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Oral Human Papillomavirus Detection in Older Adults Who Have Human Immunodeficiency Virus Infection

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Abstract

Objective—To evaluate reproducibility of oral rinse self-collection for HPV detection and investigate associations between oral HPV, oral lesions, immune and sociodemographic factors, we performed a cross-sectional study of older adults with HIV infection.

Study Design—We collected oral rinse samples from 52 subjects at two different times of day followed by an oral examination and interview. We identified HPV using PCR platforms optimized for detection of mucosal and cutaneous types.

Results—Eighty seven percent of individuals had oral HPV, of which 23% had oncogenic *alpha*, 40% had non-oncogenic *alpha*, and 46% had *beta* or *gamma* HPV. Paired oral specimens were concordant in all parameters tested. Significant associations observed for oral HPV with increased HIV viral load, hepatitis-C seropositivity, history of sexually transmitted diseases and lifetime number of sexual partners.

Conclusions—Oral cavity may be a reservoir of subclinical HPV in older adults who have HIV infection. Understanding natural history, transmission and potential implications of oral HPV warrants further investigations.

Keywords

human papillomas virus; HPV; human immunodeficiency virus; HIV; immunosuppression; oral lesions; PCR

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Introduction

Human papillomaviruses (HPV) are a group of heterogeneous double-stranded, circular DNA viruses with a predilection for squamous epithelium of the skin and mucosa.[1,2] Recent work suggest that as many as 30% of oral cancers are related to an HPV infection,[3] and like other HPV-associated tumors, rates of oral cancer are elevated (up to 6 fold) in human immunodeficiency virus (HIV) infected patients compared to the general population.[4,5] Studies also show HIV-infected individuals are more likely to harbor high-risk (oncogenic) HPV genotypes in their oral mucosa.[6-9] In view of the increased life-span of HIV patients on long-term highly active anti-retroviral therapy (HAART) and the possible etiological role of oral HPV in carcinogenesis, aging HIV/HPV co-infected patients may be at significant risk for HPV-associated oral lesions and malignancies.[10,11]

HPV detection rates in the oral cavity are reportedly higher among older individuals, men who have sex with men, and those with a prior history of sexually transmitted diseases (STDs).[6,8,12] The likelihood of oral HPV infection is also influenced by the severity of immunosuppression among HIV-positive individuals, especially with CD4 T-cell counts below 200 cells/mm.[3,8] To investigate the associations between HPV detection, oral lesions and immune-related factors in older adults, we performed a cross-sectional study of oral HPV infection in an HIV-seropositive population of adults 50 years and over in a large urban healthcare center.

Reported rates of oral HPV vary by methodologies to collect specimens and the sensitivity of assays used.[6-9, 13-16] This variability renders it difficult to compare available studies and contributes to conflicting data on the prevalence of HPV in normal oral mucosa as well as association of oral HPV infection with oral cancer.[14] HPV infects and replicates within basal epithelium, which is shed as part of normal mucosal turn over, and exfoliated cells of oral mucosa provide a representative sample for HPV-DNA detection.[14, 17] Daily oral activities can result in washout and lower recovery of oral exfoliated cells during sampling. The lack of oral functions (i.e., eating, drinking, brushing, etc.) and reduced salivary flow over night may lead to a higher yield of exfoliated cells for early morning sampling before resuming oral functions. We therefore also evaluated whether specimens obtained upon arising in the morning would provide a better sample for HPV-DNA detection by PCR. The polymerase chain reaction (PCR) is the most sensitive technique for detection of HPV DNA[18] and in the context of strict measures to prevent contamination, is highly applicable to prevalence studies of subclinical HPV infections.

Methods

Subject recruitment and sample collection

HIV-seropositive adults 50 years and over were recruited prospectively from the outpatient oral medicine clinic at New Jersey Dental School (Newark, NJ) between August 2007 and August 2008. A population of older, immunosuppressed patients was targeted due to the higher risk for HPV infection compared to general population. Pregnant females and individuals with other immunosuppressive conditions (i.e., concurrent chemotherapy, organ

transplantation, etc.) or history of treatment for head and neck cancer during the preceding year were excluded. The study protocol was approved by the Institutional Review Board at the participating institution. All subjects provided signed informed consent.

All participants received a self-collection kit including detailed written instructions and asked to provide an oral lavage sample upon waking up and before eating, drinking or brushing their teeth by swishing their mouth vigorously for 30 seconds with 10 ml of Scope ™ mouthwash and expectorating into an empty collection container. A second sample was collected in a similar manner in the afternoon of the same day by the study clinician (MF) prior to oral examination. Samples were stored in the refrigerator at 4°, and shipped by overnight mail within 24 hours of collection for processing at our lab.

Study interviews and oral exams

Each patient underwent extra- and intra-oral soft tissue assessment by the same clinician (MF) trained in oral medicine, who examined the oral mucosa for the presence of mucosal abnormalities. Participants also underwent a structured interview to collect socio-demographic information (age, gender, ethnicity, etc.), health behavior (tobacco, alcohol and drug consumption), and HIV-related medical history (mode of HIV acquisition, anti-retroviral therapy, compliance with HIV therapy, etc.). HAART was defined as a regimen that contained two nucleoside analogue reverse-transcriptase inhibitors and either a protease inhibitor or a non-nucleoside reverse-transcriptase inhibitor, or as regimen that combined three different classes of HIV medications. Smokers were defined as individuals with ≥1 pack-years of cigarette use, and alcohol users were defined as those who drank ≥1 alcoholic beverage per week for ≥1 years.

Participants also completed a self-administered questionnaire on sexual history, including questions on sexual orientation, age at first vaginal intercourse, lifetime number of intercourse partners, practice of oral sex, lifetime number of oral sex partners and in the preceding year, and history of STDs, anogenital lesions, and oral warts. Plasma $CD4^+$ T-cell count and HIV-RNA level measurements collected within the six months of the oral specimen were obtained from medical charts.

HPV DNA genotyping

Oral rinse specimens were processed in a room physically separated from where the PCR was performed. Genomic DNA was isolated by proteinase K digestion and phenol/chloroform extraction using a previously described protocol.[19] Purified DNA was amplified by MY09/MY11 and FAP59/64 L1 degenerate primer PCR systems optimized for detection of mucosal (*Alpha* species) and cutaneous (*Beta* and *Gamma* species) HPV, respectively, using Gold *Taq* Polymerase (Applied Biosystems, CA). Details of the procedures are previously described.[20-23] For both HPV PCR assays, a DNA fragment from the (β-globin gene was co-amplified during PCR to control for the presence of amplifiable genomic DNA in the specimens. For every 48 specimens tested, a negative (a 100-cell copy HUH-7 cell line DNA), a weakly positive (a 2-cell copy SiHa HPV DNA), and a strongly positive control (a 100-cell copy HUH-7 cell line DNA) were also included to monitor for amplification sensitivity. Negative control for processing contamination included water

blanks devoid of DNA template in each PCR run. All PCR assays were performed in an Applied Biosystems 9700 thermocycler. The MY09/11 PCR assays used the following thermocycling parameters: 95°C for 9 minutes, followed by 40 cycles of 95°C for 60 seconds, 55°C for 60 seconds, and 72°C for 60 seconds and then a final extension at 72°C for 5 minutes.

The PCR products were separated by gel electrophoresis and hybridized with generic HPV and β-globin probes. HPV positive samples by PCR were genotyped by dot-blot hybridization using biotinylated type-specific oligonucleotide probes.[19] Hybridization signals are recorded using a 1+ to 5+ scale for signal intensity. Strict measures to prevent cross-contamination and false positive reactions were followed. The laboratory personnel were blinded to clinical data and timing of oral samples.

MY09/11 and FAP59/64-PCR products that were positive by the generic radioactive probe mix but negative by all type-specific probes were considered to represent "uncharacterized" HPV types. PCR products with a strong HPV signal (i.e., 3+ on gel) that did not type by dot-blot were isolated using either gel purification (QIAquick Gel Extraction Kit, Qiagen, Valencia, CA) or column purification (QuickStep 2 PCR Purification Kit, Edge Biosystems) and directly sequenced. Upon completion, sequence results for all samples were inputted into a computer BLAST search against GenBank and a local database in order to identify uncharacterized HPVs or novel types.

Alpha HPV types were grouped by oncogenic potential: non-oncogenic HPVs included detected types 6/11, 32/42, 34, 43/91, 53, 62, 71, 72, 73, 81, 82, 83, 84, 86/87, 89/102, 90/106, 114, and other uncharacterized *alpha* types detected by the MY09/11 PCR primer system; and oncogenic *alpha* HPV types included: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58 59, 66 and 68, reported in recent reviews on cervical cancer.[24] Cutaneous HPVs included detected *beta* types: 5, 8, 12, 14D, 20, 24, 38b, 76, 98, 105, 110 and 124; and *gamma* types: 101/103, 121, 123, 135, 137/140 and FA1.[25-27]

Statistical analyses

Contingency tables were constructed for cross-sectional analyses. HPV detection rates in paired AM and PM samples were compared by McNemar test, and the *Kappa* statistic was used to assess agreement beyond chance for HPV detection by phylogenetic group and specific type. Individuals were considered HPV positive if at least one sample (AM or PM) contained HPV DNA. Subjects were considered HPV negative if both samples were negative. Associations between oral HPV detection and the various risk factors were estimated by Exact tests and odds ratios (ORs) with 95% confidence intervals (CI). Confounding by age, HIV viral load and immune status was assessed using a change in point estimate criterion.

Results

Fifty-two HIV-seropositive adults aged 50-69 (mean=56.3 ±4.2 years) were enrolled. The study participants were primarily male (71%) and African American (75%). Twenty four (46%) had a high school education or lower, and 36 (69%) were unemployed. Thirty-four

(65%) reported current substance abuse including tobacco, alcohol and illicit drugs (Table 1).

Oral HPV-DNA was detected in 45 (87%) subjects when morning and afternoon samples were combined. This reflects patients for whom one or both paired specimens were positive for typed or uncharacterized HPV DNA by either PCR system (MY09/11 or FAP59/64). Thirty-five individuals had a typable HPV infection (67%), of which 10 (8%) had one and 25 (71%) had multiple HPV types detected. Oncogenic *alpha* types were detected in 12 individuals (23%), of which HPV58 and 68 were the most frequently detected types, found in 8 (15%) and 5 (10%) subjects, respectively and in five (10%) subjects concurrently. Among non-oncogenic *alpha* types, HPV32/42 (8%) were the most commonly detected types. Cutaneous *beta* or *gamma* types were detected in 24 (46%) individuals, of which the most common types included *beta-1* type HPV8 (8%) and *gamma-1* types HPV101/103 (4%).

We first tested whether a specimen obtained upon arising in the morning would provide more exfoliated cells and a better substrate for HPV-DNA detection. The detection rate for HPV-DNA in oral rinse samples was 73% (n=38) and 75% (n=39) in samples collected in the morning and afternoon, respectively (Table 2). This difference was not statistically different (McNemar test p=0.782) and showed fair agreement overall (75%, kappa=0.35, 95%CI: 0.1-0.6). With respect to individual HPV types detected in AM and PM samples, there was good to excellent agreement between the most commonly detected oncogenic types 58 (94%, kappa=0.74) and 68 (98%, kappa=0.88), as well as moderate to good agreement for the vaccine types 6/11 (98%, kappa=0.79) and 16 (96%, kappa=0.49). There was also significant agreement for HPV16 phylogenetically-related (*alpha*-9, kappa=0.87) and HPV18-related types *(alpha*-7, kappa=0.70), as well as for cutaneous *beta*-1 (81%, kappa=0.46) and *gamma*-1 HPV types (92%, kappa=0.56).

Duration of HIV-disease among the study participants ranged from 1-27 years (mean=14.2 +5.4years). While most patients (94%) were on some form of antiretroviral therapy and reported full compliance, only a third (38%) were on HAART. HIV-RNA levels and CD4 T-cell count data were available for 50 and 46 patients, respectively. Seven (15%) had CD4 counts of ≤200 and 29 (74%) had undetectable HIV-RNA levels (i.e., <400 copies/ml). All cases with detectable HIV viral-load were positive for HPV and showed a non-significant dose-response relationship with increasing HIV viral-load (Wilcoxon rank-sum test p=0.131). Detection of non-oncogenic *alpha* and *beta* or *gamma* HPV was significantly higher among patients with detectable levels of HIV-RNA compared to patients with undetectable levels, but not for oncogenic *alpha* HPV (Table 3). Patients on HAART were also more likely to test positive for *beta* or *gamma* HPV types.

Forty-three (83%) subjects reported sexual exposure of any type (33 heterosexual, 5 homosexual and 5 bisexual) as the possible mode of HIV acquisition, and 41 (79%) reported a positive history of one or more STDs. In addition, 37 subjects (71%) were seropositive for hepatitis-B or C, of whom 10 (19%) were seropositive for both. Detection of oncogenic and non-oncogenic *alpha* HPV was significantly associated with the hepatitis-C seropositivity. Significant associations were also observed with history of other STDs (like Gonorrhea) for

all HPV, and non-oncogenic *alpha* and cutaneous *beta* or *gamma* types specifically. In contrast, we detected a significant positive association between any oral HPV detection and lifetime number of intercourse partners, but not with lifetime number of oral sex partners or recent oral sex (Table 3).

We also assessed whether associations persisted among males alone, which represented the majority of patients in our study (71%). Males had similar sociodemographic characteristics as females but differed with respect to history of Gonorrhea (males 62% vs. females 33%, respectively; Fisher's exact p=0.07), same sex oral sex (33% vs. 0%, p=0.02), ≥10 lifetime number of intercourse partners (92% vs. 40%, p<0.001), and ≥3 oral sex partners (65% vs. 7%, p<0.001). No significant associations were observed between these characteristics and oral HPV detection among males, nor did we observe a significant difference between males who reported having sex with men and heterosexual only men (90% vs. 92%, respectively, p=1.0). Significant associations were observed between HIV viral-load and detection of non-oncogenic *alpha* HPV (OR=6.0, 95%CI: 1.0-34.3) and *beta* or *gamma* HPV (OR=6.0, 95%CI: 1.0-34.3), as well as between hepatitis-C seropositivity and oncogenic *alpha* HPV (OR=15.3, 95%CI: 1.7-139.3).

On intraoral examination, nine patients (17%) had missing tonsils, and 15 (29%) had oral lesions including, candida-associated lesions (15%, n=8), intraoral ulcerations (12%, n=6), leukoplakia (4%, n=2), and oral hairy leukoplakia (OHL; 2%). Other oral abnormalities included coated tongue (13%), angular cheilitis (10%), intraoral herpes (2%), and linear gingival erythema (2%). There were no statistically significant associations between oral HPV detection and presence of leukoplakia, not including OHL (100% in patients with leukoplakia vs. 89% in patients without lesions, Fisher exact test p=0.803), any form of oral candidiasis (88% vs. 89%, p=1.000), or ulcerative lesions combined (83% vs. 89%, p=0.547). No significant HPV type-specific associations were observed with presence of concurrent oral lesions, although *alpha*-7, 1/8, 9 and 10 HPV types were detected more often in patients with oral candidiasis, while patients with leukoplakia were more likely to present with *beta*-1 types (Table 4). The detection of oncogenic *alpha* HPV and non-oncogenic *alpha*-10 types (HPV6/11) was also higher among subjects with a history of genital warts compared to those without (38% vs. 20%, p=0.366 and 13% vs. 5%, p=0.401, respectively), whereas subjects with a history of cutaneous warts were more likely to harbor *beta* or *gamma* HPV (37% vs. 43%, p=0.397).

Discussion

Reported prevalence rates of oral HPV in non-cancer patients vary widely. We observed a high prevalence (87%) of oral HPV in this population of older HIV-positive subjects reflecting a diverse distribution of *alpha, beta* and *gamma* HPV types. Furthermore, we detected varying associations with immune status markers, sexual behaviors and oral abnormalities for different HPV types grouped by phylogenetic relatedness and oncogenic potential.

The overall HPV DNA detection rate of 87% in our study is higher than the 14%-52% range reported in other studies of HIV positive subjects.[6-9, 13,14.28,29] Our higher rate of detection

may be attributed in part to differences in sampling methods; whereas oral brushings collect cells from specific anatomical area, oral rinses sample exfoliated cells from the entire oral cavity. The latter is a validated, non-invasive method for sampling the oral cavity for exfoliated mucosal cells for HPV detection.[14, 17] Another difference is the use of a single assay versus a combination of PCR systems optimized for detection of a broad spectrum of HPV types, including both mucosal and cutaneous, as done in our study. HPV DNA (including uncharacterized types) was detected in 20 subjects by MY09/11 (detection rate of 38%) and in 44 subjects by FAP56/64 PCR (detection rate of 85%).

Mucosal HPV infection rate of 38% in our study is between 3 and 4 times higher than 13.5% and 10% rates reported in the two retrospective analyses of published data on subclinical HPV detection in normal mucosa.[15, 16] Our higher detection rate may be explained by the characteristics of our cohort. The key contrast between our investigation and the studies included in these reviews is our focus on HIV seropositive subjects who are known to have higher rate of oral HPV carriage. The detection rate of 38% for mucosal HPV is well within the range of 14%-52% reported in studies of HIV positive subjects using assays optimized for detection of alpha species only.[6-9, 13, 14, 28, 29] Given the compelling role for HPV in dysplastic progression of oral disease suggested by the two reviews, the high prevalence of oncogenic HPV types detected in our study of older HIV-positive adults may pose potential implications for cancer risk in HIV/HPV co-infected patients as they survive longer on HAART.

Whereas primers included in the MY09/11-PCR system are designed to detect *alpha* HPV types, this may miss subjects harboring *beta* or *gamma* HPV type infections targeted by the FAP59/64-PCR system also used in this study. The combination of two PCR platforms not only improves sensitivity by allowing an exhaustive search for overlapping HPV types tested for by both assays, but also expands the range of detection allowing identification of new types not identified by the other assay alone. This renders our detection data more representative of overall oral HPV burden by including types with predilection to infect skin, mucosa or both.

A total of 146 type-specific infections were identified in 52 paired oral rinse samples collected in the morning and afternoon of the same day and to our knowledge, this is the first study to systematically assess the effect on HPV-detection of sampling in the morning versus the afternoon. Whereas discordant results would reflect differential epithelial shedding, our results indicate that early morning sampling is not significantly better in providing a substrate for HPV detection. Testing for agreement in HPV infection by type and species groups, however, did suggest mucosal (*alpha*) HPV types were more likely to 'persist' than cutaneous (*beta* or *gamma*) HPV types. This may represent a stronger propensity for *alpha* HPV types to infect and replicate within basal epithelium compared to cutaneous HPV which may more readily shed as part of normal mucosal turn over.

HIV-infected individuals are more likely to harbor high risk, multiple genotypes in their oral mucosa.[7,8,11] and the increased risk of tonsillar and oropharyngeal carcinomas observed among HIV-positive patients[4, 5, 30] may be attributed to the higher incidence of oral HPV infections in these anatomical sites with frequent high risk sexual exposures.[6,12] With

respect to high-risk types included in the recently released prophylactic HPV vaccines (HPV16 and 18), 3 patients (6%) were positive for HPV 16 and 1 (2%) for HPV 18 by PCR. The detection rate of HPV 16 in our study compares well with the 6% prevalence reported for HIV positive cohorts in other studies.[13, 29, 31]

The biological basis for high prevalence and broad genotype distribution of HPV in the oral are not fully understood and potential repercussions of these infections are yet to be determined. The high rate of consular HPV infection in the upper aero-digestive tract is attributed to the exposure of basal cells to HPV within the tonsillar crypts.[32] In a molecular-based study, high-risk HPV was present in 26% of gingival biopsies from patients with chronic periodontitis and localized to the superior component of the junctional epithelium in the gingival pocket.[33] Chronic periodontitis was also an independent risk factor for head and neck squamous cell carcinoma in a retrospective case-control study.[34] In addition, associations between history of periodontal disease and HPV positive head and neck cancer [35] as well as periodontitis and oral leukoplakia mediated through local inflammatory processes have recently been suggested.[36] We observed no statistically significant associations between evidence of concurrent periodontitis and oral HPV detection, although the direction of association differed for mucosal oncogenic (OR=1.4, 95%CI: 0.3-5.9) and non-oncogenic types (OR=2.0, 95%CI:0.6-6.5) compared to cutaneous types (OR=0.3, 95% CI:0.1-1.1). It has been speculated that chronic local inflammation may facilitate HPV entry and persistence within the basal epithelium of periodontium[34] - the only mucosal site where target cells for HPV infection are exposed to the oral environment.[33] Such novel concepts, if confirmed, could have potentially significant preventive and therapeutic implications.

Although the underlying mechanism is not clear, local and/or systemic immunosuppression may promote reactivation of latent and/or acquisition of new HPV infections.[6, 7, 11, 13] The failure of immune system to clear an HPV infection may also lead to progression of genetic alterations conducive to cancer.[37] In our study, only six patients had CD4 counts <200 cells/mm^3 consistent with AIDS. This may explain the lack of statistically significant difference between detection of oral HPV and immune status. An association between HIV viral-load and oral HPV, possibly manifested through attenuation of local immunity, has also been suggested.[13,14, 29] All cases with detectable HIV viral-load in our study tested positive for HPV in the oral cavity. Our group has previously reported significant associations between incident cervical HPV infection and combined CD4 count/HIV viral-load in HIV-positive females.[38] Such categorizations may be necessary for understanding the interaction of CD4 count/HIV viral-load on oral HPV infection. However, a larger number of subjects than those participating in our pilot project would be needed. Nonetheless, HIV viral-load was the single strongest factor for HPV overall and for cutaneous types. In contrast, detection of mucosal types was more closely associated with history of STDs and sexual activity.

Previous studies have postulated that smoking may lead to impaired mucosal immunity and promote HPV infection in the oral cavity.[8, 12, 39] We did not detect a significant association with oral HPV detection and smoking or substance abuse in this HIV-infected population. However, detection of oral HPV was associated with hepatitis-C or B seropositivity, which may be a marker of drug exposure as intravenous drug use is a well-known risk factor for

hepatitis-C transmission. An increased, albeit non-significant, increase in overall HPV was detected among subjects that reported acquiring HIV through intravenous drug use (OR=1.94, 95%CI: 0.2-17.9).

Sexual contact has been proposed as the potential route for transmission of HPV to the oral cavity, with studies reporting an association between oral HPV and earlier age at first intercourse, increase in lifetime number of sexual partners,[12,39] number of recent oral sex encounters,[12] and open-mouth kissing.[12,40] While we detected higher prevalence of oncogenic and non-oncogenic *alpha* oral HPV among sexually active subjects (engaging in both intercourse and oral sex), this was not observed for cutaneous HPV types.

With the exception of education, our study matches the profile of the surrounding urban population. While a higher educational background may render reporting error less likely, faulty recall may still contribute to misclassification of information on sexual activity. Subject misclassification may also result from confounding by unmeasured sexual behavior left out from our questionnaire. More specifically, we did not collect information on the use of barrier contraception during oro-genital sex (cunnilingus and fellatio) as well as open-mouth kissing, both of which could impact the observed associations between markers of sexual activity and oral HPV infection.

In our study, only two patients had leukoplakia on clinical examination. Some have advocated the use of a colposcope during oral examination to detect subclinical HPV infected mucosa and improve diagnostic accuracy[40] Although this device may have a role as a diagnostic adjunct in detection of HPV-infected oral lesions, no guidelines regarding its use in the oral cavity has yet been established. No significant associations were found with current oral lesion status, although we did observe suggestive associations with history of cutaneous and genital warts that support the etiologic propensities of mucosal and cutaneous HPV. Whereas mucosal HPV were positively associated with history of genital warts, *beta* and *gamma* HPV types appeared to be specifically associated with cutaneous warts. However, low-risk *alpha* types HPV6/11, known to manifest into mucocutaneous warts, were detected only in three (6%) subjects, none of whom had genital warts.

In spite of the small sample size and limited power, we identified several statistically significant associations with HIV viral load, Hepatitis-C seropositivity, history of STDs, and lifetime number of sexual partners (including intercourse and oral sex partners). However, the cross-sectional study design and lack of specific information on timing of proposed exposures did not allow us establish a temporal relationship or causality. The relative associations between oral HPV detection and the various risk characteristics were also assessed using multivariable logistic regression adjusting for age and for HIV viral load or CD4 count, but showed little statistical difference using the change in estimate criteria proposed by Greenland & Robins.[41] Due to the small sample size, we were not able to test for effect modification or combined effects of multiple covariates. This study was designed to examine the reproducibility of early morning oral rinse self-sampling for oral HPV detection and to describe putative risk factor associations with oral HPV detection in an HIV seropositive population warranting further investigations. The selective nature of our study population impacts generalizability of our findings such that data on oral HPV prevalence as

well as identified associations between oral HPV status and selected variables are only applicable to older, urban, HIV positive cohorts. The information gleaned from the analysis of HPV concordance between paired oral rinse samples is; however, relevant to any PCR-based investigation using oral lavage to sample exfoliated cells for HPV detection.

Conclusion

In this study, we observed a high prevalence of oral HPV in older HIV-positive subjects including HPV types previously associated with skin conditions, as well as mucosal types with known associations to cancer. The high burden of HPV and the broad spectrum of types detected suggest that oral cavity, in the context of aging and immunosuppression may be a reservoir of subclinical HPV infection and pose potential implications for HIV/HPV co-infected patients whose life expectancy has improved dramatically with HAART. Paired oral specimens were equivalent in all parameters tested suggesting that self-collected oral rinse sampling may be a reproducible, convenient method for monitoring oral HPV infection in high risk patients. We observed significant associations between oral HPV and increased HIV viral-load, hepatitis-C seropositivity, history of STDs, and lifetime number of sexual partners. While this study also suggests associations may exist between oral HPV and different types of oral lesions and warts detected in HIV positive patients, these remain to be confirmed. Future studies are warranted to clarify mechanism of HPV spread to the oral cavity, risk factors for its acquisition and persistence as well as the potential role of different HPV types in oral disease.

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Statement of clinical relevance

In the context of aging and immunosuppression, the oral cavity may be a reservoir of subclinical HPV infection which may be reliably and conveniently monitored by self-collected oral rinse sampling.

Table 1
Demographic, immune and sexual behavior characteristics of study sample

Demographic characteristics Age	N (%)
Age	
<56 years	25 (48%)
56+ years	27 (52%)
Gender	
Females	15 (29%)
Males	37 (71%)
Race/Ethnicity	
African-American	39 (75%)
White/Hispanic	13 (25%)
Education	
≤Grade 12	24 (46%)
>Grade 12	28 (54%)
Current employment	
Unemployed	36 (69%)
Employed/retired	16 (31%)
Substance abuse	
No	18 (35%)
Yes	34 (65%)
Current smoker	
No	32 (61%)
Yes	20 (39%)
HIV/AIDS markers	
CD4 count (cells/mm^3)[b]	
>500	20 (44%)
≤500	26 (56%)
HIV Viral load (copies/mL)[b]	
Undetectable	37 (74%)
Detectable	13 (26%)
HAART[a]	
No	29 (56%)
Yes	20 (38%)
Other infections	
History of Sexually transmitted diseases[c]	
Chlamydia	8 (15%)
Gonorrhea	28 (54%)
Genital herpes	21 (40%)

Demographic characteristics Age	N (%)
Syphilis	15 (29%)
Blood borne infections[c]	
Hepatitis-B	23 (44%)
Hepatitis-C	24 (46%)
Sexual Activity	
Age of first intercourse	
16+ years	28 (54%)
<16 years	24 (46%)
Lifetime # of intercourse partners	
<10	12 (23%)
10+	40 (77%)
Lifetime # of oral sex partners	
≤2	27 (52%)
3+	25 (48%)
Oral sex in last year	
No/Never	35 (67%)
Yes	17 (33%)
Same sex oral sex	
No	34 (76%)
Yes	11 (24%)

[a] Row numbers may not sum to 52 due to missing data or overlapping categories.

[b] Restricted to test results collected within 6 months of HPV sample.

Table 2
Detection rate of HPV by phylogenetic relatedness and oral sampling method

HPV by species group and oncogenic potential	Self Sample N (%)[a]	Clinic Sample N (%)[a]	Overall N (%)[a]	Kappa (95%CI)[b]
***Alpha* species**				
α10 (*HPV6/11 and related types*)	3 (6%)	2 (4%)	3 (6%)	0.79 (0.4-1.0)
HPV6/11 alone	3 (6%)	2 (4%)	3 (6%)	0.79 (0.4-1.0)
α9 (*HPV16 and related types*)	10 (19%)	8 (15%)	10 (19%)	0.87 (0.7-1.0)
HPV16 alone	1 (2%)	3 (6%)	3 (6%)	0.49 (-0.1-1.0)
α11	2 (4%)	1 (2%)	3 (6%)	-0.03 (-0.08-0.03)
α7 (*HPV18 and related types*)	6 (12%)	5 (10%)	7 (14%)	0.70 (0.4-1.0)
HPV18 alone	0 (0%)	1 (2%)	1 (2%)	0.00 (NA)[c]
α5/α6	3 (6%)	5 (10%)	6 (12%)	0.46 (0.02-0.9)
α15/α3/α2	11 (21%)	11 (21%)	13 (25%)	0.77 (0.6-1.0)
α1/α8	5 (10%)	5 (10%)	7 (14%)	0.56 (0.2-0.9)
HPV32/42 alone	4 (8%)	2 (4%)	4 (8%)	0.65 (0.2-1.0)
***Beta* species**				
β1	13 (25%)	11 (21%)	17 (33%)	0.46 (0.2-0.7)
β2	3 (6%)	2 (4%)	5 (10%)	0.29 (-0.2-0.8)
β6	0 (0%)	1 (2%)	1 (2%)	0.00 (NA)[c]
***Gamma* species**				
γ1	5 (10%)	5 (10%)	7 (14%)	0.56 (0.2-0.9)
By tropism and oncogenic status				
Oncogenic *alpha* types	12 (23%)	8 (15%)	12 (23%)	0.76 (0.5-1.0)
Non-oncogenic *alpha* types	17 (33%)	18 (35%)	21 (40%)	0.70 (0.5-0.9)
Cutaneous (*beta* & *gamma*) types	19 (37%)	17 (33%)	24 (46%)	0.49 (0.2-0.7)
All HPV (characterized types)[d]	31 (60%)	28 (54%)	35 (67%)	0.57 (0.3-0.8)
All HPV (total)	**38 (73%)**	**39 (75%)**	**45 (87%)**	**0.35 (0.1-0.6)**

[a] Proportion (%) of subjects found to be HPV-positive.

[b] Kappa statistic and 95% confidence intervals (CI).

[c] 95%CI were not approximated (NA) due to small number.

[d] Includes only confirmed types characterized by dot-blot hybridization or sequencing.

Table 3

Significant and putative associations between oral HPV detection and demographic, immune and sexual behavior

Characteristics	All HPV combined		Oncogenic *alpha* HPV		Non-oncogenic *alpha* HPV		*Beta & Gamma* HPV	
	(%)[a]	OR (95%CI)[b]	(%)[a]	OR (95%CI)[b]	(%)[a]	OR (95%CI)[b]	(%)[a]	OR (95%CI)[b]
Age								
<56 years	(80%)	(ref)	(28%)	(ref)	(48%)	(ref)	(36%)	(ref)
56+ years	(93%)	3.1 (0.5-17.8)	(19%)	0.6 (0.2-2.2)	(33%)	0.5 (0.2-1.7)	(56%)	2.2 (0.7-6.8)
Gender								
Females	(73%)	(ref)	(13%)	(ref)	**(20%)**	**(ref)**	(40%)	(ref)
Males	(92%)	4.1 (0.8-21.3)	(27%)	2.4 (0.5-12.6)	**(49%)**	**3.8 (0.9-15.7)**	(49%)	1.4 (0.4-4.8)
Current smoker								
No	(84%)	(ref)	(19%)	(ref)	(31%)	(ref)	(47%)	(ref)
Yes	(90%)	1.7 (0.3-9.5)	(30%)	1.9 (0.5-6.8)	(55%)	2.7 (0.8-8.5)	(45%)	0.9 (0.3-2.8)
CD4 count (cells/mm^3)[c]								
>500	(90%)	(ref)	(20%)	(ref)	(40%)	(ref)	(35%)	(ref)
≤500	(85%)	0.6 (0.1-3.7)	(27%)	1.5 (0.4-6.0)	(42%)	1.1 (0.3-3.6)	(58%)	2.5 (0.8-8.4)
HIV Viral load (copies/mL)[c]								
Undetectable	(84%)	(ref)	(24%)	(ref)	**(32%)**	**(ref)**	**(38%)**	**(ref)**
Detectable	(100%)	3.1 (0.3-72.6)[d]	(23%)	0.9 (0.2-4.2)	**(69%)**	**4.7 (1.2-18.3)**	**(77%)**	**5.5 (1.3-23.4)**
HAART								
No	(90%)	(ref)	(21%)	(ref)	(45%)	(ref)	**(34%)**	**(ref)**
Yes	(85%)	0.7 (0.1-3.6)	(20%)	1.0 (0.2-4.0)	(30%)i	0.5 (0.2-1.8)	**(65%)**	**3.5 (1.1-11.7)**
Hepatitis-B								
Negative	(83%)	(ref)	(31%)	(ref)	(34%)	(ref)	(38%)	(ref)
Positive	(91%)	2.2 (0.4-12.5)	(13%)	0.3 (0.1-1.4)	(48%)	1.7 (0.6-5.3)	(57%)	2.1 (0.7-6.5)
Hepatitis-C								
Negative	(79%)	(ref)	**(7%)**	**(ref)**	**(29%)**	**(ref)**	(36%)	(ref)
Positive	(96%)	6.3 (0.7-56.4)	**(42%)**	**9.3 (1.8-48.4)**	**(54%)**	**3.0 (0.9-9.3)**	(58%)	2.5 (0.8-7.7)
History of any STD								

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Characteristics	All HPV combined		Oncogenic *alpha* HPV		Non-oncogenic *alpha* HPV		*Beta* & *Gamma* HPV	
	(%)[a]	OR (95%CI)[b]	(%)[a]	OR (95%CI)[b]	(%)[a]	OR (95%CI)[b]	(%)[a]	OR (95%CI)[b]
No	**(64%)**	**(ref)**	(9%)	(ref)	**(9%)**	**(ref)**	**(18%)**	**(ref)**
Yes	**(93%)**	**7.2 (1.3-39.6)**	(27%)	3.7 (0.4-32.1)	**(49%)**	**9.5 (1.1-81.3)**	**(54%)**	**5.2 (1.0-27.1)**
History of Chlamydia								
No	(89%)	(ref)	(20%)	(ref)	(41%)	(ref)	(45%)	(ref)
Yes	(75%)	0.4 (0.1-2.4)	(38%)	2.3 (0.5-11.6)	(38%)	0.9 (0.2-4.1)	(50%)	1.2 (0.3-5.4)
History of Gonorrhea								
No	(71%)	(ref)	(21%)	(ref)	**(25%)**	**(ref)**	(33%)	(ref)
Yes	(100%)	12.9 (1.4-298.4)[d]	(25%)	1.3 (0.3-4.7)	**(54%)**	**3.5 (1.1-11.3)**	(57%)	2.7 (0.9-8.3)
Age of first sexual intercourse								
16+ years	(79%)	(ref)	(14%)	(ref)	(32%)	(ref)	(54%)	(ref)
<16 years	(96%)	6.3 (0.7-56.4)	(33%)	3.0 (0.8-11.7)	(50%)	2.1 (0.7-6.5)	(38%)	0.5 (0.2-1.6)
Lifetime # of intercourse partners								
<10	**(67%)**	**(ref)**	(17%)	(ref)	(33%)	(ref)	(42%)	(ref)
10+	**(93%)**	**6.2 (1.1-33.1)**	(25%)	1.7 (0.3-8.9)	(43%)	1.5 (0.4-5.7)	(48%)	1.3 (0.3-4.7)
Lifetime # of oral sex partners								
<2	(81%)	(ref)	(15%)	(ref)	(33%)	(ref)	(52%)	(ref)
3+	(92%)	1.4 (0.8-2.5)	(32%)	1.4 (0.9-2.2)	(48%)	1.2 (0.8-1.8)	(40%)	0.9 (0.6-1.2)
Oral sex in last year								
No/Never	(86%)	(ref)	(20%)	(ref)	(43%)	(ref)	(49%)	(ref)
Yes	(88%)1	1.3 (0.2-7.2)	(29%)	1.7 (0.4-6.3)	(35%)	0.7 (0.2-2.4)	(41%)	0.7 (0.2-2.4)

[a] Proportion (%) of subjects found to be HPV-positive.

[b] Odds ratio (OR) and 95% confidence intervals (CI) compared to reference (ref) category.

[c] Restricted to test results collected within 6 months of HPV sample.

[d] OR and 95%CI approximated with continuity adjustment (n+1).

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Table 4

Detection rate of HPV by phylogenetic relatedness among patients with oral lesions[a]

HPV by species group and oncogenic potential	No lesions (n=37)	Oral candidiasis (n=8)	Ulcers (n=6)	Leukoplakia (n=2)	Oral lesions (n=15)
***Alpha* species**					
α10 (*HPV6/11 and related types*)	1 (3%)	**2 (25%)**	0 (0%)	0 (0%)	2 (13%)
HPV6/11 alone	1 (3%)	**2 (25%)**	0 (0%)	0 (0%)	2 (13%)
α9 (*HPV16 and related types*)	7 (19%)	**2 (25%)**	1 (17%)	0 (0%)	3 (20%)
HPV16 alone	3 (8%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
α11	1 (3%)	1 (13%)	0 (0%)	1 (50%)	2 (13%)
α7 (*HPV18 and related types*)	4 (11%)	**2 (25%)**	1 (17%)	0 (0%)	3 (20%)
HPV18 alone	1 (3%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
α5/α6	3 (8%)	1 (13%)	**2 (33%)**	1 (50%)	3 (20%)
α15/α3/α2	8 (22%)	3 (38%)	**3 (50%)**	1 (50%)	5 (33%)
α1/α8	4 (11%)	**2 (25%)**	1 (17%)	0 (0%)	3 (20%)
HPV32/42 alone	3 (8%)	1 (13%)	0 (0%)	0 (0%)	1 (13%)
***Beta* species**					
β1	10 (27%)	3 (38%)	3 (50%)	2 (100%)	7 (47%)
β2	3 (8%)	1 (13%)	**2 (33%)**	0 (0%)	2 (13%)
β6	1 (3%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
***Gamma* species**					
γ1	6 (16%)	1 (13%)	0 (0%)	0 (0%)	1 (7%)
By tropism and oncogenic status					
Oncogenic *alpha* types	8 (22%)	**3 (38%)**	1 (17%)	0 (0%)	4 (27%)
Non-oncogenic *alpha* types	14 (38%)	**5 (63%)**	3 (50%)	1 (50%)	7 (47%)
Cutaneous (*beta* & *gamma*) types	15 (41%)	5 (63%)	**4 (67%)**	2 (100%)	9 (60%)
All HPV (characterized types)[b]	24 (65%)	6 (75%)	**5 (83%)**	2 (100%)	11 (73%)
All HPV (total)	**33 (89%)**	7 (88%)	5 (83%)	2 (100%)	12 (80%)

[a] Number of proportion (%) of patients with oral lesion found to be HPV-positive are shown.

[b] Includes only confirmed types characterized by dot-blot hybridization or sequencing.