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Bacterial vaginosis modifies the association between hormonal contraception and HIV acquisition

Lisa B. HADDAD, MD, MS, MPH[a,b,*], **Kristin M WALL, PhD**[b,c], **William KILEMBE, MD, MSc**[b], **Bellington VWAILIKA, MSc, MD**[b,e], **Naw Htee KHU, MPH**[b], **Ilene BRILL, MPH**[d], **Elwyn CHOMBA, MD**[b,f], **Amanda TICHACEK, MPH**[b], and **Susan ALLEN, MD, MPH**[b]

[a]Department of Gynecology and Obstetrics, Emory University, School of Medicine, Atlanta, Georgia, USA

[b]Rwanda Zambia HIV Research Group, Department of Pathology & Laboratory Medicine, School of Medicine and Hubert Department of Global Health, Rollins School of Public Health, Emory University, Atlanta, GA, USA

[c]Department of Epidemiology, Rollins School of Public Health, Emory University, Atlanta, Georgia, USA

[d]Department of Epidemiology, Ryals School of Public Health, University of Alabama at Birmingham, Birmingham, Alabama, USA

[e]Department of Gynecology and Obstetrics, School of Medicine, University of Zambia, Lusaka, Zambia

[f]Ministry of Community Development, Mother and Child Health, Lusaka, Zambia

Abstract

Objective—To examine BV as an effect modifier for the association between hormonal contraception (HC) and incident HIV infection

Design—Serodiscordant couples enrolled in an open longitudinal cohort in Lusaka, Zambia from 1994–2012. This analysis was restricted to couples with an HIV-positive man enrolled between1994–2002 when a quarterly genital tract examination and HIV testing was performed.

Methods—Multivariate Cox models evaluated the association between contraceptive method and HIV-acquisition, stratified by time-varying BV status.

Results—Among 564 couples contributing 1137.2 couple-years of observation, BV was detected at 15.5% of study visits. 22 of 106 seroconversions occurred during intervals after BV was detected (12 on no method/non-hormonal method (non-HC), 2 on injectables, 8 on oral contraceptive pills, (OCPs)). Unadjusted seroincidence rates per 100-couple-years for non-HC, injectable, and OCP users, respectively, during intervals with BV were 8.3, 20.8 and 31.0 and during intervals without BV were 8.2, 9.7 and 12.3. In the BV-positive model, there was a

[*]Corresponding Author: Lisa Haddad, MD MS MPH, Department of Gynecology and Obstetrics Emory University School of Medicine, 49 Jesse Hill Jr. Drive NE, 3rd Floor, Atlanta, GA 30303, Phone: 404-778-1385, lisa.haddad@emory.edu.

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significant increase in incident HIV among those using injectables (adjusted hazard ratio, aHR 6.55, 95% CI 1.14–37.77) and OCPs (aHR 5.20, 95% CI 1.68–16.06) compared to non-HC. HC did not increase the hazard of HIV acquisition in BV-negative models. These findings persisted in sensitivity analyses when all covariates from the nonstratified model previously published were included, when other genital tract findings were excluded from the model and with the addition of condom-less sex and sperm on wet-prep.

Conclusions—Future research should consider a potential interaction with BV when evaluating the impact of HC on HIV acquisition.

Keywords

Hormonal Contraception; Injectable Contraceptives; Oral Contraceptives; HIV; AIDS; Bacterial Vaginosis; Vaginal Microbiota

INTRODUCTION

Hormonal contraception is widely used globally for the prevention of unintended pregnancy. However, some have postulated that hormonal contraception may increase susceptibility to human immunodeficiency virus (HIV) infection.[1–3] While overall data on the association between combined hormonal contraceptive pills and HIV acquisition are limited, there appears to be no increased risk in women with use of these methods.[3] With injectable contraceptive use, particularly depot medroxyprogesterone acetate (DMPA), recent meta-analyses suggest a 20–70% increased risk of HIV acquisition.[3, 4] Importantly, not all studies have demonstrated a consistent link between use of DMPA and HIV risk in women, with several well-designed studies finding no statistical association.[5–7] The World Health Organization (WHO) recognizes this important knowledge gap and encourages research to clarify the association and explore mechanisms associated with hormonal contraceptives that may underlie an increase in HIV transmission risk.[8, 9]

Several local factors within the vagina may increase HIV transmission risk and mediate the association between hormonal contraception and HIV acquisition.[10] One potential factor is bacterial vaginosis (BV), a condition of a polymicrobial vaginal flora with an elevated vaginal pH and divergence from a healthy lactobaccili dominant vaginal flora.[10, 11] There is a growing body of literature supporting the role of the microbiota in altering local and systemic immune function.[12–14] Several studies have found that HIV acquisition increases in the setting of BV and non-lactobaccili-dominant flora.[15–18] While there is growing evidence of this direct association between vaginal microbiota and HIV acquisition, it is unclear if vaginal microbiota may be implicated in the noted association between hormonal contraception and HIV risk.

Most studies suggest that combined estrogen-progestin and progestin-only hormonal contraception does not increase the presence of BV and some methods may even be protective against BV.[19–21] In a recent meta-analysis, all studies reviewed showed either a statistically significant decrease or no significant difference in the incidence of BV in hormonal contraceptive users when compared to non-hormonal contraceptive users. When including the three highest quality studies, they found a 10–20% reduction in the incidence

of BV in combined oral contraceptive users and an 18–30% reduction in DMPA users versus non-hormonal method users.[22] Another meta-analysis including 55 studies reported an approximate 25% reduction in BV in hormonal contraceptive users compared with non-users, with this pattern similar for both combined estrogen-progestin and progestin-only methods.[23] These data suggest that alterations in the vaginal microflora with contraceptive use are not part of the mechanisms for the observed increased HIV acquisition risk with hormonal contraception.

Prior studies have evaluated the effect of HSV or genital tract ulcers as potential effect modifiers in the association between hormonal contraception and HIV acquisition in women.[7, 24] However, no studies to date have evaluated whether BV may modify this association. Our objective was to leverage a large HIV discordant couple cohort to explore the association between hormonal contraception and HIV acquisition in women, considering BV as a potential effect modifier. Our previous evaluation of the cohort did not demonstrate a significant increase in incident HIV with oral contraceptive, injectable contraceptive or contraceptive implant use. We hypothesized that we would see a different association between hormonal contraception and incident HIV in the presence of BV. This hypothesized interaction between two independent potential risk factors may explain some of the inconsistencies in the literature that cloud our interpretation of hormonal contraception as a potential HIV risk factor.

METHODS

Study Design, Participants and Ethics

The study is a secondary analysis of a longitudinal cohort of heterosexual HIV serodiscordant couples in which the man is HIV-positive and the woman HIV-negative (M+F-) in Lusaka, Zambia. Heterosexual married or cohabitating HIV serodiscordant couples were invited to enroll in an open cohort study between 1994–2012. The study recruitment,[25, 26] intervention design, uptake of contraception immediately after an educational intervention,[27] impact of informed consent on knowledge and concerns about contraceptive methods,[28] demographics of the cohort, rates of unintended pregnancy and impact of contraceptive method on unintended pregnancy,[29] impact of the intervention on incident pregnancy,[30] patterns of contraceptive use and discontinuation,[31] impact of hormonal contraception on HIV acquisition risk,[7] HIV transmission to partners,[32] and disease progression[33] have been previously reported. The Institutional Review Boards at Emory University and the University of Zambia approved this study. Written informed consent was obtained from all participating couples.

Exposure of interest

Contraceptive method used since last visit (none/condoms only, oral contraceptive pills (OCPs), DMPA (150mg IM dosage), copper intrauterine device (IUD), contraceptive implant (Levonorgestrel implant: Norplant, Jadelle), or permanent methods (hysterectomy/tubal ligation/vasectomy)) was recorded at baseline and three-monthly follow-up visits. The majority of OCPs were combined pills containing both an estrogen and progestin, with progesterone-only pills being primarily prescribed to breastfeeding women until children

were 6 months old or the minority of women with contraindications to estrogens. In our primary analysis, contraceptive methods were categorized as implant, injectable, or OCP versus non-hormonal (non-HC), including none/condoms only or permanent methods. All methods were provided at the research site.

Outcome of interest

The primary outcome evaluated any incident HIV infection among women, either linked or unlinked to the cohabiting male partner. HIV testing using rapid serologic tests was conducted at intervals of three-months.[26]

Baseline covariates

At enrollment, baseline demographic data was collected including age of the man and woman, years cohabiting, monthly income, and woman's literacy in Nyanja. Other possible risk factors evaluated as covariates included number of previous pregnancies, number of sexual partners for the woman in the last year, and viral load (log10 copies/mL) of the positive male partner.

Time-varying covariates

At scheduled three-monthly (or client-initiated interim) follow-up visits, time-varying exposures of interest were collected including time from enrollment (0–3 months reflecting prior to CVCT versus >3 months, reflecting those receiving CVCT), pregnancy, self-reported sex without a condom with study partner in past 3 months, self-reported sex with a condom with study partner in past 3 months, sperm present on vaginal swab wet-prep, candida by wet-prep, vaginal discharge on exam, general vaginal inflammation on exam, sexually transmitted infection (STI; gonorrhea and/or chlamydia based on purulent endocervical discharge and/or trichomonas based on wet-prep), bilateral inguinal adenopathy (BIA) on exam, and active genital or perianal ulcers for woman in past 3 months (by self-report or examination finding).

Effect modification evaluation

The effect modifier of interest was a time-varying diagnosis of BV. This was diagnosed by a wet-prep at baseline and at scheduled or client-initiated interim follow-up visits. BV was diagnosed with microscopy (wet-prep for clue cells, KOH prep for whiff test, and/or gram stain) at a routine or interim visit.

Longitudinal data collection

Participants were provided with free outpatient care and the full range of contraceptive methods at the research clinic. Data collection varied by type and frequency of data collected over 17 years of follow-up. From 1994–2002, both partners were seen every 3 months and underwent physical exams including RPR screening for syphilis, genital exams and wet-prep with repeat HIV testing of the HIV- partner. After 2002, physical exams and wet-prep diagnoses were performed at baseline and thereafter only if signs and symptoms of infections were present. Given this change in approach to only evaluating symptomatic individuals, we restricted this analysis to 1994–2002.

Data analysis

Analyses were conducted with SAS v9.3 (Cary, NC). Our analytic approach was informed by recommendations for a rigorous and consistent analysis of the association of HC and HIV acquisition described by Polis et al. [34] This approach was used previously in our evaluation with this cohort and demonstrated no statistically significant association between HC and HIV acquisition in women without consideration of effect modification with BV.[7] All analyses were stratified and separately analyzed for intervals with BV and intervals without BV.

Couple-years of follow-up were calculated from enrollment until the couple was censored. Couples were censored when either partner died, the couples separated, the positive partner started ART, or if either partner was lost to follow-up. HIV incidence rates for each contraceptive method type were compared to the non-HC reference group using univariate Cox models. These rates with corresponding 95% confidence intervals were calculated as the number of incident infections per couple-year of follow-up, stratified by whether a women had a BV infection noted at the visit prior to that study interval.

Baseline and time-varying data were described by HIV acquisition status using counts and percentages for categorical data or means and standard deviations for continuous data. These descriptive analyses were calculated across all study intervals and were stratified by BV status.

Bivariate associations between baseline and time-varying covariates and outcome infection of interest were evaluated via unadjusted Cox models to generate crude hazard ratios (HRs) and 95% confidence intervals (CIs). Additional bivariate associations between these baseline and time-varying covariate were compared for the combined outcome groups (seroconverters and non-converters) by BV status.

Multivariate Cox models estimated the total effect of time-varying contraceptive method type on time to outcome infection. Covariates significantly ($p<0{\cdot}05$) associated with the exposure and outcome of interest were considered as potential confounders. Variable multi-collinearity was assessed (condition indices of 0·30 and variance decomposition proportions of 0·05 as cutoff criteria); if any two variables were found to be collinear, the variable with the weakest association with the outcome was removed. The proportional hazards assumption using Schoenfeld residuals and graphical methods (plots of log [-log (survival probability)] versus log (time)) was confirmed for time-independent covariates. Adjusted HRs (aHRs) and 95% CIs are presented for covariates in the final multivariate models. For each model, contraceptive method was forced into the final multivariable models. Breslow-day test was used to determine the significance of an interaction by time-varying BV status.

Sensitivity analysis

We ran a sensitivity analysis including all the variables included in a non-BV stratified model we previously published on from this cohort[7] given potential for unidentified confounding in the smaller stratified samples. Similarly, we ran an additional analysis including all covariates in the stratified models if found to be a confounder (associated with exposure and outcome at <0.05) in either one or both of the strata. We also ran the analysis

including variables in the prior cohort evaluation[7] adding as covariates sperm on wet-prep and self-reported unprotected sex. We ran an analysis excluding the genital tract findings (infections and ulcerations) as confounders as BV was highly correlated with several of these findings. Further, we also ran the previously published model, excluding the genital tract findings.

RESULTS

Baseline demographics and rates for seroconversion (table 1)

Among the 564 couples, a total of 106 women seroconverted over 1137.2 couple-years of observation. BV was detected at 648 of 4183 study visits (15.5%). 22 of the seroconversions occurred during intervals where BV was detected at the visit prior to the seroconversion and 84 during intervals without BV detected. Among the 4183 visits, implants were used at 17 visits (0.4%), injectable methods at 373 visits (8.9%), OCPs at 568 visits (13.6%) and non-hormonal methods at 3225 visits (77.1%, with copper IUD at 40 visits (0.9%), tubal ligation or vasectomy at 42 visits (1.0%) and no method or only condoms at 3143 visits (75.1%)).

During intervals where BV was detected, seroincidence rates per 100-couple-years were 8.3, 20.8 and 31.0 for non-hormonal, injectable and OCP users, respectively. During intervals without BV detected, seroincidence rates per 100-couple-years were 8.2, 9.7 and 12.3, for non-hormonal, injectable and OCP users, respectively. No seroconversions occurred among implant users.

Bivariate analyses (table 2)

Covariates significantly associated with BV status included illiteracy to Nyanja (83% vs. 76%), higher number of sex partners in the last year at baseline (1.09 vs. 1.03), less injectable contraceptive usage (5% vs 10%), increased self-reported sex without a condom in the past 3 months (44% vs. 38%), higher rates of sperm on wet-prep (24% vs. 17%), increased vaginal discharge (14% vs. 9%), increased STIs inflammation (15% vs. 6%) and increased BIA in the woman (8% vs 7%).

Among intervals with BV, baseline and time-varying covariates significantly associated with HIV incidence (non-seroconverting intervals vs, seroconverting intervals) included younger age of the woman (27.46 vs. 22.68), OCP use (14% vs. 36%), being in the initial 3 months after enrollment in CVCT cohort (2% vs. 23%), vaginal inflammation (3% vs. 14%) and BIA on exam (8% vs. 23%) in the past 3 months. Among the intervals without BV, being in the initial 3 months after enrollment in the CVCT cohort (3% vs. 14%), having a STI inflammation (6% vs. 13%) and BIA (7% vs. 19%) in the past 3 months were associated with increased HIV incidence.

Contraceptive method at follow-up was significantly associated with seroconversion only among those with BV, with an increase in seroconversion among those using OCPs compared to those using non-HC.

Multivariate analyses (table 3)

Hormonal contraceptive method was associated with incident HIV in the multivariable analysis in the time-varying BV-positive models but not in the BV-negative models, where BV was a significant interaction term (Breslow-day test for interaction by BV: $p<0.001$). In the BV-positive model, when controlling for women's age (per year increase), vaginal inflammation in the past 3 months, and time interval since enrollment (0–3 months versus 3 months), there was a significant increase in HIV acquisition among those using injectable contraception (aHR 6.55, 95% CI 1.14–37.77) and OCPs (aHR 5.20, 95% CI 1.68–16.06) compared to the non-HC group. In the BV-negative model, use of the implant, injectables and OCPs did not have any increased hazards of HIV acquisition compared to the non-HC group, when controlling for time interval since enrollment (0–3 months vs. >3 months), STI in the past 3 months, and BIA in the past 3 months.

Sensitivity analysis

Overall, the results from the sensitivity analyses led to similar conclusions. In the multivariate models limited to including all the covariates that were confounders in the non-stratified evaluation previously published, we found a similar significant increase in HIV acquisition with use of injectable contraception (aHR 6.58, 95% CI 1.06–40.87) and OCPs (aHR 4.66, 95% CI 1.45–14.96) compared to non-HC use for the BV positive model. Similarly when controlling for women's age, time interval since enrollment, vaginal inflammation, inflammatory STI or BIA in last 3 months, the covariates that were identified as potential confounders in either or both strata, we found a similar increase in HIV acquisition with injectable (aHR 5.91, 95% CI 1.02–34.97) and OCP use. (aHR 4.78, 95% CI 1.52, 15.01). When excluding other genital tract findings, there was a significant increase in HIV acquisition with OCP use compared to non-HC use (aHR 5.7, 95% CI 1.9, 17.2) in the BV positive model with the association among injectable users approaching significance (aHR 5.4, 95% CI 0.96, 30.4, $p=0.056$). When sperm on wet prep and condomless sex were added to the model, a similar increase in HIV acquisition was noted with injectable (aHR 9.2, 95% CI 1.3, 62.5) and OCP use (aHR 7.6, 95% CI 1.9, 31.0) compared to non-HC use in the BV models. Lastly, with all the confounders from the published analysis included and excluding the genital tract findings, there was a significant increase in HIV acquisition among injectable (aHR 5.9, 95% CI 1.02, 33.9) and OCP (aHR 5.6, 95% CI 1.9, 17.1) users compared to non-HC users in the BV positive model. There was no association between use of HC and HIV acquisition in the non-BV models compared to non-HC use in any of the sensitivity analyses.

DISCUSSION

We present results indicating BV as a potent effect-modifier of the association between HC and HIV acquisition that may have large global health implications. While increasing evidence has supported a direct role for BV in increasing HIV acquisition risk, our results suggest that this impact is amplified in the setting of HC use, specifically injectables and OCPs, with increased hazard ratios greater than 5 for HIV acquisition in the setting of BV for injectables and OCPs compared to non-HC use. Though our numbers were small and confidence intervals large, these findings highlight the importance of acknowledging the role

of the vaginal environment in HIV acquisition risk and evaluating the genital tract environment in future studies investigating the association between hormonal contraceptives and HIV risk.

Proposed mechanisms for the association between HC and HIV have included alterations in the local genital tract immunologic milieu and the composition of key HIV target immune cells, as well as alterations in vaginal epithelial tight junctions and mucosal permeability.[35–37] Recent evidence has pointed towards the gut microbiota and the hormonal environment as working synergistically to influence the development of disease states such as obesity, diabetes and certain cancers.[38] While a significant amount of literature has been building to explore the importance of the gut microbiota, our current understanding of the significance of the vaginal microenvironment is relatively limited. HC may have a differential impact to amplify the effect of the microbiota on the vaginal epithelium and immune environment. Future studies are needed to elucidate the mechanism for this interaction.

While epidemiologic studies have not previously explored this interaction, a recent study by Fichorova et al[10] compliments our findings. Those results demonstrated that the immunomodulatory changes attributed to different hormonal contraceptives are dependent on the genital tract microenvioronment. Specifically, they report an increase in RANTES (*r*egulated upon *a*ctivation, *n*ormal *T*-cell *e*xpressed, and *s*ecreted), a chemokine that has been associated with increased HIV acquisition, among OCP users in the setting of BV, a finding that is mechanistically consistent with our observations. Although the consensus from the literature suggests no association between OCP use and HIV risk,[3] the reduction in BV among OCP users and differences in OCP adherence may have diluted any significant results. In the context of the demonstrated effect-modification potential of BV reported here, re-evaluation of those findings, if possible, is warranted.

The prevalence of BV is variable based on the clinical setting and may vary by race and behavioral practices. [39] While the prevalence may be underestimated since many women are asymptomatic, the greatest burden of BV is noted in sub-Saharan Africa.[40] Further, the sensitivity and specificity of clinical techniques are variable in the diagnosis of BV. For example, using Amsel's criteria with a wet-prep, which can be done easily as a point-of-care approach, yet is only about 70% sensitive for BV.[41] The presence of clue cells on wet prep, is highly sensitive and specific for BV.[42] Nugent scores from a gram stain, currently considered the gold standard for diagnosis for BV diagnosis, may have greater sensitivity, but can be subject to variability in interpretation. Utilizing more sensitive microbiome techniques, such as 16s gene rRNA sequencing, offers an opportunity to understand the microenvironment at the level of specific microbial species and define microbial diversity with more specific methodology. While historically symptomatic BV has been attributed to *Gardnerella vaginalis*, these newer technologies have identified other bacteria associated with dysbiotic vaginal microbial states including *Atopobium vaginae, Lactotrichia amnionii, Megasphaera*, and members of the *Clostridiales* sometimes referred to as BV-associated bacteria (BVAB).[11, 43–45] Further, as recent studies have suggested that specific microbacteria in the vaginal environment may have a differential impact on the local

immune environment and HIV risk[45] it is important to explore this interaction using these more sensitive techniques.

This study has several notable limitations. Our biggest limitation is the small number of women using contraception who seroconverted with BV detected. While even with this reduced power, we find a significant association; the stability of this finding weighs heavily on a small number of observations. Even during the years where we conducted routine examinations every 3 months, we may be missing episodes of BV, due to the possible shifts between flora considered normal and flora considered intermediate or abnormal demonstrated in some studies.[46] This misclassification bias would primarily impact the association between contraception and HIV during the interval assessment without BV detected. Additionally, we use several criteria in diagnosing BV, which may increase our sensitivity while reducing the specificity of our BV diagnoses. This could lead to misclassification. The generalizability of these results may be limited as this cohort consists of jointly tested and counseled HIV discordant couples who often adopt condom use following counseling with a corresponding reduction in transmission and seroconversion rates. While we aimed to control for the impact of condom non-use as a potential confounder, both sperm on wet-prep and self-reported unprotected sex have limitations in their ability to detect unprotected coital events, thus some degree of unmeasured confounding is possible. Given these limitations, our results should be interpreted with caution and we encourage future studies to systematically evaluate BV as a modifier in future studies.

While this evaluation begins to elucidate some of the diversity noted in epidemiologic studies, many unanswered questions remain. It is unclear if the changes attributed to BV that lead to increased acquisition risk and modification of the HC-HIV association are consistent among BV-symptomatic compared to asymptomatic women. It is also unknown if treatment of BV will alter these associations. For example, treatment of asymptomatic BV during pregnancy does reduce the risk of preterm delivery associated with BV.[47] As BV is often recurrent, exploration of the role of suppressive or periodic presumptive treatment for BV on biologic and clinical outcomes is warranted.[48] We must leverage newer sequencing approaches to effectively explore the role of individual taxa or microbial communities of these relationships utilizing rigorous epidemiologic methodology.[49] And lastly, we must explore alternative explanations for the findings we report. For example, is there something else about individuals who get BV that predisposes them to having increased risk with hormonal contraceptive use, such as biologic or behavioral factors?

In conclusion, our findings suggest an association between HIV acquisition and HC use, specifically OCPs and injectables, among individuals with BV. Current guidelines do not restrict the use of contraceptive methods for women at high-risk for HIV. The newest WHO recommendations specifically recommend that providers discuss the potential for increased acquisition with the use of DMPA.[50] An individual’s contraceptive choice is influenced by many factors, and ultimately should remain with each woman in consultation with her provider. Data on alternative contraceptive options are critical for adequate counseling on relative risks for contraceptive use. Further, the vaginal microenvironment cannot be overlooked in the field of reproductive health and HIV, especially given recent data on the

impact of *Gardenerella* on antiretroviral drug concentrations used for pre-exposure prophylaxis effectiveness as well the possible increased risk of human papilloma virus (HPV) with non-lactobacillus dominant flora.[51, 52] Future research is needed to further explore the interaction between BV and HC on HIV acquisition. If this interaction persists among other studies, identification of BV may help tailor family planning discussions to appropriately counsel individuals on their risk. Further, periodic testing and treatment among asymptotic women at high-risk for HIV and counseling women on the symptoms of BV may be important preventative strategies among reproductive aged women.

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Table 1

Unadjusted seroincidence rates for linked and unlinked HIV seroconversion, stratified by time-varying BV and contraceptive method use (N = 564 M+F− couples), 1994–2002

Contraceptive method	Number of seroconversions	Couple-years of follow-up time	Seroincidence per 100 couple-years	95%CI	
BV = YES					
Non-HC	12	145.4	8.3	4.5	14.0
OCPs	8	25.8	31.0	14.4	58.8
Injectables	2	9.6	20.8	3.5	68.8
Implant	0	1.3	0.0		
Total	*22*	*182.1*	*12.1*	*7.8*	*18.0*
BV = NO					
Non-HC	61	746.9	8.2	6.3	10.4
OCPs	15	122.1	12.3	7.1	19.8
Injectables	8	82.8	9.7	4.5	18.4
Implant	0	3.2	0.0		
Total	*84*	*955.0*	*8.8*	*7.1*	*10.8*

BV: Bacterial vaginosis; OCP: oral contraceptive pill; Non-HC: Non-hormonal contraception, includes no method use, condom use, copper intrauterine device, and permanent method; CI: confidence interval

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Table 2

Descriptive analyses and p-values from unadjusted associations (from Cox models) between covariates and time to linked and unlinked HIV seroconversion, stratified by time-varying BV status (N = 564 M+F− couples), 1994–2002

	BV = YES							BV = NO							
	Total (n = 648 intervals)		Non-seroconversion interval (n = 626 intervals)		Linked and unlinked seroconversion interval (n = 22 intervals)			Total (n = 3535 intervals)		Non-seroconversion interval (n = 3451 intervals)		Linked and unlinked seroconversion interval (n = 84 intervals)			p-value for BV Yes versus No
	N	%	N	%	N	%	p-value (2-tail)	N	%	N	%	N	%	p-value (2-tail)	
BASELINE COVARIATES (n-intervals,%intervals)															
Man age (mean, SD)	33.79	8.27	33.92	8.34	30.05	4.74	0.068	34.84	8.03	34.86	8.03	33.92	7.88	0.302	
Woman age (mean, SD)	27.30	7.92	27.46	7.97	22.68	4.64	0.014	27.83	7.38	27.87	7.40	26.35	6.61	0.083	0.528
Years cohabiting (mean, SD)	7.35	7.09	7.44	7.17	4.73	3.31	0.118	8.36	7.00	8.37	7.02	7.55	6.10	0.323	0.153
Monthly family income (mean, SD)	56.19	60.34	56.94	61.02	34.81	29.73	0.086	58.84	69.48	58.81	69.73	60.11	58.30	0.950	0.478
Woman reads Nyanja															0.027
Yes, easily	107	17%	105	17%	2	9%	0.332	831	24%	815	24%	16	19%	0.331	
No/With difficulty	541	83%	521	83%	20	91%		2704	76%	2636	76%	68	81%		
Number of previous pregnancies (mean, SD)	3.84	2.94	3.89	2.96	2.57	1.96	0.082	3.97	2.81	3.98	2.83	3.71	2.14	0.471	0.68
Number of sex partners in last year (mean, SD)	1.09	0.41	1.10	0.41	1.05	0.22	0.234	1.03	0.34	1.03	0.33	1.06	0.45	0.431	<.0.001
Log viral load of positive partner, log10 copies/ml (mean, SD) *	4.91	0.73	4.90	0.73	5.14	0.57	0.187	4.91	0.74	4.91	0.74	5.04	0.66	0.132	0.873
TIME-VARYING COVARIATES (N intervals, % interval)															
Contraceptive method															
Non-HC	517	80%	505	81%	12	55%		2708	77%	2647	77%	61	73%		
Implant	2	0%	2	0%	0	0%	n/a	15	0%	15	0%	0	0%	n/a	0.0157
Injectables	33	5%	31	5%	2	9%	0.130	340	10%	332	10%	8	10%	0.761	0.009
OCPs	96	15%	88	14%	8	36%	0.004	472	13%	457	13%	15	18%	0.312	0.298
Study interval time from enrollment															0.641

	BV = YES							BV = NO							
	Total (n = 648 intervals)		Non-seroconversion interval (n = 626 intervals)		Linked and unlinked seroconversion interval (n = 22 intervals)			Total (n = 3535 intervals)		Non-seroconversion interval (n = 3451 intervals)		Linked and unlinked seroconversion interval (n = 84 intervals)			p-value for BV Yes versus No
	N	%	N	%	N	%	p-value (2-tail)	N	%	N	%	N	%	p-value (2-tail)	
0–3 months	18	3%	13	2%	5	23%	<.0.001	100	3%	88	3%	12	14%	<.0.001	
> 3 months	630	97%	613	98%	17	77%		3435	97%	3363	97%	72	86%		
Pregnant during interval															0.554
Yes	76	12%	74	12%	2	9%	0.733	382	11%	370	11%	12	14%	0.312	
No	566	88%	546	88%	20	91%		3107	89%	3035	89%	72	86%		
Sex with study partner with a condom in past 3 months															0.057
Yes	494	76%	474	76%	20	91%	0.126	2884	82%	2818	82%	66	79%	0.572	
No	154	24%	152	24%	2	9%		648	18%	630	18%	18	21%		
Sex with study partner without a condom in past 3 months															0.026
Yes	288	44%	278	44%	10	45%	0.917	1335	38%	1293	38%	42	50%	0.081	
No	360	56%	348	56%	12	55%		2197	62%	2155	63%	42	50%		
Sperm present on wet prep															<.0.001
Yes	122	24%	117	24%	5	31%	0.958	474	17%	459	16%	15	21%	0.211	
No	377	76%	366	76%	11	69%		2390	83%	2333	84%	57	79%		
Vaginal discharge															<0.001
Yes	90	14%	86	14%	4	18%	0.462	311	9%	300	9%	11	13%	0.169	
No	558	86%	540	86%	18	82%		3253	91%	3180	91%	73	87%		
Vaginal inflammation on exam															0.522
Yes	21	3%	18	3%	3	14%	0.044	92	3%	89	3%	3	4%	0.664	
No	616	97%	597	97%	19	86%		3324	97%	3243	97%	81	96%		
Candida															0.890
Yes	129	20%	121	19%	8	36%	0.068	635	18%	614	18%	21	25%	0.081	

	BV = YES							BV = NO							
	Total (n = 648 intervals)		Non-seroconversion interval (n = 626 intervals)		Linked and unlinked seroconversion interval (n = 22 intervals)			Total (n = 3535 intervals)		Non-seroconversion interval (n = 3451 intervals)		Linked and unlinked seroconversion interval (n = 84 intervals)			p-value for BV Yes versus No
	N	%	N	%	N	%	p-value (2-tail)	N	%	N	%	N	%	p-value (2-tail)	
No	519	80%	505	81%	14	64%		2865	82%	2802	82%	63	75%		
STI															<.0.001
Yes	99	15%	95	15%	4	18%	0.368	202	6%	191	6%	11	13%	0.004	
No	549	85%	531	85%	18	82%		3333	94%	3260	94%	73	87%		
BIA of women															0.037
Yes	52	8%	47	8%	5	23%	0.048	254	7%	238	7%	16	19%	0.001	
No	589	92%	572	92%	17	77%		3169	93%	3101	93%	68	81%		
Genital ulceration in past three months															0.106
Yes	135	21%	127	20%	8	36%	0.085	730	21%	707	20%	23	27%	0.071	
No	513	79%	499	80%	14	64%		2805	79%	2744	80%	61	73%		

SD: Standard deviation; BV: Bacterial vaginosis; OCP: oral contraceptive pill; Non-HC: Non-hormonal contraception, includes no method use, condom use, copper intrauterine device, and permanent method; STI: gonorrhea and/or chlamydia based on purulent endocervical discharge and/or trichomonas based on wet-prep; BIA: Bilateral inguinal adenopathy.

* Viral load collection began in 1999.

Table 3

Multivariate models of hormonal contraception use and time to linked or unlinked HIV seroconversion, stratified by time-varying BV status (N = 564 M+F− couples), 1994–2002

	BV = YES				BV = NO			
Number of outcomes modeled:	**22 (out of 22)**				**84 (out of 84)**			
Current contraceptive method at follow-up visit	**aHR***	**95%CI**		**p-value (2-tail)**	**aHR****	**95%CI**		**p-value (2-tail)**
Non-HC	ref				ref			
Implant	n/a				n/a			
Injectables	6.546	1.135	37.767	0.036	1.348	0.637	2.853	0.435
OCPs	5.196	1.681	16.062	0.004	1.36	0.764	2.422	0.296

SD: Standard deviation; BV: Bacterial vaginosis; OCP: oral contraceptive pill; Non-HC: Non-hormonal contraception, includes no method use, condom use, copper intrauterine device, and permanent method; ref: reference; n/a: not applicable; aHR: adjusted hazards ratio; CI: confidence interval.

*Controlling for: Woman's age (per year increase), vaginal inflammation of woman in past 3 months, and time interval since enrollment (0–3 months vs. >3 months)

**Controlling for: inflammatory STI in the past 3 months, bilateral inguinal adenopathy in past 3 months, and time interval since enrollment (0–3 months vs. >3 months)