An Effective Intervention to Reduce Intravaginal Practices Among HIV-1 Uninfected Kenyan Women

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Abstract

Intravaginal practices (IVP) are common among African women and are associated with HIV acquisition. A behavioral intervention to reduce IVP is a potential new HIV risk-reduction strategy. Fifty-eight HIV-1-uninfected Kenyan women reporting IVP and 42 women who denied IVP were followed for 3 months. Women using IVP attended a skill-building, theory-based group intervention occurring weekly for 3 weeks to encourage IVP cessation. Vaginal swabs at each visit were used to detect yeast, to detect bacterial vaginosis, and to characterize the vaginal microbiota. Intravaginal insertion of soapy water (59%) and lemon juice (45%) was most common among 58 IVP women. The group-counseling intervention led to a decrease in IVP from 95% (54/58) at baseline to 0% (0/39) at month 3 ($p=0.001$). After 3 months of cessation, there was a reduction in yeast on vaginal wet preparation (22% to 7%, $p=0.011$). Women in the IVP group were more likely to have a *Lactobacillus iners*-dominated vaginal microbiota at baseline compared to controls [odds ratio (OR), 6.4, $p=0.006$] without significant change in the microbiota after IVP cessation. The group counseling intervention was effective in reducing IVP for 3 months. Reducing IVP may be important in itself, as well as to support effective use of vaginal microbicides, to prevent HIV acquisition.

Introduction

MAINTAINING A HEALTHY VAGINAL MUCOSA and microbiota is key to reducing adverse consequences such as pelvic inflammatory disease and HIV-1 infection.¹ Dysbiosis (i.e., imbalance) of the vaginal microbiota, bacterial vaginosis (BV), and vaginal candidiasis are associated with increased HIV-1 risk.^{2,3}

Natural dynamics of the vaginal microbiota lead to temporary disruptions; however, certain sexual behaviors, contraceptive methods, and intravaginal practices (IVP) can also lead to vaginal microbiota imbalance. Intravaginal practices are common throughout sub-Saharan Africa,⁴ especially among high-risk women such as female sex workers (FSW).⁵ As outlined by the World Health Organization,⁴ these include internal insertion of fingers and other substances including

fluids (douching) into the vagina.⁶ These practices are associated with a decreased abundance and proportion of *Lactobacillus* sp., increased BV, and higher risk for HIV-1.^{7,8} In longitudinal follow-up of Kenyan FSWs, IVP increased HIV-1 risk ~4-fold.⁹ The important implication of these findings is that eliminating these practices may reduce BV and HIV acquisition.

Intravaginal practices are deeply ingrained in cultural norms and beliefs. Nonetheless, a U.S. trial demonstrated that women receiving individualized counseling were more likely to stop their IVP (OR 1.60 at 12 months).¹⁰ A recent study among Kenyan FSW found that a theory-based behavioral intervention had a positive effect in reducing IVP over 1 month.¹¹ However, similar studies have not been conducted in Africa among non-FSWs. The purpose of this investigation was to evaluate an information, motivation, and behavioral

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skills (IMB) theory-based behavioral intervention to encourage cessation of IVP for 3 months in HIV-uninfected non-FSW Kenyan women. Our study also set out to include the potential impact of an IVP intervention on the composition of the vaginal microbiota, epithelium integrity, vaginal inflammatory cytokines, and activated immune cells as key biomarkers of vaginal ecology relevant to HIV-1 acquisition risk.

Materials and Methods

Participants

Recruitment. The study was conducted at Bomu Medical Centre in Mombasa, Kenya. Participants were recruited from the general medical clinic via flyers and staff information about the study. Community recruitment occurred via a study outreach staff person who was familiar with the population; potential interested individuals were sent to the clinic for assessment of eligibility. Participants were provided information about the study by trained study staff and consented. From September 2010 to July 2011, we recruited women 18 to 45 years old. Subjects were required to be asymptomatic, sexually active (at least one episode of sex in the last year), and not self-identifying as a FSW. Exclusion criteria included HIV or other infections in the past 3 months, pregnancy or being postpartum in the past 3 months, and the use of hormonal contraception in the past 2 months, as these can influence the composition of the vaginal microbiota.

Ethics. Participants signed informed consent following procedures and instruments approved by institutional review boards at New York University School of Medicine and at Kenyatta National Hospital in Kenya.

Participants who reported regular IVP ("washing or inserting substances into your vagina") were assigned to the IVP group. To compare biological outcomes between women in the intervention group versus women who did not perform IVP, we enrolled women who reported never engaging in IVP to serve as controls.

Study procedures

At baseline, women were interviewed using a standardized questionnaire. If control women reported IVP they were excluded from the study. During baseline, IVP women were asked to cease IVP for 3 months and received the intervention once a week for three consecutive weeks. Women were asked to describe substances that they used and to indicate specific washing techniques (use of fingers or cloth in introitus on a pelvic model; depth was noted by staff on study forms). Information regarding intrapersonal and interpersonal motivations for IVP were noted in the study questionnaire. All study participants were asked to return for follow-up 1- and 3-month visits.

Background, theoretical framework, description, and rationale for the intervention. A brief intervention approach was selected since the literature has shown that some brief interventions delivered in clinical settings can be effective¹² given that both patient and provider time have multiple competing demands. The behavioral framework used for the intervention included IMB skills¹³ and transtheoretical stage of change models.¹⁴ Evidence for applicability of IMB to

health conditions including and beyond HIV disease is accruing.¹⁵ The Transtheoretical Model allowed us to classify women as being at the stage of precontemplation (not ready for change to reduce or stop IVP), contemplation (ready to consider making a change soon), preparation (ready to take action toward behavior change), action (initiating behavior change), or maintenance (sustaining behavior change over time).¹⁴ These behavioral theories were selected as being most salient to the overall goal of harm reduction, to provide information about risks, to understand motivations behind behaviors, and to support less harmful behaviors or substitutions, along a continuum of readiness to make changes.

All group sessions were delivered in Kiswahili in a small group format with two trained counselors (nurses) only to the IVP-using participants. The counselors were trained by experienced staff who had delivered the intervention previously to a female sex worker population in Mombasa.¹⁶ The counselors followed an intervention manual outlining elements for each session. The group sessions focused on readiness to modify vaginal washing, barriers, facilitators, attitudes, self-efficacy to modify intravaginal practices, as well as sex-partner-related, cultural, familial, and economic factors, and healthy substitution behaviors. Staff incorporated client-centered motivational-interviewing-based communication such as the use of open-ended questions, affirmations, reflection, and summaries.

Before each weekly intervention session, women completed a survey assessing readiness to stop IVP, engagement in and type of IVP in the past week, sexual activity in the past week, gender of sexual partner, and condom use. Intervention session one provided information about female anatomy, IVP risks, HIV/STD information, and benefits of a healthy vaginal environment. It was acknowledged that women engage in these practices for many reasons, and that changing behavior can be difficult. Session two covered motivations and reasons for IVP, allowing participants to discuss the influence of sexual/emotional relationships, cultural expectations, and health beliefs on intravaginal practices and HIV/STI risk. This session introduced Kegel exercises, e.g., as a substitute for IVP for the vaginal tightness goal that was perceived to be a preference of the women's sex partners and, thus, one of the reasons that many women engaged in IVP. Session three covered strategies for reducing and/or stopping harmful IVP and problem-solving among the group; this included role plays as well as women describing how they had tried to attain their goals including talking with their partners.

Pelvic specimen collection procedures

During pelvic examinations, specimens were collected and a rapid pregnancy test (QuickVu; Quidel Corporation, San Diego, CA) and a rapid HIV test (Determine HIV-1/2, Abbot Japan Co Ltd, Tokyo and UniGold HIV, Trinity Biotech PLC, Bray) were performed.

Colposcopy at all three visits assessed epithelial and vascular lesions as classified by the Contraceptive Research and Development Program and World Health Organization. Vaginal swabs were taken for wet mount, Nugent scoring,¹⁷ vaginal pH, *Lactobacillus* culture, and whiff test. Cervicovaginal lavage (CVL) specimens were collected, as well as endocervical samples for *Chlamydia trachomatis* (CT) and *Neisseria gonorrhoeae* (NG) testing at baseline.

Blood was collected for syphilis testing (rapid plasma reagin titer confirmed by *Treponema pallidum* hemagglutination assay) and HSV-2 serology (HerpeSelect-2, Focus Diagnostics, Cypress, CA). All infections diagnosed were treated by Kenyan national guidelines.

Laboratory methods

Laboratory personnel were blinded to participants' IVP status. A detailed description of laboratory specimen handling and analysis is included in the Appendix (Supplementary Data are available online at www.liebertpub.com/aid). We defined abnormal vaginal microbiota (including intermediate and BV) as a Gram-stain Nugent score of 4–10.

Trichomonas vaginalis-positive samples were defined by the presence of motile trichomonads, and vaginal candidiasis by the presence of budding yeast or hyphae on vaginal wet mount examination. *Lactobacillus* presence was defined using colonial morphologic characteristics and Gram stain findings and classified as H₂O₂ producers if blue pigment was detectable.

Cervical cytokine and chemokine measurements. Levels (pg/ml) of six cytokines were measured using the Cytokine Bead Array kit (BD Biosciences, San Diego, CA).¹⁸

Cervical cytobrush immunophenotyping

Samples were processed using a BD FACSCalibur flow cytometer. Flow cytometry data were analyzed in FlowJo v.9.2 (Tree Star, Inc., Stanford University).

Vaginal microbiota studies

Genomic DNA was extracted from archived vaginal swabs. We used two universal primers, 27F and 534R, for PCR amplification of the V1–V3 hypervariable regions of the 16S rRNA gene.¹⁹ The purified amplicon mixtures were sequenced by 454 pyrosequencing with 454 Life Sciences primer A by the Genomics Resource Center at the Institute for Genome Sciences, University of Maryland School of Medicine. The QIIME software package²⁰ was used for quality control of the sequence reads with the split-library.pl script. Taxonomic assignments were performed using a combination of pplacer²¹ and speciateIT (<http://speciateIT.sourceforge.net>).

Statistical analysis

We used a repeated-measures study design where intravaginal practice at baseline was the key between-subjects factor and time was the key within-subjects factor. We utilized data from our quantitative questionnaire to assess changes in IVP across the three study visits. To assess women's readiness to stop IVP, we assessed any movement in the five stages of change classification from baseline to month 3.

For comparisons of vaginal microbiota types, mucosal epithelial lesions, and inflammatory markers, we compared baseline to the 3-month visit. Lesions were categorized as epithelial disruption, vascular disruption, erythema, and friability, but since relatively few lesions were observed, we combined categories into one variable indicating any non-iatrogenic lesions. We utilized generalized estimating equa-

tion (GEE) models with a logit link and exchangeable correlation structure to compare lesions and BV baseline and 3 months. GEE models with a Gaussian link and exchangeable correlation structure compared changes in cytokines between baseline and 3 months. The GEE analysis approach was selected as a single framework accommodating categorical and continuous outcome variables, confounding factors, and clustering of repeated observations within individual women. Potential confounding factors included in the models were sexual risk behaviors, genital tract infections, and reported vaginal symptoms. Cytokine data were log₁₀ transformed. Analyses were performed using Stata (Version 12, StataCorp, College Station, TX, 2011).

Vaginal microbiota community clustering analyses. The dissimilarity between community states (i.e., vaginal species composition and abundance) was measured with the Jensen–Shannon metric.²² The clustering of community states into community state types (CSTs) was done with hierarchical clustering based on the Jensen–Shannon distances between all pairs of community states and Ward linkage.²²

Results

Baseline characteristics

Between September 2010 and July 2011, 58 HIV-uninfected women were enrolled into the IVP group and 42 women into the control group. During the study, 16 IVP women and 12 control women were excluded (voluntary withdrawal $n=12$, pregnancy $n=7$, new symptoms $n=8$, or lost to follow-up $n=1$). Forty-two (72%) IVP group women and 30 (73%) control group women completed the study (Fig. 1).

Table 1 describes participant baseline characteristics. Women in the IVP group were significantly more likely to have frequent sexual intercourse ($p=0.008$) but did not report a significantly higher number of sexual partners. We found no significant differences in vaginal inflammatory cytokines, percent cervical CD4⁺ T cells expressing HIV receptors CCR5 and integrin β_7 ,²³ prevalence of *T. vaginalis*, HSV-2, NG or CT, or BV as determined by Nugent score in women in IVP and control groups during baseline. When comparing both groups of women to the nationally representative Kenya AIDS Indicator Survey (KAIS) of 2012, our sample had a somewhat higher proportion of women who said they were married/cohabiting (KAIS 62.0%), though proportions who said that they were living with a partner or spouse were similar (KAIS 2012 78.0%). Age distributions were similar to national KAIS 2012 data, as was reported number of sex partners. The level of unemployment was higher in our sample than that noted as a national average among women in KAIS 2012 (62.1%).²⁴

Women's use of IVP (motivations and use)

As summarized in Table 2, behavioral data indicated that many women learned IVP from peers and engage in these practices because they believe that their male partners prefer the sexual experience that intravaginal washing is thought to bring. At baseline 41% of the IVP women did not believe that these practices were harmful. Specific behaviors noted included use of caustic materials such as detergents and “shabu” (alum). These are likely more important to target in

FIG. 1. Flow diagram of women enrolled and excluded during the study period. STI, sexually transmitted infection.

a harm reduction approach in which women can be encouraged to substitute less risky practices, until they feel they are able to cease IVP entirely. The substitution of Kegel's exercises is an example of this approach that was incorporated into the intervention.

The 3-week behavioral intervention for IVP cessation was effective

Table 2 describes IVP reported at baseline. The average age when starting these practices was 23 years (range 15–38); 54% reported personal hygiene and 46% reported vaginal tightness/dryness to enhance male sexual pleasure as the principal reason for engaging in IVP. Over half the women (57%) reported daily IVP at a median of twice daily (range, 1–5 times daily). The most common substances inserted into the vagina were soap and water ($n=34$, 59%), lemon juice ($n=26$, 45%), soda ($n=11$, 19%), and salt ($n=10$, 17%) (numbers add up to >100%). Intravaginal practices were conducted primarily before or after sexual intercourse (43% and 33%, respectively).

Between baseline when women were actively engaged in IVP and 3 months, there was a significant shift in the stage of behavioral change (Table 3 and Fig. 2). At baseline, most women were in the first three stages of behavior change (precontemplation 1.8%, contemplation 15.8%, and preparation 78.9%). Women increased one or more stages from baseline, typically increasing from “preparation” to “early action” stages ($p<0.001$) within a month. The proportion of women reporting IVP the previous week decreased from 94% (54/58) at baseline to 0% (0/39) after 3 months ($p<0.001$).

Vaginal epithelial lesions were rare

Colposcopy revealed that vaginal and cervical lesions were rare (2% of women in both groups without significant change between visits). Over the entire cohort and study periods, there were 47 reports of any type of vaginal or cervical abnormality (cervical epithelial disruption $n=1$, epithelial friability in vagina or cervix $n=20$, cervical erythema $n=15$, cervical vascular disruption $n=2$, vaginal erythema

TABLE 1. BASELINE CHARACTERISTICS

	IVP group (n=58)	Non-IVP group (n=42)	p
Median age, years (range)	25.7 (19–44)	27.8 (18–41)	0.058 ^a
Currently married with one spouse, N (%)	43 (74)	38 (90)	0.069
Unemployed, N (%)	38 (66)	32 (76)	0.277
Reporting a single sexual partner, N (%)	55 (95)	42 (100)	0.260
Sexual intercourse 2+ /week, N (%)	40 (70)	18 (43)	0.008
Reporting history of STD symptoms, N (%)	14 (24)	7 (17)	0.459
Reporting past diagnosis of an STD, N (%)	15 (26)	9 (22)	0.644
Herpes simplex virus-2 positive, N (%)	28 (48)	18 (43)	0.685
Gonorrhea, N (%)	0 (0)	0 (0)	1.000
Chlamydia, N (%)	2 (3)	0 (0)	0.508
Trichomonas, N (%)	3 (5)	1 (2)	0.637

^aMann–Whitney test. All others are by Fisher's exact test.

IVP, intravaginal practice; STD, sexually transmitted diseases.

TABLE 2. INTRAVAGINAL PRACTICES AND MOTIVATIONS REPORTED AT BASELINE VISIT

Characteristics	IVP group (n=58)
Median age at start of IVP, years (range)	23 (15–38)
Learned IVP practices from, <i>N</i> (%)	
Friends	48 (83)
Self-taught	7 (12)
Relatives (grandmother, mother, sister)	3 (5)
Reason for starting intravaginal practices, <i>N</i> (%)	
Male sexual experience or after childbirth (for vaginal dryness or tightening)	25 (43)
Peer pressure (friends were doing it, told by relatives to do it)	20 (34)
Hygiene (to remove foul smell or to feel clean)	13 (22)
Reason for currently engaging in intravaginal practices, <i>N</i> (%) ^a	
Hygiene (to remove foul smell or to feel clean)	37 (64)
Male sexual experience or after childbirth (for vaginal dryness or tightening)	36 (62)
Female sexual experience	3 (5)
Frequency of intravaginal practice	
Daily practice (<i>n</i> =33) median #/day (range)	2 (1–5)
Weekly practice (<i>n</i> =23), median #/week (range)	2 (1–9)
Monthly practice (<i>n</i> =1), median #/month	2
Method of insertion of substances into the vagina, <i>N</i> (%) ^a	
Finger	35 (60)
Cloth	28 (48)
Substances inserted into vagina, <i>N</i> (%) ^a	
Soap and water	34 (59)
Lemon or lime juice	26 (45)
Soda (Coca-Cola or Sprite)	11 (19)
Salt or salt water	10 (17)
Detergent	9 (15)
Garlic	7 (12)
Shabu (alum powder)	4 (7)
Brewed tea	4 (7)
Other (cream, herbs)	4 (7)
Timing of intravaginal washing, <i>N</i> (%) ^a	
Before sexual intercourse	25 (43)
After sexual intercourse	19 (33)
Before and after sexual intercourse	10 (17)
After menses	9 (16)
What are the benefits of doing IVP? <i>N</i> (%) ^a	
Satisfy sexual partner	19 (33)
Feel clean	18 (31)
To keep the vagina dry and tight	13 (22)
Feel like a virgin	5 (9)
Prevents infection	3 (5)
What are the harms of doing IVP? <i>N</i> (%)	
IVP is not harmful	24 (41)
Painful sexual intercourse	19 (33)
Causes vaginal itching, burning, or bruising	13 (22)
What would it take to NOT do IVP at all?	
Believe harm outweighs good, <i>N</i> (%)	50 (86)

^aWomen reported more than one reason for engaging in intravaginal practices (IVP); therefore the total percentage is greater than 100%.

n=1). Women practicing IVP were not more likely to have epithelial lesions compared to women who did not.

The prevalence of HSV was high

Around half of the IVP women (28, 48%) and 43% (18) of the control women had positive HSV-2 serology (*p*=0.685). Two IVP women had *C. trachomatis* and none had *N. gonorrhoeae*.

Women with *C. trachomatis* or HSV-2 infection at baseline were more likely to have elevated cervicovaginal cytokines compared to women who were uninfected by both [tumor

necrosis factor (TNF)- α 3.21 pg/ml vs. 1.27 pg/ml, *p*=0.011; interleukin (IL)-6 52.29 pg/ml vs. 32.10 pg/ml, *p*=0.028; interferon (IFN)- γ 1.96 pg/ml vs. 1.03 pg/ml, *p*=0.057].

Prevalence of vaginal candidiasis significantly decreased after IVP cessation

The prevalence of vaginal candidiasis decreased in the IVP group from 22% to 7% (*p*=0.011) whereas a similar decrease was not seen in women in the control group (Table 4). The decrease in vaginal candidiasis remained significant after

TABLE 3. INTERVIEW BEFORE EACH VAGINAL EXAMINATION FOR INTRAVAGINAL AND NON-INTRAVAGINAL GROUPS

	IVP group	Non-IVP group
Engaged in IVP 7 days before pelvic examination		
Baseline	54/58 (93%)	0/41 (0%)
1 month	1/46 (2%)	1/31 (3%)
3 months	0/36 (0%)	0/29 (0%)
Median number of times engaging in IVP 7 days before pelvic examination (range)		
Baseline	4 (1–11) ^a	0
1 month	1 (1) ^b	1 (1)
3 months	0	0
Number of women reporting use of the following substances 7 days before baseline pelvic examination (n = 54), N (%)		
Water alone	9 (54)	—
Lemon or lime juice	26 (48)	—
Soap and water	21 (39)	—
Detergent	20 (37)	—
Soda (Coca-Cola or Sprite)	13 (24)	—
Garlic	7 (13)	—
Salt or salt water	5 (9)	—
Brewed tea	5 (9)	—
Shabu (alum powder)	4 (7)	—
Other (lotion, herbs, lubricant)	4 (7)	—
Sexual intercourse without a condom during the 7 days before pelvic examination?		
Baseline	33/58 (57%)	29/42 (69%)
1 month	26/48 (54%)	21/32 (66%)
3 months	25/39 (64%)	19/30 (63%)

^an = 54 participants in the IVP group.

^bn = 1 participant in the IVP group and one in the non-IVP group. IVP, intravaginal practice.

controlling for frequency of sexual activity the week before the pelvic examination. Women in the IVP group did not have a significantly higher prevalence of vaginal candidiasis at baseline compared to women who did not engage in IVP (22% vs. 12%; $p=0.199$).

Vaginal IL-6 and TNF- α levels decreased over 3 months

Women in the IVP and control groups had significant decreases in vaginal IL-6 (41 pg/ml to 24 pg/ml, $p=0.001$;

FIG. 2. Proportion of women in the intravaginal practice (IVP) group in one of five stages of behavior change.

3.61 pg/ml to 0.87 pg/ml, $p=0.005$, respectively) and TNF- α levels (2.47 pg/ml to 0.97 pg/ml, $p=0.006$; 91.2 pg/ml to 16 pg/ml, $p=0.04$) (Table 4).

Prevalence of bacterial vaginosis did not change after IVP cessation

At baseline, 10 (17%) of 58 IVP women had BV by Nugent criteria compared to 13 (31%) of 42 control group women ($p=0.149$); proportions of BV did not significantly change statistically by month 3 ($p=0.770$ among IVP women and $p=0.280$ among controls, Table 4).

Women who engage in IVP are more likely to harbor a vaginal microbiota dominated by *L. iners*

We characterized the vaginal microbiota of 97 women (55 IVP and 42 controls) using 454 pyrosequencing of barcoded 16S rRNA gene hypervariable regions V1–V3. Of 250 vaginal-swab samples processed, 226 had sufficient reads (>1,000, median reads/sample 5,462) and were analyzed. There were 56 women with samples from all three visits, 17 with samples from two visits, and 24 with samples from the baseline visit. Samples were clustered into four CSTs, three of which were most often dominated by a *Lactobacillus* spp. [*L. iners* (CST III), *L. crispatus* (CST I), and *L. gasseri* (CST II)] and the fourth by *Gardnerella vaginalis* (CST IV-B).

Figure 3 represents a heatmap of percentage abundances of microbial taxa in the vaginal communities of subjects at baseline and at 1 and 3 months. Each community was clustered using hierarchical clustering according to Gajer *et al.*²² At baseline, there were significantly more women with CST III (mostly dominated by *L. iners*) in the IVP group than the control group [odds ratio (OR), 6.4, $p=0.006$] and significantly fewer CST IV-B (often dominated by *G. vaginalis*) in the IVP group (OR, 0.08, $p=0.004$). This remained significant after controlling for age, sexual activity (within the last 4 weeks vs. >4 weeks ago), and HSV status. There was no significant change in CST between baseline and 3-month visits in control group women.

Discussion

Intravaginal practices are common in many countries in Africa where HIV is prevalent. For example, a population-level survey in KwaZulu-Natal found that over half of women reported intravaginal cleaning.⁴ Recent systematic reviews that have been published of prospective longitudinal studies examining associations between IVP and HIV infection acquisition have revealed that using IVP did not protect from HIV and led to an increased risk of BV. Furthermore, IVP may directly impact HIV prevention efforts by potentially diminishing the efficacy of vaginal microbicides. For these reasons, interventions to discourage IVP may be important to reduce individual and population spread of HIV infection.

In this study, we demonstrate that a theory-based behavioral intervention to encourage cessation of IVP among HIV-uninfected general-population Kenyan women was effective at reducing the reported practice and that this effect was sustained over 3 months. This supports the results of a 1-month pilot study using the same behavioral intervention in HIV-uninfected Kenyan FSWs engaged in IVP.²⁵ Other studies conducted in the United States have also reported some success.¹⁰

TABLE 4. CHANGES IN VAGINAL ENVIRONMENT BETWEEN BASELINE AND MONTH 1 AND MONTH 3

	Baseline	Month 1	Month 3	Baseline vs. month 3, p*
Changes After Cessation of Intravaginal Practices				
Mean vaginal pH	5.28	5.08	5.15	0.486
BV by Nugent score, <i>N</i> (%)	10/58 (17)	4/46 (9)	6/42 (14)	0.770
Positive <i>Lactobacillus</i> culture	22/58 (38)	20/46 (43)	17/42 (40)	0.863
H ₂ O ₂ producing <i>Lactobacillus</i>	7/58 (12)	5/46 (11)	8/42 (19)	0.228
Vaginal candidiasis	12/58 (22)	8/46 (17)	3/42 (7)	0.011
Cytokines [mean log ₁₀ transformed, pg/ml (SD)]				
IFN- γ	1.43 [3.21]	1.19 [1.62]	0.60 [1.10]	0.112
TNF- α	2.47 [6.10]	0.87 [1.91]	0.97 [4.02]	0.006
IL-10	0.62 [1.07]	0.50 [0.98]	0.58 [2.06]	0.172
IL-6	41.32 [62]	24.81 [56]	24.02 [68]	0.001
IL-4	0.04 [0.25]	0.22 [0.73]	0.15 [0.48]	0.171
IL-2	0.58 [0.72]	0.76 [0.93]	0.56 [0.69]	0.913
% CCR5 ⁺ CD4 ⁺ T cells	65.39	66.82	65.52	0.822
% CCR5 ⁺ β_7^{hi} CD4 ⁺ T cells	12.92	11.22	10.50	0.240
Changes Among Women Who Do Not Engage in Intravaginal Practices				
Mean vaginal pH	5.5	5.6	5.6	0.628
BV by Nugent score, <i>N</i> (%)	13/42 (31)	6/32 (19)	6/30 (20)	0.280
Positive <i>Lactobacillus</i> culture	14/42 (33)	9/32 (28)	14/30 (47)	0.166
H ₂ O ₂ producing <i>Lactobacillus</i>	4/42 (10)	5/32 (16)	2/30 (7)	0.308
Vaginal candidiasis	5/42 (12)	7/32 (22)	7/30 (23)	0.197
Cytokines [mean log ₁₀ transformed, pg/ml (SD)]				
IFN- γ	1.96 [3.12]	0.94 [1.57]	1.15 [0.89]	0.626
TNF- α	3.61 [8.82]	0.97 [1.90]	0.87 [1.59]	0.005
IL-10	0.85 [1.69]	0.47 [1.04]	0.68 [0.92]	0.856
IL-6	91.2 [182]	21.21 [31]	16 [19]	0.041
IL-4	0.38 [0.70]	0.46 [1.68]	0.27 [0.62]	0.419
IL-2	2.15 [3.57]	0.81 [0.72]	1.03 [1.04]	0.092
% CCR5 ⁺ CD4 ⁺ T cells	68 [13]	67 [16]	71 [14]	0.114
% CCR5 ⁺ β_7^{hi} CD4 ⁺ T cells	10.9 [9.7]	12.5 [9.8]	9.65 [4]	0.254

**p* values from either a binomial generalized estimating equation (GEE) with logit link or a Gaussian GEE analysis.

Figures in *bold* are statistically significant.

BV, bacterial vaginosis; SD, standard deviation; IFN, interferon; TNF, tumor necrosis factor; IL, interleukin.

We found that cessation of IVP for 3 months did not reduce BV. Another pilot study in the United States on the impact of a 12-week douching cessation on BV also did not find a statistically significant decrease in BV occurrence.²⁶

At baseline, we found that women who engaged in IVP were more likely to harbor a vaginal CST dominated by *L. iners* compared to women denying IVP, independent of frequency of sexual activity. *L. iners* has only recently been described because of fastidious culture requirements and is now recognized as one of the most common vaginal bacteria,¹⁹ especially during transition such as after metronidazole treatment.²⁷ It is possible that regular IVP leads to rapid vaginal environment fluctuations that selects *L. iners*, which is adapted to survive under a range of conditions.²⁸ We found no significant shift in the relative composition of the vaginal microbiota after IVP cessation, suggesting that 3 months after cessation of a major disturbance (IVP) may be too soon to see substantial shifts in the vaginal microbiota. Alternatively, IVP cessation may have been overreported resulting in little change in actual perturbation frequency and observed microbiota.

Our study incorporated biomarkers including CCR5 expression in order to assess the potential impact on the vaginal environment (including the microbiota) of IVPs and, thus, HIV risk. Several observations on changes in vaginal biomarkers therefore deserve mention. First, HSV-2-seropositive women were significantly more likely to have elevated vaginal

proinflammatory cytokines compared to HSV-2-negative women. This is biologically plausible as HSV-2 is associated with an increased risk of HIV acquisition.²⁹ Second, few longitudinal data exist on changes in cervical CD4 T cells in African women. We found that among women in both groups the proportion of cervical CD4⁺ expressing CCR5⁺ is high (66%) and stable over 3 months. Finally, we observed a decrease in vaginal IL-6 and TNF- α levels between baseline and 1- and 3-month visits in both IVP and control groups. The reason for this decrease is unclear.

Several caveats should be highlighted. The majority of women were in the preparatory phase of behavioral change likely reflecting selection bias, since women who may have been more resistant to IVP cessation may have declined to enroll. Future studies in Africa could randomize IVP women to the intervention, which would include women at all stages of behavioral change. This strategy was found to be feasible in a U.S. population.³⁰ Limited follow-up time precludes the assessment of the durability of the behavior change observed. Finally, the loss to follow-up rate (27%) may have reduced the power to assess differences in vaginal milieu changes between women in the IVP and control groups. Women enrolled in our study were similar in some characteristics to national adult survey data.

In summary, we describe an effective 3-week group-counseling intervention to encourage cessation of intravaginal

FIG. 3. Heatmap of percentage abundances of microbial taxa found in the vaginal communities of the subjects at baseline and at 3 and 6 months by intravaginal practice (IVP) status. Each community was clustered in community state types using hierarchical clustering.

practices and found quantitative improvements in vaginal health, looking at both behavioral and biomarker outcomes. Implications for women's health includes the benefit of reduced reproductive tract disorders including candidiasis, as well as a potential reduced HIV risk. A potential, though not yet proven, benefit may include noninterference with microbicide activity by reducing IVP. Microbicides are an important female-controlled HIV prevention method. It has been noted that because intravaginal practices present a risk of diluting efficacy through removing or interfering with microbicides, more work is needed to understand motivations and risk reduction around IVP in the context of effective microbicide use.⁶ Conducting large-scale behavioral interventions on intravaginal cessation should be an important component to future microbicide trials conducted in areas in which intravaginal practices are prevalent.

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Author Disclosure Statement

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