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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 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.

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1. 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.

2. Materials and methods

2.1. 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-s 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.

2.2. 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 $\geq$ 1000 as the main outcome [8], and the standard (lab) cutoff of MFI $\geq$ 484, as a sensitivity analysis.

DNA was purified from the oral rinse using a magnetic bead-based automated platform (QIAsymphony; 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).

2.3. 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 CD4T 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.

3. 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

Table 1
HPV antibody seropositivity among people with and without oral HPV16 infection.

	Oral HPV16 DNA		
Antibody Seropositivity by HPV Type	Oral HPV16 DNA Negative (n = 155)	Oral HPV16 DNA Positive (n = 93)	P-value[a]
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

[a] P-value from Fisher's exact test.