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A RANDOMIZED TRIAL OF METRONIDAZOLE IN ASYMPTOMATIC BV TO PREVENT ACQUISITION OF STDS

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Abstract

Objective—To determine if treatment of BV decrease the incidence of sexually transmitted diseases.

Study Design—Women with asymptomatic BV were studied prospectively to determine the effect of treatment of BV for the prevention of STD. Women were randomized to observation or treatment and prophylaxis with intravaginal metronidazole gel. Women were screened monthly for STDs.

Results—Women randomized to metronidazole gel had a significantly longer time to development of STD compared to women in the observation group (p=0.02). The 6-month STD rate was 1.58/ person-year (95% CI 1.29, 1.87) for women in the metronidazole gel group versus 2.29/person-year (95% CI 1.95, 2.63) for women in the observational group. The difference in STD rates was driven by a significant difference in the number of chlamydial infections (0.013).

Conclusion—Treatment and twice-weekly prophylactic use of intravaginal metronidazole gel resulted in significantly fewer cases of chlamydia.

Keywords

Bacterial vaginosis; STD; metronidazole; chlamydia

Introduction

Bacterial vaginosis (BV) is the most common form of vaginitis worldwide. It is characterized by a shift in the microbial flora resulting in marked decreases in protective lactobacilli and increases in anaerobes and *Gardnerella vaginalis* [1] . Bacterial vaginosis has been consistently associated with adverse outcomes including acquisition of HIV and other sexually transmitted diseases (STDs) in cross-sectional studies and in some prospective studies [2-4]. Although multiple factors may be involved, hydrogen-peroxide producing lactobacilli have been shown in-vitro to inhibit the growth of bacteria as well as HIV and their absence in women with BV is likely to be a biological risk factor for STD/HIV acquisition [5, 6]. There are two published studies on the treatment of STDs including BV to prevent HIV acquisition [7, 8] , however, there are no published studies examining the effect of BV treatment on acquisition of other STDs. Treatment of symptomatic BV is necessary to alleviate the bothersome symptoms. However,

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The data has not previously been presented

Condensation Women with BV who were treated and placed on twice weekly metronidazole gel had a significantly longer time to develop chlamydia than women not treated.

treatment of asymptomatic BV is currently not recommended except in special circumstances [9]. Therefore, we were able to conduct a prospective study on the treatment of asymptomatic BV as a means of preventing STDs.

Materials and Methods

Women attending the Jefferson County Department of Health (JCDH) STD Clinic in Birmingham, AL were invited to participate in this prospective study. Approval for the study was obtained from the Institutional Review Boards (IRB) of the University of Alabama at Birmingham (UAB) and the JCDH Department of Health. Written consent was obtained from all participants and human experimentation guidelines of the UAB IRB were followed in the conduct of this clinical research. Women were screened for vaginal infections and STDs as part of their routine clinic visit. Women with asymptomatic BV, defined by Nugent criteria and lack of report of vaginal odor and/or discharge upon direct questioning were invited to participate in the trial. Women with symptomatic BV (vaginal odor and/or discharge) were not eligible to participate since they required therapy to alleviate their symptoms. Women were randomized to receive treatment for BV versus observation alone since an appropriate placebo was not available for use. A computer generated randomization scheme was prepared with random blocks of 2 or 4 which was provided to the study pharmacist. Treatment assignments were maintained by sealed envelopes until randomization. Treatment consisted of intravaginal metronidazole gel at bedtime for 5 days, followed by twice weekly use for 6 months to prevent recurrences[10]. Women were followed monthly for the first six months, then every three months for a total of one year. No treatment was administered during the final six months unless clinically indicated for symptomatic BV. Screening for STDs, including gonorrhea, chlamydia, trichomonas and herpes simplex virus type 2, was performed at each visit. Pelvic inflammatory disease was based on a clinical diagnosis of complaints of abdominal pain and adnexal tenderness and/or cervical motion tenderness on examination. Incident HSV-2 infection was defined as a conversion from a negative to a positive serological result. Women with an STD at baseline were ineligible for the study with the exception of women who were seropositive for HSV-2. Women who developed an STD or a vaginal yeast infection during the course of the study (with the exception of asymptomatic HSV) were treated appropriately and continued in the study. Women who developed symptomatic BV during the course of the study were re-treated with a 5-day regimen of metronidazole gel and then asked to resume prophylactic twice-weekly therapy.

Microbiological Methods

Vaginal fluid pH, microscopy, and “whiff test were performed as previously described [1]. Vaginal Gram stains were interpreted according to the method of Nugent et al [11]. Scores of 0-3 were considered normal, 4-6 as evidence of intermediate flora and 7-10 as BV. Urine specimens were tested for *Neisseria gonorrhoeae* and *Chlamydia trachomatis* using nucleic acid amplification techniques (Abbott Laboratories, Abbott Park, IL and Gen-Probe, San Diego, CA). The presence of *Trichomonas vaginalis* was detected using the InPouch TV culture technique (BioMed Diagnostics, Inc, White City, OR). Type specific serology for HSV was performed using at type–specific ELISA using recombinant gG2 for HSV-2 (HerpeSelect, Focus Technologies, Herndon, VA).

Statistical Analysis

Sample Size

Sample size estimates were derived to demonstrate a difference in STD-free survival between the two groups with 80% power, 5% alpha based on a two-sided log rank test. Assumptions included that 60% of women in the observation group would not develop and STD after six

months of follow-up compared to 75% in the treatment group and a drop-out rate of 10% per month for the first six months in each group. This resulted in an estimated sample size of 146 total women.

Descriptive

Categorical variables are compared by the X^2 test for Fisher's exact test. Continuous variables are compared by the t-test or the Wilcoxon-rank sum test, if appropriate.

Time to First STD and STD Rates

For follow-up analysis, an STD was defined as either gonorrhea, chlamydia, trichomoniasis, HSV or PID. BV was categorized as positive (≥ 7), intermediate (4-6), and negative (<4). Time to first episode of STD was defined as the number of days from baseline to development of first episode of STD. STD rates per person-year were calculated as the sum of all episodes of STD divided by the sum of all the follow-up days for the same group of persons during the same time period, expressed as person-years. Because treatment was only administered for the first six months of the study, we calculated the STD rates for the first six months for comparative purposes. The 95% confidence interval for rates was calcualated assuming the Poisson distribution for incidence. The probability of remaining STD free was modeled using the Kaplan-Meier method.

Results

One hundred seven women were enrolled into the study with 54 randomized to observation and 53 to metronidazole gel (Figure I). Table 1 shows the distribution of baseline demographic and behavioral factors for women enrolled into the study. The mean age of the participants was 25.1 years and all were African-American. There were no significant differences in baseline demographic and clinical factors, including the percentage of adolescents enrolled into each arm.

Time to Development of STD and STD Rates

Women randomized to metronidazole gel experienced a significantly longer time to development of STD compared to women randomized to observation (p= 0.02) (Figure II). The median time to develop STD was 94 days in the observational group and 138 days in the metronidazole group. The 6-month rate of STDs was 2.29/person-year (95% CI 1.95, 2.63) in the observational group compared to a 6-month STD rate of 1.58/person-year (95% CI 1.29, 1.87) in the metronidazole group which was significantly lower. A comparison of STD rates for the 6-month period following the end of the intervention (months 7-12) found no significant differences between the groups. The overall STD rates for the entire 12-month period was 1.64 (95 % CI 1.20, 2.08) in the observational group and 1.31 (95% CI 0.90, 1.72) in the treatment group. Follow-up was similar in both cohorts (31.12 person-years in the placebo group and 29.73 person-years in the treatment group). The distribution of STDs in the treatment group over 12 months was as follows: 3 (8.6%) chlamydia, 8 (22.9%) gonorrhea, 16 (45.7%) trichomonas, 3 (8.6%) HSV-2, 3 (8.6%) PID and 2 (5.7%) combined infections. Among the observation group over 12 months there were 13 (27.1%) chlamydia, 4 (8.3%) gonorrhea, 22 (45.8%) trichomonas, 5 (10.4%) HSV-2, 1 (2.1%) PID and 3 (6.3%) combined infections. Thus, the only significant difference between the groups in terms of individual pathogens was chlamydia. The proportion of chlamydia infections in the observation group compared to the treatment group was significantly greater (p= 0.013).

Patterns of Vaginal Flora Over the Course of the Study

Table 2 shows the distribution of vaginal flora patterns stratified by Nugent groups over the first six months of the study. As anticipated, rates of BV during the initial six months of the study were generally higher in the women assigned to observation compared to those assigned to treatment. Prevalence of BV was not significantly different between the groups at months 9 and 12 reflecting the cessation of treatment at month 6.

Comment

Bacterial vaginosis is associated with numerous obstetrical and gynecological complications including acquisition and transmission of HIV and other STDs. It is hypothesized that the lack of hydrogen-peroxide producing lactobacilli in the vaginal flora of women with BV is the major biological risk for STD acquisition although other factors such as elevated vaginal pH and local cytokine production which accompany BV may be operative as well [5, 6, 12-14]. Cross-sectional studies have documented a significant association between BV and HIV seropositivity [15-17]. Prospective studies have shown that HIV seroconversion is significantly associated with alterations in vaginal flora[18]. In terms of BV as a risk factor for transmission of HIV, Cu-Uvin et al showed that HIV infected women with BV were significantly more likely to have high levels of HIV in the lower genital tract than women without BV[19].

BV is frequently present as a co-infection with cervical and vaginal STDs. Women with trichomoniasis are highly likely to be co-infected with BV [20-22]. In a prospective study, acquisition of trichomoniasis was associated with abnormal vaginal flora on Gram's stain (HR, 1.8; 95% CI, 1.3-2.4). Infections with *Neisseria gonorrhoeae* and *Chlamydia trachomatis* have also been significantly associated with abnormal vaginal flora. In a cross-sectional study of women attending an STD clinic, lactobacilli were present in significantly fewer women infected with gonorrhea than uninfected women. In addition, among those women who reported recent sexual contact to men with gonorrhea, 28% of the women with gonorrhea had inhibitory lactobacilli present versus 73% of the women not acquiring the infection ($p<0.05$)[23]. In a more recent cross-sectional analysis, female sexual contacts of men with either gonorrhea or chlamydia were three to four times more likely to be infected with the STD if they had BV than if they had normal vaginal flora. Further, vaginal colonization with hydrogen-peroxide producing lactobacilli was negatively associated with infection with either gonorrhea or chlamydia[24]. A prospective, longitudinal study of female sex workers in Kenya found that absence of vaginal lactobacilli was significantly associated with acquisition of gonorrhea in a multivariate model controlling for other risk factors (HR, 1.7; 95%CI,_1.3-2.4)[22]. Bacterial vaginosis has also been found to be associated with incident HSV-2 [3]. There is also a clear association between BV and upper genital tract infection including endometritits and PID [25].

Despite all of the data suggesting that abnormal vaginal flora is a risk factor for STD/HIV, treatment of women with asymptomatic BV is not recommended except in certain circumstances [9]. The lack of a recommendation for treatment is largely due to the fact that up until now, no data was available to show that treatment of BV could reduce rates of STD. A study design using a one time treatment regimen would be hampered by the fact that recurrence rates of BV are quite high, particularly in women at highest risk for STD/HIV. Thus, we used a combined approach of treatment followed by prophylactic therapy to attempt to maintain the vaginal flora as normal as possible for as long as possible. Prophylactic therapy has been shown to be beneficial for preventing recurrent BV [10]. In our study, women assigned to twice weekly metronidazole gel maintained healthier vaginal flora during the treatment phase than the observational group although rates of BV were still high. This likely reflects inadequacy of current treatment regimens as well as difficulty adhering to twice weekly intravaginal therapy for the asymptomatic patient for six months. Using this approach we were able to demonstrate

a significant reduction in the rate of chlamydia infections among women randomized to treatment and prophylaxis of asymptomatic BV versus those randomized to observation alone.

Due to limited resources we were required to halt enrollment prematurely and thus did not reach our targeted sample size. Nevertheless, the difference in chlamydia rates is highly significant. This study thus provides valuable data for use in designing similar intervention studies focused primarily on chlamydia. An additional hypothesis for the difference in chlamydial infection rates between the two groups of women in our study relates to an indole escape mechanism for chlamydia survival. One mechanism for host defense against chlamydia is the depletion of tryptophan. However, organisms associated with BV mayprovide an indole-rich environment which promotes production of tryptophan and thus enhances the ability of chlamydia to survive in the genitourinary tract [26].

In summary, this is the first study to show that treatment and prophylaxis of asymptomatic BV is associated with decreased rates of incident chlamydia infection. Based on this data, consideration should be given to routine treatment of women with asymptomatic BV; however, further studies are warranted to confirm these findings . In addition, studies enrolling more racially and ethnically diverse populations are needed. If our results are due to normalization of the vaginal flora, as we hypothesize, there is an urgent need to develop more effective treatments for BV both in terms of initial efficacy and decrease of recurrence. Development of more effective therapies for BV could add additional benefit to this approach.

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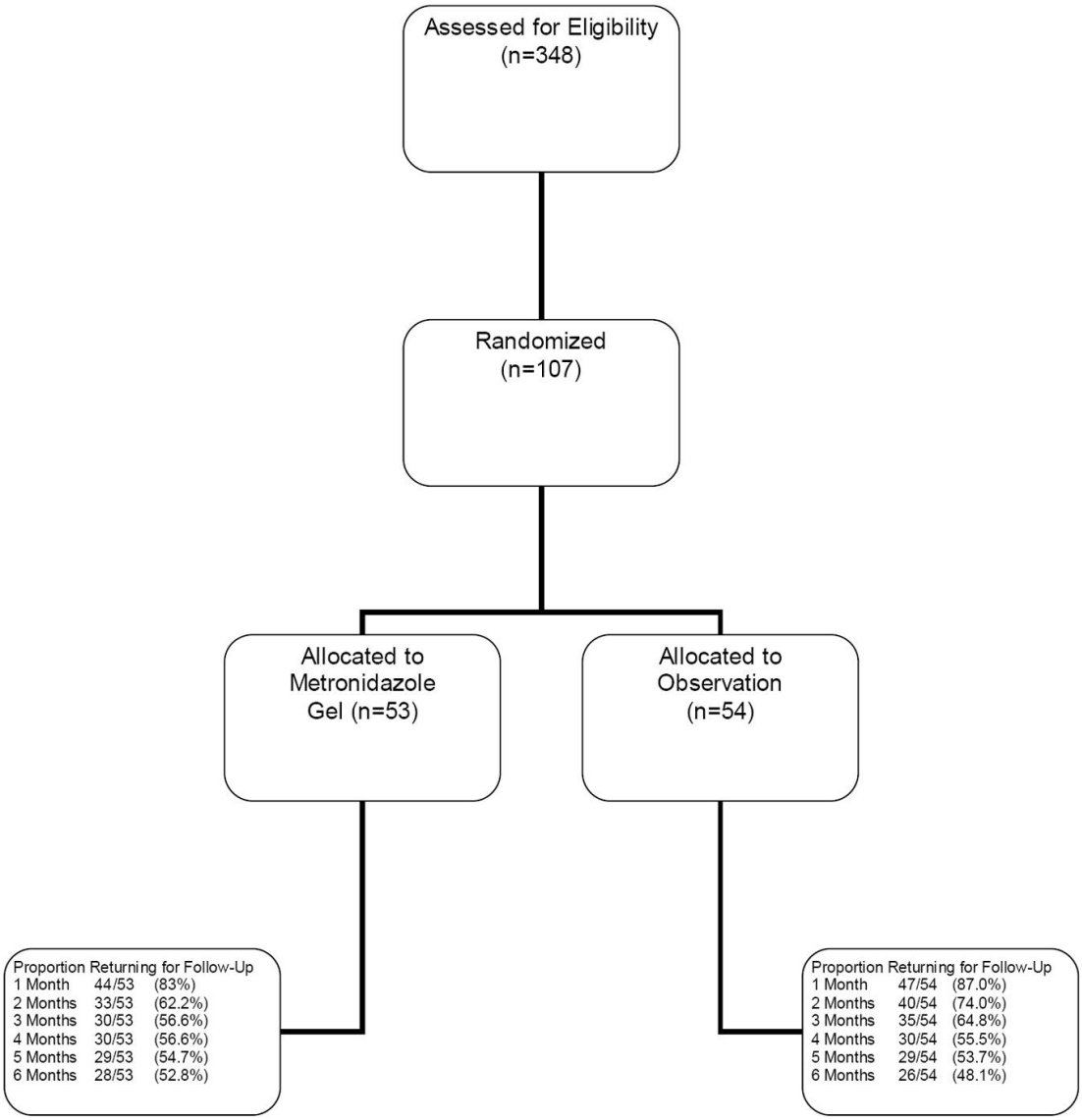

Figure I.
Flow Diagram of Subject Progress

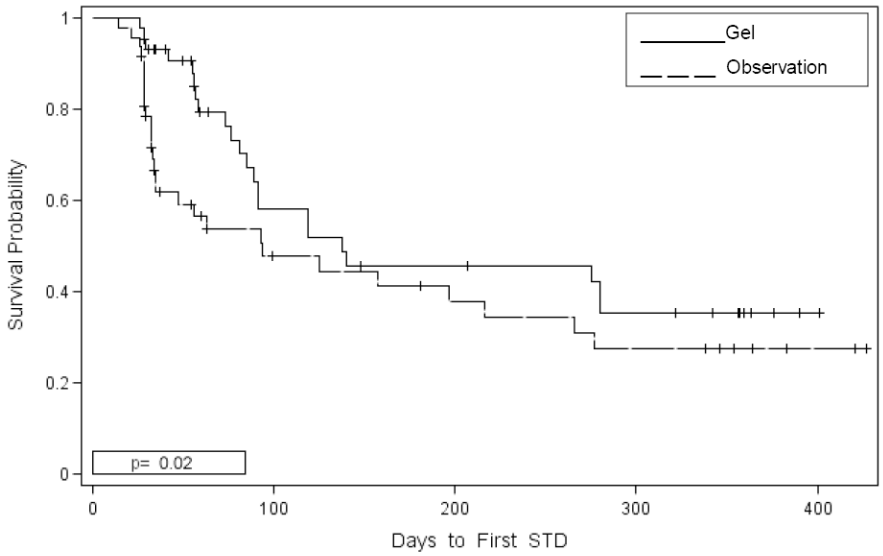

Figure II.
Percentage STD Free by Randomization Group among Women with Asymptomatic BV

Table I

Baseline Demographic and Behavioral Factors by Randomization Group

Factor	Observation n(%)	MetronidazoleGel n (%)	p
Highest grade completed			
< 12	12 (22.2)	18 (34.0)	0.29
12	30 (55.6)	22 (41.5)	
> 12	12 (22.2)	13 (24.5)	
Ever smoked			
Yes	30 (55.6)	27 (50.9)	0.43
No	24 (44.4)	26 (49.1)	
Douching in last 30 days			
Yes	29 (53.7)	36 (67.9)	0.13
No	25 (46.3)	17 (32.1)	
Ever had STD			
Yes	48 (88.9)	42 (79.2)	0.17
No	6 (11.1)	11 (20.8)	
Used BC			
Yes	44 (86.3)	43 (84.3)	0.78
No	7 (13.7)	8 (15.7)	
Condom last sex			
Yes	16 (31.4)	18 (35.3)	0.67
No	35 (68.6)	33 (64.7)	
Baseline STD			
Yes	31 (60.8)	29 (56.9)	0.69
No	20 (39.2)	22 (43.1)	
	Mean ± sd	Mean ± sd	
Current age	24.7 ± 5.9	25.5 ± 6.0	0.40
Total partners past 30 days	1.4 ± 0.8	1.3 ± 0.6	0.40
Total partners past 3 mo	1.4 ± 0.8	1.3 ± 0.6	0.18
Last sex (days)	7.9 ± 10.1	7.8 ± 7.4	0.97
Age at first Sexual intercourse	15.1 ± 2.0	15.5 ± 2.1	0.34

Table II

BV Scores in Tertiles by Study Group and Visit

Group	Nugent score	1M n(%)	2M n(%)	3M n(%)	4M n(%)	5M n(%)	6M n(%)
Observation	<4	3 (6.4)	11	2 (6.1)	5 (17.9)	5 (17.2)	3 (11.1)
	4-6	4 (8.5)	(28.2)	5 (15.2)	5 (17.9)	4 (13.8)	5 (18.5)
	≥ 7	40 (85.1)	6 (15.4)	26	18	20	19
			22	(78.7)	(64.2)	(69.0)	(70.4)
			(56.4)				
Metronidazole	<4	13 (31.0)	9 (29.0)	8 (28.6)	7 (24.1)	6 (21.4)	9 (33.3)
Gel	4-6	5 (11.9)	3 (9.7)	7 (25.0)	12	6 (21.4)	4 (14.8)
	≥ 7	24 (57.1)	19	13	(41.4)	16	14
			(61.3)	(46.4)	10	(57.2)	(51.9)
					(34.5)		
p		0.005	0.836	0.017	0.065	0.646	0.164

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