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HIV-1 is undetectable in pre-ejaculatory secretions from HIV-1-infected men on suppressive HAART

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Abstract

Objective—Pre-ejaculatory fluid, a viscous, lubricating secretion expressed by penile urethral glands during sexual excitement, may play a role in the sexual transmission of HIV-1. The urethra has been shown to be an important site of HIV infection in men and macaques, and pre-ejaculatory fluid and urethral swabs from HIV-1 infected men often contain HIV. Recent studies have shown that highly active antiretroviral therapy (HAART) reduces but does not eliminate seminal HIV shedding in infected men, and that the penile urethra remains a site of persistent SIV infection in HAART-treated macaques. The objective of this study was to determine whether HIV-infected men on stable HAART continue to shed HIV into pre-ejaculatory secretions.

Design—Single-center prospective study.

Methods—Sixty HIV-infected men on HAART were recruited to provide pre-ejaculatory fluid, semen and blood for HIV RNA quantification by RT-PCR.

Results—Eight men had detectable HIV in blood; of these 4 had HIV in semen (range: 40-96,000 copies/ml), and one had HIV in pre-ejaculate (2,400 copies/sample). Fifty-two men had undetectable HIV RNA in blood; of these 10 (19.2%) had HIV RNA in semen (range: 59-800 copies/ml) whereas none (0%) had HIV RNA in pre-ejaculate (p=0.004).

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AUTHOR CONTRIBUTIONS

Study concept: DA, KM and JP. Study design: DA, KM and JP. Experimental procedures: JP. Data analysis: JP. Initial manuscript draft: JP and DA. Manuscript revisions: JP, DA and KM.

Conclusions—This study documents for the first time high levels of HIV RNA in pre-ejaculate fluid. However, none of the men on stable HAART with undetectable blood viral load had HIV RNA in pre-ejaculate, even though many had detectable HIV in semen. The urethral glands do not appear to be a principal source of HIV in men on suppressive HAART.

Keywords

Semen; HIV-1; urethra; pre-ejaculate; HAART

INTRODUCTION

Semen is a major vector for the sexual transmission of HIV-1 [1-3]. Elevated peripheral blood HIV-1 viremia and genital inflammation/infections have been associated with increased levels of HIV in semen [4-6], whereas effective antiretroviral therapy (ART) that suppresses HIV in blood decreases seminal HIV-1 levels [7-10]. However, we and others have demonstrated that many men on highly active antiretroviral therapy (HAART) continue to shed HIV in semen [7-10]. Genital tract infections have been associated with increased seminal HIV-1 shedding in both untreated and HAART-treated men [6, 10-16]. Information concerning the source of HIV in semen from men on HAART could shed light on HIV reservoirs in HIV infected men on HAART, and could also have important implications for HIV prevention.

The human penile urethra is a primary site of HIV-1 infection [17]. This tissue contains a high number of HIV target cells [18, 19], and is readily infectable with HIV *in vitro* [20, 21]. Urethral secretions from HIV infected men often contain HIV-1 DNA [22] and RNA [23], and urethral inflammation has been associated with high concentrations of HIV in semen [13, 14, 16]. Furthermore, pre-ejaculatory fluid (PE), a viscous urethral secretion produced by the glands of Littré, Cowper's glands and the glands of Morgagni, and released during sexual excitement [24] has been implicated in HIV transmission. PE from HIV-infected men contains HIV-1-infected white blood cells (WBC) [25, 26], and delayed application of condoms is a risk factor for HIV-1 transmission among men who have sex with men (MSM), providing evidence that PE may be infectious [27, 28]. SIV-infected nonhuman primates, like men, harbor virus in the penile urethra [29], and a recent study in male macaques demonstrated that HAART does not reduce SIV RNA levels in the urethra [30]. The purpose of the present study was to determine whether HIV persists in pre-ejaculatory fluid from HIV-1 infected men on HAART, and whether it is associated with seminal HIV shedding.

METHODS

Study Participants

Study participants were a predominantly MSM cohort receiving medical care for HIV infection at Fenway Health in Boston, Massachusetts, USA. Fenway Health is the largest center caring for sexual and gender minority patients in the USA [31]. Men recruited for the study were HIV-1 infected, sexually active (i.e., having had sex in the past six months), and had been on a stable HAART regime for at least 3 months. This study was approved by the

Institutional Review Boards of both Fenway Health and Boston University Medical Campus (BUMC).

Specimen Collection and Processing

Men provided PE secretions and semen in a private room at Fenway Health after a minimum of 24 hours of sexual abstinence. All subjects were instructed to identify PE (drops of viscous secretion appearing in the opening of the penile urethra during sexual excitement prior to ejaculation), and to collect PE during masturbation using an ophthalmic wick [Merocel Eye Spear (Medtronic Xomed, Jacksonville, FL, U.S.A.)] [32, 33]. Wicks were made more permeable and softened by adding 100 μl sterile PBS prior to use [34], enabling the collection of more secretion (total wick volume ~500 μl), and to reduce the possibility of tissue irritation or damage. After sample collection, swabs were placed individually in a 5-ml sterile polystyrene test tube with a snap cap (Falcon, Becton Dickinson and Co., Franklin Lakes, NJ, U.S.A.), and participants then used a specimen container to collect the ejaculate. Ten ml of blood were collected by venipuncture in test tubes coated with EDTA at the same visit. All samples were sent to the laboratory at BUMC, and processed within 4 h of collection.

Upon receipt of PE samples by the laboratory, 1 ml PBS was added, and samples were incubated for 10 min at room temperature with intermittent vortexing. The eluate was then transferred to an Eppendorf tube and centrifuged at high speed (15,600 × g) for 40 seconds; a 250-μl aliquot that contained supernatant and pelleted cells was reserved for HIV RNA-PCR. For semen specimens, volume was measured, and semen was processed as previously described [10, 35, 36]. In brief, semen was diluted 1:1 in PBS, and the Endtz test was performed to enumerate polymorphonuclear leukocytes (PMNs) [36]. Seminal cells were pelleted by centrifugation at 600 × g for 10 minutes, and seminal plasma aliquots were taken. Semen cells were re-suspended in PBS. Whole blood was centrifuged (400 × g for 20 min) and plasma aliquots taken. Seminal plasma, semen cell, PE and blood plasma aliquots were stored with TRI Reagent/PolyAcryl Carrier (Molecular Research Center, Cincinnati, OH, USA) at –80° C for subsequent RT-PCR.

Quantitative RT-PCR

HIV-1 RNA was quantified in seminal plasma, semen cells, blood plasma and PE (fluid and cells). The details of the HIV RT-PCR assay have been described by us previously [10]. Seminal plasma HIV RNA was extracted with silica beads from NucliSens (Biomerieux, Boxtel, The Netherlands) to exclude the possibility of PCR inhibition by seminal plasma. The lowest quantitative limit for HIV-1 RNA was 1 copy/μl RNA (equivalent to 40 copies/ml for blood plasma, PE and semen cells, and 80 copies/ml for seminal plasma). HSV serostatus and seminal HSV-2 DNA were determined as described by us previously [10].

Statistical Analysis

GraphPad Prism (version 5.04, GraphPad Software, La Jolla, CA, USA) and StatView (version 5.0.1, SAS Institute, Cary, NC, USA) statistical software were used to perform the statistical computations. Categorical data were analyzed by Fisher’s Exact Test or

McNemar's Test. Differences were considered to be statistically significant when $p < 0.05$, the standard convention.

RESULTS

Subjects

The 60 men that provided samples for this study are a subset of 101 subjects for whom seminal HIV data were previously reported [10]. These men were selected for the current analysis because they had provided a complete set of blood, semen and PE samples. Protein concentrations were assessed in PE to confirm successful sample collection; PE wick eluates contained 0.3–4.8 (Median=0.9) mg/ml of protein.

Eight of the 60 men, had detectable HIV-1 RNA in blood plasma (Range: 80-640,000 copies/ml), and were removed from the principal analysis for this study. The remaining 52 subjects with undetectable HIV RNA in blood plasma had been on a stable HAART regimen for at least three months prior to sample collection, and forty subjects (77%) had been on ART for more than one year. The age range of the 52 subjects was 24 to 59 (Median=42.5), and their peripheral $CD4^+$ cell counts ranged from 108 to 1,492 cells/mm^3 (Median=518.5 cells/mm^3). Fifty (96.2%) of the men self-identified as MSM, and 44.2% of the men had engaged in unprotected insertive anal sex (UIAS) within the past 3 months. None of the subjects had a bacterial STI diagnosis at the time of sample collection. However, one subject was positive for HSV-2 DNA in semen. In addition, one had urethritis of undetermined etiology and leukocytospermia ($\geq 10^6$ PMNs/ml of semen [37]), and 9 had leukocytospermia without urethritis. Thirty-two (61.5%) of the 52 men were seropositive for HSV-1 and 30 (57.7%) were seropositive for HSV-2.

HIV-1 in Semen and PE

For the purpose of this analysis, we combined HIV RNA detection in seminal plasma and semen cells to create an overall semen HIV RNA variable which reflects potential cell-free and cell-associated HIV transmission. Pre-ejaculate HIV detection also included both fluid and cellular components. Four of the 8 men with detectable HIV in blood had HIV RNA in semen (copy numbers ranging from 40–96,000), and one man from this group with HIV RNA in semen (96,000 copies/ml) also had HIV RNA in pre-ejaculate (2,400 copies/sample). This man did not have any signs of infection or urethritis.

Of the 52 men with undetectable HIV in blood, 10 (19%) were positive for HIV RNA in semen (Range: 59-800 copies/ml), whereas none had HIV RNA in PE (p=0.004, McNemar's Test). Data are summarized in Table 1. Detection of HIV RNA in semen was associated with leukocytospermia [6/10 (60%) men with leukocytospermia vs 4/42 (9.5%) men without leukocytospermia had HIV in seminal plasma (p=0.002, Fisher's Exact Test)]. The subject with non-specific urethritis, and the subject positive for seminal HSV-2 DNA both had detectable levels of seminal HIV RNA. Detection of HIV RNA in semen was not associated with HSV-1 or HSV-2 serostatus (p's >0.10, Fisher's Exact Tests).

DISCUSSION

Treatment of HIV-infected men and women with antiretroviral drugs dramatically reduces the sexual transmission of HIV [38] and is being touted as an effective HIV prevention strategy [39]. However, a number of recent studies have shown that HIV persists in semen from a subset of men on stable HAART regimens. Evidence from several studies indicates that the male genital tract is a compartmentalized HIV environment, where genital infections promote localized HIV proliferation, evolution and shedding into semen, even in men on HAART [23, 40-43]. The penile urethra has been identified as an important HIV/SIV infection site, and HIV in urethral pre-ejaculatory fluid has been implicated in the sexual transmission of HIV. The purpose of our study was to determine whether HIV is present in pre-ejaculatory urethral fluid of men on HAART, and whether PE HIV viral loads are associated with semen viral loads in men who continue to shed HIV in semen when virus is undetectable in blood. This information would provide evidence that the penile urethra is an important HIV sanctuary in men on HAART, and also that pre-ejaculatory fluid from men on HAART may be infectious.

Whereas high levels of HIV RNA were detected in PE from a man in this study with high levels of virus in semen and blood, HIV was not detected in PE from 52 men on stable HAART with undetectable blood HIV viral load. A high percentage (19%) of men from this group, however, had detectable levels of HIV in semen as previously described [10]. None of the men in our study had been diagnosed with an STI, although one man had nonspecific urethritis at the time of the study, and nine more had leukocytospermia which is another index of male genital inflammation [44].

This study is the first to document high levels of HIV RNA in a urethral PE sample, although the detection of HIV DNA and infected cells in PE has been previously described [25, 26]. Other reports have documented HIV in urethral samples, but the methodologies differed significantly from the present study. The study by Moss et al [22] detected HIV DNA in urethral swabs from men in the pre-HAART era with a high prevalence of gonococcal urethritis. Coombs et al. [23] reported a high prevalence of HIV RNA in urethral samples from HIV^+ men, and a correlation between urethral and seminal RNA levels. However, in their study, as in the Moss study, urethral samples were collected by insertion of a wick or swab into the penile opening in non-sexually stimulated men, and all of the subjects had detectable HIV in blood and semen (the majority of their subjects were not on ART). In our study, we used a noninvasive sampling technique to detect HIV in urethral secretions that were naturally expressed from the penis as “pre-ejaculatory fluid” during sexual excitement, and all the men were on HAART. Our study provides the first evidence that pre-ejaculatory sexual secretions in men on HAART, unlike those from untreated HIV-infected men, do not contain detectable HIV. Additional research is needed to determine whether HIV shedding into pre-ejaculatory fluid is enhanced by clinical urethritis or STIs, and to determine the effect of HAART on urethral HIV in this high risk group.

In conclusion, although HIV-1 RNA was detected in semen of men on stable HAART with undetectable blood HIV viral load, it was not detected in pre-ejaculatory secretions. These

data indicate that pre-ejaculatory fluid may not contribute to HIV transmission in men on HAART, at least in men without genital infections.

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Table 1

HIV-1 Detection in Semen and Pre-ejaculatory Fluid from HIV-1-infected Men on HAART

	Blood Plasma HIV$^+$ (n=8)		**Blood Plasma HIV$^-$ (n=52)**	
	Semen	**Pre-ejaculate**	**Semen**	**Pre-ejaculate**
HIV-1 Detection Rate (%)	4/8 (50%)	1/8 (12.5%)	10/52 (19.2%)	0/52 (0%)
Significance[1]	0.25		**0.004**	

[1] McNemar's Test