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High-risk HPV types and head and neck cancer

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Abstract

Although HPV16 has been strongly implicated in oropharyngeal carcinogenesis, the role of other high-risk HPV types in the etiology of head and neck cancer remains unclear. To date, few data exist addressing the nature of the association between antibodies to oncogenic proteins of non-HPV16 HPVs in relation to head and neck cancer. We examined the relationship between multiple HPV types (HPV6, 11, 16, 18, 31, 33, 45, 52, 58) and head and neck squamous cell carcinoma (HNSCC) in a large population-based case-control study (1069 cases and 1107 controls). Serological measures for HPV types included antibodies to L1, E6 and/or E7. In a secondary analysis, we excluded HPV16 seropositive subjects to examine independent associations with other high-risk HPVs. All analyses were adjusted for age, race, sex, education, smoking, and alcohol consumption. Statistically significant associations were observed for HPV16, 18, 33, and 52 and risk of HNSCC after mutually adjusting for HPV types. Among HPV16 seronegative subjects, elevated risks of HNSCC were observed for HPV18 E6 (OR = 4.19, 95% CI = 1.26–14.0), HPV33 E6 (OR = 7.96, 95% CI = 1.56–40.5), and HPV52 E7 (OR = 3.40, 95% CI = 1.16–9.99). When examined by tumor type, associations with HPV18 and HPV33 remained statistically significant for oropharyngeal cancer, and HPV52 was associated with oral cancer. In addition, magnitude of associations for HNSCC increased dramatically with increasing number of seropositive high-risk HPV infections. High-risk HPV types, other than HPV16, are likely to be involved in the etiology of HNSCC.

Keywords

HPV infection; head and neck cancer; oropharyngeal cancer; oral cancer

Introduction

Incidence rates for oral cavity and laryngeal cancers have been decreasing rapidly over the past two decades, while oropharyngeal cancer rates have been increasing by an average of 2.9% annually between 2001–2010¹. The substantial decrease in oral cavity and laryngeal cancer rates, observed in the US and other countries^{2, 3}, is likely due to changes in cigarette smoking patterns, whereas the increase in oropharyngeal cancer rates may reflect changes in behaviors that increase exposure to human papillomavirus (HPV)⁴. The majority (>90%) of oral, pharyngeal and laryngeal cancers (collectively referred to as head and neck cancers) derive from a squamous epithelial origin⁵.

The association between HPV16 and risk of head and neck squamous cell carcinoma (HNSCC), while relatively newly discovered, is well-documented^{6–8}. In addition, HPV16-positive HNSCCs display different risk factors than HPV16-negative cancers, and are likely to be distinct from those caused by tobacco and alcohol^{9, 10}. Although HPV subtypes other than HPV16 are suspected to play a role in HNSCC, given their oncogenic potential and associations with anogenital cancers¹¹, only a few epidemiologic studies have examined HPV types in HNSCC while controlling for HPV16 infection or after excluding HPV16 seropositive subjects^{12, 13}.

We previously published results for HPV types and risk of HNSCC using the first phase of our case-control study⁶; we reported positive associations for HPV6 and 18, adjusting for HPV16. In the current analysis, we include subjects from the second phase of the study (effectively doubling the sample size) and add measures for five additional HPV types (HPV31, 33, 45, 52, and 58). Furthermore, with our large sample size (1069 cases and 1107 controls), we were able to examine high-risk, non-HPV16 HPVs among HPV16 seronegative subjects.

Methods

Study population

Incident cases of HNSCC were identified in the geographic greater Boston area and recruited from head and neck clinics and departments of otolaryngology or radiation oncology at the major teaching hospitals in the region (Brigham and Women's Hospital, Beth Israel Deaconess Medical Center, Boston Medical Center, Dana-Farber Cancer Institute, Massachusetts Eye and Ear Infirmary, Massachusetts General Hospital, and New England Medical Center). Patients with a confirmed incident diagnosis of HNSCC were included if they were residents in the study area and 18 years or older. Recurrent cases and incident cases diagnosed more than 6 months prior to contact were excluded. HNSCC cases consist of those with a diagnosis code of 141, 143–146, 148, 149, or 161 based on the International Classification of Disease, Ninth Revision (ICD-9). Greater than 90% of the incident cases reported to the state cancer registry for the study period in the catchment area were identified and approached for participation. Controls were selected from the same population and frequency-matched to cases by sex, age (+/- 3 yrs), and town of residence using the Massachusetts town lists. The study population for this analysis includes data

collected from two periods of recruitment from the same population: the first period was between December 1999 and December 2003 (Phase I) and the second was between October 2006 and June 2011 (Phase II). Participation rates for cases and controls were 78% and 47%, respectively. A total of 1069 cases and 1107 controls provided blood upon enrollment during the two phases. All cases and controls enrolled in the study provided written informed consent as approved by the Institutional Review Boards of the participating institutions.

To examine disease by site, tumors were classified as oral cavity (ICD-9 codes 141.1–141.5, 141.8, 141.9, 143–145.2, 145.5–145.9, 149.8, 149.9), pharynx (ICD-9 codes 141.0, 141.6, 145.3, 145.4, 146, 148, 149.0, 149.1), or larynx (ICD-9 code 161) in accordance with the recommendations of the American Joint Committee on Cancer. Nasopharyngeal cancers were not included in this study.

HPV serology measurement

Serum from venous blood was separated within 12–24 hr of blood drawing and stored at –80°C. To detect antibodies against HPV L1, E1, E2, E4, E6 and E7 proteins, a glutathione S-transferase capture enzyme-linked immunosorbent assay (ELISA) was used in combination with fluorescent bead^{14, 15}. This assay detects HPV antibodies with high type specificity and demonstrates assay sensitivity similar to the “gold-standard” for L1-serology that uses virus-like particles (VLP) as antigens¹⁵. L1 antibodies were measured for HPV types 6, 11, 16, 18, 31, 33, and 45. E6 antibodies were measured for all except HPV6, and E7 on all except HPV6, 31, 33, and 45. Finally antibodies for E1, E2, and E4 were measured on HPV16 and 18.

Additional exposure assessment

Cases and controls responded to a self-administered questionnaire to collect data on demographic characteristics, medical history (including asthma and allergies), family history of cancer, detailed smoking (including marijuana use) and alcohol consumption habits, occupational history, and residential history. Questionnaires were provided to cases during their initial clinic visit and were mailed to controls, and were returned and reviewed by study personnel during the second visit for cases and first in-person visit for controls.

Statistical Analysis

Case and control differences across baseline characteristics were assessed using ANOVA and controlling for sex and age. Odds ratios (OR) and 95% confidence intervals (CIs) were estimated for HPV status using unconditional logistic regression and controlling for known risk factors, including age, sex, race (white or other), smoking status (ever/never), smoking (pack-years as a continuous variable), average alcohol drinks per week (continuous), education (less/high school graduate or more), and study phase (I/II). We applied multiple imputation methods using age and sex to assign values to missing values for race (0.2% missing), education (7% missing), alcohol (7% missing), marijuana (7%) and pack-years (6.7% missing); using multiple imputation for missing data reduces bias^{16, 17}. Additional control for body mass index (BMI), income (below/above \$50K per year), or marijuana use, did not change the observed associations and were left out of the final model. To examine if

different subtypes of HPV were associated with HNSCC independently of HPV16 infection, we conducted additional analyses among those who were HPV16 seronegative for E6, E7 or L1 (611 cases; 967 controls). In addition, we conducted analyses to examine whether there are any multiplicative interactions with HPV16 seropositive status and high-risk HPV types, as well as with allergy history; tests for interaction were conducted using cross-product terms in the logistic regression models. Finally, to examine the role of co-infections, we examined associations for multiple infections.

All analyses were conducted in R (Version 2.14) and all tests were two-sided. Multiple imputations were carried out using the R package *mi*¹⁸.

Results

The distributions of known risk factors of HNSCC by case-control status for this study have been previously published¹⁹. Briefly, smoking, alcohol consumption, and HPV16 seropositivity were higher among cases than controls, and controls were more educated than cases. The mean age was 60.7 years among cases and 59.4 among controls, 74% of subjects were males, and most subjects were white (92% cases and 91% controls). The prevalence of HPV16 seropositivity for E6/E7 antibodies among controls was 6.8%, and 8% for other high-risk HPV types (Table 1); about 1% of controls were positive for both HPV16 and other high-risk HPV types. Controls were more likely to be seropositive for HPV16 or other HPV types if they were older, women, non-Caucasian, high-school graduates (vs. some college), and smokers (for HPV16 only). While HPV16 seropositive controls were more likely to be high alcohol consumers, the opposite trend was observed for other high-risk HPV types.

Table 2 provides population characteristics by case/control status. As expected, smoking and alcohol, and HPV16 were strongly associated with case status. In addition, cases were less educated than controls, and had a slightly earlier age at first intercourse (p-value 0.02). Table 3 provides correlation coefficients between different HPV types; HPV33 and HPV58 were mostly strongly correlated with HPV16 ($r=0.41$ and $r=0.27$, respectively).

In the main analyses, we observed positive associations for HNSCC with all HPV subtypes after adjusting for HPV16 seropositivity for non-HPV16 subtypes (although HPV31 and HPV45 were not statistically significant; Table 4). After mutually controlling for all HPV types, statistically significant associations remained for all HPV16 viral protein antibodies, HPV18 E6, HPV33 E6, and HPV52 E7 (Table 4). With the exception of HPV31, all HPV types were strongly associated with pharyngeal cancer (Table 5; HPV16 E6 seropositive: OR = 81.2, 95% CI = 51.1–128.9). For oral cavity cancers, statistically significant 2-fold or greater increases in risk were observed for HPV16 (HPV16 seropositive, E6: OR = 6.76, 95% CI = 3.95–11.6; E7: OR = 2.93, 95% CI = 1.87–4.59), HPV18 E6, HPV33 E6, HPV52 E7 and HPV58 E6 (Table 5). For laryngeal cancers, statistically elevated risks were observed for HPV16 (E6: OR = 5.64, 95% CI = 2.89–11.0; E7: OR = 2.95, 95% CI = 1.65–5.26), and HPV33 (E6: OR = 14.2, 95% CI = 4.43–45.8); Table 5). Including L1-positive serology in the analysis (vs. only E6/E7 positivity) increased the overall number of subjects who were HPV seropositive (e.g., 12.5% of controls positive for HPV16 E6, E7 or L1 vs.

6.8% of controls positive for HPV16 E6 or E7), but attenuated the associations between the different HPVs and risk of HNSCC (e.g., HPV16 OR = 1.40, 95% CI = 1.33–1.47).

Since the primary association with non-16 HPV types was of interest, we sought to remove any potential residual confounding by HPV16 infection by conducting a secondary analysis excluding all subjects with HPV16 seropositivity for L1, E6 and E7 (n=598). In this secondary analysis with 1578 subjects, an 8-fold increase in HNSCC risk was observed for HPV33 E6, a 4-fold increase in HNSCC risk for HPV18 E6, and a 3-fold increase in HNSCC risk for HPV52 E7 (Table 6). While most of the increase in HNSCC risk associated with non-HPV16 types was driven by pharyngeal cancer (e.g., HPV33 E6: OR = 18.9, 95% CI = 3.22–110.8; table 7), a statistically significant association was also observed for HPV52 and oral cavity cancer (E7 seropositive, OR = 4.79, 95% CI = 1.47–15.6) (Table 7).

We investigated whether there was any evidence of an interaction between HPV16 seropositivity and high-risk non-HPV16 types. A multiplicative interaction was observed between HPV16 E6 and high-risk HPV ($p=0.007$); subjects with a positive serology for HPV16 and non-HPV16 high-risk HPV E6 types had a 85-fold increase in risk of HNSCC compared to those with no high-risk HPV infection (OR = 84.6, 95% CI = 20.3–352.4), while positive serology for HPV16 E6 or other high-risk HPV E6 alone conferred lower increases in risk (HPV16 E6 only, OR = 20.5, 95% CI = 12.8–33.0; high-risk non-HPV16 E6 only, OR = 1.80, 95% CI = 1.17–2.76). Similarly, we observed a dose-response increase in risk with additional high-risk HPV infections (OR = 5.04, 95% CI = 3.85–6.59, for seropositive with 1 high risk HPV; OR = 19.3, 95% CI = 10.5–36.0, for seropositive with 2 high risk HPVs; OR = 169, 95% 23–1226, for seropositive with 3 or more HPVs compared to no seropositive HPV infections).

Since we^{19, 20} and others^{21–23} have shown that a history of allergy and a history of periodontal disease are independently associated with head and neck cancer risk (and since a humoral response could play a role in disease among those infected with HPV), we examined the data for evidence of a potential interaction between these two medical conditions and high-risk HPV infection (including HPV16). However, we found no evidence of any significant interaction between history of allergy or history of periodontal disease with HPV infection (data not shown).

Discussion

In this large US case-control study, we observed positive associations for several high-risk, non-HPV 16 types (i.e., HPV18 E6, HPV33 E6, HPV52 E7) and risk of HNSCC among seronegative HPV16 individuals. Statistically significant associations were observed for HPV18 E6, HPV33 E6 and HPV58 E7 and pharyngeal cancer, but not for other tumor sites. However, a statistically significant association was observed for HPV52 E7 and risk of oral cavity cancer. Finally, we observed a strong interaction between HPV16 E6 and other high-risk non-HPV16 types (E6), and increasing magnitude of risk for HNSCC with additional number of high-risk HPV seropositive infections.

The largest study, to date, to examine serology for non-HPV16 types (HPV6, 11, 18, 31, 33, 35, 45, 52, 58) and risk of HNSCC, reported statistically significant positive associations for HPV31, 33, 35, 52, and 58; moreover, after excluding subjects seropositive for HPV16 E6/E7, a strong association remained for HPV52 and oropharyngeal cancer (OR = 9.15, 95% CI = 1.87–44.8)¹². Statistically significant associations for non-HPV16 types, among HPV16 seronegative subjects, were also observed in a large pooled case-control study (ARCAGE) for HPV18 E6 and HPV52 E7 and risk of oropharyngeal cancer¹³. Serology for HPV58 was not measured in the ARCAGE study, and no increase in risk was observed with HPV33 E7; however a statistically non-significant positive association was observed for HPV33 L1 and oropharyngeal cancer (OR = 1.97, 95% CI = 0.78–4.97)¹³. In a European prospective cohort study, a statistically significant association between HPV33 E6 positive serology and risk of larynx cancer was observed among HPV16 seronegative subjects²⁴, which is consistent with the 7-fold increased risk for laryngeal cancer in this study, although our finding was not statistically significant (p=0.07; Table 5). However, small numbers of oropharyngeal cases limited power to analyze non-HPV16 associations among seronegative HPV16 subjects in that cohort study²⁴.

Three additional studies reported positive associations for HNSCC risk (overall or for oropharyngeal) and serology of non-HPV 16 types (L1/L2 antibodies), including HPV18, 31²⁵, 33²⁶, and 35⁹, although another study reported no associations for HPV18, 33, and 73²⁷. In a previous report based on Phase I of this case-control study, we found positive associations for both HPV6 (a ‘low-risk’ virus) and HPV18 and risk of HNSCC after adjusting for HPV16 seropositivity⁶. In our current analysis, which includes an additional 521 incident HNSCC cases (and 559 controls), we observed a 54% increase risk for HPV6 L1 after controlling for HPV16, but the association was no longer present after controlling for other HPV types (Table 3).

Associations between HPV16 and oropharyngeal cancer have been consistently observed, and HPV16 has been confirmed by IARC as Group 1 carcinogen (evidence is convincing) for oropharyngeal and oral cancer²⁸. In contrast, HPV16 has been less consistently linked to laryngeal cancer and the 2012 IARC monographs concludes that “evidence is not conclusive” for this cancer²⁸. In this study, we observed statistically significant association for HPV16 and laryngeal cancer, suggesting that HPV infection is indeed etiologically important in this tumor type; strong associations with HPV16 and laryngeal cancer were also recently reported in the ARCAGE study¹³.

Viral proteins produced by HPVs are known to interact with key tumor suppressor proteins, such as p53 and RB, resulting in dysregulation of cell cycle control and delayed epithelial cell differentiation¹¹. In addition, expression of viral oncoproteins E6 and E7 can lead to genomic instability, which may contribute to HPV persistence and accumulation of oncogene mutations²⁹. HPVs also have a unique ability to evade the immune response, using a battery of different mechanisms, including blocking interferon activity, establishing cytotoxic T lymphocyte (CTL) tolerance, reducing CD8⁺-mediated response by downregulating IL18 expression, and disrupting antigen processing and presentation in antigen-presenting cells (APCs)³⁰. Mechanisms by which different HPV early proteins interact with host proteins and impact the immune response and other cellular processes are

still being unraveled. New data suggest that similar viral proteins (e.g., E7) may behave differently within the same HPV genus³¹. Consequently, the presence of more than one type of high-risk HPV could provide opportunity for tumorigenesis through interaction of multiple viral proteins having different impacts on the host; this may explain the 85-fold increase in HNSCC risk that we observed for combined infection with high-risk HPVs and HPV16 and for the increase in magnitude of risk with additional number of infections.

The strengths of this study include the second largest study to date measuring multiple high-risk HPV types and risk of head and neck cancer. Furthermore, detailed data on potential confounders, including smoking, alcohol consumption and HPV16 status, allowed for examination of independent associations and potential interactions by these risk factors. Seroprevalence of HPV16 infection based on serology in this population was as expected and similar to previous studies^{9, 26}. Serology-based measures of HPV16 (E6/E7) have been shown to have high specificity for oncogenic HPV infections and provide similar or better estimates of clinical outcome in the oropharynx when compared with DNA based measures^{32, 33}. Moreover, given that DNA-based measures are unreliable in the larynx, with a reproducibility of 32%³⁴, serology provides a more sensitive test that is necessary to allow for the detection of lower relative risks.

This study had some limitations, including a retrospective study design with a lower response rate in the controls than in the cases, a largely Caucasian population, and potential confounding by other types of HPV that were not measured. While we cannot exclude the possibility that the results were influenced by the lower response rates among controls, we observed associations for HPV16 that are consistent with the existing literature, suggesting the response rate did not substantially alter the associations. Another limitation to our study was the small number of cases/controls that were infected with some of the subtypes of HPV; while some of findings were statistically significant for rarer subtypes of HPV, large risk estimates should be interpreted with caution and larger or pooled studies should provide more stable risk estimates for those associations. In this study, we chose HPV types that have been associated with cancer; we were not able to measure all HPV subtypes and regions due to budgetary constraints. Consequently, we cannot rule out the possibility that unmeasured HPV types were confounding the associations reported. More studies will be necessary to examine these associations in non-Caucasian populations.

In this large population-based case-control study, we confirm strong associations between HPV16 E6 seropositivity and risk of oral cavity, pharyngeal and laryngeal cancers. In addition, HPV types 18, 33, and 52, were strongly associated with HNSCC, and particularly pharyngeal cancer, and associations remained statistically significant after excluding HPV16 seropositive subjects, supporting the role for non-HPV16 subtypes in the etiology of HNSCC.

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Novelty

Our findings suggest that HPV18, 33 and 52 seropositivity is strongly associated with HNSCC independently of HPV16, and that HPV16 seropositivity is associated with oral cavity and laryngeal cancers, as well as pharyngeal cancers

Impact: Understanding role of HPV in HNSCC is crucial to developing prevention strategies and providing targeted treatment.

Table 1

Descriptive demographic and lifestyle statistics among controls, stratified by HPV16 status, and separately by non-HPV16, high risk HPV status.

Characteristic	HPV 16* seronegative (N = 1031)	HPV 16 seropositive (N = 75)	High Risk** seronegative (N = 1018)	High Risk seropositive (N = 89)
Age				
Mean (sd)	60.5 (11.0)	62.6 (12.2)	60.7 (11.2)	60.9 (10.5)
Sex				
Male	760 (73.7)	59 (78.7)	749 (73.6)	71 (79.8)
Female	271 (26.3)	16 (21.3)	269 (26.4)	18 (20.2)
Race				
White	944 (91.6)	62 (82.7)	927 (91.1)	80 (89.9)
Other	87 (8.4)	13 (17.3)	91 (8.9)	9 (10.1)
Education				
High school graduate	280 (27.2)	25 (33.3)	276 (27.1)	29 (32.6)
At least some college	747 (72.5)	50 (66.7)	738 (72.5)	60 (67.4)
Smoking, pack years				
None	420 (40.7)	22 (29.3)	408 (40.1)	34 (38.2)
First tertile (>0 to 18)	215 (20.9)	14 (18.7)	214 (21.0)	15 (16.9)
Second tertile (18 to 41)	199 (19.3)	20 (26.7)	198 (19.4)	21 (23.6)
Third tertile (>41)	197 (19.1)	19 (25.3)	198 (19.4)	19 (21.3)
Average alcohol drinks/wk				
First quartile (2)	252 (24.4)	16 (21.3)	245 (24.1)	24 (27.0)
Second quartile (2–6)	298 (28.9)	20 (26.7)	292 (28.7)	26 (29.2)
Third quartile (6–14)	236 (22.9)	11 (14.7)	227 (22.3)	20 (22.5)
Fourth quartile (>14)	242 (23.5)	28 (37.3)	252 (24.8)	18 (20.2)
Age of first intercourse				
Mean (sd)	18.8 (3.6)	18.7 (3.8)	18.8 (3.8)	18.7 (2.9)
Allergy				
No	596 (57.8)	45 (60.0)	593 (58.3)	48 (53.9)
Yes	389 (37.7)	29 (38.7)	381 (37.4)	38 (42.7)

* HPV16 positive for E6, and/or E7.

** Positive for HPV18 (E6/E7), 31 (E6), 33 (E6), 45 (E6), 52 (E6/E7), and/or 58 (E6/E7).

Table 2

Descriptive demographic and lifestyle statistics by HNSCC case-control status.

Characteristic	Cases (N = 1069)	Controls (N = 1107)	P-value*
Age			
Mean (sd)	59.4 (11.4)	60.7 (11.1)	
Sex			
Male	798 (74.6)	820 (74.1)	
Female	271 (25.4)	287 (25.9)	
Race			
White	985 (92.1)	1007 (91.0)	0.23
Other	82 (7.7)	100 (9.0)	
Education			
High school graduate	376 (35.2)	305 (27.6)	<0.001
At least some college	547 (51.2)	276 (25.1)	
Smoking, pack years			
None	230 (21.5)	442 (39.9)	<0.001
First tertile (>0 to 18)	205 (19.2)	255 (23.0)	
Second tertile (18 to 41)	222 (20.8)	227 (20.5)	
Third tertile (>41)	268 (25.1)	283 (25.6)	
Average alcohol drinks/wk			
First quartile (3)	210 (19.6)	348 (31.4)	<0.001
Second quartile (3–7)	167 (15.6)	293 (26.5)	
Third quartile (7–21)	226 (21.1)	291 (26.3)	
Fourth quartile (>21)	318 (29.7)	172 (15.5)	
Age at first intercourse			
Mean (sd)	18.2 (3.5)	18.8 (3.6)	0.02
Allergy			
No	602 (56.3)	641 (57.9)	0.001
Yes	292 (27.3)	419 (37.9)	
HPV 16**			
Negative	674 (63.0)	1031 (93.1)	<0.001
Positive	395 (37.0)	75 (6.8)	

* Adjusted for age and gender.

** HPV16 positive for E6, and/or E7.

Table 3

Correlation coefficients for measures of HPV (any positive E6 and/or E7 vs. all negative) among cases and controls (N = 2176).

HPV type	6	11	16	18	31	33	45	52	58
6	1								
11	0.07	1							
16	0.12	0.10	1						
18	0.04	0.11	0.12	1					
31	0.00	0.03	0.05	0.14	1				
33	0.08	0.09	0.41	0.10	0.12	1			
45	0.02	0.14	0.08	0.13	0.16	0.17	1		
52	0.01	0.06	0.06	0.00	-0.01	0.13	0.04	1	
58	0.07	0.02	0.27	0.01	0.05	0.47	0.07	0.33	1

Table 4

Odds ratios and 95% CI for HPV 6, 11, 16, 18, 31, 33, 45, 52, and 58 positive serology in relation to head and neck cancer.

HPV type	Case/Control Seropositive No.	OR (95% CI)*	OR (95% CI)**	p-value**
HPV 6 L1	140/118	0.83 (0.62, 1.13)	0.88 (0.63, 1.23)	0.45
HPV 11 L1	149/93	0.75 (0.53, 1.05)	0.82 (0.55, 1.21)	0.31
HPV 11 E6	11/10	0.82 (0.27, 2.48)	0.59 (0.18, 1.89)	0.43
HPV 11 E7	14/2	6.75 (1.46, 30.9)	NA	NA
HPV 16 L1	298/73	6.80 (5.10, 9.06)	7.06 (4.03, 9.89)	<0.0001
HPV 16 E6	346/25	30.6 (19.9, 47.2)	21.7 (13.9, 34.0)	<0.0001
HPV 16 E7	137/51	3.69 (2.59, 5.25)	5.35 (3.39, 8.46)	<0.0001
HPV 18 L1	245/177	0.93 (0.72, 1.21)	1.00 (0.74, 1.35)	0.99
HPV 18 E6	31/4	8.73 (2.88, 26.4)	7.38 (2.32, 23.5)	0.001
HPV 18 E7	54/20	2.72 (1.57, 4.70)	1.70 (0.31, 9.40)	0.54
HPV 31 L1	174/95	1.20 (0.87, 1.64)	1.11 (0.51, 2.39)	0.80
HPV 31 E6	50/38	1.20 (0.71, 2.02)	0.79 (0.44, 1.41)	0.42
HPV 33 L1	160/81	1.07 (0.75, 1.52)	1.31 (0.93, 1.85)	0.12
HPV 33 E6	121/5	7.40 (2.77, 19.8)	4.82 (1.57, 14.8)	0.006
HPV 45 L1	145/90	0.74 (0.52, 1.06)	0.73 (0.48, 1.11)	0.14
HPV 45 E6	20/8	1.82 (0.68, 4.88)	0.64 (0.19, 2.22)	0.48
HPV 52 E6	16/0	NA	NA	NA
HPV 52 E7	19/6	3.56 (1.32, 9.60)	3.31 (1.08, 10.1)	0.04
HPV 58 E6	87/4	4.89 (1.60, 15.0)	1.97 (0.58, 6.70)	0.28
HPV 58 E7	31/17	1.62 (0.82, 3.21)	1.11 (0.51, 2.39)	0.80

* Adjusted for age, race, gender, education, tobacco smoking, alcohol use, study phase, and HPV16 (for all non-HPV16 types). HPV 52 and 58 E7 not adjusted for phase because they were only measured for phase 1.

** Additionally mutually adjusting for HPV types in table; p-values presented are for these associations

Table 5

Odds ratios and 95% CI for HPV 6, 11, 18, 31, 33, 45, 52, and 58 positive serology (E6, E7) in relation to different sites of head and neck cancer.

HPV type	Controls, Seropositive No.	Oral cavity (N=400) Seropositive No.	Oral cavity OR* (95% CI)	Oral cavity p-value	Pharyngeal (N=486) Seropositive No.	Pharyngeal OR* (95% CI)	Pharyngeal p-value	Laryngeal (N=168) Seropositive No.	Laryngeal OR* (95% CI)	Laryngeal p-value
HPV 16 E6	25	42	6.76 (3.95, 11.6)	<0.001	281	81.2 (51.1, 128.9)	<0.001	19	5.64 (2.89, 11.0)	<0.001
HPV 16 E7	51	44	2.93 (1.87, 4.59)	<0.001	68	3.33 (2.25, 4.93)	<0.001	22	2.95 (1.65, 5.26)	<0.001
HPV 18 E6	4	10	5.47 (1.58, 18.9)	0.01	18	10.7 (3.53, 32.4)	<0.001	2	3.23 (0.56, 18.6)	0.19
HPV 18 E7	9	20	1.23 (0.54, 2.81)	0.62	40	5.01 (2.87, 8.77)	<0.001	4	0.92 (0.28, 3.04)	0.90
HPV 31 E6	38	19	1.53 (0.85, 2.75)	0.16	24	1.61 (0.94, 2.75)	0.08	7	1.13 (0.47, 2.72)	0.79
HPV 33 E6	5	19	14.2 (5.12, 39.2)	<0.001	92	52.8 (21.2, 131.5)	<0.001	10	14.2 (4.43, 45.8)	<0.001
HPV 45 E6	8	2	0.63 (0.13, 3.16)	0.57	17	5.05 (2.14, 11.9)	<0.001	1	0.56 (0.06, 5.25)	0.61
HPV 52 E7	6	8	3.80 (1.21, 11.9)	0.02	8	4.03 (1.30, 12.5)	0.02	3	2.13 (0.45, 10.1)	0.34
HPV 58 E6	4	9	8.71 (2.52, 30.2)	<0.001	76	56.3 (20.4, 155.8)	<0.001	2	2.71 (0.41, 18.1)	0.30
HPV 58 E7	17	13	1.43 (0.64, 3.21)	0.38	11	2.24 (0.96, 5.23)	0.06	6	1.53 (0.54, 4.35)	0.42

* Adjusted for age, race, gender, education, tobacco smoking, alcohol drinking, study phase and HPV16 (for all non-HPV16 types), HPV 52 and 58 E7 not adjusted for phase because they were only measured for phase 1.

Table 6

Odds ratios and 95% CI for HPV 6, 11, 18, 31, 33, 45, 52, and 58 positive serology in relation to head and neck cancer among subjects who were seronegative for HPV16 (L1, E6, and E7).

HPV type*	Case/Control Seropositive No.	OR (95% CI)**	p-value
HPV 6 L1	61/90	0.90 (0.62, 1.32)	0.60
HPV 11 L1	37/53	0.91 (0.56, 1.47)	0.70
HPV 11 E6	4/8	0.81 (0.20, 3.18)	0.76
HPV 11 E7	2/0	NA	NA
HPV 18 L1	86/122	1.17 (0.85, 1.61)	0.35
HPV 18 E6	12/4	4.19 (1.26, 14.0)	0.02
HPV 18 E7	13/15	1.25 (0.56, 2.77)	0.59
HPV 31 L1	41/63	1.20 (0.78, 1.85)	0.42
HPV 31 E6	20/32	1.10 (0.59, 2.05)	0.77
HPV 33 L1	29/49	0.98 (0.60, 1.62)	0.95
HPV 33 E6	8/2	7.96 (1.56, 40.5)	0.01
HPV 45 L1	31/45	1.25 (0.76, 2.07)	0.39
HPV 45 E6	4/8	0.79 (0.21, 3.01)	0.74
HPV 52 E6	2/0	NA	NA
HPV 52 E7	15/6	3.40 (1.16, 9.99)	0.03
HPV 58 E6	5/3	2.44 (0.51, 11.7)	0.26
HPV 58 E7	20/13	1.47 (0.64, 3.37)	0.36

* High-risk HPV include HPV18, 31, 33, 45, 52, and/or 58.

** Adjusted for age, race, gender, education, smoking, alcohol drinking, and study phase.

Odds ratios and 95% CI for HPV 6, 11, 18, 31, 33, 45, 52, and 58 positive serology (E6, E7) in relation to different sites of head and neck cancer, among subjects who were seronegative for HPV16 (L1, E6, and E7).

Table 7

HPV type*	Controls, Seropositive No.	Oral cavity Seropositive No.	Oral cavity OR (95% CI)	Oral cavity p-value	Pharyngeal Seropositive No.	Pharyngeal OR (95% CI)	Pharyngeal p-value	Laryngeal Seropositive No.	Laryngeal OR (95% CI)	Laryngeal p-value
HPV 11 E6	8	0	NA	NA	3	2.32 (0.49, 11.0)	0.29	1	1.34 (0.16, 11.3)	0.79
HPV 18 E6	4	6	2.96 (0.72, 12.1)	0.13	5	8.25 (2.10, 32.4)	0.002	1	2.14 (0.23, 19.9)	0.50
HPV 18 E7	15	6	1.14 (0.42, 3.12)	0.79	4	1.69 (0.53, 5.42)	0.38	3	1.19 (0.32, 4.46)	0.79
HPV 31 E6	32	12	1.37 (0.67, 2.81)	0.39	2	0.32 (0.07, 1.55)	0.16	6	1.35 (0.51, 3.55)	0.55
HPV 33 E6	2	2	4.99 (0.64, 38.9)	0.13	4	18.9 (3.22, 110.8)	0.001	2	7.62 (0.84, 69.3)	0.07
HPV 45 E6	8	0	NA	NA	3	2.77 (0.66, 11.7)	0.17	1	0.60 (0.06, 5.90)	0.66
HPV 52 E7	6	8	4.79 (1.47, 15.6)	0.01	4	3.02 (0.71, 12.9)	0.14	3	2.82 (0.57, 13.9)	0.20
HPV 58 E6	3	1	0.81 (0.08, 8.60)	0.86	4	7.30 (1.42, 37.7)	0.02	0	NA	NA
HPV 58 E7	13	11	1.65 (0.65, 4.21)	0.29	5	1.62 (0.47, 5.59)	0.45	4	1.31 (0.36, 4.69)	0.68

* High-risk HPV include HPV 18, 31, 33, 45, 52, and/or 58. HPV 52 E6 had no seropositive controls.

** Adjusted for age, race, gender, education, smoking, alcohol drinking and study phase.