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Trichomonas Vaginalis Treatment Reduces Vaginal HIV-1 Shedding

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Abstract

Background: Vaginal HIV-1 shedding has been associated with *Trichomonas vaginalis* (TV) infection and could play a role in HIV transmission. The purpose of the study was to examine if effective TV treatment reduces the presence of vaginal HIV-1 RNA.

Methods: TV+ women attending an HIV outpatient clinic in New Orleans, LA, who resolved infection (n = 58) and TV-negative controls (n = 92), matched on antiretroviral therapy (ART) were examined and interviewed at baseline, 1, and 3 months. TV status was tested by culture and the amount of cell free HIV-1 RNA in the vaginal fluids was determined by the Amplicor HIV-1 Monitor ultrasensitive assay.

Results: Most women (81.3%) were black and the mean age was 37.5 (SD 8.7). At baseline, 46.0% had plasma HIV-1 RNA 10,000 copies/mL, 26.4% had CD4<200 cells/μL, 54.7% were taking ART, and only 26.0% had detectable HIV-1 RNA in their vaginal fluids. TV-positive women who were effectively treated for TV were less likely to shed HIV vaginally at 3-months post-treatment compared to baseline (R.R. 0.34, 95% CI: 0.12–0.92, *P* = 0.03), whereas there was no change for TV-negative women.

Conclusion: This study provides additional support that reducing TV infection among HIV-positive women may have an impact on the prevention of HIV transmission. Reasons for the delayed treatment effect and the effect on cervical shedding need further investigation.

TRICHOMONAS VAGINALIS (TV) INFECTION IS THE most common nonviral sexually transmitted disease (STD) among HIV-infected women with prevalence rates ranging from 10% to 30%.¹⁻³ Because of this high prevalence, the role that TV infection may play in influencing HIV transmission from HIV-infected women to their male sex

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partners is important. Whereas several studies have demonstrated that TV increases the risk of HIV acquisition,⁴⁻⁶ few studies have demonstrated the association between TV and HIV transmission.^{7,8} In addition, most published studies come from African cohorts before there was access to antiretroviral therapy (ART), so the relevance of these studies for women who are receiving ART is limited.

Whereas neither the quantity of HIV necessary to cause transmission nor the part of the genital tract that is most likely to facilitate transmission (i.e., cervical or vaginal) is known, several studies have identified factors found to be associated with genital shedding of HIV-1 among women. These factors HIV disease,^{7,10,11,14-16} hormonal contraception,^{17,18} ART,^{11,14,19} higher plasma viral levels,^{7,10,13,15,17,20-27} presence of cytokines,²⁵ basic pH,²² pregnancy,²⁸ cervical inflammation,²⁹ selenium,³⁰ and vitamin A deficiencies,^{16,17,24} and any genital tract infection^{7,11,16,31} including genital ulcers,¹¹ human papillomavirus,⁷ bacterial vaginosis,³² TV,³³ and vaginal candidiasis.^{14,23,34} Two studies demonstrated reductions in genital HIV levels after treatment for vaginal infections^{33,35} and conversely, other studies have reported increased vaginal HIV-1 shedding (or levels) after treatment for cervical dysplasia³⁶ and interepithelial lesions.³⁷ Most of these studies, however, were cross-sectional and examined only cervical or genital shedding of HIV through either eudocervical swabs and/or cervico-vaginal lavage. Because female genital shedding of HIV can be either continuous or intermittent^{9,31} prospective studies with control groups are needed. Because TV is a vaginal infection, we sought to isolate the effect of TV on HIV vaginal shedding.

If TV infection among HIV-positive women causes increased presence of HIV-1 in the vaginal fluids, and treatment reduces this prevalence, then more aggressive testing and treatment of TV could cause a reduction of HIV transmission and should become a higher priority as a public health strategy to reduce the spread of HIV.⁸ The purpose of this study was to determine if effective treatment for TV infection among HIV-positive women is, associated with a reduction in detectable vaginal HIV-1 RNA levels.

Methods

HIV-infected women were recruited from the HIV Outpatient Program (HOP) in New Orleans, LA from June 2002 to January 2005. HOP is a public HIV clinic that serves a large, urban, predominantly indigent population. To be eligible, women had to be 18 years of age, scheduled to undergo a gynecological examination, return for at least one follow-up visit and provide informed consent. Menstruating women were generally not given a pelvic exam and were rescheduled to be seen later. Control women were excluded from the study if they had been treated with metronidazole in the last 2 weeks or had a medical contraindication to taking metronidazole (e.g., allergies or chronic alcoholism). Because of the known high recurrence rate for TV, women who retested positive at the 1-month visit were also excluded from analyses.^{1,38,39} The study was approved by the institutional review boards of Tulane and Louisiana State Universities Health Sciences Centers and all women provided informed consent before participation in the study.

Eligible women were screened at baseline for TV using wet mount microscopy that was then confirmed by culture (as described below). Women who were negative on wet mount but were later found to be positive on culture were considered positive and called back to the clinic for treatment and offered participation the study. Women who tested positive for TV and were negative at follow-up visits were matched on ART status (yes/no) and date of enrollment (within 6 months) to women who tested negative at all time points and also came back for at least 1 follow-up visit (1:2 ratio). Participants were then followed at 1 and 3 months. Treatment failures and those who acquired TV were not included so that the effects

of TV treatment could be studied, given the known high treatment failure rate for TV among HIV-positive women (e.g., 18.3%).⁴⁰

Specimen Collection and Laboratory Testing

Physical and gynecological examinations were performed by patients' medical providers following a standard protocol. Physical findings were recorded using a standard data collection tool. Gynecological specimens were collected in a specific sequence. Nonlubricated specula were utilized for the pelvic examinations. After speculum insertion, 5 Dacron vaginal swabs were obtained for various tests. The first swab was placed into the vaginal vault and rolled three times on the vaginal wall for testing of HIV-1 RNA levels. Four subsequent swabs were placed into the posterior fornix to obtain vaginal secretions for additional testing (one swab for *Mycoplasma genitalium* (MG) and Gram stain preparation, one swab for TV culture, one swab for the wet mount preparation and one swab for the vaginal pH). Subsequently, dacron endocervical swabs for Papanicolaou smear, HPV, and MG were collected. Swabs were always taken in the same order and were not randomized. A urine specimen was taken to test for CT, GC, and MG.

Trichomonas Culture

Specimens intended for TV isolation were inoculated into the InPouch™ system (Biomed Diagnostics, White City, OR) and were transported in insulated carriers to the Louisiana State University Infectious Diseases Laboratory where they were incubated and monitored daily at 37° C for 5 days. Readings were done at days 1, 3 and 5 by trained technicians.

Determination of HIV Levels

Vaginal secretions were collected for analysis of HIV levels on Dacron swabs as described above and were immediately placed in 1 mL of sterile saline and put on ice. Samples were transported to the laboratory, weighed, processed, and stored at -70° C within 3 hours of collection. Upon receipt in the laboratory, the tube was vortexed, the swab was removed, and the sample was centrifuged at $400 \times g$ for 10 minutes to separate cells from the supernatant. The cell-free fraction was stored at -70° C. Blood samples collected in EDTA anticoagulant were received and processed in the laboratory within 3 hours of collection. The sample was centrifuged at $400 \times g$ for 10 minutes and the plasma removed and stored frozen in 1 mL aliquots at -70° C.

To quantify the amount of HIV-1 RNA found in the cell free fraction of the vaginal secretions and in the plasma, samples were thawed and analyzed with the Amplicor HIV-1 Monitor ultrasensitive protocol. In this protocol, virus contained in the sample was purified by high-speed centrifugation, followed by lysis and PCR amplification as directed in the protocol. This protocol can detect as few as 50 copies of HIV-1 RNA per sample. HIV levels were reported as copies per vaginal swab or milliliter of plasma. Undetectable/not quantifiable samples were reported as <50 copies.

HIV Specimen Collection Variability

To standardize the amount of vaginal fluids that were collected, which could cause variability in the amount of HIV-1 RNA found in the fluids and thus measurement error, swabs rather than vaginal lavage were collected. Secretions collected on the swabs were eluted in 1 mL of PBS, providing a smaller and more concentrated specimen. To evaluate the variability in the amount of secretions collected, weights of secretions and PBS recovered from the swab were measured for 296 randomly selected specimens. The mean weight of the TV+ specimens (n = 80) was 0.77 g (S.D. 0.19) compared to the mean weight

of the TV– specimens ($n = 216$) 0.78 g (S.D. 0.17) and was found to be equivalent (t -test, -0.222 , 294 d.f. $p < 0.84$).

Other Vaginal Specimen Testing

Additional laboratory studies including the following: detection of *Neisseria gonorrhea* (GC) and *Chlamydia trachomatis* (CT) using the ProbTec assay (Becton Dickinson Inc, Sparks MD), detection of *Mycoplasma genitalium* using a locally developed PGR method,⁴¹ assessment of bacterial vaginosis (BV) per Nugent's criteria using 7 or greater as a cut-off point, determination of vaginal vulvovaginal candidiasis by culture; and CD4 lymphocyte counts/ μ L.

Behavioral Data

Respondents were also interviewed at all visits using computer-assisted self-administered interviews (CASI) or computer-assisted personal interviews (CAPI), depending on the woman's preference, and were conducted either before or after the clinical examination, depending on the clinic flow. The interviews captured various socio-demographic, clinical and behavioral characteristics of the women. Behavioral information included sexual abstinence, unprotected vaginal sex and douching in the last month. Additionally, adherence to ART was determined by asking the woman "did you take your medications yesterday, as prescribed," with a yes/no response. Behavioral data have been published elsewhere.⁴²

Clinical Data

Additional clinical data on factors were collected by clinicians using a standard form or were abstracted from the medical records. These variables included vaginal characteristics (e.g., discharge, erythema, presence of blood on swabs, etc.) and HIV clinical parameters at baseline (e.g., CD4, ART status, and plasma HIV-1 RNA viral level). Phase of menstrual cycle was estimated for women who had intact uteruses and who were less than 50 years of age by calculating how many days it was from the beginning of the last menstrual cycle until the data of the specimen collection (1–4 menses, 5–14 luteal/ovulation, 15–30 luteal). Any women who had not had a period in the last 30 days was considered to be amenorrheic.

TV Treatment

TV-infected women were treated with either 2 g of metronidazole by mouth in a single dose (88.2%) or 500 mg metronidazole orally twice a day for 7 days (11.8%) depending on provider preference. Direct observation to assure that the women actually took the medication was performed for 74.1% of those who received the 2 g dose. All women were offered 2 g of metronidazole to deliver to each of their named sex partners, and of the 82 women who had partners, 70% reported they gave the therapy to their partners. Partner packs were distributed in plastic containers with childproof caps that contained warning labels and a contact number to call for questions or medical emergencies. Women were given appointments to return at 1 month for posttreatment testing and again at 3 months.

Follow-Up Visits

A window of 2 to 6 weeks for the first month visit and 10 to 14 for the 3 month visit was allowed. Exams and interviews followed an identical protocol at all visits. Data were collected at baseline and again at 1 and 3 months using identical protocols. CASI data were entered directly into QDS software and all other data were double-entered into Access software. The maximum duration of each visit was 3 hours and participants received \$25.00 (baseline) or \$50.00 (follow-up) reimbursement for their time and effort. To improve follow-up, study staff sent out reminder appointment cards and called the women both the

day before their visit and the day of the visit. If women needed transportation, cab rides were arranged by study staff at no cost to the participant.

Statistical Analyses

Baseline associations between TV status, vaginal HIV-1 RNA presence, and selected clinical, laboratory and behavioral data were examined through likelihood ratio χ^2 or Fisher exact test of independence as appropriate. The primary outcome in this study was detectable HIV-1 in the vaginal fluids. Because only 26.0% of the women had detectable HIV-1 in their vaginal fluids, this outcome was treated as binary (present or absent). The minimum level of detection based on the Amplicor assay is 50 copies per mL, or per swab. Therefore, HIV shedding was defined as a categorical variable – detectable HIV-1 RNA or >50 copies/swab versus undetectable or ≤50 copies/swab. The primary independent variable of interest was culture confirmed TV. Pearson χ^2 , χ^2 test of trends and adjusted risk ratios were performed.

Treatment effect was examined using multivariate logistic regression within a generalized estimating equations (GEE) framework to account for repeated measurement on the same subject.⁴³ Models were adjusted for ART usage using an exchangeable correlation matrix and the visit was considered the unit of analysis. All analyses were conducted using SAS version 9.0 (SAS Institute, Cary, NC).

Results

Study Sample

A total of 436 women were screened for TV. Of these, 101 (23.2%) were positive and were invited into the study. Of the 101, 33 (32.7%) did not return for their follow-up visit within the allotted window-time or did not have a valid TV or HIV specimen and were excluded. Of the remaining 68 who were followed to 1 month, 10 (14.7%) tested TV-positive and were excluded from the analyses. After a case was invited, completed their first visit and was found to be TV negative at 1 month follow-up, 2 controls were invited. The controls (n = 136) were frequency matched by ART status for each case followed (n = 68). Of these controls invited, 44 (32.3%) did not return within the allotted window time and were excluded from analyses. This left 58 successfully treated women compared to 92 TV negative control women for the analyses (see Fig. 1).

Characteristics of the Cohort

HIV-infected TV-positive women who were successfully treated (n = 58) were compared to TV-negative women (n = 92). At baseline, most women were black (81.3%), with a median age of 37.0 (range 20–61), a median CD4 of 410 cells/μL (range 6–1823), and the median plasma HIV-1 RNA level among those who had detectable plasma virus (n = 120) was 17,001 copies/mL (range 51–818,575). The remaining 20.0% had undetectable plasma HIV. Of those who were shedding vaginally (n = 39), the median viral load was 553 (range 52–89300). The remaining 74% had undetectable vaginal HIV. Of the 30 women who had undetectable plasma virus, 1 (3.3%) was shedding vaginally. Of the 81 who had plasma viral loads <10,000 copies, 6 (7.4%) were shedding vaginally. The majority of women (54.7%) reported they were taking ART and 89.6% of reported they took it as prescribed on the day before the visit.

At baseline, 15.5% of the women had no intact uterus because of a prior hysterectomy and there was a nonstatistical trend that women who were TV-positive were more likely to have had a hysterectomy compared to women who were TV-negative (22.8% vs. 11.0%, $P < 0.06$). Of the remaining 127 women with intact uteruses, 4.7% were in menses, 26.8% were

in the follicular phase, 34.6% were in the luteal phase, and 33.9% were amenorrheic. At visit 2, 59.1% of the women were in the same phase of the menstrual cycle and at visit 3, 56.5% were in the same phase. Of the 43 with intact uteruses who were amenorrheic, 6 (14.0%) were taking DepoProvera and 7 (16.3%) were over 50 years of age. For women with intact uteruses who were under 50 ($n = 120$) there were no differences in the phase of the menstrual cycle by TV status ($P = 0.32$) or by HIV vaginal shedding status ($P = 0.88$).

Baseline Differences by TV Status and by Vaginal HIV-1 Detection

Univariate analysis of baseline data demonstrated that TV-positive women ($n = 58$) were more likely than TV-negative women ($n = 92$) to be black, younger, to have douched in the last month, to have higher plasma and vaginal viral loads, to have *C. trachomatis*, *M. genitalium*, and candidiasis. (Table 1). Women who had detectable vaginal HIV-1 RNA levels ($n = 39$) compared to women who did not ($n = 111$) were more likely to have higher plasma viral loads, to be less likely to be taking ART, and more likely to have a lower pH (Table 2).

Factors Associated With HIV-1 RNA Presence in Vaginal Fluids and the TV Treatment Effect

The mean time of follow-up to the first visit ($n = 137$) was 30.9 days (S.D. 9.4) and to the second visit ($n = 112$) was 87.2 days (S.D. 10.2). There was no difference in mean time of follow-up by TV status ($P = 0.82$ follow-up 1 and $P = 0.41$ follow-up 2) or by vaginal shedding status ($P = 0.18$ follow-up 1 and $P = 0.91$ follow-up 2). The prevalence of detection of HIV-1 RNA in vaginal secretions over time by TV status revealed TV-positive women to have higher prevalence of detection at baseline (36.2% vs. 19.6%, $P = 0.02$) which remained at 1 month (34.6% vs. 14.1%, $P = 0.01$) and then diminished at 3 months (18.4% vs. 12.2%, $P = 0.91$). These data are depicted in Figure 2. After adjusting for ART status and accommodating the intraperson correlation in GEE, women who were successfully treated for TV-positive were less likely to be vaginally shedding at visit 2 and visit 3 compared to visit 1 but TV-negative women were equally as likely to be vaginally shedding at all time points (see Table 3). There was no effect of TV treatment on plasma viral loads.

Discussion

This study demonstrated that effective TV treatment was associated with a reduction, albeit delayed, in vaginal HIV-1 RNA detection posttreatment. This delayed effect was surprising given that TV likely clears after about 3 weeks.⁴⁴ However, TV is known to induce a strong proinflammatory cytokine reaction and the length of this reaction is unknown.⁴⁵ Wang et al. found a more immediate effect of TV treatment.³³ Differences in the study populations especially exposure to ART could account for this. Wang et al.'s African cohort was likely not receiving ART. Over half of our cohort was receiving ART and most reported good adherence. In our study, the majority of women (74%) had undetectable levels of HIV-1 in their vaginal fluids. It is not clear what percentage of the women had detectable vaginal virus in the Wang cohort. Wang's study also did not have a TV- control, so it is difficult to say if the cohort would have improved irrespective of treatment.

Whereas our study has a very strong design (prospective cohort with a control matched on the strongest potential confounders) and had meticulous care taken to measurement of both exposure and outcome variables, there are a few weaknesses. We were not able to collect the samples at the same phase of the menstrual cycle for all women. Whereas hormonal differences in different phases of the menstrual cycle have been shown to have an influence on cervical HIV shedding,^{12,13,46} less is known about its influence on vaginal shedding and

at least 1 study suggests that there is no influence.¹³ In our study, over half of the cohort was measured in the same phase of the menstrual cycle and there was no difference in the phases of the cycle by TV or vaginal shedding status for those who were not measured in the same phase. This suggests that the error was random and, therefore, not an important confounder of our results.

We chose to measure vaginal shedding over cervical because, in general, TV is a lower genital tract infection. However, in a subset of women, TV can cause an erosive inflammation of the ectocervical epithelium resulting in cervicitis.⁴⁷ The transformation zone at the cervix has a greater concentration of HIV-receptor cells, indicating it may be a more important point of entry for HIV infection compared to vaginal epithelium.^{48,49} Less, however, is known about the expression of HIV from the cervical compared to the vaginal areas of the female genital tract because most studies examine cervico-vaginal shedding. One study that examined cervical and vaginal HIV-1 expression separately found that cervicitis was not associated with endocervical HIV-1 RNA shedding.⁵⁰ Nonetheless, future studies of the effect of successful TV treatment on HIV genital shedding should also examine both vaginal and cervical viral loads to fully explore the mechanism by which TV increases infectiousness.

Overall, we believe that this is one of the most thorough investigations of the association between TV and HIV-1 RNA vaginal shedding conducted to date.

In summary, TV treatment was associated with a protective, yet somewhat delayed, effect on vaginal shedding of HIV-1. This reinforces the notion that reducing TV infection among HIV-positive women can have an impact on the prevention HIV transmission. Reasons for the delay in the effect and the influence of treatment on cervical shedding need further investigation.

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Fig. 1.
Flow diagram of screened, invited and women included in analyses.

Fig. 2.
Presence of vaginal HIV-1 RNA by TV status.

TABLE 1

Baseline Characteristics According to TV Status (N = 150)

	TV+ % (n = 58)	TV- % (n = 92)	P-Value
Demographic			
African American race	89.7	76.1	0.04
Mean age (S.D.)	35.4 (8.1)	38.8 (8.8)	0.02
Employed	21.1	22.5	0.84
Married/living with steady partner	23.9	26.8	0.73
Behavioral			
Abstinent in last month	15.8	27.0	0.12
Unprotected sex at last vaginal sex	33.8	33.7	0.70
Douched in last month	27.6	13.0	0.03
HIV clinical			
Taking ART	55.2	54.3	0.92
CD4 <200 cells/ μ l	34.5	21.3	0.08
Plasma viral load >10,000 copies/ml	58.6	38.0	0.01
Detectable vaginal viral load	36.2	19.6	0.02
STIs/other vaginal factors			
<i>Chlamydia trachomatis</i>	7.0	0	0.02
<i>Neisseria gonorrhea</i>	1.8	0	0.39
<i>Mycoplasma genitalium</i>	14.0	1.1	0.01
Presence of other vaginal infections			
Bacterial vaginosis	48.3	51.7	0.69
Vulvovaginal Candidiasis	31.0	14.1	0.01
Any hormonal contraceptive	11.1	6.5	0.36
Depo-provera	7.4	5.8	0.44
Prior hysterectomy	22.8	11.0	0.06
PMNs present on vaginal swab	68.8	76.1	0.41
Bleeding on vaginal swab	62.2	50.0	0.19
Vaginal pH >4.5	83.3	85.0	0.82

TABLE 2

Baseline Characteristics According to Presence of HIV-1 RNA in Vaginal Fluids (N = 150)

	Present % (n = 39)	Absent % (n = 111)	P-Value
Demographic			
African American race	87.2	79.3	0.28
Mean age (S.D.)	35.8 (9.5)	38.1 (8.3)	0.17
Employed	13.2	25.6	0.98
Married/living with steady partner	25.8	25.6	0.98
Behavioral			
Abstinent in last month	13.2	25.9	0.11
Unprotected sex at last vaginal sex	36.8	34.3	0.41
Douched in last month	23.1	17.1	0.41
HIV clinical			
Taking ART	25.6	64.9	0.001
CD4 <200 cells/ μ l	32.4	24.3	0.33
Plasma viral load >10,000 copies/ml	84.6	32.4	0.001
STIs/other vaginal factors			
<i>Chlamydia trachomatis</i>	5.3	1.8	0.26
<i>Neisseria gonorrhea</i>	0	0.9	1.00
<i>Mycoplasma genitalium</i>	7.7	5.5	0.70
Presence of other vaginal infections			
Bacterial vaginosis	55.3	48.6	0.48
Vulvovaginal Candidiasis	30.8	17.1	0.07
Any hormonal contraceptive	7.9	8.3	1.00
Depo-provera	8.3	5.8	0.73
Prior hysterectomy	15.4	15.6	0.40
PMNs present on vaginal swab	83.8	70.0	0.11
Bleeding on vaginal swab	62.5	51.6	0.28
Vaginal pH >4.5	97.0	78.3	0.02

TABLE 3

Predictors of Presence of HIV-1 in Vaginal Fluids by TV Status

	TV+ (n = 58, Observations = 147) R.R. (95% C.I.)	TV– (n = 92, Observations = 251) R.R. (95% C.I.)
Visit 2 vs. Visit 1	1.24 (0.69–2.23)	0.67 (0.32–1.42)
Visit 3 vs. Visit 1	0.34 (0.12–0.92) *	0.59 (0.23–1.51)
ART (yes vs. no)	0.25 (0.10–0.62) †	0.53 (0.19–1.50)

* $P < 0.05$.† $P < 0.01$.