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Research Article

Cancer Epidemiology, Biomarkers & Prevention

The Epidemiology of Oral HPV Infection among a Multinational Sample of Healthy Men

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Abstract

Background: Oral human papillomavirus type-16 (HPV16) infection is a risk factor for oropharyngeal cancer. We examined oral HPV infection among healthy men.

Methods: Oral rinse/gargle specimens and questionnaire data were collected from 1,688 healthy men aged 18 to 74 (median = 31 years), from the United States, Mexico, and Brazil. HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58 and 59, and noncarcinogenic HPV types were detected using Roche Linear Array.

Results: Oral HPV DNA was detected in 67 of 1,680 (4.0%, 95% CI = 3.1%–5.0%) β-globin–positive specimens; carcinogenic HPVs were detected in 1.3% (95% CI = 0.8%–2.0%; $n = 22$) and HPV16 was the most commonly detected carcinogenic HPV type (0.6%, 95% CI = 0.2%–1.1%; $n = 10$). The prevalence of oral HPV infection was similar by country except for HPV55, which had notably higher prevalence in Mexico (3.0%) than Brazil (0%) or the United States (0.2%). Oral HPV prevalence nonsignificantly increased over increasing age categories ($P_{trend} = 0.096$). The strongest predictor of oral HPV was current tobacco use, which increased the odds 2.5-fold (95% CI = 1.4–4.4). Oral sexual behaviors were not associated with oral HPV infection.

Conclusions: Oral HPV16 infection was rare in healthy men, especially at younger ages, and was positively associated with current tobacco use.

Impact: Oral HPV appears to be about 10-fold less prevalent than infection at genital sites in men (4% vs. ~40%, respectively). It remains unclear whether this reflects reduced exposure or if the oral region is more resistant to HPV infection compared with anogenital sites. *Cancer Epidemiol Biomarkers Prev; 20(1); 172–82. ©2011 AACR.*

Introduction

Human papillomavirus type 16 (HPV16) infection is one of the most important human carcinogens, causing greater than 300,000 deaths per year (1, 2). Approximately, 25% to 50% of oropharynx cancers are caused by HPV infection (3, 4), greater than 90% of which are caused by HPV16. A recent case–control study showed that detection of HPV16 infection in oral exfoliated cells was significantly associated with oropharyngeal cancer (OR ≈ 13; ref. 4). However, despite the established link between HPV16 and some oropharyngeal cancers, little is known about the epidemiology of oral HPV infection among healthy individuals such as the country-specific and age-specific prevalence of oral HPV infections.

A recent systematic review of the literature showed oral HPV16 prevalence was 1.3% among healthy individuals and appeared to differ by geographic region, although significant heterogeneity between studies due to differences in specimen collection, processing, and testing limited conclusive interpretation of the data (5). Small sample size among each study was also a limitation. Although it was not possible to evaluate the age-specific oral HPV prevalence in the context of this recent review, individual smaller studies have addressed this question. Interestingly, and in contrast to what has been observed for the cervix, it appears that oral HPV infection may either increase or remain stable with increasing age (6–8).

To expand upon previous publications, we conducted a study to evaluate oral HPV prevalence among healthy adult men from 3 countries utilizing a shared protocol for specimen collection, as well as centralized specimen processing and HPV testing. The aim of this study was to describe the epidemiology and natural history of HPV infection in oral specimens collected from healthy men internationally. In this first report, we describe the baseline oral HPV prevalence among

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the first 1,688 men to have an oral specimen collected in this cohort.

Materials and Methods

This work was nested within the ongoing HPV in Men (HIM) Study, which has been previously described (9). Briefly, men were recruited from Sao Paulo, Brazil; Cuernavaca, Mexico; Tampa, FL (and its surrounding areas), from March, 2005, to December, 2006. The oral component of this research was initiated in June, 2007; the specific recruitment period for oral specimen collection at each of the clinic sites for data included in this manuscript was as follows: Brazil, December 13, 2007, to January 16, 2009; Mexico, April 7, 2008, to February 29, 2009; United States, June 13, 2007, to November 11, 2008. The Human Subjects Committees of the University of South Florida, the Centro de Referencia e Tratamento de Doencas Sexualmente Transmissiveis e AIDS, Brazil, and the National Institute of Public Health of Mexico approved all study procedures. All participants gave written informed consent.

Population

The cohort consisted of men who met the following eligibility criteria: (a) ages 18 to 70 years; (b) residents of 1 of 3 recruitment sites in Brazil, Mexico, or the United States; (c) reported no prior diagnosis of penile or anal cancers (Note: no exclusion was based on head and neck cancer); (d) have never been diagnosed with genital or anal warts; (e) currently, report no symptoms of a sexually transmitted infection or treatment for a sexually transmitted infection; (f) not participating in an HPV vaccine study; (g) no history of HIV or AIDS; (h) no history of imprisonment, homelessness, or drug treatment during the past 6 months; and (i) willing to comply with 10 scheduled visits every 6 months for 4 years with no plans to relocate within the next 4 years.

Men were recruited from different population sources to increase access to a broad range of ages, sexual behaviors, and HPV risk. In Brazil, men were recruited from the general population at a facility for urogenital care (Centro de Referencia e Tratamento de Doencas Sexualmente Transmissiveis e AIDS) and through general media advertising. Men presenting for conditions related to nonsexually transmitted infection were enrolled in the present study. In addition, the spouses and partners of women participating in a large cohort study of the natural history of HPV infection and risk of cervical neoplasia conducted in Sao Paulo since, 1993, were also recruited. At the Mexico site, the population was composed of employees and beneficiaries of the Instituto Mexicano de Seguro Social, factory employees, and officials of the Mexican army that are permanently assigned to this geographic area. In the United States, the population was recruited from the University of South Florida and the greater Tampa metropolitan area. Flyers and posters were distributed throughout the campus and community; monthly educational presentations were given. In addition, men from the broader Tampa Bay, FL, community were recruited through the mail and media using brochures and flyers as well as advertisements in local and university papers.

Study protocol

The HIM Study protocol includes a preenrollment run-in visit, a baseline (enrollment) visit, and 8 additional visits after enrollment scheduled 6 months apart. Of note, because the oral component was initiated approximately 2 years after enrollment into the HIM cohort commenced, the first oral specimen collected (not necessarily at the enrollment visit) was utilized in the current study. Here, we report results for the first 1,688 men with at least 1 archived oral gargle specimen.

Risk factor questionnaire. An extensive computer-assisted self-interview was administered and queried on sociodemographic characteristics, sexual history (e.g., recent and lifetime numbers sexual partners for vaginal and anal sex, oral sexual practices), condom use practices, alcohol and tobacco use, and history of abnormal Pap smears in female partners.

Specimen collection. For the oral gargle specimens, we chose to use locally available mouthwashes after pilot work indicated there was abundant human DNA with similar purity measures regardless of the brand (Brazil: PLAX; Mexico: Oral-B; US: Target Brand). Fifteen milliliters of mouthwash was swished in the oral cavity for 15 seconds, gargled for 15 seconds, and expectorated into a specimen cup. Specimens were refrigerated until processing, which occurred before the end of the day. To process, the oral rinse was centrifuged at $3{,}000 \times g$ for 10 minutes at 4°C, the supernatant was decanted and the pellet was resuspended in 10 mL of sterile normal saline; the centrifugation was repeated and the pellet was resuspended in 1 mL of saline with repeated pipetting and vortexing to ensure even sample distribution. The sample was then stored at −70°C until PCR analyses and genotyping were conducted.

DNA extraction and HPV testing

Because of the previous reports of the importance of DNA extraction for oral HPV DNA detection (10), 2 protocols were compared in the early stages on this research: Puregene (the gold-standard established by the publication) and the QIAGen automated DNA extraction method. All available oral specimens with known HPV positivity that were initially extracted using the manual method of DNA extraction (with a PCR input volume of 30 ng of total DNA) were used ($n = 6$). Further, to compensate for low viral load of HPV DNA in oral specimens, 4 concentrations of the total input DNA were compared [10 ng, 30 ng (the amount specified by the Roche PCR protocol), 50 ng, and 100 ng]; each specimen was therefore tested 8 additional times (2 methods of DNA extraction × 4 concentrations of total DNA input) after the initial round of testing. Similar amounts of DNA

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were available using each method; however, the automated method, within each input concentration, always detected more HPV infections than the manual method. Further, in varying the input DNA, 10 ng appeared inadequate as the majority of the known oral HPV infections were not detected (4/6). For the highest DNA input (100 ng), known oral HPV positives were also not detected (2/6). DNA input of 50 ng not only detected the highest number of HPV infections but also detected 1 multiple infection. Although limited by the available number of positive oral samples on which to conduct this methods work, we concluded that automated DNA extraction using 50 ng of total DNA per PCR reaction was optimal for detecting oral HPV infections and implemented these procedures accordingly.

DNA extraction was conducted using the Robotic MDx Media Kit (Qiagen) according to the instructions of the manufacturer; if a sample was β-globin negative, it was manually re-extracted. Briefly, 200 μL of aliquots of clinical material were digested with 20 μL of proteinase K solution and lysed with 200 μL of lysis buffer at 56°C. Specimens were tested for the presence of HPV by amplifying 50 ng of DNA with the PGMY09/11 L1 consensus primer system. HPV genotyping was conducted using the linear array method on all samples, regardless of HPV PCR result (Roche Molecular Diagnostics; refs. 11, 12). Samples were amplified using MJ Research PTC-200 thermocycler.

Classification of HPV types. The following 12 HPV types were categorized as carcinogenic: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59 (2). The other (noncarcinogenic) HPV types detected with the Linear Array system of Roche were 6, 11, 26, 40, 42, 44, 53, 54, 55, 61, 62, 64, 66, 67, 68, 69, 70, 71, 72, 73, 81, 82, 83, 84, IS39, and CP6108. A participant was considered positive for "any HPV infection" if his sample amplified HPV on PCR and hybridized with a specific HPV type upon genotyping [8 samples (0.5%) were PCR negative but genotype positive upon hybridization]. The category of "any carcinogenic type" included those who were positive either for only carcinogenic genotypes and those who were positive for both carcinogenic and noncarcinogenic types. Only single or multiple infections with noncarcinogenic HPV types were classified as "any noncarcinogenic type."

Statistical analysis

For this analysis, the first 1,688 men who provided an oral rinse gargle specimen collected were included. β-Globin was detected in 99.5% of oral samples tested (1,680/1,688); only β-globin–positive samples were included in these analyses. The majority (59%) of the baseline samples from this work came from either the run-in or enrollment study visit. These men were representative of the entire HIM cohort ($n = 4,074$) with regard to country, age distribution, sociodemographic characteristics, and sexual behaviors; no major exceptions were noted (data not shown).

We evaluated the distribution of participant characteristics, including demographics and sexual behavior, in Table 1; participants were given the option of refusing to answer each of the questions on the web-based survey, and these refusals were treated as missing observations. For all time-dependant covariates, such as age, data from the questionnaire corresponding to the visit when the oral specimens were collected was used for this analysis.

Prevalence of HPV infections was evaluated by country and by age; significant heterogeneity was assessed by use of the χ^2 test. Logistic regression models were used to estimate ORs and 95% CIs for associations between demographic and exposure variables and the presence of oral HPV DNA. Trend tests were conducted across ordered groups. Variables that were important in univariate analysis were evaluated in a multiple logistic regression model, as were variables that were considered to be relevant on the basis of the literature. The final model was created by the inclusion of variables with potential biological significance, as well as those that remained statistically significant after adjustment. All P values reported were 2-sided and were considered to be statistically significant at $P < 0.05$.

Results

A total of 1,680 men (475 from Brazil, 591 from Mexico, and 614 from the United States) with β-globin–positive oral specimens were included in this analysis (Table 1). At the time of oral specimen collection, men ranged in age from 18 to 74 years, with a median age of 31 years; men from the United States were younger than men from the 2 other sites. The majority of study participants were nonwhite. Almost one-half (46.8%) reported Hispanic ethnicity. Men from Brazil were more likely to have less than or equal to 12 years of education whereas U.S. participants were more likely to have 13 or more years of schooling. Nearly half of the participants (47.1%) were either married or cohabiting, and 44.3% reported being single or never married. Less than 25% of the population reported current smoking; although, the proportion of smokers was higher in Mexico (29.9%) compared with Brazil and the United States (18.2% and 18.8%, respectively). Most men reported only having sex with women (91.8%) in their lifetime; 3.5% and 4.7% reported having sex with men only (MSM) and with both men and women (MSMW), respectively. The majority of men reported 2 or more female sexual partners during the lifetime; men from Brazil reported more lifetime sexual partners than men from Mexico and the United States. Ever participating in oral sex was common for men in each country (94.3% in Brazil, 83.6% in Mexico, and 95.6% in the United States), as was participating in oral sex over the most recent 6 months (Table 1).

The prevalence of HPV DNA in the oral region was 4.0% (95% CI = 3.1%–5.0%) and differed significantly by country [Brazil 2.1% (95% CI = 1.0%–3.8%), Mexico 5.9% (95% CI = 4.2%–8.1%), United States 3.6% (95% CI =

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Table 1. Sociodemographic characteristics, tobacco use, and sexual behavior of HIM Study participants by country

	Brazil (*N* = 475)	Mexico (*N* = 591)	United States (*N* = 614)	Total (*N* = 1,680)
Sociodemographic characteristics				
Age, y				
18–24[a]	86 (18.1)	85 (14.4)	334 (54.4)	505 (30.1)
25–34	171 (36.0)	226 (38.2)	112 (18.2)	509 (30.3)
35–44	174 (36.6)	159 (26.9)	82 (13.4)	415 (24.7)
45–54	34 (7.2)	88 (14.9)	47 (7.7)	169 (10.1)
55+	10 (2.1)	33 (5.6)	39 (6.3)	82 (4.8)
Median	33.0	34.0	24.0	31.0
Race[b]				
White	306 (64.4)	37 (6.3)	423 (68.9)	766 (45.6)
Black	124 (26.1)	2 (0.3)	105 (17.1)	231 (13.8)
Other	45 (9.5)	552 (93.4)	86 (14.0)	683 (40.6)
Ethnicity[c]				
Hispanic	104 (22.2)	589 (99.8)	88 (14.4)	781 (46.8)
Non-Hispanic	364 (77.8)	1 (0.2)	523 (85.6)	888 (53.2)
Marital status[d]				
Single	201 (42.5)	128 (21.7)	414 (67.6)	743 (44.3)
Married	223 (47.0)	431 (73.1)	135 (22.1)	789 (47.1)
Divorced	50 (10.5)	31 (5.2)	63 (10.3)	144 (8.6)
Education, y				
<12	74 (15.7)	251 (42.6)	16 (2.6)	341 (20.4)
12	195 (41.3)	145 (24.6)	69 (11.2)	409 (24.4)
13 –16	177 (37.5)	149 (25.3)	468 (76.3)	794 (47.4)
17+	26 (5.5)	44 (7.5)	61 (9.9)	131 (7.8)
Tobacco				
Tobacco use				
Never	295 (62.5)	269 (46.3)	387 (66.2)	951 (58.1)
Former	91 (19.3)	138 (23.8)	88 (15.0)	317 (19.3)
Current	86 (18.2)	174 (29.9)	110 (18.8)	370 (22.6)
Age at initiation of smoking, y				
<15	40 (23.0)	65 (20.8)	61 (31.3)	166 (24.4)
15–17	69 (39.7)	150 (48.1)	72 (36.9)	291 (42.7)
18+	65 (37.4)	97 (31.1)	62 (31.8)	224 (32.9)
Years of smoking				
Zero	296 (63.0)	270 (46.6)	392 (67.1)	958 (58.7)
1–<10	75 (16.0)	152 (26.3)	105 (18.0)	332 (20.3)
10+	99 (21.0)	157 (27.1)	87 (14.9)	343 (21.0)
Sexual behavior				
Sexual behavior with women and/or men				
MSW	373 (81.8)	531 (95.5)	560 (96.2)	1,464 (91.8)
MSM	32 (7.0)	8 (1.4)	15 (2.6)	55 (3.5)
MSWM	51 (11.2)	17 (3.1)	7 (1.2)	75 (4.7)
Age at sexual debut, y				
Virgin	13 (2.9)	32 (5.9)	27 (5.2)	72 (4.8)
9–14	126 (28.4)	71 (13.2)	77 (15.2)	274 (18.4)
15–17	190 (42.8)	209 (38.8)	236 (46.5)	635 (42.6)
18+	115 (25.9)	227 (42.1)	168 (33.1)	510 (34.2)
Lifetime number of female sexual partners				
0–1	52 (11.7)	89 (16.2)	99 (17.4)	240 (15.4)
2–9	145 (32.5)	296 (53.9)	233 (41.0)	674 (43.1)

(Continued on the following page)

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Table 1. Sociodemographic characteristics, tobacco use, and sexual behavior of HIM Study participants by country (Cont'd)

	Brazil (*N* = 475)	Mexico (*N* = 591)	United States (*N* = 614)	Total (*N* = 1,680)
10+	249 (55.8)	164 (29.9)	237 (41.6)	650 (41.5)
Performed oral sex[c]				
Never	24 (5.7)	87 (16.4)	21 (4.4)	132 (9.2)
Ever	400 (94.3)	443 (83.6)	460 (95.6)	1,303 (90.8)
Recently performed oral sex[e]				
No	88 (20.1)	219 (37.2)	164 (28.3)	471 (29.3)
Yes	350 (79.9)	370 (62.8)	416 (71.7)	1,136 (70.7)
Number of times performed oral sex in the past 6 months				
0–1	110 (25.9)	248 (43.6)	187 (33.6)	545 (35.2)
2–9	134 (31.6)	160 (28.1)	137 (24.6)	431 (27.8)
10+	180 (42.5)	161 (28.3)	233 (41.8)	574 (37.0)
Insertive anal sex[c]				
Never	99 (21.6)	252 (47.6)	311 (60.6)	662 (44.1)
Ever	360 (78.4)	277 (52.4)	202 (39.4)	839 (55.9)
Receptive anal sex[c]				
Never	376 (82.5)	492 (93.9)	490 (95.5)	1,358 (91.0)
Ever	80 (17.5)	32 (6.1)	23 (4.5)	135 (9.0)

NOTE: The given values are represented as number (percentages).
Abbreviations: MSW, men who have sex with women; MSM, men who have sex with men; and MSMW, men who have sex with men and women.
[a]Category contains one 17 year old.
[b]In the race variable, the "other" category includes Asian, Pacific Islander, American Indian, and Mixed race individuals. In Mexico, most participants in our study self-identify as "Mestizo" which is typically considered a mix of European white and indigenous races.
[c]Indicates at least 10% of the data were missing.
[d]In the marital status variable, the "married" category includes cohabiting and living together and "divorced" includes separated and widowed.
[e]For variables indicating recent sexual activity, recent was defined as activity occurring over the past 6 months

2.3%–5.4%); $P = 0.005$; Table 2]. When HPV types were grouped by carcinogenicity, 1.3% (95% CI = 0.8%–2.0%) of the overall population had a carcinogenic HPV infection. The prevalence of these carcinogenic infections was similar across countries [Brazil 1.3% (95% CI = 0.5%–2.7%), Mexico 1.0% (95% CI = 0.4%–2.2%), United States 1.6% (95% CI = 0.8%–3.0%); $P = 0.642$]. Multiple infections were rare [detected in 0.3% of the population or 4.5% of the oral HPV-positive population (5 of 67); Table 2].

The most commonly detected carcinogenic HPV type in each country was HPV16 [0.6% (95% CI = 0.3%–1.1%); $n = 10$; Table 3]. Other carcinogenic types detected included HPV 31, 35, 39, 52, 56, 58, and 59. Individual noncarcinogenic infections were rarely detected, except for HPV55 (1.1%, 95% CI = 0.7%–1.8%; $n = 19$), which had

Table 2. Summary results for baseline oral specimen collection, grouped by HPV type and country

	Brazil (*N* = 475)	Mexico (*N* = 591)	USA (*N* = 614)	Total (*N* = 1.680)	*P* for differences across countries
Any HPV	10 (2.1)	35 (5.9)	22 (3.6)	67 (4.0)	0.005
Any carcinogenic[a] HPV	6 (1.3)	6 (1.0)	10 (1.6)	22 (1.3)	0.642
Any noncarcinogenic HPV	4 (0.8)	29 (4.9)	12 (2.0)	45 (2.7)	<0.001
Multiple HPV infections	1 (0.2)	1 (0.2)	3 (0.5)	5 (0.3)	0.307

NOTE: The given values are represented as number (percentages).
[a]Carcinogenic HPV types defined as HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59.

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Table 3. Baseline type-specific HPV infection distribution by country

	Brazil (*N* = 475)	Mexico (*N* = 591)	United States (*N* = 614)	Total (*N* = 1,680)
Carcinogenic[a]				
16	3 (0.6)	3 (0.5)	4 (0.7)	10 (0.6)
31	1 (0.2)			1 (0.1)
35			1 (0.2)	1 (0.1)
39		1 (0.2)		1 (0.1)
51	1 (0.2)	1 (0.2)	1 (0.2)	3 (0.2)
52			1 (0.2)	1 (0.1)
56		1 (0.2)	1 (0.2)	2 (0.1)
58	1 (0.2)			1 (0.1)
59			2 (0.3)	2 (0.1)
Noncarcinogenic[a]				
6	1 (0.2)		1 (0.2)	2 (0.1)
11		1 (0.2)		1 (0.1)
53			1 (0.2)	1 (0.1)
55		18 (3.0)	1 (0.2)	19 (1.1)
61	1 (0.2)	3 (0.5)		4 (0.2)
62		3 (0.5)	1 (0.2)	4 (0.2)
64			1 (0.2)	1 (0.1)
66	1 (0.2)	1 (0.2)	1 (0.2)	3 (0.2)
69	1 (0.2)			1 (0.1)
70	1 (0.2)		1 (0.2)	2 (0.1)
71		1 (0.2)	2 (0.3)	3 (0.2)
72			4 (0.7)	4 (0.2)
82		1 (0.2)		1 (0.1)
84	1 (0.2)		3 (0.5)	4 (0.2)
89		1 (0.2)		1 (0.1)

NOTE: The given values are represented as number (percentages).

[a]HPV types 18, 33, 45, 68, 26, 40, 42, 54, 67, 73, 81, 82, and 83, were not detected in any of the oral specimens and were excluded from the table.

a notably high prevalence in Mexico (3.0%; $n = 18/19$ infections detected). Because of concerns that this finding might be an artifact despite rigorous clinical and laboratory methods to avoid contamination, we investigated these specimens further: the HPV55-positive specimens were collected at several recruitment sources (and not a single possibly contaminated source) over a period of several months (and not limited to a short time period). They were extracted on different days and the PCR and genotyping also occurred on different days. The HPV55-positive specimens were then re-extracted and genotyped in a blinded fashion which resulted in identical findings to the first run; throughout, all positive and negative controls performed as expected.

Because of the limited number of outcomes of individual HPV type infections, men from the 3 countries were combined to evaluate factors associated with oral HPV prevalence. Men in the youngest age category of 18 to 24 years ($n = 505$; Note: category includes one 17 year old) had the lowest oral HPV infection prevalence (3.2%; 95% CI = 1.8%–5.1%). The prevalence of oral HPV nonsignificantly increased over increasing age categories ($P_{trend} = 0.096$), men in the oldest age strata, aged 55 to 74 years ($n = 82$), had the highest oral HPV prevalence (6.1%, 95% CI = 2.0%–13.7%; Fig. 1); similar patterns by country were observed. Behavioral factors were also investigated; only smoking-related variables were associated with a significant increase in the odds of detecting oral HPV infection (OR for current smoking: 2.5, 95% CI = 1.4–4.4). No significant associations were observed for the following variables: alcohol measured as drinks per day and lifetime consumption, other forms of tobacco use such as chew and snuff, age at vaginal sexual debut, lifetime number of vaginal sexual partners, performing oral sex, and anal sex (insertive and receptive). Individuals who reported never performing oral sex had a similar prevalence of oral HPV infections compared with those who reported ever having oral sex (3.8% vs. 4.1%, respectively). In the multivariate model, current tobacco use independently increased odds of oral HPV

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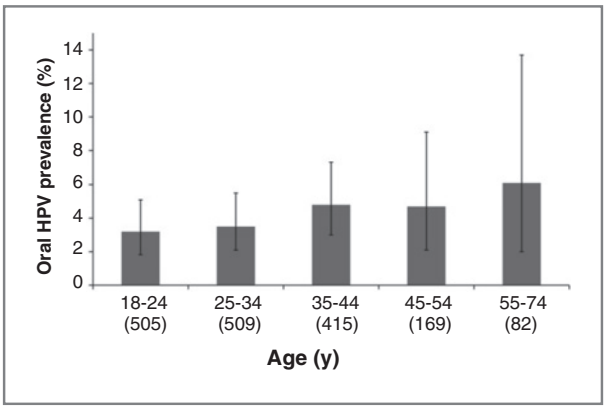

Figure 1. Age-specific prevalence of any oral HPV infection. Oral HPV prevalence was lowest in the youngest age category of 18 to 24 years ($n = 505$; 3.2%; 95% CI = 1.8%–5.1%; Note: category includes one 17 year old). The prevalence of oral HPV nonsignificantly increased over increasing age categories ($P_{trend} = 0.096$), men in the oldest age strata, aged 55 to 74 years ($n = 82$), had the highest oral HPV prevalence (6.1%, 95% CI = 2.0%–13.7%).

infection; the borderline significant trend of increasing oral HPV infection noted with increasing age was no longer present; these associations were adjusted for country (Table 4).

Discussion

We observed, in the largest study to date, that oral HPV infection is present in a subset of healthy men (~4%) and that oral HPV16 infection, a risk factor for oropharyngeal cancer, is rare in our cohort (<1%). However, oral HPV infection was present in 6% of healthy men over the age of 55 years. This finding is in contrast to what is typically observed at the cervix, where cervical HPV infection decreases with increasing age into the fourth or fifth decades of life throughout most of the world (13) but is similar to the epidemiology of HPV infection at the anus (14,15) and the penis (16–18), where HPV prevalence remains constant across the age span. Because these were prevalently detected infections, a function of both incidence and duration, it is not possible to determine whether new infections were either occurring more commonly or were more likely to persist in older age groups, thereby creating the observed age effect. Immune waning over the aging process could explain both increased acquisition and persistence at older ages, but does not account for the differences between the trends observed for oral compared with cervical HPV infection. Instead, it may be that immune surveillance differs at different anatomic sites and perhaps other co-factors, such as oral hygiene and microbiome (19), may play an important role in the immune response to HPV infection in the oral region. Oral HPV natural history is not well studied or understood. More research is needed that evaluates rates of HPV persistence across multiple anatomic sites within a cohort.

Current tobacco use was significantly associated with detection of oral HPV infection and was the factor most strongly associated with oral HPV infection; a finding previously shown in another study of oral HPV infection among healthy individuals (20). Cigarette smoking alters a wide range of immunologic functions in the oral cavity including adaptive and innate immune responses (21). It may be that current tobacco use inhibits immune function in a way that allows for increased HPV persistence in addition to the direct genetic damage to cells; persistence would in turn increase the opportunity for detection cross-sectionally. From the cervical literature, tobacco smoking consistently increases the duration of HPV infections and the risk of progression to cervical precancer and cancer, even after controlling for the effects of HPV infection (22–24). This effect may be more profound in the oral cavity, where the mucosal epithelium is directly exposed to the carcinogens in tobacco compared with the indirect path to the cervical mucosa (although the 2-fold elevation in risk of infection is comparable to what is reported in the cervical cancer literature). Prospective studies of oral HPV infection that investigate acquisition and persistence can best address the association between tobacco use and the natural history of oral HPV infections.

Performing oral sex on a partner, and other sexual behaviors, did not appear to play a significant role in detection of prevalent oral HPV infection. When we stratified by sexual practices, men who had sex with men had no cases of oral HPV infection, whereas men who have sex with both men and women had nonsignificantly higher oral HPV infection than men who have sex only with women. Despite the hypothesis that HPV is transmitted to the oral region via sexual behaviors, there has been reporting by some (9, 20) but not others (6) of an association between oral sex and oral HPV infection among HIV-negative individuals. In our study, it may be that individuals are misreporting their sexual behaviors, although previous work in this cohort looking at sexual behaviors and risk of penile and anal HPV infections show clear associations and therefore good internal validity of the survey instrument. In addition, the reliability of reporting sensitive sexual behaviors has been demonstrated for this questionnaire (25). Behaviors not presently queried, such as kissing (20), may be more relevant to the transmission of HPV infection in this setting.

To our knowledge, this is the first study to systematically collect and test oral specimens from healthy men from multiple countries. The epidemiology of oral HPV infection appeared quite similar across the 3 countries. Specifically, HPV16 and other carcinogenic HPV infections were similarly prevalent in each country, and the associations between age and tobacco held for each country. However, there was an unexpectedly high prevalence of HPV55 in men from Mexico (18/19

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Table 4. Analysis of associations with an oral HPV infection

	Oral HPV Infection *N* (%)	Univariate OR (95% CI)	Multivariate[a] OR (95% CI)
Sociodemographic characteristics			
Country			
Brazil	475 (2.1)	0.5 (0.3–1.2)	0.5 (0.2–1.1)
Mexico	591 (5.9)	1.7 (0.98–2.9)	1.3 (0.8–2.3)
United States	614 (3.4)	1.0	1.0
Age, y			
17–24[b]	505 (3.2)	1.0	1.0
25–34	509 (3.6)	1.1 (0.6–2.2)	0.9 (0.4–1.9)
35–44	415 (4.8)	1.6 (0.8–3.0)	1.3 (0.6–2.6)
45–54	169 (4.7)	1.5 (0.7–3.6)	1.2 (0.5–3.0)
55+	82 (6.1)	2.0 (0.7–5.6)	1.7 (0.6–5.0)
P_{trend}		0.096	0.218
Marital status[c]			
Single	743 (3.2)	1.4 (0.7–2.6)	
Married	789 (4.3)	1.0	
Divorced	144 (6.3)	2.5 (1.1–5.9)	
Tobacco			
Tobacco use			
Never	951 (2.7)	1.0	1.0
Former	317 (4.7)	1.8 (0.9–3.4)	1.7 (0.9–3.2)
Current	370 (7.0)	2.7 (1.5–4.7)	2.5 (1.4–4.4)
P_{trend}		<0.001	0.001
Number of cigarettes per day in the last 6 months (among current smokers)			
0.5–9	211 (8.1)	1.0	
10+	8 (5.1)	0.6 (0.3–1.5)	
Age at initiation of smoking, y			
9–14	166 (5.4)	1.0	
15–17	291 (5.5)	1.0 (0.4–2.4)	
18+	224 (7.1)	1.3 (0.6–3.1)	
Years of smoking[d]			
Zero	958 (2.7)	1.0	
1–<10	332 (6.0)	2.3 (1.3–4.2)	
10+	343 (6.1)	2.3 (1.3–4.2)	
Sexual behavior			
Sexual behavior			
MSW	1,464 (4.2)	1.0	
MSM	55 (0)	0	
MSMW	75 (5.3)	1.3 (0.5–3.6)	
Age at sexual debut, y			
Virgin	72 (1.4)	0.2 (0.03–1.9)	
9–14	274 (5.5)	1.0	
15–17	635 (3.3)	0.6 (0.3–1.2)	
18+	510 (4.5)	0.8 (0.4–1.6)	
Lifetime number of female sexual partners			
0–1	240 (2.1)	1.0	
2–9	674 (3.7)	1.8 (0.7–4.8)	
10+	650 (4.8)	2.4 (0.9–6.1)	
Performed oral sex			
Never	132 (3.8)	1.0	
Ever	1,303 (4.1)	1.1 (0.4–2.8)	

(Continued on the following page)

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Table 4. Analysis of associations with an oral HPV infection (Cont'd)

	Oral HPV Infection *N* (%)	Univariate OR (95% CI)	Multivariate[a] OR (95% CI)
Recently performed oral sex[e]			
No	471 (4.2)	1.0	
Yes	1,136 (4.0)	1.0 (0.5–1.8)	
Number of times performed oral sex in the past 6 months			
0–1	545 (4.0)	1.0	
2–9	431 (3.9)	1.0 (0.5–1.9)	
10+	24 (4.2)	1.0 (0.6–1.9)	
Insertive anal sex			
Never	662 (4.1)	1.0	
Ever	839 (4.1)	1.0 (0.6–1.7)	
Receptive anal sex			
Never	1,358 (4.1)	1.0	
Ever	135 (3.0)	0.7 (0.3–2.0)	

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

[a]In the multivariate model, current tobacco use independently increased the odds of oral HPV infection; the borderline significant trend of increasing oral HPV infection noted with increasing age was no longer present; these associations were adjusted for country.

[b]Category contains one 17 year old.

[c]In the marital status variable, the "married" category includes cohabitating and living together and "divorced" includes separated and widowed.

[d]Tobacco use and years of smoking were highly collinear and yielded the same estimates when controlling for country and age, as in the multivariate model. Tobacco use was a slightly more robust factor, and therefore was retained in the final model, although similar estimates were present when we looked at years of tobacco use.

[e]For variables indicating recent sexual activity, recent was defined as activity occurring over the past 6 months.

HPV55 infections detected), which explains the significantly higher overall HPV prevalence observed for Mexico compared with the United States and Brazil. HPV55 is in the α10 clade, which contains noncarcinogenic HPV types such as HPV6 and HPV11 (26). Although contamination at either the clinic or laboratory cannot be definitively ruled out, recent data presented by Chaturvedi and colleagues showed a similarly high prevalence of this previously unreported HPV type in oral specimens collected from HIV-infected men and women (27). It may be that in the changing landscape of the HPV epidemic, types not previously reported as having infected the oral region will be detected there in the future.

Finally, coinfection with multiple HPV types among men with oral HPV infection was a rare phenomenon (5%) compared with that which was observed at male external genital (26%; ref. 9) and anal (39%; ref. 14) samples from the same cohort or cervical (32%) samples from the published literature (28). Multiple HPV types may be less likely to infect the oral mucosa compared with genital epithelia as a function of the rarity of any type of HPV infection. Alternatively, immune surveillance in the oral region may contribute to an overall lower prevalence as well as fewer multiple infections.

One important limitation to note for this study is the generalizability of our findings to other populations. Although men from Mexico and the United States were recruited from either the general population or factories, specialized populations were also targeted, such as college students and military recruits. Further, men from Brazil were enrolled from a clinic specializing in the diagnosis and treatment of sexually transmitted diseases and HIV/AIDS; although enrolled men were presenting for conditions related to nonsexually transmitted infection, we cannot dismiss the fact that these individuals were at a different risk of oral HPV infection compared with the population at large. It was reassuring to note that despite the differences in the populations by country, the country-specific oral HPV prevalence as well as the associations with age and behavior variables were consistent across countries.

HPV16 is one of the most common sexually transmitted infections detected in the anogenital region (29) yet is relatively rare in the oral region. It remains unknown why oral HPV infection is rare especially considering the similarity of the mucosal epithelium in the oral and anogenital regions—it may be partly explained by differences in specimen collection, in that oral specimens are often collected in a large volume (via oral rinse), thereby

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diluting the HPV DNA; this may be compounded by lower viral load in oral specimens despite the sensitive PCR-based assay used for oral HPV detection. Alternatively, it could be a function of exposure, although in this population, oral sexual contact was common. Finally, it may be that the oral region is resistant to this infection. Understanding the intervening steps in the natural history of oral HPV to cancer in the oropharynx is important; specifically, the rates of clearance and progression will provide insight into the carcinogenic process. This is especially timely in light of recent reports suggesting that the incidence of oropharynx cancer is increasing (30).

Disclosure of Potential Conflicts of Interest

Publication and report contents are solely the responsibility of the authors and do not necessarily represent the official views of NCI/NIH. L. L. Villa is consultant of Merck Sharp & Dohme for the quadrivalent HPV vaccine.

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