

Human Papillomavirus 16, 18, and 33 Infections and Risk of Prostate Cancer: A Nordic Nested Case-Control Study

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Abstract

Epidemiologic evidence of sexual history has emerged as a consistently found risk factor for prostate cancer. Some studies have reported an association between human papillomavirus (HPV) infections and prostate cancer. We did a nested case-control study within cohorts of more than 200,000 men enrolled in three Nordic biobanking projects. Follow-up using cancer registry linkages identified 804 prospectively occurring prostate cancer cases. Four control subjects per case were randomly selected from eligible sets of matched subjects that were alive and free of cancer at the time of diagnosis of the corresponding case and were matched to cases on biobank cohort, age (± 2 years), county of residence, and date of blood sampling (± 2 months in the

Finnish and Swedish cohorts, ± 6 months in the Norwegian cohort). The serum samples were analyzed by standard ELISAs for the presence of immunoglobulin G antibodies against HPV types 16, 18, and 33. The joint HPV-16/HPV-18/HPV-33 seroprevalence in the joint cohort was 13.4% (107 of 799) among cases and 14.0% (363 of 2,596) among controls (odds ratio, 0.94; 95% confidence interval, 0.74-1.19). There were no noteworthy differences when the data were analyzed by different HPV type, country, or antibody levels. Our data do not support an association between serologic markers of HPV-16, HPV-18, and HPV-33 infections and risk of prostate cancer. (Cancer Epidemiol Biomarkers Prev 2005;14(12):2952-5)

Introduction

A number of epidemiologic studies of prostate cancer have found a positive association between risk of prostate cancer and indices of sexual risk-taking behavior (e.g., age at first intercourse and lifetime number of sexual partners) and history of sexually transmitted infections (1, 2). However, it is difficult to obtain accurate information on sexual risk taking behavior. In addition, sexual history is not directly related to exposure to specific infectious agents and such associations may vary between populations. An involvement of specific sexually transmitted infections in prostate cancer has been sought for decades. Positive associations have been reported with gonorrhea, syphilis, and human papillomavirus (HPV) infections (1, 3-6). There has been a special interest in HPV infections as a possible prostate cancer risk factor because of their link to cervical carcinoma (7, 8) and other anogenital tumors (8). HPV types 16 and 18 are the major oncogenic HPV types (7, 8). HPV-18, in particular, has tropism for glandular epithelium and can immortalize prostate epithelial cells (9). Several studies have reported high rates (up to 100%) of detection of HPV DNA in prostate cancer cells by PCR (10-14)

but other studies have not reproduced this finding, even reporting no HPV DNA at all (15, 16).

Because of the difficulties to obtain representative tumor samples with intact DNA and without PCR contaminations, serologic studies offer an alternative with readily standardized sampling from both cases and controls. In addition, because prior exposure is not necessarily reflected in current presence of HPV DNA, serology can provide a means to measure the cumulative exposure to the virus.

Several seroepidemiologic studies of HPV infection in relation to prostate cancer have been done (3, 4, 17, 18). A previous nested case-control study found increased relative risk for prostate cancer associated with HPV-18 (3) whereas some case-control studies found a tendency for association with HPV-16 (3, 18, 19). A population-based case-control study found an association with HPV-33, but not with HPV-16 or HPV-18 (4). There are also case-control studies that found no association of prostate cancer with antibodies to HPV-16 or HPV-18 (18, 20).

To further investigate the association of HPV serology with prostate cancer risk, we did a considerably larger case-control study nested in three prospectively followed Nordic biobank cohorts.

Materials and Methods

Cohorts. Cohorts of more than 200,000 men are included in the biobank network "Nordic Biological Specimen Banks working group on Cancer, Causes and Control." The cohorts and their study design have previously been described in detail (21). Briefly, the Janus project in Norway contains serum samples, stored at -25°C , from $\sim 160,000$ men who participated in population-based health examinations, mostly for

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cardiovascular diseases. The biobanks also contains a blood donor cohort, ~10% in size compared with the health examination cohort. The Finnish cohort contained ~19,000 men participating in the Helsinki Heart Study, a clinical drug trial. Serum was drawn from all participants at first screening visit and stored at -20°C . In the Swedish cohort, ~30,000 men have been recruited to the Northern Sweden Health and Disease Study as a representative population sample from the counties of Västerbotten and Norrbotten in Northern Sweden and plasma samples were stored at -80°C . The characteristics of the subjects enrolled in the study are described by cohort in Table 1. In the Finnish and Swedish cohorts, measurements of body mass index ($= \text{weight} / \text{height}^2$) were available. Each respective local research ethics committee approved the study.

Case Ascertainment and Control Selection. The distribution of cases and study characteristics is described in Table 1. All incident cases of prostate cancer and all cases of death in the cohorts were identified through the national or regional cancer and mortality registries. The local tumor stage according to Unio Internationale Contra Cancrum was used (22). Four controls were randomly selected for each case from sets of all subjects alive and free of cancer at the time of diagnosis of the case and were matched to cases on cohort, age (± 2 years), county of residence (only in Norwegian and Swedish cohorts), and date of blood sampling (± 2 months in the Finnish and Swedish cohorts, ± 6 months in the Norwegian cohort).

Laboratory Methods. The serum samples were analyzed at the Department of Medical Microbiology, Malmö University Hospital. The Finnish and Norwegian samples consisted of serum and the Swedish samples consisted of heparin plasma. No one in the analyzing laboratory had any knowledge about the identity of the samples.

HPV seropositivity was determined by the standard ELISA assay using baculovirus-expressed capsids (23) with disrupted capsids of bovine papillomavirus as negative control (24, 25). The HPV-16 capsids were obtained from Dr. John T. Schiller (National Cancer Institute, Bethesda, MD) and the HPV-18 and HPV-33 capsids were obtained from Dr. Martin Sapp (University of Mainz, Mainz, Germany). To ensure that the cutoff levels used to assign positivity from continuous values were representative, the evaluation was based on cutoff levels already established from previous work. A cutoff level of 0.100 absorbance unit for HPV-16 was able to distinguish HPV-16-infected and virgin women (26). Patients with cervical

cancer and normal subjects were, however, more clearly distinguished using a higher cutoff level (which, relative to internal standards, corresponds to 0.277 absorbance unit in the present study; ref. 24) presumably because rapidly cleared (transient) infections induced lower levels of antibodies than do persistent infections (27). For the other HPV types, comparable alternatives of cutoff levels for HPV-18 (low, 0.100; high, 0.200) and HPV-33 (low, 0.113; high, 0.224) absorbance units were used.

There was a high specificity of the serology for the sexually transmitted HPV types because no antibodies could be found in panels of serum samples from virginal women analyzed in parallel with the sera of the present study (26).

Statistical Analysis. The serum samples were analyzed without knowledge of the identity of the samples and the results were transmitted to the Finnish Cancer Registry where the code was broken and the odds ratio (OR) of developing prostate cancer given HPV-16, HPV-18, and HPV-33 seropositivity was estimated using conditional logistic regression.

Results

The ORs for the development of prostate cancer among HPV-seropositive men in the Nordic countries are shown in Table 2. The HPV seroprevalence between cases and controls did not show any statistically significant differences in the full cohort (Table 2). Among the cases in the joint cohort, the HPV-16 seroprevalence was 5.9% (47 of 799) whereas it was 6.2% (160 of 2,596) for the controls. The HPV-33 seroprevalences were also similar among cases and controls (Table 2). The HPV-18 seroprevalence was 2.9% (23 of 799) among the cases and 3.6% (95 of 2,595) among controls. Analysis of the association with prostate cancer risk in each cohort separately did not reveal any significant differences (Table 2). There was a substantial variation of HPV-18 seroprevalences among controls, resulting in the associated ORs for prostate cancer ranging from 0.55 (Finland) to 2.00 (Sweden). The Swedish result was attributable to a very low prevalence of 1% in the HPV-18 infection among the population-based controls, which is unusually low in comparison with both the two other countries and the two other HPV infections (Table 2).

To investigate whether the risk of prostate cancer is associated with high antibody levels, an alternative higher cutoff level was used. We found no evidence of association

Table 1. Characteristics of prostate cancer cases occurring in Nordic biobank cohorts

Characteristics	All cohorts, n (%)	Norway, n (%)	Finland, n (%)	Sweden, n (%)
Age at enrollment (y)				
<45	184 (23)	171 (30)	12 (9)	1 (1)
45-50	401 (50)	353 (61)	39 (28)	9 (10)
51-60	206 (26)	48 (8)	89 (64)	69 (79)
>60	13 (2)	5 (1)	—	8 (9)
Lag time between enrollment and diagnosis (y)				
<1	15 (2)	1 (0)	1 (1)	13 (15)
1-5	99 (12)	24 (4)	15 (11)	60 (69)
6-10	117 (15)	60 (10)	43 (31)	14 (16)
>10	573 (71)	492 (85)	81 (58)	—
Calendar year of recruitment				
Before 1985	690 (86)	550 (95)	140 (100)	—
1985-1989	35 (4)	26 (5)	—	9 (10)
After 1989	79 (10)	1 (0)	—	78 (90)
Tumor stage				
Localized	539 (67)	409 (71)	67 (48)	63 (72)
Nonlocalized	192 (24)	140 (24)	37 (26)	15 (17)
Not determined	73 (9)	28 (5)	36 (26)	9 (10)
Total number of cases	804	577	140	87

NOTE: Controls are not presented as they were matched to cases on age and date of recruitment.

Table 2. Odds ratios for the development of prostate cancer among HPV-seropositive men in the Nordic countries

Infectious agent/ cutoff level	Finland		Norway		Sweden		All cohorts	
	Cases/controls	OR (95% CI)	Cases/controls	OR (95% CI)	Cases/controls	OR (95% CI)	Cases/controls	OR (95% CI)
HPV-16								
0-0.99	132/466	Reference	541/1,651	Reference	79/319	Reference	752/2,436	Reference
0.100+	4/32	0.44 (0.15-1.26)	35/101	0.97 (0.64-1.46)	8/27	1.18 (0.53-2.66)	47/160	0.90 (0.64-1.26)
HPV-18								
0-0.99	132/470	Reference	559/1,688	Reference	85/342	Reference	776/2,500	Reference
0.100+	4/28	0.55 (0.19-1.60)	17/63	0.81 (0.47-1.40)	2/4	2.00 (0.37-10.92)	23/95	0.79 (0.49-1.26)
HPV-33								
0-0.112	129/472	Reference	539/1,633	Reference	81/320	Reference	749/2,425	Reference
0.113+	7/26	1.04 (0.45-2.44)	38/119	1.00 (0.69-1.47)	6/26	0.89 (0.36-2.22)	51/171	0.99 (0.72-1.38)

between prostate cancer risk and high HPV antibody levels (Table 3). Rather, there was a tendency for inverse association between prostate cancer risk and high HPV-18 antibody levels (OR, 0.49; Table 3).

Finally, we investigated whether HPV antibodies would be associated with prostate cancer risk depending on age at enrollment (<45, 45-50, and >50 years), lag between enrollment and cancer diagnosis (0-5, 6-10, or >10 years), calendar year of enrollment (before 1985, 1985-1989, 1990, or later), and tumor stage at diagnosis (localized, nonlocalized, and unknown). There were no noteworthy differences and only in one case was the null hypothesis of homogeneous ORs rejected, in case of HPV-18 antibodies and stage ($P = 0.04$, likelihood ratio statistic). The HPV-18-related ORs were 0.9 [95% confidence interval (95% CI), 0.5-1.6] for localized cancer, 0.2 (95% CI, 0.1-1.0) for nonlocalized cancer, and 2.1 (95% CI, 0.6-7.2) for cancer with unknown stage. Because 12 independent tests were conducted, it is likely that one borderline significant finding of inhomogeneity could have occurred by chance.

Discussion

An association of prostate cancer with sexual history, particularly sexually transmitted diseases like HPV, has been reported in several studies (1, 3, 4, 6). Our Nordic case-control study, nested within a prospectively followed population-based cohort, found no evidence for association between serologic markers of HPV-16, HPV-18, and HPV-33 infections and risk for prostate cancer. Most of the studies on the effect of HPV infection on prostate cancer risk have been case series using PCR methods to detect the presence of HPV DNA, thus not providing much of evidence on etiology of prostate cancer (10, 11, 13, 28-30). Cross-sectional case-control studies based on detection of HPV DNA have been rather inconsistent (10). Serologic studies based on biobanks with long follow-up were used because they were based on readily standardized samples (serum), all taken from healthy subjects who only later developed disease or remained healthy, thus minimizing risks for biases induced by the disease process affecting sampling or assay performance.

HPV seropositivity is a marker of past or present HPV infection of the body as a whole, although only ~50% to 60% of infected subjects will seroconvert (31). In the case of cervical cancer epidemiology, studies focusing on persistence of the virus have found much higher relative risks for cancer than studies of the risk associated with the infection per se (8) because past infections that have cleared do not seem to be associated with increased risk (8). Possibly, studies of viral persistence as risk factor could have had better possibilities to detect any possible association. On the other hand, our studies addressing exposure per se provide a more readily interpretable measure, unaffected by unknown determinants of viral clearance.

Two seroepidemiologic studies have found positive associations between HPV and prostate cancer although they have reported associations with different HPV types (3, 4). One study found a highly significant association with HPV-18 but no association with HPV-33 (3) whereas the other study found a strong association with HPV-33 but no association with HPV-18 (4). Inconsistent associations with different HPV types seen in different studies could be the result of chance, bias, or confounding by some unknown risk factor that may associate with different HPV infections in different populations.

Our study, to date, is the largest case-control study nested within a cohort on possible etiologic role of HPV-16, HPV-18, and HPV-33 infections on prostate cancer. The fact that no positive associations were seen when a large study with strong study design was used indicates that HPV infection does not increase the risk of prostate cancer. If anything, there was a tendency for HPV-18 to associate with decreased risk [particularly for high HPV-18 antibody levels; OR, 0.5 (95% CI, 0.2-1.1)]. A previous study on HPV and esophagus cancer found that HPV-18 antibodies were protective against esophageal adenocarcinoma [OR, 0.2 (95% CI, 0.1-0.7); ref. 32], a finding that has remained unexplained.

A previous study in the same cohort as the present study found that *Chlamydia trachomatis* exposure was inversely associated with prostate cancer (OR, 0.69; 95% CI, 0.51-0.94). This also speaks against the possibility that sexual history and exposure to sexually transmitted infections would increase the prostate cancer risk (33). Formal meta-analysis of the association of HPV and prostate cancer has reported that, taking the entire literature into account, there is a slight effect [OR, 1.5 (95% CI, 1.1-2.1); ref. 6]. However, the literature is very heterogeneous both in terms of study designs and exposure assessment methods making the reliability of meta-analysis questionable. In addition, the addition of the present large and prospective study to the literature is likely to affect the conclusions of future meta-analysis.

Table 3. Risk of prostate cancer among men with high HPV antibody levels

Infectious agent/ cutoff level	All cohorts	
	Cases/controls	OR (95% CI)
HPV-16		
0-0.276	772/2,516	1 (Reference)
0.277+ HPV-18	27/80	0.99 (0.63-1.57)
HPV-18		
0-0.199	792/2,549	1 (Reference)
0.200+	7/46	0.49 (0.22-1.09)
HPV-33		
0-0.223	769/2,495	1 (Reference)
0.224+	31/101	1.04 (0.68-1.57)

Overall, the body of evidence suggests that there is no increased effect for common sexually transmitted diseases, such as HPV or *Chlamydia*, on prostate cancer risk. This does not exclude that more high-risk sexually transmitted diseases (such as gonorrhea) may be associated (6, 18). However, also, this literature is not entirely consistent (6). By contrast, there are presently no data that would be inconsistent with an inverse association with *Chlamydia trachomatis* and studies that instead investigate the consistency of possible protective associations of sexually transmitted diseases and their possible explanations may be more informative in the continuing elucidation of prostate cancer etiology.

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