ORIGINAL ARTICLE

BACTERIAL VAGINOSIS, ALTERATIONS IN VAGINAL FLORA AND HIV GENITAL SHEDDING AMONG HIV-1-INFECTED WOMEN IN MOZAMBIQUE

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Objectives. We investigated whether abnormal vaginal flora, including bacterial vaginosis (BV), are associated with detection of cervical HIV-1 RNA among HIV-infected women in Mozambique.

Methods. We obtained clinical data and vaginal specimens from HIV-infected women registering for their first visit at one of two HIV care clinics in Mozambique. We compared women with detectable cervical HIV viral load (≥40 copies/ml) with women with undetectable cervical HIV.

Results. We enrolled 106 women. Women with abnormal vaginal flora (intermediate Nugent scores, 4 - 6) were more likely to have detectable cervical HIV RNA than women with normal vaginal flora (adjusted odds ratio 7.2 (95% confidence interval 1.8 - 29.1), adjusted for CD4 count). Women with BV had a non-significantly higher likelihood of detectable cervical HIV than women with normal flora.

Conclusions. Abnormal vaginal flora were significantly associated with cervical HIV expression. Further research is needed to confirm this relationship.

HIV genital shedding enhances HIV transmission to sexual partners.[1] We investigated whether abnormal vaginal flora, including bacterial vaginosis (BV), are associated with cervical HIV-1 RNA expression among HIV-infected women in Mozambique.

METHODS

Women were enrolled from October 2007 to March 2008 as part of an evaluation of reproductive tract infections among HIV-infected individuals registering for a first visit at one of two HIV care clinics in Mozambique: Xai Xai Provincial Hospital and Mavalane General Hospital.[2] We collected demographic and clinical data, and plasma and vaginal specimens. Cervical lavage specimens were obtained by application of 10 ml of sterile saline to the cervix and collection from the posterior fornix after 3 minutes. Diagnosis of BV was based on Nugent's criteria. Diagnoses of *Trichomonas vaginalis*, *Chlamydia trachomatis*, *Neisseria gonorrhoeae* and *Mycoplasma genitalium* were done by real-time multiplex polymerase chain reaction testing. HIV RNA levels were determined by nucleic acid sequence-based amplification assay, with a lower limit of detection of 40 copies/ml. Patients were treated according to the 2006 Mozambique Ministry of Health treatment guidelines and offered partner notification cards. The evaluation protocol, consent form and questionnaires were approved by Mozambique's National Health Bioethics Committee and the CDC Institutional Review Board.

Data were analysed using SAS version 9.2 (SAS Institute, Cary, NC). Nugent scores were categorised as normal (0 - 3), intermediate vaginal flora (4 - 6) and BV (7 - 10). Cervical HIV RNA was dichotomised as detectable (≥40 copies/ml) or undetectable. Women with detectable cervical HIV were compared with women without detectable cervical HIV by the chi-square or Fisher's exact test for categorical variables, and the

t-test for continuous variables. We used multivariable logistic regression models to test whether abnormal vaginal flora (intermediate vaginal flora or BV) were associated with detectable cervical HIV RNA. Plasma viral load, CD4 count, trichomoniasis and age were considered for inclusion; variables were excluded if they were not significant ($p \geq 0.05$) or disrupted the model's goodness of fit (e.g. CD4 count was included in the final model and plasma viral load was not). Missing data were excluded from the analyses.

RESULTS

Of 258 women enrolled in the larger study, 106 agreed to have cervical lavage specimens collected; there were no significant differences between women who agreed and did not agree to cervical lavage. The mean age of the 106 participating women was 33 years and most of them were from Xai Xai (Table I). None of the women was receiving antiretroviral therapy.

Lower CD4 counts ($p=0.01$) and abnormal vaginal flora ($p=0.04$) were associated with cervical HIV RNA detection (Table I). In multivariable logistic regression modelling, women with intermediate vaginal flora had higher odds of detectable cervical HIV RNA than women with normal vaginal flora (adjusted odds ratio (aOR) 7.2 (95% confidence interval (CI) 1.8 - 29.1), adjusted for CD4 count). Women with BV had non-significantly higher odds of detectable cervical HIV RNA compared with women with normal vaginal flora (aOR 2.7 (95% CI

TABLE I. CHARACTERISTICS OF ENROLLED WOMEN AND ASSOCIATION WITH DETECTION OF CERVICAL HIV RNA

		Detection of cervical HIV RNA		
	N	**Yes (*N* (%))**	**No (*N* (%))**	***p***
Total	106	75 (71)	31 (29)	--
Study site (*N*=106)				
Xai Xai	70	48 (69)	22 (31)	0.49
Maputo	36	27 (75)	9 (25)	
Education (*N*=84)				
No education	2	1 (50)	1 (50)	0.51
Primary	66	45 (68)	21 (32)	
Secondary or mid-level	16	10 (63)	6 (37)	
Marital status (*N*=81)				
Single	38	24 (63)	14 (37)	0.93
Unmarried, in relationship	32	22 (69)	10 (31)	
Married	2	1 (50)	1 (50)	
Widowed	9	6 (67)	3 (33)	
Prior antiretroviral therapy (*N*=84)				
Yes	1	0 (0)	1 (100)	0.15
No	83	56 (67)	27 (33)	
CD4 count (cells/µl) (*N*=106)				
<50	14	12 (86)	2 (14)	0.01
50 - 199	31	25 (81)	6 (19)	
200 - 349	26	21 (81)	5 (19)	
≥350	35	17 (49)	18 (51)	
HIV-1 plasma viral load (copies/ml) (*N*=76)				
<10 000	12	5 (42)	7 (58)	0.06
10 000 - 99 999	31	24 (77)	7 (23)	
≥100 000	33	24 (73)	9 (27)	
Abnormal vaginal flora categories* (*N*=106)				
Bacterial vaginosis	47	32 (68)	15 (32)	0.04
Intermediate vaginal flora	34	29 (85)	5 (15)	
Normal vaginal flora	25	14 (56)	11 (44)	
Mycoplasma genitalium† (*N*=103)				
Positive	14	9 (64)	5 (36)	0.62
Negative	89	63 (71)	26 (29)	
Trichomonas vaginalis† (*N*=103)				
Positive	54	41 (76)	13 (24)	0.16
Negative	49	31 (63)	18 (37)	
Chlamydia trachomatis† (*N*=103)				
Positive	1	1 (100)	0 (0)	0.51
Negative	102	71 (70)	31 (30)	
Neisseria gonorrhoeae† (*N*=103)				
Positive	1	1 (100)	0 (0)	0.51
Negative	102	71 (70)	31 (30)	

*Diagnosed by Nugent's criteria (normal 0 - 3, intermediate 4 - 6, bacterial vaginosis 7 - 10).

†Diagnosed by polymerase chain reaction of vaginal specimens.

0.8 - 8.7), adjusted for CD4 count). Mean Nugent scores of women with detectable cervical HIV were comparable to those of women without detectable cervical HIV (5.7 v. 5.3, *p*=0.64).

Compared with women with plasma viral loads of ≥100 000 copies/ml, women with plasma viral loads of <10 000 copies/ml more often had abnormal vaginal flora (58% v. 21%, *p*=0.049). On stratified analysis, there was a non-significant trend towards higher plasma viral load and cervical HIV RNA detection among women with normal Nugent's scores or BV, yet there was no association between plasma viral load and cervical HIV among women with intermediate vaginal flora.

CONCLUSIONS

We found positive associations between intermediate vaginal flora and BV and detection of cervical HIV RNA among HIV-infected women in Mozambique, suggesting that abnormal vaginal flora might enhance HIV genital viral shedding. Previously published work demonstrated associations between the presence of BV and detection of cervical HIV RNA,[3,4] possibly because of immune activation.[5] It was surprising that although intermediate vaginal flora were significantly associated with genital HIV expression, BV was not. It is possible that intermediate vaginal flora are more conducive to HIV viral shedding than BV, yet the explanatory mechanism is unclear. It is also possible that our study lacked adequate power to detect a significant association between BV and detectable cervical HIV due to a small sample size: this may be reflected in the wide confidence intervals. Limitations include that we did not control for menstrual cycle timing, contraceptive use, possible semen contamination of cervical specimens and herpes simplex viral infection, and cold-chain interruptions may have occurred. We did not find a clear overall association between plasma HIV viral load and cervical HIV expression, although the association may have been confounded by the presence of abnormal vaginal flora. Despite these limitations, these data suggest that abnormal vaginal flora might enhance HIV genital shedding and thus potentially enhance HIV transmission to sexual partners. Further research is needed to confirm this association.

This analysis has not been presented at scientific conferences or published elsewhere. External funding was not used to support this work.

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