

Long-term Persistence of Oral Human Papillomavirus Type 16: The HPV Infection in Men (HIM) Study

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Abstract

Persistent infection with oral HPV16 is believed to drive the development of most oropharyngeal cancers. However, patterns of oral HPV16 persistence remain understudied, particularly among HIV-negative individuals. Oral HPV16 persistence was evaluated among 1,626 participants of the HPV Infection in Men (HIM) Study. Twenty-three oral HPV16-positive men who provided an oral gargle sample on ≥ 2 study visits were included in the analysis. Archived oral samples from all follow-up visits were tested for HPV16 using Linear Array and INNO-LiPA detection methods. Persistence was evaluated using consecutive HPV16-positive visits held approximately 6 months apart and using the Kaplan–Meier method. Oral HPV16-positive men were aged 18 to 64 years [median, 36 years; interquartile range (IQR), 25–42] and were followed for a median of 44.4 months (IQR, 29.9–49.5). Of 13 incident

infections, 4 (30.8%) persisted ≥ 12 months, 1 (10.0%) persisted ≥ 24 months, and none persisted ≥ 36 months [median infection duration, 7.3 months; 95% confidence interval (CI), 6.4–NA]. Of 10 prevalent infections, 9 (90.0%) persisted ≥ 12 months, 8 (80.0%) persisted ≥ 24 months, 4 (57.1%) persisted ≥ 36 months, and 2 (40.0%) persisted ≥ 48 months (median infection duration, NA). Twelve-month persistence of incident infections increased significantly with age ($P_{\text{trend}} = 0.028$). Prevalent oral HPV16 infections in men persisted longer than newly acquired infections, and persistence appeared to increase with age. These findings may explain the high prevalence of oral HPV observed at older ages. Understanding oral HPV16 persistence will aid in the identification of men at high-risk of developing HPV-related oropharyngeal cancer. *Cancer Prev Res*; 8(3); 190–6. ©2015 AACR.

Introduction

Mounting evidence in some economically developed countries suggests that human papillomaviruses (HPV) cause the majority of oropharyngeal cancers among men (1, 2), with HPV type 16 implicated in most cases (3). In the United States, oropharyngeal cancers incidence is 4-fold higher among men than women (4).

Though still considered a rare cancer (<10 per 100,000 individuals; ref. 4), HPV-positive oropharyngeal cancer incidence has increased significantly in recent decades among men (1, 4), with the annual number of overall oropharyngeal cancer cases now surpassing that of cervical cancers (4).

Most HPV-driven oropharyngeal cancer cases are diagnosed at an advanced clinical stage (III–IV; ref. 5), and treatment often causes substantial morbidity, including facial disfigurement and permanent difficulties in speaking, swallowing, and breathing. Unfortunately, there are no clinically validated screening tests available to detect oropharyngeal cancers at an earlier stage or to predict the risk of developing HPV-driven oropharyngeal cancers. Though primary prevention of oral HPV through prophylactic vaccination is promising (6), there is no definitive evidence that HPV vaccines will prevent new oral HPV infection and subsequent disease. Furthermore, generations of men and women will remain unprotected, including individuals currently outside of the recommended age range for vaccination and those already infected with oral HPV (7), emphasizing the need for the development of oral HPV prevention and early oropharyngeal cancer detection methods, particularly among high-risk individuals.

Advancing our knowledge of the natural history of genital HPV infection has led to important innovations in the prevention of cervical cancer (e.g., cervical HPV testing and prophylactic HPV vaccination). Similar data on oral HPV natural history are needed to advance the development of oropharyngeal cancer interventions. Unfortunately, oral HPV natural

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history remains understudied. Like cancers at other anatomic sites, persistent oral HPV infection may be the driving factor in oropharyngeal cancer carcinogenesis; therefore, it is crucial to examine each step along the continuum of carcinogenesis, from initial HPV infection to development of cancer. Previously, we described the prevalence (8) and acquisition (9) of oral HPV detected among otherwise healthy men enrolled in a large HPV natural history study. Here, we supplement these data with an evaluation of long-term oral HPV16 persistence among the same cohort of men.

Materials and Methods

Study population

The HPV Infection in Men (HIM) Study is an ongoing, multinational cohort study of the natural history of genital HPV infections (10, 11). More than 4,000 men ages 18 to 70 years were recruited from a variety of population sources within Brazil, Mexico, and the United States beginning in 2005. Men reported no previous diagnosis of anogenital cancers, had never been diagnosed with anogenital warts, and reported no symptoms of or treatment for a sexually transmitted infection, including HIV/AIDS to be eligible for the study. In 2007, oral sample collection was initiated, and details of the HIM Study oral HPV substudy can be found elsewhere (8, 9). Briefly, the first consecutively recruited 1,626 men who provided oral gargle samples on ≥ 2 study visits were tested for oral HPV, none of whom reported a history of head and neck cancer (9). Twenty-eight men were positive for oral HPV16 and 23 men had sufficient data to evaluate oral HPV16 persistence (i.e., ≥ 6 months of follow-up after the initial infection).

The Human Subjects Committees of the University of South Florida (Tampa, FL), Ludwig Institute for Cancer Research (São Paulo, Brazil), Centro de Referência e Treinamento em Doenças Sexualmente Transmissíveis e AIDS (São Paulo, Brazil), and Instituto Nacional de Salud Pública de México (Cuernavaca, Mexico) approved all study procedures, and all participants provided written informed consent.

Procedures

Participants attended a pre-enrollment (baseline) visit, an enrollment visit (2 weeks postbaseline), and follow-up visits every 6 months for up to 4 years. At each visit, participants completed a computer-assisted self-interview with questions about sociodemographic characteristics and potential risk factors, and underwent a clinical examination in which genital and oral samples were collected. Each participant was asked to rinse with and gargle 15 mL of locally available mouthwash for a total of 30 seconds (8). The sample was centrifuged at $3,000 \times g$ for 15 minutes at 4°C, the supernatant was decanted, and the cell pellet was resuspended in 20 mL of sterile saline. To ensure even sample distribution, centrifugation was repeated, and the cell pellet was resuspended in 1.2 mL of saline with repeated pipetting and vortexing. Samples were then stored at -80°C for PCR and genotyping analyses.

HPV DNA extraction and genotyping have been described previously (8, 9). Briefly, oral cells underwent robotic DNA extraction using the QIAamp Media MDx Kit (Qiagen) in accordance with the manufacturer's instructions. Samples were tested for the presence of HPV DNA with PGMY09/11 PCR, and HPV genotyping was conducted with Linear Array (Roche Molecular

Diagnostics), irrespective of HPV PCR results. The Linear Array assay detects 37 mucosal HPV types, classified as high-risk (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68) or low-risk [6, 11, 26, 40, 42, 53, 54, 55, 61, 62, 64, 66, 67, 69, 70, 71, 72, 73, 81, 82, 82 subtype IS39, 83, 84, and 89 (CP6108; ref. 12)].

To evaluate long-term infection persistence among oral HPV16-positive study participants, we identified and retrieved all available oral gargle samples that were archived through the end of the study. Oral HPV genotyping of all archived samples from these men was completed using Linear Array. For 11 men, at least one intervening negative result occurred among a series of positive results, suggesting that oral HPV16 viral load fell below the lower limit of detection of the assay. We used a more sensitive assay, INNO-LiPA HPV Genotyping Extra (Fujirebio, Ghent, Belgium), to confirm the results of 14 specimens that, according to Linear Array, were intervening negatives (single or multiple negatives) between positive visits. Eleven specimens (78.6%) tested HPV16-positive using INNO-LiPA, although three specimens remained HPV16-negative using INNO-LiPA. If a visit tested HPV16-positive by INNO-LiPA, that visit was considered HPV16-positive, overriding the results of the Linear Array assay. If an intervening negative tested HPV16-negative by both Linear Array and INNO-LiPA, but had prior and subsequent HPV16-positive visits, the intervening negative visit was considered a false negative; this occurred for three men (two with prevalent HPV and one with incident HPV). We also used INNO-LiPA to test a random sample of five HPV16-positive and five HPV16-negative specimens previously determined using Linear Array; all (100%) of the HPV16-positive specimens remained positive and all (100%) of the HPV16-negative specimens remained negative. Using results from both assays, we evaluated oral HPV16 persistence and clearance for up to 56.4 months of follow-up.

Statistical analysis

Persistence was first evaluated using a definition based on consecutive oral HPV16-positive visits. Persistence was estimated at 6-month intervals, as study visits occurred approximately 6 months apart; however, as pre-enrollment and enrollment visits occurred 2 weeks apart, oral HPV16 results from these visits were combined into one visit (i.e., the combined visit was considered positive if either visit was positive). Six-month type-specific persistence was defined as the presence of oral HPV16 DNA on two or more consecutive study visits, 12-month type-specific persistence as the presence of oral HPV16 DNA on three or more consecutive study visits, and so on. If a single HPV-negative visit occurred between two HPV-positive visits, that visit was deemed a false negative and was treated as though it were an HPV-positive visit. Persistence was reported as a proportion and was calculated as the number of persistent HPV16 infections divided by the total number of HPV16 infections detected among men who had been followed for that period of time or longer. As not all men were followed for 48 months, the total number of infections (i.e., denominator) changed over time.

Persistence was also evaluated using the Kaplan–Meier method to estimate the cumulative probability of infection throughout the entire study period and median time to clearance. Infection clearance was defined as two or more HPV16-negative visits following an HPV16-positive visit. However, an infection was considered cleared if a single HPV-negative occurred at the final study visit, as there was insufficient evidence to consider

this a false negative (i.e., the absence of an HPV16-positive visit following the HPV16-negative visit); this occurred for one man with a prevalent infection. Men whose infections did not clear by the final study visit were censored in the Kaplan–Meier analysis. Log-rank tests were used to identify significant differences between the cumulative probabilities of prevalent versus incidence infections.

Factors associated with oral HPV16 infection persistence were assessed if they were believed to be relevant on the basis of previous work (8, 9). At 12, 24, 36, and 48 months, estimates of persistence were stratified by each categorical risk factor, and exact Pearson χ^2 tests and Cochran–Armitage tests for trend were used to identify differences and linear trends, respectively, in the proportions of persistent infections. Because of low numbers of oral HPV16 infections, factors associated with persistence were also assessed using Cox proportional-hazards regression.

All analyses were conducted separately for prevalent and incident HPV infections. All statistical tests were two-sided and attained statistical significance at $\alpha = 0.05$. Analyses were performed using SAS version 9.3 (SAS Institute).

Results

Twenty-three men were evaluated for long-term oral HPV16 persistence in this prospective analysis. These men were followed for a median of 44.4 months [interquartile range (IQR) 29.9–49.5] and ranged in age from 18 to 64 years (median 36 years; IQR, 25–42). One man (4.3%) only completed four follow-up visits, 4 men (17.4%) completed five visits, 8 men (34.8%) completed six visits, one man (4.3%) completed seven visits, 6 men (26.1%) completed eight visits, and 3 men (13.0%) completed nine visits. The sociodemographic and behavioral profile of men with oral HPV16, stratified by prevalent or incident infection, is presented in Table 1. Oral HPV16-positive men were predominantly young, white, non-Hispanic, never smokers, and well educated. No significant differences were observed between men with incident versus prevalent oral HPV16 infections.

Ten men (43.5%) entered the study with a prevalent oral HPV16 infection, and throughout the study period, 13 men (56.5%) acquired a new, incident infection. Thirteen men (56.5%) cleared their infection by the end of the study: 3 (30.0%) with prevalent and 10 (76.9%) with incident infections.

Overall, oral HPV16 persistence was substantially greater for prevalent versus incident infections (Table 2). Among men with prevalent infections, nine (90.0%) were found to persist ≥ 12 months, eight (80.0%) persisted ≥ 24 months, four (57.1%) persisted ≥ 36 months, and two (40.0%) persisted ≥ 48 months. Among men with incident oral HPV16 infections, four (30.8%) persisted ≥ 12 months, one (10.0%) persisted ≥ 24 months, and none persisted beyond 36 months. Using the Kaplan–Meier method, the cumulative probability of continued infection among men with prevalent oral HPV16 was consistently higher than that among men with an incident infection across the follow-up period (log-rank, $P = 0.014$; Fig. 1).

Age appeared to be associated with oral HPV16 persistence. The proportion of incident infections persisting ≥ 12 months increased significantly with increasing age ($P_{\text{trend}} = 0.028$; Fig. 2); men ≥ 45 years of age were more likely to have a persistent oral HPV16 infection (100%) than men ages 31 to 44 years (50.0%) or those men ages 18 to 30 years (0%).

Though not statistically significant, 12-month persistence of prevalent infections also increased with age ($P_{\text{trend}} = 0.604$). No statistically significant age trends were observed in the persistence of incident or prevalent infections at 24, 36, or 48 months (data not shown). Furthermore, no associations were observed between oral HPV16 persistence at 12, 24, 36, or 48 months and cigarette smoking, alcohol intake, country, educational attainment, marital status, sexual orientation, lifetime numbers of sexual partners, or ever/recent oral sexual behaviors (data not shown). In analyses using Cox regression, oral HPV16 persistence was not associated with any socio-demographic or behavioral characteristic, including age (data not shown).

Discussion

In this study, we describe patterns of long-term oral HPV16 persistence among otherwise healthy men. Using two sensitive HPV genotyping assays and analyzing additional specimens from a longer follow-up period allowed us to more accurately estimate long-term persistence compared with our previous studies (9). Our results indicate that prevalent HPV16 infections of the oral cavity are more likely to persist than newly acquired infections, and persistence appears to increase with age among men.

Few studies conducted to date have evaluated oral HPV16 persistence, none of which have reported data separately for prevalent and incident infections over such a long time span and across such a broad age range among otherwise healthy individuals. Furthermore, persistence estimates have varied widely, likely due to differences in definitions of persistence, study populations, study visit intervals, length of follow-up, oral sampling techniques, and HPV DNA detection methods. In a recent study by Mooij and colleagues conducted among 413 HIV-negative men who have sex with men (MSM), 3 of 9 (33%) prevalent oral HPV16 infections persisted 6 months (13). In a small study of 59 HIV-negative women, D'Souza and colleagues determined that three of five (60%) prevalent oral HPV infections persisted 6 months (14); however, the authors did not provide separate estimates for oral HPV16. As part of the large, prospective Finnish Family HPV (FFHPV) Study, an analysis of oral HPV among 121 male and 216 female spouses observed that 11 of 20 (55%) prevalent high-risk oral HPV infections detected at baseline persisted two years among men, and 15 of 31 (50%) persisted 2 years among women (15); however, these estimates were likely overestimated, as persistence analyses were not genotype-specific and relied only on prevalently detected infections. In subsequent FFHPV Study analyses, HPV16 was identified as the most frequent type to persist at the oral cavity, with longer persistence observed among men (mean, 22 months; ref. 16) than among women (mean, 19 months; ref. 17), although these analyses combined prevalent and incident HPV infections. In our study of 1,626 healthy, adult men, we report higher rates of persistence, with 100% of prevalent oral HPV16 infections persisting ≥ 6 months and 80% persisting ≥ 2 years, and 39% of incident infections persisting ≥ 6 months and 10% persisting ≥ 2 years.

Existing oral HPV16 infections (i.e., prevalent infections detected at baseline) were more likely to persist than newly acquired infections (i.e., incident infections detected at a follow-up visit), as predicted. Prevalent HPV infections are

Table 1. Characteristics of men with oral HPV16 at the time of enrollment into the oral HPV substudy

Characteristic	Total (n = 23) n (%)	Prevalent (n = 10) n (%)	Incident (n = 13) n (%)	P ^a
Country				0.241
USA	8 (34.8%)	2 (20.0%)	6 (46.2%)	
Brazil	7 (30.4%)	5 (50.0%)	2 (15.4%)	
Mexico	8 (34.8%)	3 (30.0%)	5 (38.5%)	
Age, years				0.721
Median (IQR)	36 (25-42)	37 (25-43)	32 (30-37)	
18-30	10 (43.5%)	4 (40.0%)	6 (46.2%)	
31-44	10 (43.5%)	4 (40.0%)	6 (46.2%)	
45-64	3 (13.0%)	2 (20.0%)	1 (7.7%)	
Race				1.000
White	12 (52.2%)	5 (50.0%)	7 (53.8%)	
Black	2 (8.7%)	1 (10.0%)	1 (7.7%)	
Asian/Pacific Islander	1 (4.3%)	0 (0%)	1 (7.7%)	
Mixed race	8 (34.8%)	4 (40.0%)	4 (30.8%)	
Ethnicity				1.000
Non-Hispanic	15 (65.2%)	7 (70.0%)	8 (61.5%)	
Hispanic	8 (34.8%)	3 (30.0%)	5 (38.5%)	
Marital status				0.594
Single	10 (43.5%)	3 (30.0%)	7 (53.8%)	
Married or cohabiting	10 (43.5%)	5 (50.0%)	5 (38.5%)	
Divorced, separated, or widowed	3 (13.0%)	2 (20.0%)	1 (7.7%)	
Education, y				0.754
≤12	7 (30.4%)	4 (40.0%)	3 (23.1%)	
13-15	6 (26.1%)	2 (20.0%)	4 (30.8%)	
≥16	10 (43.5%)	4 (40.0%)	6 (46.2%)	
Smoking status				1.000
Never	16 (69.6%)	8 (80.0%)	8 (61.5%)	
Former	1 (4.3%)	0 (0%)	1 (7.7%)	
Current	5 (21.7%)	2 (20.0%)	3 (23.1%)	
Missing data	1 (4.3%)	0 (0%)	1 (7.7%)	
Alcohol intake (drinks per month)				0.861
0	4 (17.4%)	2 (20.0%)	2 (15.4%)	
1-30	10 (43.5%)	5 (50.0%)	5 (38.5%)	
≥31	9 (39.1%)	3 (30.0%)	6 (46.2%)	
Sexual orientation				1.000
MSW	20 (87.0%)	9 (90.0%)	11 (84.6%)	
MSM	1 (4.3%)	0 (0%)	1 (7.7%)	
MSWM	2 (8.7%)	1 (10.0%)	1 (7.7%)	
Lifetime number of sex partners				0.934
Median (IQR)	10 (4-26)	11 (4-26)	8 (5-20)	
0-2	3 (13.0%)	1 (10.0%)	2 (15.4%)	
3-7	6 (26.1%)	2 (20.0%)	4 (30.8%)	
8-19	7 (30.4%)	4 (40.0%)	3 (23.1%)	
≥20	7 (30.4%)	3 (30.0%)	4 (30.8%)	
Ever had oral sex				0.277
No	4 (17.4%)	3 (30.0%)	1 (7.7%)	
Yes	19 (82.6%)	7 (70.0%)	12 (92.3%)	
Ever had oral sex with a man				0.622
No	18 (78.3%)	7 (70.0%)	11 (84.6%)	
Yes	5 (21.7%)	3 (30.0%)	2 (15.4%)	
Had oral sex in the past 6 months ^b				0.640
No	7 (31.8%)	4 (40.0%)	3 (23.1%)	
Yes	15 (68.2%)	6 (60.0%)	9 (69.2%)	
Missing data	1 (4.3%)	0 (0%)	1 (7.7%)	
Number of oral sex acts in the past 6 months ^b				0.579
0	9 (39.1%)	4 (40.0%)	5 (38.5%)	
1-6	1 (4.3%)	0 (0%)	1 (7.7%)	
7-24	8 (34.8%)	3 (30.0%)	5 (38.5%)	

(Continued on the following column)

Table 1. Characteristics of men with oral HPV16 at the time of enrollment into the oral HPV substudy (Cont'd)

Characteristic	Total (n = 23) n (%)	Prevalent (n = 10) n (%)	Incident (n = 13) n (%)	P ^a
≥25	4 (17.4%)	3 (30.0%)	1 (7.7%)	
Missing data	1 (4.3%)	0 (0%)	1 (7.7%)	
Time since last oral sex (days) ^b				0.519
0-3	6 (26.1%)	3 (30.0%)	3 (23.1%)	
>3-10	2 (8.7%)	0 (0%)	2 (15.4%)	
>10-30	3 (13.0%)	2 (20.0%)	1 (7.7%)	
>30	6 (26.1%)	2 (20.0%)	4 (30.8%)	
Never	4 (17.4%)	3 (30.0%)	1 (7.7%)	
Missing data	2 (8.7%)	0 (0%)	2 (15.4%)	

Abbreviations: MSW, men who have sex with women; MSM, men who have sex with men; MSWM, men who have sex with women and men.

^aExact Pearson χ^2 P value using Monte Carlo estimation.

^bQuestion not asked among all men because the study questionnaire was revised in 2007 after oral sample collection began.

more likely to be long-term, persistent infections; the longer the duration of an HPV infection, the higher the likelihood of continued persistence, and the higher the probability of detecting HPV DNA at any one time point. In our study, 40% of prevalent oral HPV16 infections persisted beyond 4 years, whereas none of the incident infections persisted beyond 3 years. These findings are consistent with the cervical HPV literature, which supports the hypothesis that prevalent high-risk cervical HPV infections are associated with a high likelihood of persistence (18).

Recent studies have sought to understand rates and determinants of oral HPV acquisition and clearance and, in doing so, have raised important questions about the effects of gender and age. In a large, population-based study of oral HPV prevalence (19), men were three times more likely than women to have a prevalent infection, mirroring the gender ratio observed in HPV-positive oropharyngeal cancers. In the same study, a significant bimodal age distribution was observed among men, with the peak prevalence occurring among men over 50 years, which is contrary to the established decline in cervical HPV prevalence with age (20). In a previous analysis conducted among men in the HIM Study (9), we showed that the risk of acquiring a new oral HPV infection remained constant across the lifespan, suggesting that the peak in prevalence at older ages is likely due to an increase in infection persistence. Here, we suggest that persistence of incident oral HPV16 infections increases with age among men, a finding consistent with one study conducted among women (14). Altogether, these data support the hypothesis that increased prevalence of oral HPV16 detected among men at older ages is likely due to increased duration of infections and not increased acquisition, a pattern consistent with that of cervical HPV infection persistence (21-23). Similar to factors thought to influence HPV persistence at the cervix, our observation of increased oral HPV persistence with age may be due to impairment of host immune responses (24) and elevation of inflammatory cytokines (25) that occur throughout the aging process. In a recent FFHPV study, Louvanto and colleagues demonstrated that oral high-risk HPV infections persist three times longer among women with long-term, persistent cervical HPV infections compared with cervical HPV-negative women (26), suggesting that similar factors may be responsible for increased HPV persistence,

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Table 2. Long-term persistence of prevalent and incident oral HPV16 infections in men ($n = 23$)

Persistence	Prevalent ($n = 10$)		Incident ($n = 13$)	
	Number of persistent infections/ number of total infections ^a	%	Number of persistent infections/ number of total infections ^a	%
≥6 months	10/10	100	5/13	38.5
≥12 months	9/10	90.0	4/13	30.8
≥18 months	9/10	90.0	4/13	30.8
≥24 months	8/10	80.0	1/10	10.0
≥30 months	6/8	75.0	1/10	10.0
≥36 months	4/7	57.1	0/9	0
≥42 months	3/6	50.0	0/9	0
≥48 months	2/5	40.0	0/9	0

^aAs not all men were followed for 48 months, the number of total infections changes over time.

regardless of epithelial site of infection. Larger cohort studies are needed to elucidate the biologic and behavioral mechanisms underlying oral HPV persistence.

The role of tobacco use in oral HPV natural history remains unclear. In our previous analysis, cigarette smokers were significantly more likely to acquire a new oral high-risk HPV infection (9); however, here we report no association between smoking and oral HPV16 persistence. This finding is contrary to two other studies that demonstrated that any (14) and high-risk (16) oral HPV were more likely to persist among current smokers. Smoking is known to cause local inflammation and immunosuppression (27) and is thought to increase susceptibility to a new HPV infection at the oral epithelium. However, smoking has also been found to induce expression of secretory leukocyte protease inhibitor (SLPI; ref. 28), an innate immunity-associated protein that has been hypothesized to protect against HPV infection (29). Future studies are needed to understand the complex role of smoking and SLPI with respect to oral HPV natural history before definitive interpretations are made.

This is one of the first studies to provide detailed estimates of long-term oral HPV16 persistence using data from a large cohort of healthy, adult men. These findings are more likely to represent oral HPV16 natural history than previous reports, due to increased sensitivity of HPV DNA detection and longer follow-up of study participants. However, the following limitations should be considered. First, despite having such a large cohort, we acknowledge that oral HPV16 infection is rare

among otherwise healthy men, thus limiting our ability to identify factors significantly associated with infection persistence. However, to our knowledge, the HIM Study is the only existing HPV natural history study with the ability to describe long-term oral HPV persistence in healthy men. Second, our definition of persistence was based on consecutive study visits and assumed that men returned every 6 months, on schedule. While most men adhered to this protocol, some may have returned for the next visit later than scheduled, increasing the time between visits. As a result, we performed an additional time-to-event analysis based on the Kaplan–Meier method. Third, we may have underestimated persistence among one individual who had a single HPV16-negative result at his final study visit. This individual's infection was considered cleared in our analysis, although it is possible that this final specimen contained a low viral load that was undetectable by Linear Array. Finally, intermittent negativity was observed among some HPV16-positive men, which has been described in other studies (30). These negative visits may indicate a low copy number infection that fell below the lower limit of assay detection (31); however, we acknowledge that this pattern may also represent reactivation of a latent infection or reinfection after a previously cleared infection. In the current study, we utilized a more sensitive assay, INNO-LiPA, to re-test samples that appeared to have fallen below the lower limit of detection, and indeed found that we would have misclassified several specimens as negative and therefore underestimated our estimates of infection persistence, had we relied on the linear array method alone.

Understanding patterns of oral HPV persistence is essential in the development of HPV-driven oropharyngeal cancers prevention and early detection strategies. Long-term persistence of high-risk genital HPV infection is the strongest predictor of cervical precancer and cancer development in women (32). Among all high-risk types, HPV16 has been shown to persist longest and confer the highest risk of developing precancerous lesions. More than 40% of women with cervical HPV16 infections persisting at least one year develop cervical intraepithelial neoplasia grade 2 or more severe lesions within 3 years (33). Given that the overwhelming majority of prevalent oral HPV16 infections detected here persisted beyond one year, and 40% persisted beyond 4 years, there is a clear need to evaluate whether long-term persistent oral HPV16 infection can predict future oropharyngeal cancer risk. With a constant rate of acquisition of new oral HPV infections (9) and higher likelihood of persistence with age, mid-adult and older men appear to be at the highest risk of oral HPV infection and should be the focus of prevention interventions.

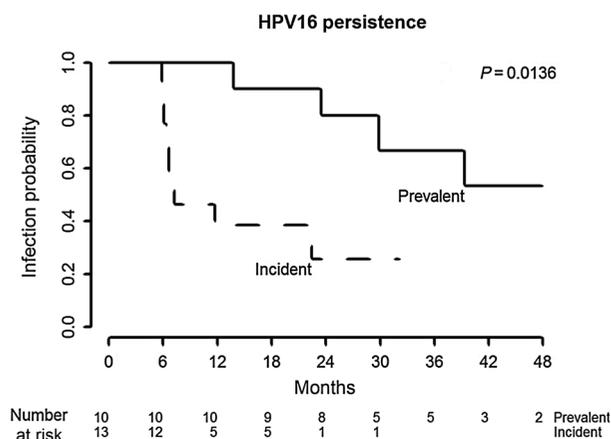
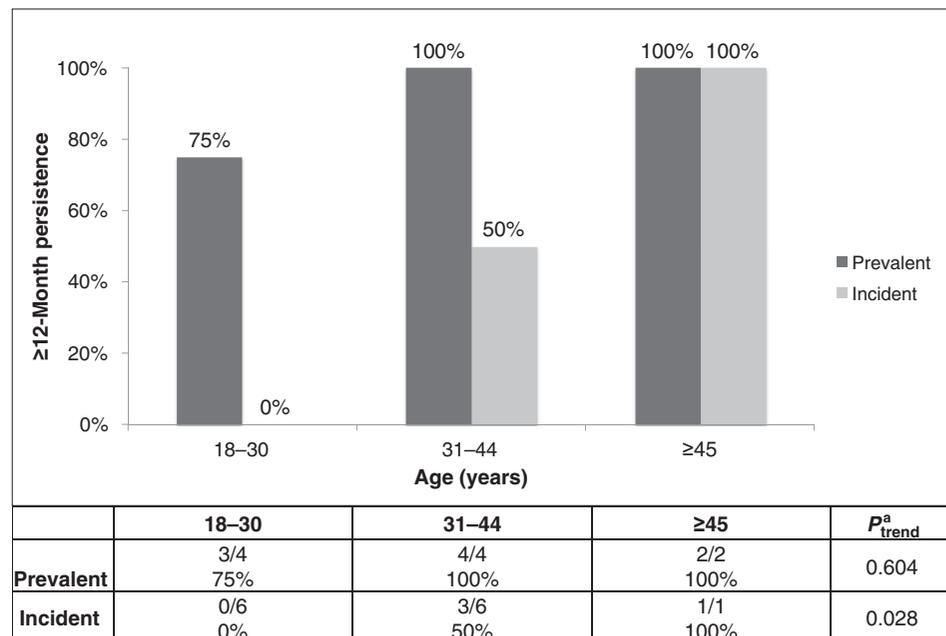


Figure 1. Kaplan–Meier estimates of prevalent and incident oral HPV16 persistence in men ($n = 23$).

Figure 2.
 ≥ 12 -Month persistence of oral HPV16 in men ($n = 23$), by age. ^aCochran-Armitage test for trend using exact Monte Carlo methods.



Disclosure of Potential Conflicts of Interest

L.L. Villa has received speakers bureau honoraria from Merck Sharp & Dohme and is a consultant/advisory board member for Merck Sharp & Dohme and BD Technologies. No potential conflicts of interest were disclosed by the other authors.

Disclaimer

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