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ORIGINAL ARTICLE

Epidemiology of human herpesvirus type 8 and parvovirus B19 infections and their association with HIV-1 among men who have sex with men and injection drug users in Taiwan

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Background/Purpose: Human herpesvirus 8 (HHV-8), the causal agent of Kaposi's sarcoma (KS), is transmitted sexually among men who have sex with men (MSM), but little is known of its transmission among injection drug users (IDUs). By contrast, human parvovirus B19 (B19), a causative agent for anemia, is most frequently detected in IDUs. The aim of this study was to investigate the associations between HHV-8 infection and human immunodeficiency virus type 1 (HIV-1), and between B-19 and HIV-1 among MSM and IDUs patients.
Methods: Serum samples from 553 IDUs and 231 MSM were analyzed for anti-HHV-8 lytic and anti-B19 viral structural capsid protein 2 (VP-2) antibodies using enzyme immunoassay, indirect immunofluorescence, and immunoblot assays. Odds ratios (ORs) and 95% confidence intervals (CIs) were used to evaluate the associations between different viral infections.

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Results: HIV-1-seropositive MSM had significantly higher rates of HHV-8 infection than seronegative MSM (32.3% and 15.4%, respectively; OR = 2.62, 95% CI = 1.37−5.02). Among HIV-1/AIDS patient groups, MSM had significantly higher HHV-8 seropositive rates (32.3% vs. 6.6%, $p < 0.0001$) and lower B19 infection rates (35.4% vs. 78.8%, $p < 0.001$) than IDUs. In addition, HIV-1-infected MSM were 5.95 times (95% CI = 3.38−10.46) more likely to be infected with HHV-8 than male HIV-1-infected IDUs. By contrast, male IDUs were 6.74 times odds (95% CI = 4.28−10.61) more likely to contract B19 infection than MSM.
Conclusion: In Taiwan, MSM have a significantly higher prevalence for HHV-8 than IDUs. The contrasting risks of HHV-8 and B19 infections between different HIV-1/AIDS groups suggest that the efficiency of viral infection is affected by their distinct transmission routes.

Introduction

Kaposi's sarcoma-associated herpesvirus (KSHV), also known as human herpesvirus 8 (HHV-8), was discovered in 1994 and is the causative agent of Kaposi's sarcoma (KS), primary effusion lymphoma (PEL), and multicentric Castleman's disease.[1] Among HIV-1 exposure categories at risk of AIDS KS in North America and Europe, the prevalence of HHV-8 shows a distinct spread. Within a defined geographical area, this prevalence is highest among HIV-1-infected men who have sex with men (MSM), lower among HIV-1-infected injection drug users (IDUs) or HIV-1-infected non-IDU heterosexual individuals, and lowest among children.[2,3] Interestingly, in one US region, the HHV-8 prevalence rates among young MSM (15−22 years old) were comparable with the rates among young heterosexual men, and both were noticeably lower than rates among older MSM in that region.[4] Moreover, HHV-8 acquisition has been associated with multiple sex partners among both MSM and heterosexuals, and practices involving saliva are thought to increase transmission, which may account for differences in incidence of HHV-8 and HIV.[5,6] International studies have provided some evidence that HHV-8 can be transmitted by blood or blood products.[7,8] Atkinson et al also showed that longer duration of injection drug use is associated with an increase in the risk of HHV-8 infection that is not explained by sexual behavior or demographic differences.[7] Among IDUs, while syringe sharing is a common means of transmitting HIV-1, hepatitis B virus (HBV), and hepatitis C virus, it may also result in the spread of HHV-8.[9]

Human parvovirus B19 (B19) can cause a common childhood disease with symptoms of exanthema and fever known as erythema infectiosum.[10] B19 has a strong tissue tropism for erythroid progenitor cells and can cause anemia. Study of occult B19 infection is recommended in patients with hematological disease.[11] In common with other latent viruses such as herpesviruses, infections with parvovirus B19, HBV, and hepatitis GB virus C (HGBV-C) are contained successfully by the immune response and persist in the host. When immune control breaks down, reactivation of both latent and persistent viruses occurs.[12,13] The background seroprevalence in blood donors is high for B19 (≥64%), HBV (≥70%), CMV, and EBV (≥90%), and is significantly increased in individuals infected with HIV, HBV, cytomegalovirus (CMV), varicella-zoster virus (VZV; symptomatic HIV), and HHV-8 (asymptomatic and symptomatic HIV).[14,15] Persistent parvovirus B19 infection has been reported in patients both with and without underlying immunodeficiencies.[16−18] Antibody prevalence provided an estimate of viral exposure and allowed adjustment for the prevalence of potential reactivation to be calculated as well as the odds ratios (ORs) for reactivation in susceptible individuals. Levels of exposure among susceptible HIV-infected patients to parvovirus B19 are comparable to those for HIV-negative controls, because this infection is transmitted via the respiratory route.[19] Previous studies also demonstrated that prolonged parvovirus infection with anemia in HIV-infected patients was typically associated with the absence of an antibody response to B19.[20] In this study, we compared the seroprevalence of HHV-8 and B19 infection between MSM and IDU groups among HIV-1/AIDS patients in Taiwan.

Materials and methods

Subjects

This cross-sectional study was conducted among subjects from the following three populations: 553 inmates who had history of using injection drugs were recruited from detention centers and prisons in Taiwan[21,22]; 127 HIV-1-infected MSM were selected randomly from 879 patients attending the outpatient clinics of Taipei City Hospital from 2004 to 2006, and 104 HIV-1 seronegative MSM were randomly selected from 1093 patrons from gay saunas in Taiwan.[23] Their HIV status and demographic data were recorded by reviewing their medical history or assessed through a self-administered questionnaire. Informed consent was obtained from all participants and this study was approved by the Institutional Review Board of the National Yang-Ming University. Plasma samples were obtained from the subjects for serological testing. The samples were processed and stored at −80 °C.

Serological testing

Sera were tested for HHV-8 antibodies by HHV-8 whole virus lytic immunoglobulin G (IgG) enzyme-linked immunosorbent assay (ELISA; Advanced Biotechnologies Inc., Columbia, MD, USA)[24,25] and indirect fluorescent assay (IFA; Advanced Biotechnologies Inc., Columbia, MD, USA),[26,27] which

detected antibodies against lytic antigens. Sera from 784 subjects were also screened for viral protein 2 (VP-2) antibodies against human parvovirus B19 by Parvo B19 IgG EIA and Parvo Blot IgG (Biotrin international Ltd., Dublin, Ireland).[28,29] These assays were performed according to the protocols provided by manufacturers.

Statistical analysis

Univariate analysis was performed using either Pearson χ^2 test or Fisher's exact test as appropriate in order to detect statistical significances when comparing HIV-1-seropositive and HIV-1-seronegative subjects from the MSM and IDU groups. Student *t* test was used to compare the mean ages between the HIV-1-seropositive and HIV-1-seronegative subjects from the MSM and IDU groups. ORs and 95% confidence intervals (CIs) were used to evaluate the association between HIV-1 and HHV-8 or HIV-1 and B19. The Breslow-Day method was used to test the homogeneity of the ORs between the MSM and IDU groups. SAS statistical software (SAS version 9.1; SAS Institute Inc., Cary, North Carolina, USA) was used for all analyses. Statistical significance was set at $p < 0.05$.

Results

Demographic characteristics of the subjects who participated in this study

In total, 784 subjects (504 HIV-1-seropositive and 280 HIV-1-seronegative) participated in this study. Table 1 compares the demographic characteristics of the MSM and IDU groups. The male to female ratio of the IDU population was 3.8:1. The mean age of the study population was 33.8 ± 7.9 years

Table 1 Demographic characteristics of the study subjects

	MSM ($N = 231$)			IDUs ($N = 553$)		
	HIV-1(−) ($N = 104$) *n* (%)	HIV-1(+) ($N = 127$) *n* (%)	*p*	HIV-1(−) ($N = 176$) *n* (%)	HIV-1(+) ($N = 377$) *n* (%)	*p*
Gender						
Male	104 (100)	127 (100)	—	127 (72.2)	310 (82.2)	0.0067
Female	0 (0)	0 (0)		49 (27.8)	67 (17.8)	
Age (y)	$n = 80$	$n = 127$	0.0124	$n = 176$	$n = 374$	0.2515
20—29	27 (33.8)	56 (44.1)		47 (26.7)	117 (31.3)	
30—39	35 (43.8)	61 (48.0)		76 (43.2)	166 (44.4)	
40—49	10 (12.5)	8 (6.3)		50 (28.4)	80 (21.4)	
≥50	8 (10.0)	2 (1.6)		3 (1.7)	11 (2.9)	
Mean age	34.6 ± 10.4	31.0 ± 7.0	0.0076	35.0 ± 7.0	34.7 ± 7.7	0.1694
Education	$n = 68$	$n = 125$	0.0003	$n = 176$	$n = 368$	0.0258
Informal/primary	1 (1.5)	1 (0.8)		12 (6.8)	39 (10.6)	
Junior high	4 (5.9)	3 (2.4)		82 (46.6)	191 (51.9)	
Senior high	38 (55.9)	35 (28.0)		73 (41.5)	108 (29.4)	
College/university /graduate school	25 (36.8)	86 (68.8)		9 (5.1)	30 (8.2)	
Marital status	$n = 68$	$n = 124$	0.0495	$n = 176$	$n = 374$	0.3245
Single	57 (83.8)	117 (94.4)		92 (52.3)	222 (59.4)	
Married/cohabiting	9 (13.2)	5 (4.3)		32 (18.2)	64 (17.1)	
Divorced/separated	2 (2.9)	2 (1.6)		52 (29.6)	87 (23.3)	
Widowed	0 (0)	0 (0)		0 (0)	1 (0.3)	
CD4 counts		$n = 126$	—		$n = 159$	0.0423*
≤200	—	3 (2.4)		—	16 (10.1)	
201—350	—	35 (27.8)		—	37 (23.3)	
351—500	—	36 (28.6)		—	52 (32.7)	
>500	—	52 (41.3)		—	54 (34.0)	
Mean ± SD		498.9 ± 234.8			441.5 ± 194.2	0.0280*
HIV-1 viral load		$n = 125$	—		$n = 45$	0.2419*
≤50	—	60 (48.0)		—	17 (37.8)	
51—500	—	22 (17.6)		—	5 (11.1)	
501—10,000	—	13 (10.4)		—	8 (17.8)	
>10,000	—	30 (24.0)		—	15 (33.3)	
Mean ± SD		17,635 ± 51,110			27,094 ± 83,614	0.4791*
ART		$n = 125$	—		$n = 356$	<0.0001*
Yes	—	88 (70.4)		—	62 (17.4)	
No	—	37 (29.6)		—	294 (82.6)	

ART = antiretroviral therapy; IDUs = injection drug users; MSM = men who have sex with men; — = not detected.
*The *p* values are only presented as the statistical significance between HIV-1-seropositive MSM and IDUs.

old, but the IDUs were significantly older than the MSM (34.3 ± 7.5 vs. 32.4 ± 8.6, $p = 0.0047$). Approximately half of MSM [111/193 (57.5%)] had college degree or post-graduate education, but 92.8% of IDUs had received education up to senior high school. A larger percentage of MSM [174/192 (90.6%)] were single, while more than 20% of IDUs were divorced or separated. Of the HIV-1-seropositive subjects, 70.4% (88/125) of the MSM group were receiving antiretroviral therapy (ART), but only 62 IDUs (17.4%) were receiving ART ($p < 0.0001$). Although HIV-1 viral load did not differ significantly among two groups, MSM group had a significantly higher CD4 than IDUs ($p = 0.0280$).

The associations between HHV-8 and HIV-1, and between B19 and HIV-1 infection among MSM and IDUs

Overall, HHV-8 prevalence was 24.68% (57/231) among MSM, and 4.88% (27/553) among IDUs ($p < 0.0001$). HHV-8 infection was more prevalent among MSM, particularly in young people, while HHV-8 seroprevalence in IDUs increased in middle-aged people (Fig. 1A). The prevalence of B19 in IDUs [428/553 (77.40%)] was twice as high as that in MSM [92/231 (39.83%)] ($p < 0.0001$). Across different age groups, B19 infection remained relatively common in IDUs (Fig. 1B). The associations between HHV-8 and HIV-1 infection in MSM and IDUs were determined. As shown in Table 2, the HIV-1-seropositive subjects had significantly higher HHV-8 infection rate than the HIV-1-seronegative subjects among the MSM (32.3% vs. 15.4%, OR = 2.62, 95% CI = 1.37–5.02) and IDUs (6.6% vs. 1.1%, OR = 6.18, 95% CI = 1.45–26.38). Although the ORs were not statistically significant in B19 infection, the infection rate was lower among HIV-1-seropositive MSM (35.4% vs. 45.2%, OR = 0.67, 95% CI = 0.39–1.13), while it was higher among HIV-1-seropositive IDUs (78.8% vs. 74.4%, OR = 1.28; 95% CI = 0.84–1.94). When we analyzed the associations only in male subjects, MSM had 5.95 times higher (95% CI = 3.38–10.46) odds of being infected with HHV-8 than IDUs (Table 3). By contrast, IDUs had 6.74 times higher odds (95% CI = 4.28–10.61) of being infected with B19 than MSM. Of the 504 HIV-positive patients from whom HHV-8 and B19 titers were determined, CD4 cell counts and HIV viral load were available for only 285 (56.5%) and 170 (33.7%), respectively. After adjustment for age, CD4 cell counts, and receipt of highly active antiretroviral therapy (HAART) among male HIV-1-infected subjects, MSM had 3.34 times higher (95% CI = 1.45–7.66) odds of being infected with HHV-8 than IDUs, and IDUs had 4.31 times higher (95% CI = 2.31–8.07) odds of being infected with B19 than MSM.

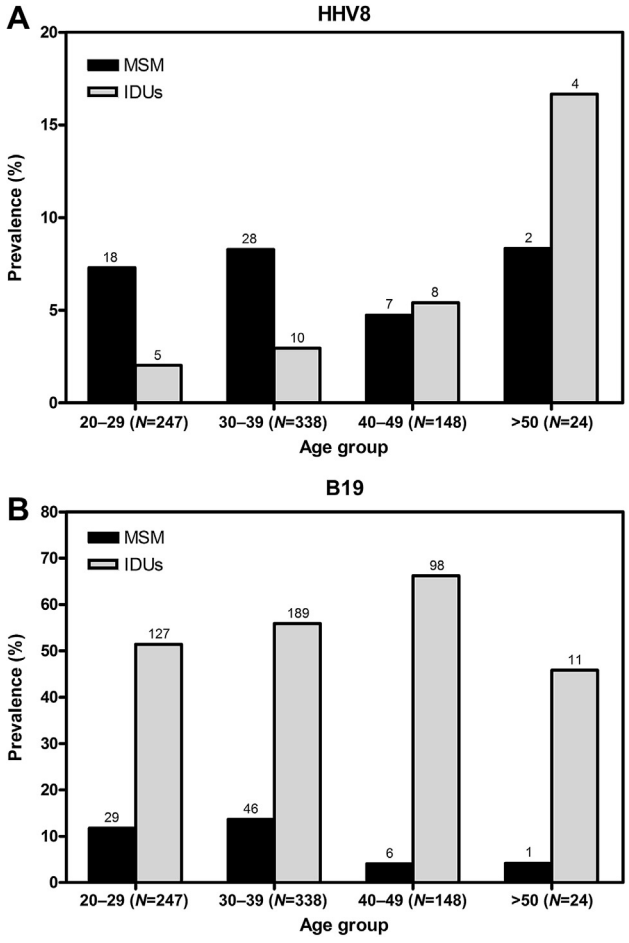

Figure 1. Age-dependent seroprevalence of viruses for men who have sex with men (MSM) and injection drug users (IDUs) in Taiwan ($N = 784$). Bar graphs depict the seroprevalence of (A) human herpesvirus 8 (HHV-8) and (B) parvovirus B19 (B19) infection. The number of subjects in each group is shown above each bar.

Discussion

In this study, we conducted a seroepidemiological study to investigate the prevalence of anti-HHV-8 lytic and anti-B19 VP-2 antibodies among HIV-1/AIDS patients. Antibody testing in this cross-sectional study provides snapshots of the MSM and IDUs populations that are the main high risk groups in Taiwan. We found that HIV-1-seropositive subjects had a higher seroprevalence rate for HHV-8 than HIV-1-seronegative subjects [66/504 (13.1%) vs. 18/280 (6.4%)], while the prevalence of anti-B19 antibodies was almost the same between HIV-1-seropositive and HIV-1-seronegative subjects [342/504 (67.9%) vs. 178/280 (63.6%)] (Table 2). In addition, MSM had a significantly higher HHV-8 infection rate than IDUs among HIV-1/AIDS patients (32.3% vs. 6.6%). Previous reports have shown that high HHV-8 seroprevalence rates occur among HIV-infected patients in Africa, Western countries, and Taiwan.[2,3,30,31] HHV-8 seroprevalence varies greatly depending on the study population in Taiwan, specifically 83.3% in KS(+)/HIV(−) patients, 40% in KS(−)/HIV(+) patients, 24.5% in patients with hematological disorders, 23% in blood donors, 20.6% when there is sexual contact, 19.5% in hemodialysis patients, and 12.9% in non-KS cancer patients.[31–34] In previous studies of Taiwan, IFA was the common method used to detect HHV-8 antibody titers against latent, lytic antigens, or recombinant proteins [open reading frame 16 (ORF16), ORF57, and ORF71] of HHV-8.[31,33–35] However, one study demonstrated that HHV-8 antibodies are detected more frequently in HIV-infected IDUs using the recombinant antigen ORF66 (lytic and latent antigen)- and ORFK12 (latent antigen)-based Western blot strip assays and ELISA.[32] They reported that

Table 2 The association between HIV-1 and HHV-8 and HIV-1 and B19 in MSM and IDUs in Taiwan

	HHV-8		OR (95% CI)	Parvovirus B19		OR (95% CI)
	+	−		+	−	
MSM						
HIV-1(+) (N = 127)	41 (32.3%)	86 (67.7%)	2.62 (1.37−5.02)	45 (35.4%)	82 (64.6%)	0.67 (0.39−1.13)
HIV-1(−) (N = 104)	16 (15.4%)	88 (84.6%)	1	47 (45.2%)	57 (54.8%)	1
IDUs						
HIV-1(+) (N = 377)	25 (6.6%)	352 (93.4%)	6.18 (1.45−26.38)	297 (78.8%)	80 (21.2%)	1.28 (0.84−1.94)
HIV-1(−) (N = 176)	2 (1.1%)	174 (98.9%)	1	131 (74.4%)	45 (25.6%)	1

HHV-8 = human herpesvirus 8; IDUs = injection drug users; MSM = men who have sex with men; OR = odds ratio.

Table 3 HHV-8 and B19 infection rates among male HIV-1-infected subjects in Taiwan

	HHV-8		OR (95% CI)	Parvovirus B19		OR (95% CI)
	+	−		+	−	
MSM (N = 127)	41 (32.3%)	86 (67.7%)	5.95 (3.38−10.46)[a]	45 (35.4%)	82 (64.6%)	1
IDUs (N = 310)	23 (7.4%)	287 (92.6%)	1	244 (78.7%)	66 (21.3%)	6.74 (4.28−10.61)[a]

[a] Before adjustment. If adjusted by age, CD4 cell counts, and receipt of HAART, the ORs for HHV-8 and B19 seropositivity were 3.34 (95% CI = 1.45−7.66) and 4.31 (95% CI = 2.31−8.07), respectively.
HHV-8 = human herpesvirus 8; IDUs = injection drug users; MSM = men who have sex with men; OR = odds ratio.

the recombinant ORF66 showed higher levels of sensitivity and specificity, and surveyed the seroprevalence of HHV-8 antibodies in HIV-1-infected subjects who attended hospital in the central region of Taiwan in 2005. In the present study, we used commercially available ELISA and IFA, which detected antibodies against lytic antigens of HHV-8, and conducted a seroepidemiological study among subjects recruited from detection centers, gay saunas, and STD clinics in different geographic areas of Taiwan. Yang et al reported that 79.4% of HHV-8 antibody-positive sera belonged to IDUs,[32] while only 37.9% of HHV-8 antibody-positive sera belonged to IDUs in our study. The difference in the HHV-8 seroprevalence rates reported in the present study and previous reports in Taiwan is probably due to changes in the antigens and diagnostic methods used for the detection of HHV-8 antibodies, or alternatively the study population recruited in different geographic areas of Taiwan.

Among HIV-infected individuals, increased seroprevalence may be due to a sharing of transmission routes. In the present study, we found that HHV-8 antibodies were detectable more frequently among MSM (OR = 5.95), but B19 seroprevalence was higher among IDUs (OR = 6.74) (Table 3). Based on sexual behavior and method of drug use, MSM and IDUs in Taiwan are two entirely different groups. Sexual behavior is considered as the predominant route of HHV-8 transmission in MSM, while blood-borne transmission is considered the main route of B19 transmission in IDUs. Although HHV-8 can be transmitted by both sexual contact and blood,[2,3,8] the efficiencies of HHV-8 and B19 infection seems to have opposite levels of efficiency between the different transmission routes. However, it should be noted that the order of the viral infection is unknown in this cross-sectional study. Thus, in terms of this limitation, individuals could have been infected with HHV-8 or B19 before being infected with HIV-1 in a sexual relationship with a virus-bearing patient or by direct contact with respiratory secretions or blood. Another limitation was that there was too much missing data of the virologic and immunologic parameters among IDUs. Further longitudinal follow-up studies that overcome the methodological limitations described above are needed to address the causal relationship between HIV-1 and HHV-8/B19 infection.

In conclusion, the results show that MSM had significantly higher prevalence rates of HHV-8 infection than IDUs in Taiwan. The opposing levels of risk that are present for HHV-8 and B19 infection in different groups suggest that the efficiency of viral infection is affected by the distinct transmission routes of the two viruses.

Conflicts of interest

All contributing authors declare no conflicts of interest.

Acknowledgments

LYM performed the analysis of the data and drafted the manuscript. LYT and CSY revised the manuscript and did supplementary analysis. WSF participated in the design of the study. CYM participated in the design, revised the manuscript, and coordinated the study. They all approved the final version of the manuscript. We thank staff from the Genome Research Center at National Yang-Ming University for their technical support. We also thank colleagues at the AIDS Prevention and Research Center of the National Yang-Ming University, and Division of Clinical Virology, Department of Pathology, and Laboratory Medicine at Taipei-VGH for helpful discussion and support. This research was supported in part by a grant from the Republic of China (ROC) National Health Research Institutes (NHRI-EX101-10149SI).

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