Human Herpesvirus 8: Seroepidemiology among Women and Detection in the Genital Tract of Seropositive Women

Denise Whitby, Nicola A. Smith, 1 Steve Matthews, Siobhan O'Shea, Caroline A. Sabin, Ranjaban Kulasegaram, Chris Boshoff, Robin A. Weiss, Annemiek de Ruiter, and Jennifer M. Best

An indirect IFA to detect antibodies against latent nuclear antigens of human herpesvirus 8 (HHV-8) was used to determine the prevalence of HHV-8 antibodies in 169 women attending a sexually transmitted diseases clinic and a human immunodeficiency virus (HIV) clinic at a London hospital. Nested polymerase chain reaction was used to detect HHV-8 DNA in 93 blood samples and 89 cervical brush scrapes (CBS). Another 96 CBS from women attending a colposcopy clinic were also analyzed. The overall seroprevalence of HHV-8 was 18.3%. The seroprevalence was higher among women born in Africa (24.7%) than among women born elsewhere (11.5%; \(P = .06\)) and was independent of HIV serostatus. HHV-8 DNA was detected in 3 CBS and 6 peripheral blood samples from 11 HHV-8-seropositive women but not in CBS from 78 seronegative women, 96 women from the colposcopy clinic, or in blood samples from 82 seronegative women.

Human herpesvirus 8 (HHV-8) is strongly associated with Kaposi's sarcoma (KS). Epidemiologic studies of KS in AIDS patients suggest a sexual route of transmission, since KS occurs more frequently in those who acquire human immunodeficiency virus (HIV) sexually than parenterally [1]. Sexual transmission of HHV-8 has been confirmed in homosexual men [2]. Preliminary serologic studies among heterosexuals indicate that HHV-8 antibodies are more common in sera of those attending sexually transmitted diseases (STD) clinics than in blood donors, which suggests a sexual transmission route of infection [3, 4], as does the finding of HHV-8 DNA in the semen of KS patients and HIV-positive men [5, 6]. It is not yet clear if HHV-8 may be transmitted from mothers to infants and from females to males. Since KS occurs in children in Africa, mother-to-child transmission is likely in KS-endemic regions. One serologic study in Haiti, where KS is not endemic, showed no mother-to-child transmission of HHV-8 [7]. We have found serologic evidence for transmission of HHV-8 from mother to child in South Africa [8], but the route of such transmission remains to be elucidated. To date, studies on HHV-8 in the female genital tract have yielded conflicting, mainly negative, data [6, 9].

In this study, we sought to determine the rate of detection of HHV-8 DNA in genital tract samples and the prevalence of HHV-8 infection in women from different KS risk groups attending an STD clinic and an HIV clinic within a genitourinary medicine department in a central London hospital.

Patients and Methods

Study participants and specimens. Seventy-nine HIV-positive and 90 HIV-negative women were recruited from the HIV unit and STD clinic, respectively, at St. Thomas’ Hospital, London, Serum was available from all 169 women, and whole blood for DNA extraction was available from 92 of the 169. Cervical brush scrapes (CBS) were obtained from 32 of the HIV-positive women and 57 of the HIV-negative women using an Axibrush (Colgate Medical, Windsor, UK) after swabs were taken for routine STD screen and cervical cytology as clinically indicated. Clinical and epidemiologic data, including country of birth, were collected at the time samples were obtained. We also analyzed 96 CBS from women attending the colposcopy clinic at the same hospital.

The samples were eluted in 8 mL of PBS and centrifuged at 400 g for 5 min prior to DNA extraction from the pellet. The pellet was resuspended in 150 μL of lysis buffer (10 mM Tris, pH 8, 10 mM EDTA, 5 mM NaCl, 2% SDS, and 10 mg/mL protease K) and incubated for 2 h at 65°C before phenol-chloroform extraction.

IFA and polymerase chain reaction (PCR). HHV-8 antibodies against latent nuclear antigens were detected in serum using indirect immunofluorescence [4, 10]. The negative controls were HIV-positive, HHV-8-negative sera.
HIV-8 DNA was detected using nested PCR as previously described [5, 11]. About 200 ng of sample DNA was tested with two sets of primers from nonoverlapping regions of the HHV-8 genome, and only those positive with both sets were considered positive. Negative controls for the PCR assay of genital tract samples were an unused Axibrush eluted in PBS and sterile water for the blood PCR. A titration of DNA extracted from the primary effusion lymphoma cell line, HBL6, was included in each assay as a positive control. The DNA quality was determined using PCR for ERV-3, a single-copy endogenous retrovirus gene [12]. All negative controls gave negative results.

Data analysis. Women were divided into 4 risk groups: HIV-positive African-born, HIV-positive non–African-born, HIV-negative African-born, and HIV-negative non–African-born. Statistical analyses were performed using the Statistical Analysis System (SAS Institute, Cary, NC) software package. We used the \( \chi^2 \) test or, where numbers were small, Fisher’s exact test to compare the rate of HHV-8 detection in the 4 groups. Confidence intervals (CIs) for detection rates were calculated using the normal approximation to the binomial distribution when numbers were large enough (>10 positive samples) and exact methods when numbers were small.

### Results

**Antibodies to HHV-8.** The overall prevalence of antibodies to HHV-8 was 18.3% (95% CI, 12.5%–24.2%). The detection rates of antibodies to HHV-8 were 23.2%, 17.4%, 25.7%, and 9.1% in the 4 groups, respectively (table 1). Women born in Africa were more likely to have HHV-8 antibodies (22 [24.7%] of 91) than women not born in Africa (9 [11.5%] of 78), although this was of only borderline significance (\( P = .06, \chi^2 \) test). There was no relationship between HIV status and HHV-8 seropositivity among African-born women; however, there was a significant association between HHV-8 serostatus and the region of birth within Africa. Women born in western, eastern, and central Africa had a greater risk of HHV-8 infection (22 [29.3%] of 75 women tested) than those born in southern Africa (none of 14 women from Zambia, Angola, South Africa, and Zimbabwe were HHV-8 seropositive; \( P = .04, \) Fisher’s exact test of overall comparison between 3 groups). Among women not born in Africa, there was a trend toward more HHV-8 antibodies among HIV-positive women than among those who were HIV-negative (\( P = .44, \) Fisher’s exact test). Two African women had KS at the time of this study; both had detectable HHV-8 antibodies but declined to give genital tract samples.

**HHV-8 DNA in CBS.** HHV-8 DNA was detected in 3 of 11 CBS obtained from HHV-8–seropositive women attending the genitourinary medicine department (table 2). Two of 3 women with a positive CBS had detectable HHV-8 DNA in a blood sample taken at the same time. In comparison, HHV-8 DNA was not detected in any of the 78 CBS from HHV-8–seronegative women or in 96 CBS from women of unknown HHV-8 serostatus attending the colposcopy clinic.

**HHV-8 DNA in blood.** DNA from whole blood was available from 93 women. Eleven of these women were seropositive for HHV-8, and HHV-8 DNA was detected in 6 of these (table 2) but in none of the seronegative patients. Two of the 6 women had HHV-8 detected in CBS.

### Discussion

Among women born in Africa, the seroprevalence of HHV-8 was 24.7%. These findings are consistent with recent reports of high HHV-8 seroprevalence in several West African countries [13, 14]. In addition, the incidence of KS in Africa is the highest in the world, both HIV- and non-HIV–related [15]. We found no difference in seroprevalence (23.2%) between 56 women from West Africa (Ghana, Nigeria, Ivory Coast, Senegal) and 23 women from central and East Africa (Uganda, Tanzania, Kenya, and former Zaire; 17.4%), although the incidence of KS is much lower in West Africa, even after adjusting for the lower prevalence of HIV infection. We found no association between HIV status and HHV-8 status, although the power to detect any difference was low.

Among women attending the STD clinic who were not born in Africa, the seroprevalence of HHV-8 was 9.1%. This compares with 3.4% among females attending an STD clinic in San Francisco [16] and 3% among blood donors in London [4] and suggests that some sexual transmission may occur among non-African heterosexual patients.

HHV-8 DNA was detected in whole blood DNA from 6 of 11 seropositive women from whom whole blood DNA was available for testing. Epidemiologic studies of KS have not

<table>
<thead>
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<th>Group</th>
<th>Cervical brush scrapes</th>
<th>Whole blood</th>
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<tbody>
<tr>
<td></td>
<td>HHV-8–seropositive</td>
<td>HHV-8–seronegative</td>
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<tr>
<td>HIV-positive</td>
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<td></td>
</tr>
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<td>African</td>
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</tr>
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<tr>
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<td></td>
</tr>
<tr>
<td>African</td>
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<td>0/3</td>
</tr>
<tr>
<td>Non-African</td>
<td>0/4</td>
<td>0/48</td>
</tr>
<tr>
<td>Total</td>
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NOTE. Data are no. positive/no. tested.
shown blood transfusion, intravenous drug use, or blood products as high risk factors for developing KS in AIDS patients [1], but this may relate to low levels of virus in blood. Our results suggest that HHV-8 might be transmitted by blood, but the number of women studied was small. Martin et al. [2] showed an increased risk for HHV-8 infection in men who had received a blood transfusion, but few transfused subjects were studied.

HHV-8 DNA was detected in 3 CBS from 11 HHV-8–sero-positive women. HHV-8 DNA was not detected in the genital tract of 78 women known to be seronegative or in 96 samples from women whose HHV-8 serostatus was unknown. These findings suggest that sexual transmission of HHV-8 via vaginal contact is possible, although detection of viral DNA does not prove the presence of infectious virus or that virus is contained within or replicating in the genital tract. However, it is possible that the detection rate of HHV-8 DNA in the genital tract was underestimated, because women were sampled only once and viral shedding may be intermittent. HHV-8 DNA may have been present as a result of blood contamination resulting from sample collection. Alternatively, virus may have been present in semen remaining from recent sexual intercourse; it was not possible to test for the Y chromosome, as insufficient samples remained. Nevertheless, sexual transmission may occur regardless of the source of virus in the genital tract.

The 27% detection rate of HHV-8 DNA in CBS from sero-positive women is similar to the detection rate in semen of HIV-infected gay men, ~20% in most studies [5, 9]. This suggests that sexual transmission is not common and not the only route of transmission. Transmission of HHV-8 from infected mothers to infants may also occur via the female genital tract. KS occurs in very young children in Africa, suggesting early infection with HHV-8. Of interest, all 3 of our HHV-8–positive cervical samples were from African women.

Transmission may also occur via saliva, as HHV-8 was detected in the saliva of 15% of HIV-positive subjects and 75% of AIDS-KS patients, although the numbers studied were small [17]. Larger, more detailed studies are needed to assess the role of HHV-8 shedding at different sites for the transmission of the virus in the general population. However, this study and others [4, 16] suggest a low prevalence of HHV-8 in the general heterosexual populations in the United States and United Kingdom, which makes the study of routes of transmission within this setting difficult. Further studies in Africa are indicated.

Acknowledgments

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References