CONCISE COMMUNICATION

Correlates of Prevalent and Incident Kaposi's Sarcoma–Associated Herpesvirus Infection in Men Who Have Sex with Men

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Infection with Kaposi's sarcoma–associated herpesvirus (KSHV) is common among men who have sex with men (MSM). To determine correlates of infection, 578 human immunodeficiency virus (HIV)–negative MSM were assessed by serologic assays, questionnaires, and physical examinations. At baseline, 76 (16%) of 474 participants were KSHV seropositive. Prevalent KSHV infection was significantly associated with hepatitis A (odds ratio [OR], 3.3; 95% confidence interval [CI], 1.5–7.5), hepatitis B seropositivity (OR, 2.6; 95% CI, 1.4–4.8), herpes simplex virus (HSV)–2 (OR, 2.4; 95% CI, 1.3–4.4), and >4 male partners in the previous 6 months (OR, 1.9; 95% CI, 1.1–3.2). Fifteen KSHV seroconversions (4%) were observed for an incidence of 3.8/100 person-years, similar to HSV-1 incidence in this cohort and more frequent than incidence of HIV and HSV-2. Reporting ⩾1 HIV-positive partner (OR, 5.9; 95% CI, 1.8–19.3), amyl nitrite use (OR, 7.0; 95% CI, 2.1–23.0), and lymphadenopathy in the past 6 months (OR, 7.7; 95% CI, 1.9–31.0) correlated with KSHV seroconversion.

Since the identification of Kaposi's sarcoma–associated herpesvirus (KSHV), epidemiologic studies have attempted to identify risk factors associated with KSHV acquisition. In North America and Western Europe, men who have sex with men (MSM) have a prevalence of KSHV infection of 15%–30% that is associated with a large number of sex partners and a history of sexually transmitted infections (STIs) [1]. However, findings in these investigations regarding specific sexual behaviors that may transmit KSHV are conflicting and have led to the lack of a consensus about modes of acquisition [2, 3].

Failure to definitively identify behaviors that may predispose to infection with KSHV is due to many factors, including a focus on prevalent KSHV infections and imperfect serologic tools. To determine risk factors associated with prevalent and incident KSHV infection and clinical symptoms associated with KSHV acquisition, we studied a cohort of 474 human immunodeficiency virus (HIV)–negative men for KSHV infection by use of an indirect fluorescent antibody (IFA) test to define prevalent KSHV infection and EIA to confirm seroconversion. Detailed semiannual questionnaires about risk behaviors and clinical symptoms were used to determine risk factors associated with KSHV seroprevalence and seroincidence.

Methods

Study cohort. A cohort of 578 HIV-negative MSM was recruited from the Seattle area from March through October 1995 as part of the HIV Network for Prevention Trials [4]. To be eligible to enroll in the study, men had to be ⩾18 years old and HIV negative and to have engaged in anal sex in the prior 12 months. Information was obtained regarding sexual behavior, drug use, STIs, and clinical symptoms in the prior 6 months. Serum was tested for KSHV, HIV, syphilis, herpes simplex virus (HSV)–1 and -2, and hepatitis B serologies. Information from the initial study visit was used to characterize demographics and baseline behavioral risk factors among participants. Participants were prospectively followed at scheduled semiannual visits for 12 months, at which time HSV, HIV, syphilis, and hepatitis B serologies were repeated and interim sexual behavior and clinical histories assessed. Specific questions were asked about sexual practices, including orogenital, oroanal, and anogenital sex.

Serologic methods. KSHV serologic testing was performed by an IFA test and the body cavity–based lymphoma–1 cell line, as described elsewhere [5]. Seropositivity was defined as the presence of both latent and lytic antibodies or lytic antibodies alone at a serum dilution ⩾1:40, a strategy found to have a specificity of ~95% [6].

Subjects identified as KSHV seroconverters also had their baseline sera tested by whole virus KSHV EIA. We used 96-well plates coated with KSHV whole virus lysate (ABI). In all, 10 μL of each serum sample was diluted in 1 mL of 4% goat serum in PBS, and

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Written informed consent was obtained from each study participant. The Human Subjects Division, University of Washington, approved the study protocol, and human experimentation guidelines of the US Department of Health and Human Services were followed.

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100 μL was added to each well of the plate. Every plate contained positive and negative controls. Plates were incubated for 30 min at 37°C, washed twice with a PBS–0.05% Tween 20 solution (BioRad) and once with PBS, and then dried.

We added 100 μL of conjugate solution of anti-human IgG–horseradish peroxidase at a 1:2000 dilution in sample diluent (4% goat serum in PBS) to each well and incubated the plates at 37°C. Plates were washed, as above, and incubated with 50 μL of TMB peroxidase substrate and 50 μL of peroxidase solution B (H_2O_2) per well at room temperature for 8 min. The reaction was stopped by the addition of 100 μL of stop solution (1 *M* H_3PO_4) to each well. Optical densities were measured with a BioKinetics EL 340 plate reader (Bio-Tek Instruments).

Statistical analysis. Correlates of prevalent KSHV infection were assessed by use of univariate and multivariate logistic regression. In all, 398 KSHV-seronegative participants at study entry were eligible for the analysis of incident KSHV infection, and 378 men had serum samples available from both baseline and 12-month study points. All serum samples from the 12-month time point were tested for KSHV antibody status, and those with positive results had serum samples from the 6-month visit tested for KSHV antibodies to determine the 6-month interval during which seroconversion occurred. Time of acquisition was defined as the midpoint between the last negative and first positive specimen. Risk factors for KSHV acquisition were assessed by univariate and multivariate logistic regression. Age was modeled continuously and number of male partners was modeled dichotomously, split at the median number of partners.

Results

KSHV seroprevalence. Of the initial 578 participants, 474 had consented to stored serum samples for testing, and 76 (16%) had detectable antibodies against KSHV at study entry. By univariate analysis, a history of hepatitis A, chlamydia or nongonococcal urethritis, gonorrhea, syphilis, anogenital herpes, and anogenital warts all were significantly correlated with prevalent KSHV infection ($P < .05$; table 1). In addition, HSV-2–seropositive subjects were more likely to be KSHV seropositive (odds ratio [OR], 3.8; 95% confidence interval [CI], 2.3–6.3), as were participants reporting amyl nitrite use in the prior 6 months (OR, 3.0; 95% CI, 1.8–5.0).

Behavioral correlates of prevalent KSHV infection included reporting higher than the median number of partners in the prior 6 months, compared with those reporting fewer than the median (OR, 2.2; 95% CI, 1.3–3.6) and having had sex with a known HIV-positive partner (OR, 1.6; 95% CI, 1.0–2.8). We did not find a significant correlation between KSHV serostatus and receptive or insertive unprotected anal sex or oral-anal contact ("rimming") in the 6 months prior to study entry.

In multivariate analysis, a history of hepatitis A, serologic evidence of infection with hepatitis B or HSV-2, and reporting >4 sex partners in the preceding 6 months were independent predictors of KSHV seropositivity.

KSHV seroincidence. Of the initial 474 participants, 378 were KSHV seronegative at study entry and provided serum samples at baseline and 12-month time points. Among these participants, 15 (4%) were KSHV IFA positive at the 12-month study visit, for a calculated incidence of KSHV infection of 3.8/100 person-years. All seroconversions were confirmed by the whole virus EIA. The incidence of KSHV infection was higher than the incidence of HIV-1 (1.3/100 person-years) and HSV-2

Table 1. Association of selected characteristics with baseline Kaposi's sarcoma–associated herpesvirus (KSHV) serostatus.

	KSHV[a]			
Risk factor	Seropositive (*n* = 76 [16%])	Seronegative (*n* = 398 [84%])	OR (95% CI)	Adjusted OR[b]
Mean age, years	36	33	1.0 (1.0–1.1)	1.0 (1.0–1.0)
Chlamydia/nongonococcal urethritis[c]	29 (38)	95 (24)	2.0 (1.2–3.3)	
Gonorrhea[c]	32 (42)	78 (20)	3.1 (1.8–5.1)	
Syphilis[d]	5 (7)	9 (2)	3.0 (1.0–9.4)	
Genital/rectal warts[e]	25 (33)	85 (21)	1.8 (1.1–3.1)	
Hepatitis A[c]	14 (18)	19 (5)	4.7 (2.2–9.8)	3.3 (1.5–7.5)
Hepatitis B[f]	39 (51)	87 (22)	3.7 (2.2–6.1)	2.6 (1.4–4.8)
HSV-1 antibody positive at baseline	44 (58)	216 (54)	1.1 (0.7–1.9)	
HSV-2 antibody positive at baseline	39 (51)	87 (22)	3.8 (2.3–6.3)	2.4 (1.3–4.4)
Used amyl nitrites in prior 6 months	33 (43)	82 (21)	3.0 (1.8–5.0)	
⩾1 HIV-positive partner in past 6 months	24 (32)	86 (22)	1.6 (1.0–2.8)	
>4 Sex partners in past 6 months	44 (58)	154 (39)	2.2 (1.3–3.6)	1.9 (1.1–3.2)

NOTE. CI, confidence interval; HSV, herpes simplex virus; OR, odds ratio.

[a] Data are no. (%) of patients.

[b] Adjusted for age, HSV-2 status, no. of male partners, hepatitis A virus history, and hepatitis B serology. Adjusted estimates for other variables were not statistically significant.

[c] Self-reported.

[d] Either positive VDRL- or self-reported.

[e] Either self-reported or physical examination finding.

[f] Either hepatitis B core antibody positive or hepatitis B surface antigen positive.

(1.0/100 person-years) infection, but it was slightly lower than the incidence of bacterial syndromes of urethritis and proctitis (5.7/100 person-years) and HSV-1 (4.3/100 person-years), in the same cohort [7].

Men reporting > 4 sex partners in the past 6 months (OR, 2.2; 95% CI, 0.8–6.2), a known HIV-positive partner (OR, 6.3; 95% CI, 2.1–19.0), or unprotected insertive anal sex with an HIV-positive partner (OR, 8.8; 95% CI, 2.1–36.7) were more likely to acquire KSHV infection (table 2). Participants who had a sex partner with AIDS in the prior 6 months were more likely to become KSHV seropositive (OR, 4.5; 95% CI, 1.2–17.6), although the relationship failed to remain significant after adjustment with multivariate modeling. The use of recreational drugs other than amyl nitrites in the preceding 6 months was also associated with KSHV seroconversion (OR, 3.8; 95% CI, 1.1–13.7), and men reporting the use of amyl nitrites were especially at risk (OR, 6.6; 95% CI, 2.3–19.1).

Four (27%) of 15 participants who seroconverted to KSHV reported swollen or painful lymph nodes in the prior 6 months, compared with 19 (5%) of 363 participants who remained seronegative for KSHV (OR, 6.5; 95% CI, 1.9–22.5). None of these participants seroconverted to HIV, HSV-1, or HSV-2 during this interval. No other systemic or local symptoms were associated with KSHV acquisition. In multivariate analysis adjusted for age, sexual activity with an HIV-positive partner (OR, 5.9; 95% CI, 1.8–19.3), use of amyl nitrites (OR, 7.0; 95% CI, 2.1–23.2), and report of swollen lymph nodes (OR, 7.7; 95% CI, 1.9–31.1) were independent correlates of KSHV seroconversion.

The relationship of amyl nitrite use to KSHV seroincidence was further investigated by adding the significant univariate variables to the model, one at a time. The OR did not change after adding either HSV-2 infection or bacterial STIs to the model, but it declined from 7.0 (95% CI, 2.0–24.9) to 5.5 (95% CI, 1.4–20.8) after adding a reported history of bathhouse use. Thus, these variables did not mitigate the association between amyl nitrite use and KSHV seroconversion.

Discussion

We identified a high prevalence (16%) and incidence (3.8 cases/100 person-years) of KSHV infection among a cohort of HIV-negative men in Seattle. Both markers of other STIs and high number of sex partners were significantly associated with prevalent KSHV infection. In contrast to studies reported elsewhere [2, 3], no specific sexual behavior was significantly associated with KSHV seroprevalence. Two behaviors that were associated with seroconversion to KSHV in a multivariate model were having an HIV-positive partner and the use of amyl nitrites in the past 6 months. The few longitudinal cohort studies that have assessed KSHV incidence found similar risk factors for KSHV seroconversion, including number of years of homosexual activity [2], number of partners with whom orogenital sex is performed [8, 9], older age [9], and HIV serostatus [2, 9].

To our knowledge, the association between having an HIV-positive partner and seroconversion to KSHV has not been reported, although we previously found an association between deep kissing with an HIV-positive partner and prevalent KSHV infection [10]. HIV-positive persons are more likely to be infected with KSHV [1] and may shed the virus at higher quantities in their saliva [11]. Too few KSHV seroconverters reported partners with AIDS to determine if the clinical stage of HIV infection was significantly correlated with KSHV seroconversion, although an association was suggested. Amyl nitrite use as a risk factor for prevalent KSHV infection has been reported elsewhere [10], although not as a risk factor for incident KSHV infection.We explored potential confounding by amyl nitrites, which may serve as a risk marker for exposure to KSHV as the users are likely to meet partners at high-risk venues (e.g., bath-

Table 2. Association of selected characteristics with Kaposi's sarcoma–associated herpesvirus (KSHV) seroconversion.

	KSHV[a]			
Risk factor (in 6 months before last visit)	Seroconverters (n = 15 [4%])	Seronegative (n = 363 [96%])	OR (95% CI)	Adjusted OR[b]
>4 Sex partners in the past 6 months	7 (47)	103 (28)	2.2 (0.8–6.2)	
Perceived exposure to HIV	6 (40)	69 (19)	2.8 (1.0–8.1)	
⩾1 HIV-positive partner	8 (53)	58 (16)	6.3 (2.1–19.0)	5.9 (1.8–19.3)
Sex partner with diagnosis of AIDS	3 (20)	19 (5)	4.5 (1.2–17.6)	
Self-reported diagnosis of chlamydia	2 (13)	10 (3)	5.4 (1.1–27.1)	
Use of amyl nitrites	8 (53)	53 (15)	6.6 (2.3–19.1)	7.0 (2.1–23.2)
Use of other drugs	12 (80)	186 (51)	3.8 (1.1–13.7)	
Sex in a bathhouse	5 (33)	41 (11)	3.5 (1.1–11.6)	
Swollen/painful lymph nodes	4 (27)	19 (5)	6.5 (1.9–22.5)	7.7 (1.9–31.1)
Burning with urination	3 (20)	12 (3)	7.3 (1.8–29.2)	

NOTE. CI, confidence interval; HIV, human immunodeficiency virus; OR, odds ratio.

[a] Data are no. (%) patients.

[b] Adjusted for age at enrollment, lymphadenopathy, ⩾1 HIV-positive partner, and amyl nitrite use.

houses), have unprotected sex, and use other drugs. However, adjusting for these factors did not eradicate the association. A biologically plausible model for amyl nitrite use and acquisition of KSHV remains unclear, although amyl nitrites may suppress the activity of human lymphocytes in vitro, increase the duration of ejaculation, reduce sphincter tone, and dilate blood vessels, leading to increased exposure to KSHV during sex between KSHV-discordant partners [12].

The increased frequency of swollen lymph nodes among KSHV seroconverters is intriguing. Primary infection with KSHV in HIV-positive persons can cause cervical lymphadenopathy, fever, arthralgia, and splenomegaly [13] and, in transplant patients, fever, thrombocytopenia, and splenomegaly [14]. Given the similarities between KSHV and Epstein-Barr virus, which classically causes lymphadenopathy during primary infection, further studies of the clinical manifestations of primary KSHV infection should be pursued.

This study was primarily designed to assess risk factors for HIV acquisition and, therefore, may have failed to identify risk behaviors specific to KSHV infection. We did not include questions about lifetime number of partners or partners with HIV, behaviors leading to exposure to saliva, and other risk behaviors found to be associated with prevalent KSHV infection, subsequent to the initiation of this study [3, 10]. In addition, because the study only enrolled men who engaged in anal sex in the past year, this correlate of KSHV infection could not be examined. Although the KSHV incidence rate in this cohort was similar to that reported from other cohorts of MSM [8, 9], the absolute number of seroconverters was small. Limited data about the dynamics of the immune response to KSHV latent and lytic antigens that serve as the basis for serodiagnostic testing complicate serologic studies of KSHV acquisition [15].

We found KSHV to be a commonly acquired STI with an incidence rate similar to HSV-1 and higher than HIV or HSV-2. The common practice of various sexual behaviors and the exchange of saliva between partners could cause erroneous conclusions associating sexual behavior with KSHV serostatus and should be examined in future studies. Such studies are needed to determine the most common mode of KSHV transmission and risk factors for KSHV acquisition. Current uncertainty limits our ability to provide a clear and effective public health message to high-risk populations about methods to reduce KSHV transmission and acquisition.

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