Published in final edited form as:

Sex Transm Dis. 2015 December ; 42(12): 677–685. doi:10.1097/OLQ.0000000000000362.

Epidemiology of Human Papillomavirus (HPV) Detected in the Oral Cavity and Fingernails of Mid-Adult Women

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Abstract

Background—Oral and fingernail human papillomavirus (HPV) detection may be associated with HPV-related carcinoma risk at these non-genital sites and foster transmission to the genitals. We describe the epidemiology of oral and fingernail HPV among mid-adult women.

Methods—Between 2011–2012, 409 women aged 30–50 years were followed for 6 months. Women completed health and behavior surveys and provided self-collected oral, fingernail, and vaginal specimens at enrollment and exit for type-specific HPV DNA testing. Concordance of type-specific HPV detection across anatomic sites was described with kappa statistics. Using generalized estimating equations or exact logistic regression, we measured the univariate associations of various risk factors with type-specific oral and fingernail HPV detection.

Results—Prevalence of detecting HPV in the oral cavity (2.4%) and fingernails (3.8%) was low compared to the vagina (33.1%). Concordance across anatomic sites was poor ($\kappa < .20$ for all comparisons). However, concurrent vaginal infection with the same HPV type (OR=101.0;95%CI: 31.4–748.6) and vaginal HPV viral load (OR per one $\log_{10}$ viral load increase=2.2;95%CI:1.5–5.5) were each associated with fingernail HPV detection. Abnormal Pap history (OR=11.1;95%CI: 2.8-infinity), lifetime number of male vaginal sex partners ≥ 10 (OR vs. 0–3 partners=5.0;95%CI: 1.2-infinity), and lifetime number of open-mouth kissing partners ≥ 16 (OR vs. 0–15 partners=infinity;95%CI:2.6-infinity, by exact logistic regression) were each associated with oral HPV detection.

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The authors have declared no conflict of interest.

Conclusions—While our findings support HPV DNA deposition or autoinoculation between anatomic sites in mid-adult women, the rarity of HPV in the oral cavity and fingernails suggests that oral/fingernail HPV does not account for a significant fraction of HPV in genital sites.

Keywords

human papillomavirus; women; oral; fingernail

INTRODUCTION

Although alpha human papillomavirus (HPV) types are detected most commonly in genital sites, they are also detected in the oral cavity^{1, 2} and fingernails.^{3–5} While HPV-related carcinoma of the fingers or nailbed is rare, the incidence of HPV-related oropharyngeal cancer (primarily HPV-16-related⁶) is higher and increasing.⁷ Furthermore, HPV detection in non-genital sites is potentially important for viral transmission to the genitals.⁸ Therefore, it is important to elucidate the epidemiology of HPV in the oral cavity and fingernails. Utilizing data from a 6-month longitudinal study of mid-adult women, we evaluated the frequency of and risk factors for HPV detection in the oral cavity and fingernails. We also described the concordance of HPV detection among oral, fingernail, and vaginal samples.

MATERIAL AND METHODS

Study Design and Population

Between March 2011 and January 2012, we enrolled 409 mid-adult women (aged 30–50 years) affiliated with the University of Washington into a 6-month longitudinal study. Exclusion criteria included current pregnancy, hysterectomy, and serious medical conditions that would prohibit protocol adherence. Women provided self-collected vaginal, oral, and fingernail samples (in order) for HPV genotyping at in-clinic enrollment and 6-month exit visits. Demographics, health, and sexual behaviors were collected at enrollment and exit via online surveys. In February 2012, the exit survey was expanded to capture additional potential oral HPV risk factors (including open mouth kissing and oral sex), with questions covering the time periods before enrollment and between enrollment and exit. For example, “before you enrolled in this study, about how many different partners did you perform oral sex on?” and “since you enrolled in the study, did you perform oral sex on any new partners?” Women exiting before February 2012 were invited to complete a post-exit online supplemental survey with these additional questions. A total of 323 women (79%) provided information on these additional potential risk factors for oral HPV.

Sample Collection

Vaginal specimens were collected with two sequential sterile Dacron swabs (to enhance sensitivity for HPV detection⁹) and stored in 1.5 mL of specimen transport medium (STM) (Qiagen, Gaithersburg, MD).¹⁰ Two types of oral specimens were collected (swab and rinse) to maximize sensitivity for HPV detection.¹¹ Women were instructed and supervised in mirror-assisted collection of two oral swabs, with the first swabbing the tonsils or palatine arches and the second swabbing the back of the tongue and pharynx. Swabs were stored in 1.5 mL STM. For the rinse, women gargled 10 mL of Scope mouthwash for 30 seconds and

expectorated into a collection cup. The sample was centrifuged for 5 minutes and the remaining pellet stored in 1 mL of Qiagen cell lysis solution. Fingernail sample collection was implemented in June 2011. Women rubbed their fingertips and the underside of the tip of their fingernails on both hands with a cytology brush. The brush was stored in 1 mL STM.

HPV Genotyping

All specimens were digested with 20 µg/mL proteinase K at 37°C for one hour. DNA was isolated from 200 µL of digested swab samples using the QIAamp DNA blood mini kit (Qiagen, Cat. No. 51104). Genomic DNA from the oral lavage cell pellet was isolated using the Gentra Puregene Buccal Cell kit (Qiagen, Cat. 158845). For oral and fingernail samples, HPV and β -globin were PCR-amplified simultaneously using the MY09/11 system, dotted onto nylon filters, and probed with both a biotin-labeled HPV generic probe and β -globin probe. Specimens negative for β -globin were considered insufficient and excluded, and specimens β -globin positive but HPV-negative were considered negative for HPV. Specimens positive by both probes were genotyped for 37 HPV types (6/11/16/18/26/31/33/35/39/40/42/45/51/52/53/54/55/56/58/59/61/62/64/66/67/68/69/70/71/72/73/81/82/83/84/CP6108/IS39) via the Roche Linear Array. Samples HPV-positive by dot blot but HPV-negative by Roche (1 oral swab, 5 oral rinse, and 3 fingernail samples) were considered HPV-negative in analyses. Samples HPV-positive by dot blot but negative for β -globin by Roche assay (2 fingernail samples) were considered insufficient and excluded. All vaginal samples were directly HPV genotyped by Roche Linear Array.

HPV Viral Load Testing

Viral load testing was conducted on vaginal samples only using duplex real-time PCR for quantification of cellular (β -actin) and HPV E7 DNA, described in detail previously.¹² Samples positive for type-specific high-risk HPV by the Roche assay were selected. Testing was conducted for 16 high-risk types: 16/18/31/35/39/45/51/52/53/56/58/59/66/68/73/82.

HPV Variant Sequencing

Type-specific HPV variant sequencing was conducted on samples positive for the same HPV type in different anatomic sites within a woman. Variant testing was available for 12 types: 16/18/31/35/39/45/51/52/56/58/59/68. Methods for PCR sequencing and analysis were described previously.^{13, 14} If 1 nucleotide alteration was detected among type-specific isolates in different samples from one woman, they were considered to be different variants. In analyses, paired samples with different variants were considered discordant; if variant results were unavailable, paired samples positive for the same HPV type were assumed to be concordant.

Statistical Analysis

Women were considered oral HPV-positive if either the rinse or the swab tested positive. Prevalence, 6-month cumulative incidence, and 6-month persistence and clearance (with 95% confidence intervals [CIs]) were estimated by anatomic site. Prevalence was defined as the proportion of women with sufficient enrollment samples who tested DNA-positive for

any HPV type. Six-month cumulative incidence was calculated by dividing the number of women positive for any new type at exit by the number with sufficient exit samples. Six-month persistence (or clearance) was defined as the proportion of type-specific prevalent infections positive (or negative) for the same type at exit.

We used proportion positive agreement (PPA) and un-weighted kappa statistics based on PPA with percentile bootstrapped 95% CIs using women-level clustered sampling¹⁵ to describe concordance of type-specific (or variant-specific, if variant testing was completed) HPV detection between sites. Enrollment and exit samples were pooled for this analysis.

We generated odds ratios for associations between selected factors and type-specific HPV using generalized estimating equations logistic regression. Separate models were constructed for oral and fingernail HPV detection. At each anatomic site, both enrollment and exit samples were included in the same model. Each woman contributed multiple “woman-types” to the analysis, equal to the number of HPV types assessed (37 for most analyses) multiplied by the number of samples contributed (1 or 2, depending on whether the woman contributed samples at both enrollment and exit). Demographic, health, and sexual behavior variables and presence and viral load of concurrent type-specific (or variant-specific, if variant testing was completed) vaginal HPV were assessed as time-varying covariates at the time of each sample collection. Robust variance estimates were used to account for correlation within women due to dual measurements at enrollment and exit and also multiple HPV types. Due to the small number of oral and fingernail HPV detections, we calculated 95% CIs using percentile bootstrap methods (5,000 repetitions) for all variables without zero cells. For variables with zero cells, exact logistic regression was used to estimate 95% CIs, without accounting for correlation within women.

RESULTS

A total of 409 women were enrolled and submitted vaginal and oral samples at enrollment; 269 enrolled after May 2011 also submitted fingernail samples at enrollment. The majority of enrolled women were white (79%) and reported a history of sex with male partners only (80%) (Table 1). Three hundred and eighty-one women had an exit visit; 379 women submitted vaginal samples, 381 submitted oral samples, and 380 submitted fingernail samples at exit.

Four of 787 (0.5%) oral swab, 16 (2.0%) of 789 oral rinse, 140 (21.8%) of 649 fingernail, and 1 (0.1%) of 788 vaginal samples were insufficient and excluded from analyses. Oral HPV prevalence was 2.4% for any HPV, 2.2% for high-risk HPV, and 0.7% for HPV-16 (Table 2). Fingernail HPV prevalence was 3.8% for any HPV and 2.4% for high-risk HPV (HPV-16 was not detected). Vaginal HPV prevalence was 33.1% for any HPV, 21.8% for high-risk HPV, and 3.7% for HPV-16. The 6-month cumulative incidence of detecting a new HPV type was 0.3% for oral, 0.6% for fingernail, and 16.1% for vaginal samples. No cases of type-specific HPV in fingernail samples were persistently detected at the 6-month exit visit, whereas 23.1% of type-specific HPV in oral and 67.1% of type-specific HPV in vaginal samples were persistently detected at exit.

Only one pair of oral samples tested positive for HPV (type 16) in both the rinse and swab; in all 17 other cases, oral HPV was detected in only one sample of a pair (Table 3). Variant testing was conducted on 14 samples representing 6 cases where the same type was detected across anatomic sites. Variant characterization was not completed for 2 of 6 sets due to failure to PCR-generate target DNA fragments for sequencing. Samples from 3 of 4 (75%) of the remaining sets tested positive for the same type-specific variant within a woman, and one (25%) set of HPV-16 positive samples had a different variant detected in the enrollment oral sample versus the enrollment and exit vaginal samples.

PPA was lowest between oral and vaginal samples (0.4%) and highest between fingernail and oral samples (7.7%), though PPA was low for all comparisons and concordance poor ($\kappa < 0.20$) (Table 4).

For oral HPV, history of an abnormal Pap test (OR = 11.1; 95% CI: 2.8-infinity), reporting a lifetime number of male sex partners 10 (OR vs. 0–3 partners = 5.0; 95% CI: 1.2-infinity) and open-mouth kissing partners 16 (OR vs. 0–15 partners = infinity; 95% CI: 2.6-infinity, by exact logistic regression) were each positively associated with oral HPV detection (Table 5). For fingernail HPV, having concurrent vaginal HPV infection with the same type (OR = 101.0; 95% CI: 31.4–748.6) and vaginal HPV viral load (OR per one $\log_{10}$ increase in viral load = 2.2; 95% CI: 1.5–5.5) were each positively associated with fingernail HPV detection (Table 6).

DISCUSSION

In this study of mid-adult women, oral HPV prevalence was low. Prior studies in immunocompetent populations reported substantial variation in oral HPV prevalence across populations and geographic areas.^{1, 2} A systematic review of 18 studies worldwide estimated oral HPV prevalence at 4.5% for any HPV, 3.5% for high-risk HPV, and 1.3% for HPV-16, with similar estimates between men and women.² A U.S. population-based study (2009–2010 National Health and Nutrition Examination Survey [NHANES]) reported that HPV prevalence in oral rinse samples from 14–69 year old participants was 6.9% for any HPV, 3.7% for high-risk HPV, and 1.0% for HPV-16, but prevalence in males was higher than in females (10.1% vs. 3.6% for any HPV).¹ In the U.S., oral HPV prevalence demonstrates a bimodal age pattern, and our estimates are comparable to the modeled female estimates within the same age range in NHANES.¹ Varying prevalence estimates may also be attributable to differences in study populations, sampling methods (oral HPV specimen collection methods are not standardized¹⁶), and laboratory assays and protocols.¹⁷ We used both oral rinse and swab samples to increase sensitivity,¹¹ and found that most oral HPV was detected in one sample type only. Our laboratory methods were identical to those used in our previous study of oral HPV in 18–25 year old men, which also detected discordance between oral swab and rinse samples.¹⁸ Differences in HPV detection in oral rinse versus swab samples may reflect different infection sites within the oral cavity.¹⁸

There are limited data on oral HPV incidence and persistence in immunocompetent cohorts. The Finnish HPV Family Study reported that in 331 women, the 24-month cumulative incidence of oral HPV was ~10%, and none of 40 women with prevalent high-risk HPV

showed evidence of clearance at 24 months.¹⁹ D'Souza et al. reported that 60% of oral HPV detected in 63 HIV-negative women was persistently detected at 6 months.²⁰ In general, oral HPV incidence in immunocompetent populations has been reported to be higher in male than in female cohorts (although 24-month cumulative incidence was similar in males and females enrolled in the Finnish study¹⁹). In our previous study of young men, the 12-month cumulative incidence of oral HPV was 12.3%, and 28.6% of oral HPV was repeatedly detected within 4 months to 1.5 years.¹⁸ In another multi-center U.S. study, the two-year cumulative incidence of oral HPV was 19% in HIV-uninfected individuals, with 20% still detected at one year.²¹ In our cohort, incident detection was rare (<1%), but follow-up was limited to 6 months. Less than 25% of prevalent types were redetected at 6-month exit.

HPV prevalence in fingernail samples was also low, and type-specific HPV was not repeatedly detected in any fingernail sample at 6 months (but only 9 of 12 prevalent HPV samples had a paired exit sample sufficient for testing). In our previous study of 128 women aged 18–22 years, we reported that HPV prevalence was 14.3% in fingertip samples and that 14.5% were repeatedly detected at the subsequent visit ~4 months later.³ The low prevalence and transient nature of HPV in fingernails suggests detection may not represent true infection. Incidence of fingernail HPV detection was also low in the present study (<1%). We previously reported a 24-month cumulative incidence of 30% for detecting any HPV in the fingernails of young men.⁴

While apparently uncommon, HPV detection at multiple anatomic sites does occur^{22, 23} and is likely due to increased host susceptibility to HPV infection, a single source of infection (e.g. an infected sexual partner), autoinoculation, or DNA deposition between sites. Transmission or DNA deposition between partners may occur via direct contact between anatomic sites. Autoinoculation or DNA deposition within an individual may occur via direct (e.g. DNA deposition from genitals to fingers) or indirect (e.g. DNA deposition from genitals to the oral cavity via fingers) contact. We observed poor concordance of type-specific HPV detection between oral, fingernail, and vaginal sites. Of the studies that have addressed concurrent oral and genital HPV infections, findings have been mixed, with a wide range of concordance estimates reported (ranging from 0% to 60%).^{24, 25} A meta-analysis of 10 published studies in women with cervical infection estimated that type-specific HPV concordance was 27% between oral and genital sites.²⁴ Another study among 1,812 U.S. women in NHANES reported low type-specific concordance (6.6%) between oral and cervical sites, suggesting differences in the natural history of infection at the two sites.²⁵

Plausibility for a finger-genital route of HPV transmission was first suggested by a previous report that genital HPV types were found on the fingernails of 12 of 22 patients with genital warts.⁵ Furthermore, in a previous study of 25 heterosexual couples, sequential detection of HPV in the hands and genitals (within individuals and between partners) occurred.²² We previously reported low type-specific concordance between fingertips and genital sites ($\kappa=0.17$) among young women.³ Despite low concordance in the present study, we observed that concurrent vaginal HPV infection with the same HPV type was associated with fingernail HPV. These results are consistent with our previous study in young men, whereby concurrent genital infection was associated with a nearly 12-fold increase in

incident detection with the same type in the fingernails.¹⁸ We also previously reported a higher likelihood of detecting concurrent infections between genital sites and fingernails if both cervical and vaginal/vulvar sites were HPV-positive, and hypothesized that infection at multiple genital sites may reflect infections of higher viral load that in turn increase the likelihood of DNA deposition or transmission between sites.³ Our results further support this hypothesis with the observation that higher HPV viral load in concurrent vaginal samples was associated with fingernail HPV. We did not observe any notable associations between any demographic, health, or sexual behavior variables and fingernail HPV detection. However, a limitation is that we did not collect any data on self or partnered finger-genital contact.

We observed that higher lifetime numbers of open-mouth kissing partners and male vaginal sex partners were each positively associated with oral HPV detection, consistent with prior studies.^{1, 26, 27} Although multivariate analyses were not performed as part of this exploratory study, we conducted a post-hoc analysis including both variables in a simplified, women-level exact logistic regression model (necessitated by the lack of outcomes in women with fewer than 16 kissing partners). Although not directly comparable to the univariate models, the association between kissing partners and oral HPV remained significant in this analysis, whereas the association with vaginal sex partners was attenuated. We did not observe a significant association between lifetime number of oral sex partners and oral HPV, in contrast to some prior studies.^{26, 27} Given that sexual behaviors tend to correlate, teasing out the contributions of individual risk behaviors to oral HPV infection is methodologically challenging.

Several other limitations should be noted. First, the protocol for testing vaginal samples was different than that for oral and fingernail samples, because vaginal samples were tested directly with the Roche Linear Array. Oral and fingernail samples were first tested by less costly dot blot hybridization, given the expected low prevalence of oral and fingernail versus vaginal HPV. Therefore, concordance between vaginal and oral or fingernail samples for detecting HPV DNA may have been underestimated. On the other hand, when variant specific results were unavailable, we assumed that type-specific concordant samples between anatomic sites were variant specific. However, our limited variant data suggest that 25% of type-specific concordant samples within a woman are not variant specific. Therefore, we may have also overestimated concordance. Second, incidence and persistence estimates were limited by the short follow-up time with only two assessments 6 months apart. In addition, the low numbers of oral and fingernail HPV positives limited power and precluded an analysis of risk factors for persistent or incident oral or fingernail HPV. Specifically, we were unable to evaluate recent sexual behaviors as risk factors for incident HPV. In addition, oral risk behavior data were subject to varying lengths of recall and not available for all women, due to the mid-study addition of these questionnaire items. Power for analyses of fingernail HPV was further limited by the fact that fingernail sample collection was added to the protocol after the study started, and the proportion of fingernail samples that were negative for β -globin and deemed insufficient for HPV DNA testing was relatively high (21.8%). In our previous studies that used the same laboratory methods, the proportion of insufficient fingernail samples was considerably lower (1.4% in young men⁴ and 6.2% in young women³). In post-hoc experiments, we quantified total genomic DNA in

insufficient fingernail samples using a highly sensitive quantitative PCR assay against the repetitive sequence Alu, and concluded that insufficiency in fingernail samples was due to low levels of genomic DNA rather than PCR inhibition. The low genomic DNA level might explain the variability of insufficiency rates across studies. We did compare insufficient fingernail samples from the present mid-adult cohort and our previous young adult female cohort, but no appreciable differences in genomic DNA level were observed to shed light on the differences in the proportion of insufficient samples. However, the possibility that the samples from the young adult cohort (collected from 2000–2007) had degraded cannot be ruled out. It is also possible differences in the order of specimen collection could have contributed to differences in insufficiency rates across studies. Specifically, fingernail samples were collected first in the earlier studies, and last in the present study. It is therefore conceivable that hand washing before and after the vaginal self-collection could have contributed to reduced levels of genomic DNA in fingernail samples, thereby increasing the number of insufficient samples in the present study. In a post-hoc analysis in the mid-adult cohort, vaginal HPV prevalence in women with sufficient versus insufficient fingernail samples was similar (34.7% versus 31.4%, $p=.47$), supporting the validity of the fingernail HPV results in women with sufficient fingernail samples.

In conclusion, detection of HPV in the oral cavity and fingernails was uncommon among this cohort of mid-adult women, and persistent detection was rare. While results suggest that deposition or autoinoculation between anatomic sites is plausible in mid-adult women, the rarity of HPV in the oral cavity and fingernails suggests that oral/fingernail HPV does not account for a significant fraction of HPV in genital sites. To further understand HPV transmission between anatomic sites, future studies of couples with sampling at multiple anatomic sites and detailed collection of partnered and non-partnered sexual behaviors are warranted.

Acknowledgments

Funding: This work was financially supported by a National Institutes of Health P01 grant (AI083224-01A1) to LAK and RLW.

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Table 1

Enrollment characteristics of mid-adult women in Seattle, Washington, 2011–2012 (N = 409)

<i>Characteristics</i>	<i>Mean</i>	<i>(SD)</i>
Age (years)	38.3	(6.1)
Age at first sexual intercourse with a male partner (years)*	18.8	(4.1)
	<i>Median</i>	<i>(IQR)</i>
Lifetime number of male sex partners	7	(3 – 15)
	<i>n[†]</i>	<i>(%)</i>
Race		
African American	11	(2.7)
Asian	46	(11.2)
White	323	(79.0)
Other [‡]	29	(7.1)
Education		
Some college or less	68	(16.6)
College bachelor's degree	152	(37.2)
College master's or doctoral degree	189	(46.2)
Marital status		
Unmarried or separated	157	(38.6)
Married or living with a partner	250	(61.4)
Ever had a non-HPV-related sexually transmitted disease [§]		
No	327	(80.3)
Yes	80	(19.7)
Ever had genital warts		
No	363	(88.8)
Yes	46	(11.2)
Ever had an abnormal Pap test		
No	234	(57.2)
Yes	175	(42.8)
Ever had 1 dose of HPV vaccine		
No	378	(92.6)
Yes	30	(7.4)
Ever been pregnant		
No	168	(41.1)
Yes	241	(58.9)
Currently using hormonal birth control methods [¶]		
No	270	(66.0)
Yes	139	(34.0)
Currently have an immunosuppressive condition		
No	401	(98.0)
Yes	8	(2.0)
Smoking status ^{**}		

<i>Characteristics</i>	<i>Mean</i>	<i>(SD)</i>
Never	301	(73.8)
Former	88	(21.6)
Current	19	(4.7)
Currently consumes alcoholic beverages		
No	63	(15.4)
Yes	346	(84.6)
Type of vaginal sex partners (lifetime)		
No sex partners	4	(1.0)
Male only	327	(80.0)
Female only	8	(2.0)
Both male and female	70	(17.1)
Sex with male partners within 12 months before enrollment		
No sexual activity	63	(15.6)
Sex with non-new male partners only	217	(53.8)
Sex with 1 new male partner	123	(30.5)
Ever open-mouth or tongue kissed ^{††}		
Never	4	(1.3)
Male partners only	207	(68.1)
Both male and female partners	93	(30.6)
Ever performed oral sex ^{††}		
Never	12	(3.9)
Male partners only	234	(77.0)
Female partners only	6	(2.0)
Both male and female partners	52	(17.1)

* Restricted to 397 women (97.1%) who reported ever having had sex with a male partner

[†] Numbers may not add up to total due to missing data.

[‡] Includes individuals indicating the following: American Indian/Alaska Native, Native Hawaiian/Other Pacific Islander, other race, or multiple races

[§] Includes chlamydia, gonorrhea, genital herpes, and HIV

[¶] Includes birth control pills, hormonal patches, vaginal rings, implanted contraception, injectable contraception, and hormonal intrauterine devices

^{//} Includes HIV positivity (n=1) or currently taking immunosuppressive medications (n=7)

^{**} Smoking was defined as smoking at least one cigarette a day for one month or longer; former smokers reported ever smoking but not currently smoking, and current smokers reported currently smoking.

^{††} Restricted to 323 women who provided information on these additional potential risk factors for oral HPV. Questions were added only to the exit survey after the start of the study. Questions on these additional potential risk factors covered both the period prior to enrollment, and the period between enrollment and exit; numbers reported in this table reflect the time period prior to enrollment. 135 women who exited before the new questions were incorporated were invited to complete a post-exit online supplemental survey consisting of these questions; 81 responded. 246 women exited after the questions were added, and 242 completed an updated exit survey with the additional questions incorporated.

Prevalence, incidence, and persistence of oral, fingernail, and vaginal HPV DNA among mid-adult women in Seattle, Washington, 2011–2012

Table 2

	Oral*			Fingernail			Vaginal		
	N	n	(%)	N	n	(%)	N	n	(%)
<i>Woman-level</i>									
Prevalence at enrollment	409 [†]			213 [‡]			408 [§]		
Any HPV [¶]	10	(2.4)		8	(3.8)		135	(33.1)	
High-risk HPV**	9	(2.2)		5	(2.3)		89	(21.8)	
HPV-16	3	(0.7)		0	(0.0)		15	(3.7)	
6-month cumulative incidence ^{††}	381	1 ^{‡‡}	(0.3)	161	1 ^{‡‡}	(0.6)	378	61 ^{‡‡}	(16.1)
<i>Infection-level (type-specific)</i>									
6-month persistence ^{§§}	13	3 ^{¶¶}	(23.1)	11	0	(0.0)	249	167	(67.1)
6-month clearance	13	10	(76.9)	11	11	(100.0)	249	82	(32.9)

* Detection of HPV in either oral swab or rinse sample

[†] Women with a sufficient oral sample either through swab or rinse were considered sufficient. One sample was insufficient on oral swab, and 10 samples were insufficient on oral rinse.

[‡] 56 fingernail samples were insufficient; we did not add fingernail sampling to the study protocol until June 2011, therefore fingernail samples were not collected at enrollment for 140 women. There were 296 sufficient fingernail samples at exit, of which 5 samples tested positive for any HPV type, translating to a prevalence estimate at exit of 1.7%.

[§] One vaginal sample was insufficient.

[¶] Any of the following 37 HPV types: 6, 11, 16, 18, 26, 31, 33, 35, 39, 40, 42, 45, 51, 52, 53, 54, 55, 56, 58, 59, 61, 62, 64, 66, 67, 68, 69, 70, 71, 72, 73, 81, 82, 83, 84, CP6108, and IS39

^{||} Represents 15 type-specific oral infections, 12 type-specific fingernail infections, and 274 type-specific vaginal infections.

^{**} Any of the following 19 high-risk HPV types: 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73, 82, and IS39

^{††} Incident infection defined as testing HPV DNA positive for a HPV type not detected at enrollment. The denominator is restricted to women with sufficient samples at enrollment and exit.

^{‡‡} Represents 1 type-specific oral infection, 1 type-specific fingernail infection, and 81 type-specific vaginal infections

^{§§} Persistent infection defined as testing HPV DNA positive at the exit visit for the same HPV type detected at enrollment, among prevalent detections at enrollment with a sufficient exit sample.

^{¶¶} Persistent oral HPV consisted of two HPV-16 cases and one HPV-61 case.

Table 3

Type-specific HPV DNA status across anatomic sites in mid-adult women with oral or fingernail HPV detected at enrollment or 6-month exit, Seattle, Washington, 2011–2012

ID no.	Enrollment			Exit		
	Oral	Fingernail	Vaginal*	Oral	Fingernail	Vaginal*
1	–		–	–	6	6
2	–		–	–	58	–
3†	16§		16	–	16	16
4	–		66	–	66	66
5	16§		–	16†§	–	–
6	45†		–	–	–	–
7	18†, 39†		–			
8	39†		39	–	–	–
9	31†		–	–	–	–
10	–	51, 66	–	–	–	–
11	18†, 35†, 39†, 52†		35	–	–	–
12	–	35, 53	35, 53	–	–	53
13	–	83	83	–	–	83
14	51§, 66§	51, 66	–	–	–	–
15	–	58	–	–		–
16	–	54, 61	54, 61	–	–	54, 61
17	–	72	72	–	–	72
18	–	–	–	61†	–	–
19	–	56	56	–	–	56
20	–	–	62	–	62	62
21	61†	–	–	61†		–
22	16§	–	–	16§	–	–

Note: Bolded HPV types indicate that variant testing was completed on the woman-type. Cells shaded black indicate samples not collected; cells shaded gray indicate insufficient samples for HPV DNA testing.

* Only HPV types detected in oral or fingernail samples at either enrollment or exit are included.

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† Different HPV-16 variants were detected in the oral and vaginal samples (the same variant was detected in the 2 vaginal samples); the fingernail sample tested negative for HPV-16 by PCR during variant sequencing.

‡ HPV DNA positive in oral rinse sample

§ HPV DNA positive in oral swab sample

Concordance among oral, fingernail, and vaginal samples^{*} for type-specific HPV DNA detection among mid-adult women in Seattle, Washington, 2011–2012

Table 4

	No. of pairs [†]			PPA (%) [‡]	Kappa [§]	(95% CI) [¶]
	+/+	+/-	-/+			
Oral / vaginal	2	17	520	28,580	0.37	0.01 (0.00, 0.01)
Vaginal / fingernail	11	334	6	18,408	3.13	0.03 (0.01, 0.06)
Fingernail / oral	2	15	9	18,807	7.69	0.08 (0.00, 0.29)

^{*} Enrollment and exit samples combined to calculate concordance between anatomic sites

[†] Represents the number of women multiplied by the number of HPV types evaluated per woman multiplied by the number of samples evaluated per woman.

[‡] PPA is proportion positive agreement (number DNA-positive in both samples) / (number DNA-positive in either sample).

[§] Kappa is calculated by (observed PPA – expected PPA) / (1 – expected PPA).

[¶] Confidence intervals estimated using percentile bootstrap methods with 1,000 repetitions to account for correlation due to multiple HPV types and dual visits within women

^{||} One pair with HPV-16 in oral and vaginal samples was considered (+/-) due to detection of different variants in the oral versus the vaginal sample.

Table 5

Univariate analysis of factors associated with oral HPV detection among mid-adult women in Seattle, WA, 2011–2012, using GEE logistic regression (N=409)

<i>Characteristics</i>	Oral HPV detection (N_{Total} = 29,230[†])			
	<i>N</i> [*]	<i>n</i> [§]	<i>OR</i>	<i>(Bootstrap 95% CI)</i>
Age (years)	29,230	19	1.0	(0.9, 1.1)
Marital status				
Unmarried or separated	10,804	7	1.0	–
Married or living with a partner	18,204	12	1.0	(0.2, 10.6)
Ever had a non-HPV related STD [¶]				
No	23,162	15	1.0	–
Yes	5,846	4	1.1	(0.0, 4.3)
Ever had genital warts				
No	25,530	19	1.0	–
Yes	3,552	0	0.0	(0.0, 1.5)*
Ever had an abnormal Pap test				
No	16,502	2	1.0	–
Yes	12,654	17	11.1	(2.8, Inf)
Ever been pregnant				
No	11,803	5	1.0	–
Yes	17,390	14	1.9	(0.5, 13.4)
Currently using hormonal contraceptives				
No	22,977	11	1.0	–
Yes	6,105	8	2.7	(0.3, 9.6)
Smoking status ^{**}				
Never	21,682	10	1.0	–
Former	6,031	7	2.5	(0.0, 12.2)
Current	1,293	2	3.4	(0.0, 16.8)
Currently consumes alcoholic beverages				
No	4,662	1	1.0	–
Yes	24,420	18	3.4	(0.8, Inf)
Type of vaginal sex partners (lifetime)				
Male only	23,421	16	1.0	–
Female only or both male and female	5,513	3	0.8	(0.0, 3.2)
Age at first sexual intercourse with a male partner (yrs) ^{††}	28,046	18	0.8	(0.7, 1.0)
Lifetime number of male sex partners (tertiles)				
0 – 3	7,955	2	1.0	–
4 – 9	8,806	2	0.9	(0.0, Inf)
10+	11,840	15	5.0	(1.2, Inf)
Lifetime number of male or female open-mouth kissing partners (median split) ^{‡‡}				
0 – 15	11,174	0	1.0	–

<i>Characteristics</i>	Oral HPV detection ($N_{\text{Total}} = 29,230^{\dagger}$)			
	<i>N</i> [*]	<i>n</i> [§]	<i>OR</i>	<i>(Bootstrap 95% CI)</i>
16+	10,730	11	Inf	(2.6, Inf) [*]
Lifetime number of male or female oral sex partners (median split) ^{††}				
0 – 5	11,729	5	1.0	–
6+	10,397	6	1.4	(0, Inf)
Type of oral sex partners ^{††§§}				
Male only	17,094	8	1.0	–
Female only or both male and female	4,292	3	1.5	(0.0, 8.8)
Vaginal HPV infection of the same type				
No	28,597	17	1.0	–
Yes	522	2	6.5	(0.0, 19.2)
Vaginal HPV viral load of the same type (continuous) ^{¶¶}	256	2	1.1	(0.6, 1.6)

* indicates 95% confidence interval generated using exact logistic regression due to 0 in one cell; cannot account for correlation within women

[†] Both enrollment and exit samples were included. 409 women contributed a total of 29,230 women-types to the oral HPV analysis.

[‡] Indicates the total number of women multiplied by the number of HPV types evaluated per woman multiplied by the number of samples evaluated per woman.

[§] Indicates the number of women–types positive for oral HPV

[¶] Includes chlamydia, gonorrhea, genital herpes, and HIV

^{//} Includes birth control pills, hormonal patches, vaginal rings, implanted contraception, injectable contraception, and hormonal intrauterine devices

^{**} Smoking was defined as smoking at least one cigarette a day for one month or longer; former smokers reported ever smoking but not currently smoking, and current smokers reported currently smoking.

^{††} Restricted to 397 women who reported ever having had sex with a male partner

^{††} Restricted to 323 women who filled out either a modified exit survey or a supplemental exit survey with additional questions on open-mouth kissing and oral sex.

^{§§} Twelve women who reported no history of oral sex were excluded.

^{¶¶} Restricted to 16 high-risk HPV types with available viral load results, including HPV 16, 18, 31, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73, and 82, and to samples with a vaginal infection of the same HPV type detected

Table 6

Univariate analysis of factors associated with fingernail HPV detection among mid-adult women in Seattle, WA, 2011–2012, using GEE logistic regression (N=348)

<i>Characteristics</i>	Fingernail HPV detection (N_{Total} = 18,833[†])			
	<i>N</i> [*]	<i>n</i> [§]	<i>OR</i>	<i>(Bootstrap 95% CI)</i>
Age (years)	18,833	17	1.0	(0.9, 1.1)
Marital status				
Unmarried or separated	6,845	6	1.0	–
Married or living with a partner	11,877	11	1.1	(0.3, 5.2)
Ever had a non-HPV related STD [¶]				
No	14,911	9	1.0	–
Yes	3,848	8	3.4	(0.8, 12.4)
Ever had genital warts				
No	16,465	17	1.0	–
Yes	2,294	0	0.0	(0.0, 1.7) [*]
Ever had an abnormal Pap test				
No	10,101	8	1.0	–
Yes	8,695	9	1.3	(0.3, 5.7)
Ever been pregnant				
No	7,289	6	1.0	–
Yes	11,544	11	1.2	(0.3, 4.6)
Currently using hormonal contraceptives				
No	15,022	14	1.0	–
Yes	3,737	3	0.9	(0.0, 3.2)
Smoking status ^{**}				
Never	14,060	13	1.0	–
Former	3,811	4	1.1	(0.2, 3.8)
Current	851	0	0.0	(0.0, 5.1) [*]
Currently consumes alcoholic beverages				
No	3,108	1	1.0	–
Yes	15,651	16	3.2	(0.8, Inf)
Type of vaginal sex partners (lifetime)				
Male only	15,096	15	1.0	–
Female only or both male and female	3,515	2	0.6	(0.0, 2.1)
Age at first sexual intercourse with a male partner (yrs) ^{††}	18,204	16	0.9	(0.8, 1.0)
Lifetime number of male sex partners (tertiles)				
0 – 3	4,884	2	1.0	–
4 – 9	5,772	4	1.7	(0.0, Inf)
10+	7,807	11	3.4	(0.9, Inf)
Lifetime number of male or female open-mouth kissing partners (median split) ^{‡‡}				
0 – 15	7,437	2	1.0	–

<i>Characteristics</i>	Fingernail HPV detection ($N_{\text{Total}} = 18,833^{\dagger}$)			
	<i>N</i> [*]	<i>n</i> [§]	<i>OR</i>	<i>(Bootstrap 95% CI)</i>
16+	7,511	11	5.5	(0.3, Inf)
Lifetime number of male or female oral sex partners (median split) ^{‡‡}				
0 – 5	7,807	5	1.0	–
6+	7,289	8	1.7	(0.0, Inf)
Type of oral sex partners ^{‡‡§§}				
Male only	11,766	12	1.0	–
Female only or both male and female	2,738	1	0.4	(0.0, 1.7)
Vaginal HPV infection of the same type				
No	18,414	6	1.0	–
Yes	345	11	101.0	(31.4, 748.6)
Vaginal HPV viral load of the same type (continuous) ^{¶¶}	168	5	2.2	(1.5, 5.5)

* indicates 95% confidence interval generated using exact logistic regression due to 0 in cells; cannot account for correlation within women

[†] Both enrollment and exit samples were included. 348 women contributed a total of 18,833 women-types to the fingernail HPV analysis.

[‡] Indicates the total number of women multiplied by the number of HPV types evaluated per woman multiplied by the number of samples evaluated per woman.

[§] Indicates the number of women–types positive for fingernail HPV

[¶] Includes chlamydia, gonorrhea, genital herpes, and HIV

^{//} Includes birth control pills, hormonal patches, vaginal rings, implanted contraception, injectable contraception, and hormonal intrauterine devices

^{**} Smoking was defined as smoking at least one cigarette a day for one month or longer; former smokers reported ever smoking but not currently smoking, and current smokers reported currently smoking.

^{‡‡} Restricted to 337 women who reported ever having had sex with a male partner

^{‡‡} Restricted to 251 women who filled out either a modified exit survey or a supplemental exit survey with additional questions on open-mouth kissing and oral sex

^{§§} Twelve women who reported no history of oral sex were excluded.

^{¶¶} Restricted to 16 high-risk HPV types with available viral load results, including HPV 16, 18, 31, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73, and 82, and to samples with a vaginal infection of the same HPV type detected