Infection With Kaposi’s Sarcoma–Associated Herpesvirus Among Families of Patients With Classic Kaposi’s Sarcoma

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Background: Classic Kaposi’s sarcoma (CKS) primarily affects elderly Mediterranean or Eastern European men. Incidence rates of CKS in Israel are among the world’s highest. In practically all cases, antibodies against Kaposi’s sarcoma–associated herpesvirus (KSHV) can be detected. A relatively high seroprevalence rate of KSHV in Israel generally correlates with the incidence of CKS. A sexual mode of virus transmission is recognized among homosexual men, whereas the precise transmission routes in the heterosexual population and those with CKS are still unclear.

Objective: To better assess the transmission routes of KSHV in Israeli patients with CKS and their first-degree relatives as compared with a control group.

Design: Serum was collected from all study participants and tested for KSHV antibodies by means of latent and lytic immunofluorescence assays. An open reading frame 65 (ORF65) Western blot assay was applied as a confirmatory tool.

Setting: Three dermatological departments in Israel.

Patients: Sixty-four Jewish patients with CKS, 143 of their first-degree relatives, and 186 hospital-based control subjects.

Results: Seropositivity to KSHV was detected in 62 (96.9%) of the patients with CKS, in 56 (39.2%) of their first-degree relatives, and in only 21 (11.3%) of the hospital controls (P < .001). The specific relationship with the index patient (spouse, offspring, or sibling) had no significant effect on the prevalence of seropositivity in the family members.

Conclusion: Our serologic evidence of familial clustering of KSHV infection suggests a predominantly non-sexual horizontal transmission route of the virus.

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demonstrated an association between KSHV seropositivity, multiple sex partners, and sexually transmitted diseases.\textsuperscript{12,13} In North America and Western Europe, infection is rare in childhood and the acquisition of KSHV infection occurs mainly after puberty.\textsuperscript{15} However, sexual activity has not been specifically associated with an increased risk of KSHV infection among heterosexuals.\textsuperscript{4,5} Likewise, in the developing world, children become infected with Epstein-Barr virus within the first decade of life, whereas in the more developed western world, up to half of children remain seronegative at the end of their first decade of life and become infected in adolescence or young adulthood.\textsuperscript{15}

In Italy, KSHV DNA has been found in the peripheral blood mononuclear cells of up to 50% of individuals with AIDS-associated KS\textsuperscript{16,17} and in 8% to 10% of healthy subjects.\textsuperscript{17,18} Infection in children is rare in the developed world, but the acquired immune deficiency syndrome (AIDS) epidemic causes a marked increase in the incidence of KSHV infection.\textsuperscript{19,20} Viral DNA was detected in saliva in patients with KS;\textsuperscript{19,20} and viral DNA was detected in semen and prostatic fluid of patients with KS.\textsuperscript{21,22} Accordingly, KSHV could potentially be transmitted through blood contact, sexual contact, saliva, and transplanted organs.

Classic KS (CKS) is found mostly among elderly people of Mediterranean or Jewish origin with no apparent immunodeficiency.\textsuperscript{23} Between 1960 and 1998, the overall age-standardized incidence rates (\pm SD) of CKS in the Israeli Jewish population were 20.7 \pm 9.3 per million among men and 7.5 \pm 3.4 per million in women.\textsuperscript{24} These rates represent one of the highest incidence rates of CKS in the developed world. Seroprevalence surveys\textsuperscript{25,26} found 6.5\% to 9.9\% of the Israeli Jewish population to be infected with KSHV. In view of the intermediate infection rates with KSHV and the high living standards, Israel presents a unique opportunity to investigate the epidemiology of CKS and to evaluate the transmission modes of KSHV.

The objective of the present study was to assess the intrafamilial patterns of KSHV transmission to obtain insight into the transmission modes of KSHV among heterosexuals. We have focused on first-degree family members of patients with CKS and compared this population with a randomly selected hospital-based control population. In what we believe to be the largest CKS family study conducted thus far, we present evidence of intrafamilial transmission of KSHV in families of Israeli CKS patients.

**METHODS**

**STUDY POPULATION**

Enrollment of CKS patients to the study was based on Israel Cancer Registry information and used a systematic sampling approach of Jewish patients from 3 dermatological departments in Israel (Rambam Medical Center in Haifa, HaEmek Medical Center in Afula, and Rabin Medical Center in Petah-Tikva) whose diagnosis was ascertained. Patients with AIDS-associated KS were excluded from the study, and selected patients had at least a 2-year history of disease. In total, 200 CKS patients were sampled; 64 (48 men and 16 women) were located and agreed to participate in the study. Concurrently, 146 of their first-degree family members—spouses, offspring, and siblings, all of them living in Israel—agreed to participate in the study. Because the CKS patients were rather old, no children were included in the family member group. For the control population, 189 subjects were consecutively recruited from the emergency and orthopedic departments. Written informed consent was obtained from all study participants, and ethical approval was obtained from the Helsinki committee of the Israeli Ministry of Health.

**DATA COLLECTION AND STATISTICAL ANALYSIS**

Blood samples were collected during 1999-2002, and serum was separated and stored at –20°C until testing. All study participants underwent a face-to-face interview. Data were recorded on standardized forms that were linked to the serum samples by numeric code. Statistical analyses were performed using SPSS statistical software (version 10.0, SPSS Inc, Chicago, Ill). The null hypothesis for the final stage of the analysis was that there would be no association between the serostatus of KSHV and the category of familial relationship to the CKS patients. A 2-sided \(P<.05\) was considered statistically significant and sufficient to refute the null hypothesis.

**SEROLOGIC ASSAYS**

All serologic assays were performed and interpreted while the serum samples remained coded. We adopted a unique flowchart approach for defining KSHV seropositivity (Figure). Each serum sample was first tested for KSHV antibodies by means of a latent immunofluorescence assay (IFA) and a lytic IFA. If the results were concordant, a final positive or negative serologic diagnosis was made (I and II). A KSHV ORF65 (open reading frame 65) Western blot (WB) assay was applied for samples that were negative by latent IFA but positive by lytic IFA (III). A Ramos IFA was applied for samples that were negative by lytic IFA but positive by latent IFA (IV).

**Figure.** Flowchart for defining the serostatus of Kaposi’s sarcoma–associated herpesvirus (KSHV). Each serum sample was first tested for KSHV antibodies by means of a latent immunofluorescence assay (IFA) and a lytic IFA. If the results were concordant, a final positive or negative serologic diagnosis was made (I and II). A KSHV ORF65 (open reading frame 65) Western blot (WB) assay was applied for samples that were negative by latent IFA but positive by lytic IFA (III). A Ramos IFA was applied for samples that were negative by lytic IFA but positive by latent IFA (IV).
tive by latent IFA were also tested by the KSHV-negative Ramos cell line to exclude nonspecific nuclear reactivity. Serum samples that tested negative by this assay and positive by latent IFA were scored as positive.

## RESULTS

### SEROPREVALENCE OF KSHV

#### CKS Patients

Sixty-four CKS patients were studied, including 48 men and 16 women. The mean ± SD age of the CKS patients was 71.8 ± 11.0 years, and is comparable (*P* = .16) to that of Jewish Israeli CKS patients, which was 69.2 ± 14.8 years during the period between 1970 and 2000.24 Our serologic evaluation strategy indicated that 62 (96.9%) of the patients were seropositive. Of all 64 patients, 62 (95.3%) were seropositive by lytic IFA. One 70-year-old woman with CKS presented a seropositive response by lytic IFA and seronegative responses by latent IFA and the ORF65 Western blot assay; based on our serostatus evaluation strategy, this patient was designated as being seronegative. In addition, a 53-year-old woman with CKS, who was also diagnosed as having pulmonary KS, was found to be seronegative by all assays and therefore was designated as being seronegative as well. The concordance rate between latent and lytic IFAs among CKS patients was 96.9% (62 of 64).

#### Family Members of CKS Patients and Hospital Controls

We next tested the seroprevalence of KSHV among 146 family members (1-7 family members for each of 60 patient with CKS) and 189 hospital controls. Following the completion of the serologic evaluation, we excluded from the statistical analysis 3 control subjects and 1 family member who reported homosexual or bisexual sexual preferences, as well as 2 family members with indeterminate KSHV serostatus. Characteristics of the 2 groups are shown in Table 1. Because the characteristics of the 2 groups differed, we repeated all statistical analyses separately for each group.

The overall seroreactivity to KSHV in family members of CKS patients was 39.2% (56 of 143 family members). A significantly lower overall seroreactivity to KSHV was found among the hospital controls (11.3% [21 of 186 hospital controls], *P* < .001). The estimated KSHV prevalence rate in our control population was similar to the adjusted rate reported previously in Israel.26 The presence of antibodies to KSHV was strongly associated with clinical CKS, whether we were comparing the CKS patients with their family members (*P* < .01) or with the hospital controls (*P* < .01).

### EFFECT OF SEX AND AGE ON KSHV SEROPREVALENCE

Similar frequencies of KSHV seropositivity were observed among men and women in the family members and the hospital controls (Table 1). The KSHV seropositivity peaked in family members 65 years and older and in the 50- to 64-year age group in the hospital controls (Table 1). Among family members, the mean ± SD age of those who were seropositive was 53.1 ± 17.0 years, and that of those who were seronegative was 46.8 ± 15.2 years. Among the hospital controls, the mean age of the seropositive persons was 59.9 ± 15.5 years, and that of those who were seronegative was 53.6 ± 19.2 years. Overall, seropositive individuals among family members of CKS patients and hospital controls tended to be older than seronegative individuals. This trend, however, was not statistically significant for hospital controls (Table 1).

### INTRAFAMILIAR TRANSMISSION OF KSHV

The first-degree family members of CKS patients included spouses, offspring, and siblings (Table 2). Hav-
families in which the mother had CKS or was KSHV-stratified clustering of serologic status, especially among these differences were not statistically significant (Table 2). Among the relatives of CKS patients, seroprevalence rates varied between 56% in siblings, 49% in spouses, and 34% in offspring. However, patients, seroprevalence rates varied between 56% in siblings, 49% in spouses, and 34% in offspring. However, the high seroprevalence of KSHV among family members demonstrated clustering of seropositive individuals within families of CKS patients, with no significant difference between seroprevalence in spouses compared with seroprevalence in other family members. The correlation of KSHV serostatus between mother and child (Table 3), although only marginally significant, supports an increased risk of mother-to-child KSHV transmission compared with father-to-child transmission. This correlation between the serostatus of the mother and that of her offspring could reflect the greater role of the mother in intimate care and does not necessarily represent transmission during pregnancy, delivery, or breastfeeding. Significant correlations in KSHV serostatus between mothers and offspring, between siblings, and between spouses were reported in Israel and in Tanzania in families not affected with KS.26,30 In a study of 20 CKS patients and 36 of their first-degree family members demonstrated clustering of seropositive individuals within families of CKS patients, with no significant difference between seroprevalence in spouses compared with seroprevalence in other family members. The correlation of KSHV serostatus between mother and child (Table 3), although only marginally significant, supports an increased risk of mother-to-child KSHV transmission compared with father-to-child transmission. This correlation between the serostatus of the mother and that of her offspring could reflect the greater role of the mother in intimate care and does not necessarily represent transmission during pregnancy, delivery, or breastfeeding. Significant correlations in KSHV serostatus between mothers and offspring, between siblings, and between spouses were reported in Israel and in Tanzania in families not affected with KS.26,30 In a study of 20 CKS patients and 36 of their first-degree family members demonstrated clustering of seropositive individuals within families of CKS patients, with no significant difference between seroprevalence in spouses compared with seroprevalence in other family members. The correlation of KSHV serostatus between mother and child (Table 3), although only marginally significant, supports an increased risk of mother-to-child KSHV transmission compared with father-to-child transmission. This correlation between the serostatus of the mother and that of her offspring could reflect the greater role of the mother in intimate care and does not necessarily represent transmission during pregnancy, delivery, or breastfeeding.

Salivary shedding of KSHV may provide a possible mode of intrafamilial transmission, because exposure to saliva may occur while playing, sharing eating implements, and kissing during childhood and between adults. Sexual and vertical transmission among family members of CKS patients may also take place. Still, the similarity in the risk of KSHV infection among spouses, offspring, and siblings supports similar modes of virus infection among first-degree family members with different relationships (spouse, sibling, parent or child).

### Table 2. KSHV Seroprevalence by Relationship to Patient With CKS

<table>
<thead>
<tr>
<th>Relationship</th>
<th>No. Seropositive/No. Analyzed (% Seropositive)</th>
<th>OR (95% CI)*</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Offspring</td>
<td></td>
<td>1.00 (Referent)</td>
<td></td>
</tr>
<tr>
<td>Spouse</td>
<td></td>
<td>0.99 (0.33-2.93)</td>
<td>.98</td>
</tr>
<tr>
<td>Sibling</td>
<td></td>
<td>1.07 (0.21-5.38)</td>
<td>.94</td>
</tr>
</tbody>
</table>

Abbreviations: CI, confidence interval; CKS, classic Kaposi’s sarcoma; KSHV, Kaposi’s sarcoma–associated herpesvirus; OR, odds ratio.*Adjusted for sex and age of family members.

### Table 3. Association Between Parental and Offspring KSHV Serostatus

<table>
<thead>
<tr>
<th>Parental KSHV Status</th>
<th>No. of All Offspring (No. of Families)</th>
<th>No. of KSHV-Seropositive Offspring (No. of Families)</th>
<th>KSHV-Seropositive, %*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mother has CKS</td>
<td>30 (13)</td>
<td>11 (7)</td>
<td>37</td>
</tr>
<tr>
<td>Father has CKS</td>
<td>65 (32)</td>
<td>21 (14)</td>
<td>32</td>
</tr>
<tr>
<td>Father has CKS, mother is KSHV-seropositive†</td>
<td>30 (11)</td>
<td>13 (7)</td>
<td>43</td>
</tr>
<tr>
<td>Father has CKS, mother is KSHV-seronegative†</td>
<td>21 (12)</td>
<td>4 (4)</td>
<td>19</td>
</tr>
</tbody>
</table>

Abbreviations: CKS, classic Kaposi’s sarcoma; KSHV, Kaposi’s sarcoma–associated herpes virus.

COMMENT

We identified a relatively high seroprevalence of antibodies to KSHV among family members of CKS patients. Because the mean ± SD age of the hospital controls in our study (54.3 ± 18.8 years) was significantly higher than that of the family members (49.2 ± 16.1 years) (P = .01), and because the seroprevalence of KSHV increases with age, this difference further amplifies the inequality of KSHV seroprevalence between the 2 groups studied. Overall, we found that family members were 6.44-fold (95% confidence interval, 3.36-12.32) more likely to be infected with KSHV than were control subjects. Although it is unknown whether familial clustering is due to direct intrafamilial transmission or due to shared risk factors for infection, the latter seems unlikely because of the high seroprevalence of KSHV among family members of CKS patients (39.2% vs 11.3% in hospital controls). The seroprevalence of KSHV was higher among spouses than offspring, suggesting possible sexual transmission of KSHV, but this difference was not statistically significant (Table 2). Likewise, a study from Sardinia29 of 20 CKS patients and 36 of their first-degree family members demonstrated clustering of seropositive individuals within families of CKS patients, with no significant difference between seroprevalence in spouses compared with seroprevalence in other family members. The correlation of KSHV serostatus between mother and child (Table 3), although only marginally significant, supports an increased risk of mother-to-child KSHV transmission compared with father-to-child transmission. This correlation between the serostatus of the mother and that of her offspring could reflect the greater role of the mother in intimate care and does not necessarily represent transmission during pregnancy, delivery, or breastfeeding. Significant correlations in KSHV serostatus between mothers and offspring, between siblings, and between spouses were reported in Israel and in Tanzania in families not affected with KS.26,30 In a study of people of African origin living in French Guyana, similar correlations were noted between the serostatus of children and that of their mothers and between siblings, but not between the serostatus of children and that of their fathers nor between spouses.
Therefore, our study suggests a predominantly non-sexual, person-to-person spread among family members. The increased correlation between seropositivity of the mother with that of the offspring further supports this notion and could suggest that mothering responsibilities result in increased exposure to maternal transmission. The relatively limited transmission of KSHV from KSHV-seropositive fathers to their offspring is similar to the intrafamilial transmission patterns of hepatitis B virus infection.1,32

We found no significant sex difference in the prevalence of KSHV antibodies among family members or hospital controls. This suggests that some factor other than an increased exposure to virus infection must account for the observed male-female ratio of 2.8:1 in the incidence of CKS in Israel.24 A similar trend has been reported worldwide.23

In conclusion, our data underscore the issue of intrafamilial spread of KSHV infection among family members of CKS patients. Our findings may also apply, although to a lesser extent, to KSHV-seropositive individuals who have not developed the disease. Exposure to the saliva of infected individuals, especially in the familial setting, may be a major mode of horizontal KSHV spread. Nevertheless, assuming that KSHV, like Epstein-Barr virus, reactivates periodically in infected individuals and is shed in the saliva, and given that infection with KSHV could occur years before disease onset, it is reasonable to conclude that, although we have found an increased risk for virus infection among family members of CKS patients, the transmission of KSHV is inefficient. This may also explain the nonubiquitous nature of KSHV infection in most populations.

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REFERENCES


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News and Notes

20th Continuing Medical Education Course for Practical Dermatology and Venereology. Munich, Germany, July 23 to 28, 2006. Lectures will be held in German. Information and registration: Prof Dr Gerd Plewig (Congress President), Priv-Doz Dr Peter Thomas (Organizing Committee), Mrs Gertrud Hammel (Congress Office). Address: Fortbildungswwoche fu¨r praktische Dermatologie und Venerologie e.V. c/o Department of Dermatology, Ludwig-Maximilians-University Munich, Frauenlobstrasse 9-11, D-80337 Munich, Germany; telephone, 49-89-5160 6065; fax, 49-89-5160 6066; www.fortbildungswwoche.de. Registration will start in November 2005.