Elevated Seroprevalence of Human Herpesvirus 8 among Men with Prostate Cancer

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Background. To investigate any epidemiological association between human herpesvirus (HHV)-8 and prostate cancer, we determined the prevalence of HHV-8 seropositivity among prostate cancer case and control subjects in the United States and Trinidad and Tobago.

Methods. Antibodies against HHV-8 were detected in 2 independent laboratories using either indirect immunofluorescence assay (IFA) or a combination of enzyme-linked immunosorbent assay and IFA.

Results. Among 138 Tobago men with prostate cancer, HHV-8 seroprevalence was 39.9%—significantly higher than that among 140 age-matched control subjects (22.9%; odds ratio [OR], 2.24; 95% confidence interval [CI], 1.29–3.90). Among 100 US men with prostate cancer, seroprevalence was 20%—significantly higher than that of 177 blood donors (5.1%; OR, 4.67; 95% CI, 1.91–11.65) and higher than that of 99 men with cancer not related to HHV-8 (13%; OR, 0.253; 95% CI, 0.77–3.54).

Conclusions. HHV-8 seropositivity is elevated among men with prostate cancer compared with control subjects, which suggests that HHV-8 plays a role in the development of prostate cancer.
able candidate for an infectious agent cofactor, as suggested by Hayes et al. [8]. If HHV-8 serves as a cofactor for the development of prostate cancer, then HHV-8 seroprevalence may be increased among men with the cancer, compared with age-matched control subjects. For the present article, we tested this hypothesis by determining the seroprevalence rate of HHV-8 among patients with prostate cancer and control subjects in populations of differing ethnicity and environment in Trinidad and Tobago and in the United States.

SUBJECTS AND METHODS

Subject populations. A case-control study was conducted with participants from the Caribbean nation of Trinidad and Tobago and from the United States. Informed, written consent was obtained using forms and procedures approved by the institutional review boards of the University of Pittsburgh, the Tobago Ministry of Health and Social Services, and Caroni (1975) Trinidad.

The Trinidad and Tobago participants consisted of 3 groups: 138 men from Tobago with biopsy-confirmed prostate cancer, 140 age-matched control subjects consisting of men from Tobago with no evidence of prostate cancer (normal digital rectal examination [DRE] results and prostate-specific antigen [PSA] values <4.0 ng/mL), and 174 men from Trinidad with no evidence of prostate cancer. Among the Tobagonians, 97% were of African descent, whereas, among the Trinidadians, 90% were of Asian Indian descent, with the remaining 10% being of African descent. Asian Indian ancestry in this population reflects the immigration of agricultural workers from India in the late 1800s to early 1900s. Aspects of these groups have been reported elsewhere [4, 24].

The US participants consisted of 3 groups: 100 men with advanced prostate cancer who were seen in a prostate cancer clinic at the University of Pittsburgh Medical Center, 99 men with a diagnosed cancer not related to (or suspected of being related to) HHV-8, and 177 male blood donors. The Pittsburgh control subjects with cancer were selected from a serum repository of patients with cancer that is maintained by the University of Pittsburgh Cancer Institute. Selected individuals were aged ≥40 years, male, and, at the time of the blood draw, diagnosed with a cancer that was not associated with HHV-8. We excluded men with a diagnosis of prostate cancer, KS, multicentric Castleman disease, primary effusion lymphoma, and multiple myeloma. Detailed medical information, such as previous history of cancer, PSA values, and DRE, was not available for the Pittsburgh control subjects with cancer. The US blood donors were selected from a subset of a larger repository collected from 5 major US centers by the National Heart, Lung, and Blood Institute Retrovirus Epidemiology Donor Study [25] and represent all of the men whose age was within the age range of the Pittsburgh patients with prostate cancer (46–99 years) [26].

HHV-8 serological testing. The detection of HHV-8-specific antibodies was done in 2 separate laboratories, the University of Pittsburgh and the Centers for Disease Control and Prevention (CDC). Serum samples from 21 patients with histologically confirmed KS were included as an HHV-8–positive laboratory control.

The University of Pittsburgh used a HHV-8 monoclonal antibody–enhanced immunofluorescent assay (IFA) [27] with a cutoff value for seropositivity of 1:100. HHV-8 antibody titers were determined using the same IFA on serially diluted serum samples. End-point titers were reported as the reciprocal of the last positive dilution. All samples were analyzed blinded, in duplicate, a minimum of 3 times by the same reader.

HHV-8 serological testing done at CDC involved 3 separate assays. The first 2 used a peptide-based EIA. The peptides used for detection by EIA were based on proteins encoded by HHV-8 open-reading frames 65 and K8.1 (serum dilution, 1:100) [28, 29]. Samples that showed negative results by both EIAs were then screened by the monoclonal antibody–enhanced IFA at a serum dilution of 1:40 [29, 30]. A specimen was defined as seropositive if positive results were obtained in any of the 3 assays.

Data analyses. Differences in HHV-8 seroprevalence rates were examined by χ² analysis or Fisher’s exact test, as appropriate. Comparisons of HHV-8 titer levels by categorical risk-factor variables and mean PSA values by HHV-8 serostatus were done with the Mann-Whitney U test. Student’s t test was used to examine differences in age, height, weight, and body mass index (BMI), by HHV-8 serostatus. Logistical regression analyses were used to analyze interactions of HHV-8 serostatus and various categorical variables on the presence of prostate cancer.

RESULTS

HHV-8 seropositivity. The HHV-8 seroprevalence rate was higher among both of the prostate cancer groups than in their corresponding control groups (table 1). In Tobago, men with prostate cancer were more likely to be HHV-8 seropositive than their age-matched cancer-free control subjects (odds ratio [OR], 2.24; 95% confidence interval [CI], 1.29–3.90) or healthy men from the neighboring island of Trinidad (OR, 2.63; 95% CI, 1.54–4.50). HHV-8 seropositivity was also significantly higher among Pittsburgh men with prostate cancer than among US blood donors (OR, 4.67; 95% CI, 1.9–11.65) and was also higher compared with the Pittsburgh control subjects with cancer, although this difference was not significant (OR, 1.65; 95% CI, 0.77–3.54).

To confirm these results, HHV-8 serological testing was done on all of the Trinidad, Tobago, Pittsburgh, and KS specimens at CDC using the serology screening algorithm described in

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Table 1. Human herpesvirus (HHV)-8 prevalence among study participants.

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of HHV-8–positive subjects/total no. of subjects (%)</th>
<th>P</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tobago patients with prostate cancer</td>
<td>55/138 (39.9)</td>
<td>.003</td>
<td>2.24 (1.29–3.90)</td>
</tr>
<tr>
<td>Tobago control subjects</td>
<td>32/140 (22.9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trinidad control subjects</td>
<td>35/174 (20.1)</td>
<td>&lt;.001b</td>
<td>2.63 (1.54–4.50)</td>
</tr>
<tr>
<td>Pittsburgh patients with prostate cancer</td>
<td>20/100 (20)</td>
<td>&lt;.001</td>
<td>4.67 (1.91–11.65)</td>
</tr>
<tr>
<td>US blood donors</td>
<td>9/177 (5.1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pittsburgh control subjects with cancer</td>
<td>13/99 (13)</td>
<td>.253c</td>
<td>1.65 (0.77–3.54)</td>
</tr>
</tbody>
</table>

**NOTE.** CI, confidence interval; OR, odds ratio.

'a' Fisher's exact test.

'Comparison with Tobago patients with prostate cancer.

'c' Comparison with Pittsburgh patients with prostate cancer.

Materials and Methods. There was strong agreement between the results from the University of Pittsburgh and CDC laboratories (κ = 0.742). As shown in table 2, the HHV-8 serological assay from the University of Pittsburgh detected an average of 6.5 percentage points more HHV-8 seropositive samples among both case and control subjects than that of the CDC, but these differences were not significant. In addition to the concordant results within the epidemiological groups, results from the CDC laboratory independently confirmed the significant differences between the Tobago patients with prostate cancer and Tobago control subjects (P = .020; OR, 1.94; 95% CI; 1.12–3.34). In addition, the elevated HHV-8 seroprevalence in the Pittsburgh prostate cancer group, compared with that in the Pittsburgh cancer control group, was also confirmed by the second laboratory. Thus, the association of HHV-8 seropositivity with prostate cancer in both Trinidad and Tobago and the United States was confirmed by 2 independent laboratories.

**HHV-8 antibody titers.** To compare the levels of HHV-8 antibodies among the different groups, antibody titers were measured using an end-point IFA. Although the mean HHV-8 antibody titer was higher in each of the prostate cancer groups than in their corresponding control groups (table 3), with one exception, the differences observed were not statistically significant. In addition, the average antibody titer of the Tobago prostate cancer group was significantly higher than any of the US cancer or control groups (P < .02). This difference was not seen when we compared the mean titers from either the Tobago or Trinidad control groups with any of the US groups.

**HHV-8 seropositivity by age.** We determined the average age of the HHV-8–seropositive and –seronegative men in each group. As shown in table 4, with the exception of the US blood donors, there was no significant difference in the average age of the HHV-8–seropositive men, compared with that of the HHV-8–seronegative men.

**HHV-8 seroprevalence by population.** The background rate of seropositivity (i.e., seropositivity in control subjects), was lower in the US blood donors (5%) and Pittsburgh control subjects with cancer (13%) than in the Tobago (25%) and Trinidad (20%) control subjects. Within the Trinidad group, the HHV-8 seroprevalence rate among men of African descent was 33% (6/18), compared with 19% (29/156) among men of Asian Indian descent. In the Pittsburgh prostate cancer group, ethnicity data were available for 87 men. Within this subgroup, 42.9% (37/87) of the black men were HHV-8 positive, compared with 18.8% of white men.

Table 2. Comparison of human herpesvirus (HHV)-8 serological assay result.

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of HHV-8–positive subjects/total no. of subjects (%)</th>
<th>University of Pittsburgh</th>
<th>CDC</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tobago patients with prostate cancer</td>
<td>55/138 (39.9)</td>
<td>45/138 (32.6)</td>
<td>.214</td>
<td></td>
</tr>
<tr>
<td>Tobago control subjects</td>
<td>32/140 (22.9)</td>
<td>28/140 (20)</td>
<td>.565</td>
<td></td>
</tr>
<tr>
<td>Pittsburgh patients with prostate cancer</td>
<td>20/100 (20)</td>
<td>12/100 (12)</td>
<td>.129</td>
<td></td>
</tr>
<tr>
<td>US blood donors</td>
<td>9/177 (5.1)</td>
<td>ND</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>Pittsburgh control subjects with cancer</td>
<td>13/99 (13)</td>
<td>8/99 (8.1)</td>
<td>.261</td>
<td></td>
</tr>
<tr>
<td>Patients with KS</td>
<td>21/21 (100)</td>
<td>19/21 (90.5)</td>
<td>.244</td>
<td></td>
</tr>
</tbody>
</table>

**NOTE.** CDC, Centers for Disease Control and Prevention; KS, Kaposi sarcoma; ND, not determined.

'a' Fisher's exact test.
(15/80) of the white men. This elevated HHV-8 seroprevalence rate among black men was not present among the US blood donors (0/7). The Pittsburgh control subjects with cancer did not include any black men. Future studies of larger populations will be required to determine the significance of these interesting results.

**Association between HHV-8 seropositivity and PSA values and Gleason scores.** Gleason scores from prostate tissues were available for 55% of the Pittsburgh subjects with prostate cancer and 100% of the Tobago men with prostate cancer. The Gleason score is a histological grading system used in the United States that assigns histological patterns on a scale of 1–5 on the basis of the degree of differentiation. The distribution of the Gleason scores for the HHV-8-seropositive individuals in Pittsburgh and Tobago was not different from that of the corresponding seronegative subjects (data not shown).

PSA levels were available for every Tobago subject with prostate cancer and for 84% of the Pittsburgh subjects with prostate cancer. The mean PSA values (excluding 2 values that were ±2 SD from the mean) for the HHV-8-seropositive men in Pittsburgh (124.2 ± 183.0) and Tobago (39.4 ± 107.31) were not significantly different from the corresponding seronegative men in either Pittsburgh (70.20 ± 127.02) or Tobago (65.24 ± 243.58) on the basis of the Mann-Whitney U test. There was also no correlation between PSA values and HHV-8 antibody titer levels in either the Pittsburgh or Tobago cancer groups (data not shown). The higher PSA values seen in the Pittsburgh men likely reflects the fact that these men had advanced prostate cancer.

**Associations between HHV-8 seropositivity and medical history parameters in the Tobago cohorts.** Among the Tobago patients with prostate cancer and control subjects, self-reported information for several medical history variables was available, including a history of benign prostatic hyperplasia, prostatitis, syphilis, gonorrhea, smoking, and family history of cancer, as well as their BMI (kg/m²). This information was available on a majority of the Tobago subjects—81%–99%, depending on the particular variable. There was no association between HHV-8 seropositivity and any of the medical history variables (data not shown). In addition, using logistical regression analyses, we determined that there were no interactions between HHV-8 seropositivity and these different health variables on the presence of prostate cancer (data not shown).

**DISCUSSION**

To our knowledge, our study is the first to document elevated HHV-8 seropositivity among men with prostate cancer compared with controls. This relationship was seen in serological test results

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**Table 3. Human herpesvirus–8 antibody end-point titers.**

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Mean ± SE</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tobago patients with prostate cancer</td>
<td>56</td>
<td>5416.96 ± 1461.51</td>
<td>&lt;.106</td>
</tr>
<tr>
<td>Tobago control subjects</td>
<td>32</td>
<td>4634.38 ± 1796.89</td>
<td></td>
</tr>
<tr>
<td>Trinidad control subjects</td>
<td>49</td>
<td>1006.12 ± 291.79</td>
<td>&lt;.001,b,c</td>
</tr>
<tr>
<td>Pittsburgh patients with prostate cancer</td>
<td>20</td>
<td>870.00 ± 214.73</td>
<td>&lt;.285</td>
</tr>
<tr>
<td>US blood donors</td>
<td>9</td>
<td>466.67 ± 158.9</td>
<td></td>
</tr>
<tr>
<td>Pittsburgh control subjects with cancer</td>
<td>13</td>
<td>553.85 ± 11.30</td>
<td>&lt;.842,d</td>
</tr>
</tbody>
</table>

a Mann-Whitney U test.

b Comparison with Tobago patients with prostate cancer.

c Comparison with Tobago control subjects.

d Comparison with Pittsburgh patients with prostate cancer.

e Comparison with US blood donors.

**Table 4. Age and human herpesvirus (HHV)–8 serostatus.**

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Age, mean years (±SD)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Total group</td>
<td></td>
<td>66.64 ± 7.98</td>
<td>67.25 ± 6.86</td>
</tr>
<tr>
<td>HHV-8-seropositive</td>
<td></td>
<td>66.07 ± 8.16</td>
<td>67.07 ± 7.74</td>
</tr>
<tr>
<td>HHV-8-seronegative</td>
<td></td>
<td>68.96 ± 9.04</td>
<td>67.85 ± 7.89</td>
</tr>
</tbody>
</table>

a Student’s t test comparing means of HHV-8-seropositive with –seronegative individuals.

b Age data were missing for 1 Pittsburgh control subject with cancer.

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from 2 independent laboratories, using assays and algorithms that have been demonstrated to be reliable in identifying the meaningful risks associated with HHV-8 infection [31–35]. We analyzed populations from Trinidad and Tobago and the United States, which have dramatically different rates of prostate cancer. In both populations, there was an elevation in HHV-8 seroprevalence between the cancer and their associated control groups that corresponded to a 2–4-fold higher prostate cancer risk in men with either a past or current HHV-8 infection.

Among the Tobago patients with prostate cancer, there was a 2-fold higher rate of HHV-8 seropositivity, compared with a well-defined, age-matched control group. An increase in HHV-8 seropositivity between patients with prostate cancer and control subjects was also seen in the United States, even though the control groups were not as well-defined as the Tobago groups. Among the Pittsburgh patients with prostate cancer, there was a 4-fold higher rate of HHV-8 seropositivity, compared with that in a control group selected from US blood donors. However, the US blood donor population is a selected group that excludes individuals with specific infections and behaviors [36]. Thus, this group does not fully represent the general population, in contrast to the recruitment for the Trinidad and Tobago control subjects. The Pittsburgh cancer control group was composed of ill men who presented to the University of Pittsburgh hospital system with a non–HHV-8–related cancer. Detailed medical information was not available on these subjects, and, although we knew that at the time of the blood draw, they were not diagnosed with an HHV-8–related cancer, we cannot determine whether they had a prior history of prostate or any other HHV-8–related cancer. Nonetheless, our serological test results showed a trend in the association of HHV-8 to prostate cancer in the United States using this control group. Although the rate of HHV-8 seropositivity among the US control subjects with cancer (13%) was higher than that of the US blood donors (5.1%), it was not significantly higher than the HHV-8 seroprevalence rate of 100 University of Pittsburgh students (10%) [27]. The lower seroprevalence rate in the blood donor group is probably a result of the screening process used in selecting blood donors.

Thus, it appears that the association of prostate cancer with HHV-8 is similar across populations with different HHV-8 seroprevalence rates. In addition, HHV-8 seropositivity is higher among men of African descent both in the United States and Trinidad. These results suggest that HHV-8 infection may be more endemic in these populations and therefore may help explain the higher prevalence (Tobago) or incidence (United States) of prostate cancer in men of African descent.

The fact that not all men with prostate cancer are HHV-8 seropositive probably indicates that the virus is not associated with all cases of prostate cancer. In addition, these results may explain discrepancies reported in the literature regarding the presence of HHV-8 in semen and prostate tissue. The presence of HHV-8 DNA or its protein expression in semen or prostate tissue has varied greatly among reports [37]. None of these studies has examined the seroprevalence rate of HHV-8 among the individuals tested; the discrepancies of virus detection in these reports may reflect the fact that the seroprevalence rate in the general population is low (5%–10%) and that HHV-8 may not be associated with every case of prostate cancer. This lack of serological data prompted the current study.

One interpretation of our data is that HHV-8 directly elevates the risk for prostate cancer. Under this scenario, we would speculate that the virus serves to increase either the risk of developing prostate cancer or the tumorigenicity of an existing prostate cancer through autocrine or paracrine paths. There was no significant difference in HHV-8 antibody titers between seropositive men in the prostate cancer groups and their corresponding control groups. This suggests that the virus is not actively replicating (beyond the basal rate needed to maintain a latent repository) in men with prostate cancer. If vigorous viral replication was occurring, we would have expected to see much higher levels of HHV-8 antibodies, as is seen in patients with KS [38–40].

An alternative interpretation of our results is that HHV-8 antibodies are a marker for some other infectious agent or a genetic, behavioral, or environmental factor that is responsible for cancer development. This scenario is similar to that seen with HSV-2 and cervical cancer, where the rate of HSV-2 seroprevalence was higher among women with cervical cancer compared with age-matched control subjects [41, 42]. The elevated HSV-2 seroprevalence rate was not due to a direct role of HSV-2 in development of the cancer but instead reflected similar risk factors in the acquisition of HSV-2 and the actual viral etiological agent of cervical cancer, human papillomavirus. Distinguishing between a causative role for HHV-8 and its being a marker for the actual causative agent of prostate cancer will require an assessment of whether HHV-8 is present in either the tumor, in the surrounding normal cells, or in the infiltrating B cells of HHV-8–seropositive men with prostate cancer.

In conclusion, although the data presented in the present study do not explain what role(s) HHV-8 may play in prostate cancer, they do suggest an association with this cancer. Thus, the detection of HHV-8 seropositivity may prove to be a useful marker for an increased risk of prostate cancer.

Acknowledgments

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References