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The epidemiology of oral HPV infection among a multinational sample of healthy men

Aimee R. Kreimer¹, Alessandro Villa¹, Alan G. Nyitray², Martha Abrahamsen², Mary Papenfuss², Danelle Smith², Allan Hildesheim¹, Luisa L. Villa³, Eduardo Lazcano-Ponce⁴, and Anna R. Giuliano²

¹National Cancer Institute, NIH, Bethesda, Maryland ²H. Lee Moffitt Cancer Center and Research Institute, Tampa, FL ³Ludwig Institute for Cancer Research, São Paulo, Brazil ⁴Instituto Nacional de Salud Pública, Cuernavaca, México

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 non-carcinogenic HPV types were detected using Roche Linear Array.

Results—Oral HPV DNA was detected in 67 of 1680 (4.0%, 95%CI 3.1% to 5.0%) β -globin positive specimens; carcinogenic HPVs were detected in 1.3% (95%CI 0.8% to 2.0%; n=22) and HPV16 was the most commonly detected carcinogenic HPV type (0.6%, 95%CI 0.2% to 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 US (0.2%). Oral HPV prevalence non-significantly increased over increasing age categories (p for 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 ~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 to anogenital sites.

Keywords

oral; human papillomavirus; HPV; normal mucosa; men

Introduction

Human papillomavirus type 16 (HPV16) infection is one of the most important human carcinogens, causing >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 (odds ratio ~13) (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 in part to differences in specimen collection, processing and testing limited conclusive interpretation of the data (5); small sample size among these studies was also a limitation. While 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 three 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 the first ~1600 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 São Paulo, Brazil; Cuernavaca, Mexico; Tampa, Florida (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; US, June 13, 2007 to November 11, 2008. The Human Subjects Committees of the University of South Florida, the Centro de Referencia e Tratamento de Doenças Sexualmente Transmissíveis 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 one of three recruitment sites in Brazil, Mexico, or the United States (US); (c) reported no prior diagnosis of penile or anal cancers (n.b: 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 non–sexually transmitted infection–related conditions 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 São Paulo since 1993 were also recruited. At the Mexico site, the population was comprised 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 pre-enrollment run-in visit, a baseline (enrollment) visit, and eight additional visits after enrollment scheduled 6 months apart. Of note, because the oral component was initiated approximately two 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 1688 men with at least one 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 mL 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 3,000g for 10 min 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 1ml of saline with repeat 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

Due to previous reports of the importance of DNA extraction for oral HPV DNA detection (10), two 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 30ng of total DNA) were used (n=6). Further, to compensate for low viral load of HPV DNA in oral specimens, four concentrations of the total input DNA were compared (10ng, 30ng [the amount specified by the Roche PCR protocol], 50ng and 100ng); each specimen was therefore tested eight additional times (two methods of DNA extraction × four

concentrations of total DNA input) after the initial round of testing. Similar amounts of DNA 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, 10ng appeared inadequate as the majority of the known oral HPV infections were not detected (4 of 6). For the highest DNA input (100ng), known oral HPV positives were also not detected (2 of 6). DNA input of 50ng not only detected the highest number of HPV infections but also detected one multiple infection. Although limited by the available number of oral positive samples on which to conduct this methods work, we concluded that automated DNA extraction using 50ng 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 μ L aliquots of clinical material were digested with 20 μ L of proteinase K solution and lysed with 200 μ L of lysis buffer at 56°C. Specimens were tested for the presence of HPV by amplifying 50ng 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, Alameda, CA) (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 (non-carcinogenic) 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 (eight 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 non-carcinogenic types. Only single or multiple infections with non-carcinogenic HPV types were classified as “any non-carcinogenic 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 (1680 of 1688); 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=4074) with regard to country, age distribution, socio-demographic 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 co-variates 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 odds ratios (ORs) and 95% confidence intervals (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 US were on average seven years younger than men from the two other sites. The majority of study participants were non-white. Almost one-half (46.8%) reported Hispanic ethnicity. Men from Brazil were more likely to have less than 12 years of education whereas US participants were more likely to have 17 or more years of schooling. 47.1% of participants 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 (30.0%) compared to Brazil and the US (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 one to nine female sexual partners during the lifetime; men from Brazil reported more lifetime sexual partners than men from Mexico and the US. 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 US), as was participating in oral sex over the most recent six months (Table 1).

The prevalence of HPV DNA in the oral region was 4.0% (95%CI 3.1% to 5.0%) and differed significantly by country (Brazil 2.1% [95%CI 1.0 to 3.8%], Mexico 5.9% [95%CI 4.2% to 8.1%], US 3.6% [95%CI 2.3% to 5.4%]; $p = 0.005$; Table 2). When HPV types were grouped by carcinogenicity, 1.3% (95%CI 0.8% to 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% to 2.7%], Mexico 1.0% [95%CI 0.4% to 2.2%], US 1.6% [95%CI 0.8% to 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% to 1.1%], $n = 10$) (Table 3). Other carcinogenic types detected included: HPV 31, 35, 39, 52, 56, 58, and 59. Individual non-carcinogenic infections were rarely detected, except for HPV55 (1.1%, 95%CI 0.7% to 1.8%; $n = 19$), which had a notably high prevalence in Mexico (3.0%, $n = 18$ of the 19 infections detected). Due to concerns that this finding might be an artifact despite rigorous clinic 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.

Due to limited number of outcomes of individual HPV type infections, men from the three countries were combined to evaluate factors associated with oral HPV prevalence. Men in the youngest age category of 18–24 ($n = 505$; n.b: category includes one 17 year old) had the lowest oral HPV infection prevalence (3.2%; 95%CI 1.8 to 5.1%). The prevalence of oral HPV non-significantly increased over increasing age categories (p for trend 0.096)—men in

the oldest age strata, aged 55 to 74 (n=82), had the highest oral HPV prevalence (6.1%, 95%CI 2.0 to 13.7%) (Figure 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 to 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 to 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 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 4th or 5th 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 to 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 immunological 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 to 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 non-significantly 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 mis-reporting 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 three 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 of the 19 HPV55 infections detected), which explains the significantly higher overall HPV prevalence observed for Mexico compared to the US and Brazil. HPV55 is in the $\alpha 10$ clade, which contains non-carcinogenic HPV types such as HPV6 and 11 (26). Although contamination at either the clinic or laboratory cannot be definitively ruled out, recent data presented by Chaturvedi *et al* 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, co-infection with multiple HPV types among men with oral HPV infection was a rare phenomenon (5%) compared to that which was observed at male external genital (26%) (9) and anal (39%) (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 to 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. While men from Mexico and the US 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 non-sexually transmitted infection-related conditions, we cannot dismiss the fact that these individuals were at a different risk of oral HPV infection compared to 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 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).

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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 18–24 (n=505; 3.2%; 95%CI 1.8 – 5.1%; n.b: category includes one 17 year old). The prevalence of oral HPV non-significantly increased over increasing age categories (p for trend 0.096)—men in the oldest age strata, aged 55 to 74 (n=82), had the highest oral HPV prevalence (6.1%, 95%CI 2.0 – 13.7%)

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=1680)
	<i>n</i> (%)	<i>n</i> (%)	<i>n</i> (%)	<i>n</i> (%)
SOCIODEMOGRAPHIC CHARACTERISTICS				
Age				
18–24 ¹	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)
<i>Median</i>	<i>33.0</i>	<i>34.0</i>	<i>24.0</i>	<i>31.0</i>
Race²				
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)
Ethnicity³				
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 status⁴				
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 (years)				
Less than 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 to 16	177 (37.5)	149 (25.3)	468 (76.3)	794 (47.4)
17 or more	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 (years)				
<15	40 (23.0)	65 (20.8)	61 (31.3)	166 (24.4)
15 to 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 to <10	75 (16.0)	152 (26.3)	105 (18.0)	332 (20.3)

	Brazil (N=475)	Mexico (N=591)	United States (N=614)	Total (N=1680)
	n (%)	n (%)	n (%)	n (%)
10 or more	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)	1464 (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 (years)				
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 to 1	52 (11.7)	89 (16.2)	99 (17.4)	240 (15.4)
2 to 9	145 (32.5)	296 (53.9)	233 (41.0)	674 (43.1)
10 or more	249 (55.8)	164 (29.9)	237 (41.6)	650 (41.5)
Performed oral sex³				
Never	24 (5.7)	87 (16.4)	21 (4.4)	132 (9.2)
Ever	400 (94.3)	443 (83.6)	460 (95.6)	1303 (90.8)
Recently performed oral sex⁵				
No	88 (20.1)	219 (37.2)	164 (28.3)	471 (29.3)
Yes	350 (79.9)	370 (62.8)	416 (71.7)	1136 (70.7)
Number of times performed oral sex in the past 6 months				
0 to 1	110 (25.9)	248 (43.6)	187 (33.6)	545 (35.2)
2 to 9	134 (31.6)	160 (28.1)	137 (24.6)	431 (27.8)
10 or greater	180 (42.5)	161 (28.3)	233 (41.8)	574 (37.0)
Insertive anal sex³				
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 sex³				
Never	376 (82.5)	492 (93.9)	490 (95.5)	1358 (91.0)
Ever	80 (17.5)	32 (6.1)	23 (4.5)	135 (9.0)

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

¹Category contains one 17 year old

²In 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.

³Indicates at least 10% of the data were missing

⁴In the Marital Status variable, the 'married' category includes cohabiting and living together and 'divorced' includes separated and widowed.

⁵For variables indicating recent sexual activity, recent was defined as activity occurring over the past six months

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=1680)		P for differences across countries
	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	
Any HPV	10 (2.1)	35 (5.9)	22 (3.6)	67 (4.0)	0.005				
Any carcinogenic [/] HPV	6 (1.3)	6 (1.0)	10 (1.6)	22 (1.3)	0.642				
Any non-carcinogenic 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				

[/] Carcinogenic HPV types defined as HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68

Table 3

Baseline type-specific HPV infection distribution by country

	Brazil (N=475)	Mexico (N=591)	USA (N=614)	Total (N=1680)
	<i>n</i> (%)	<i>n</i> (%)	<i>n</i> (%)	<i>n</i> (%)
Carcinogenic ^I				
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)
Non-carcinogenic ^I				
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)
CP6108		1 (0.2)		1 (0.1)

^I HPV types 18, 33, 45, 68, 26, 40, 42, 54, 67, 73, 81, 83, IS39 were not detected in any of the oral specimens and were excluded from the table

Table 4

Analysis of associations with an oral human papillomavirus (HPV) infection.

	Oral HPV Infection N (%)	Univariate Odds Ratio (95%CI)	Multivariate ^I Odds Ratios (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)
USA	614 (3.4)	1.0	1.0
Age			
17–24 ²	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)
<i>p for trend</i>		0.096	0.218
Marital status³			
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)
<i>p for trend</i>		<0.001	0.001
Number of cigarettes per day in the last 6 months (among current smokers)			
0.5 to 9	211 (8.1)	1.0	
10 or more	8 (5.1)	0.6 (0.3–1.5)	
Age at initiation of smoking (years)			
9 to 14	166 (5.4)	1.0	
15 to 17	291 (5.5)	1.0 (0.4–2.4)	
18+	224 (7.1)	1.3 (0.6–3.1)	
Years of smoking⁴			
Zero	958 (2.7)	1.0	
1 to <10	332 (6.0)	2.3 (1.3–4.2)	
10 or more	343 (6.1)	2.3 (1.3–4.2)	
SEXUAL BEHAVIOR			
Sexual behavior			
MSW	1464 (4.2)	1.0	
MSM	55 (0)	0	
MSMW	75 (5.3)	1.3 (0.5–3.6)	

	Oral HPV Infection N (%)	Univariate Odds Ratio (95%CI)	Multivariate ¹ Odds Ratios (95%CI)
Age at sexual debut (years)			
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 to 1	240 (2.1)	1.0	
2 to 9	674 (3.7)	1.8 (0.7–4.8)	
10 or greater	650 (4.8)	2.4 (0.9–6.1)	
Performed oral sex			
Never	132 (3.8)	1.0	
Ever	1303 (4.1)	1.1 (0.4–2.8)	
Recently performed oral sex⁵			
No	471 (4.2)	1.0	
Yes	1136 (4.0)	1.0 (0.5–1.8)	
Number of times performed oral sex in the past 6 months			
0 to 1	545 (4.0)	1.0	
2 to 9	431 (3.9)	1.0 (0.5–1.9)	
10 or greater	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	1358 (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; MSMW: Men who have sex with men and women.

¹ 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

² Category contains one 17 year old

³ In the Marital Status variable, the 'married' category includes cohabitating and living together and 'divorced' includes separated and widowed.

⁴ 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.

⁵ For variables indicating recent sexual activity, recent was defined as activity occurring over the past six months