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Risk Factors Influencing Antibody Responses to Kaposi's Sarcoma-Associated Herpesvirus Latent and Lytic Antigens in Patients Under Antiretroviral Therapy in South Texas

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Abstract

Background—Kaposi's sarcoma-associated herpesvirus (KSHV) seropositivity and lytic antibody titer are predictors for Kaposi's sarcoma (KS).

Methods—We examined demographic, viral and immunological factors that influence KSHV latent and lytic antibodies in HIV-infected patients.

Results—Detection rate of KSHV latent but not lytic antibodies was lower in patients with CD4 cells/mm^3 ≤200 than >200 (odds ratio [OR], 0.26; 95% confidence interval [CI], 0.11–0.61) and CD8 cells/mm^3 ≤400 than >400 (OR, 0.26; 95% CI, 0.07–0.67). Overall seropositivity rate was higher in patients with CD4 cells/mm^3 ≤200 than >200 (OR, 2.34; 95% CI, 1.37–4.02) and HIV copies/mL >400 than ≤400 (OR, 1.70; 95% CI, 1.09–2.65). Lytic antibody level was inversely correlated with CD4 count (P<0.001). Lytic seropositivity (OR, 2.47; 95% CI, 1.35–4.50) and

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antibody level (adjusted difference mean optical density [admOD], 0.324; 95% CI, 0.16–0.46) were higher in patients with HIV infection >15 than ≤15 years. Hispanics had higher lytic seropositivity rate (OR, 1.71; 95% CI, 1.07–2.73) and antibody level (admOD, 0.111; 95% CI, 0.03–0.18) than non-Hispanics.

Conclusions—Lower CD4 and CD8 counts impair antibody response to KSHV latent antigens. Immune deterioration, long-term HIV infection and Hispanic status are risk factors for KS predictors.

Keywords

KSHV; Kaposi's sarcoma; Latent and lytic antibodies; Risk factors; HIV/AIDS

INTRODUCTION

Kaposi's sarcoma-associated herpesvirus (KSHV) is associated with Kaposi's sarcoma (KS), a common malignancy in HIV-infected patients[1]. Individuals at a higher risk for KS have a higher KSHV seroprevalence[2–6]. Detection of KSHV DNA and antibodies precedes KS onset[2,3,7,8].

While HIV infection accelerates KS development[9], the time from KSHV seroconversion to KS onset varies from months to years[2,3], suggesting involvements of cofactors. Higher KSHV lytic antibody titers are associated with advanced disease[10,11] and patients with lytic antibodies have higher KS incidence rates[12,13]. Furthermore, KS incidence and disease status are positively correlated with detection and load of peripheral blood KSHV DNA[2,7,8,12,14–17], and KS regressed following anti-herpesviral treatments that inhibit lytic replication[18–20]. Thus, KSHV lytic replication and lytic antibody titer are predictors for KS development.

The advent of highly active antiretroviral therapy (HAART) has reduced KS incidence[21]. AIDS-KS regression due to HAART is associated with decreased blood KSHV load and lytic antibody titers[10,14,17,22–25]. Nevertheless, some HIV patients continue to develop KS[26–28]. As HIV patients live longer, their likelihood of developing KS becomes higher. Identification of cofactors for KS development in the HAART era is of particular importance.

While serologic assays are useful for examining association of KSHV serostatus with KS progression, detection of latent and lytic antibodies remains inconsistent[29–31]. It is unclear what factors might affect KSHV seropositivity. Extensive characterizations of KSHV infection have been performed in AIDS-KS patients, but fewer were in HIV patients without KS[2,3,13,32]. We investigated clinical correlates and risk factors for antibody responses to KSHV antigens in a cohort of HIV patients receiving HAART.

METHODS

HIV patients under HAART without KS were recruited from the Family Focused AIDS Clinical Treatment and Services Clinic, San Antonio Military Medical Center HIV Unit and Audie L. Murphy Memorial Veterans Hospital Immunosuppression Clinic in San Antonio. The protocol was approved by the Institutional Review Boards of participating sites. Written informed consent was obtained from patients. CD4 and CD8 T cell counts and HIV loads were obtained from the Frederic C. Bartter General Clinical Research Center. Demographic, medical information, other coinfections and comorbid conditions was collected.

Latency-associated nuclear antigen (LANA) antibodies were detected by an immunofluorescence antibody assay using BCP-1 cells[3], and confirmed with recombinant KSHV-infected rat precursor cells[33]. Besides using genuine uninfected controls, these cells have minimal cross-reactivity with human autoantibodies. Lytic antigen ORF65 antibodies were detected by an enzyme-linked immunosorbent assay[6]. Samples with optical density (O.D.) values ≥3 times of average O.D. of a panel of negative controls plus 5 times of standard deviations were defined as positive. Sera were tested at 1:50 dilution. Sets of serum samples from KS patients and blood donors previously tested seropositive and seronegative, respectively, were included as controls[34,35]. Both assays have been extensively evaluated in previous studies[29,34,35]. Serostatus was scored based on the presence of antibodies to LANA alone (“*LANA*”), ORF65 alone (“*ORF65*”), any of *LANA* and *ORF65* including *LANA* and *ORF65* dually positive samples (“*ANY*”), and both *LANA* and *ORF65* only (“*BOTH*”).

Given their non-normal distribution, differences of variables between seropositive and seronegative patients were examined using the Mann-Whitney U test. The magnitude of association between outcome and dichotomous independent predictors of KSHV seropositivity was estimated using odds ratio (OR) and corresponding 95% confidence interval (CI), and multivariate analyses were conducted using unconditional logistic regression analysis adjusted for age and ethnicity. We included preselected first order interaction terms to assess potential effect measure modification.

Relative ORF65 antibody level was analyzed using a general linear model (GLM) after log transformation of data with reference groups identified for each factor. We performed univariate analysis examining association between predictor variables and antibody level as well as multivariate analyses adjusted for age and ethnicity. Analyses were performed with STATA/SE 10.0 (StataCorp., College Station, TX).

RESULTS

We determined KSHV serostatus in 383 HIV patients (Supplementary Table). The overall *LANA*, *ORF65*, *ANY* and *BOTH* seropositivity rates were 21%, 30%, 36% and 13%, respectively. Logistic regression analysis with *ANY* serostatus adjusted for age and ethnicity showed a higher seropositivity rate in males than females (40% *vs* 13%; OR, 4.94; 95% CI, 2.14–11.44; $P<0.001$) (Table 1). Similar results were observed with other serostatus, which were consistent with previous studies[2–4,6]. Surprisingly, Hispanics had a higher seropositivity rate than Non-Hispanics (32% *vs* 25%; OR, 1.71; 95% CI, 1.07–2.73; $P=0.024$) when analyzed by *ORF65* serostatus.

Analysis of HIV-related factors and coinfections based on *ANY* serostatus revealed a higher seropositivity rate in patients with CD4 T cells/mm^3 ≤200 than >200 (53% *vs* 33%; OR, 2.34; 95% CI, 1.37–4.02; $P=0.002$), HIV copies/mL >400 than ≤400 (42% *vs* 32%; OR, 1.70; 95% CI, 1.09–2.65; $P=0.019$), with than without syphilis (56% *vs* 34%; OR, 2.48; 95% CI, 1.28–4.79; $P=0.007$), and with than without hepatitis (47% *vs* 33%; OR, 1.76; 95% CI, 1.07–2.90; $P=0.027$) (Table 1). No association was found between KSHV seropositivity and any comorbid conditions (data not shown).

The association of CD4 T cell count and HIV load with KSHV seropositivity persisted when serostatus was defined by *ORF65* but not by *LANA* and *BOTH*, indicating *ORF65* seropositivity as the main contributing factor (Table 1). A higher seropositivity rate was also found in patients with duration of HIV infection >15 years than ≤15 years when defined by *ORF65* (40% *vs* 25%; OR, 2.47; 95% CI, 1.35–4.50; $P=0.003$).

We analyzed the interactions of variables. When adjusted for other factors, lower CD4 T cell count remained as a risk factor for *ORF65* and *ANY* serostatus (data not shown). Association

of HIV load with *ORF65* and *ANY* serostatus was not affected by duration of HIV infection and CD8 T cell count but disappeared after adjusting for CD4 T cell count. Association of duration of HIV infection with *ORF65* serostatus was not altered by other factors. In contrast, association of Hispanic status with *ORF65* serostatus disappeared after adjusting for other factors. Interestingly, Hispanics had lower CD4 and CD8 T cell counts than Non-Hispanics ($P<0.001$ and $=0.025$, respectively) but no difference was found for HIV load and duration of HIV infection (Supplementary Fig. 1A). Lower CD4 T cell count persisted in Hispanics regardless *ORF65* serostatus ($P=0.004$ and 0.001, respectively), and in *ORF65*+ patients regardless Hispanics status ($P<0.001$ and $=0.001$, respectively) (Supplementary Fig. 1B). In contrast, the difference of CD8 T cell count between Hispanics and Non-Hispanics disappeared when *ORF65* serostatus was considered.

The results thus far indicated an association of CD4 T cell count, HIV load, or duration of HIV infection with *ORF65* but not *LANA* serostatus. We examined effects of these factors on antibody detection in KSHV-infected patients by logistic regression adjusting for age and ethnicity (Table 2). HIV load had no effect on detection of latent or lytic antibodies. However, detection rate of latent antibodies was lower in those with CD4 T cells/mm^3 ≤200 than >200 (35% *vs* 67%; OR, 0.26; 95% CI, 0.11–0.61; $P=0.002$), CD8 T cells/mm^3 ≤400 than >400 (28% *vs* 64%; OR, 0.22; 95% CI, 0.07–0.67; $P=0.007$) and duration of HIV infection >15 years than ≤15 years (45% *vs* 62%; OR, 0.42; 95% CI, 0.18–1.02; $P=0.057$) though the later was not statistically significant. Thus, lower CD4 and CD8 T cell counts impeded antibody responses to latent antigens. In contrast, lower CD4 T cell count (92% *vs* 71%; OR, 3.41; 95% CI, 0.93–12.45; $P=0.064$) and longer duration of HIV infection (87% *vs* 73%; OR, 5.28; 95% CI, 1.50–18.59; $P=0.010$) increased detection rates of lytic antibodies (Table 2).

The *ORF65* serostatus may reflect KSHV lytic replication status. We examined the main and interaction effects of KSHV-associated risk factors on relative ORF65 antibody levels in *ANY*+ patients (Table 3). HIV load, CD8 T cell count or other coinfections had no effect on ORF65 antibody level. In contrast, ORF65 antibody level was higher in patients with duration of HIV infection >15 than ≤15 years (adjusted difference mean O.D. [admOD]=0.324; 95% CI, 0.16–0.46; $P=0.001$) and with CD4 T cells/mm^3 ≤200 than >200 (admOD=0.105; 95% CI, −0.01–0.19; $P=0.063$) though the later was not statistically significant. ORF65 antibody level was negatively correlated with CD4 T cell counts ($r=0.407$; $P<0.001$) and positively with duration of HIV infection at a marginal level ($r=0.157$; $P=0.065$) but not correlated with CD8 T cell count ($P=0.827$) nor with HIV load ($P=0.135$) (Supplementary Fig. 2). Consistent with *ORF65*+ rate, Hispanics had higher ORF65 antibody levels than Non-Hispanics (admOD=0.111; 95% CI, 0.03–0.18; $P=0.012$) (Table 3).

Analysis of risk factor interactions showed a lower CD4 T cell count as a strong factor for a higher ORF65 antibody level when adjusted for other factors (data not shown). Duration of HIV infection remained a factor for a higher ORF65 antibody level while CD8 T cell count and HIV load showed no association. The association of Hispanic status with higher ORF65 antibody levels was not affected by CD8 T cell count, HIV load and duration of HIV infection but was marginally influenced by CD4 T cell count (admOD=0.074; 95% CI, −0.01–0.14; $P=0.071$).

DISCUSSION

A serologic assay with one antigen may be insufficiently sensitive to identify all KSHV-infected patients. Indeed, inconsistencies were observed among assays based on single antigen[30,31]. Cross-examination with multiple assays including both latent and lytic antigens

may increase the sensitivity and specificity for identifying KSHV-infected patients[29,35]. As expected, our *LANA*, *ORF65* and *BOTH* seropositivity rates are within the reported ranges; however, the *ANY* rate (36%) is at the higher estimates[2–6].

We found an overall higher KSHV seropositivity rate among patients with lower CD4 T cell counts or higher HIV loads (Table 1). Both factors could influence immune surveillance, and hence KSHV lytic replication and serostatus. Indeed, both factors were associated with lytic seropositivity. However, a higher ORF65 antibody level was only associated with a lower CD4 T cell count (Table 3). Furthermore, association of HIV load with *ORF65* seropositivity was marginally affected by CD4 T cell count (data not shown). Thus, immune status is likely a better predictor than HIV load for opportunistic diseases, confirming the observation that HIV load does not always predict immune status including CD4 T cell count[36].

In contrast to KSHV lytic antibodies, lower CD4 and CD8 T cell counts, and longer duration of HIV infection affected detection of latent antibodies (Table 2). Whether this observation can be extended to all latent antigens remain unclear. A previous report has also shown dependence of detecting LANA antibodies on CD4 T cell counts[37]. These findings explain why previous studies failed to observe an association of *LANA* seropositivity with CD4 T cell count and HIV load[4,11,15,38].

In the early AIDS epidemic, patients rapidly progressed to KS following KSHV seroconversion with over half developing KS within 12 months[2,3,39]. We found higher KSHV seropositivity rates and lytic antibody levels in patients with duration of HIV infection >15 years than ≤15 years (Table 3). These associations were not confounded by other factors, indicating that longer duration of HIV infection is an independent predictor for KSHV seropositivity and higher lytic antibody levels. Of note, classical KS is commonly found in elderly men[1]. While HIV infection and resulting immunosuppression were dominant factors controlling KS development in early HIV epidemics, HAART has reduced their effects as manifested by the reduced KS incidence in the last decade[40]. As patients live longer, other factors such as duration of HIV infection have emerged as cofactors.

We found higher *ORF65*+ rates and higher *ORF65* antibody levels in Hispanics than in non-Hispanics (Table 1 and 3). KSHV epidemiology in South Texas is distinct with a slightly higher seroprevalence in the general population than other US regions (15% *vs* ≤12%)[34], and a unique spectrum of KSHV genotypes including 75% K15M subtype and 50% K1C3/K15M mosaic genotype. These predominant genotypes are associated with Hispanics and an advanced KS stage[41], suggesting a viral factor might contribute to an increased risk and a more advanced KS stage. Nevertheless, association of Hispanics with *ORF65* seropositivity, though not *ORF65* antibody level, was confounded by other factors. While Hispanics and Non-Hispanics had similar duration of HIV infection and HIV load suggesting their comparable treatments, Hispanics had lower CD4 and CD8 T cell counts than Non-Hispanics (Supplementary Fig. 1A). Thus, genetic or environmental factors might contribute to worse HIV-induced immune deterioration resulting in higher risks for KSHV seropositivity and higher lytic antibody levels.

A limitation of this study is its cross-sectional nature. Our subjects were enrolled in a prospective cohort study; however, we lacked sufficient cumulative follow-up experience to elucidate the direct relationship between KSHV infection or lytic replication, and incident KS. Although we utilized three referral clinics, our population might not be reflective of the general HIV population. Strengths include the diversity of the population with a high proportion of Hispanics, and simultaneous examination of KSHV latent and lytic antibodies.

In summary, besides high HIV load and deteriorated immunity, extended duration of HIV infection, probably a result of HAART, increased the risk for KSHV seropositivity and lytic antibody level, and thus may contribute to a higher risk for developing KS. South Texas Hispanic HIV patients appear at a higher risk for KS than other US regions. Results of this study should be considered for long-term management of HIV patients in the HAART era.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Greene W, Kuhne K, Ye FC, et al. Molecular biology of KSHV in relation to AIDS-associated oncogenesis. Cancer Treat Res 2007;133:69–127. [PubMed: 17672038]
2. Gao SJ, Kingsley L, Hoover DR, et al. Seroconversion to antibodies against Kaposi's sarcoma-associated herpesvirus-related latent nuclear antigens before the development of Kaposi's sarcoma. N Engl J Med 1996;335:233–241. [PubMed: 8657239]
3. Gao SJ, Kingsley L, Li M, et al. KSHV antibodies among Americans, Italians and Ugandans with and without Kaposi's sarcoma. Nat Med 1996;2:925–928. [PubMed: 8705864]
4. Kedes DH, Operskalski E, Busch M, Kohn R, Flood J, Ganem D. The seroepidemiology of human herpesvirus 8 (Kaposi's sarcoma-associated herpesvirus): distribution of infection in KS risk groups and evidence for sexual transmission. Nat Med 1996;2:918–924. [PubMed: 8705863]
5. Miller G, Rigsby MO, Heston L, et al. Antibodies to butyrate-inducible antigens of Kaposi's sarcoma-associated herpesvirus in patients with HIV-1 infection. N Engl J Med 1996;334:1292–1297. [PubMed: 8609946]
6. Simpson GR, Schulz TF, Whitby D, et al. Prevalence of Kaposi's sarcoma associated herpesvirus infection measured by antibodies to recombinant capsid protein and latent immunofluorescence antigen. Lancet 1996;348:1133–1138. [PubMed: 8888167]
7. Whitby D, Howard MR, Tenant-Flowers M, et al. Detection of Kaposi sarcoma associated herpesvirus in peripheral blood of HIV-infected individuals and progression to Kaposi's sarcoma. Lancet 1995;346:799–802. [PubMed: 7674745]
8. Moore PS, Kingsley LA, Holmberg SD, et al. Kaposi's sarcoma-associated herpesvirus infection prior to onset of Kaposi's sarcoma. AIDS 1996;10:175–180. [PubMed: 8838705]
9. Beral V, Peterman TA, Berkelman RL, Jaffe HW. Kaposi's sarcoma among persons with AIDS: a sexually transmitted infection? Lancet 1990;335:123–128. [PubMed: 1967430]
10. Cattelan AM, Calabro ML, Aversa SM, et al. Regression of AIDS-related Kaposi's sarcoma following antiretroviral therapy with protease inhibitors: biological correlates of clinical outcome. Eur J Cancer 1999;35:1809–1815. [PubMed: 10673996]
11. Cattelan AM, Calabro ML, Gasperini P, et al. Acquired immunodeficiency syndrome-related Kaposi's sarcoma regression after highly active antiretroviral therapy: biologic correlates of clinical outcome. J Natl Cancer Inst Monogr 2001:44–49. [PubMed: 11158206]
12. Engels EA, Biggar RJ, Marshall VA, et al. Detection and quantification of Kaposi's sarcoma-associated herpesvirus to predict AIDS-associated Kaposi's sarcoma. AIDS 2003;17:1847–1851. [PubMed: 12891072]

13. Newton R, Carpenter L, Casabonne D, et al. A prospective study of Kaposi's sarcoma-associated herpesvirus and Epstein-Barr virus in adults with human immunodeficiency virus-1. Br J Cancer 2006;94:1504–1509. [PubMed: 16705315]
14. Bourboulia D, Aldam D, Lagos D, et al. Short- and long-term effects of highly active antiretroviral therapy on Kaposi sarcoma-associated herpesvirus immune responses and viraemia. AIDS 2004;18:485–493. [PubMed: 15090801]
15. Campbell TB, Fitzpatrick L, MaWhinney S, Zhang X, Schooley RT. Human herpesvirus 8 (Kaposi's sarcoma-associated herpesvirus) infection in men receiving treatment for HIV-1 infection. J Acquir Immune Defic Syndr 1999;22:333–340. [PubMed: 10634194]
16. Cannon MJ, Dollard SC, Black JB, et al. Risk factors for Kaposi's sarcoma in men seropositive for both human herpesvirus 8 and human immunodeficiency virus. AIDS 2003;17:215–222. [PubMed: 12545082]
17. Cattelan AM, Calabro ML, De Rossi A, et al. Long-term clinical outcome of AIDS-related Kaposi's sarcoma during highly active antiretroviral therapy. Int J Oncol 2005;27:779–785. [PubMed: 16077928]
18. Jones JL, Hanson DL, Chu SY, Ward JW, Jaffe HW. AIDS-associated Kaposi's sarcoma. Science 1995;267:1078–1079. [PubMed: 7855583]
19. Mocroft A, Youle M, Gazzard B, Morcinek J, Halai R, Phillips AN. Anti-herpesvirus treatment and risk of Kaposi's sarcoma in HIV infection. Royal Free/Chelsea and Westminster Hospitals Collaborative Group. AIDS 1996;10:1101–1105. [PubMed: 8874626]
20. Martin DF, Kuppermann BD, Wolitz RA, Palestine AG, Li H, Robinson CA. Oral ganciclovir for patients with cytomegalovirus retinitis treated with a ganciclovir implant. Roche Ganciclovir Study Group. N Engl J Med 1999;340:1063–1070. [PubMed: 10194235]
21. Jones JL, Hanson DL, Dworkin MS, Ward JW, Jaffe HW. Effect of antiretroviral therapy on recent trends in selected cancers among HIV-infected persons. Adult/Adolescent Spectrum of HIV Disease Project Group. J Acquir Immune Defic Syndr 1999;21 (Suppl 1):S11–17. [PubMed: 10430212]
22. Jones JL, Hanson DL, Dworkin MS, Jaffe HW. Incidence and trends in Kaposi's sarcoma in the era of effective antiretroviral therapy. J Acquir Immune Defic Syndr 2000;24:270–274. [PubMed: 10969352]
23. Lebbe C, Blum L, Pellet C, et al. Clinical and biological impact of antiretroviral therapy with protease inhibitors on HIV-related Kaposi's sarcoma. AIDS 1998;12:F45–49. [PubMed: 9619797]
24. Pellet C, Chevret S, Blum L, et al. Virologic and immunologic parameters that predict clinical response of AIDS-associated Kaposi's sarcoma to highly active antiretroviral therapy. J Invest Dermatol 2001;117:858–863. [PubMed: 11676823]
25. Pellet C, Chevret S, Frances C, et al. Prognostic value of quantitative Kaposi sarcoma--associated herpesvirus load in posttransplantation Kaposi sarcoma. J Infect Dis 2002;186:110–113. [PubMed: 12089670]
26. Maurer T, Ponte M, Leslie K. HIV-associated Kaposi's sarcoma with a high CD4 count and a low viral load. N Engl J Med 2007;357:1352–1353. [PubMed: 17898112]
27. Nasti G, Talamini R, Antinori A, et al. AIDS-related Kaposi's Sarcoma: evaluation of potential new prognostic factors and assessment of the AIDS Clinical Trial Group Staging System in the Haart Era--the Italian Cooperative Group on AIDS and Tumors and the Italian Cohort of Patients Naive From Antiretrovirals. J Clin Oncol 2003;21:2876–2882. [PubMed: 12885804]
28. Power DG, Mulholland PJ, O'Byrne KJ. AIDS-related Kaposi's sarcoma in a patient with a normal CD4 count. Clin Oncol (R Coll Radiol) 2008;20:97. [PubMed: 17954026]
29. Laney AS, Peters JS, Manzi SM, Kingsley LA, Chang Y, Moore PS. Use of a multiantigen detection algorithm for diagnosis of Kaposi's sarcoma-associated herpesvirus infection. J Clin Microbiol 2006;44:3734–3741. [PubMed: 17021103]
30. Rabkin CS, Schulz TF, Whitby D, et al. Interassay correlation of human herpesvirus 8 serologic tests. HHV-8 Interlaboratory Collaborative Group. J Infect Dis 1998;178:304–309. [PubMed: 9697708]
31. Spira TJ, Lam L, Dollard SC, et al. Comparison of serologic assays and PCR for diagnosis of human herpesvirus 8 infection. J Clin Microbiol 2000;38:2174–2180. [PubMed: 10834972]

32. Biggar RJ, Engels EA, Whitby D, Kedes DH, Goedert JJ. Antibody reactivity to latent and lytic antigens to human herpesvirus-8 in longitudinally followed homosexual men. J Infect Dis 2003;187:12–18. [PubMed: 12508141]
33. Zhou FC, Zhang YJ, Deng JH, et al. Efficient infection by a recombinant Kaposi's sarcoma-associated herpesvirus cloned in a bacterial artificial chromosome: application for genetic analysis. J Virol 2002;76:6185–6196. [PubMed: 12021352]
34. Baillargeon J, Deng JH, Hettler E, et al. Seroprevalence of Kaposi's sarcoma-associated herpesvirus infection among blood donors from Texas. Ann Epidemiol 2001;11:512–518. [PubMed: 11557184]
35. Fu B, Sun F, Li B, et al. Seroprevalence of Kaposi's sarcoma-associated herpesvirus and risk factors in Xinjiang, China. J Med Virol 2009;81:1422–1431. [PubMed: 19551832]
36. Rodriguez B, Sethi AK, Cheruvu VK, et al. Predictive value of plasma HIV RNA level on rate of CD4 T-cell decline in untreated HIV infection. JAMA 2006;296:1498–1506. [PubMed: 17003398]
37. de Souza VA, Pierrotti LC, Sumita LM, Freire WS, Segurado AA, Pannuti CS. Seroreactivity to Kaposi's sarcoma-associated herpesvirus (human herpesvirus 8) latent nuclear antigen in AIDS-associated Kaposi's sarcoma patients depends on CD4+ T-cell count. J Med Virol 2007;79:1562–1568. [PubMed: 17705173]
38. Corchero JL, Mar EC, Spira TJ, Pellett PE, Inoue N. Comparison of serologic assays for detection of antibodies against human herpesvirus 8. Clin Diagn Lab Immunol 2001;8:913–921. [PubMed: 11527803]
39. O'Brien TR, Kedes D, Ganem D, et al. Evidence for concurrent epidemics of human herpesvirus 8 and human immunodeficiency virus type 1 in US homosexual men: rates, risk factors, and relationship to Kaposi's sarcoma. J Infect Dis 1999;180:1010–1017. [PubMed: 10479125]
40. Engels EA, Pfeiffer RM, Goedert JJ, et al. Trends in cancer risk among people with AIDS in the United States 1980–2002. AIDS 2006;20:1645–1654. [PubMed: 16868446]
41. Zhang YJ, Davis TL, Wang XP, et al. Distinct distribution of rare US genotypes of Kaposi's sarcoma-associated herpesvirus (KSHV) in South Texas: implications for KSHV epidemiology. J Infect Dis 2001;183:125–129. [PubMed: 11106539]

TABLE 1

Multivariable Logistic Regression Analysis of KSHV Serostatus and Risk Factors in HIV Patients (n=383)[a]

		LANA+				*ORF65+*				*ANY+*				*BOTH+*			
		%	**OR**	**95% CI**	***P***	**%**	**OR**	**95% CI**	***P***	**%**	**OR**	**95% CI**	***P***	**%**	**OR**	**95% CI**	***P***
Demographics																	
Gender	Female[b]	2	1.00	-	-	11	1.00	-	-	13	1.00	-	-	0	-	-	-
	Male	25	17.99	(2.43–133.12)	**.005**	31	4.29	(1.74–10.55)	**.002**	40	4.94	(2.14–11.44)	**<.001**	15	-	-	-
Age (yr)	≤35[b]	22	1.00	-	-	29	1.00	-	-	34	1.00	-	-	17	1.00	-	-
	35–45	21	.87	(0.48–1.56)	.634	29	0.92	(0.54–1.57)	.757	38	1.10	(0.66–1.82)	.718	12	0.59	(0.30–1.19)	.140
	>45	21	0.88	(0.45–1.72)	.711	26	0.75	(0.40–1.39)	.359	37	1.01	(0.57–1.81)	.960	10	0.48	(0.21–1.12)	.089
Ethnicity	Non-H[b]	22	1.00	-	-	25	1.00	-	-	36	1.00	-	-	12	1.00	-	-
	H	20	1.08	(0.65–1.80)	.772	32	1.71	(1.07–2.73)	**.024**	38	1.33	(0.85–2.06)	.207	14	1.66	(0.89–3.07)	.110
HIV-related factors																	
CD4 (cells/mm3)	>200[b]	22	1.00	-	-	23	1.00	-	-	33	1.00	-	-	12	1.00	-	-
	≤200	19	0.83	(0.43–1.63)	.595	49	2.96	(1.71–5.14)	**<.001**	53	2.34	(1.37–4.02)	**.002**	14	1.08	(0.50–2.34)	.836
CD8 (cells/mm3)	>400[b]	23	1.00	-	-	27	1.00	-	-	36	1.00	-	-	14	1.00	-	-
	≤400	12	0.47	(0.18–1.24)	.127	36	1.42	(0.72–2.81)	.313	43	1.36	(0.71–2.62)	.356	5	0.28	(0.06–1.20)	.087
HIV load (copies/ml)	<400[b]	20	1.00	-	-	23	1.00	-	-	32	1.00	-	-	10	1.00	-	-
	>400	24	1.26	(0.75–2.10)	.385	35	1.96	(1.21–3.16)	**.006**	42	1.70	(1.09–2.65)	**.019**	16	1.57	(0.84–2.96)	.160
HIV duration (yr)	≤15[b]	21	1.00	-	-	25	1.00	-	-	34	1.00	-	-	12	1.00	-	-
	>15	21	0.88	(0.44–1.74)	.714	40	2.47	(1.35–4.50)	**.003**	46	1.61	(0.91–2.83)	.101	15	1.64	(0.72–3.73)	.238
Coinfections																	
Syphilis	N[b]	20	1.00	-	-	27	1.00	-	-	34	1.00	-	-	12	1.00	-	-
	P	37	2.37	(1.18–4.75)	**.015**	39	1.89	(0.96–3.73)	.067	56	2.48	(1.28–4.79)	**.007**	20	1.98	(0.85–4.65)	.116
Gonorrhea	N[b]	21	1.00	-	-	28	1.00	-	-	36	1.00	-	-	13	1.00	-	-
	P	24	1.15	(0.47–2.82)	.761	31	1.30	(0.56–2.98)	.539	41	1.29	(0.59–2.80)	.527	14	1.17	(0.38–3.58)	.781
HPV	N[b]	22	1.00	-	-	29	1.00	-	-	38	1.00	-	-	13	1.00	-	-
	P	16	0.66	(0.19–2.34)	.520	16	0.48	(0.14–1.69)	.252	16	0.31	(0.09–1.10)	.069	16	1.29	(0.36–4.65)	.701
Chlamydia	N[b]	21	1.00	-	-	29	1.00	-	-	37	1.00	-	-	13	1.00	-	-

		LANA+				*ORF65+*				*ANY+*				*BOTH+*			
		%	**OR**	**95% CI**	***P***	**%**	**OR**	**95% CI**	***P***	**%**	**OR**	**95% CI**	***P***	**%**	**OR**	**95% CI**	***P***
HSV2	P	23	1.05	(0.37–2.99)	.921	18	0.56	(0.18–1.73)	.316	27	0.66	(0.25–1.75)	.404	14	0.99	(0.28–3.54)	.986
	N[b]	21	1.00	-	-	28	1.00	-	-	37	1.00	-	-	13	1.00	-	-
Hepatitis	P	24	1.41	(0.63–3.17)	.408	24	1.19	(0.54–2.60)	.669	36	1.14	(0.55–2.38)	.723	12	1.71	(0.66–4.43)	.269
	N[b]	20	1.00	-	-	26	1.00	-	-	33	1.00	-	-	13	1.00	-	-
	P	25	1.30	(0.72–2.32)	.383	34	1.49	(0.87–2.54)	.144	47	1.76	(1.07–2.90)	**.027**	11	0.92	(0.43–1.99)	.840

[a] Analyses for HIV-related factors and coinfections were adjusted for age and ethnicity.

[b] Reference category.

Abbreviations: OR, odds ratio; CI, confidence interval; yr, year; Non-H, Non-Hispanics; H, Hispanics; Y, years; N, negative; P, positive; HPV, human papilloma virus; HSV2, herpes simplex virus 2.

TABLE 2

Multivariable Logistic Regression Analysis Assessing the Effects of HIV-Related Factors on the Detection Rates of KSHV Latent and Lytic Antibodies in KSHV-Infected Patients[a]

		LANA+				*ORF65+*			
		%	**OR**	**95% CI**	***P***	**%**	**OR**	**95% CI**	***P***
CD4 (cells/mm3)	>200[b]	67	1.00	-	-	71	1.00	-	-
	≤200	35	0.26	(0.11–0.61)	**.002**	92	3.41	(0.93–12.45)	.064
CD8 (cells/mm3)	>400[b]	64	1.00	-	-	75	1.00	-	-
	≤400	28	0.22	(0.07–0.67)	**.007**	83	1.29	(0.34–4.98)	.707
HIV load (copies/ml)	<400[b]	62	1.00	-	-	70	1.00	-	-
	>400	56	0.75	(0.37–1.49)	.411	82	1.91	(0.82–4.47)	.135
HIV duration (yr)	≤15[b]	62	1.00	-	-	73	1.00	-	-
	>15	45	0.42	(0.18–1.02)	.057	87	5.28	(1.50–18.59)	**.010**

[a] Analyses were adjusted for age and ethnicity.

[b] Reference category.

Abbreviations: OR, odds ratio; CI, confidence interval; yr, year.

TABLE 3

Mean ORF65 Antibody Levels and Risk Factors for Higher ORF65 Antibody Levels in KSHV-Infected Patients[a]

		Freq.	mOD	admOD	95% CI	*P*
Demographics						
Gender	Female[b]	7	0.280	-	-	-
	Male	133	0.304	0.084	(−0.16–0.24)	.431
Age (yr)	≤35[b]	39	0.338	-	-	-
	35–45	65	0.276	0.070	(−0.02–0.14)	.121
	>45	36	0.316	0.026	(−0.09–0.12)	.634
Ethnicity	Non-H[b]	80	0.269	-	-	-
	H	60	0.352	0.111	(0.03–0.18)	**.012**
HIV-related factors						
CD4 (cells/mm3)	>200[b]	103	0.278	-	-	-
	≤200	37	0.382	0.105	(−0.01–0.19)	.063
CD8 (cells/mm3)	>400[b]	121	0.295	-	-	-
	<400	18	0.330	0.034	(−0.13–0.16)	.640
HIV load (copies/ml)	≤400[b]	71	0.285	-	-	-
	<400	68	0.321	0.056	(−0.04–0.14)	.245
HIV duration (yr)	≤15[b]	108	0.282	-	-	-
	>15	31	0.387	0.324	(0.16–0.46)	**.001**
Other coinfections						
Syphilis	N[b]	117	0.310	-	-	-
	P	23	0.268	0.038	(−0.08–0.19)	.553
Gonorrhea	N[b]	128	0.302	-	-	-
	P	12	0.307	0.061	(−0.15–0.21)	.524
HPV	N[b]	137	0.302	-	-	-
	P	3	0.320	0.021	(−0.22–0.50)	.901
Chlamydia	N[b]	134	0.301	-	-	-
	P	6	0.335	0.085	(−0.22–0.27)	.514

		Freq.	mOD	admOD	95% CI	*P*
HSV2	N[b]	127	0.299	-	-	-
	P	13	0.333	0.078	(−0.12–0.22)	.390
Hepatitis	N[b]	98	0.291	-	-	-
	P	42	0.331	0.083	(−0.04–0.18)	.168

[a] Analyses for HIV-related factors and coinfections were adjusted for age and ethnicity.

[b] Reference category.

Abbreviations: mOD, mean optical density; admOD, adjusted difference mean O.D.; CI, confidence interval; Non-H, Non-Hispanics; H, Hispanics; yr, year; N, negative; P, positive; HPV, human papilloma virus; HSV2, herpes simplex virus 2.