Null Results in Brief

Cancer Epidemiology, Biomarkers & Prevention

Human Papillomavirus Types 16, 18, and 31 Serostatus and Prostate Cancer Risk in the Prostate Cancer Prevention Trial

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Abstract

Since human papillomavirus (HPV) infection was first identified as a risk factor for cervical cancer, several seroepidemiologic and tissue-based studies have investigated HPV in relation to prostate cancer, another common genitourinary malignancy, with mixed results. To further inform this potential association, we conducted a large, prospective investigation of HPV types 16, 18, and 31 in relation to risk of prostate cancer in the Prostate Cancer Prevention Trial. Cases were a sample of men diagnosed with prostate cancer after visit 2 or on their end-of-study biopsy (n = 616). Controls were men not diagnosed with prostate cancer during the trial or on their end-of-study biopsy (n = 616). Controls were frequency matched to cases by age, treatment arm, and family history of prostate cancer. Sera from visit 2 were tested for IgG antibodies against HPV types 16, 18, and 31. No associations were observed for weak or strong HPV-16 [odds ratio (OR), 0.94; 95% confidence interval (95% CI), 0.53-1.64 and OR, 1.07; 95% CI, 077-1.48, respectively], HPV-18 (OR, 0.75; 95% CI, 0.27-2.04 and OR, 0.87; 95% CI, 0.47-1.63, respectively), or HPV-31 seropositivity (OR, 0.76; 95% CI, 0.45-1.28 and OR, 1.15; 95% CI, 0.80-1.64, respectively) and risk of prostate cancer. Considering this finding in the context of the HPV and prostate cancer literature, HPV does not appear to be associated with risk of prostate cancer, at least by mechanisms proposed to date, and using epidemiologic designs and laboratory techniques currently available. *Cancer Epidemiol Biomarkers Prev; 19(2); 614–8. ©2010 AACR.*

Introduction

Since human papillomavirus (HPV) infection was first identified as a risk factor for cervical cancer, several studies have investigated HPV in relation to prostate cancer with mixed results (1-7). When Taylor et al. (2) combined the results of 10 of these studies, they observed a significant positive association between HPV and prostate cancer; however, subsequent investigations have observed null associations (3-6) or have detected minimal/no evidence of HPV in prostate tissue (7-12). To further inform HPV and prostate cancer, we conducted a prospective investigation of HPV types 16, 18, and 31 and prostate cancer in the Prostate Cancer Prevention Trial (13). The unique design of this trial allowed us to investigate both HPV and screen-detected cancer among annually screened men as well as HPV and end-of-study biopsy-detected cancer to rule out differential likelihood of screening or biopsy as noncausal explanations for study findings.

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doi: 10.1158/1055-9965.EPI-09-1080

Materials and Methods

Study Design

We conducted a nested case-control study among Prostate Cancer Prevention Trial participants with adequate serum at visit 2 (14). Cases were men with a confirmed diagnosis of prostate cancer after visit 2 (n = 616). Approximately equal numbers of cases diagnosed by "for-cause" and "end-of-study" biopsy were selected as well as equal numbers with low-grade (Gleason sum <7) and high-grade (Gleason sum ≥7) disease. The mean time from blood draw to diagnosis was 3.4 years for "for-cause" cases and 5.0 for "end-of-study" cases. Controls were men not diagnosed with prostate cancer during the trial or on end-of-study biopsy (n = 616). Controls were frequency matched to cases by age, treatment

arm, and family history of prostate cancer and enriched for non-Whites.

This study was approved by the Johns Hopkins Bloomberg School of Public Health and Fred Hutchinson Cancer Research Center institutional review boards.

HPV Antibody Assessment

Sera were tested for IgG antibodies against HPV types 16, 18, and 31 virus-like particles using ELISAs specific for each HPV type (15). Samples were tested in random order, and laboratory personnel were blinded to case-control status. Each sample was tested in duplicate with repeat duplicate testing for duplicates with absorbance (*A*) coefficients of variation >25% and at least one value above the *A* cutoff point for seropositivity. Mean *A* values were calculated based on duplicate test values or based on the mean of the three values in closest agreement for men with repeat duplicate testing. *A* cutoff points of 0.080 (3 SDs above the mean for control children), 0.100 (3 SDs), and 0.065 (5 SDs) were initially used to define seropositivity for HPV types 16, 18, and 31, respectively.

Assay reproducibility was investigated by including 12 sets of approximately six blinded replicate samples each in the testing sequence (14). Eleven sets had 100% and

Table 1. HPV types 16, 18, and 31 antibody distributions for 616 prostate cancer cases and 616 frequency-matched controls in the Prostate Cancer Prevention Trial

	Cases*	Controls	*P*†
HPV-16			
Mean *A*	0.06	0.06	0.60
Geometric mean *A*	0.05	0.05	0.56
Serostatus (%)			
Seronegative ($A \leq 0.080$)	81.3	81.8	0.90
Weak seropositive ($0.080 < A \leq 0.092$)	4.1	4.5	
Strong seropositive ($A > 0.092$)	14.5	13.7	
HPV-18			
Mean *A*	0.04	0.04	0.81
Geometric mean *A*	0.03	0.03	0.87
Serostatus (%)			
Seronegative ($A \leq 0.100$)	95.6	94.8	0.78
Weak seropositive ($0.100 < A \leq 0.117$)	1.1	1.5	
Strong seropositive ($A > 0.117$)	3.3	3.7	
HPV-31			
Mean *A*	0.05	0.05	0.48
Geometric mean *A*	0.04	0.04	0.48
Serostatus (%)			
Seronegative ($A \leq 0.065$)	83.5	83.6	0.41
Weak seropositive ($0.065 < A \leq 0.077$)	4.3	5.6	
Strong seropositive ($A > 0.077$)	12.3	10.8	
HPV types 16, 18, and/or 31			
All seronegative (%)	70.7	70.7	
At least one weak seropositive but no strong seropositives (%)	5.9	7.7	
One strong seropositive (%)	17.7	15.9	0.75‡
Two strong seropositives (%)	4.6	4.8	
Three strong seropositives (%)	1.0	0.9	
At least one strong seropositive (%)	23.4	21.6	0.72§

NOTE: Standardized by age, treatment arm, family history of prostate cancer, and race (non-White versus White) using linear regression.

*Cases were a sample of men diagnosed with prostate cancer on any biopsy after visit 2 or on their end-of-study biopsy (1996-2003).

†*P* values were calculated by linear regression for continuous variables and by generalized logit models for categorical variables.

‡*P* value for the comparison of all seronegatives, at least one weak seropositive but no strong seropositives, one strong seropositive, two strong seropositives, and three strong seropositives.

§*P* value for the comparison of all seronegatives, at least one weak seropositive but no strong seropositives, and at least one strong seropositive.

Table 2. OR (95% CI) of prostate cancer by HPV types 16, 18, and 31 serostatus in 616 prostate cancer cases and 616 frequency-matched controls in the Prostate Cancer Prevention Trial

	HPV-16		HPV-18		HPV-31	
	Cases/ controls	OR* (95% CI)	Cases/ controls	OR* (95% CI)	Cases/ controls	OR* (95% CI)
Total prostate cancer						
Seronegative†	503/502	1.00	590/583	1.00	516/513	1.00
Weak seropositive‡	25/28	0.94 (0.53-1.64)	7/9	0.75 (0.27-2.04)	27/34	0.76 (0.45-1.28)
Strong seropositive§	88/86	1.07 (0.77-1.48)	19/24	0.87 (0.47-1.63)	73/69	1.15 (0.80-1.64)
Prostate cancer diagnosed by for-cause biopsy‖						
Seronegative†	272/502	1.00	313/583	1.00	274/513	1.00
Weak seropositive‡	13/28	0.89 (0.45-1.76)	6/9	1.21 (0.42-3.45)	16/34	0.82 (0.44-1.52)
Strong seropositive§	42/86	0.92 (0.62-1.37)	8/24	0.69 (0.31-1.58)	37/69	1.05 (0.68-1.62)
Prostate cancer diagnosed by end-of-study biopsy¶						
Seronegative†	231/502	1.00	277/583	1.00	242/513	1.00
Weak seropositive‡	12/28	1.01 (0.50-2.06)	1/9	0.22 (0.03-1.72)	11/34	0.65 (0.32-1.31)
Strong seropositive§	46/86	1.25 (0.84-1.86)	11/24	1.08 (0.51-2.28)	36/69	1.27 (0.82-1.99)
Gleason sum <7 prostate cancer						
Seronegative†	261/502	1.00	299/583	1.00	269/513	1.00
Weak seropositive‡	16/28	1.24 (0.65-2.38)	5/9	1.11 (0.36-3.42)	14/34	0.72 (0.38-1.38)
Strong seropositive§	36/86	0.82 (0.54-1.26)	9/24	0.83 (0.37-1.86)	30/69	0.94 (0.59-1.50)
Gleason sum ≥7 prostate cancer						
Seronegative†	242/502	1.00	291/583	1.00	247/513	1.00
Weak seropositive‡	9/28	0.69 (0.32-1.49)	2/9	0.41 (0.09-1.91)	13/34	0.77 (0.40-1.49)
Strong seropositive§	52/86	1.31 (0.89-1.91)	10/24	0.90 (0.42-1.92)	43/69	1.33 (0.88-2.03)
Organ-confined ($\leq T_2N_0M_0$) prostate cancer						
Seronegative†	459/502	1.00	540/583	1.00	473/513	1.00
Weak seropositive‡	24/28	0.98 (0.56-1.74)	7/9	0.83 (0.30-2.25)	26/34	0.79 (0.47-1.35)
Strong seropositive§	83/86	1.10 (0.79-1.53)	19/24	0.95 (0.51-1.77)	67/69	1.16 (0.80-1.67)

NOTE: Cases were a sample of men diagnosed with prostate cancer on any biopsy after visit 2 or on their end-of-study biopsy (1996-2003).

*Calculated by unconditional logistic regression, including terms for age (continuous), treatment arm, family history of prostate cancer, and non-White race.

†HPV-16: $A \leq 0.080$, HPV-18: $A \leq 0.100$, and HPV-31: $A \leq 0.065$.

‡HPV-16: $0.080 < A \leq 0.092$, HPV-18: $0.100 < A \leq 0.117$, and HPV-31: $0.065 < A \leq 0.077$.

§HPV-16: $A > 0.092$, HPV-18: $A > 0.117$, and HPV-31: $A > 0.077$.

‖Refers to a biopsy done because of an elevated prostate-specific antigen concentration or an abnormal digital rectal examination.

¶Refers to a biopsy done without indication after 7 y of participation in the study as part of the study protocol.

1 set had 66.7% agreement for HPV-16, 10 had 100% and 2 had 66.7% agreement for HPV-18, and 10 had 100% and 2 had 83.3% agreement for HPV-31. Based on these data, we defined additional strong seropositive cutoff points to better distinguish likely seronegatives from seropositives [0.092 (>4 SD), 0.117 (>4 SD), and 0.077 (>7 SD) for HPV types 16, 18, and 31, respectively].

Statistical Analysis

Age, treatment arm, family history, and race standardized A means, geometric means, and proportions were calculated by prostate cancer status. Odds ratios (OR) and 95% confidence intervals (95% CI) were calculated by logistic regression adjusting for age, treatment arm, family history, and race. Confounding was investigated by adding terms for ELISA plates, other HPV types, and other variables (14) individually to the model and comparing the results to the base model. Separate analyses were done for prostate cancer diagnosed by for-cause and end-of-study biopsy, low- and high-grade cancer, organ-confined disease, and combinations thereof. Stratified analyses were done to evaluate effect modification.

A priori, we had ≥80% power to detect an OR ≥ 1.6 for a control seroprevalence of 10%.

Results

No differences were observed in the distribution of anti-HPV types 16, 18, and 31 antibodies between cases and controls or when the data for all three HPV types were combined (Tables 1 and 2). Generally null results were also observed after adjustment for potential confounders as well as for prostate cancer diagnosed by for-cause biopsy, Gleason sum <7 cancer, and organ-confined cancer. Very slight, nonsignificant positive findings were observed for HPV types 16 and 31 with cancer diagnosed by end-of-study biopsy and with Gleason sum ≥7 cancer (Table 2). No effect modification was observed by treatment arm, age at cancer diagnosis, family history of prostate cancer, or race.

Discussion

In this large study of older American men, no associations were observed between HPV types 16, 18, and 31 and overall prostate cancer risk. Very slight, nonsignificant positive findings were observed for HPV types 16 and 31 with prostate cancer diagnosed by end-of-study biopsy and Gleason sum ≥7 cancer; the reasons for which are unclear and could reflect chance findings. Our generally null results are consistent with those from most serologic studies conducted to date (3-6, 16-20).

As in most previous serologic studies, we assessed HPV serostatus using virus-like particle ELISAs and serum collected before but near cancer diagnosis, raising the possibility that antibody titers could have diminished since earlier "hit and run" infection(s) or during invasive cancer development. The former possibility was suggested by Strickler and Goedert (1) based on discrepancies between observed positive associations in studies that collected serum decades before diagnosis (21, 22) and null associations in studies that collected serum at diagnosis (16, 17), whereas the latter was suggested by Rosenblatt et al. (20) based on stronger observed serologic associations for *in situ* than invasive HPV-associated cancers, which are likely closer in time to productive (capsid-expressing) HPV infections than invasive cancers. Since these studies were conducted, however, null results were observed in another study with early serum collection (3), and minimal/no evidence of HPV DNA was observed in most recent tissue-based studies (7-12), suggesting that waning capsid antibody titers are unlikely to explain null serologic results.

Thus, viewing the HPV and prostate cancer literature as a whole, HPV does not appear to be associated with prostate cancer risk, at least by mechanisms proposed to date, and using epidemiologic designs and laboratory techniques currently available.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

We thank Robert Dayton, Jr., for preparing serum specimens for testing, Barbara Silver for HPV antibody testing, the Baltimore City Health Department (Dr. Emily J. Erbelding, Vincent Marsiglia, and Sarah Norman) for the generous provision of quality-control serum specimens, Dr. Charlotte A. Gaydos for earlier discussions on the rationale for investigating HPV infection in relation to prostate cancer and serologic testing, and Dr. Catherine M. Tangen for general statistical discussions.

Grant Support

National Cancer Institute, NIH grants P01 CA108964 (Biology of the Prostate Cancer Prevention Trial) and CA37429. The content of this work is solely the responsibility of the authors and does not necessarily represent the official views of the NIH.

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Received 10/15/09; revised 10/27/09; accepted 11/23/09; published online 2/8/10.

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