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Herpes simplex virus 2 serostatus and viral loads of HIV-1 in blood and semen as risk factors for HIV transmission among men who have sex with men

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Abstract

Objective—Human immunodeficiency virus type 1 blood plasma viral load is correlated with the sexual transmission of HIV, although transmission from men involves virus from semen instead of blood. We quantified HIV-1 RNA in the blood and semen of men who did or did not transmit HIV to their sex partners. We compared the relationships of HIV-1 transmission risk with blood plasma viral load, seminal plasma viral load, herpes simplex virus 2 serostatus and other factors.

Design—A case–control study.

Methods—Participants were men evaluated for primary HIV infection and their recent male sex partners. They were interviewed, and clinical specimens were collected. Epidemiologic and phylogenetic linkages were determined by history and molecular techniques. Couples were grouped on the basis of transmission after exposure. Fisher's exact test and Wilcoxon tests were used for comparisons between groups. Multivariable logistic regressions were fit to identify independent predictors of transmission.

Results—HIV-transmitting partners ($n=15$) had a higher median seminal plasma viral load ($P<0.015$) and median blood plasma viral load ($P<0.001$) than nontransmitting partners ($n=32$). Herpes simplex virus 2 serostatus was associated with transmission only when the HIV-infected source partner was herpes simplex virus 2 seropositive and the HIV-exposed partner was not (odds ratio 16, $P<0.03$). Adjusting for other factors, HIV transmission was significantly associated with blood plasma viral load (odds ratio 13.4, $P<0.02$) but not seminal plasma viral load (odds ratio 0.69, $P=\text{not significant}$).

Conclusion—Blood and seminal plasma viral load were both associated with human immunodeficiency virus type 1 transmission, but blood plasma viral load was the stronger predictor in this cohort. Herpes simplex virus 2 coinfection was associated with the risk of transmission but not acquisition of human immunodeficiency virus type 1.

Keywords

herpes simplex virus 2; HIV; men who have sex with men; seminal viral load; transmission

Introduction

The majority of new HIV infections originate from sexual exposures to virus transmitted from semen [1]; however, not every exposure results in new infection. Many virological, biological, and behavioral factors are associated with risk of transmission, like human immunodeficiency virus type 1 (HIV-1) subtype [2,3], mode of sexual exposure [4], condom use [5], penile circumcision [6,7], mucosal inflammation [8], use of drug therapy, stage of infection [9], hormonal factors [10] or host genetic background [11,12]. The HIV-seropositive source partner's infectiousness may be the most important determinant. Stage of infection [9,13] and HIV-1 blood plasma viral load (BPVL) [14] correlate strongly with the risk of sexual transmission even though it involves virus from genital secretions rather than blood. Although the seminal plasma viral load (SPVL), reflecting the concentration of cell-free HIV-1 in semen, roughly correlates to BPVL [15,16], no empirical studies to date have directly examined the relationship between SPVL and HIV-1 transmission [17].

Materials and methods

Participants

Men who have sex with men (MSM) being evaluated for possible HIV-1 infection were recruited for participation in the San Diego HIV Transmission Study or the San Diego cohort of the Acute Infection and Early Disease Research Program (AIEDRP). Consent was obtained from eligible participants, observing all guidelines pertaining to research involving individuals as outlined by the US Department of Health and Human Services and the University of California, San Diego, Human Research Protection Program. Participants were interviewed and examined by research staff, who administered pretest and posttest HIV counseling and treatment for infection with *Neisseria gonorrhea*, *Chlamydia trachomatis*, or *Treponema pallidum*, as indicated.

Biological samples

Participants submitted urine specimens and then blood and semen samples at specified intervals. Samples of blood and semen were collected and processed as described previously [18]. Reference laboratories measured Rapid Plasma Reagin titers, total CD4+ T-cell counts, and performed nucleic acid amplification tests (LabCorp, San Diego, California, USA) on urine to assess for gonorrhea or Chlamydia. HSV-2 serostatus was determined as previously described [19].

Human immunodeficiency virus type 1 RNA

HIV-1 RNA was extracted and quantified from 500 µl of blood plasma (Amplicor HIV-1 Monitor Test; Roche Molecular Systems Inc., Alameda, California, USA) and from 500 µl of seminal plasma (qc-HIV Assay; GenProbe Inc., San Diego, California, USA) according to the manufacturers' protocols.

Transmission confirmation

Population-based *pol* sequencing of HIV-1 RNA extracted from blood plasma was used to confirm transmission with maximum-likelihood phylogenetic analysis as previously described [18]. Direction of transmission was based upon clinical history and the estimated duration of infection of the partners, with the more recently infected partner termed as 'recipient' and the more chronically infected partner as 'source.'

Estimations of timing

The stage of infection was defined as chronic (>12 months) or recent, the latter incorporating both acute and early infections, using the previously described AIEDRP algorithm for estimating the date of infection (EDI) [20,21]. The number of days after HIV-1 exposure until collection of HIV-infected source partners' samples was estimated for each pair. For the group in which viral transmission occurred, the date of the sexual exposure was taken to be the same as the EDI. When viral transmission between putative partners was not confirmed, there was no EDI. For HIV-seronegative partners in the nontransmitting group, the date of sexual exposure was taken as 14 days before screening, on the basis of participants having been instructed to recruit only people with whom they had had sex within 2 weeks of screening. Some men identified sex partners who were already participants in the study and had biological samples in storage. Samples collected closest to the estimated date of exposure were used in the analysis. Thus, sometimes a HIV-infected source partner's samples were collected before the HIV-exposed seronegative partners' enrollment. In such cases, the number of days 'after' enrollment until collection was assigned a negative value.

Statistical analysis

All viral load data were log-transformed. Bivariate comparisons for both groups were tested using Fisher's exact test and Wilcoxon tests (Table 1). We examined bivariate relationships between HIV-infected source partner characteristics and HIV transmission in contingency table analysis, and multivariable logistic regressions were then fit. Vectors of HSV-2 serostatus and presence of sexually transmitted infection (STI) were created to ascertain influence of partner discordance. STAT version 9.1 (SAS Institute Inc., Cary, North Carolina, USA) was used.

Results

Sexual partners were characterized and sorted into groups of transmitting and nontransmitting pairs. For the transmitting group ($n=15$ pairs) and the non-transmitting group ($n=32$ pairs) the respective mean ages of HIV-infected source (33 and 30 years) and HIV-exposed (34 and 35 years) partners, median CD4 cell counts (469 and 548 cells/ μ l), and rates of antiviral (HIV-1 and HSV-2) treatment were similar (Table 1).

Bacterial STI were more common in the group of transmitting pairs, particularly when the HIV-exposed partner tested positive ($P<0.003$). HSV-2 serostatus was associated with HIV-1 transmission only when the HIV-infected source partner was HSV-2 seropositive and the HIV-exposed partner was not ($P<0.014$). No factor was associated with transmission in multivariate analysis.

The median BPVL of the transmitting group was higher than that of the nontransmitting group ($\log_{10}$ 4.74 vs. $\log_{10}$ 4.07, $P<0.001$), as was the median SPVL ($\log_{10}$ 3.63 vs. $\log_{10}$ 2.58, $P<0.015$) (Fig. 1). The SPVL range, however, for the transmitting group ($\log_{10}$ 2.04–4.84) was entirely within the range of the nontransmitting group ($\log_{10}$ 1.68–5.05). This was not the case with BPVL, wherein the transmitting group demonstrated a narrower range of values with a higher maximum ($\log_{10}$ 4.12–6.66) than the nontransmitting group ($\log_{10}$ 1.70–5.83). SPVL correlated with BPVL in both the nontransmitting and transmitting groups of HIV-infected source partners (R^2 correlations of 0.49 and 0.22, respectively). These correlations were similar to previous report [17]. Multivariate logistic regression analysis adjusted for race, ethnicity, age, BPVL, SPVL, stage of infection with HIV, and source-positive and recipient-negative HSV-2 serostatus with an area under the ROC curve (AUROC) of 0.92. Using multivariate analysis to identify independent risk associations, higher BPVL was associated with

transmission [odds ratio (OR) 13.4, confidence interval (CI) 1.52–118, $P<0.02$] and SPVL was not (OR 0.99, CI 0.16–2.93, $P>0.05$) (Table 1).

The estimated time between exposures and collecting HIV-infected source partners' semen differed ($P<0.001$, Table 1) between the two groups. For the HIV-transmitting partners, samples were collected after a median of 103 days (range 20–146 days). For the nontransmitting partners, samples were collected after a median of just 24 days (range 107 days before to 42 days after the exposure).

Discussion

We describe two groups of MSM in which a recently HIV-exposed individual identified an HIV-infected sex partner: one group in which HIV transmission occurred and one group in which it did not. As participants in this case-control study were identified recently after exposure, we could characterize and compare the relationships between SPVL, BPVL, and other biologic factors with the risk of HIV-1 transmission. Here, HSV-2 infection was associated with HIV-1 transmission, but only in HSV-2 serodiscordant couples whose HIV-1-infected source partner was HSV-2 seropositive and HIV-exposed partner was not. HIV-uninfected participants who were HSV-2 seropositive were not more likely to acquire HIV-1 from their sex partners than the HSV-2-seronegative participants, consistent with recent reports in which treatment of HSV-2-seropositive participants with acyclovir did not reduce their rate of HIV-1 acquisition [22,23]. These results suggest that asymptomatic HSV-2 infection may have an impact on the risk of transmission rather than acquisition of HIV-1.

Symptomatic HSV-2 episodes, with genital ulceration and viral shedding, are associated with a greater increase in the risk of HIV-1 acquisition [24] than the risk during asymptomatic reactivations of chronic HSV-2 [25]. The relative risk of transmitting HIV-1 during an asymptomatic reactivation of chronic HSV-2 is unknown. An increase in HIV-1 RNA levels has been reported in the genital secretions of persons with untreated HSV-2 infection [26,27], but what effect treatment of asymptomatic HSV-2 may have on SPVL, or whether a threshold SPVL exists below which transmission does not occur, has not been determined. Future studies will need to evaluate shedding of HSV-2 in genital secretions among transmitting and nontransmitting HIV-infected source partners and correlate HSV-2 shedding with SPVL. A role for suppressive therapy of asymptomatic HSV-2 infection may exist, but it may be indicated for HIV-infected people to reduce their risk of transmitting HIV-1 rather than for HIV-uninfected people to reduce their risk of acquiring HIV-1, as has been investigated to date. This must be determined empirically.

Although sexual transmissions of HIV-1 are mediated by exposure to virus in genital secretions and not the blood, BPVL statistically correlated better than SPVL with the risk of HIV-1 transmission in this study. This may well be artifactual owing to the lag between sexual exposures and specimen collections. Longitudinal quantification of HIV-1 from paired blood and semen samples collected from HIV-infected men on antiretroviral therapy (ART) have shown that SPVL vary over time and over a broader range than matched BPVL [28]. Because SPVL may fluctuate so much over time, it is crucial to report the time intervals between sexual exposure, sample collection, and the estimated date of infection of the source partner. Current methods for EDI are not precise, but reporting the EDI is important nonetheless. In this report, we utilized the AIEDRP algorithm, which is well suited for our cohort, although other methods [29,30] may be more appropriate in other contexts. Consistent reporting of these time intervals will be necessary to enable meaningful comparisons between studies when considering the relationships between BPVL, SPVL, and transmission risk. More study participants with collections timed more closely to the time of transmission are needed to better delineate these relationships.

With our report, the concentration of cell-free HIV-1 in the semen of HIV-infected source partners has been correlated with the risk of HIV-1 transmission between MSM. The circumstances under which HIV-1 transmission occurs from seminal cell-associated virus and those under which it is mediated by seminal plasma virus, however, have yet to be defined. This study has only correlated SPVL with transmission; should transmitted HIV-1 prove to have its origin among the cell-associated virus instead, then the amount of cell-free virus (SPVL) would be less relevant and may explain why BPVL was a better predictor of HIV-1 transmission in this cohort. This has obvious implications for efforts directed at preventing transmission of HIV-1 by reducing SPVL through pharmacotherapy or by targeting cell-associated virus with the use of a cytotoxic T lymphocyte (CTL)-based vaccine. Other limitations of our study includes the relatively small size of the cohort, the lack of information about sexual positioning, frequency of intercourse in the partner pairs, HSV-2 shedding, and the human leukocyte antigen (HLA) similarity within partner pairs. A better understanding of these determinants of HIV-1 infection should provide crucial insights for the prevention of sexual transmissions from the male genital tract and for reducing the worldwide burden of disease caused by HIV/AIDS.

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Fig. 1. Comparisons of viral loads of HIV-1

Vertical bars represent the ranges of seminal plasma viral loads (SPVL) and blood plasma viral loads (BPVL) for the two groups. Horizontal bars denote the first and third quartiles. Diamonds indicate the median viral load for the group.

Table 1

Comparisons between groups.

MSM	Transmitting	Nontransmitting
Number of pairs	15	32
Age of source, years (mean, range)	33 (22–41)	30 (20–51)
Age of recipient, years (mean, range)	34 (20–47)	35 (19–57)
Source taking ART	1/15	5/31
HSV-2(+) source taking (val)acyclovir	1/8	1/14
HSV-2(+) recipient taking (val)acyclovir	1/5	1/13
HSV-2 serostatus, source (+) recipient (+)	3/15	10/29
HSV-2 Serostatus, source (+) recipient (–) ^a	5/15	1/29
HSV-2 Serostatus, source (–) recipient (–)	5/15	16/29
HSV-2 Serostatus, source (–) recipient (+)	2/15	2/29
Bacterial STI, infected source	1/14	4/30
Bacterial STI, infected recipient ^a	7/14	2/29
Estimated number of days from sexual exposure that source partners' genital secretions were collected, (median, range) ^a	103 days (20–146 days after)	24 days (107 days before–42 days after)
Recently HIV-infected source ^a	4/15	19/32
CD4, cells/μl (median, range)	469 (147–737)	548 (229–927)
Log ₁₀ BPVL, copies/ml (median, range) ^a	4.74 (4.12–6.66)	4.07 (1.70–5.83)
Log ₁₀ SPVL, copies/ml (median, range)	3.63 (2.04–4.84)	2.58 (1.68–5.05)

ART, antiretroviral therapy; BPVL, blood plasma viral load; HSV-2, herpes simplex virus 2; SPVL, seminal plasma viral load; STI, sexually transmitted infection.

^aDifferences between transmitting and nontransmitting groups were not statistically significant ($P>0.05$) except for these. Bivariate analysis determined an odds ratio (OR) of 16 [confidence interval (CI) 1.49–171, $P<0.03$] for HIV transmission with the source (+) recipient (–) pattern of HSV-2 serotypes. Bacterial STI, when the HIV-exposed recipient partner tested positive, was associated with HIV transmission ($P<0.003$) using Fisher's Exact test. The estimated number of days between sexual exposure and collection of genital secretions was longer for the transmitting group ($P<0.001$) by the Wilcoxon Test. Multivariate analysis determined that more recent HIV infection (OR=0.09, CI 0.01–0.93, $P<0.05$) and higher BPVL (OR=13.4, CI 1.52–118, $P<0.02$) were each independently associated with an increased risk of HIV transmission.