Effects of Urethritis on Human Immunodeficiency Virus (HIV) in Semen: Implications for HIV Prevention and Cure

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Background. Prior to the widespread availability of antiretroviral therapy (ART), men living with human immunodeficiency virus (HIV) with urethritis had increased concentrations of HIV in semen. This study aims to better evaluate HIV shedding in men with urethritis receiving ART, and its implications for the cure of HIV.

Methods. Men living with HIV with urethritis taking ART ≥12 weeks were enrolled at a sexually transmitted infections clinic in Lilongwe, Malawi. Study follow-up included visits at 1, 2, 4, 8, 12, 24, 36, and 48 weeks after urethritis diagnosis and treatment. Matched blood and semen samples were collected at all visits, and all additional episodes of urethritis were followed with extra visits 1, 2, and 4 weeks after treatment.

Results. There were 111 men enrolled in the study between January 2017–March 2019, and 77 (69%) were suppressed in the blood (<400 copies/mL). Among the 77 men, 87 episodes of urethritis were evaluated during follow-up. Of the 87 episodes, 15 episodes (17%) had instances of seminal viral shedding ≥400 copies/mL despite viral suppression in the blood. During nonurethritis follow-up, ≤6% of men at each visit had a viral load ≥400 copies/mL in the semen while maintaining viral suppression in the blood.

Conclusions. An HIV cure requires the elimination of HIV from every body compartment, but available ART does not currently accomplish this. Our study highlights the male genital tract as a local source of HIV that can be reversibly activated. A better understanding of this phenomenon is important to advance the HIV cure field.

Keywords. HIV; urethritis; antiretroviral therapy; semen; Malawi.

Human immunodeficiency virus (HIV) RNA can be readily detected in seminal plasma in men living with HIV [1]. Blood and semen are compartmentalized, with some local HIV replication [2–4]. The concentrations of HIV in blood and semen correlate strongly with the probability of HIV transmission [5, 6]. After initiation of antiretroviral treatment (ART), the HIV concentration gradually decreases in blood [7] and semen [8, 9], and renders the person living with HIV no longer contagious in most circumstances [10–14].

Many people living with HIV infection are at risk for acquisition of classical sexually transmitted infections (STIs) that cause mucosal inflammation and/or ulcers [15]. In studies conducted before ART was widely available, men living with HIV who had urethritis had increased concentrations of HIV in semen [16–18], amplifying the risk of HIV transmission [6, 19].

Among people on ART, urethritis can cause breakthrough shedding of HIV in the genital tract [20, 21] while the blood viral load remains suppressed. This observation suggests that local mucosal inflammation has led to the detection of persistent or "latent" HIV [22]. Further study of this phenomenon is important for our understanding of HIV transmission prevention and for the cure of HIV, which requires elimination of HIV from all body compartments.

The current study was undertaken to better evaluate the shedding of HIV in men with urethritis receiving ART. We studied men in an STI clinic in Lilongwe, Malawi.

METHODS

Men with HIV with acute urethritis, defined as observed signs and symptoms of urethral discharge, who reported taking ART for at least 12 weeks were recruited from Bwaila District Hospital's STI Clinic in Lilongwe, Malawi. Bwaila District Hospital is a secondary care health facility under the Lilongwe District Health Office, and is the largest public hospital in Lilongwe District. Care is provided for all patients seeking STI services.

Study recruitment and enrollment took place during a single clinic visit for men being treated for urethritis. The HIV diagnosis was confirmed using confirmatory rapid tests, per the Malawian standard of care [23]. Participants were followed for 48 weeks after enrollment, with visits at 1, 2, 4, 8, 12, 24, 36,

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and 48 weeks. Blood and semen samples were collected for HIV RNA testing at each visit, and a brief behavioral survey was administered. Participants received HIV education, ART adherence counseling, and risk reduction counseling at all visits. Participants who had additional episodes of urethritis during follow-up had additional visits 1, 2, and 4 weeks after each urethritis diagnosis and treatment. Genital swabs were collected at all episodes of urethritis for etiologic testing.

HIV RNA concentrations in blood and semen were measured using the Abbott RealTime HIV-1 RNA assay. Blood specimens were centrifuged, and the plasma was harvested and frozen (−80°C freezer). The semen specimen volume was measured using precision pipettes, and viral transport media (Gibco Roswell Park Memorial Institute (RPMI) 1640 Medium [Mediatech Inc.], 1000 U penicillin-streptomycin solution [Mediatech Inc.], and 10 000 U/mL Nystatin [Sigma-Aldrich]) was added prior to freezing in 1 ml aliquots (−80°C freezer). Plasma and semen samples were thawed and tested in batch. The semen dilution factor was added to the onboard run template of the assay and was accounted for automatically in each viral load result. HIV viral suppression was defined as <400 copies/mL for both blood and genital compartments. Because the population of interest was men virally suppressed on ART, those who had viremia in the blood ≥400 copies/mL were excluded from analysis after their last suppressed visit.

Etiological testing for *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, *Trichomonas vaginalis*, and *Treponema pallidum* was performed for each episode of urethritis during follow-up. Gonorrhea was confirmed via GeneXpert and culture, chlamydia via GeneXpert, trichomonas via the OSOM®-Trichomonas Rapid Test, and syphilis via the BD Macro-Vue rapid plasma reagin (RPR) and Serodia Treponema pallidum particle agglutination (TP-PA) assay. All patients were treated for urethritis with gentamicin at 240 mg intramuscular single dose, doxycycline at 100 mg orally twice daily for 7 days, and metronidazole at 2 g orally as a single dose, per the Malawian standard of care [17]. Participants diagnosed with syphilis were treated per Malawian STI treatment guidelines. We considered any urethritis within 4 weeks of the initial presentation as a single episode. We used frequency distributions and descriptive statistics to characterize the study population, as well as generalized estimating equations to calculate confidence intervals (CI). All analyses were conducted using SAS version 9.4 (Cary, NC) or R version 3.6.1 (Vienna, Austria).

This study was approved by the Malawi National Health Sciences Research Ethics Committee and the University of North Carolina Institutional Review Board. All participants provided written informed consent for study participation.

RESULTS

Between January 2017 and March 2019, 111 men living with HIV who reported taking ART for at least 12 weeks presented for care with acute urethritis and enrolled in the study. The median age was 35 years (interquartile range [IQR], 31–39). Men reported having urethritis for a median of 4 days (IQR, 3–6). Participants had been taking ART for a median time of 36 months (IQR, 19–76). Most men were married (73%), employed full-time (55%), and had less than secondary education (74%). Of the participants, 97 men (87%) reported taking TDF/3TC/EFV, 6 reported Tenofovir (TDF)/Lamivudine (3TC) + Nevirapine (NVP) (5%), 3 reported Zidovudine (AZT)/3TC + Atazanavir (ATV)/Ritonavir (r) (3%), 3 reported AZT/3TC/NVP (3%), and 2 reported taking another regimen.

Gonorrhea was detected in 89 men at enrollment (80%) via GeneXpert, and 61% were culture positive. There were 7 men (6%) who tested positive for chlamydia, 3 (3%) who tested positive for trichomoniasis, and 10 (9%) who tested positive for syphilis. Of the 3 predominant etiologies for urethritis in Malawi—gonorrhea, chlamydia, and trichomoniasis—85 men (77%) tested positive for a single etiology, 7 (6%) tested positive for multiple etiologies, and 19 (17%) were not positive for any.

Among the 111 enrolled participants, 77 (69%) were virally suppressed in the blood at enrollment, with <400 copies/mL. Of these 77 men, 18 (23%) developed viremia (≥400 copies/ml) during follow-up visits; these participants' outcomes were excluded beyond that time point.

We evaluated 87 episodes of urethritis during the study among the 77 participants with a suppressed blood viral load at enrollment. Of these, 68 men had a single episode of urethritis, 8 men had 2 episodes, and 1 man had 3 episodes. Among the 87 episodes of urethritis, we detected HIV RNA ≥400 copies/mL in the semen of 15 men (17%), despite viral suppression in their blood. We noted 6 episodes (7%; 95% CI, 2–13%) of viral shedding in the semen at urethritis diagnosis and treatment, 9 episodes (11%; 95% CI, 4–19%) at 1 week after treatment of urethritis, and 6 episodes (8%; 95% CI, 2–13%) at 2 weeks after treatment (Table 1; Figure 1).

By Week 4 after urethritis treatment, HIV was suppressed in both blood and semen for all study participants. The median seminal viral load among those that were not suppressed was highest at the time of urethritis diagnosis and antibiotic treatment (median, 7376 copies/mL; IQR, 1229–50 666 copies/mL), and fell to a median of 1528 copies/mL (IQR, 868–2006 copies/mL) at Week 2 (Table 1; Figure 2).

After the first episode of urethritis, 68 participants (88%) continued follow-up while maintaining viral suppression in the blood. During the subsequent weeks (8 to 48), HIV was detected (≥400 copies/mL) in semen in 8 (12%) men (Table 1). The time to detection of shedding of HIV in semen was distributed across every follow-up visit between Week 8 and 48,

Table 1. Viral Suppression in Semen Among Those Suppressed in the Blood After Urethritis Diagnosis and Treatment

Week	Total Visits With Semen VL	Semen Viral Load		
		Suppressed,[a] n (%)	Unsuppressed, n (%)	Unsuppressed VL, copies/mL, median (IQR)
Urethritis episodes				
0	84	78 (93)	6 (7)	7376 (1229–50 666)
1	80	71 (89)	9 (11)	3898 (1458–11 145)
2	80	74 (93)	6 (8)	1528 (868–2006)
4	76	76 (100)	0 (0)	…
Nonurethritis follow-up				
8	62	61 (98)	1 (2)	426
12	62	61 (98)	1 (2)	656
24	60	58 (97)	2 (3)	4632; 394 839
36	56	55 (98)	1 (2)	1 667 295
48	52	49 (94)	3 (6)	550; 577; 2450

Data are from 87 episodes in 77 participants.

Abbreviations: IQR, interquartile range; VL, viral load.

[a]Suppressed VL was defined as <400 copies/mL.

and each participant only had 1 instance of shedding of HIV in semen despite suppressed blood viremia during follow-up. At a given study visit, HIV was detected in semen in only 1–3 participants (≤6%). Semen viral concentrations in these subjects ranged from 426 copies/mL to 1667295 copies/mL (Table 1).

CONCLUSIONS

In the current study, we enrolled men with urethritis in Malawi receiving ART. The results demonstrate that HIV can be detected in semen in a substantial percentage of men, in spite of viral suppression in the blood.

HIV in semen has been a source of concern since the inception of the HIV epidemic [24], and it has been studied extensively [1, 25]. In the absence of ART, the concentration of HIV in semen is closely correlated with the probability of HIV transmission to a sexual partner [6, 19].

STIs are common in people with HIV infection and, in the absence of ART, facilitate transmission [1, 26]. Before the availability of ART, we and others demonstrated that urethritis greatly increases the concentration of HIV in semen, and that the treatment of urethritis reduces the HIV concentration, albeit over several weeks [16–18].

Antiretroviral medications suppress blood and semen viremia, and some antiretrovirals concentrate in semen [27, 28]. In a meta-analysis, STIs appear to have a negligible effect on viremia [29]. But the effects of urethritis on HIV in the genital tract in people treated with antiretrovirals have remained unclear [20, 21, 30]. In

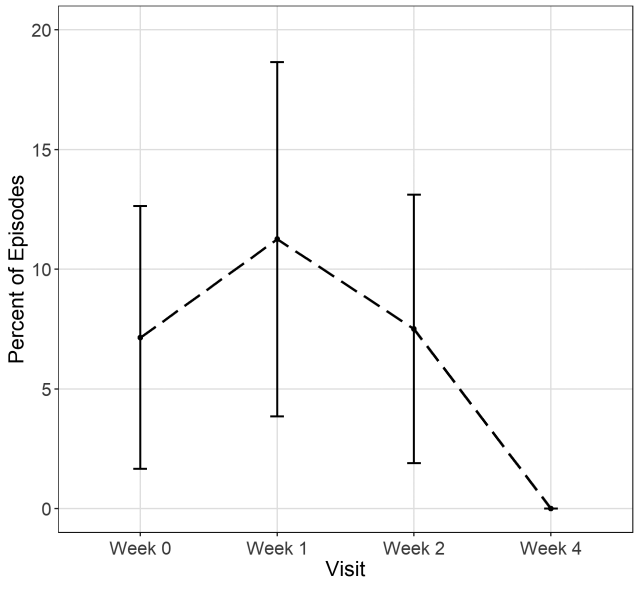

Figure 1. Percent of episodes with unsuppressed seminal viral loads ≥400 copies/mL within 4 weeks of urethritis diagnosis and treatment, with 95% confidence intervals (n = 87 episodes).

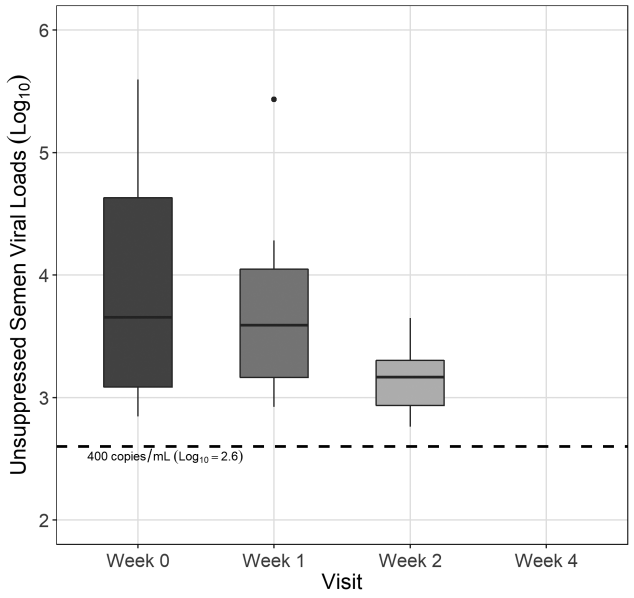

Figure 2. Medians and interquartile ranges of unsuppressed semen viral loads ≥400 copies/mL within 4 weeks of urethritis diagnosis and treatment.

1 small study examining the effects of urethritis on HIV in seminal plasma, HIV was rarely detected in the semen in the absence of viremia [21]. But proviral DNA was identified in seminal cells, suggesting a potential source of HIV in seminal plasma.

The detection of HIV in blood plasma in treated people has been divided into blips (detection of HIV on 1 occasion) or low-level viremia (detection of HIV on more than 1 occasion) [31]. We detected HIV in the semen related to episodes of urethritis and genital inflammation over 4 weeks, followed by random blips in semen. Our detection of HIV in semen in 2–6% of men at follow-up visits after urethritis episodes is similar to previous estimates of 7% [32]. However, we were unable to monitor viral loads continuously, so viral loads in both the blood and the semen may have been higher between study visits.

The exact source of HIV in semen is unknown. HIV can be detected in multiple anatomic sites in the genital tract [33, 34]. The detection of different HIV variants in blood and semen suggest compartmentalization [2, 4, 35, 36]. Viruses in the male genital tract may result from HIV replication or cellular clonal expansion [34, 36].

The detection of HIV in semen in spite of ART has important implications. First, it raises concern about the possibility of HIV transmission. Many of the seminal viral sheddings observed are above the threshold required for transmission; however, any transmission probability would be affected by when sex occurred relative to the urethritis and the elevated seminal viral load [5, 6]. The viral variants detected may not be replication competent [37]. In addition, the antiretrovirals leading to HIV suppression in blood are also found in genital secretions [27], and would be expected to further compromise HIV transmission. Most importantly, extensive clinical and epidemiological studies have found virtually no sexual transmission of HIV from treated people, regardless of the urethritis observed in many people living with HIV who were participating in these studies [10–14, 38].

The cure for HIV will require the elimination of HIV from every compartment in the body. But clearly, available ART does not eliminate the virus from the male genital tract. The current results show that antimicrobial therapy provided to treat urethritis slowly reversed shedding of HIV, similar to what we observed before ART was available[16], although the magnitude of virus detected with subjects on ART was markedly lower. The results affirm a local source of HIV in the male genital tract that can be reversibly activated. A better understanding of the exact mechanism(s) by which this occurs is important for strategies designed to cure HIV infection [39].

Notes

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