prevalence of TV found in the lower SES category (10.6% vs. 6.4% with higher SES). While a large majority (69.1%) of women reported at least 13 sex acts in the three months prior to the baseline visit, prevalence of TV did not vary across sexual activity categories. Very few women reported having a partner who had other sex partners, or using an intrauterine device for contraception, leading to unstable estimates of the prevalence of TV in these strata. No participants reported smoking, using hormonal contraception or vaginal douching. These latter measures were thus not included as potential confounders in the multivariable GEE model.

Table 1 shows TV risk by change in vaginal flora status between the baseline and three-month visits. For ease of reporting, only the baseline- to three-month visit results are tabulated; results from the three- to six-month visits (data not shown) demonstrate a similar risk gradient. A majority of women had normal flora at both visits (54.9%), and few of these women (1.4%) tested positive for TV at three months. Fewer women had normal flora at baseline and abnormal flora at the three-month visit (12.4%), though a higher proportion tested positive for TV at three months (6.2%). Of the women who had abnormal flora at

baseline and normal flora at the three-month visit (13.2%), a similar proportion (7.8%) tested positive for TV at three months. Approximately one in five women had abnormal vaginal flora at both the baseline and three-month visit (20.6%), and these women were at greatest risk for testing positive for TV at the three-month visit (13.0%).

Prevalence and incidence of TV infection and abnormal vaginal flora

Prevalence of TV declined by study visit, from 8.5% at baseline to 5.5% at three months and 3.0% at six months. Of the 775 women who were TV negative at baseline, 23 (3.0%) had TV diagnosed at their three month follow-up visit and eight (1.1%) had TV at the six-month visit; of those 8, 5 were first-time TV diagnoses. In comparison, of the 73 women who had TV at baseline, 24 (32.9%) were TV infected again at the three-month visit, and 10 had TV at all three visits, despite having received repeated metronidazole treatment. The prevalence of abnormal vaginal flora also decreased by visit, from 33.7% at baseline to 32.4% at three months and 28.7% at six months. Of the 526 women with normal vaginal flora at baseline, 35/526 (6.6%) were Amsel positive for BV, and 97/526 (18.4%) had abnormal flora at three months. In comparison, of the 264 women with abnormal flora at baseline, 60/264 (22.7%) were Amsel positive, and 161/264 (61.0%) had abnormal flora again at three months, and 94 participants had abnormal flora at all three visits. For all visits, Amsel clinical diagnosis resulted in treatment of 4.1% of those with normal flora (Nugent score 0–3), 18.3% with intermediate flora (Nugent score 4–6), and 34.7% with BV (Nugent score 7–10).

TV risk by multivariable GEE analysis (Table 2)

Participants with abnormal flora across any two consecutive visits had nine times higher risk (95% CI 4.1, 20.0) of TV infection at the latter visit relative to those women with persistently normal flora, adjusted for covariates. Women whose flora became abnormal after being normal at the previous visit had a higher risk of TV (aRR 7.11, 95% CI 2.7, 18.2), as did those whose flora became normal after being abnormal at the previous visit (aRR 4.50, 95% CI 1.7, 11.7). Of the covariates examined, Muslim religion (aRR 0.44, 95% CI 0.2, 1.1), having a partner for at least seven years (aRR 1.94, 95% CI 0.9, 4.0) and HSV-2 infection (aRR 1.68, 95% CI 0.9, 3.0) each had weaker evidence for an association with TV infection.

Table 3 shows results from the alternate GEE models. The GEE models using different definitions for abnormal flora provided results that were all consistent in direction, though with reduced magnitude relative to the original GEE model. For the three longitudinal exposure categories Normal-Abnormal, Abnormal-Normal and Abnormal-Abnormal, the model using Nugent scores of seven or higher to indicate abnormal flora resulted in aRR of 4.12 (95% CI 1.9, 8.9), 4.44 (95% CI 2.0, 10.1) and 3.15 (95% CI 1.4, 7.0) respectively and the model using Amsel score of three or higher resulted in aRRs of 2.81 (95% CI 1.5, 5.3), 1.94 (95% CI 0.7, 5.8; $p=0.215$) and 4.89 (95% CI 2.3, 10.5) respectively. The GEE model using our original abnormal flora definition of Nugent scores of four or higher but only including incident TV infection resulted in aRR of 8.59 (95% CI 3.2, 22.7), 3.15 (95% CI 0.9, 10.5; $p=0.043$) and 4.75 (95% CI 1.8, 12.6), respectively. Unless otherwise indicated, all p -values were less than 0.005.

DISCUSSION

We found evidence for a 4- to 9-fold increased risk of TV infection among women who had abnormal vaginal flora within a three month span, with the highest risk among those women found to have abnormal flora at consecutive visits. Our alternate GEE models indicate the relationships are generally maintained when other widely accepted abnormal flora measurement criteria are used, and for incident TV infection. This finding is consistent with

the findings of cross-sectional studies showing that a disturbed vaginal flora is associated with an increased risk of sexually transmitted infections, including HIV and TV(13,30–33). It also confirms the findings from longitudinal studies in Kenya and the United States(20,22), which show that abnormal vaginal flora is associated with subsequent acquisition of TV infection, and thus strengthens the evidence for a causal role. Thurman, Doncel and others have suggested a number of mechanisms whereby disturbed vaginal flora might increase the risk of HIV and other STI infections, including: initiation of a clinical or subclinical mucosal inflammatory response; alteration of innate mucosal immunity; alteration of normal vaginal microflora and pH; and weakening or breach of the cervico-vaginal mucosa(22,34–38). As other studies have suggested, all of these mechanisms could also plausibly pertain to the relationship between abnormal vaginal flora and acquisition of TV infection.

The declining prevalence of TV infection and abnormal flora status over time in this cohort is unsurprising, given that the women were treated with metronidazole, which has been shown to be effective in treating both conditions(39,40). As TV infection is typically self-limiting in men(41), and reported extra-marital partnerships were low among participants, the reduction in prevalence from 8.5 to 3.0% after two rounds of diagnosis and treatment was expected, and demonstrates that control of this infection with metronidazole in resource-limited settings is possible. In contrast, the small reduction in the prevalence of abnormal flora is of concern, as is the high rate of repeat diagnosis. Treatment based on the Amsel diagnostic criteria identified only 35% of women who were found to have BV based on Nugent test (and 18% of women with Intermediate Nugent scores). In this setting, the Amsel criteria did not appear to be sufficiently a sufficiently sensitive method for diagnosis of BV. This is also a notable finding because BV has been found to increase the risk for STIs and adverse birth outcomes, which are commonly found in resource constrained settings(42).

Consistent with other studies that found Muslim religion to be associated with a lower risk of HIV and STI, our study found that the risk of TV was lower among Muslim women, which could be due to cultural practices, such as male circumcision among their sex partners(43,44). We also found some evidence of an increased risk of TV infection associated with HSV-2 infection, consistent with the findings of other studies of TV(16,45). HSV-2 infection has been shown to increase the risk of acquisition of STIs because of genital ulcers(46), and may be a proxy of past sexual risk behavior(47).

This study has a number of strengths, including: a large sample size, standardized laboratory testing for TV and BV, minimal loss to follow up, and the use of longitudinal measures. On the other hand, there were also several limitations: First, we did not use a population-based sample, so our findings may not be generalizable. Second, as vaginal flora are highly dynamic(48), it is possible that we underestimated new abnormal flora episodes between visits. Third, because we did not do a ‘test of cure’ for women treated in the study, we do not know whether TV infections were new or persistent. Because metronidazole treatment is thought to be 95% effective(49), it is unlikely that this limitation would significantly affect the findings. Furthermore, although all women diagnosed with TV were provided with treatment for their sex partners, we could not ascertain whether partners were offered or accepted the treatment. What appears to be repeated TV infection may be prevalent infections from the perspective of the sexual dyad, and as such the results from this analysis cannot fully rule out reverse causation. However, the final GEE model, using only incident TV infection, does provide additional evidence of abnormal flora preceding TV infection. Finally, since a minority of participants with abnormal vaginal flora was treated based on Amsel criteria, the aRR estimates are likely biased towards the null.

In conclusion, the findings of this study emphasize the need to screen for TV when women are found to have abnormal vaginal flora. More research is needed to help ascertain the mechanisms or co-factors involved in the relationship between BV and acquisition of TV including common exogenous factors, such as other sexually transmitted infections and host immune factors. It will be important to understand the mechanisms by which abnormal vaginal flora enhances susceptibility to TV.

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For i =participants 1 to 853 and j =visits 2 to 3:

$$\log[\Pr(TV)_{ij}=1 \mid \text{covariates}] = \beta_0 + \beta_1(\text{abnormal flora})_{ij} + \beta_2(\text{abnormal flora})_{ij-1} + \beta_3(\text{age of sexual debut})_{i1} + \beta_4(\text{years with partner})_{i1} + \beta_5(\text{years of education})_{i1} + \beta_6(\text{religion})_{i1} + \beta_7(\text{vaginal sex acts in past 3 months})_{ij} + \beta_8(\text{asset index score})_{i1} + \beta_9(\text{HSV2})_{ij}$$

Figure 1.
GEE model specification

Table 1

Stratum-specific *Trichomonas vaginalis* infection by baseline to three-month visit vaginal flora status, Mysore, India 2005–2006

	Retained at three-month visit (n=790) *		Of which TV positive (n=41) at three-month visit	
	n	%	n	%
Vaginal flora status **				
Normal-Normal	429	54.9	6	1.4
Normal-Abnormal	97	12.4	6	6.2
Abnormal-Normal	103	13.2	8	7.8
Abnormal-Abnormal	161	20.6	21	13.0

* 58 women were missing one or both vaginal flora specimens, of which 6 (10.3%) were positive for TV at the three-month visit.

** Normal flora is defined as a Nugent score of 0–3, Abnormal flora is a Nugent score of 4–10.

Table 2

Log-linear multivariable GEE model results for *Trichomonas vaginalis* infection at three- or six-month visits, Mysore, India 2005–2006

	Adjusted RR	95% CI	p
Vaginal flora status change ⁺			
Normal-Normal	1.00		
Normal-Abnormal	7.11	2.77, 18.24	***
Abnormal-Normal	4.50	1.72, 11.77	***
Abnormal-Abnormal	9.00	4.05, 20.02	***
Age of sexual debut (years)			
7–14	1.00		
15–29	0.96	0.56, 1.64	
Years with partner			
0–6	1.00		
7–19	1.94	0.94, 4.00	*
Years of education			
0	1.00		
1–17	1.29	0.76, 2.18	
Religion			
Hindu/Christian	1.00		
Muslim	0.44	0.17, 1.13	*
Vaginal sex acts in past 3 months			
0–12	1.00		
13–120	0.80	0.47, 1.36	
SES			
Low	1.00		
High	0.72	0.43, 1.20	
HSV-2 positive			
No	1.00		
Yes	1.68	0.94, 3.03	*

*
p < 0.10

p < 0.005

⁺ Normal flora is defined as a Nugent score of 0–3, Abnormal flora is a Nugent score of 4–10.

Sensitivity of log-linear multivariable GEE model results for *Trichomonas vaginalis* infection at three- or six-month visits, Mysore, India 2005–2006

Table 3

	Abnormal flora is Nugent ≥ 7 , all TV infection			Abnormal flora is Amsel ≥ 3 , all TV infection			Abnormal flora is Nugent ≥ 4 , incident TV infection		
	aRR	95% CI	p	aRR	95% CI	p	aRR	95% CI	p
Vaginal flora status change									
Normal-Normal	1.00			1.00			1.00		
Normal-Abnormal	4.12	1.91, 8.90	***	2.81	1.49, 5.26	***	8.59	3.24, 22.71	***
Abnormal-Normal	4.44	1.96, 10.07	***	1.94	0.65, 5.75		3.15	0.94, 10.50	*
Abnormal-Abnormal	3.15	1.41, 7.03	***	4.89	2.27, 10.54	***	4.75	1.79, 12.59	***

aRR = Adjusted Risk Ratio

*
p < 0.10

p < 0.005