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The Effect of Vaginal Douching Cessation on Bacterial Vaginosis: A Pilot Study

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Abstract

Objective—To evaluate the risk for bacterial vaginosis (BV) in a douching cessation trial.

Study design—Thirty-nine reproductive-age women who reported use of douche products were enrolled into a 20-week study consisting of a 4-week douching observation (phase I) followed by 12-weeks of douching cessation (phase II). In phase III, participants then chose to resume douching or continue cessation for the remaining 4 weeks. Self-collected vaginal samples were obtained twice-weekly in the first 16 weeks and one sample was collected during week 20 (1,107 samples total). BV was diagnosed by Nugent score ≥ 7 . Conditional logistic regression was used to evaluate douching cessation on the risk of BV.

Results—The adjusted odds ratio (aOR) for BV in the douching cessation phase as compared to the douching observation phase was 0.76 (95% CI:0.33–1.76). Among women who reported their

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Table for inclusion in the article summary: Table 4, Risk for bacterial vaginosis comparing the observational douching phase (4 weeks) and the douching cessation phase (12 weeks), Baltimore, MD, 2005–2007, n=39

Overview:

Vaginal douching cessation may reduce the risk of bacterial vaginosis in a subset of women.

Clinical implications:

- Limited data are available describing the effects of douching on vaginal microbial flora or risk for bacterial vaginosis (BV).
- This pilot study suggests cessation of vaginal douching may reduce the risk for BV in a subset of women.
- Women who report douching after menstruation or frequent douching may benefit from douching cessation interventions.
- Our findings suggest that further study of vaginal douche products in an adequately powered study is warranted.

Condensation:

Douching cessation yielded a significantly reduced risk of bacterial vaginosis in a subset of women who douched as a hygienic measure after menstruation.

primary reason for douching was to cleanse after menstruation, BV was significantly reduced in douching cessation (aOR:0.23; 95% CI:0.12–0.44).

Conclusion—Vaginal douching cessation may reduce the risk for BV in a subset of women.

Keywords

vaginal douching; bacterial vaginosis; intravaginal cleansing; intravaginal washing

INTRODUCTION

Vaginal douching is commonly practiced. The 2001 National Health and Nutrition Examination Survey, found that the overall prevalence of douching in the United States was 22.4%, with highest rates reported by non-Hispanic black women (50.2%).¹ Douching has been associated with a number of adverse outcomes including changes in vaginal ecology², and in largely cross-sectional studies, increased risk of bacterial vaginosis (BV),^{3–5} cervical infection,^{6–9} pelvic inflammatory disease,^{10–12} ectopic pregnancy,^{13–15} and cervical cancer¹⁶. Intravaginal washing has also been demonstrated to be an independent risk factor for HIV acquisition.¹⁷

Despite the widespread practice of douching¹, and the findings of increased BV in populations who douche¹⁸, there are no data to demonstrate a direct causal relationship. Observational epidemiologic studies have suggested a strong association between douching and BV.^{3;4;19–22} However, the reported association may be due to reverse causality—that is, it is not known if douching causes BV or BV symptoms lead women to douche.^{23;24} Little is known about the impact of douching on vaginal ecology. Studies assessing the microbiology of vaginal flora in response to douching are limited by short observation periods.^{2;25;26} To our knowledge, the only reported randomized controlled trial of douching and risk for BV is a pilot study which concluded that douching interventions are feasible and future trials should have a duration of more than eight weeks.²⁷

We report here on a douching cessation intervention with twice-weekly sampling for 16 weeks and the resulting changes in vaginal flora.

Materials and Methods

The study population was comprised of women (≥ 18 years) who responded to recruitment through newspaper advertisements, posted fliers, and referral from the Johns Hopkins Bayview Department of Obstetrics and Gynecology between December 2005 and March 2007. Inclusion criteria were report of regular menstrual cycle and any douching in the two months before screening.

Because of the theoretical risk associated with douching and no known health benefit, we chose a cessation design for the preliminary study. A 4-week douching observation interval (phase I), in which participants were expected to douche at least once, was initiated on day one of the menstrual cycle and was followed by a 12-week douching-cessation interval (phase II). During the douching cessation phase, women were asked to discontinue the use of all feminine hygiene products including douches, wipes, creams, sprays, vaginal films and powders. Participants were encouraged to use unscented soaps and to only perform external genital washing. In the final four weeks of the study, participants chose to resume douching or continue douching cessation (phase III). At the end of the study (week 20), participants were contacted by phone and surveyed on their current douching practice. Participants were instructed to douche at their preferred frequency in phase I and phase III and to choose products that they normally used.

All were asked to return for a visit at week 4 for the douching cessation educational intervention and a final visit at week 16.

Participants used daily diaries, in the form of a yes/no check-off list, to report menstrual bleeding, vaginal douching, sexual activity (including vaginal intercourse, receptive oral sex, digital penetration, rectal sex, sex toys, condoms, spermicides, lubricants), thong undergarment, medications, or use of a diaphragm, sanitary napkin and/or tampons. Trained female staff conducted 15-minute questionnaires at baseline and at the end of phases II and III. At baseline, women were surveyed on demographic factors, including age, race, educational attainment, mother's education, and contraceptive use. In addition, baseline and follow-up surveys included a collection of information on sexual activity and douching practices. A douche product was described to participants as "washing or cleansing inside your vagina." At all visits, women were asked about their frequency, and with multiple selections, reasons for douching and product types used.

Self-collected vaginal samples were obtained twice weekly in phases I and II and a final sample in week 20. Self-collected swabs demonstrate high overall and morphotype-specific validity compared to provider-collected swabs.²⁸ Participants were instructed to insert the vaginal swab 1–2 inches into the vagina, twist the swab to collect material on all sides of the cotton tip, wipe in several full circles on the vaginal wall, keep in the vagina for 20 seconds, and then roll each swab across a slide and allow the material to air-dry. The slides were labeled only with a unique study identifier and date of sample. Participants placed the slide into a storage container and mailed the slides and behavioral diaries to our laboratory weekly. Study staff logged incoming postal mail, and if a participant failed to submit her weekly samples, she was immediately contacted and reminded to send in the samples. The slides were heat-fixed and Gram-stained, read in random order and blinded to phase of study. A microscopy score of 0 to 10 was assigned using the standardized method described by Nugent²⁹. A Nugent Gram stain score of 0–3 was normal, 4–6 intermediate and 7–10 categorized BV. One experienced microbiologist scored all slides.

At baseline and final visits, we conducted beta human chorionic gonadotropin (hCG) tests on urine and nucleic acid amplification tests (NAATs) for *Chlamydia trachomatis* (CT) and *Neisseria gonorrhoeae* (GC) (Becton Dickinson, Sparks, Maryland, BD ProbeTec ET) on self-collected swabs. Women who tested positive for GC or CT were referred to the Baltimore City STD clinic for treatment and partner notification procedures and were permitted study enrollment at a later date. Participants were excluded if they were pregnant. Women not reporting the use of intrauterine device or hormonal contraception or a history of tubal ligation were asked to return for hCG pregnancy testing if the daily diaries demonstrated more than 35 days between menstrual cycles. One participant tested positive for CT at the screening visit, was treated successfully, but she subsequently was lost to follow-up before she began study sampling. One participant was not eligible due to pregnancy at the screening visit.

Participants were instructed to contact the study investigator if they experienced any genital or pelvic symptoms during the study. Symptomatic women were offered an appointment with the Johns Hopkins Center for Reproductive Tract Infections, or were referred to the Baltimore City Health Department free STD clinics or their personal clinician. Medications were noted by the participant on the daily diary forms.

Conditional logistic regression (CLR) was used in this crossover design to evaluate the risk of BV in a participant's douching cessation phase compared to her douching observation (phase II vs phase I).³⁰ Each woman acts as her own control, and therefore the effect of variation (and confounding) that occurs between participants is eliminated. The matched strata were made up of observations from each study phase and the matching factor was participant identification

number. CLR accounts for within subject correlations. Time-varying factors occurring in the three days before sampling that had been identified on the basis of previous literature, biologic plausibility and preliminary univariable analyses as possible confounders were included in the multivariable models. Tests for multiplicative interaction were performed by examining stratum-specific results. One participant reported douching during the final week of the cessation phase; however, data were analyzed according to the intent to treat principle. 95% CIs are based on robust estimations of the standard errors. We also evaluated the data using random effects statistical models and the results were similar (data not shown). Data were analyzed using STATA/SE 9.2 for Windows (Stata Corporation, College Station, Texas).

The protocol was approved by the Institutional Review Board of the Johns Hopkins University School of Medicine. All participants provided written informed consent.

Results

Follow-up

Figure 1 describes participant enrollment and withdrawals. Forty-seven women consented to participate in the study. However, because study sampling started on the first day of the menstrual cycle, there was a delay between study consent and study initiation. Thirty-nine women initiated the study and are the focus of these analyses. Of the 39 women, six were lost to follow-up. Five women discontinued within three weeks of study entry and one woman discontinued at 12 weeks. Eight-five percent (n=33) were interviewed at their final scheduled contact.

We longitudinally collected 1,107 vaginal smears from the 39 participants. Among women who completed the study, the average number of specimens per woman was 32.3 (SD: 1.2, range: 29–34). Daily diaries were completed from 99.9% of enrollment weeks.

Baseline participant description

The mean age of participants was 37.4 (range 22.2–53.4); 56.4% reported African-American ethnicity and 35.9% reported white. Forty-six percent reported a history of tubal ligation, 12.8% oral contraceptive use, 20.5% condom use only, 2.6% intrauterine device, 2.6% ring, 7.7% no contraceptive method and 7.7% combination methods. Eight-five percent reported being in a monogamous sexual relationship with a male partner.

At study entry, participants reported their mutually-exclusive primary reason for douching was to remove menstrual blood (33.3%), to feel clean or fresh (51.3%), or remove vaginal odor (5.1%). Seventy-four percent reported douching 1–2 times monthly; 12.8% reported douching more often. Demographic characteristics by frequency of observed douching during phase I are listed in Table 1. There were few differences between women by frequency of observed douching. However, women who douched less frequently tended to have a higher educational attainment ($p<0.08$). Nineteen women (48.7%) had BV in the first 4 weeks of observation.

As there is no independent means for verifying use of feminine hygiene products, our study relied on daily diaries and questionnaires. Observed frequency of douching as documented by the daily diaries in phase I had excellent agreement with the monthly frequency reported at the baseline survey (Table 2, kappa = 0.85, $p=0.0001$). At baseline, women who reported frequent douching (3 or more times per month) were observed to douche more frequently than those who reported douching 1–2 times per month (Table 2). With multiple selections possible, 69% of participants reported using commercial douche products and 8% reported using a water-only douche. (Table 3)

Vaginal flora comparison in Phase I and Phase II

Eighteen percent of specimens in the douching observation phase and 15% in the douching cessation phase were scored as BV positive (Figure 2). The average Nugent scores in phase I and phase II (2.02 vs 1.97, respectively) were not statistically different (paired t-test, p-value=0.8). Among women who had a Gram stain indicative of BV at any time during follow-up, 33% of specimens were categorized as BV in the douching observation phase compared to 27% in the douching cessation phase.

In univariable analysis, comparing a participant's douching cessation to her douching observation phase, the odds ratio for BV during douching cessation was 0.73 (95% CI: 0.33–1.61). Multivariable analysis, controlling for potentially confounding factors occurring in the 3 days before sampling (receptive oral sex, menstrual bleeding, and candidiasis and BV treatment) resulted in a similar estimate (adjusted OR (aOR) 0.76, 95% CI: 0.33–1.76). (Table 3) Other time-varying factors reported in the three days before sampling including vaginal intercourse, condom use, rectal sex, sex toy use, digital penetration and use of thong underwear were not statistically associated with risk of BV in multivariable modeling. Additionally, varying lengths of lag for the interval between phase I (douching observation) and phase II (douching cessation) produced similar results (35 day lag: OR 0.75 (95% CI: 0.30–1.87); 70 day lag: OR 0.75, (95% CI: 0.30–1.88)).

Because the first week of the menstrual cycle is a known interval for disruption in vaginal flora,^{31–33} we also conducted additional analyses relative to the menstrual cycle. There was a reduced risk of BV during douching cessation when samples collected during menstruation or 3 days following menstrual bleeding (peri-menstrual samples) were not included in the model (aOR 0.44, 95% CI: 0.20–1.00). In a separate model among women who reported at the baseline visit that their primary reason for douching was to clean after menstruation, BV was significantly reduced during douching cessation (aOR 0.23; 95% CI: 0.12–0.45, p-value for interaction = 0.002).

Eleven of 33 women (33.3%) elected to resume douching in phase III. Among these 11 participants, when the last sample from phase II was evaluated in comparison to the sample in phase III, one participant converted from BV negative to BV positive and one participant cleared BV, (McNemar's exact p-value=1.0).

Comment

This pilot study suggests that douching cessation may reduce the odds of BV by approximately 24%, although the study was not adequately powered and the results did not reach statistical significance. However, among women who routinely use vaginal douches after menstruation, there was a significant 77% reduction in the odds of BV during douching cessation, suggesting that the interaction between douching and menstrual bleeding may result in a synergistic disturbance on the vaginal flora. Similarly, when peri-menstrual samples were excluded from the model, there was a significant 56% reduction in BV during douching cessation. Previous longitudinal studies have identified the first seven days of the menstrual cycle as a time of significant risk for BV.^{31–39} Acidity is thought to be important in controlling the overgrowth of BV-associated bacteria and menstrual blood in the vagina may play a role in onset of BV by raising the vaginal pH. Additionally, changes in estrogen levels during the menstrual cycle may influence susceptibility to BV.³³ Our finding of an interaction between menstrual cycle and douching suggests that douching during the time of vaginal flora instability may increase the risk for BV. This finding corroborates a report by Schwebke et al. of a cross-sectional study of 250 adolescent women in which douching after menses was associated with a five-fold risk of BV (OR:5.11, CI:1.99–13.15).²²

We believe that this pilot study presents preliminary findings that can be utilized in the planning of future trials. This small sample suggests that a short education intervention on douching is effective and compliance with self-collected vaginal sampling and weekly submissions is excellent. The study also reinforces the importance of collecting menstrual bleeding information on daily diaries. Based on our results, any future cross-over trial that attempts to assess the relationship between douching and BV may require a sample size of approximately 400 women, based upon the degree of uncertainty surrounding the maximum likelihood estimate of 20% risk, a probability of BV in the douching period of 48% (p_1), and correlation coefficient for exposure between the paired case and control periods of 0.33. The sample size calculation is a conservative estimate based on McNemar's test⁴⁰ with 0.05 type I error and 80% power.

The only significant time-varying behavioral factor associated with BV in our cross-over study was the use of vaginal lubricants. Vaginal lubricant use referred to application that occurred in the three days prior to vaginal smear collection. Few studies have examined the association between BV and use of vaginal lubricants. Two large cross-sectional studies, one among women in London⁴¹ and the other among women in Sweden⁴², found no association between vaginal lubricant use and BV; however, a cross-sectional study of HIV-1-seronegative Kenyan female sex workers reported that intravaginal lubrication with petroleum jelly was associated with an increased odds of BV (aOR: 2.8, 95% CI: 1.4–5.6).⁴³

The strengths of our study include the availability of 1,107 sequentially self-collected vaginal swabs and daily diaries which were mailed to our laboratory weekly. Additionally, the design of the study allowed an individual-level analysis in which a woman's douching phase samples are compared directly to her douching cessation phase samples. By matching on the individual, we reduce unmeasured confounding and eliminate confounding of time-invariant factors. For example, demographics and other time-invariant factors, such as individual lifestyle and behavioral factors, are difficult to measure, may result in residual confounding and factor significantly in reproductive health research. Population-based studies of sexually transmitted infections and BV have demonstrated substantially higher prevalence rates in African-American women as compared to white women^{18;44–47}, and elevated risk estimates persist after controlling for behavioral and demographic risk factors. Similarly, there is racial disparity in U.S. douching prevalence rates: 2–3 times more non-Hispanic black women report douching than non-Hispanic white women (22% vs 50%).¹ The crossover analysis provides an estimate of the risk from douching which is not influenced by such confounding effects.

There are several limitations to our study. These include small number of enrolled women, missing data due to loss to follow-up, and lack of information such as daily reports of vaginal symptoms (discharge, irritation or odor). There may have been some residual confounding by the douche product used as each commercial product has varying ingredients and detergents²⁶, however, 69% of our participants reported using commercial vinegar and water products without additives. There are no commercial or research assays for detecting vaginal hygiene product use. However, participants were compensated regardless of adherence to douching cessation, thereby removing an incentive to misreport. Studies have also indicated that self-reported adherence to treatment is generally accurate.⁴⁸ The finding of reduced risk of BV in the douching cessation phase may reflect spontaneous improvement, regression to the mean, measurement bias, and/or unidentified parallel interventions.^{49;50} Our causal inference is limited in this study by the ethically dictated omission of the reverse cross-over in which we would ask women to resume douching – that is, a randomized two-treatment two-period (2×2) cross-over design in which half of the participants would be randomized to stop douching in the first phase followed by planned resumption of douching in second phase and half of the participants would complete the study in the reverse as outlined in this report. However, our inclusion of all women, and not just women with BV at study entry, means

regression to the mean is less likely. The short time interval between 2 measurements (3–4 days) also makes substantial regression to the mean less likely. We observed vaginal flora fluctuating in each menstrual cycle, within each treatment phase, (data not shown) which increases the plausibility that the significant decreases in BV incidence that we observed were in fact due to the douching cessation intervention. Additionally, our study design broadly defined douching exposure by study phase (douching cessation or observation) and therefore, the analysis of douching practice was not conducted at the specimen-level of observation as a time-varying exposure. However, a longitudinal specimen-level analysis of time-varying douching may be confounded by a problem of reverse causality such that it is difficult to determine from the observational analysis if douching causes BV or BV symptoms lead women to douche. Our crossover design and the analysis by intervention phase obviate this problem. Also, interactions between study phase and condom use (or other sexual behaviors) could not be evaluated because the number of women whose behavior varied between phases was too small to evaluate as an interaction term. Lastly, because of the small sample size and only two observed transitions on BV status between phase II and phase III, we were unable to determine whether the women who resumed douching in phase III experienced an increase in BV risk.

Nevertheless, our findings and others²⁷ suggest that further study of vaginal douche products in an adequately powered randomized two-treatment two-period (2×2) trial, or a douching cessation trial with randomization to prompt or delayed cessation, is warranted. Women who report douching after menstruation or frequent douching may benefit from douching cessation interventions.

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Figure 1. Douching cessation trial enrollment

Figure 1 is a display of trial enrollment and loss to follow-up.

study design phase	PHASE I	PHASE II	PHASE III	
	4 weeks	12 weeks	4 weeks	
	douching observation	douching cessation	optional return to douching	
sampling frequency	twice weekly sampling	twice weekly sampling	one sample at the end of the phase	
			resumed douching	did not resume douching
ALL ENROLLED WOMEN				
number of specimens collected	285	789	11	22
average Nugent Gram's Stain score (SD) [†]	2.02 (2.4)	1.97 (2.4)	1.64 (2.7)*	2.23 (3.1)*
percent of specimens by vaginal flora status:				
normal flora	70.5%	72.9%	81.8%	63.6%
intermediate flora	11.6%	12.0%	9.1%	22.7%
BV flora	17.9%	15.1%	9.1%	13.6%
AMONG WOMEN WHO EXPERIENCED AT LEAST ONE EPISODE OF BV				
number of specimens collected	155	445	7	12
average Nugent Gram's Stain score (SD) [†]	3.36 (2.5)	3.29 (2.4)	2.43 (3.1)*	4.00 (3.3)*
percent of specimens by vaginal flora status:				
normal flora	49.0%	52.6%	71.4%	33.3%
intermediate flora	18.1%	20.7%	14.3%	41.7%
BV flora	32.9%	26.7%	14.3%	25.0%

* only one sample in phase III and therefore this is not an average

[†] paired t-test comparing average score per woman in phase I and phase II was non-significant, p-value >0.8

Figure 2. Summary of the protocol and the distribution of BV-associated vaginal flora in each phase of the study among women who completed the study and stratified by women who are at risk for BV as defined by having had at least one episode of BV under observation (n=33)

Figure 2 is a display of the protocol, demonstrating the study design of Phase I, II and III and the respective percent of normal and BV-associated flora in each phase.

TABLE 1

Descriptive characteristics of women who were observed to douche up to twice per month or more often, Baltimore, MD, 2005–2007, n=39*

	Observed douching frequency in the first 4 weeks of observation, (phase I)			
	0–2 n=28		3+ n=11	
	n	%	n	%
Age (years)				
18–29	8	28.6	0	0.0
30–39	9	32.1	6	54.6
40+	11	39.3	5	45.5
Race				
Black	14	50.0	8	72.7
White	11	39.3	3	27.3
Hispanic	2	7.1	0	0.0
Other	1	3.6	0	0.0
Educational attainment [†]				
Less than high school	3	10.7	4	36.4
High school graduate (or GED)	11	39.3	5	45.5
More than high school	14	50.0	2	18.2
Mother's educational attainment				
Less than high school	6	21.4	4	36.4
High school graduate (or GED)	11	39.3	5	45.5
More than high school	8	28.6	1	9.1
Don't know	3	10.7	1	9.1
Ever smoked	16	57.1	3	27.3
STD history in 12 months prior	3	10.7	0	0.0
History of genital herpes or ulcers [‡]	1	3.7	2	18.2
Lifetime number of sexual partners				
1–6	18	64.3	5	45.5
7+	10	35.7	6	54.6
Bacterial vaginosis in the first 4 weeks of observation	12	42.9	6	54.6

* Of the 6 women who were lost to follow-up, 2 did not report douching in phase I and 4 douched once in phase I

[†] p<0.08 by Fisher's Exact test

[‡] One participant who used 0–2 products declined response to genital herpes/ulcer history

TABLE 2

Comparison of self-reported douching at baseline interview and observed douching in the first 4 weeks of study (phase I), Baltimore, MD, 2005–2007, n=39

	Observed monthly frequency of douching by daily diaries in phase I			
	0–2		3+	
	n	%	n	%
Monthly douching frequency reported on baseline survey				
0–2	28	100.0	6	54.6
3+	0	0.0	5	45.5

Table 3

Types of vaginal douche products reported at study entry with multiple selections possible, Baltimore, MD, 2005–2007, n=39*

	n	%
Homemade vinegar and water mixtures	1	2.6
Water only	3	7.7
Commercial products:		
Vinegar and water	27	69.2
Vinegar with cleanser additive	9	23.1
Medicated	3	7.7
Scented	13	33.3
Betadine	1	2.6
Baking soda	5	12.8
Other	2	5.0

* One woman reported rectal douching and 1 woman reported Melaleuca plant-based soap and water douche

Table 4

Risk for bacterial vaginosis comparing the observational douching phase (4 weeks) and the douching cessation phase (12 weeks), Baltimore, MD, 2005–2007, n=39

	aOR*	95% CI		p-value
Phase				
douching	reference	-	-	-
non-douching	0.76	0.33	1.76	0.52
Factors occurring within 3 days before sampling				
receptive oral sex	1.33	0.91	1.96	0.15
vaginal lubricant	2.65	1.59	4.40	0.00
menstruation	1.20	0.71	2.01	0.50
candidiasis and bacterial vaginosis treatment	3.12	0.73	13.36	0.13

* Adjusted for variables listed in the table.

** Medications include oral/topical antifungal or antibiotics for bacterial vaginosis. One woman received vaginal metronidazole gel at the end of phase I, three women applied antifungal treatment in phase I and five applied antifungal in phase II.