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Exploratory study of an oral screening dysplasia program for HIV-infected men who have sex with men

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Background: HIV-infected men who have sex with men (MSM) are at high risk to develop human papilloma virus (HPV)-related oropharyngeal cancer. The aim of our study was to assess the usefulness of a pilot oral dysplasia screening program and its correlation with an anal dysplasia screening program.

Methods: This was a prospective study with HIV-infected MSM. Oral and anal screenings were performed based on HPV determination, liquid cytology, direct and microscopy oral examinations, high-resolution anoscopy and biopsies, if necessary.

Results: A total of 103 patients were included. The mean age of the patients was 44.6 years, 55.3% were smokers, and 57.3% had a history of previous anal high-grade squamous intraepithelial lesions (HSILs). The prevalence of oral HPV infections was 14% (9% HPV-high risk), the prevalence of abnormal cytology was 25.2%, and in 4.8% of the patients, oral examinations showed suspicious HSILs. Oral microscopy did not detect additional lesions that visual inspection. Five oral biopsies were performed and the results were normal. No risk factors for oral HPV infections were identified. The prevalence of anal HPV infections was 88.3% (76.7% HPV-high risk), 52.9% of the patients had altered cytology, and in 45.6% anoscopy showed changes suggestive of HSILs. Seventy-two anal biopsies were performed, detecting 25 cases of HSILs (24.3%).

A poor correlation was observed between oral and anal HPV infections ($\kappa = 0.037$).

Conclusions: The prevalence of oral HPV infections, abnormal cytology and lesions in HIV-infected MSM was low, and their correlation with anal HPV-related lesions was slight. These results confirm the current barriers to oral dysplasia screening techniques.

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Introduction

Infection by oncogenic human papilloma virus (HPV) is the cause of 96% of cervical cancers, 93% of anal cancers, and 63% of oropharyngeal cancers [1]. An HPV infection is able to induce a series of changes that lead to the development of squamous intraepithelial lesions (SILs), which may spread and evolve to invasive cancer [2]. Although the incidences of these HPV-associated neoplasms are high in the overall population, they are of particular concern in people with HIV (PWH) infections. In this high-risk population, the incidence of HPV cancer is 10 times higher than that in the general population and currently represents one of the most frequently identified neoplasms [3,4]. Screening strategies based on the detection of these SILs in the cervix by cytology, HPV determination and colposcopy and early treatment have represented one of the main prevention strategies and have resulted in a significant decline in the incidence of invasive cervical cancer.

Although there are no universal recommendations for the prevention of anal cancer, the strategy of anal dysplasia screening in high-risk populations, such HIV-infected men who have sex with men (MSM), is increasingly widespread. This screening program is based on anal cytology and high-resolution anoscopy with biopsies of suspected lesions, analogous to established cervical screening, for the early diagnosis and treatment of premalignant lesions [5].

In the case of oral and oropharyngeal cancers, and despite the similarities to cervical and anal cancers and the increasing incidence in PWH, no screening strategies have been explored. In fact, scarce data are available about the prevalence and predictors of oral HPV infections and related lesions and their correlations with anal HPV infections in this at-risk group. Moreover, the usefulness of oral cytology and oroscopy to detect oral cancer precursors is unknown [6].

The aim of our study was to describe the prevalence and predictors of oral HPV infections and the prevalence of oral premalignant lesions and their correlations with anal HPV infections and anal lesions in a cohort of HIV-infected MSM who were attended in an anal dysplasia screening program. We also explored the usefulness of a pilot oral screening program based on oral cytology, HPV detection and direct oral microscopy to identify oral HPV-related lesions in HIV-infected MSM.

Methods

Population

The Screening and Treatment of Anal Dysplasia Unit of the University Hospital Vall d'Hebron (Barcelona, Spain) was created in May 2009 and has attended more than 1000 patients. MSM with HIV infections who were treated in the Anal Dysplasia Unit were informed about the pilot oral screening program, and participation was offered. Patients were consecutively included. All included patients provided informed consent for the use of information available in the database and their medical records. The study was approved by the Commission of Medical Ethics of Hospital Vall d'Hebron (PR(AG)433/2018).

Study variables and data collection

From each patient, the following variables were recorded: sociodemographic data (age, current tobacco smoker, alcohol consumption, country of origin); HIV infection-related data (date of HIV diagnosis, AIDS-defining illnesses, hepatitis C virus antibodies, nadir and current $CD4^+$ cell count, zenith and actual HIV viral load, antiretroviral treatment (ART), and time on ART); sexual behavior data (age at which the individuals became sexually active, number of sexual partners in their lifetime, stable sexual partners, use of condoms for anal intercourse, and history of sexually transmitted diseases); and results of the anal and oral screening programs (oral and anal symptoms, oral and anal cytology, oral and anal HPV detection, high-resolution anoscopy, direct oral microscopy and histology results if biopsies were performed).

Anal dysplasia screening procedure

The anal and oral screening procedures were performed at the same visit. Anal dysplasia screening included anal cytology with HPV detection followed by digital anorectal examination and high-resolution anoscopy (HRA), as previously described [7,8].

Oral dysplasia screening procedure

Our pilot oral dysplasia screening program included an exploration of the oral cavity (direct oroscopy and microscopy), oral liquid cytology, HPV testing and oral biopsies, if necessary. All procedures were performed at the same visit and by a trained otolaryngologist.

In the first step, each patient underwent oral and oropharyngeal examinations. Visual inspection was undertaken in bright daylight and with the additional use of a flashlight. A tongue depressor was used for correct exploration. The labial and buccal mucosa, gingiva, tongue, floor of the mouth, hard palate, soft palate, retromolar area, anterior tonsil pillar (palatoglossal muscle), posterior tonsil pillar (palatopharyngeal muscle), uvula, posterior pharyngeal wall and palatine tonsils were carefully inspected. The findings were recorded as normal, benign lesions, oral precancerous lesions (homogeneous leucoplakia, nonhomogeneous leucoplakia, oral submucous fibrosis, erythroplasia, and erythroleukoplakia) [9], or invasive cancer. The presence, type, and location of each oral lesion were recorded.

In the second step, an oral examination was carried out by direct oral microcopy (DOM), with the oral application of the colposcopy technique used in gynecology. A stereo zoom binocular microscope and magnifications of 10×, 15× and 30× were used to explore the oral cavity. The topical application of acetic acid and Lugol solution on the oral and oropharyngeal mucosa was performed to improve the visualization of possible lesions. All lesions with abnormal tinctorial characteristics (acetowhite or Lugol negative) and/or an atypical vascular profile (punctuation, mosaicism and atypical vessels) were recorded.

To collect cytological samples, soft scraping of the oral cavity and oropharynx (with special attention to the tonsils) was initially carried out using a cotton swab. Subsequently, the used swab was introduced into 20 ml of PreservCyt/ThinPrep maintenance solution (Cytyc Iberia SL, Barcelona, Spain) and shaken for 60 s. Next, the participants rinsed and gargled with 10–15 ml of Listerine mouthwash (active ingredients: eucalyptol 0.092%, menthol 0.042%, methyl salicylate 0.060%, thymol 0.064%) for a total time of 30 s [10]. The rinses were mixed with the same 20 ml PreservCyt solution medium in which the swab had been shaken.

These samples were used to perform cytology analysis and HPV testing. The cytology results were classified as normal, atypical squamous cells of uncertain significance (ASCUS), atypical squamous cells-cannot exclude HSILs (ASC-H), low-grade squamous intraepithelial lesions (LSILs), high-grade squamous intraepithelial lesions (HSILs), or squamous cell carcinoma (SCC), in accordance with the Bethesda classification [11].

For HPV detection, DNA was extracted from the cell suspensions using the 'QIAamp Viral DNA minikit' (QIAGEN, Hilden, Germany). The DNA quality was tested for amplification of the actin gene, and specific sequences of papillomavirus were amplified by the specific 'CLART Genomic HPV-4' protocol in accordance with the manufacturer's instructions. The kit enables the detection of 35 HPV types (6, 11, 16, 18, 26, 31, 33, 35, 39, 40, 42, 44, 45, 51, 52, 53, 54, 56, 58, 59, 61, 62, 66, 68, 68, 70, 71, 72, 73, 81, 82, 83, 84, 85, and 89). Oncogenic HPV (HR-HPV) testing results were considered positive if some of the HPV genotypes associated with the highest risk of malignancy were detected (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, and 82); other results were considered as low-risk HPV (LR-HPV).

Oral and oropharyngeal biopsies were performed on suspicious lesions of oral dysplasia that were revealed by DOM. Suspicious lesions were defined as potential oral precancerous lesions, and lesions that had abnormal tinctorial characteristics and/or an atypical vascular profile. Oral biopsies were carried out under local anesthesia with 10% lidocaine spray (Xylonibsa), which was applied 2–5 min before the procedure. The samples were obtained by means of biopsy forceps (Weil-Blakesley) in the anatomical areas mentioned above. Any adverse effects were recorded. Pathologists were blinded to the clinical characteristics of the patients.

Statistical analysis

Categorical variables were described as numbers (percentages), and continuous variables were described as medians [interquartile ranges (IQRs)], unless otherwise specified. Student's *t* test or the Mann–Whitney *U* test for paired data was used for quantitative variables, and a chi square test or McNemar test was performed to compare categorical variables. The interassay agreement between the tests was estimated by calculating Cohen's unweighted kappa coefficient (κ).

A univariate analysis was performed to identify variables associated with the risk of having abnormal oral cytology or the presence of an oral HPV infection. Multivariate risk models for HPV detection were built using stepwise selection, assuming a P value cut point of <0.2, with a priori adjustment for age.

All statistical tests were two-tailed and were performed at a level of significance of 0.05. IBM SPSS statistics software for Windows (version 19.0; IBM Corp, Armonk, New York, USA) was used for the statistical analyses.

Results

Patients' characteristics

From May 2019 to May 2021, 103 patients who participated in the anal screening program were included in the oral screening program. The median age of the patients was 45 years (IQR 37–51 years), 55.3% were smokers and 55.4% usually drank alcohol. Regarding HIV infections, the median duration of infection was 11 years (IQR 7–15 years), and the median nadir $CD4^+$ cell count was 297 cells/μl (IQR 218–460), with 23% of the patients having a nadir $CD4^+$ cell count $<$ 200 cells/μl. At study entry, all of the patients were receiving ART and had HIV viral loads below 200 copies/ml, and the median $CD4^+$ cell count was 760 (IQR 560–940) cells/μl.

Regarding sexual habits, the median number of different lifetime sexual partners was 100 (IQR 50–250), and 67% of the patients reported a previous diagnosis of a sexually transmitted disease (other than an HIV infection).

Abnormal cytology, human papillomavirus infection, and histological dysplasia in anal screening

The patients had a median of 6 years of participation in the anal screening program and 57.3% were diagnosed previously with anal HSILs.

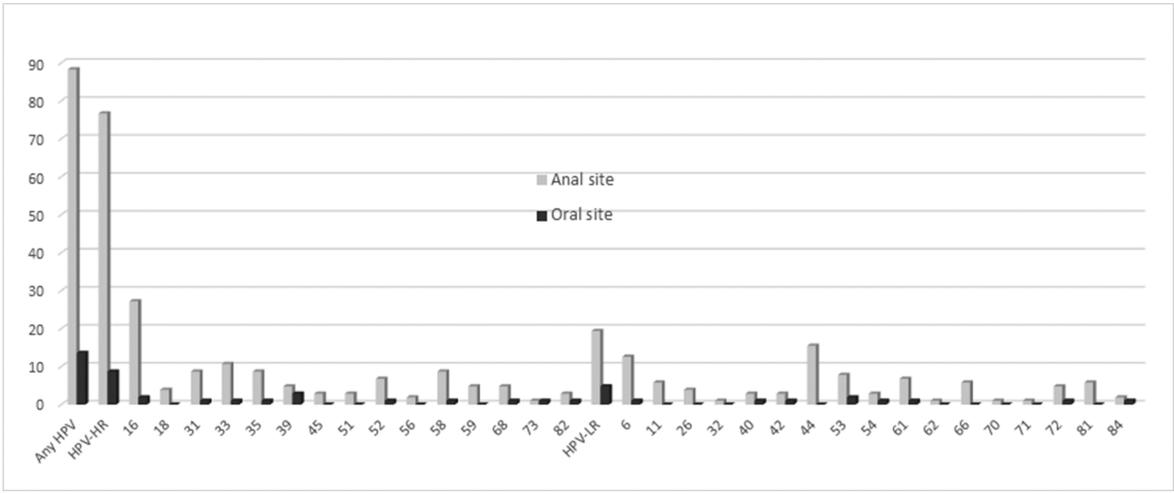

Fig. 1. Distribution of the HPV genotypes detected in oral and anal sites. HPV, human papillomavirus; LR, HPV infection sustained by only low-risk HPV types; HR, HPV infection sustained by at least one high-risk HPV type.

In the anal screening examinations, the cytology was normal in 40 (38.8%) patients, LSILs were found in 18 (17.5%) patients, ASCUS were found in 29 (28.2%) patients, HSILs/ASC-H were found in seven (6.8%) patients and the cytology results were not valuable for nine (8.8%) patients. HPV genotypes were detected in 91 (88.3%) patients, with multiple HPV types in 63.1% of the patients and a median of two genotypes (IQR: 1–4). Oncogenic HPV genotypes were found in 79 patients (76.7%), HPV 16 was found in 28 patients (27.2%), and HPV 18 was found in four patients (3.9%). Figure 1 shows the distribution of the detected HPV genotypes, and Table 1 shows the cytological results according to the presence of an HPV infection.

Seventy (67.9%) patients had abnormal appearances in the HRA. In total, 72 patients had biopsy samples taken, with 25 histological HSIL cases (24.3%) found.

Oral screening program results

No patients had oral symptoms at the beginning of the screening program.

Oral cytology was normal in 76 (73.8%) patients. Atypia was detected in 26 patients: 24 (23.3%) with ASCUS and 2 (1.9%) with LSILs. HPV genotypes were only detected in 14 patients (13.6%), and HPV 16 was detected in two patients (1.9%). Figure 1 shows the distribution of the detected HPV genotypes and Table 1 shows the cytological results according to the presence of an HPV infection.

Regarding oral inspection, although characteristics of the lesions (surface, color, demarcation) were more easily seen with DOM than routine clinical examination, DOM did not detect additional oral lesions that were not previously observed on visual inspection. Twenty-three patients (21.4%) had abnormal appearances on oral inspection. Seventeen patients had benign lesions, including frictional keratosis in the jugal mucosa (12 patients), geographic tongue (2 patients), reticular lichen planus (2 patients), and oral papillomas (1 patient). Five patients showed potential oral precancerous lesions, including amigdalar ulcers (1 patient), hyperpigmented lesions (1 patient), leucoplakia (2 patients) and oral papilloma with atypical vascularization (1 patient). No lesions were observed with abnormal tinctorial characteristics, and only one (oral papilloma) had an atypical vascular profile. Five biopsy samples were taken from these lesions, and none of them showed oral dysplasia.

Correlation between the oral and anal screening programs

In total, HPV detection was concordant between the oral and anal sites in 26 patients (26.2%). All patients with oral HPV detection had anal HPV infections; however, only in four of the 14 patients (28.6%) with HPV detection, the same HPV types in different sites were detected (2 patients with HPV 16 detection, one patient with HPV 35 and another with HPV 11 and 42). The agreement between the HPV detection tests at both sites was poor ($\kappa = 0.037$; 95% confidence interval [CI], −0.015–0.07).

Regarding cytology, abnormal oral and anal cytology was detected in 16.1% of the patients, and 33.3% had normal cytology. No correlation was found between the abnormal results at either site. The agreement between the cytology for both sites was poor ($\kappa = 0.112$; 95% CI, −0.028 to 0.252). Table 2 shows the agreement between HPV detection and the cytological results.

With respect to the patients with biopsy, of the five patients with oral biopsy, four also underwent anal biopsy and in two cases HSIL histological was diagnosed. None

Table 1. Results of screening tests at oral and anal sites.

Results of anal screening tests

				Anal biopsies		
HPV detection	Anal cytology	High-resolution anoscopy	Number of cases	Not adequate	No HSIL	HSIL
No HPV	Not adequate	Abnormal (suggestive of HSIL)	1	0	1	0
	Normal	Normal	3	–	–	–
		Abnormal (suggestive of HSIL)	4	0	4	0
	LSIL	–	0	–	–	–
	ASC-US	Abnormal (suggestive of HSIL)	4	1	2	1
	ASC-H	–	0	–	–	–
	HSIL	–	0	–	–	–
HPV LR	Not adequate	Normal	1	–	–	–
		Abnormal (suggestive of HSIL)	1	0	1	0
	Normal	Normal	3	–	–	–
		Abnormal (suggestive of HSIL)	1	0	1	0
	LSIL	Abnormal (suggestive of HSIL)	1	0	1	0
	ASCUS	Normal	1	–	–	–
		Abnormal (suggestive of HSIL)	3	0	1	2
	ASC-H	–	0	–	–	–
	HSIL	–	0	–	–	–
HPV HR	Not adequate	Normal	1	–	–	–
		Abnormal (suggestive of LSIL)	1	0	1	0
		Abnormal (suggestive of HSIL)	3	0	1	2
	Normal	Normal	13	–	–	–
		Abnormal (suggestive of LSIL)	5	0	5	0
		Abnormal (suggestive of HSIL)	11	1	7	3
	LSIL	Normal	4	–	–	–
		Abnormal (suggestive of LSIL)	6	0	5	1
		Abnormal (suggestive of HSIL)	7	0	2	5
	ASCUS	Normal	5	0	1	0
		Abnormal (suggestive of LSIL)	5	0	2	3
		Abnormal (suggestive of HSIL)	11	0	5	6
	ASC-H	Normal	1	0	1	
		Abnormal (suggestive of HSIL)	2	0	1	1
	HSIL	Abnormal (suggestive of LSIL)	2	1	1	0
		Abnormal (suggestive of HSIL)	2	0	1	1

Results of oral screening tests

				Oral biopsies	
HPV detection	Oral cytology	Oral oroscopy	Number of cases	No HSIL	HSIL
No HPV	Normal	Normal	48	–	–
		Abnormal (suggestive of LSIL)	11	–	–
		Abnormal (suggestive of HSIL)	4	4	0
	LSIL	Abnormal (suggestive of LSIL)	1	–	–
	ASC-US	Normal	18	–	–
		Abnormal (suggestive of LSIL)	4	–	–
	ASC-H	–	0	–	–
	HSIL	–	0	–	–
HPV LR	Normal	Normal	3	–	–
	LSIL	–	0	–	–
	ASC-US	Normal	2	–	–
	ASC-H	–	0	–	–
	HSIL	–	0	–	–
HPV HR	Normal	Normal	7	–	–
		Abnormal (suggestive of LSIL)	1	–	–
	LSIL	Abnormal (suggestive of HSIL)	1	1	0
	ASCUS	–	0	–	–
	ASC-H	–	0	–	–
	HSIL	–	0	–	–

ASC-H, atypical squamous cells cannot exclude HSILs; ASC-US, atypical squamous cells of undetermined significance; HPV, human papillomavirus; HR, HPV infection sustained by at least one high-risk HPV type; HSILs, high-grade squamous intraepithelial lesions; LR, HPV infection sustained by only low-risk HPV types; LSILs, low-grade squamous intraepithelial lesions.

Table 2. Agreement in HPV detection and cytological results at oral and anal sites.

		Anal HPV results		
		HPV HR	HPV LR	No HPV
Oral HPV results	HPV HR	7 (7%)	2 (2%)	0
	HPV LR	4 (4%)	1 (1%)	0
	No HPV	65 (65%)	9 (9%)	12 (12%)

		Anal cytology results					
		Not adequate	Normal	LSILs	ASCUS	ASC-H	HSILs
Oral cytology results	Not adequate	0	0	0	1	0	0
	Normal	6 (5.9%)	31 (30.4%)	13 (12.7%)	18 (17.6%)	3 (2.9%)	4 (3.9%)
	LSILs	0	0	2 (2%)	0	0	0
	ASCUS	2 (2%)	9 (8.8)	3 (2.9%)	10 (9.8%)	0	0
	ASC-H	0	0	0	0	0	0
	HSILs	0	0	0	0	0	0

The agreement between the tests was slight ($\kappa = 0.037$; 95% CI, −0.015–0.07). The agreement between the tests was slight ($\kappa = 0.112$; 95% CI, −0.028 to 0.252). ASC-H, atypical squamous cells cannot exclude HSILs; ASC-US, atypical squamous cells of undetermined significance; HPV, human papillomavirus; HR, HPV infection sustained by at least one high-risk HPV type; HSILs, high-grade squamous intraepithelial lesions; LR, HPV infection sustained by only low-risk HPV types; LSILs, low-grade squamous intraepithelial lesions.

of the five patients had oral HPV infection, but all of them had anal HR HPV detection.

Risk factors for oral human papilloma virus infections

In the multivariate analysis, after adjustment for age, no independent risk factor for oral HPV infections was identified. Nadir $CD4^+$ T-cell counts <200 cells/μl was the factor closest to statistical significance (odds ratio [OR] 2.4; 95% CI, 0.73–9.29). Regarding abnormal oral cytology, a nadir $CD4^+$ T-cell count <200 cells/μl was found to be a predictive factor (OR 3.1; 95% CI, 1.02–9.35). The results are shown in Table 3.

Discussion

Although several screening strategies for HPV-associated oral cancer have been proposed, currently, no program exists that is accepted overall [12,13]. Moreover, few data exist regarding the usefulness of oral dysplasia screening tools in populations at high risk for this disease, such as HIV-infected MSM.

In this study, we evaluated the usefulness of a pilot oral dysplasia screening program for HIV-infected patients, comparing the results with the well established anal dysplasia screening program that was simultaneously performed. The results of this pilot program should be discussed.

The first point that we want to emphasize is that oral examinations by microscopy did not allow us to identify more lesions than those observed by direct visual examination. This differs from cervical and anal dysplasia screening, where colposcopy and high-resolution anoscopy are considered the gold standard tests and allow for the identification of lesions that would go unnoticed in a simple examination [5,6]. In our study, despite an exhaustive examination by a specialist and the use of several stains, colposcopy did not detect more lesions. Only in 20% of the patients was the oral examination considered abnormal, in contrast to the 64% of patients who had abnormal appearances in the HRA, and none of the patients had dysplasia confirmed. Few studies have previously evaluated the usefulness of oral colposcopy, and all of them were carried out with patients with previously diagnosed oral lesions [14–16]. In this situation, oral colposcopy helped to characterize the lesions and define the best area to perform a biopsy. However, according to our results, it did not increase the detection of premalignant lesions. Currently, it is believed that oral HPV-related carcinogenesis occurs at the bottom of the tonsil crypt, a place that is difficult to see with visual examination. Furthermore, a histological premalignant lesion has not been identified in oral HPV-related cancer [12]. These results preclude the utility of oral microscopy examination as a screening tool.

Oral cytology has also been investigated as a method to detect HPV-associated morphological changes in asymptomatic individuals. Different cytological techniques have been evaluated, although with low success [17–20]. A study based on oral liquid cytology with rinsing and shaving carried out with HIV-infected patients showed only 6% of the cytological alterations [19]. Another recent study in which conventional cytology was performed directly on the amygdala also observed 6% of atypia cases [20]. In our study, despite using liquid cytology with rinse-and-gargle and scraping directly on the amygdala, only 25% of alterations in the specimens were obtained. Our results stand out with 60% of cytological alterations that were found in paired anal

Table 3. Risk factors for oral HPV infections and abnormal oral cytology.

Risk factors for oral HPV infections		Univariate		Multivariate	
		n/N (%)	*P*	OR (95% CI)	*P*
Age (years)	≥50	7/25 (28%)	0.062	2.42 (0.73–9.29)	0.120
	<50	8/70 (11.5%)			
Previous STI	Yes	8/62 (12.9%)	0.37		
	No	6/30 (20%)			
Nadir $CD4^+$ T-cell count (cells/μl)	≥200	7/68 (10.3%)	0.07	1.95 (0.49–7.77)	0.344
	<200	6/21 (28.6%)			
Smoking exposure, lifetime pack-years	≥20	4/20 (20%)	0.42		
	<20	4/41 (9.8%)			
Current $CD4^+$ T-cell count (cells/μl)	≥500	12/76 (15.8%)	0.20		
	<500	2/19 (10.5%)			
Anal HPV infection	Yes	14/83 (16.9%)	.20		
	No	0/12 (0)			
Risk factor for abnormal oral cytology		**Univariate**		**Multivariate**	
		n/N (%)	*P*	OR (95% CI)	*P*
Age (years)	≥50	8/26 (30.8%)	0.603	1.11 (0.36–3.40)	0.855
	<50	18/76 (23.7%)			
Previous STI	Yes	15/66 (22.7%)	0.627		
	No	9/33 (27.3%)			
Nadir $CD4^+$ T-cell count (cells/μl)	≥200	15/73 (20.5%)	0.054	3.1 (1.02–9.35)	0.043
	<200	10/23 (43.5%)			
Smoking exposure, lifetime pack-years	≥20	6/22 (27.3%)	1		
	<20	14/31 (31.1%)			
Current $CD4^+$ T-cell count (cells/μl)	≥500	19/82 (23.2%)	0.391		
	<500	7/20 (35%)			
Oral HPV infection	Yes	3/14 (21.4%)	1		
	No	23/86 (26.7%)			

CI, confidence interval; HPV, human papillomavirus; OR, odds ratio; STI, sexually transmitted infection.

samples. Moreover, no correlation was observed between the oral and anal cytological abnormalities. To our knowledge, there are no other studies that have evaluated anal and oral cytology obtained at the same visit. It seems that oral cytology should be ruled out as a screening test, due to its low yield and inability to access target regions (i.e., the base of the tonsils).

Oral HPV testing appeared to be one of the most promising tools; however, recent results have shown important barriers to its incorporation in screening programs. In our study, we found approximately 15% of oral HPV infections, a low percentage compared to the 90% observed in the same patients at the anal site. These differences between the prevalence of oral and anal HPV detection are consistent with those observed in other studies [18,21–23]. Parisi *et al.* [21] also reported a prevalence of HPV infections of 9.4 and 65.4% in oral and anal sites in 1712 HIV-infected MSM. In fact, two meta-analyses situated the prevalence of oral HPV infections in HIV-infected MSM to be approximately 16–17% [24,25], in contrast with the 75% anal HPV infection prevalence [26]. However, more importantly, the correlation between oral high-risk HPV infections and abnormal cytology and abnormal exploration was poor. In fact, studies comparing HPV detection in oral rinse samples with HPV detection in the tonsils have shown poor concordance [27,28]. In a recent study by Combes *et al.* [28], the percentage of positive agreement in HPV 16 status between oral rinsing and direct tonsil brushing ex vivo was <10%, suggesting that the oral HPV status does not accurately represent the HPV status in the biological site of interest. It is possible that the detection of HPV in the oral cavity is more likely an exposure assessment than a (future) disease assessment, whereas we were not able to sample the relevant areas of carcinogenesis.

Another obtained result was that nadir $CD4^+$ cell count was found to be the only predictive factor of abnormal oral cytology but was not a predictive factor of oral HPV infection. Previous studies have reported the nadir $CD4^+$ cell count as a determinant of oral HPV infections in HIV-infected patients [23,29,30]. The size of the screening cohort and the low prevalence of HPV infections may explain the difficulty in finding these same results.

In summary, we suggest that differences in HPV infections and related diseases between oral and anogenital sites explain the results of our study. Different susceptibility to HPV infection in the oral cavity, the lack of identifiable oral clinical precursor lesions and the carcinogenesis process that seems to occur in an

inaccessible place for screening techniques are current barriers to carrying out oral screening programs.

Some limitations of this study should be considered. First, because HPV-associated cancer is rare, our sample size may have been too small to detect HPV-induced lesions of a dysplastic nature, despite the inclusion of HIV-infected individuals who were at increased risk for HPV-associated diseases. Second, although we performed a screening program that included the main available screening tests, including oral microscopy, we did not include serological testing for HPV 16 E6 protein, one of the most promising tools [31,32]. The serological HPV antibody test is not commercially available, which prevented its inclusion in the present study. The strengths of the study were the assessment of the oral screening program in a population at high risk for HPV diseases and its correlation with the anal screening program.

In conclusion, in this study of a novel oral screening program with HIV-positive MSM, a low prevalence of HPV infections, abnormal cytology and oral lesions was detected. Moreover, their correlations with anal HPV-related lesions were poor. These results confirm the current barriers to oral screening techniques, even in selected high-risk populations. More studies focused on finding a useful oral screening tool are mandatory.

Acknowledgements

Informed Consent: All included patients provided informed consent for the use of information available in the database and their medical records. The study was approved by the Commission of Medical Ethics of Hospital Vall d'Hebron (PR(AG)433/2018).

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Conflicts of interest

There are no conflicts of interest.

References

1. Wu X, Watson M, Wilson R, Saraiya M, Cleveland JL, Markowitz L. **Human papillomavirus-associated cancers-United States, 2004–2008.** *MMWR Morb Mortal Wkly Rep* 2012; **61**:258–261.
2. Burd EM. **Human papillomavirus laboratory testing: the changing paradigm.** *Clin Microbiol Rev* 2016; **29**:291–319.
3. Silverberg MJ, Lau B, Justice AC, Engels E, Gill MJ, Goedert JJ, *et al*. **Risk of anal cancer in HIV-infected and HIV-uninfected individuals in North America.** *Clin Infect Dis* 2012; **54**:1026–1034.
4. Shiels MS, Pfeiffer RM, Gail MH, Hall HI, Li J, Chaturvedi AK, *et al*. **Cancer burden in the HIV-infected population in the United States.** *J Natl Cancer Inst* 2011; **103**:753–762.
5. Sawaya GF, Huchko MJ. **Cervical cancer screening.** *Med Clin North Am* 2017; **101**:743–753.
6. Cachay ER, Mathews WC. **Human papillomavirus, anal cancer, and screening considerations among HIV-infected individuals.** *AIDS Rev* 2013; **15**:122–133.
7. Burgos J, Curran A, Landolfi S, Navarro J, Tallada N, Guelar A, *et al*. **The effectiveness of electrocautery ablation for the treatment of high-grade anal intraepithelial neoplasia in HIV-infected men who have sex with men.** *HIV Med* 2016; **17**:524–531.
8. Burgos J, Curran A, Tallada N, Guelar A, Navarro J, Landolfi S, *et al*. **Risk of progression to high-grade anal intraepithelial neoplasia in HIV-infected MSM.** *AIDS* 2015; **29**:695–702.
9. Warnakulasuriya S, Johnson NW, van der Waal I. **Nomenclature and classification of potentially malignant disorders of the oral mucosa.** *J Oral Pathol Med* 2007; **36**:575–580.
10. Rollo F, Latini A, Pichi B, Colafigli M, Benevolo M, Sinopoli I, *et al*. **Prevalence and determinants of oral infection by human papillomavirus in HIV-infected and uninfected men who have sex with men.** *PLoS One* 2017; **12**:e0184623.
11. Solomon D, Nayar R. *The Bethesda system for reporting cervical cytology: definitions. Criteria and explanatory notes.* 2nd ed. New York: Springer; 2004.
12. Kreimer AR, Shiels MS, Fakhry C, Johansson M, Pawlita M, Brennan P, *et al*. **Screening for human papillomavirus-driven oropharyngeal cancer: considerations for feasibility and strategies for research.** *Cancer* 2018; **124**:1859–1866.
13. Kreimer AR, Chaturvedi AK. **HPV-associated oropharyngeal cancers—are they preventable?** *Cancer Prev Res (Phila)* 2011; **4**:1346–1349.
14. Gynther GW, Rozell B, Heimdahl A. **Direct oral microscopy and its value in diagnosing mucosal lesions: a pilot study.** *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2000; **90**:164–170.
15. Chomik P, Michcik A, Michajłowski I, Starzyńska A. **Application of direct oral microscopy in evaluating mucosal margins around invasive oral squamous cell carcinoma.** *Postepy Dermatol Alergol* 2015; **32**:349–357.
16. Ujwala N, Singh NA, Milind N, Prafulla P, Vidhya P, Bhushan B, *et al*. **Colposcopy in premalignant lesions and oral squamous cell carcinoma: linking threads of clinical, histopathological and colposcopic inferences.** *J Cancer Res Ther* 2016; **12**:295–301.
17. Reboiras-López MD, Pérez-Sayáns M, Somoza-Martín JM, Gayoso-Diz P, Barros-Angueira F, Gándara-Rey JM, *et al*. **Comparison of the Cytobrush®, dermatological curette and oral CDx® brush test as methods for obtaining samples of RNA for molecular analysis of oral cytology.** *Cytopathology* **2012; 23:192–197**.
18. Vergori A, Garbuglia AR, Piselli P, Del Nonno F, Sias C, Lupi F, *et al*. **Oral human papillomavirus DNA detection in HIV-positive men: prevalence, predictors, and co-occurrence at anal site.** *BMC Infect Dis* 2018; **18**:25.
19. Benevolo M, Rollo F, Giuliani M, Pichi B, Latini A, Pellini R, *et al*. **Abnormal cytology in oropharyngeal brushings and in oral rinses is not associated with HPV infection: the OHMAR study.** *Cancer Cytopathol* 2020; **128**:648–655.
20. Fakhry C, Rosenthal BT, Clark DP, Gillison ML. **Associations between oral HPV16 infection and cytopathology: evaluation of an oropharyngeal 'pap-test equivalent' in high-risk populations.** *Cancer Prev Res (Phila)* 2011; **4**:1378–1384.
21. Parisi SG, Basso M, Scaggiante R, Andreis S, Mengoli C, Cruciani M, *et al*. **Oral and anal high-risk human papilloma virus infection in HIV-positive men who have sex with men over a 24-month longitudinal study: complexity and vaccine implications.** *BMC Public Health* 2019; **19**:645.
22. Shiboski CH, Lee A, Chen H, Webster-Cyriaque J, Seaman T, Landovitz RJ, *et al*. **Human papillomavirus infection in the oral cavity of HIV patients is not reduced by initiating antiretroviral therapy.** *AIDS* 2016; **30**:1573–1582.
23. Videla S, Darwich L, Cañadas MP, Coll J, Piñol M, García-Cuyás F, *et al*. **Natural history of human papillomavirus infections involving anal, penile, and oral sites among HIV-positive men.** *Sex Transm Dis* 2013; **40**:3–10.
24. King EM, Oomeer S, Gilson R, Copas A, Beddows S, Soldan K, *et al*. **Oral human papillomavirus infection in men who have sex with men: a systematic review and meta-analysis.** *PLoS One* 2016; **11**:e0157976.

25. Farahmand M, Monavari SH, Tavakoli A. **Prevalence and genotype distribution of human papillomavirus infection in different anatomical sites among men who have sex with men: a systematic review and meta-analysis.** *Rev Med Virol* 2021; **31**:e2219.
26. Wei F, Gaisa MM, D'Souza G, Xia N, Giuliano AR, Hawes SE, *et al*. **Epidemiology of anal human papillomavirus infection and high-grade squamous intraepithelial lesions in 29 900 men according to HIV status, sexuality, and age: a collaborative pooled analysis of 64 studies.** *Lancet HIV* 2021; **8**:e531–e543.
27. Kreimer AR, Alberg AJ, Daniel R, Gravitt PE, Viscidi R, Garrett ES, *et al*. **Oral human papillomavirus infection in adults is associated with sexual behavior and HIV serostatus.** *J Infect Dis* 2004; **189**:686–698.
28. Combes JD, Dalstein V, Gheit T, Clifford GM, Tommasino M, Clavel C, *et al*. **Prevalence of human papillomavirus in tonsil brushings and gargles in cancer-free patients: the SPLIT study.** *Oral Oncol* 2017; **66**:52–57.
29. Muller K, Kazimiroff J, Fatahzadeh M, Smith RV, Wiltz M, Polanco J, *et al*. **Oral human papillomavirus infection and oral lesions in HIV-positive and HIV-negative dental patients.** *J Infect Dis* 2015; **212**:760–768.
30. Ablanedo-Terrazas Y, Romero-Mora K, Gómez-Palacio M, Alvarado-de la Barrera C, Ruiz-Cruz M, Hernández-Juan R, *et al*. **Prevalence and risk factors for oral human papillomavirus infection in Mexican HIV-infected men.** *Salud Publica Mex* 2018; **60**:653–657.
31. Lang Kuhs KA, Pawlita M, Gibson SP, Schmitt NC, Trivedi S, Argiris A, *et al*. **Characterization of human papillomavirus antibodies in individuals with head and neck cancer.** *Cancer Epidemiol* 2016; **42**:46–52.
32. Hibbert J, Halec G, Baaken D, Waterboer T, Brenner N. **Sensitivity and specificity of human papillomavirus (HPV) 16 early antigen serology for HPV-driven oropharyngeal cancer: a systematic literature review and meta-analysis.** *Cancers (Basel)* 2021; **13**:3010.