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The seroprevalence of human herpesvirus 8 (HHV-8) was studied in 326 human immunodeficiency virus (HIV)–positive and –negative persons from Honduras; women constituted 77% (n = 251) of the subjects. Sera were tested for lytic HHV-8 antibodies by an IFA, and positive samples were confirmed by a radioimmunoprecipitation assay. Of the 326 persons tested, 58 (17.8%) had HHV-8 antibodies. Among the HIV-infected women, 22.7% were seropositive; 11.3% of the HIV-negative women were seropositive. HHV-8 seroprevalence was almost four times higher in HIV-positive female commercial sex workers (36%) than in HIV-negative female non–commercial sex workers (9.9%; odds ratio = 3.8, 95% confidence interval = 1.1–13; P = 0.01), suggesting that commercial sex work is a risk factor for HHV-8 infection. In the men studied, the overall HHV-8 seroprevalence was 22.6%, with a seropositivity rate of 28% for HIV-positive men compared with 12% for HIV-negative men.

Human herpesvirus 8 (HHV-8) has been detected in Kaposi's sarcoma (KS) tumors of all clinical forms [1], in lymphomas [2], in Castleman's disease [3], and in skin cancers [4]. Several B lymphoma cell lines that harbor HHV-8 have been established and used for serologic assays [5, 6]. IFA showed that most KS patients have specific antibodies to HHV-8–related antigens [7]. In another study, which used a Western blot assay, an HHV-8 seroprevalence of 80% was reported for KS patients but for only 18% of human immunodeficiency virus (HIV)–positive homosexual men without KS. No positive samples were identified among healthy blood donors or HIV-positive men with hemophilia [8]. Similar results were found using a recombinant-based Western blot assay: 84% in KS patients, 1.19% in HIV-positive hemophilic patients, and 3.17% in HIV-positive intravenous drug users [9].

HIV-positive homosexual men develop KS at a rate 20,000-fold higher than that in the general population [10]. In contrast, the incidence of KS in HIV-positive women is only 1%–3% [10, 11]. Recently, a study by Kedes et al. [12] found HHV-8 antibodies in only 4% of HIV-positive versus 1.2% in HIV-negative American women [12]. In contrast, another study showed HHV-8 antibodies in 21% of a small group of HIV-positive women, in >95% of HIV-infected homosexual men without KS, and in 25% of healthy adults [13].

The aim of our work was to determine the HHV-8 seroprevalence and associated risk factors in a population of women and men in Honduras; in Latin America, the incidence of KS is low. Determination of the HHV-8 infection in men and women of this area should provide important information about the distribution of and risk factors for HHV-8 infection.

Materials and Methods

Patients. Men and women (n = 326) attending public clinics in five cities in Honduras (Tegucigalpa, San Pedro Sula, Comayagua, La Ceiba, and Puerto Tela) were enrolled in the study, which is part of an ongoing study on HIV infection by the University of Miami. The clinics belong to the Honduran Ministry of Health and provide services free of charge to the general population. Participants represented a cross-sectional sampling of the population and were recruited from the five previously mentioned major public care centers. We deliberately recruited known HIV-positive individuals when they came for health care so they would comprise ~50% of the study population. Individuals were enrolled consecutively into the study during their routine medical checkups at the clinics. All eligible participants were included in the study; however, subjects who attended the clinics more than once were not reenrolled in the study.

Participants with AIDS, intravenous drug users, and hemophiliacs were excluded by chart review because the aim of the study was to assess factors contributing to sexual transmission of HHV-8. Intravenous drug usage and blood transfusion would add a noncontrolled additional risk to the study, such as acquiring HHV-8 by needle sharing or by contaminated blood. Patients with AIDS were excluded because the purpose was to evaluate persons with intact immune systems in an asymptomatic population.

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All participants signed informed consent forms, including for HIV testing, and the study received prior approval from the Honduran Ministry of Health.

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A questionnaire was administered to collect sociodemographic characteristics and HIV and HHV-8 risk factor information. It provided information on the age of first sexual intercourse, type, condom use, and history of sexually transmitted diseases. The lifetime number of sex partners was not available; however, the number of partners in the prior 12 months was self-reported by study participants.

Blood samples were collected for HHV-8 serology and other tests. Blood samples from all self-reported HIV-positive individuals were tested by ELISA and Western blot at the University of Miami School of Medicine. Patients who tested positive for HIV received counseling at the clinics. HHV-8 testing was not available at the clinics at that time, so information about HHV-8 serologic status was not given to the patients.

HHV-8 IFA. IFA was performed to detect HHV-8 antibodies according to described procedures [13]. An HHV-8–positive cell line (BCBL-1; from M. McGrath and D. Ganem via the AIDS Repository Program [6], NIH), an HHV-8–negative cell line (BJAB), and an Epstein-Barr virus (EBV) producer cell line (P3HR-1) were used. Cells were induced with tetradecanoyl phorbal acetate (TPA) and spotted onto slides. Samples were examined using an epifluorescent microscope, and technicians were not aware of the IFA or radioimmunoprecipitation (RIP) results. All samples were coded, and an inverse antibody titer ≥ 10 was considered positive.

A reference patient serum (HIV-positive, KS-positive) showed distinctive IFA lytic patterns of bright, punctuated nuclear and cytoplasmic staining in induced BCBL-1 cells. Control normal serum showed no staining. Lytic antigen expression was further confirmed with an HHV-8–specific anti–ORF 59 monoclonal antibody (11D1). Due to the discrepancies in previous seroprevalence studies, 1:10 and 1:40 cutoff dilutions were both used, and the positive samples were titrated (1:10–1:1280). To eliminate the possibility of EBV cross-reaction, the 1:10 dilution–positive samples were preadsorbed using lysates from an EBV producer cell line (P3HR1). The diluted sera were incubated with cell lysates from 1×10^7 TPA-stimulated P3HR1 cells, and absorbed versus unabsorbed samples were compared. HHV-8–specific fluorescent patterns were maintained after absorption, indicating that reactivities at the 1:10 dilution were not due to cross-reaction with EBV.

HHV-8 RIP and SDS-PAGE analysis. Ten million uninduced or TPA-induced (4–5 days after induction) BCBL-1 cells were labeled for 20 h with 25 μCi/mL [^{35}S]methionine (Tran^{35}S label, specific activity, 1177 Ci/mmol; ICN, Irvine, CA). Immunoprecipitation was done as described elsewhere [14]. RIP was performed with all 1:10 IFA–positive samples to confirm these results.

Statistical analysis. The univariate analysis was used (Epi Info, version 6.0; CDC, Atlanta) to assess the relative importance of various risk factors for HHV-8 infection. Medians and percentages were calculated to compare variables across sexual groups and their serologic status. Associations with HHV-8, as well as with HIV and dual HIV plus HHV-8 seropositivity, were measured with odds ratios (ORs) and 95% confidence intervals (CIs).

Results

Demographics and risk factors. Of the 326 participants recruited, 77% were women (median age, 27 years), and 91% were single. They had <6 years of formal education, and the majority were of low socioeconomic status. Of all individuals, 56% lived on the Atlantic coast area, and 44% lived in the country's interior. First sexual contact was reported at a median age of 15 years. More than 52.4% of the participants acknowledged the use of noninjected drugs, mainly cocaine and alcohol. Almost all knew about condoms, but only 37% acknowledged consistent usage. A history of sexually transmitted diseases was reported by 34.4% of the participants.

Table 1. HHV-8 and human immunodeficiency virus (HIV) seroprevalences in selected groups.

		HHV-8–positive[†]	
		Cutoff point	
	HIV serologic status*	1:10	1:40
Women (total = 251)	(+) 110 (43.8)	25 (22.7)	7 (6.4)
	(−) 141 (56.2)	16 (11.3)	5 (3.5)
Non–sex workers	(+) 85 (33.9)	16 (18.8)	3 (3.5)
	(−) 71 (28.3)	7 (9.9)	2 (2.8)
Commercial sex workers	(+) 25 (10.0)	9 (36.0)	4 (16.0)
	(−) 70 (27.9)	9 (12.9)	3 (4.3)
Men (total = 75)	(+) 50 (66.7)	14 (28.0)	4 (8.0)
	(−) 25 (33.3)	3 (12.0)	0
Homosexual	(+) 20 (26.7)	7 (35.0)	2 (10.0)
	(−) 2 (2.7)	0	0
Heterosexual	(+) 30 (40.0)	7 (23.3)	2 (6.7)
	(−) 23 (30.7)	3 (13.0)	0
Total study population = 326	(+) 157 (48.2)	39 (24.8)	11 (7.0)
	(−) 169 (51.8)	19 (11.2)	5 (3.0)

NOTE. Data are no. (%).
* Percentages were calculated based on total no. of women or men.
[†] Percentages were calculated based on HIV serologic status of subgroup.

HHV-8 seroprevalence. The frequency of HHV-8–positive samples at the 1:10 and 1:40 dilutions was determined (table 1). Of the 326 persons tested, 58 (17.8%) were positive: 41 (16.3%) were women, and 17 (22.6%) were men (table 2). Among the HIV-infected women, an overall HHV-8 seropositivity of 22.7% (25/110) at the 1:10 dilution and 6.4% (7/110) at the 1:40 dilution was found (table 1). In contrast, the seropositivity of HHV-8 among HIV-negative women was 11.3% (16/141) at the 1:10 and 3.5% (5/141) at the 1:40 cutoff. In the univariate analysis, there were differences between the proportion of HIV-positive and HIV-negative women who were infected with HHV-8. The HHV-8 seroprevalence was almost four times higher among HIV-positive female sex workers (36%) than among HIV-negative non–commercial sex workers (9.9%; OR = 3.8, 95% CI = 1.1–13; $P = .01$), suggesting that prostitution and HIV are risks associated with HHV-8 infection. Information about the numbers of lifetime sex partners from these two groups of women were not available; however, the correlation between the number of sex partners in the prior 12 months and HHV-8 serologic status was not significant. Our interpretation could still be affected due to

Table 2. Study groups and selected HHV-8 risk factors.

	Total	HIV-positive	HHV-8–positive*	HIV/HHV-8–positive
Women	251	110 (43.8)	41 (16.3)	25 (9.9)
Men	75	50 (66.6)	17 (22.6)	14 (18.6)
Total	326	160 (49.0)	58 (17.8)	39 (12.0)
Risk factors				
Homosexual male contact	22	20 (90.9)	7 (31.8)	7 (31.8)
Female sex worker	85	25 (29.4)	18 (21.2)	9 (10.6)
Drug use history	171	89 (52.0)	37 (21.6)	23 (13.4)
Never use condoms	143	80 (55.9)	25 (17.5)	17 (11.9)
History of STDs	112	76 (67.8)	25 (22.3)	18 (16.1)
Abnormal Pap smear	76	43 (56.6)	14 (18.4)	13 (17.1)

NOTE. STDs, sexually transmitted diseases; HIV, human immunodeficiency virus.
* Based on 1:10 dilutions of serum samples.

the limited data on the types of sex partners and the duration of commercial sex work of these women. In men, the overall HHV-8 seroprevalence was 22.6% (17/75) (table 2). The rate of HHV-8 infection in HIV-positive men was more than twice as high as in HIV-uninfected men at the 1:10 dilution (28% vs. 12%) (table 1), but these results were not statistically significant due to the small sample size.

There were also differences in the univariate analysis of the risks associated with different behaviors (table 2): Being a homosexual male (OR = 10.0, 95% CI = 1.3–92.9, $P = .009$), engaging in sexual intercourse at early age (comparison of means, 14.6 years, 16.4 years; $P = .01$), history of sexually transmitted diseases (OR = 3.5, 95% CI = 1.6–8.0; $P = .01$), and abnormal Pap smear (OR = 5.2, 95% CI = 1.7–15.9; $P = .006$) were significantly associated with dual HIV/HHV-8 seropositivity. All variables associated with HIV/HHV-8 seropositivity or with an OR < 0.75 or >1.5 were then entered into a logistic regression model. First sexual activity at $\geqslant 16$ years old (adjusted OR = 0.4; 95% CI = 0.2–0.8; $P = .011$) and being male (adjusted OR = 3.0, 95% CI = 1.3–7.0; $P = .012$) were independently associated with dual HIV/HHV-8 infection. Among the small group of HIV-infected men studied, sexual orientation was not statistically associated with HHV-8 serologic status (OR = 1.7, 95% CI = 0.5–6.1; $P = .4$).

RIP. To confirm that weak positive sera (1:10 titer) were indeed positive, RIP was performed. Positive sera recognized at least 20 polypeptides in induced BCBL-1 cells, which are probably proteins associated with lytic HHV-8 antigens (figure 1, lanes 1, 5, 7, 9, 11 [uninduced] and lanes 2, 6, 8, 10, 12 [TPA-induced]). IFA-negative sera did not show a specific reaction in RIP (figure 1, lanes 3, 4). All sera recognized 155-, 116-, 105-, 90-, 55-, 50-, 38-, 29-, and 23-kDa polypeptides from induced cells. Studies indicate that the 116-, 110-, 90-, 70-, 55-, and 50-kDa proteins are HHV-8 glycoproteins [14]. Only a weak reaction was seen in the uninduced BCBL-1 cells, and only a limited number of polypeptides (225-, 145-, 74-, 70-, 55-, and 23-kDa) were immunoprecipitated. Positive RIP criteria were established on the basis of the appearance of reacting viral proteins of the expected sizes: 225, 145, 74, 70, 55, and 23 kDa.

Discussion

To our knowledge, this is the first comprehensive study to determine the prevalence of HHV-8 infection in a Latin American country. Our data extend previous serologic reports [7] linking high HHV-8 seroprevalence with HIV coinfection. Evidence of strong association between HHV-8 and HIV infections is supported in this study by the fact that HIV-positive women have almost twice the risk of HHV-8 infection than a similar group of HIV-negative women. We chose this group because the HIV-positive women were very likely to be infected heterosexually and were available for follow-up studies to correlate the incidence of HHV-8 infection with KS development.

A noticeable lack of consistency in the reported literature of HHV-8 seroprevalence could be related to poor sampling methods, different levels of test sensitivity (1:10 dilutions vs. 1:40 as a final cutoff point), and possible variations in assay formats, including the detection of lytic or latent antibodies. Nevertheless, the unique epidemiology of KS seems to support the hypothesis that the cause of KS is multifactorial and that one of the cofactors (necessary or enhancing factors) is HHV-8, which predisposes HIV-positive persons to KS.

We chose to consider all sera showing reactivities at a 1:10 dilution or above as seropositive in this study because this dilution includes more infected patients and, consequently, shows a more realistic distribution of HHV-8–infected persons. Because all these samples were first absorbed with EBV cell lysate to remove cross-reactivity, we are confident that they reacted solely with HHV-8 antigens. Furthermore, all samples were tested blindly by IFA at different sites (Nebraska and Kansas), and all weakly reactive samples were confirmed by RIP. Finally, our study included a negative control group, providing additional confidence that any bias found in the HIV-positive group would also be found in the control group.

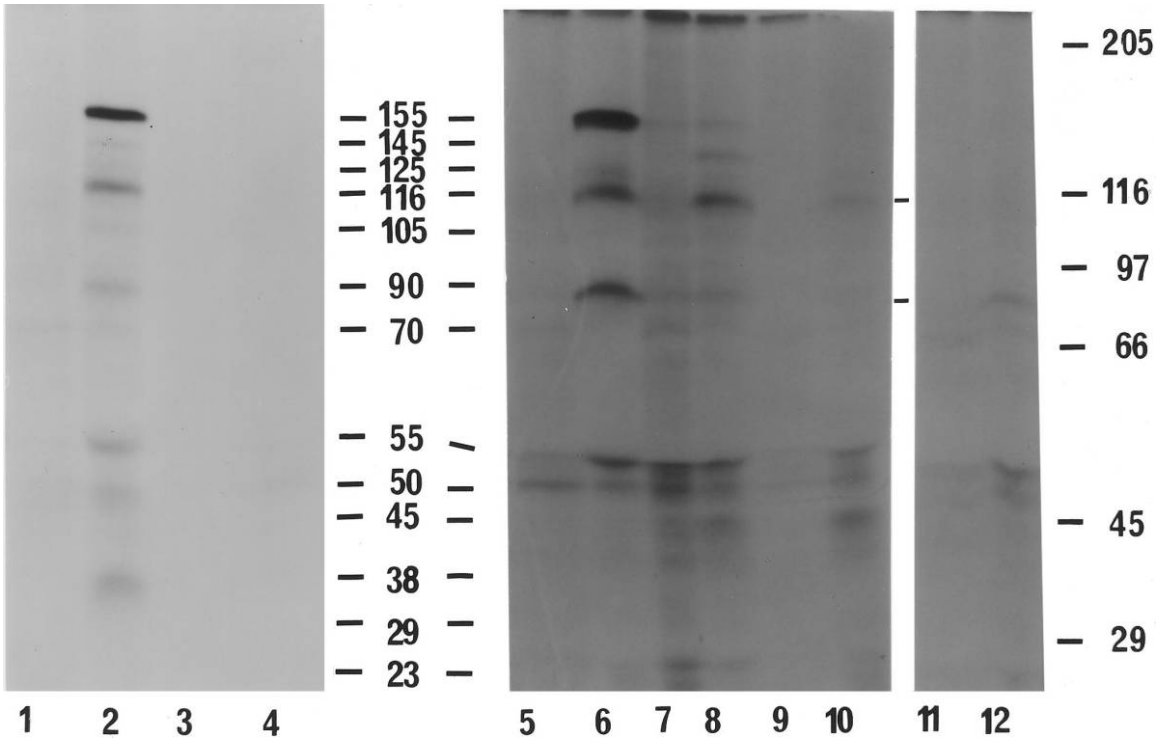

Figure 1. Radioimmunoprecipitation of selected samples: Lanes 1, 3, 5, 7, 9, and 11 are uninduced BCBL-1 cells. Lanes 2, 4, 6, 8, 10, and 12 are tetradecanoyl phorbal acetate–induced BCBL-1 cells. All cells were labeled with [^{35}S]methionine for 20 h. Lanes 1 and 2 are sera that were human immunodeficiency virus–positive (HIV) and HHV-8 positive by IFA at titer of 1:640. Lanes 3 and 4 are HIV-positive and HHV-8–negative (IFA $<$ 1:10). Lanes 5–12 are sera positive for HHV-8 by IFA.

Lennette et al. [10] analyzed a small number of HIV-positive women and reported an HHV-8 seroprevalence (21%) similar to that in our study. However, in the HIV-negative group, they detected a higher rate (28%). Our study with HIV-positive women showed higher rates than those reported by Kedes et al. [13]. This may be explained by the cutoff (1:40) used in that study.

The analysis of the occupation of women in our study demonstrated no increase in the risk of HHV-8 infection among HIV-negative prostitutes. In contrast, the HIV-positive prostitutes had a strikingly increased risk of HHV-8 infection. We found a rate of 35% in our population of HIV-positive homosexual men. The higher rate of KS among HIV-infected homosexual men may be explained by a higher incidence of HHV-8 infection among these persons as well as the association with specific sexual practices that are typical for this population [15]. Two other IFA studies [8, 9] gave results concordant with those of our investigation. However, these rates contrast with the 18% seropositivity found by the study of Gao et al. [7], in which latent antigens were measured, and suggests that the titer of lytic antibodies may differ from those of the latency-associated antigens.

Our study has determined the HHV-8 seroprevalence in a Latin American country and suggests that HIV-positive women have almost twice the risk of acquiring HHV-8 than an HIV-negative group. These results could be biased because a significant proportion of the HIV-positive women were commercial sex workers; however, having multiple sex partners does seem to be a major risk factor for HHV-8 infection, suggesting that HHV-8 can be transmitted heterosexually.

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