

Kaposi's Sarcoma–Associated Herpesvirus and Kaposi's Sarcoma in Africa

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Background: Endemic Kaposi's sarcoma (KS) is a clinically and epidemiologically distinct human immunodeficiency virus negative form of KS occurring in Africa. Kaposi's sarcoma is now the most frequently reported cancer in some areas of Africa.

Objective: To determine if a KS-associated herpesvirus (KSHV) is present in both endemic HIV-seronegative and HIV-seropositive KS lesions from African patients.

Methods: Paraffin-embedded tissue specimens from Ugandan patients with KS and non-KS tumor control patients attending a university-based oncology clinic were examined in a blinded case-control study. Tissue DNA specimens were examined for detectable KSHV genome by nested polymerase chain reaction performed at two independent laboratories.

Results: We identified KSHV in 17 (85%) of 20 KS tissue specimens from HIV-seronegative patients and 22 (92%) of 24 KS tissue specimens from HIV-infected persons. Kaposi's sarcoma lesions from four HIV-infected persons and four HIV-seronegative persons were positive for KSHV. Unlike previous studies in North America and Europe, three (14%) of 22 non-KS cancer control patients' tissue specimens were also positive for KSHV that resulted in an overall odds ratio of 49.2 (95% confidence interval, 9.1 to 335) for detecting KSHV in KS lesions from patients in Uganda.

Conclusion: As in North America and Europe, KSHV infection is strongly associated with both HIV-seropositive and HIV-seronegative KS in Africa. However, it is likely that infection with this virus is more highly prevalent in Uganda.

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K APOSI'S SARCOMA (KS) is a spindle cell, vascular tumor that occurs in skin, lymphoid, respiratory, and gastrointestinal tract tissues. It is a common tumor in both human immunodeficiency virus (HIV)–seropositive and HIV-seronegative persons in parts of Africa.^{1,2} This represents approximately half of all the tumors reported to the Kampala Cancer Registry in 1989 through 1991 in Kampala, Uganda.³ The HIV seronegative form of African KS is called endemic KS and is pathologically indistinguishable from other clinical forms of KS. Patients with endemic KS do not have immunodeficiency as measured by CD4⁺/CD8⁺ ratios, antigen skin tests, and other measures of immunologic status.⁴ Endemic KS is generally an indolent tumor in HIV-seronegative adults, but it also occurs in children where it has a fulminant and often fatal course.²

Herpesvirus-like DNA sequences have been found in both acquired immunodeficiency syndrome (AIDS)–associated and

HIV-seronegative classical KS tissue specimens.⁵⁻⁸ Isolation and characterization studies have confirmed that these DNA sequences belong to a new human herpesvirus, tentatively termed KS-associated herpesvirus (KSHV).⁹ To determine if this virus is also present in both endemic and HIV-associated KS lesions from African patients, formalin-fixed, paraffin-embedded tissue specimens from both HIV-seropositive and HIV-seronegative Ugandan patients with KS were compared with cancer tissue specimens from patients without KS in a blinded case-control study.

RESULTS

Of 66 tissue specimens examined, 24 were from AIDS-KS cases, 20 were from endemic HIV-seronegative KS cases, and 22

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METHODS

PATIENT ENROLLMENT

Archival KS biopsy specimens were selected from approximately equal numbers of HIV-associated and endemic HIV-negative patients with KS enrolled in an ongoing case-control study of cancer and HIV infection at Makerere University in Kampala. Control tissues were consecutive archival biopsy specimens from patients with various malignancies enrolled in the same study, chosen without prior knowledge of HIV serostatus. All patients were tested for HIV antibody (measured by Cambridge Bioscience Recombigen enzyme-linked immunosorbent assay, Cambridge, England).

TISSUE PREPARATION

Each sample examined was from an individual patient. Approximately 10 tissue sections (10 μ m each) were cut from each paraffin block using a cleaned knife blade for each specimen. Tissue sections were deparaffinized by extracting the sections twice with 1 mL of xylene for 15 minutes followed by two extractions with 100% ethanol for 15 minutes. The remaining pellet was then resuspended and incubated overnight at 50°C in 0.5 mL of lysis buffer (25-mmol/L potassium chloride, 10-mmol/L Tris-HCl [pH 8.3], 1.4-mmol/L magnesium chloride, 0.01% gelatin, and 1-mg/mL proteinase K). DNA was extracted with phenol chloroform and ethanol, precipitated, and resuspended in 10-mmol/L Tris-HCl and 0.1-mmol/L edetic acid at a pH of 8.3.

POLYMERASE CHAIN REACTION AMPLIFICATION

DNA (0.2 to 0.4 μ g) was used in polymerase chain reactions (PCRs) with KS330₂₃₃ primers as previously described.⁷ The samples that tested negative were retested by nested PCR amplification, which is approximately 10²- to 10³-fold more sensitive in detecting the KS330₂₃₃ sequence than the previously published KS330₂₃₃ primer set.⁷ These samples were tested twice and samples showing discordant results were retested a third time. Fifty-one of 74 samples initially examined were available for independent extraction and testing at Chester Beatty Laboratories in London, England, using identical nested PCR primers and conditions to ensure fidelity of the PCR results. Results from eight samples were discordant between laboratories and were removed from the analysis as uninterpretable (four positive samples from each laboratory). Statistical comparisons were made using EPI-INFO version 5 (USD, Stone Mountain, Ga) with exact confidence intervals.

were from control patients with cancer without KS. Seven of the control patients with cancer were HIV seropositive and 15 were HIV seronegative (**Table**). Tumors examined in the control group included carcinomas of the breast, ovaries, rectum, stomach, and colon; fibrosarcoma; lymphocytic lymphomas; Hodgkin's lymphomas; choriocarcinoma; and anaplastic carcinoma of un-

Polymerase Chain Reaction Analysis of KS330₂₃₃ in DNA Samples From Patients With Kaposi's Sarcoma and Tumor Controls*

	No. Tested	KS KS330 ₂₃₃ Positive (%)
KS tissue specimens		
AIDS-KS	24	22 (92)
Endemic KS	20	17 (85)
Total	44	39 (89)
Control tumors		
HIV seropositive	7	1 (14)
HIV seronegative	15	2 (13)
Total	22	3 (14)

*KS indicates Kaposi's sarcoma; AIDS, acquired immunodeficiency syndrome; and HIV, human immunodeficiency virus.

known primary site. The median age of the patients with AIDS-KS was 29 years (age range, 3 to 50 years) compared with 36 years (age range, 3 to 79 years) for patients with endemic KS and 38 years (age range, 21 to 73 years) for control patients with cancer.

Among KS lesions, 39 (89%) of 44 were positive for KS330₂₃₃ PCR product that included KS tissue specimens from 22 (92%) of 24 HIV-seropositive patients and 17 (85%) of 20 HIV-seronegative patients. In comparison, three (14%) of 22 non-KS cancer control tissue specimens were positive that included specimens from one (14%) of seven HIV-seropositive and two (13%) of 15 HIV-seronegative control patients (Table). The control patients included a 73-year-old HIV-seronegative man and a 29-year-old HIV-seronegative woman with breast carcinomas, and a 36-year-old HIV-seropositive woman with ovarian carcinoma. The odds ratios for detecting the sequences in tissue specimens from HIV-seropositive and HIV-seronegative cases and controls was 66 (95% confidence interval, 3.8 to 3161) and 36.8 (95% confidence interval, 4.3 to 428), respectively. The overall weighted Mantel-Haenszel odds ratio stratified by HIV serostatus was 49.2 (95% confidence interval, 9.1 to 335). The KS tissue specimens from four HIV-seropositive children (aged 3, 5, 6, and 7 years) and four HIV-seronegative children (aged 3, 4, 4, and 12 years) were all positive for KS330₂₃₃.

All discordant results (ie, KSHV-negative KS or KSHV-positive non-KS cancers) were reviewed microscopically. All KS330₂₃₃ PCR-negative KS samples were confirmed to be KS. Likewise, histopathologically, all KS330₂₃₃ PCR-positive non-KS cancers were found not to have occult KS.

COMMENT

Our results indicate that KSHV DNA sequences are found not only in AIDS-KS,³ classical KS,⁶ and transplant KS⁷ but also in African KS from both HIV-seropositive and HIV-seronegative patients. Despite differences in clinical and epidemiological features, KSHV DNA sequences are present in all major clinical subtypes of KS from widely dispersed geographic settings.

Our study was performed on banked, formalin-

fixed tissue specimens that prevented the use of specific detection assays such as Southern hybridization. DNA extracted after such treatment is often fragmented, which reduces the detection sensitivity of PCR and may account for the five PCR-negative KS samples found in our study. Our results, however, are unlikely to be due to PCR contamination or nonspecific amplification. Specimens were tested blindly and a subset of samples were independently extracted and tested at a physically separate laboratory. Specimen blinding is essential to ensure the integrity of results based solely on PCR analyses. A subset of amplicons was sequenced and found to be more than 98% identical to the published KS330₂₃₃ sequence, thus confirming their specific nature and, because of minor sequence variation, making the possibility of contamination unlikely (data not shown).

In contrast to previous studies in North American and European populations, three of our 22 control tissue specimens showed evidence of KSHV infection. Since these cancers represent a variety of tissue types, it is unlikely that KSHV has a causative role in these tumors. One possible explanation for our findings is that these results reflect the rate of KSHV infection in the non-KS population in Uganda. Four independent controlled studies from North America,^{5,10} Europe,⁷ and Asia⁸ have failed to detect evidence of KSHV infection in more than 200 cancer control tissue specimens, with the exception of an unusual AIDS-associated, body cavity-based lymphoma.¹⁰ Taken together, these studies indicate that DNA-based detection of KSHV infection is rare in most non-KS cancer tissue specimens from developed countries. The KSHV infection has been reported in posttransplantation skin tumors, although well-controlled studies are needed to confirm these findings.¹¹ Since the rate of HIV-negative KS is much more frequent in Uganda than in the United States, detection of KSHV in control tissue specimens from patients with cancer in our study may reflect a relatively high prevalence of infection in the general Ugandan population.

While KS is extremely rare among children in developed countries,² the rate of KS in Ugandan children has risen dramatically over the past three decades: age-standardized rates (per 100 000) for boys aged 0 to 14 years were 0.25 in 1964 through 1968 and 10.1 in 1992 and 1993 (J.Z. and E.K.-M., unpublished data). Detection of KSHV genome in KS lesions from prepubertal children suggests that the virus has a nonsexual mode of transmission among Ugandan children. The fact that five of these children were 5 years old or younger raises the possibility that the agent can be transmitted perinatally. Whether or not immune tolerance due to perinatal transmission accounts for the more fulminant form of KS occurring in African children remains to be investigated. A similar study has demonstrated KSHV sequences in endemic KS lesions from patients from Tanzania.¹²

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REFERENCES

1. Oettle AG. *Symposium on Kaposi's Sarcoma*. Basel, Switzerland: Karger; 1962; 18:330-363.
2. Beral V. Epidemiology of Kaposi's sarcoma. In: *Cancer, HIV and AIDS*. London, England: Imperial Cancer Research Fund; 1991:5-22.
3. Wabinga HR, Parkin DM, Wabwire-Mangen F, Mugerwa J. Cancer in Kampala, Uganda, in 1989-91: changes in incidence in the era of AIDS. *Int J Cancer*. 1993;54:26-36.
4. Kestens L, Melbye M, Biggar RJ, et al. Endemic Kaposi's sarcoma is not associated with immunodeficiency. *Int J Cancer*. 1985;36:49-54.
5. Chang Y, Cesarman E, Pessin M, et al. Identification of herpesvirus-like DNA sequences in AIDS-associated Kaposi's sarcoma. *Science*. 1994;266:1865-1869.
6. Moore PS, Chang Y. Detection of herpesvirus-like DNA sequences in Kaposi's sarcoma lesions from persons with and without HIV infection. *N Engl J Med*. 1995;332:1181-1185.
7. Boshoff C, Whitby D, Hatzioannou T. Kaposi's sarcoma-associated herpesvirus in HIV negative Kaposi's sarcoma. *Lancet*. 1995;345:1043-1044.
8. Su I-J, Hsu Y-S, Chang Y-C, Wang I-W. Herpesvirus-like DNA sequence in Kaposi's sarcoma from AIDS and non-AIDS patients in Taiwan. *Lancet*. 1995; 345:722-723.
9. Moore PS, Gao S-J, Dominguez G, et al. Primary characterization of a herpesvirus-like agent associated with Kaposi's sarcoma. *J Virol*. In press.
10. Cesarman E, Chang Y, Moore PS, Said JW, Knowles DM. Kaposi's sarcoma-associated herpesvirus-like DNA sequences are present in AIDS-related body cavity based lymphomas. *N Engl J Med*. 1995;332:1186-1191.
11. Rady PL, Yen A, Rollefson JL. Herpesvirus-like DNA sequences in non-Kaposi's sarcoma skin lesions of transplant patients. *Lancet*. 1995;345:1339-1340.
12. Schalling M, Ekman M, Kaaya EE, Linde A, Bieberfeld P. A role of a new herpesvirus (KSHV) in different forms of Kaposi's sarcoma. *Nature Med*. 1995; 1:707-708.