Increasing Kaposi's sarcoma-associated herpesvirus seroprevalence with age in a highly Kaposi's sarcoma endemic region, Zambia in 1985

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Background: Kaposi's sarcoma (KS)-associated herpesvirus (KSHV) is a newly discovered virus found in all forms of KS. In the United States, KSHV infection appears to be most common amongst individuals at high-risk for KS. Preliminary data from Africa suggest that KSHV infection may be much more common in the general population.

Objective: To examine the KSHV seroprevalence and age-specific patterns of infection in an African country with high rates of KS.

Design: Cross-sectional seroprevalence study.

Methods: Sera were taken for a hospital-based HIV seroprevalence study conducted in August 1985 in Lusaka, Zambia at a time when HIV was just becoming epidemic in this area. A total of 251 sera were randomly sampled and examined for antibodies against latent and lytic antigens to KSHV. KSHV seroprevalence was compared with demographic and clinical variables using χ^2 test for linear trend and odds ratios and 95% confidence intervals.

Results: Overall, 58% of persons aged 14–84 years were KSHV-seropositive. KSHV seroprevalence increased linearly with age ($P = 0.04$) and was inversely related to years of education ($P = 0.015$). In contrast, HIV infection peaked in those aged 20–29 years and was positively related to years of education ($P = 0.015$). No association between KSHV and gender, marital status, or HIV serostatus was seen.

Conclusions: KSHV infection was significantly more common in this region of Zambia in 1985 than it currently is in the United States. Our data are consistent with KSHV being well-established in this region prior to 1985 and that continued adult transmission of the virus was occurring. The high seroprevalence in the adolescent age-group and the relatively linear increase in prevalence with age suggest that non-sexual modes of transmission may be important for KSHV transmission in Africa.

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Introduction

Since the initial discovery of Kaposi's sarcoma (KS)-associated herpesvirus (KSHV) in 1994 [1], KSHV DNA has been identified in 95% of all KS tissues examined, but is nearly always absent from control tissues from patients without KS or AIDS [2]. Although such tissue-based studies provide critical evidence

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linking KSHV to KS, they leave many epidemiologic questions unanswered. Serologic studies permit detection of KSHV antibodies presumed to identify past and present infections and are particularly suitable to study the natural history of KSHV infection, notably the extension of spread and risk factor profiles for transmission.

Current assays to detect antibodies to lytic and latent KSHV-related antigens are approximately 80–90% sensitive [3–6]. In North America the seroprevalence appears to be very low in blood donors (0–1%), moderate in HIV-infected homosexual and bisexual men (30–35%), and high in KS patients with AIDS (82–88%) [4,5]. In contrast, in Milan, Italy where KS is endemic, blood donors have a 4% seropositivity rate [4]. Preliminary data suggest that even higher rates of KSHV infection are present in Central Africa [4,6,7]. The high KSHV seroprevalence rates in Africa suggest that either the virus has been present in Africa longer than in North America and thus has had more time to establish itself, or, if it is new, that some cofactor present in Africa dramatically increases KSHV transmission efficiency. A traditional method used to examine whether an infectious agent is endemic or epidemic is to examine the age-specific seroprevalence. A linear increase with age is suggestive of continuous or steady-state transmission in a population, which is characteristic of an endemic pathogen [8].

To further characterize the pattern of KSHV infection in African populations, we conducted a seroprevalence study in a hospital-based population in Lusaka, Zambia using serum taken in 1985. Zambia, like several other equatorial African countries, has a high incidence of endemic KS. With the advent of the HIV epidemic the KS rate has dramatically increased [9–11].

Materials and methods

Study design and patients

We conducted a retrospective, cross-sectional seroprevalence study on a sample of 251 African subjects. A randomly selected subset of sera were chosen from 1078 serum samples taken during a hospital-based HIV seroprevalence study conducted in August 1985 at the University Teaching Hospital, Lusaka, Zambia [12]. General demographic data including sex, age, and education were collected at the time of blood draw. Educational data were not available for the medical staff, although it is likely that this group had obtained a higher educational level on average than the patient population. Sera had remained frozen at −80°C.

Serologic assays

Two serologic assays were used to detect latent and lytic antibodies to KSHV. An immunofluorescence assay performed on whole BCP-1 cells [4] was used to detect antibodies against the latency-associated nuclear antigen encoded by open reading frame (ORF) 73 [13]. Sera were diluted 1 : 160, fluorescein isothiocyanate-labeled goat anti-human IgG (Molecular Probes, Eugene, Oregon, USA) was diluted 1 : 100, and IgM (Sigma, St Louis, Missouri, USA) was diluted at 1 : 50, as previously reported [4]. A Western blot assay was used to detect antibodies to the ORF 65.2 recombinant lytic antigen. The KSHV truncated ORF 65.2 was expressed in bacteria, purified using the Xpress System Protein Purification (Invitrogen Corporation, San Diego, California, USA) and immunoblotted as previously described [6]. Because we did not know the true specificity of these tests in African populations, we used a conservative definition of seropositivity requiring a positive result on both assays. Subjects with an indeterminate result on either or both assays were excluded from the analysis. All sera were tested in a blinded fashion.

Statistical analysis

The χ^2 test for linear trend was computed to examine the association of age and education, independently, with KSHV seroprevalence using EpiInfo version 6.02 (Centers for Disease Control and Prevention, Stone Mountain, Georgia, USA). Odds ratios and 95% confidence intervals were computed for univariate analyses examining the association between KSHV seropositivity and various demographic and clinical variables. Mean age was compared using the t-test. To examine the effect of KSHV and HIV serostatus by clinic, we used analysis of variance. These statistics were computed using SPSS version 6.0 (Chicago, Illinois, USA).

Results

KSHV serology was complete for 211 (84%) of the 251 subjects. Forty patients had indeterminate results on one or both tests and were excluded from the main study. The mean age of the 211 subjects was 31 years (range, 14–84 years), 98 (46.4%) were men, and 39 (18.5%) had antibodies to HIV. The 40 excluded patients were slightly younger (mean age, 26 years; $P = 0.03$) but did not differ significantly in gender [odds ratio (OR), 0.87; $P = 0.68$] or HIV seropositivity (OR, 0.78; $P = 0.60$). There was no significant difference in the proportion of staff and patients excluded from the analysis (OR, 1.99; $P = 0.11$).

Overall, 122 (57.8%) subjects were seropositive for KSHV on both assays. Hospital staff and patients were equally likely to be KSHV-seropositive [OR, 1.28; 95% confidence interval (CI), 0.56–2.94] and did not differ by age (mean age, 27 versus 32 years; $P = 0.08$).

Table 1. Kaposi's sarcoma-associated herpesvirus (KSHV) and HIV seroprevalence and mean age of study subjects by hospital group.

Hospital population	Total (n)	n (%)		Mean (range) age (years)
		KSHV-seropositive	HIV-seropositive	
Skin clinic*	18	13 (72.2)	7 (38.9)	28 (18–44)
Sexually transmitted disease clinic	40	18 (45.0)	12 (30.0)	25 (15–47)
Medical ward	62	31 (50.0)	8 (12.9)	37 (15–84)
Orthopedics/surgery	33	23 (69.7)	4 (12.1)	39 (14–65)
Antenatal clinic	31	20 (64.5)	2 (6.5)	27 (16–42)
Total patients	184	105 (57.1)	33 (17.9)	32 (14–84)
Medical staff	27	17 (63.0)	6 (22.2)	27 (19–42)
Total patients and staff	211	122 (57.8)	39 (18.5)	31 (14–84)

*Includes three KS patients who were KSHV-seropositive.

KSHV seroprevalence did not vary significantly ($P = 0.09$) between patient populations but ranged from a low of 45% in the sexually transmitted disease clinic to a high of 72% in the skin clinic (Table 1). Three of the patients from the skin clinic had KS and all three were KSHV-seropositive, contributing to the high KSHV seroprevalence in this group. The seroprevalence among doctors and nurses was 63%. HIV serostatus differed significantly between hospital clinics ($P = 0.007$); the skin clinic had the highest seroprevalence (39%) and the antenatal clinic had the lowest seroprevalence (7%; Table 1). At the time of sampling, 22% of the hospital staff were HIV-seropositive.

KSHV seroprevalence increased linearly with age ($P = 0.04$) rising from 47% in those aged 14–19 years to 71% amongst those persons aged 50 years and older (Fig. 1). A similar age-specific increase in KSHV infection was observed in HIV-negative individuals ($P = 0.04$; data not shown). In contrast, HIV seroprevalence peaked in the 20–29-year age-group, consistent with the well-documented cohort effect due to HIV transmission among young adults in the early 1980s [14]. HIV infection was entirely absent in persons aged 50 years and older.

Amongst the patients, education was significantly inversely associated with KSHV seroprevalence ($P = 0.015$), although those with more education were more likely to be HIV-seropositive ($P = 0.015$; Fig. 2). No associations were found for KSHV seropositivity and being female (OR, 0.90; 95% CI, 0.52–1.56), having ever been married (OR, 1.50; 95% CI, 0.83–2.70), or HIV seropositivity (OR, 0.72; 95% CI, 0.36–1.45).

Discussion

Whereas KS is a rare neoplasm in developed countries, Zambia has historically had some of the highest incidence rates of KS in the world. KS rates dramatically increased with the AIDS epidemic in the early 1980s [9]. For example, KS incidence rates nearly quadrupled

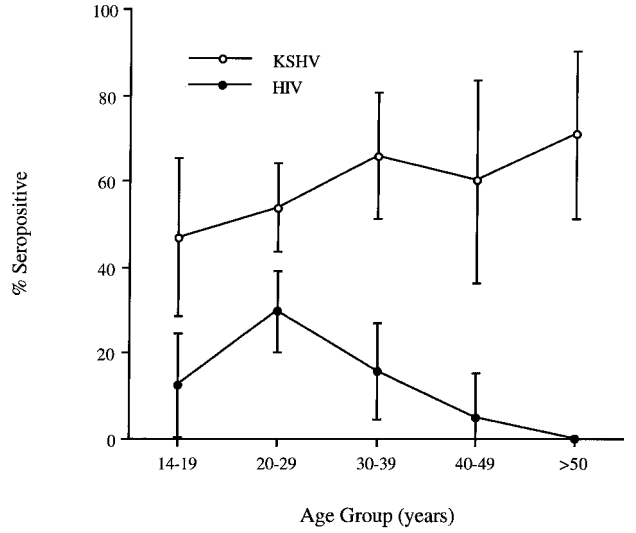

Fig. 1. Kaposi's sarcoma-associated herpesvirus (KSHV) and HIV seroprevalence and 95% confidence intervals by age-group.

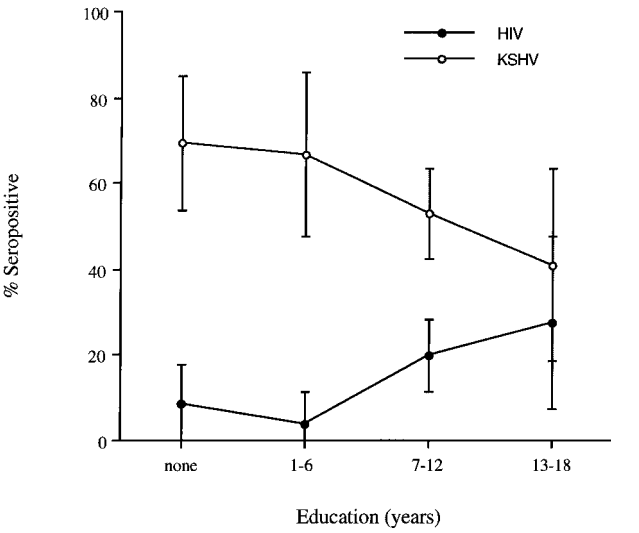

Fig. 2. Kaposi's sarcoma-associated herpesvirus (KSHV) and HIV seroprevalence and 95% confidence intervals among the patients by years of education.

in Zambian children and nearly doubled in Zambian adults between 1983–1984 and 1989 [10,11].

Fifty eight per cent of our subjects were KSHV-seropositive in 1985 on both latent and lytic antigen assays, suggesting that the KSHV infection is hyperendemic in this part of equatorial Africa. These findings are in agreement with a previous report of high KSHV seroprevalence in a group of HIV-positive and negative Ugandan cancer patients without KS (> 50%) [4]. Sera used in this study were drawn in 1985 when the HIV epidemic was newly appearing in Zambian populations and thus our estimates may be an indicator of the pre-AIDS KSHV seroprevalence rate in this population.

Our findings demonstrate marked differences in the epidemiology of HIV and KSHV in Zambia in 1985. HIV peaked in persons aged 20–29 years and was entirely absent in persons aged over 50 years, consistent with a cohort effect due to the recent introduction of HIV into the sexually active population and its eventual spread to the wider population [12]. KSHV infection, however, was high in adolescents (47% in those aged 14–19 years) and increased linearly with age among the study subjects. The high KSHV seroprevalence in adolescents (persons just entering their sexual debut) is consistent with non-sexual transmission being prominent. The linear increase with age demonstrates continued transmission during adulthood, which may in part be due to sexual transmission. In contrast to the marked cohort effect of HIV, the linear increase with age is also consistent with environmental or other sources of transmission. Our data therefore suggest multiple modes of transmission for this virus. In contrast to HIV, the relatively constant increase in KSHV seroprevalence with age (0.34% per year), suggests that a steady-state of transmission has been achieved in this population. Antibodies to KSHV are long-lived [4], and thus Fig. 1 probably reflects the cumulative prevalence of KSHV infection in this population.

KSHV was more common in less educated populations, in contrast with HIV seroprevalence, which increased with education amongst the patient population [12]. In the early African AIDS epidemic, more highly educated individuals (a potential surrogate for socioeconomic status) were at a greater risk of exposure to HIV, possibly through increased travel. Thus, risk factors for acquisition of KSHV and HIV are likely to differ in this setting. The inverse association between KSHV and education is similar to that seen for other herpesvirus infections [e.g., Epstein–Barr virus (EBV)], which are related to socioeconomic status in that more crowded living conditions and less hygienic conditions may enhance transmission. We note, however, that KSHV seroprevalence was high amongst the hospital staff (63%), despite their presumably higher educational level than the patient population. KSHV seroprevalence was similar in men and women. This is interesting because prior to AIDS, men were approximately 10 times more likely than women to have endemic KS in equatorial Africa [15]. Similar KSHV rates and dissimilar KS rates among men and women perhaps point toward sex-specific risk factors (e.g., male hormones) that may be necessary for disease progression. However, neither being single nor HIV-seropositive was related to increased risk of KSHV seropositivity. High rates of KSHV infection have also recently been found in Gambia, a West African country with much lower rates of KS [16].

Risk factor studies of homosexual men with AIDS suggest that KSHV in developed countries is primarily transmitted though sexual practices [5,17]. It has been hypothesized that KSHV could be transmitted through oral contact as with EBV. KSHV DNA has been isolated from both saliva and nasal secretions of persons with KS [18,19], although transmission by this route has not been documented. A recent PCR-based study using peripheral blood from Zambian infants presenting at the same hospital with a first-time fever found that four (8%) out of 53 HIV-negative infants without KS were KSHV-positive [20]. These results suggests that KSHV can be acquired very early in life and most likely point to non-sexual modes of transmission, although this requires confirmation using techniques less prone to technical artefact. The epidemiology of KSHV may have parallels with that of hepatitis B virus (HBV) which has multiple modes of transmission. In North America and western Europe, HBV is primarily transmitted through percutaneous or intimate sexual contact. In contrast, HBV appears to be primarily transmitted by non-sexual modes is Africa (e.g., perinatal and sib to sib) [21]. However, the precise mode of transmission of HBV among children and adolescents remains unknown.

Our study provides additional insight into the epidemiologic pattern of hyperendemic KSHV infection in an African country. This serosurvey shows that in contrast to HIV in Zambia, KSHV was likely to have non-sexual modes of transmission and had achieved a steady-state by 1985.

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