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No Serological Evidence of Association between Prostate Cancer and Infection with Herpes Simplex Virus Type 2 or Human Herpesvirus Type 8: A Nested Case-Control Study

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Sexual history has consistently been found to be a risk factor for the development of prostate cancer. An association between prostate cancer and herpes simplex virus type 2 (HSV-2) or Kaposi sarcoma-associated herpesvirus/human herpesvirus type 8 (HHV-8) infections has also been reported. Linkage of data on a cohort of 20,243 healthy Finnish men identified 165 cases of prostate cancer that were diagnosed up to 24 years after donation of a serum sample. Two control subjects were matched by age, sex, and municipality of residence to each case patient. Serum levels of immunoglobulin G against HSV-2 and HHV-8 were determined. Neither HSV-2 infection (odds ratio [OR], 0.93 [95% confidence interval {CI}, 0.44–1.96]) nor HHV-8 infection (OR, 0.74 [95% CI, 0.19–2.88]) was associated with prostate cancer.

Sexual history and sexually transmitted infections (STIs) have consistently been found to be risk factors in the etiology of prostate cancer [1]. Direct evidence of the involvement of an infectious agent in prostate cancer has been sought for many years.

Human papillomavirus (HPV)—in particular, types 16 and 18—is recognized as the primary cause of intraepithelial neo-

plasias of the anogenital tract, which are precursor lesions of anogenital cancers (most notably cervical cancer) [2]. Although several studies have found an association between HPV infection and prostate cancer, the association has not been consistently found [3, 4].

Herpes simplex virus type 2 (HSV-2) has been reported to infect prostate tissue [5]. However, there are technical difficulties in conducting virus detection studies. Furthermore, these studies typically lack control samples of prostate tissue from healthy persons. Serological studies may be preferable for studying the possible association between infectious agents and prostate cancer, because serum samples can be easily obtained from both case patients and healthy control subjects and because serological markers for past or present infection exist. Seroprevalences of pathogens that cause STIs are also commonly used as objective markers of sexual history in populations [6]. An increase in HSV-2 antibodies in patients with prostate cancer has been reported in several studies [7].

Kaposi sarcoma-associated herpesvirus/human herpesvirus type 8 (HHV-8)—which is the cause of Kaposi sarcoma, primary effusion lymphoma, and multicentric Castleman disease [8]—has been reported to be present in prostate tissue [9]. HHV-8 is transmitted through sexual intercourse, but it may also have other modes of transmission [6]. A recent case-control study performed in both the United States and Tobago reported a highly significant association between prostate cancer and HHV-8 seropositivity (odds ratio [OR], 2.24 [95% confidence interval {CI}, 1.3–3.9] in Tobago; OR, 4.67 [95% CI, 1.9–11.6] in the United States) [10]. However, case-control studies may be subject to selection biases and reverse causality biases, and it is therefore important to investigate the consistency of this association using studies that have strong epidemiological designs. In the present study, we report results from a prospective and population-based nested case-control study of prostate cancer risk in relation to HSV-2 and HHV-8 infection assessed by serological methods.

Materials and methods. Between 1968 and 1972, the Mobile Clinic of the Social Insurance Institution of Finland performed a health screening examination—which included a health interview (including questions about marital status and smoking status), some routine physiological measurements (such as body-mass index), and collection of a serum sample—on 20,243 men residing in most parts of Finland [11]. Linkage of data in the serum bank with that in the files of the nationwide Finnish Cancer Registry led to the identification of 165 cases of prostate cancer that were diagnosed

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during follow-up through the end of 1991. The histological types of prostate cancer identified were adenocarcinoma ($n = 127$), unspecified carcinoma ($n = 14$), transitory carcinoma ($n = 1$), and no reported histological type ($n = 23$). The mean time between the health screening examination and the diagnosis of prostate cancer was 17 years (range, 1–24 years). Serum volumes permitting successful analysis were not available for 2 case patients and 2 control subjects.

Two control subjects were matched to each case patient by sex, age, and municipality of residence. Control subjects were free of any cancer at baseline and remained alive and free of prostate cancer up to the time of diagnosis of cancer in the corresponding case patient. Because of the way the cohort was assembled, matching for the municipality of residence resulted in matching for the time of the sample collection. In total, 290 control subjects with serum samples were included in the study. Age was matched as closely as possible: in 129 sets of case patients and control subjects, ages were exactly matched; 27 sets had an age discrepancy of a maximum of 3 years; and 9 sets had an age discrepancy of up to 11 years. Mean ages at the health screening examination were 58.6 years in case patients and 58.3 years in control subjects (range, 18–78 years).

The presence of antibodies specific for HSV-2 was determined using an established ELISA [12]. Microtiter plates were coated with HSV-2 antigen from infected cells and negative antigen from cell cultures not inoculated with virus. Cross-reactivity with HSV-1 was blocked with rabbit anti-HSV-1 (diluted to a concentration of 1:8 in 10% horse serum in PBS [HS-PBS]; DAKO) at room temperature for 2 h. Human serum samples in dilutions of 1:31.6, 1:100, and 1:316 in HS-PBS were added. After incubation with horseradish peroxidase-conjugated rabbit anti-human IgG (diluted to a concentration of 1:1000 in HS-PBS; DAKO) at room temperature for 1 h and addition of substrate, the absorbance at 415 nm was measured. The absorbance value for each serum sample incubated with negative antigen was subtracted from the absorbance value for each serum sample that had HSV-2 reactivity. The absorbance values were then transformed into ELISA units by use of the parallel line method [13]. Each set of experiments included a validation panel of HSV-1-positive serum samples from children (that were always negative for HSV-2 reactivity). An initial comparison of the results of this ELISA with the results of ELISAs that used purified glycoprotein G from HSV-2 (gift from T. Bergström) found that the infected-cell ELISA with anti-HSV-1 blocking had identical specificity but slightly better sensitivity, as determined with validation panels of serum samples from individuals with isolation-verified HSV-2 infections.

Antibodies against HHV-8 were detected by an ELISA that used the HHV-8 small viral capsid protein encoded by open-reading frame (ORF) 65. The ORF65 protein is highly immunogenic in humans and is the serological marker for HHV-

8 that is most widely established to be HHV-8 specific [8]. The ORF65 protein (gift from T. F. Schulz) was diluted to a concentration of 1:1200 in 100 mmol/L NaHCO₂ (pH 9.6), plated onto microtiter plates (Costar), and incubated at room temperature overnight. The plates were blocked with 5% powdered milk in PBS at room temperature for 2 h. Serum samples were incubated at a dilution of 1:100 in 5% powdered milk in PBS at room temperature for 1 h, and then bound IgG was detected by a 2-step ELISA that used monoclonal antibody against IgG (γ -chain specific, diluted to a concentration of 1:800; Eurodiagnostica) and a horseradish peroxidase-conjugated goat antibody to mouse IgG (diluted to a concentration of 1:2000; Southern Biotechnology). The substrate was added, and the mixture was incubated for 2 h at room temperature. The absorbance value measured at 415 nm for each serum sample incubated with buffer was subtracted from the absorbance value measured at 415 nm for each serum sample incubated with antigen. Experiments that had positive results were repeated at least twice. The interassay coefficient of variation of the absorbances was 20.5%.

The sensitivity and specificity of the HHV-8 ELISA were evaluated using a positive control panel consisting of serum samples from 40 patients with AIDS and Kaposi sarcoma [14] and a negative control panel consisting of serum samples from 50 healthy subjects who had >2 sex partners over their lifetime and were seronegative for a panel of other pathogens that cause STIs (HSV-2, *Chlamydia trachomatis*, HPV16, HPV18, and HPV33) [6]. We determined the cutoff value for classification of a negative response to HHV-8 to be 0.123 absorbance units, a level at which all negative control samples were negative but 21 positive control samples were positive. The sensitivity of the HHV-8 test was thus 48%, and the specificity was 100%, which is comparable to what has been reported in other studies [8, 14, 15].

The serum samples were analyzed by examiners without knowledge of the identity of the samples, and the results were transmitted to the Social Insurance Institution of Finland, where the code was broken. The relative risks of developing prostate cancer in case patients and control subjects who were seropositive for HSV-2 or HHV-8 were estimated as odds ratios (ORs), which were calculated using conditional logistic regression.

Results. Only 3 of 163 (1.8%) case patients and 7 of 288 (2.4%) control subjects (table 1) were seropositive for HHV-8. The OR of developing prostate cancer in case patients and control subjects who were seropositive for HHV-8 was 0.74 (95% CI, 0.19–2.88) (table 1).

The proportion of case patients and control subjects who were seropositive for HSV-2 was also small, and there were no appreciable differences between case patients and control subjects in their rates of seropositivity (table 1). The OR of developing prostate cancer in case patients and control subjects who were seropositive for HSV-2 was 0.93 (95% CI, 0.44–1.96).

Table 1. Risk of developing prostate cancer in men seronegative or seropositive for herpes simplex virus type 2 (HSV-2) or Kaposi sarcoma–associated herpesvirus/human herpesvirus type 8 (HHV-8).

Virus, serological status	No. of		OR (95% CI)
	Case patients (n = 163)	Control subjects (n = 288)	
HSV-2			
Seronegative	152	268	1
Seropositive	11	20	0.93 (0.44–1.96)
HHV-8			
Seronegative	160	281	1
Seropositive	3	7	0.74 (0.19–2.88)
HHV-8 serological status corrected for misclassification			
Seronegative	157	274	1
Seropositive	6	14	0.74 (0.23–2.12)

NOTE. The risks for developing prostate cancer were estimated as odds ratios (ORs) and were calculated using conditional logistic regression. Correction for the low sensitivity of the HHV-8 assay was performed by dividing positive counts by the assay sensitivity. For the misclassification-corrected analyses, the ORs could be calculated only by using unconditional logistic regression. CI, confidence interval.

Although the sensitivity of the HHV-8 ELISA (~50%) was in accordance with that in previous studies [8, 14, 15], the effect of the low sensitivity was a concern, and we therefore performed a correction for misclassification. The resulting OR was quite close to the uncorrected OR (table 1).

To investigate whether seropositivity for pathogens that cause STIs, as a more general marker of sexual history, would be associated with the risk of developing prostate cancer, we combined the present data on HSV-2 and HHV-8 seropositivity with data published elsewhere on seropositivity for pathogens that cause STIs (*Chlamydia trachomatis*, HPV16, HPV18, and HPV33) in the same serum samples [3]. The point estimates of the risk of developing prostate cancer were above unity, both when being seropositive for 1 pathogen was compared with being seropositive for no pathogens (OR, 1.25 [95% CI, 0.80–1.97]) and when being seropositive for >1 pathogen was compared with being seropositive for no pathogens (OR, 1.38 [95% CI, 0.50–3.79]) (table 2). However, the ORs were not significantly elevated, and there was also no significant trend for an increasing risk with an increasing number of seropositive responses ($P > .1$).

Discussion. An association between prostate cancer and STIs such as HSV-2 and HHV-8 infections has been reported in several case-control studies [7, 10]. Our nested case-control study, performed within a prospectively followed population-based cohort, found no evidence for an association between serological markers of HSV-2 or HHV-8 infection and the risk of developing prostate cancer. Although several studies have found evidence of HSV and/or HHV-8 DNA in prostate tissue, these observations have not been reported consistently [1]. Possible reasons for discrepancies in observations include differences in sensitivities, specificities, and/or reproducibility of as-

says and epidemiological sources of bias, such as confounding, selection, and reverse causality biases.

The prospective serum bank–based study design, when used in countries that have complete nationwide case ascertainment, minimizes most epidemiological sources of bias. For example, reverse causality biases are not likely to occur when there are long follow-up times between the obtainment of serum samples and the diagnoses of cancer, and selection biases because of incomplete attendance or inadequate study base definitions are also unlikely. It should be noted, however, that only a small proportion of subjects in our study were seropositive for either virus, and we thus had rather limited statistical power to detect any possible differences between case patients and control subjects. Although the present cohort study is one of the largest to investigate a possible association between prostate cancer and HSV-2 or HHV-8 infection, we cannot exclude the possibility that associations may exist in more highly exposed populations.

The low seroprevalences of HSV-2 and HHV-8 can be attributed to the assemblage of the cohort in 1968–1972, at a

Table 2. Risk for developing prostate cancer related to seropositive responses to pathogens that cause sexually transmitted infections

No. of seropositive responses	No. of		OR (95% CI)
	Case patients	Control subjects	
0	109	207	
1	47	70	1.25 (0.80–1.97)
2–4	7	9	1.38 (0.50–3.79)

NOTE. Pathogens tested were human papillomavirus (HPV) 16, HPV18, HPV33, herpes simplex virus type 2, human herpesvirus type 8, and/or *Chlamydia trachomatis*. CI, confidence interval; OR, odds ratio.

time when the increase in STIs that occurred in other countries in association with the sexual revolution had not yet occurred in Finland. In the general population of Scandinavia, we have reported elsewhere that the seroprevalences of HSV-2 and HHV-8 in recent years were ~10%–20% and 5%–15%, respectively [6].

Our understanding of the etiology of prostate cancer is still limited, and it is likely that several factors are involved and may be interacting. Although our study provides evidence against an involvement of HSV-2 or HHV-8 in prostate carcinogenesis, it is possible that other infectious agents may be involved. Because interactions with other possible cofactors—such as androgen metabolism, androgen receptors, genetic polymorphisms, and other environmental factors—are likely, additional studies that investigate all these factors at once may be warranted.

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