Prevalence of infection with human herpesvirus 8/Kaposi's sarcoma herpesvirus in rural South Africa

David Wilkinson, Julie Sheldon, Charles F Gilks, Thomas F Schulz

Objective. To determine prevalence of infection with human herpesvirus 8 (HHV-8)/Kaposi's sarcoma herpesvirus (KSHV) and to gain some insight into possible transmission dynamics of this novel virus in South Africa.

Methods. Stored, anonymous serum from 50 patients with a sexually transmitted disease (STD), 50 adult medical ward patients (25 male, 25 female), and 36 paediatric ward patients in Hlabisa Hospital, KwaZulu-Natal, was screened by enzyme-linked immunosorbent assay (ELISA) for antibodies to the small capsid-related protein encoded by HHV-8/KSHV orf65. Antibodies to the latency-associated nuclear antigen (LANA) were measured by immunofluorescence, and sera that were reactive in the ELISA but negative by immunofluorescence were re-tested by Western blot against the recombinant orf65 protein to exclude nonspecific reactivity.

Results. Overall, 47 patients tested positive (34.6%), 76 tested negative (55.9%) and 13 (9.5%) had indeterminate results. Among those with a definite result, prevalence was similar among males (47.2%) and females (52.8%) and increased in later adulthood (< 18 months 37.5%, 19 - 120 months 38.5%, 15 - 34 years 32.1%, 35 - 69 years 62.8%). Prevalence was highest among medical patients (58.1%); among those with an STD it was 31.1% ($P = 0.01$), and among children it was 22.8% ($P = 0.001$). When age-adjusted, prevalence among medical patients (23.7%) was similar to that among patients with an STD.

Conclusion. Prevalence of HHV-8/KSHV is high in this setting and transmission appears to be occurring in childhood as well as among adults. Larger population-based studies are required to detail the transmission dynamics of HHSV-8/KSHV.

S Afr Med J 1999; **89:** 554-557.

Centre for Epidemiological Research in Southern Africa, Medical Research Council, Mtubatuba, KwaZulu-Natal

David Wilkinson, BSc, MB ChB, MSc

Molecular Virology Group, Departments of Medical Microbiology and Genitourinary Medicine, University of Liverpool, UK

Julie Sheldon, BSc

Thomas F Schulz, MD

Liverpool School of Tropical Medicine, University of Liverpool, UK

Charles F Gilks, D Phil, FRCP

The new human herpesvirus (HHV-8), or Kaposi's sarcoma-associated herpesvirus (KSHV), is consistently detected in AIDS-related, 'classic', endemic and post-transplant Kaposi's sarcoma (KS).[1-5] The detection of KSHV in peripheral blood, as well as the presence of antibodies to KSHV, are strongly associated with having, or being at risk for, KS.[6-8] KSHV infects the endothelial tumour (KS spindle) cells where it expresses a set of latent genes; it also occasionally undergoes lytic replication.[9-12] Several of the KSHV genes expressed in KS tissue have the potential to affect the control of normal cell proliferation. Taken together, this evidence strongly suggests that KSHV is the infectious cause of KS and a new human tumour virus. On its own the virus rarely leads to the development of KS tumours; however, infection with HIV-1 dramatically increases both the frequency and the clinical severity of KS.

KSHV is not thought to be ubiquitous, and is believed to be mainly sexually transmitted in the USA and northern Europe. Antibodies to KSHV are much more frequent among homosexual HIV-infected men than among HIV-infected patients with haemophilia or intravenous drug users; they are also more common among HIV-uninfected heterosexual STD clinic attendees than in healthy blood donors.[13-18]

Most sero-epidemiological studies have shown that the prevalence of KSHV is low (< 5%) in the general population of Britain and North America, a little higher in Mediterranean populations where 'classic' KS is more often observed, and highest in parts of Africa, where it may reach 50%.[16-19] KS is relatively infrequent in South Africa,[20] but more cases are being diagnosed in association with the rapidly expanding HIV epidemic (personal observations). Although KSHV has been detected in South African KS specimens,[21] its prevalence in South African populations is unknown. In order to investigate the prevalence of KSHV infection in South Africa and to begin to gain some insights into its likely routes of transmission we studied the prevalence of KSHV among patients attending a rural district hospital in KwaZulu-Natal.

Methods

Setting

Hlabisa health district is situated in northern KwaZulu-Natal and is home to around 210 000 Zulu-speaking people. The district is relatively poor and underdeveloped. The HIV epidemic has spread rapidly in KwaZulu-Natal; in Hlabisa HIV prevalence among women attending antenatal clinics increased from 4.2% in 1992[22] to 28.9% in 1998 (A Harrison — unpublished data). Sexually transmitted diseases (STDs) are also highly endemic in the area — we have estimated that around 25% of women of reproductive age have at least one STD on any given day.[23]

Survey

We selected spare serum left over from routine clinical tests done on 136 patients that had been stored and made anonymous. Fifty consecutive adult medical inpatients who were tuberculosis suspects but had no active STD (25 women and 25 men), 50 patients with a proven STD, and 36 paediatric inpatients with a variety of common illnesses (diarrhoeal disease, acute respiratory infection and malnutrition) were chosen. Serum samples had all patient identifiers removed; only age and sex identifiers were retained. Serum was stored frozen at minus 20°C until tested in the Department of Genito-urinary Medicine, University of Liverpool, UK, under a South African Department of Health permit.

Serological methods

As described previously,[16] sera were screened by enzyme-linked immunosorbent assay (ELISA) in a dilution of 1:100 for antibodies to the small capsid-related protein encoded by KSHV orf65, using the average of 10 KSHV seronegative UK blood donors plus 5 standard deviations (SDs) as a cut-off value. As a control antigen we used a purified recombinant dihydrofolate reductase protein, the fusion partner of the recombinant orf65 protein.[16] Antibodies to the 'latency-associated nuclear antigen' (LANA)[13,19] were measured by immunofluorescence on paraformaldehyde fixed B-cell precursor-1 cells, using a serum dilution of 1:150.[16] Sera that were reactive in the ELISA but negative by immunofluorescence (IFA) were re-tested by Western blot against the recombinant orf65 protein to exclude nonspecific reactivity.[16] A positive result was recorded if both ELISA and IFA were positive, if IFA alone was positive, or if samples positive by ELISA but negative by IFA were confirmed positive on Western blot with the recombinant orf65 protein (Fig. 1). Negative samples were negative on both ELISA and IFA, and indeterminate samples were those with a nonspecific immunofluorescence or Western blot pattern.

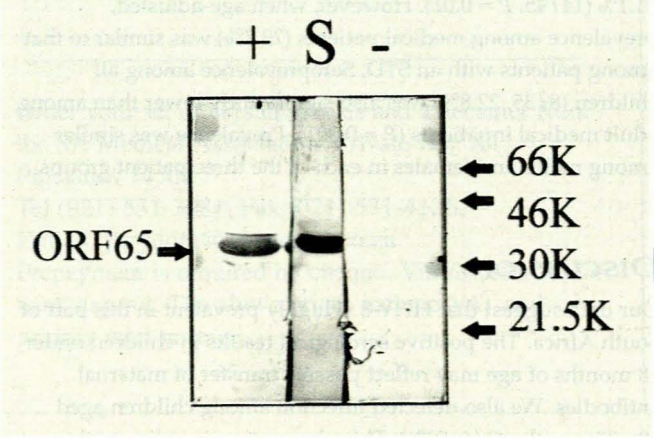

Fig. 1. Western blot examination displaying orf65 protein.

Analysis

Data were entered into an EpiInfo database and analysed with the same software. Proportions were compared with the chi-square test, with $P < 0.05$ defined as the level of statistical significance. Indirect age-standardisation was done for comparison of medical and STD patient groups.

RESULTS

Overall, 47 (34.6%) of the 136 patients tested positive for KSHV, 76 (55.9%) tested negative and 13 (9.5%) had indeterminate results. Among those with a definite result, prevalence was similar among males (47.2%) and females (52.8%). Among adults, prevalence increased with age (Table I), and was significantly lower among young adults aged 15 - 34 years (17/53, 32.1%) than among older adults aged 35 - 69 years (22/35, 62.8%, $P = 0.004$). Among those aged under 18 months, 3 (37.5%) had antibodies to KSHV, but it is possible that some of this reactivity reflected persisting maternal antibodies. One of the 3 children was aged between 12 and 18 months. Among children aged 19 - 20 months 5 of 13 (38.5%) had antibodies.

Table I. Age-specific prevalence rates of infection with human herpesvirus 8/Kaposi's sarcoma herpesvirus among selected patients in Hlabisa, KwaZulu-Natal

	Positive	
Age group	No./total	%
< 18 months	3/8	37.5
19 - 120 months	5/13	38.5
15 - 24 years	10/32	31.3
25 - 34 years	7/21	30.0
35 - 44 years	10/18	55.6
45 - 54 years	3/5	60.0
55 - 69 years	9/12	75.0

Prevalence was highest among medical inpatients (25/43, 58.1%). Among those with an STD it was significantly lower at 31.1% (14/45, $P = 0.01$). However, when age-adjusted, prevalence among medical patients (23.7%) was similar to that among patients with an STD. Seroprevalence among all children (8/35, 22.8%) was also significantly lower than among adult medical inpatients ($P = 0.001$). Prevalence was similar among males and females in each of the three patient groups.

DISCUSSION

Our data suggest that HHV-8 is highly prevalent in this part of South Africa. The positive serological results in children under 18 months of age may reflect passive transfer of maternal antibodies. We also detected infection among children aged 19 - 35 months (3/6, 50%). This observation is in line with a recent report from Uganda, where seroprevalence increased steeply in children over 2 years of age to reach adult levels before puberty.[24] This age-dependent increase suggests that horizontal transmission plays an important role in young children in Africa, but vertical transmission cannot be excluded at present. While the exact mechanism of horizontal transmission remains to be identified, KSHV has been detected in saliva by both polymerase chain reaction and culture,[25,26] suggesting that, as for other herpesviruses, transmission via saliva under conditions of crowding and poor hygiene may play an important role.

Prevalence of infection was similar in males and females in all age groups. We noted a significantly higher seroprevalence in adults over 34 years of age compared with younger adults. In a recent study of Italian blood donors we noted a similar increase in donors older than 55 years. The pattern of age-specific increase in seroprevalence among these donors is more suggestive of a reactivation of KSHV infection at higher age, or of a cohort effect, than of sexual transmission. The small number of sera tested in the present study does not allow us to discriminate between these two possibilities and a more extensive study is required.

When we adjusted the prevalence among adult patients in this study for age, prevalence was found to be similar to that of patients presenting with an STD. The higher crude prevalence in medical patients therefore simply reflects their higher age.

The serum samples that we studied were not randomly selected from the community, but were a convenience sample of patients admitted to or presenting to hospital. As such the prevalence rates reported here may overestimate community prevalence; however they do suggest that KSHV is prevalent in the area and that further study is warranted. It will be important to perform large-scale community-based sero-epidemiological studies to determine age- and sex-specific prevalence and incidence, as well as to determine risk factors for transmission, association with other viruses such as hepatitis B, the association with KS and other malignancies, and the extent to which vertical transmission occurs.

In North America most people dually infected with HIV and KSHV go on to develop KS. In Uganda, KS has become the most common tumour, now accounting for 48% of reported tumours compared with only 2% 20 years ago.[21] If KSHV is as widespread in South Africa as our findings suggest, it is possible that a similar epidemic of HIV-associated KS could also emerge here, providing yet another HIV-related care challenge. Clinicians working in high-prevalence settings have already noted an increase in cases. In addition, therefore, to sero-epidemiological studies of KSHV, sentinel cancer surveillance sites should rapidly be established to monitor the emergence of an epidemic of KS.

We thank the South African Medical Research Council, the Medical Research Council of Great Britain (project G9517856PB),

the RL Gardner Fund, and the NHS Biomedical Research Fund (project: RDO/22/9), the European Concerted Action on the Pathogenesis of Kaposi's Sarcoma (BMH4-97-2301), and the Department for International Development of the UK Government, who all supported this work.

References

1. Chang Y, Cesarman E, Pessin MS, *et al.* Identification of herpesvirus-like DNA sequences in AIDS-associated Kaposi's sarcoma. *Science* 1994; **266:** 1865-1869.
2. Boshoff C, Whitby D, Hatzioannou T, *et al.* Kaposi's sarcoma associated herpesvirus in HIV-negative Kaposi sarcoma. *Lancet* 1995; **345:** 1043-1044.
3. Moore PS, Chang Y. Detection of herpesvirus-like DNA sequences in Kaposi's sarcoma in patients with and without HIV infection. *N Engl J Med* 1995; **332**: 1181-1185.
4. Schalling M, Ekman M, Kaaya EE, Linde A, Biberfeld P. A role for a new herpesvirus (KSHV) in different forms of Kaposi's sarcoma. *Nat Med* 1995; **1:** 707-708.
5. Chang Y, Ziegler J, Wabinga H, *et al.* Kaposi's sarcoma-associated herpesvirus DNA sequences are present in African endemic and AIDS-associated Kaposi's sarcoma. *Arch Intern Med* 1996; **156:** 202-204.
6. Whitby D, Howard MR, Tenant-Flowers M, *et al.* Detection of Kaposi sarcoma-associated herpesvirus (KSHV) in peripheral blood of HIV-infected individuals predicts progression to Kaposi's sarcoma. *Lancet* 1995; **346:** 799-802.
7. Moore PS, Kingsley LA, Holmberg SD, *et al.* KSHV infection prior to onset of Kaposi's sarcoma. *AIDS* 1996; **10:** 175-180.
8. Lefrere JJ, Meyohas MC, Mariotti M, Meynard JL, Thauvin M, Frottier J. Detection of human herpesvirus 8 DNA sequences before the appearance of Kaposi's sarcoma in human immunodeficiency virus (HIV)-positive subjects with a known date of HIV seroconversion. *J Infect Dis* 1996; **174:** 283-287.
9. Boshoff C, Schulz TF, Kennedy MM, *et al.* Kaposi's sarcoma associated herpesvirus infects endothelial and spindle cells. *Nat Med* 1995; **1:** 1274-1278.
10. Staskus KA, Zhong W, Gebhard K, *et al.* Kaposi's sarcoma-associated herpesvirus gene expression in endothelial (spindle) tumour cells. *J Virol* 1977; **71:** 715-719.
11. Sturzl M, Blasig C, Schreier A, *et al.* Expression of HHV-8 latency associated To. 7 RNA in spindle cells and endothelial cells of AIDS-associated, classical and African Kaposi's sarcoma (KS). *Int J Cancer* 1997; **72:** 68-71..
12. Orenstein JM, Alkan S, Blauvelt A, *et al.* Visualization of human herpesvirus type 8 in Kaposi's sarcoma by light and transmission electron microscopy. *AIDS* 1997; **11:** F35-745.
13. Kedes DH, Operskalski E, Busch M, Kohn R, Flood J, Ganem D. The seroprevalence of human herpesvirus 8 (HHV 8): Distribution of infection in Kaposi's sarcoma risk groups and evidence of sexual transmission. *Nat Med* 1996; **2:** 918-924.
14. Kedes DH, Ganem D, Ameli N, Bacchetti P, Greenblatt R. The prevalence of serum antibody to human herpesvirus 8 (Kaposi sarcoma-associated hepesvirus) among HIV-seropositive and high risk HIV-seronegative women. *JAMA* 1997; **277:** 478-481.
15. Gao SJ, Kingsley L, Hoover DR, *et al.* Seroconversion of antibodies to Kaposi's sarcoma-associated herpesvirus-related latent nuclear antigens before the development of Kaposi's sarcoma. *N Engl J Med* 1996; **335:** 233-241.
16. 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.
17. Lennette ET, Blackbourn DJ, Levy JA. Antibodies to human herpesvirus type 8 in the general population and in Kaposi's sarcoma patients. *Lancet* 1996; **348:** 858-861.
18. Melbye M, Cook PM, Hjalgrim H, *et al.* Risk factors for Kaposi's-sarcoma-associated herpesvirus (KSHV / HHV-8) seropositivity in a cohort of homosexual men, 1981 - 1996. *Int J Cancer* 1998; 77: 543-548.
19. Gao SJ, Kingsley L, Li M, *et al.* Seroprevalence of KSHV antibodies among North Americans, Italians, and Ugandans with and without Kaposi's sarcoma. *Nat Med* 1996; **2:** 925-928.
20. National Cancer Registry. *Cancer in South Africa, 1992.* Johannesburg: National Cancer Registry, South African Institute for Medical Research 1997.
21. Sitas F, Taylor L, Madhoo J, Cooper K, Carrara H. Occurrence of human herpes virus 8 in Kaposi's sarcoma and other tumours in South Africa. *S Afr Med J* 1997; **87:** 1020.
22. Coleman RL, Wilkinson D. Increasing HIV prevalence in a rural district of South Africa: 1992 - 1995. *J Acquir Immune Defic Syndr Hum Retrovirol* 1997; **16:** 50-53.
23. Wilkinson D, Abdool Karim SS, Harrison A, *et al.* Unrecognised sexually transmitted infections in rural South African women — the hidden epidemic. *Bull World Health Organ* 1999 (in press).
24. Mayama S, Cuevas L, Sheldon J, *et al.* Prevalence of Kaposi's sarcoma associated herpesvirus (Human herpesvirus 8) in a young Ugandan population. *Int J Cancer* 1998 (in press).
25. Boldough I, Szaniszlo P, Bresnahan WA, Flaitz CM, Nichols MC, Albrecht T. Kaposi's sarcoma herpesvirus-like DNA sequences in the saliva of individuals infected with human immunodeficiency virus. *Clin Infect Dis* 1996; **23:** 406-407.
26. Vieira JD, Hunag L, Koelle DM, Corey L. Transmissible Kaposi's-associated herpesvirus (human herpesvirus 8) in saliva of men with a history of Kaposi's sarcoma. *J Virol* 1997; **71:** 7083-7087.

Accepted 15 Nov 1998.