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Elevation of cervical CXCL10 levels is associated with HIV-1 acquisition in pregnant and postpartum women

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Abstract

Objective: To evaluate the relationship between cervical cytokine/chemokine concentrations and HIV-1 acquisition in peripartum Kenyan women.

Design: Nested case-control study

Methods: Women participating in a prospective study of peripartum HIV acquisition in Kenya (the *Mama Salama Study*), were tested for HIV-1 at 1–3 month intervals during pregnancy and through 9 months postpartum. Cases positive for HIV-1 RNA during follow-up (N=14), were matched 3:1 with HIV-negative controls (N=42) based on age, marital status, partner HIV-1 status, transactional sex, and timing of cervical swab collection. Concentrations of 5 cytokines (interleukin (IL)-1 β , IL-6, IL-10, interferon gamma (IFN γ), and tumor necrosis factor alpha (TNF α)) and 4 chemokines (IL-8, C-X-C motif chemokine ligand 10 (CXCL10), macrophage inflammatory protein (MIP)-1 alpha, and MIP-1 beta) were measured from cervical swabs collected at the visit prior to HIV-1 diagnosis (cases) or matched gestational/postpartum time

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Contributors: ALD, JK, DM, BAR, JO, and GJS were involved in design of the parent studies, and SMG conceived the present analysis. JK, ALD, DM, LO, GJS performed data collection. MCS, JCP, DAL, BW, BAR, JO and SMG participated in data analysis. BW and DAL generated cytokine data. JO and GJS acquired funding for this study. BAR provided statistical guidance. MCS and SMG wrote the initial draft of the manuscript. All authors contributed to the final draft of the manuscript and approved the final draft for submission.

(controls). Cytokine/chemokine concentrations were compared between cases and controls using Wilcoxon rank-sum tests. Principal component analysis was used to create a summary score for closely correlated cytokines/chemokines. Associations with HIV-1 acquisition were analyzed using conditional logistic regression. Path analysis was used to evaluate hypothesized relationships between CXCL10, vaginal washing, Nugent score, and HIV-1 acquisition.

Results: Conditional logistic regression analysis demonstrated an association between increased concentrations of CXCL10 and HIV-1 acquisition (OR=1.74, 95% CI 1.04, 2.93; $p=0.034$). Path analysis confirmed a positive independent association between higher concentrations of CXCL10 and HIV-1 acquisition (path coefficient=0.37, 95% CI 0.15, 0.59; $p<0.001$).

Conclusions: HIV-1 acquisition was associated with increased cervical concentrations of CXCL10 in pregnant and postpartum women.

Keywords

pregnancy; HIV-1; cervical; cytokines; chemokines; CXCL10; Kenya; female

INTRODUCTION

Globally, over half of individuals living with HIV-1 are women, most of whom became infected with HIV-1 through vaginal sex. ^[1, 2] Pregnancy increases the risk of HIV-1 acquisition, and HIV-1 incidence rates during pregnancy and the postpartum period are comparable to those seen in serodiscordant couples and female sex workers. ^[3–5] This increased risk remains after adjusting for total number of sex acts, age, condom use, partner viral load, and use of pre-exposure prophylaxis. ^[5] Multiple social and biomedical factors likely contribute to increased HIV susceptibility during pregnancy, ^[6] including physiologic changes that occur during pregnancy. ^[7]

While pregnancy has traditionally been viewed as an immunologically quiescent state, recent scientific advances suggest it is an immunologically dynamic period. ^[8] Both implantation and labor require inflammation, ^[8] and concentrations of proinflammatory cervical cytokines are increased in pregnant women. ^[9, 10] Cervicovaginal inflammation has been associated with HIV-1 acquisition in non-pregnant women; ^[11] however, the role of genital tract inflammation in influencing HIV-1 risk among pregnant and postpartum women is not well elucidated. In this case-control study, we aimed to assess whether concentrations of cervical cytokines and chemokines were associated with HIV-1 acquisition in pregnant and postpartum Kenyan women.

MATERIALS AND METHODS

Study Design and Participants

A nested case-control study was performed utilizing data collected in the *Mama Salama Study*, a prospective cohort of pregnant, HIV-1 uninfected women presenting to the Ahero sub-District and Bondo District Hospitals in Western Kenya between May 2011 and June 2013. ^[6] The study was approved by the Kenyatta National Hospital/University of Nairobi

Ethics and Research Committee and the University of Washington Institutional Review Board. All participants provided written informed consent for participation.

The *Mama Salama Study* has been previously described. [6] In brief, eligibility criteria included age 14, current pregnancy, and a documented negative HIV-1 rapid test at enrollment or within the prior three months. Women were asked to return for follow-up visits during pregnancy (20, 24, 32, and 36 weeks gestation) and the postpartum period (2, 6, 10, 14 weeks, 6 and 9 months). At study visits, women: i) provided blood for HIV-1 testing by nucleic acid amplification test (NAAT) and self-collected vaginal swabs for evaluation of bacterial vaginosis (BV), vulvovaginal candidiasis (VVC), and *Trichomonas vaginalis* infection; ii) underwent a standardized physical examination by a health care professional; and iii) completed a questionnaire on sexual practices, including vaginal washing. Testing for *Chlamydia trachomatis* and *Neisseria gonorrhoeae* was performed at enrollment; women who tested positive were treated according to Kenyan guidelines. [12] At enrollment, 28 weeks gestation, 6 weeks postpartum and 6 months postpartum, women underwent speculum-assisted pelvic examination and collection of cervical swabs for planned measurement of cervical cytokines and chemokines.

Cases (defined as having both a negative NAAT and negative HIV-1 rapid test at enrollment and a positive HIV-1 RNA at a follow-up visit) were matched 3:1 with controls (defined as women that remained HIV-negative throughout follow-up and had cervical swabs available for testing). Cervical cytokine/chemokine concentrations were measured from cervical swabs collected at the visit prior to HIV-1 seroconversion (cases) or within 2 weeks of the matched case's gestational age/time postpartum (controls). Controls were also matched on age (± 3 years, continuous variable), current marital status (married, not married), partner HIV-1 status (positive, negative, unknown), and history of transactional sex (yes, no).

Laboratory Procedures

Clinical tests: At all visits, patients self-collected up to three vaginal swabs. The first vaginal swab was used for diagnosis of *T. vaginalis* infection (wet preparation) and BV (gram stain using the method of Nugent and Hillier [13]) and the second vaginal swab was used for detection of VVC (KOH preparation). Additional vaginal swabs were stored for future studies of factors associated with HIV-1 acquisition risk. Diagnosis of *N. gonorrhoeae* and *C. trachomatis* infection was performed using the Gen-Probe APTIMA Combo2 kit (Hologic, San Diego CA, USA) on cervical swabs that were collected and stored according to the manufacturer's instructions. Testing for incident HIV-1 infection was performed as previously described. [6] Briefly, HIV-1 NAAT was performed on pooled samples (10 total) using the Cobas AmpliPrep-Cobas TaqMan (CAPCTM) platform (Roche Molecular Systems, Branchburg NJ, USA). If the pooled NAAT test was positive, individual samples were re-tested and the viral load quantified using the same platform.

Measurement of cervical cytokines: Cervical samples were collected using Dacron swabs from Fitzco Inc. To minimize variability, samples were collected by trained staff according to standard operating procedures for the *Mama Salama Study*. Swabs were transferred immediately to freezing media (70% RPMI medium, 20% fetal bovine serum,

10% dimethylsulfoxide) and stored at -80°C until use. Measurement of interleukin (IL)-1 β , IL-6, IL-8, C-X-C motif chemokine ligand (CXCL10, also known as interferon gamma-induced protein 10 [IP-10]), interferon gamma (IFN γ), tumor necrosis factor alpha (TNF α), macrophage inflammatory protein 1-alpha (MIP-1 α), and macrophage inflammatory protein 1-beta (MIP-1 β) concentrations from cervical samples was performed using the V-Plex Custom Human Cytokine panel from Meso Scale Discovery (MSD; Rockville, MD, USA), as previously described. [14]

Statistical Analysis

Demographic and behavioral data reported at the time of cervical swab collection were summarized using descriptive statistics. Generalized estimating equations (GEE) with a Gaussian link (continuous variables) or log binomial link (categorical variables) and exchangeable correlation structure were used to compare unmatched variables between cases and controls. The primary exposure was the $\log_2$ transformed concentration of cytokines/chemokines analyzed (IL-1 β , IL-6, IL-8, IL-10, CXCL10, IFN γ , TNF α , MIP-1 α , and MIP-1 β). Transformation to the $\log_2$ scale was performed to i) normalize the data; and ii) increase biological relevance, as a 1 $\log_2$ increase corresponds to a doubling of concentration, and a 1 $\log_2$ decrease corresponds to a halving of concentration. The primary outcome was HIV-1 acquisition. Cytokine concentrations were compared between cases and controls using Wilcoxon rank-sum tests.

To reduce the number of statistical comparisons and evaluate the correlation between cytokines/chemokines, principal component analysis (PCA) was performed on $\log_2$ -transformed concentrations of cytokines/chemokines to pool together cytokines/chemokines that were strongly correlated (i.e., factors with an eigenvalue >1). Cytokines/chemokines with uniqueness scores $>70.0\%$ (indicating weak correlation with other cytokines/chemokines) were excluded, and the remaining cytokines/chemokines underwent repeat PCA to confirm generation of one or more factors.

Conditional logistic regression was then performed to determine if any of the factors generated by PCA, or individual cytokines/chemokines excluded in the PCA due to high uniqueness scores, were independently associated with HIV-1 acquisition. The relationship between the following variables measured at the visit of interest and HIV acquisition were also evaluated, based on their potential associations with HIV risk and with cervicovaginal inflammation: i) frequency of sex acts in the past week (continuous variable) and any condomless sex in the past week (categorical variable, yes/no); [15] ii) Nugent score (modeled as a continuous variable, with higher scores indicating greater dysbiosis); [6, 14, 16, 17] and iii) vaginal washing, defined as using a cloth or finger to wash beyond the introitus. [18] Variables were included in multivariable modeling if associated with HIV-1 acquisition at $p \leq 0.20$.

Path analysis was performed to examine possible causal pathways between variables. [19] A hypothesized model of the relationships between variables and the primary outcome (HIV acquisition) was generated. Maximum likelihood estimation was used to calculate estimates of the relationships between variables, expressed as path coefficients which are interpreted as β coefficients generated by linear regression. Due to limited sample size and some non-

normality of the data, goodness of fit was evaluated using the root mean squared error of approximation (RMSEA), a highly informative method for evaluating model paths; the RMSEA cut-off indicating good model fit was <0.05 .^[19] Equation-level goodness of fit was used to estimate the percent contribution of each variable to the primary outcome and evaluate direct and indirect paths. All statistical analyses were conducted using Stata V.15.1 (College Station, Texas, USA).

RESULTS

Cervical samples were analyzed for 14 eligible cases and 42 matched controls. The median age was 20 (interquartile range (IQR) 18–24), and the majority of women (44/56, 78%) were postpartum, at the time of sample collection (Table 1). The median time between cervical sample collection and diagnosis of HIV-1 acquisition by NAAT for cases was 4 weeks (IQR 0–4 weeks), with a maximum time between sample collection and HIV-1 diagnosis of 9 weeks. One case was excluded because the time between swab collection and seroconversion was 36 weeks. Additional demographic and clinical characteristics are summarized in Table 1.

In comparisons of cytokine/chemokine concentrations between cases and controls using Wilcoxon rank-sum tests, only higher concentrations of CXCL10 were associated with HIV-1 acquisition ($p=0.02$) (Figure 1). In an initial PCA, the $\log_2$ concentration of CXCL10 had a uniqueness score of 88.7%, suggesting that this chemokine was not highly correlated with the other cytokines/chemokines. After exclusion of CXCL10, the remaining cytokines/chemokines (IL-1 β , IL-6, IL-8, IFN γ , TNF α , MIP-1 α , and MIP-1 β) underwent repeat PCA, which confirmed generation of a single factor (eigenvalue 6.19, explaining 77.4% variability), representing highly correlated cytokines/chemokines that are better analyzed as a single variable. A composite summary statistic based on the resulting factor (labeled “cytokine score”) was generated for each woman based on PCA results and used in further analyses.

In unadjusted conditional logistic regression analysis, higher concentrations of $\log_2$ transformed cervical CXCL10 (odds ratio [OR]=1.73, 95% CI 1.05, 2.86; $p=0.03$), but not the cytokine score (OR=1.20, 95% CI 0.65, 2.21; $p=0.57$) were associated with HIV-1 acquisition (Table 2). Higher concentrations of CXCL10 remained significantly associated with HIV-1 acquisition in multivariable models adjusting for Nugent score only (CXCL10 adjusted odds ratio [aOR]=2.92, 95% CI 1.27, 6.72; $p=0.01$), vaginal washing only (CXCL10 aOR=2.34, 95% CI 1.07, 5.11; $p=0.03$), or both Nugent score and vaginal washing (CXCL10 aOR=3.65, 95% CI 1.09, 12.16; $p=0.04$). Sensitivity analyses excluding participants with diagnosed *T. vaginalis* ($n=1$) or *C. trachomatis* ($n=1$) infection at the time of sample collection did not change the overall results (data not shown).

Path analysis was used to evaluate hypothesized relationships between vaginal washing, Nugent score, cervical CXCL10 levels and HIV-1 acquisition (Figure 2). Maximum likelihood estimation confirmed significant positive associations between cervical CXCL10 concentration and HIV-1 acquisition (path coefficient=0.37, 95% CI 0.15, 0.59; $p<0.001$), Nugent score and HIV-1 acquisition (path coefficient=0.24, 95% CI 0.01, 0.47; $p=0.04$) and

vaginal washing and HIV-1 acquisition (path coefficient=0.29, 95% CI 0.07, 0.52; $p=0.009$). No significant relationship was seen between vaginal washing and CXCL10 concentration (path coefficient=-0.03, 95% CI -0.29, 0.23; $p=0.82$) or Nugent score and CXCL10 concentration (path coefficient=-0.22, 95% CI -0.47, 0.04; $p=0.10$). Analysis of the hypothesized relationship between Nugent score and vaginal washing demonstrated no significant covariance (covariance=0.17, 95% CI -0.08, 0.43; $p=0.19$).

Evaluation of global goodness of fit using RMSEA (0.0), suggested the proposed model fits the data. Equation-level goodness of fit results indicated that vaginal washing and Nugent score accounted for only 5% of the variability in cervical CXCL10 levels ($R^2=0.05$), and that 25% of the variability in HIV-1 acquisition risk ($R^2=0.25$) could be attributed to all three predictors (Nugent score, vaginal washing and CXCL10 concentration). Analysis of direct effects (pathways directly connecting a variable to the outcome) and indirect effects (pathways that connect a variable to the primary outcome via an intermediate variable) yielded results similar to those described for maximum likelihood estimation (Supplemental Table 1).

DISCUSSION

This is the first study to demonstrate an association between higher concentrations of cervical CXCL10 and HIV-1 acquisition in pregnant and postpartum women. All cytokines/chemokines examined were highly correlated except for CXCL10. Only CXCL10 was significantly associated with HIV-1 acquisition in multivariable conditional logistic regression. Furthermore, path analysis suggested that cervical CXCL10 concentration contributes to HIV risk independently of both Nugent score and vaginal washing status.

The proinflammatory chemokine CXCL10 is produced by immune (e.g., natural killer cells, T cells, monocytes) and non-immune (e.g., endothelial cells, stromal cells) cell types in response to secretion of IFN γ and other proinflammatory cytokines. [20] The primary function of CXCL10 is to activate and recruit lymphocytes to sites of inflammation. [20] Blockade of CXCL10 signaling suppresses T helper-1 (Th1) mediated inflammation in murine models of multiple sclerosis (autoimmune encephalitis) [21] and colitis, [22, 23] demonstrating the importance of CXCL10 in producing an inflammatory response. Higher levels of serum [24] and genital [4, 11, 25] CXCL10 have been associated with increased HIV-1 acquisition risk in serodiscordant couples and young non-pregnant women, respectively. One possible explanation for these findings is that higher systemic and/or cervicovaginal CXCL10 increases the number of activated cervicovaginal T cells, which are highly susceptible to HIV-1 infection. [26]

Several studies have demonstrated an increased risk of HIV-1 acquisition during pregnancy and the postpartum period, [3, 5, 27] possibly due to physiologic changes associated with pregnancy. For example, during pregnancy, ascension of bacteria from the vaginal tract to the uterus is prevented by increased cervical expression of proinflammatory cytokines, antimicrobial peptides [28] and generation of a mucus plug. [29] Furthermore, both implantation and labor require proinflammatory signaling. [8] Together, these changes may facilitate HIV acquisition by increasing recruitment of CD4⁺ T cells to the genital tract,

[30, 31] or by disrupting the mucosal barrier. [32] Although we were not able to compare genital CXCL10 levels in our pregnant cohort to non-pregnant women, other studies have shown increases in genital levels of proinflammatory cytokines (e.g., IL-1 β , IL-6 and IL-8) in pregnant versus non-pregnant women. [9, 10] Future studies comparing genital CXCL10 levels and total numbers of activated CD4⁺ T cells in the genital mucosa between pregnant and non-pregnant women may help further illuminate the biologic changes during pregnancy that influence HIV-1 acquisition risk.

Vaginal washing was highly prevalent in this cohort [33] and was associated with HIV acquisition in the analyses presented here. Prior studies have demonstrated an association between vaginal washing and increased HIV risk. [18, 34] The mechanism linking vaginal washing to HIV risk remains unclear, but may be due to washing-related changes in vaginal microbiota [35] or cervical inflammation. [36] In this study, however, the association between vaginal washing and HIV acquisition was independent of both Nugent score and concentration of CXCL10. These data suggest vaginal washing may influence HIV risk by a mechanism unrelated to alterations in vaginal microbiota, such as mucosal disruption due to traumatic washing or use of strong detergents. [37, 38] Further studies evaluating how vaginal washing influences HIV pathogenesis, as well as methods to promote vaginal washing cessation, are needed.

This study had several strengths. The case-control study design minimizes potential confounding by gestational age or time since delivery. Additionally, HIV-1 testing was performed at regular intervals using a highly sensitive NAAT-based platform, allowing for accurate detection of incident HIV-1 infection. Finally, the independent association between elevated cervical CXCL10 concentration and HIV-1 acquisition in this study was confirmed using several methods, including multivariable regression and path analysis.

This study also has several limitations. First, the time between cervical sample collection and HIV-1 diagnosis varied, and cytokine concentrations measured may not accurately reflect levels at the time of HIV-1 acquisition. Second, despite the large size of the parent cohort (>1300 women), the number of incident HIV cases with proximate sampling was relatively small; although there were 3 matched controls for each case, the study was not powered to identify small effect sizes. Third, infection with *N. gonorrhoeae*, *C. trachomatis* and human papillomavirus (HPV) can increase cervical inflammation [39–41] and HIV risk. [6, 41, 42] However, most women were not tested for *N. gonorrhoeae* and *C. trachomatis* at the time of cervical sampling, and none were tested for HPV. Fourth, diagnosis of BV using Nugent score may misclassify some women with specific species of sub-optimal microbiota (e.g., *Prevotella* species) as having normal vaginal flora. [43] Errors in diagnosis of BV, however, would be expected to be distributed evenly between cases and controls, which would thus tend to decrease the observed association of CXCL10 on HIV risk. Finally, although path analysis supported the finding that cervical CXCL10 levels independently contribute to HIV-1 acquisition, there could be residual confounding. More frequent measurement of cervical markers of inflammation and periodic evaluation for sexually transmitted infections (STIs) could help overcome many of these limitations in the future.

In conclusion, we detected increased concentrations of cervical CXCL10 among pregnant and postpartum women who acquired HIV-1. Future studies focusing on the effects of CXCL10-mediated recruitment of activated T cells are needed to define the mechanism underlying increased genital tract inflammation and HIV-1 acquisition risk. Additionally, studies comparing inflammatory markers and lymphocyte recruitment in pregnant and non-pregnant women will be critical for understanding how pregnancy-related inflammatory changes contribute to increased HIV-1 acquisition risk in pregnant and postpartum women.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1:

Wilcoxon rank-sum analysis was used to compare log_2 concentrations of cytokines and chemokines (measured in picograms/mL) in cases versus controls. The median is indicated by a black line and the interquartile range (IQR) is marked by dashed lines.

Path	Path Coefficient (95% CI)	p-value
CXCL10 concentration to HIV acquisition	0.37 (0.15, 0.59)	<0.001
Vaginal washing to HIV acquisition	0.29 (0.07, 0.52)	0.009
Nugent score to HIV acquisition	0.24 (0.01, 0.47)	0.040
Vaginal washing to CXCL10 concentration	-0.03 (-0.29, 0.23)	0.824
Nugent score to CXCL10 concentration	-0.22 (-0.47, 0.04)	0.100
	Covariance 95% CI	
Vaginal washing and Nugent score	0.17 (-0.08, 0.43)	0.187

Figure 2:

Figure of path model. Predictors are represented by light gray squares, and the primary outcome (HIV-1 acquisition) is represented by a dark gray square. Unidirectional arrowheads represent hypothesized cause and effect relationships between predictors and/or the outcome. Bidirectional arrows represent proposed correlations between two variables. Path coefficients were calculated using maximum likelihood estimation and are shown below each arrow path and in the accompanying table. Significant relationships are noted with an asterisk (*). The standardized covariance between vaginal washing and Nugent score is also shown adjacent to the bidirectional arrow and in the table.

Table 1:

Characteristics at the Time of Sample Collection for 56 Participating Women

Characteristic	N (%) or median (IQR)		p-value ⁵
	Cases (N=14)	Controls (N=42)	
Age (range, 14–37 years)	20.5 (18,24)	20 (18, 24)	
Years of education	8.5 (8, 11)	8 (7, 11)	0.3
Pregnant	3 (21.4%)	9 (21.4%)	
<20 weeks	1 (7.1%)	3 (7.1%)	
21 weeks	2 (14.3%)	6 (14.3%)	
Postpartum	11 (78.6%)	33 (78.6%)	
6 weeks postpartum	8 (57.1%)	24 (57.1%)	
6 months postpartum	3 (21.4%)	9 (21.4%)	
Married	9 (64.3%)	27 (64.3%)	
Partner HIV-1 status			
Positive	0 (0.0%)	0 (0.0%)	
Negative	7 (50.0%)	21 (50.0%)	
Unknown	7 (50.0%)	21 (50.0%)	
Transactional sex ¹	4 (28.6%)	12 (28.6%)	
Number of sex acts per week	0 (0,1)	0 (0,1)	0.7
Any condomless sex in the past week	4 (28.6%)	10 (23.8%)	0.7
Nugent score			
Normal (0–3)	6 (42.9%)	26 (61.9%)	
Intermediate (4–6)	1 (7.1%)	3 (7.1%)	
Bacterial vaginosis (7–10)	7 (50.0%)	13 (31.0%)	0.2
Sexually Transmitted Infections			
<i>Chlamydia trachomatis</i> ²	0 (0.0%)	1 (11.1%)	
<i>Trichomonas vaginalis</i>	1 (7.1%)	0 (0.0%)	
<i>Neisseria gonorrhoeae</i> ²	0 (0.0%)	0 (0.0%)	

Characteristic	N (%) or median (IQR)		p-value ⁵
	Cases (N=14)	Controls (N=42)	
Vulvovaginal candidiasis ³	4 (28.6%)	8 (20%)	
Vaginal washing ⁴	10 (71.4%)	15 (35.7%)	0.005
Log ₂ CXCL10 concentration	8.9 (7.3, 9.5)	7.9 (6.4, 8.7)	0.01

¹ Defined as trading sex for money, food, rent, or other goods.

² Data on infection with *C. trachomatis* and *N. gonorrhoeae* were available for only 12 women at the time of sample collection.

³ Defined as a positive KOH test on wet preparation.

⁴ Defined as insertion of cloth or finger to wash beyond the introitus.

⁵ Comparison of unmatched variables was performed as described in the methods. For this analysis, BV was categorized as present (Nugent score 7–10) versus absent (Nugent score 0–6). Comparison of the presence versus absence of sexually transmitted infections (STIs) and vulvovaginal candidiasis between groups was not performed due to the small number women with these diagnoses.

Table 2:
Conditional Logistic Regression Analysis of CXCL10, Cytokine Score, and Potential Confounders

Characteristic	Unadjusted OR (95% CI)	P value	Model 1: Adjusted OR (95% CI) ⁶	P value	Model 2: Adjusted OR (95% CI) ⁷	P value	Model 3: Adjusted OR (95% CI) ⁸	P value
Cytokine score ¹	1.20 (0.65, 2.21)	0.57	0.32 (0.09, 1.22)	0.10	0.89 (0.38, 2.10)	0.78	0.31 (0.05, 1.69)	0.17
CXCL10 concentration ²	1.73 (1.05, 2.86)	0.03	2.92 (1.27, 6.72)	0.01	2.34 (1.07, 5.11)	0.03	3.65 (1.09, 12.16)	0.04
Nugent Score ³	1.11 (0.96, 1.29)	0.16	1.53 (1.05, 2.23)	0.03			1.43 (0.91, 2.26)	0.12
Vaginal washing ⁴	6.84 (1.37, 34.22)	0.02			20.71 (1.57, 273.14)	0.02	10.20 (0.64, 163.09)	0.10
Frequency of sex acts in the past week	1.20 (0.46, 3.12)	0.70						
Unprotected sex ⁵	1.42 (0.28, 7.07)	0.67						

¹The cytokine score was generated by principal component analysis, and can be interpreted as a summary statistic based on the concentrations of the following cytokines for each woman at the visit of interest: IL-1 β , IL-8, IL-10, IFN γ , TNF α , MIP-1 α , and MIP-1 β .

²Log2 concentration of cytokine

³Nugent score was modeled as a continuous variable ranging from 0 to 10. Scores of 0–3 are indicative of normal flora, scores of 4–6 are considered abnormal, and scores of 7–10 are diagnostic of BV.

⁴Defined as insertion of cloth or finger to wash beyond the introitus.

⁵Defined as any instance of condomless sex in the past week. This value was set to 1 if the reported number of sex acts in the past week was greater than the reported number of sex acts in the past week during which a condom was used.

⁶Multivariable model with adjustment for primary predictors (cytokine score and CXCL10 concentration) and Nugent score (based on a bivariable association with p value <0.20).

⁷Multivariable model with adjustment for primary predictors (cytokine score and CXCL10 concentration) and vaginal washing (based on a bivariable association with p value <0.20).

⁸Multivariable model with adjustment for primary predictors (cytokine score and CXCL10 concentration) and both confounders (Nugent score and vaginal washing).

Cases and controls were matched for the following variables at the time of swab collection: gestational age, weeks postpartum, age, marital status, partner HIV-1 status, history of transactional sex.

Abbreviations: OR, odds ratio; CI, confidence interval.