Differences in Factors Associated With High- and Low-Risk Oral Human Papillomavirus Genotypes in Men

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Background. Human papillomavirus (HPV) causes oral warts and oropharyngeal cancer (OPC). Human papillomavirus-attributable OPC incidence among men is significantly increasing worldwide, yet few studies have reported oral HPV across multiple countries or examined factors associated with low- and high-risk HPV separately.

Methods. Oral gargles from 3095 men in the multinational HPV Infection in Men (HIM) Study were HPV genotyped. Multivariable models assessed factors independently associated with high-risk and low-risk HPV prevalence.

Results. The prevalence of high-risk and low-risk HPV was 6.0% and 2.8%, respectively. Greater number of sexual partners was only associated with high-risk HPV (1.88; 95% confidence interval [CI], 1.22–2.90) prevalence. In multivariable models, residing in Mexico (1.66; 95% CI, 1.15–2.40) and smoking (1.66; 95% CI, 1.13–2.44) were significantly associated with high-risk HPV, and history of consistent gum bleeding (2.16; 95% CI, 1.35–3.45) was significantly associated with low-risk HPV. Gender of the sexual partner did not alter the results for either high- or low-risk HPV endpoints.

Conclusions. Different factors were independently associated with high- and low-risk oral HPV. Oral sexual behaviors were associated with high-risk HPV, and oral health was associated with low-risk HPV. High-risk HPV prevalence differed by country of residence, highlighting the need for additional studies in multiple countries.

Keywords. HPV; international; men; oral; risk factor.

Incidence of human papillomavirus (HPV)-attributable oropharyngeal cancers (OPCs) is on the rise across the globe, especially among men residing in high-income countries [1]. In the United States, given the high access to cervical cancer screening and treatment, the number of OPC cases due to HPV has exceeded the number of cervical cancer cases in the past few years [2]. Hence, it is important to have an estimate of oral HPV prevalence and associated factors among men residing in multiple countries.

Many studies have examined factors associated with oral HPV, mostly in North America and Europe [3]. Studies conducted using the US National Health and Nutrition Examination Survey (NHANES) have reported associations with age [4], cigarette smoking [4], number of sexual partners [4], oral sexual behavior [5], and oral health factors [6]. However, almost all of these studies were underpowered to examine associations with specific groups of HPV; therefore, the majority examined

association with all HPV types combined. In addition, prior studies were limited to a single country or geographic region within a country.

In studies of HPV at other anatomic sites, high- and low-risk HPV type were associated with different factors [7–9]. For example, at the cervix, high-risk HPV infections were associated with younger age and measures of sexual activity, whereas low-risk HPV was associated with use of barrier protection and recent number of sex partners. In this study, we examined factors associated with high- and low-risk oral HPV prevalence among healthy men residing in Brazil, Mexico, and the United States.

MATERIALS AND METHODS

Study Population

The HPV Infection in Men (HIM) Study participants were men recruited from São Paulo (Brazil), Cuernavaca (Mexico), and Tampa, Florida (United States) from March 2005 to December 2009. The human subject committees of the Centro de Referência e Treinamento de Doenças Sexualmente Transmissíveis e AIDS in Brazil, the National Institute of Public Health in Mexico, and the University of South Florida in the United States approved the study procedures before the start of this study. All participants gave written consent.

The HIM study eligibility criteria were as follows: (1) age 18 to 73 years; (2) Resident of Brazil, Mexico, or United States; (3)

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did not report previous diagnoses of anal or penile cancer; (4) never diagnosed with genital or anal warts before the study; (5) no current sexually transmitted infection; (6) never participated in a HPV vaccine study; (7) no history of human immunodeficiency virus or acquired immune deficiency syndrome; (8) no history of imprisonment, homelessness, or drug treatment during the past 6 months; (9) willing to comply with 10 scheduled visits every 6 months for 4 years with no plans to relocate within the next 4 years. A full description of the cohort and cohort procedures has been previously published [10].

Study Protocol

The HIM study protocol included a pre-enrollment visit, a baseline (enrollment visit), and 8–13 visits 6 months apart after enrollment. Demographic information and risk factor history was obtained using computer-assisted self-interviewing at each visit. The HIM study participants answered a series of oral health-related questions including whether they had been diagnosed with gingivitis as an adult and whether their gums consistently bled when they brushed their teeth or were their gums swollen. Oral gargle collection initiated after the start of the study (2007/2008). Only study participants with 2 or more oral gargle specimens collected ≥ 6 months apart were included in this study analysis. The first oral gargle collected and survey data from the same visit were analyzed.

Deoxyribonucleic Acid Extraction and Human Papillomavirus Genotyping

Deoxyribonucleic acid (DNA) was extracted from oral gargle cell pellets using the automated BioRobot MDx (QIAGEN, Inc.) following the manufacturer's instructions, and HPV was genotyped using the SPF₁₀ PCR-DEIA-LiPA₂₅ system (DDL Diagnostic Laboratory, Rijswijk, the Netherlands), an in vitro reverse hybridization assay. Details regarding this assay are available elsewhere [11].

Classification of Human Papillomavirus Types

Thirteen oncogenic and potentially oncogenic HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68/73) were considered “high-risk” HPV, whereas the remaining HPV types detected by the assay (types 6, 11, 34, 40, 42, 43, 44, 53, 54, 66, 70, and 74) were considered “low-risk” HPV. If the sample amplified HPV on polymerase chain reaction and hybridized with a specific HPV type on genotyping, it was considered positive for “any HPV.” All samples positive for either only high-risk HPV or both high- and low-risk HPV were categorized as “high-risk HPV,” whereas samples positive for only low-risk HPV were categorized as “low-risk HPV.”

Statistical Analysis

Samples positive for RNase P were deemed adequate for HPV analyses (N = 3127) and from those, men with available survey data were included in the analyses (N = 3095). Bivariate associations between sociodemographic characteristics, tobacco

and alcohol use, oral health, and sexual behavioral characteristics with high- and low-risk HPV and HPV16 prevalence were examined using log binomial models. HPV16 was the only individual genotype examined in the models because it alone had a large enough sample size (n = 50). Variables that had significant bivariate associations with oral HPV prevalence were included in multivariable models with high- and low-risk HPV and HPV16 prevalence as outcomes. Due to multiple, highly correlated factors associated with oral HPV identified in bivariate analyses for high- or low-risk oral HPV infection, factors were included individually in separate, multivariable models with each model adjusted for the same set of noncorrelated factors (ie, demographics) and one of the highly correlated factors at a time. RStudio (version 1.2) was used for all analyses.

RESULTS

Demographic and oral HPV risk factors of the HIM Study oral subcohort are described in Table 1. Prevalence of oral low-risk and high-risk HPV was 2.8% and 6.0%, respectively. The median age of participants was 33 years. Approximately 39% resided in Brazil, 32% resided in Mexico, and 28% resided in the United States. Most were men who had sex with women only (80%) and married or cohabiting (50%). Thirty-five percent of participants reported performing no oral sex in the past 6 months.

In bivariate analyses, high-risk oral HPV was significantly associated with mid-adult age (31–40 years [prevalence ratio {PR}, 1.7; 95% confidence interval {CI}, 1.2–2.3]; 41–50 years [PR, 1.7; 95% CI, 1.2–2.5]). Low-risk HPV was significantly associated with older ages (41–50 years [PR, 1.8; 95% CI, 1.0–3.0]; 51–73 years [PR, 2.5; 95% CI, 1.3–4.6]). Divorced/separated/widowed status was significantly associated with high-risk (PR, 1.6; 95% CI, 1.02–2.5) and low-risk HPV (PR, 2.4; 95% CI, 1.4–4.2) compared with single men (Table 2).

Assessment of smoking and oral health variables in bivariate analyses found smoking associated with both low- and high-risk HPV and oral health associated with low-risk HPV only. Specifically, high-risk HPV was significantly associated with current (PR, 2.0; 95% CI, 1.4–2.8) and former smoking (PR, 1.7; 95% CI, 1.2–2.4) (Table 2). More than 5 pack years of smoking was significantly associated with high-risk (PR, 2.0; 95% CI, 1.4–2.8) and low-risk HPV (PR, 1.7; 95% CI, 1.03–2.9), and only high-risk HPV and HPV16 were associated with >0–5 pack years of smoking (high-risk PR, 1.7; 95% CI, 1.2–2.3; HPV16 PR, 1.9; 95% CI, 1.1–3.5). Low-risk HPV was significantly associated with having 1 or more teeth extracted due to gingivitis, gum disease, or decay (PR, 1.7; 95% CI, 1.04–2.6) and consistent gum bleeding after brushing or presence of swollen gums (PR, 2.1; 95% CI, 1.4–3.3).

The unadjusted associations of sexual activity factors differed for prevalence of high-risk HPV compared with low-risk HPV. High-risk HPV was significantly associated with having 8–19 lifetime female sex partners (PR, 1.9;

Table 1. Sociodemographic Characteristics of Study Participants (N = 3095)

Characteristic	N (%)
HPV Type	
Any	272 (8.8)
High risk	184 (6.0)
Low risk	88 (2.8)
HPV16	50 (1.6)
Age (in Years)	
Mean (SD)	34.1 (11.3)
Median (range)	33 (18–73)
18–30	1317 (42.6)
31–40	918 (29.7)
41–50	592 (19.1)
51–73	266 (8.6)
Missing	2 (0.1)
Country of Residence	
Brazil	1222 (39.5)
Mexico	1004 (32.4)
USA	869 (28.1)
Marital Status	
Single	1241 (40.1)
Divorced/separated/widowed	274 (8.9)
Married/cohabiting	1556 (50.3)
Missing	24 (0.8)
Sexual Orientation	
MSW	2465 (79.6)
MSWM	194 (6.3)
MSM	122 (3.9)
Never had sex ^a	135 (4.4)
Missing	179 (5.8)
Lifetime Number of Sex Partners	
≤2	739 (23.9)
3–7	777 (25.1)
8–19	759 (24.5)
>19	797 (25.8)
Refused	15 (0.5)
Missing	8 (0.3)
Lifetime Number of Female Partners	
≤2	653 (21.1)
3–7	794 (25.7)
8–19	715 (23.1)
>19	698 (22.6)
Refused	229 (7.4)
Missing	6 (0.2)
Lifetime Number of Male Partners	
≤2	2778 (89.8)
3–7	105 (3.4)
8–19	58 (1.9)
>19	94 (3.0)
Refused	47 (1.5)
Missing	13 (0.4)
Number of New Female Sexual Partners in the Past 6 Months	
0	796 (25.7)
1	1343 (43.4)
2+	878 (28.4)
Refused	75 (2.4)
Missing	3 (0.1)
Number of New Male Sexual Partners in the Past 6 Months	
0	2844 (91.9)

Table 1. Continued

Characteristic	N (%)
1	87 (2.8)
2+	118 (3.8)
Refused	14 (0.5)
Missing	32 (1.0)
Number of Times Oral Sex Performed in the Past 6 Months	
0	1082 (35.0)
1–6	838 (27.1)
7–24	583 (18.8)
25+	338 (10.9)
Refused	132 (4.3)
Missing	122 (3.9)
Number of Days Since Last Oral Sex	
Never had oral sex	335 (10.8)
>30 days	1023 (33.1)
>10–30 days	556 (18.0)
>3–10 days	485 (15.7)
0–3 days	485 (15.7)
Refused	75 (2.4)
Missing	136 (4.4)
Cigarette Smoking	
Never ^b	1614 (52.1)
Former	738 (23.8)
Current	666 (21.5)
Refused	5 (0.2)
Missing	72 (2.3)
Pack Years of Cigarette Smoked	
0	1752 (56.6)
>0–5	791 (25.6)
>5	499 (16.1)
Refused	52 (1.7)
Missing	1 (0.0)
Currently Chewing Tobacco or Using Snuff	
None	2893 (93.5)
Sometimes	113 (3.7)
Daily	41 (1.3)
Missing	48 (1.6)
Number of Drinks of Any Alcoholic Beverage Consumed in the Past 1 Month	
None	742 (24.0)
1–30	1388 (44.8)
>30	852 (27.5)
Refused	66 (2.1)
Missing	47 (1.5)
Ever Had Gingivitis	
No	2445 (79.0)
Yes	513 (16.6)
Missing	137 (4.4)
Number of Teeth Extracted Due to Gum Disease, Gingivitis or Decay	
0	2262 (73.1)
1+	659 (21.3)
Missing	174 (5.6)
Consistent Bleeding of Gums After Brushing or Presence of Swollen Gums	
No	2329 (75.3)
Yes	630 (20.4)
Missing	136 (4.4)

Abbreviations: HPV, human papillomavirus; MSM, men who have sex with men only; MSW, men who have sex with women only; MSWM, men who have sex with women and men; SD, standard deviation.

^aNever had sex: never had oral, vaginal, or anal sex during their lifetime.

^bNever smoked: smoked less than 100 cigarettes in their lifetime.

Table 2. Bivariate Associations Between Sexual and Health Behaviors and Infection With High-Risk or Low-Risk HPV Types

Characteristic	High-Risk HPV ^a		Low-Risk HPV ^a	
	n (%)	PR (95% CI)	n (%)	PR (95% CI)
Demographics				
Age (in Years) (N = 3093)				
18–30	59 (4.5)	Ref	28 (2.1)	Ref
31–40	68 (7.4)	1.65 (1.18–2.33)^b	24 (2.6)	1.23 (0.72–2.11)
41–50	45 (7.6)	1.70 (1.17–2.50)	22 (3.7)	1.75 (1.01–3.03)
51–73	12 (4.5)	1.01 (0.55–1.85)	14 (5.3)	2.48 (1.32–4.64)
P trend	0.10		<.01	
Country of Residence (N = 3095)				
Brazil	65 (5.3)	Ref	43 (3.5)	Ref
Mexico	73 (7.3)	1.37 (0.99–1.89)	26 (2.6)	0.74 (0.46–1.19)
United States	46 (5.3)	1.00 (0.69–1.44)	19 (2.2)	0.62 (0.36–1.06)
Marital Status (N = 3071)				
Single	68 (5.5)	Ref	34 (2.7)	Ref
Divorced/separated/widowed	24 (8.8)	1.60 (1.02–2.50)	18 (6.6)	2.40 (1.37–4.18)
Married/cohabiting	91 (5.9)	1.07 (0.79–1.45)	33 (2.1)	0.77 (0.48–1.24)
Tobacco and Alcohol Consumption				
Cigarette Smoking (N = 3023)				
Never	69 (4.3)	Ref	40 (2.5)	Ref
Former	53 (7.2)	1.68 (1.19–2.38)	24 (3.3)	1.31 (0.80–2.16)
Current	56 (8.4)	1.97 (1.40–2.77)	23 (3.5)	1.39 (0.84–2.31)
Pack Years of Cigarette Smoked (N = 3094)				
0	77 (4.4)	Ref	43 (2.5)	Ref
>0–5	58 (7.3)	1.67 (1.20–2.32)	23 (2.9)	1.18 (0.72–1.95)
>5	43 (8.6)	1.96 (1.37–2.81)	21 (4.2)	1.71 (1.03–2.86)
P trend	<.01		0.05	
Currently Chewing Tobacco or Using Snuff (N = 3047)				
None	167 (5.8)	Ref	83 (2.9)	Ref
Sometimes	8 (7.1)	1.23 (0.62–2.43)	2 (1.8)	0.62 (0.15–2.48)
Daily	7 (17.1)	2.96 (1.48–5.90)	1 (2.4)	0.85 (0.12–5.96)
Number of Alcoholic Drinks Consumed in the Past 1 Month (N = 3048)				
0	39 (5.3)	Ref	18 (2.4)	Ref
1–30	73 (5.3)	1.00 (0.69–1.46)	40 (2.9)	1.19 (0.69–2.06)
>30	64 (7.5)	1.43 (0.97–2.10)	25 (2.9)	1.21 (0.67–2.20)
P trend	0.05		0.50	
Oral Health				
Presence of Gingivitis (N = 2958)				
No	148 (6.1)	Ref	65 (2.7)	Ref
Yes	29 (5.7)	0.93 (0.63–1.37)	17 (3.3)	1.25 (0.74–2.11)
Number of Teeth Extracted Due to Gum Disease, Gingivitis, or Decay (N = 2921)				
0	136 (6.0)	Ref	54 (2.4)	Ref
1+	40 (6.1)	1.01 (0.72–1.42)	26 (4.0)	1.65 (1.04–2.62)
Consistent Bleeding of Gums After Brushing or Presence Of Swollen Gums (N = 2959)				
No	145 (6.2)	Ref	52 (2.2)	Ref
Yes	32 (5.1)	0.82 (0.56–1.18)	30 (4.8)	2.13 (1.37–3.31)
Sexual Behavior				
Sexual Orientation (N = 2916)				
MSW	145 (5.9)	Ref	61 (2.5)	Ref
MSWM	16 (8.3)	1.40 (0.85–2.30)	12 (6.2)	2.50 (1.37–4.56)
MSM	8 (6.6)	1.11 (0.56–2.22)	2 (1.6)	0.66 (0.16–2.68)
Never had sex	5 (3.7)	0.63 (0.26–1.51)	6 (4.4)	1.80 (0.79–4.08)
Lifetime Number of Sex Partners (N = 3087)				
≤2	32 (4.3)	Ref	16 (2.2)	Ref
3–7	30 (3.9)	0.89 (0.55–1.45)	17 (2.2)	1.01 (0.51–1.99)
8–19	48 (6.3)	1.46 (0.94–2.26)	16 (2.1)	0.97 (0.49–1.93)
>19	70 (8.8)	2.03 (1.35–3.04)	37 (4.6)	2.14 (1.20–3.82)
P trend	<.01		<.01	

Table 2. Continued

Characteristic	High-Risk HPV ^a		Low-Risk HPV ^a	
	n (%)	PR (95% CI)	n (%)	PR (95% CI)
Lifetime Number of Female Sex Partners (N = 3089)				
≤2	22 (3.4)	Ref	10 (1.5)	Ref
3–7	35 (4.4)	1.31 (0.78–2.21)	18 (2.3)	1.48 (0.69–3.18)
8–19	46 (6.4)	1.91 (1.16–3.14)	16 (2.2)	1.46 (0.67–3.20)
>19	59 (8.5)	2.51 (1.56–4.05)	33 (4.7)	3.09 (1.53–6.21)
P trend	<.01		<.01	
Lifetime Number of Male Sex Partners (N = 3082)				
≤2	160 (5.8)	Ref	73 (2.6)	Ref
3–7	4 (3.8)	0.66 (0.25–1.75)	7 (6.7)	2.54 (1.20–5.37)
8–19	2 (3.5)	0.60 (0.15–2.36)	1 (1.7)	0.66 (0.09–4.64)
>19	12 (12.8)	2.22 (1.28–3.84)	5 (5.3)	2.02 (0.84–4.89)
P trend	0.06		0.08	
Number of New Female Sexual Partners in the Past 6 Months (N = 3092)				
0	49 (6.2)	Ref	21 (2.6)	Ref
1	70 (5.2)	0.85 (0.59–1.21)	29 (2.2)	0.82 (0.47–1.43)
2+	58 (6.6)	1.07 (0.74–1.55)	36 (4.1)	1.55 (0.92–2.64)
Number of New Male Sexual Partners in the Past 6 Months (N = 3063)				
0	163 (5.7)	Ref	77 (2.7)	Ref
1	6 (6.9)	1.20 (0.55–2.64)	6 (6.9)	2.55 (1.14–5.69)
2+	11 (9.3)	1.63 (0.91–2.91)	3 (2.5)	0.94 (0.30–2.93)
Number of Times Oral Sex Performed in the Past 6 Months (N = 2973)				
0	49 (4.5)	Ref	24 (2.2)	Ref
1–6	56 (6.7)	1.48 (1.02–2.14)	19 (2.3)	1.02 (0.56–1.85)
7–24	41 (7.0)	1.55 (1.04–2.32)	21 (3.6)	1.62 (0.91–2.89)
25+	25 (7.4)	1.63 (1.02–2.60)	11 (3.3)	1.47 (0.73–2.96)
P trend	0.02		0.1	
Number of Days Since Last Oral Sex (N = 2959)				
Never had oral sex	14 (4.2)	Ref	8 (2.4)	Ref
>30 days	49 (4.8)	1.15 (0.64–2.05)	25 (2.4)	1.02 (0.47–2.25)
>10–30 days	35 (6.3)	1.51 (0.82–2.76)	16 (2.9)	1.21 (0.52–2.79)
>3–10 days	32 (6.6)	1.58 (0.86–2.91)	18 (3.7)	1.55 (0.68–3.53)
0–3 days	47 (9.7)	2.32 (1.30–4.14)	12 (2.5)	1.04 (0.43–2.51)
P trend	<.01		0.51	

Abbreviations: CI, confidence interval; HPV, human papillomavirus; MSM, men who have sex with men only; MSW, men who have sex with women only; MSWM, men who have sex with women and men; N, number of observations without missing data for the covariates; PR, prevalence ratio; Ref, reference category.

^aHigh risk (n) = 184; low risk (n) = 88.

^bBolded values were statistically significant at $\alpha = 0.05$ level.

NOTE: Sample size per variable may be lower than 3095 due to missing or incomplete data from participants for 1 or more variables.

95% CI, 1.2–3.1), >19 male sexual partners (PR, 2.2; 95% CI, 1.3–3.8); performing oral sex in the past 6 months 1–6 times (PR, 1.5; 95% CI, 1.0–2.1), 7–24 times (PR, 1.6; 95% CI, 1.0–2.3), and more than 25 times (PR, 1.6; 95% CI, 1.0–2.6); and 0–3 days since last oral sex performed (PR, 2.3; 95% CI, 1.3–4.1). Low-risk HPV was significantly associated with sex with both men and women (PR, 2.5; 95% CI, 1.4–4.6); 3–7 lifetime male sex partners (PR, 2.5; 95% CI, 1.2–5.4); and reporting 1 new male sexual partner in the past 6 months (PR, 2.6; 95% CI, 1.1–5.7). Both high- and low-risk HPV were significantly associated with >19 lifetime sexual partners (high-risk HPV PR, 2.0; 95% CI, 1.4–3.0; low-risk HPV PR, 2.1; 95% CI, 1.2–3.8); and >19 lifetime female sexual partners (high-risk PR, 2.5; 95%

CI, 1.6–4.1; low-risk PR, 3.1; 95% CI, 1.5–6.3) (Table 2). HPV16 was significantly associated with >19 lifetime male sexual partners (PR, 2.9; 95% CI, 1.1–7.9) (results not shown).

Multivariable analyses for high-risk oral HPV were adjusted for one of the following: (1) lifetime number of sex partners, (2) lifetime number of female sex partners, (3) lifetime number of male sex partners, or (4) number of days since last oral sex performed. All models for high-risk HPV also adjusted for age, country of residence, marital status, pack-years of cigarettes smoked, and use of tobacco or snuff. Association of these covariates with high-risk HPV did not vary between the 4 models and results of the multivariate adjustment with lifetime number of sex partners, which had the most significant impact

Table 3. Multivariate Analysis of Factors Independently Associated With High-Risk Oral HPV (N = 2910)

Risk Factors	aPR (95% CI)
Age (Years)	
18–30	Ref
31–40	1.65 (1.12–2.44)^a
41–50	1.62 (1.05–2.50)
51–73	0.80 (0.40–1.58)
Country of Residence	
Brazil	Ref
Mexico	1.66 (1.15–2.40)
USA	1.18 (0.80–1.73)
Marital Status	
Single	Ref
Divorced/separated/widowed	0.99 (0.60–1.63)
Married/cohabiting	0.73 (0.50–1.06)
Pack-Years of Cigarettes Smoked	
0	Ref
>0–5	1.57 (1.11–2.22)
>5	1.66 (1.13–2.44)
Currently Chewing Tobacco or Using Snuff	
None	Ref
Sometimes	0.89 (0.43–1.85)
Daily	1.93 (0.95–3.92)
Lifetime Number of Sex Partners	
≤2	Ref
3–7	0.83 (0.51–1.35)
8–19	1.29 (0.83–2.00)
>19	1.88 (1.22–2.90)
Lifetime Number of Female Sex Partners ^b	
≤2	Ref
3–7	1.15 (0.68–1.95)
8–19	1.66 (1.00–2.75)
>19	2.28 (1.38–3.76)
Lifetime Number of Male Sex Partners ^b	
≤2	Ref
3–7	0.66 (0.25–1.76)
8–19	0.64 (0.16–2.48)
>19	2.11 (1.14–3.89)
Number of Days Since Last Oral Sex Performed ^b	
Never had oral sex during lifetime	Ref
>30	0.97 (0.54–1.75)
>10–30	1.32 (0.72–2.43)
>3–10	1.35 (0.73–2.50)
0–3	2.07 (1.15–3.72)

Abbreviations: aPR, prevalence ratio adjusted for age, country of residence, marital status, smoking, tobacco use, and lifetime number of sex partners; CI, confidence interval; HPV, human papillomavirus; Ref, reference category.

^aBolded values were statistically significant at $\alpha = 0.05$ level.

^bPrevalence ratio adjusted for the same variables but with the listed variable in place of lifetime number of sex partners in separate models.

on high-risk oral HPV and was retained in the final model (Table 3). In the final multivariable adjusted model, ages 31–40 (adjusted PR [aPR], 1.7; 95% CI, 1.1–2.4) and 41–50 years (aPR, 1.6; 95% CI, 1.1–2.5), living in Mexico compared with Brazil (aPR, 1.7; 95% CI, 1.2–2.4), and reporting >0–5 packs years (aPR, 1.6; 95% CI, 1.1–2.2) and >5 pack-years (aPR, 1.7; 95% CI,

Table 4. Multivariate Analysis of Factors Independently Associated With Low-Risk Oral HPV (N = 2735)

Risk Factors	aPR (95% CI)
Age (Years)	
18–30	Ref
31–40	1.13 (0.59–2.15)
41–50	1.69 (0.87–3.30)
51–73	1.74 (0.72–4.20)
Country of Residence	
Brazil	Ref
Mexico	0.96 (0.53–1.73)
USA	0.69 (0.37–1.32)
Marital Status	
Single	Ref
Divorced/separated/widowed	1.49 (0.73–3.05)
Married/cohabiting	0.60 (0.33–1.09)
Pack-Years of Cigarettes Smoked	
0	Ref
>0–5	1.30 (0.75–2.27)
>5	1.29 (0.71–2.33)
Number of Teeth Extracted Due to Gum Disease, Gingivitis, or Decay	
0	Ref
1+	1.25 (0.75–2.07)
Consistent Bleeding of Gums After Brushing or Presence of Swollen Gums	
No	Ref
Yes	2.16 (1.35–3.45)^a
Lifetime Number of Sex Partners	
≤2	Ref
3–7	1.06 (0.50–2.23)
8–19	0.85 (0.40–1.80)
>19	1.47 (0.75–2.89)
Lifetime Number of Female Sex Partners ^b	
≤2	Ref
3–7	1.61 (0.69–3.76)
8–19	1.27 (0.52–3.06)
>19	2.33 (1.04–5.25)
Lifetime Number of Male Sex Partners ^b	
≤2	Ref
3–7	1.85 (0.83–4.15)
8–19	0.62 (0.09–4.34)
>19	1.60 (0.63–4.08)
Sexual Orientation	
MSW	Ref
MSWM	2.19 (1.19–4.04)
MSM	0.57 (0.14–2.33)
Never had sex ^c	2.16 (0.87–5.38)

Abbreviations: aPR, prevalence ratio adjusted for age, country of residence, marital status, smoking, number of teeth extracted due to gum disease/gingivitis/decay, and consistent bleeding of gums after brushing or presence of swollen gums, and lifetime number of sex partners; CI, confidence interval; MSM, men who have sex with men only; MSW, men who have sex with women only; MSWM, men who have sex with women and men; Ref, reference category.

^aBolded values were statistically significant at $\alpha = 0.05$ level.

^bPrevalence ratio adjusted for the same variables but with the listed variable in place of lifetime number of sex partners in separate models.

^cNever had sex: never had oral, vaginal, or anal sex during their lifetime.

1.1–2.4) of cigarettes smoked were independently significantly associated with high-risk oral HPV when adjusting for lifetime number of sex partners only. Reporting >19 lifetime number of sex partners (aPR, 1.9; 95% CI, 1.2–2.9) was associated with

increased prevalence of high-risk oral HPV infection regardless of whether adjustment was for male or female partners. When adjusting for days since last oral sex was performed, those who did so 0–3 days ago had higher prevalence of high-risk oral HPV (aPR, 2.1; 95% CI, 1.2–3.7) (Table 3). The significantly higher oral high-risk HPV prevalence in Mexico was driven by higher prevalence of HPV33 and HPV39 (data not shown).

Multivariate analyses for prevalence of low-risk oral HPV infection each adjusted for one of the following: (1) lifetime number of sex partners, (2) lifetime number of female sex partners, (3) lifetime number of male sex partners, or (4) sexual orientation. All models for low-risk oral HPV also adjusted for age, country of residence, marital status, pack-years of cigarettes smoked, number of teeth extracted from gum disease, and consistent bleeding or swollen gums. Results were similar between the 4 models with the most parsimonious multivariate model being the one adjusting for lifetime number of sex partners.

Consistent bleeding of gums after brushing or presence of swollen gums was significantly independently associated with low-risk HPV when adjusting for lifetime number of sexual partners (aPR, 2.2; 95% CI, 1.4–3.5). Prevalence of low-risk oral HPV infection was not significantly associated with lifetime number of sex partners (Table 4). This did not differ with adjustment for male partners, although >19 female lifetime sex partners was significant (aPR, 2.3; 95% CI, 1.0–5.3), but did not change the effect of other variables in the model. Other sexual activity variables did not significantly impact prevalence of low-risk oral HPV infection (Table 4).

Multivariate analyses for HPV16 were adjusted for country of residence, pack years of cigarettes smoked, and lifetime male sex partners. In the final model, >0–5 pack years of cigarettes smoked (aPR, 1.9; 95% CI, 1.0–3.5) and >19 male lifetime sexual partners (aPR, 3.0; 95% CI, 1.1–8.7) were significantly and independently associated with HPV16 prevalence (results not shown).

DISCUSSION

This is the first study to report factors associated with low- and high-risk oral HPV prevalence separately among men from 3 countries using a common protocol. In general, oral HPV prevalence was much lower than anogenital HPV prevalence among men. High-risk oral HPV was associated with mid-adult age, more pack-years of cigarettes smoked, and lifetime number partners, regardless of the partner's gender. In contrast, low-risk HPV was only associated with poor oral health.

The results of our study are predominantly consistent with results from a prior US NHANES study [4] that found any oral HPV to be independently associated with age, number of sexual partners, number of oral sex partners, and current number of cigarettes smoked per day. However, results from the current study suggest that the nature of the association with age may be different by type of HPV, whereby age was independently associated with high-risk

but not low-risk HPV. In addition, we previously observed that the association with age is different by country [12]. Differences in associations with age between studies may be due to inclusion of men from outside the United States as well as differences in the methods utilized to detect HPV in the 2 studies. Although prevalence of HPV33 and HPV39 was higher in Mexico, they rarely progress to OPC because HPV16 is responsible for ~90% of HPV-attributable OPC. It is unclear why oral HPV33 and HPV39 were elevated in Mexico. This is the first study to examine recent sexual behaviors in detail, such as number of days since last oral sex. Although number of female sexual partners was associated with both low- and high-risk oral HPV, total number of lifetime sexual partners was only associated with high-risk oral HPV regardless of partner gender. Furthermore, oral sex behaviors, such as very recent oral sex acts performed (past 3 days), were associated with oral high-risk HPV, but not low-risk HPV. Other studies have reported (1) positive associations of any oral HPV infection with number of oral sexual partners and kissing [13, 14] and (2) a positive association between oral sex and HPV16 prevalence [5]. However, a study by Mooij et al [15] conducted among men who have sex with men only (MSM) did not observe an association with oral sexual behaviors, potentially due to collinearity of oral sexual behaviors with other sexual behaviors. Differences in oral HPV risk associated with performing oral sex may be due to differing modes of transmission such as direct from oral sex for high-risk HPV compared with autotransmission for low-risk HPV as have been described from external genitals to anal among heterosexual men [16].

Oral HPV prevalence has been consistently reported to be higher in males compared with females [4, 17]. It has been hypothesized that the risk of oral HPV acquisition is higher from females to males, similar to what has been observed for genital HPV transmission [18, 19]. However, this higher rate of oral HPV transmission from women to men does not appear to explain the large difference in oral HPV observed by sex. In a study by D'Souza et al [20] that compared women who had sex with women (lesbian) to heterosexual men, lesbian women still had lower oral HPV prevalence than heterosexual men. Our study showed that the risk of oral high-risk HPV was similar regardless of the sex of past and current sexual partners. A likely factor that explains increased susceptibility to oral HPV among men is their very low rate of antibody production in response to natural HPV infection and therefore continued susceptibility to HPV at multiple anatomic sites throughout the lifespan [21].

Several studies [22, 23] have shown significant associations between measures of oral health and the risk of OPCs. As such, an interesting hypothesis has emerged as to whether oral health influences risk of oral HPV prevalence and progression. Our study is the first to report the differential association of oral health with low-risk but not high-risk HPV. In our study, history of gum bleeding after brushing or persistent swelling of gums, a known precursor of periodontitis, was associated with low-risk

HPV but not high-risk HPV. Previously, studies reported significant associations between clinical periodontitis and prevalence of any oral HPV infection [24, 25]. Inflammatory cytokines produced due to chronic inflammation in gingivitis and periodontitis may facilitate acquisition and persistence of oral HPV infection, or increased oral HPV acquisition may be due to direct effects of periodontal bacteria [26]. Further studies are needed to assess which of these 2 mechanisms influence oral HPV prevalence and whether indeed the association is limited to low-risk HPV only. Tobacco smoking is consistently associated with higher HPV prevalence and persistence among both men and women at multiple anatomic sites of infection [27]. In the current study, use of cigarettes and smokeless tobacco were both significantly associated with high-risk but not low-risk HPV. Others have reported that biological measures of tobacco exposure such as mean cotinine levels were significantly higher in individuals with oral HPV16 infection compared with individuals without oral HPV16 [28]. Given the potential for tobacco use to induce local and systemic immunosuppressive effects that may facilitate HPV incidence, persistence, or reactivation, it is unclear why this effect would only be observed for high-risk but not low-risk HPV [27, 29].

Overall, lifetime number of female partners was associated with both high-risk and low-risk oral HPV infection, whereas smoking was associated with high-risk oral HPV only, and history of consistent bleeding was significantly associated with low-risk oral HPV only. These findings are somewhat similar to the Chan et al [8] study that examined differential risk factors between high-risk and low-risk cervical HPV and found that lifetime number of sexual partners was significantly associated with both high-risk and low-risk HPV, whereas smoking was associated with high-risk HPV infection only, and use of barrier contraceptives was protective against low-risk HPV only. These findings support the hypothesis that smoking promotes HPV persistence. Lack of a physical barrier (consistent bleeding of gums for oral HPV and lack of barrier contraceptive usage for cervical HPV) seems to promote low-risk HPV infection but not high-risk HPV infection. Further studies are required to examine whether different transmission mechanisms exist for low-risk HPV and high-risk HPV.

Like all epidemiological studies, there are several strengths and limitations that must be considered in interpreting results. A key finding was the difference in risk associated with the gender of the participants' sexual partner, specifically higher prevalence associated with more female partners. However, the small number of men who reported a sexual orientation of MSM or men who have sex with women and men potentially limits our ability to assess oral HPV risk associated with having sex with men. This study had a large enough sample size, however, to allow examination of many other factors associated with high-risk and low-risk oral HPV. We utilized a common protocol for conducting the study in 3 countries and used a

sensitive HPV genotyping assay. Because this is a cross-sectional analysis, causal inference cannot be made. The subset of HIM participants included in this study differed significantly from those not included in terms of sociodemographic characteristics (age, country of residence and marital status), smoking, and sexual behavioral (lifetime number of male and female sexual partners, number of new sexual partners in the past 6 months, frequency of oral sex in the past 6 months, number of days since last oral sex, and sexual orientation). There was no statistically significant difference between the 2 subcohorts in terms of oral health factors (ever had gingivitis, number of teeth extracted due to dental diseases, and consistent bleeding of gums/presence of swollen gums). These differences may have biased the high-risk prevalence in the oral HIM subcohort towards null.

CONCLUSIONS

The findings from this study highlight the differences in risk factor profiles of high- and low-risk oral HPV and demonstrate the importance of specifying the type of HPV in studies of oral HPV natural history. Future studies are needed to inform factors associated with oral HPV incidence and persistence by type.

Notes

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Potential conflicts of interest. A. R. G. is a member of several scientific advisory boards of Merck & Co, Inc, and her institution has received funding to conduct investigator-initiated studies as well as Merck-sponsored vaccine clinical trials. L. L. V. provides occasional consultancy on Human papillomavirus (HPV) vaccines to Merck & Co., Inc., BD, Roche, and QIAGEN concerning HPV tests. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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