Published in final edited form as:

Cancer Epidemiol. 2016 August ; 43: 30–34. doi:10.1016/j.canep.2016.06.002.

Human Papillomavirus (HPV) 16 E6 seropositivity is elevated in subjects with oral HPV16 infection

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Abstract

Introduction—Human Papillomavirus (HPV) 16 E6 serum antibodies are common in people with HPV-related oropharyngeal cancers (HPV-OPC), but not the general population. We explored HPV16 seroprevalence in people with and without oral HPV16 infection, the cause of HPV-OPC.

Methods—Oral rinse samples were collected semiannually and tested for 36 types of HPV DNA by PCR. HPV16 E6 serum antibodies were tested at the visit of first oral HPV detection in participants with prevalent (n=54), or incident (n=39) oral HPV16 DNA; or at baseline in matched

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Conflicts of Interest: Other authors have no conflicts to report.

participants with no oral HPV16 DNA (n=155) using multiplex serology assay. Predictors of seropositivity were examined using logistic regression.

Results—HPV16 E6 seropositivity (7.5% vs 0.7%; $p=0.005$) but not seropositivity to the other HPV16 antigens, was significantly more common in those with than without oral HPV16 infection. There were only 8 HPV16 E6 seropositive participants, but oral HPV16 DNA remained a strong predictor of E6 seropositivity after adjustment for other risk factors (aOR=14.6 95%CI, 1.7-122.5). Seroprevalence was similar in those with prevalent (7.4%; 4/54), and incident (7.7%; 3/39) oral HPV16 infection ($p=1.00$). E6 seroprevalence was associated with reduced oral HPV16 clearance, but was not statistically significant (HR=0.65 95% CI, 0.16-2.70).

Seropositive participants were primarily male (87.5%), HIV-positive (75.0%; median CD4 cell-count of 840) and had oral HPV16 DNA (87.5%). History of an HPV-related cancer (0/8) or HPV-related anogenital dysplasia (1/8) was rare, and 4 participants had recent screening showing no anogenital dysplasia.

Discussion—HPV16 E6 seropositivity was higher among people with than without oral HPV16 infection, despite no known anogenital disease in these participants.

Keywords

Antibodies; oral HPV; seroprevalence; biomarker; HPV16 E6

Introduction

Human papillomavirus (HPV) infection causes the majority of oropharyngeal squamous cell cancer (OPC) in the U.S (1). Most oral HPV infections are transient, clearing within 2 years (2,3). The incidence of HPV-related OPC (HPV-OPC) has increased over the past 20 years, and is projected to surpass that of invasive cervical cancer in the U.S. by 2020 (4). As OPC are usually detected at a late stage (5), biomarkers for oropharyngeal pre-cancer are needed.

Given oropharyngeal pre-cancerous lesions are difficult to detect, HPV16 E6 antibodies have been suggested as a potentially specific marker for HPV-OPC screening. HPV16 E6 antibodies are detected in most HPV-OPC cases, but are rare in the general population (6–9). Furthermore, one study detected HPV16 E6 antibodies in OPC cases more than 10 years before cancer diagnosis (8). To evaluate the potential utility of HPV16 E6 antibodies, we explored their seroprevalence among participants with oral HPV16 infection, the presumed cause of HPV-OPC, compared to those without oral HPV16 infection.

Materials and Methods

Study Design and Population

This study included 248 participants from the Persistent Oral Human Papillomavirus Study (POPS) study, a cohort study of oral HPV natural history nested within two cohorts of HIV-infected and high-risk HIV-uninfected individuals: the Multicenter AIDS Cohort Study (MACS), and the Women's Interagency of HIV study (WIHS). In brief, a representative sample of participants were enrolled beginning in 2009, stratified by HIV status and study cohort (2). Oral exfoliated epithelial cells were collected semiannually for four years using a

30-second oral rinse and gargle sample with Scope® mouthwash (Procter & Gamble, Cincinnati, Ohio). Information on risk behaviors was collected using a computer assisted self-interview (CASI). Both the MACS and WIHS have cancer registry matching with confirmed cancer diagnosis information. In addition, in the WIHS, cervical cytology data was performed semiannually in the study, including colposcopy and biopsy, and in the MACS, anal cytology was performed at least once, concurrent with their oral rinse sample (10).

All participants with either prevalent (n=54) or incident (n=39) oral HPV16 DNA detected in the POPS study were included in this analysis (for prevalence of other oral HPV types see our previous publication).(11) Serum from these participants was tested for HPV16 antibodies at the visit of first oral HPV16 detection. A sample of 155 participants with no oral HPV infection (of any type) who had available blood was selected, frequency matched by gender, as a comparison group and tested for HPV16 seropositivity at their baseline visit.

Laboratory Testing Methods

Serum samples were sent to the German Cancer Research Center (DKFZ, Heidelberg, Germany) for HPV serologic testing (HPV16 E6, E7, E1, E2, E4, and L1) using glutathione S-transferase multiplex assay (12). Median fluorescence intensity (MFI) values were dichotomized to indicate HPV16 E6 seropositivity using the more specific cutoff of MFI 1000 as the main outcome (8), and the standard (lab) cutoff of MFI 484, as a sensitivity analysis.

DNA was purified from the oral rinse using a magnetic bead-based automated platform (QIAAsymphony; QIAGEN, Germantown, Maryland), and evaluated for 36 different HPV DNA genotypes using PGMY09/11 PCR primer pools, followed by reverse line blot hybridization to the Roche linear array HPV Genotyping Test (Roche Molecular Systems, Pleasanton, California).

Statistical Analysis

Seroprevalence of HPV16 E6 and other antibodies was explored overall, and compared by oral HPV16 DNA status and gender, using Fisher's exact test. Characteristics of HPV16 E6 seropositive and seronegative participants were compared using Wilcoxon rank-sum tests for continuous variables and Fisher's exact test for categorical variables. Oral HPV16 infection was classified as prevalent if detected at baseline, and as incident if oral HPV16 DNA was only later detected after a prior negative test. Oral HPV16 clearance was defined as detection of oral HPV16 infection followed by two consecutive negative visits (e.g. +, +, -, -), with time of clearance defined as the first negative visit.

Predictors of seropositivity were examined using univariate and multivariate logistic regression models. Covariates explored included age (continuous), gender, race/ethnicity, HIV status, current CD4 T cell count, any oral HPV16 infection, number of recent (past six months) and lifetime oral sexual partners, current cigarette and alcohol use, tonsillectomy, and pre-cancer/cancer history. The final multivariate model included gender, oral HPV16 infection, and number of lifetime oral sexual partners.

Results

Among the 248 participants tested for HPV16 E6 antibodies, 8 participants were seropositive. HPV16 E6 seroprevalence was significantly more common in those with than without oral HPV16 infection (7.5% vs 0.7%, $p=0.005$). In contrast, HPV16 L1 (41% vs 34%, $p=0.28$), E7 (6% vs 10%, $p=0.36$), and other E antibodies tested had similar seroprevalence by oral HPV16 DNA status (Table 1). Among those with oral HPV16 infection, HPV16 E6 seroprevalence was similar in those with prevalent (7.4%, 4/54), and incident (7.7%, 3/39) oral HPV16 DNA ($p=1.00$), Table 2. Men also had higher HPV16 seroprevalence than women (5.2% vs 0.9%, $p=0.07$). Results were similar when the lower E6 seropositivity cutoff was used. Participants with and without HPV16 E6 antibodies were similar with regards to age, race, HIV status, current CD4 T cell count, and history of tonsillectomy.

In univariate analysis, HPV16 E6 seroprevalence was significantly more common in those with than without oral HPV16 DNA (OR=12.5; 95% CI, 1.5–103.6). Male sex, older age, no current cigarette use and more lifetime oral sexual partners were associated with increased odds of HPV16 E6 seropositivity (Table 2). After adjusting for gender and lifetime number of oral sexual partners, oral HPV16 infection remained a strong predictor of E6 seropositivity (aOR=14.6; 95% CI, 1.7–122.5). Clearance of oral HPV16 was reduced but not significantly different in those with HPV16 E6 antibodies (HR=0.65; 95% CI, 0.16–2.70).

As most participants were HPV16 E6 seronegative, characteristics of seropositive participants were of interest. These 8 participants had a median age of 56 years, 6 (75%) were HIV-infected, most were male (88%), with 75 (88%) lifetime oral sexual partners. Only 1 seropositive participant had a history of anogenital dysplasia, a man with recent biopsy-confirmed anal HSIL. Among the remaining 7 seropositive participants, 4 had recent anal cytology tests suggesting no HPV-related anal dysplasia, and 3 were unscreened.

Discussion

HPV16 E6 seroprevalence was elevated among participants with compared to those without oral HPV16 DNA, despite no known anogenital disease. While most people with oral HPV16 infection were seronegative, some had HPV16 E6 antibodies. These participants were mostly male and had a higher number of lifetime oral sexual partners; after controlling for these risk factors having oral HPV16 DNA remained a significant predictor of HPV16 E6 seropositivity. It is of note that seropositivity to the other HPV16 antigens was not related to oral HPV16 infection. This suggests that history of ever HPV16 exposure was similar regardless of current oral HPV16 infection, but that HPV16 E6 oncogene expression was higher in those with oral HPV16 infection. It is unknown whether E6 seropositivity in any of these participants might represent undetected oropharyngeal pre-cancer.

Overall, HPV16 E6 seroprevalence (3.2%) in this high-risk population was higher than in previous studies of cancer free populations which found 0.6%–1.2% (6,8,13). However, seroprevalence among oral HPV16 DNA negative participants (0.7%) was in this expected range. A previous study of HPV16 seropositivity in our same cohort, used a different

laboratory for testing, and yielded a higher overall HPV16 E6 seropositivity (7.6% among all participants) (14). Reasons for differences in these laboratory assays are unclear; the testing reported in this paper uses the DKFZ gold-standard laboratory. HPV16 E7 seroprevalence was surprisingly high among those with (6%) and without (10%) oral HPV16 infection, suggesting limited specificity (i.e. some false positives) with this test, as observed in some previous studies.(8)

HPV16 E6 seroprevalence is known to be high among patients with oropharyngeal cancer (range 46%–90%)(15). However, few studies have been able to explore seroprevalence in cancer cases before they were diagnosed (8,16). One nested case control study of 135 oropharyngeal cancers found 35% of cases had HPV16 E6 antibodies detected years before cancer diagnosis (8). This study adds to that finding by demonstrating HPV16 E6 antibodies are detected in some cancer-free individuals with oral HPV16 infection, more than would be expected by chance. We would expect HPV16 E6 seropositivity to be associated with oral HPV16 persistence, if it is representative of a pre-malignancy; while there were a limited number of infections in this study, clearance did appear to be decreased, although not statistically significant, among those with HPV16 E6 antibodies.

This study has several limitations including a limited number of participants with oral HPV16 DNA, and with HPV16 E6 seropositivity. The high-risk cohort of people studied does not represent the general population, and indeed HIV-infected individuals have increased acquisition and decreased clearance of oral HPV.(2) Strengths of the study include centralized testing of oral HPV DNA and serology, longitudinal oral rinse sample collection, and information on risk factors and screening history.

Conclusions

New screening methods for oropharyngeal cancer are important, as the incidence of this cancer continues to increase, and many cancers are detected at a late stage. This study suggests that HPV16 E6 antibodies are not detected in most people with oral HPV16 infection, as expected given most people will clear these infections within 2 years (2). This is consistent with HPV16 E6 seropositivity being a specific marker, and the possibility that some of the participants with oral HPV16 infection who are HPV16 E6 seropositive might have undiagnosed oropharyngeal pre-cancer. However further research is clearly needed to better understand the sensitivity of this marker in people with oral HPV16 infection, and its association with oral HPV natural history.

Acknowledgements

Data in this manuscript were collected by the Multicenter AIDS Cohort Study (MACS). MACS (Principal Investigators): Johns Hopkins University Bloomberg School of Public Health (Joseph Margolick), U01-AI35042; Northwestern University (Steven Wolinsky), U01-AI35039; University of California, Los Angeles (Roger Detels), U01-AI35040; University of Pittsburgh (Charles Rinaldo), U01-AI35041; the Center for Analysis and Management of MACS, Johns Hopkins University Bloomberg School of Public Health (Lisa Jacobson), U01-AI35043. The MACS is funded primarily by the National Institute of Allergy and Infectious Diseases (NIAID), with additional co-funding from the National Cancer Institute (NCI), the National Institute on Drug Abuse (NIDA), and the National Institute of Mental Health (NIMH). The MACS website is located at <http://aidscohortstudy.org/>. Data in this manuscript were also collected by the Women's Interagency HIV Study (WIHS). WIHS (Principal Investigators): U01-AI-103408; Bronx WIHS (Kathryn Anastos), U01-AI-035004; Brooklyn WIHS (Howard Minkoff and Deborah Gustafson), U01-AI-031834; Chicago WIHS (Mardge Cohen and Audrey French), U01-

AI-034989; WIHS Data Management and Analysis Center (Stephen Gange and Elizabeth Golub), U01-AI-042590. The WIHS is funded primarily by the National Institute of Allergy and Infectious Diseases (NIAID), with additional co-funding from the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development (NICHD), the National Cancer Institute (NCI), the National Institute on Drug Abuse (NIDA), and the National Institute on Mental Health (NIMH).

Financial support: This work was supported by grant R01DE021395 (NIDCR, NIH; Gypsyamber D'Souza). The MACS and WIHS cohorts receive primary funding from NIAID, with additional funding from NCI, NIDA, NIMH and NICHD (full acknowledgements at end of paper).

GD, DJW and RDC have/had research support from Merck & Co., Inc. DJW is a member of the speakers bureau for Merck & Co., Inc. MLG has been a consultant for Merck & Co., Inc and GSK. RDC also reports institutional grant funding and royalties from UpToDate (on HPV related topics).

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Table 1

HPV antibody seropositivity among people with and without oral HPV16 infection

Antibody Seropositivity by HPV Type	Oral HPV16 DNA		P-value [§]
	Oral HPV16 DNA Negative (n=155)	Oral HPV16 DNA Positive (n=93)	
HPV16 E6	1%	8%	0.005
HPV16 E7	10%	6%	0.36
HPV16 E1	3%	2%	0.71
HPV16 E2	2%	6%	0.09
HPV16 E4	11%	18%	0.13
HPV16 L1	34%	41%	0.28

[§]P-value from Fisher's exact test

Table 2

Characteristics and association with HPV16 E6 seropositivity, measured at visit of first oral HPV16 detection or at baseline visit (for those with no oral HPV infection)

Characteristics of participants	N (Col %)		P-value ^{\$}	OR (95%CI)
	Seropositive (n=8)	Seronegative (n=240)		
Oral HPV16 DNA				
None	1 (13%)	154 (64%)	0.005	1.0
Present	7 (88%)	86 (36%)		12.5 (1.5–103.6)
<i>Incident</i>	3 (43%)	36 (42%)	1.00	1.0
<i>Prevalent</i>	4 (57%)	50 (58%)		1.0 (0.2–4.6)
DEMOGRAPHICS				
Gender				
Women (WIHS)	1 (12%)	113 (47%)	0.07	1.0
Men (MACS)	7 (88%)	127 (53%)		6.2 (0.8–51.4)
Age (in years)				
< 45	1 (13%)	73 (30%)	0.23	1.0
45–54	2 (25%)	89 (37%)		1.6 (0.1–18.5)
55	5 (62%)	78 (33%)		4.7 (0.5–41.0)
Age per 10 year increase, median (IQR)	56 (49–58)	50 (43–57)	0.19	1.6 (0.8–3.2)
Race/ethnicity				
White, non-Hispanic	5 (63%)	91 (38%)	0.29	1.0
Black, non-Hispanic	2 (25%)	119 (50%)		0.3 (0.1–1.6)
Hispanic/other ^{\$}	1 (13%)	30 (12%)		0.6 (0.1–5.4)
BIOLOGIC & BEHAVIORAL				
HIV-status				
Uninfected	2 (25%)	69 (29%)	1.00	1.0
Infected	6 (75%)	171 (71%)		1.2 (0.2–6.1)
Current CD4 T cell count (among HIV-infected)				
< 300	0 (0%)	48 (28%)	0.37	500 vs < 500 (ref);
300–499	2 (33%)	39 (23%)		2.1 (0.4–11.6)
500	4 (67%)	84 (49%)		
Ever had a tonsillectomy				
No/not sure	5 (63%)	162 (69%)	0.71	1.0
Yes	3 (37%)	73 (31%)		1.3 (0.3–5.7)
Current cigarette use				
No	8 (100%)	135 (57%)	0.02	Undefined
Yes	0 (0%)	103 (43%)		
Recent alcohol use (1/week)				
No	2 (25%)	83 (35%)	0.72	1.0
Yes	6 (75%)	155 (65%)		1.6 (0.3–8.1)

Characteristics of participants	N (Col %)		P-value [§]	OR (95%CI)
	Seropositive (n=8)	Seronegative (n=240)		
Number of lifetime oral sexual partners				
0	0 (0%)	17 (7%)	0.05	10 vs < 10 (ref);
1–9	1 (13%)	55 (24%)		3.1 (0.4–25.9)
10–74	0 (0%)	75 (32%)		75 vs < 75 (ref);
75	7 (88%)	86 (37%)		12.0 (1.5–98.9)
Number of recent oral sexual partners				
0	4 (50%)	152 (63%)	0.08	1 vs 0 (ref);
1	0 (0%)	44 (18%)		1.7 (0.4–7.1)
2	4 (50%)	44 (18%)		
PRE-CANCER/ CANCER HISTORY				
Ever diagnosed with HPV-related cancer ~				
No	8 (100%)	239 (99%)	1.00	Undefined
Yes	0 (0%)	2 (1%)		
Ever diagnosed with anogenital dysplasia				
No/unknown	7 (88%)	207 (86%)	1.00	1.0
Yes (anal/cervical HSIL)	1 (12%)	33 (14%)		0.9 (0.1–7.5)
Ever anal HSIL (among men)	1 (14%)	10 (8%)	0.46	2.0 (0.2–17.8)
Ever cervical HSIL (among women)	0 (0%)	23 (20%)	1.00	Undefined

OR: odds ratio; CI: confidence interval; IQR: interquartile range

[§]P-value from Wilcoxon rank-sum tests for continuous variables and Fisher's exact test for categorical variables

[§]Includes 22 Hispanic and 9 other race/ethnicity.

~Includes cervical, anal or head and neck cancer.