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Prevalence and incidence of human papilloma virus-related dysplasia of oropharyngeal, cervical, and anal mucosae in Spanish people with HIV

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Background: Objectives were to determine the prevalence/incidence of HPV-related dysplasia and clearance/acquisition rates of high-risk HPV (HR-HPV) genotypes in genital mucosa of women with HIV (WWHIV) and oropharyngeal and anal mucosa of people with HIV (PWH) and to evaluate factors related to HR-HPV infection in oropharyngeal mucosa at 12-months.

Material and methods: Prospective, longitudinal study with 12-month follow-up, enrolled PWH between December 2022 and April 2023. At baseline and 12 months, HIV-related clinical and analytical variables were recorded, oropharyngeal mucosa exudates were taken for PCR studies for human papilloma virus (HPV) and other sexually transmitted infections, whereas anal and female genital samples were self-sampled for HPV detection and genotyping by PCR and thin-layer cytology.

Results: Two hundred and seventy-six PWH with mean age of 45.3 years, 79% men, 24.3% with history of AIDS, 100% under antiretroviral therapy (ART), and 30.1% with completed HPV vaccination. HPV infection prevalence in oropharyngeal mucosa was 11.6% at baseline, most frequently by genotype 16 (2.2%), without dysplasia. No oropharyngeal dysplasia was observed at 12 months, and HR-HPV clearance and acquisition rates were 5.5 and 4.4%, respectively. Incidence of anal high grade squamous intraepithelial lesion (HSIL) was 1811.6 cases × 100 000 people-year, and HR-HPV clearance and acquisition rates were 16.2 and 25.6%, respectively. Incidence of CIN2/CIN3 or cervical cancer was zero, and HR-HPV clearance and acquisition rates were 11.3 and 7.5%. HIV-RNA viral load less than 50 copies/ml protected against HPV infection in oropharyngeal mucosa [97.2 vs. 87%, hazard ratio 0.044; 95% confidence interval (95% CI 0.042 – 0.956)].

Conclusion: Among PWH, HSIL incidence and HR-HPV acquisition rate are higher in anal *versus* oropharyngeal and genital mucosae. Nondetectability protects against oropharyngeal HPV infection.

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Keywords: cancer, cervical intraepithelial neoplasia (CIN), high grade squamous intraepithelial lesion (HSIL), oropharyngeal human papilloma virus, people with HIV, women with HIV

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Introduction

Over the past two decades, the availability of antiretroviral therapy (ART) has increased the life expectancy of people with HIV (PWH), which is approaching that of the general population [1], although differences remain among geographic regions and between the sexes [2]. ART is a cost-effective health strategy that delivers superior survival rates in comparison to chemotherapy, surgery, ischemic cardiopathy, and bone marrow transplant transplantation [3]. However, PWH under ART with undetectable viral load have persistent chronic inflammation because of the presence of latent infection (e.g. cytomegalovirus), bacterial translocation, antiretroviral toxicity, cell dysregulation, or the constant replication of viral particles of HIV itself [4]. This inflammatory status favors the emergence of comorbid non-AIDS-defining diseases, including non-AIDS neoplasms [5], and cardiovascular disease [6].

It has been estimated that HPV, the most frequent sexually transmitted infection (STI) in people, is acquired by more than 80% of sexually active individuals during their lifetime [7]. Because that HIV infection is one of the main risk factors for HPV infection [8], the associated disease load is greater in PWH than in the general population [9], not only for AIDS-related, neoplasms such as cervical cancer [10], but also for non-AIDS neoplasms, such as anal [11], oropharyngeal, and head-and-neck squamous cell [12] carcinomas. A comparative study between PWH (North American AIDS Cohort Collaboration on Research and Design, NA-ACCORD) and the general North American population described incidences of 60.1 versus 1.2/100 000 people-year, respectively, for anal squamous cell carcinoma and 34.3 versus 18.4/100 000 people-year, respectively, for oropharyngeal cancer [13]. The main risk factors for HPV infection in PWH were found to be immunosuppression and specific sexual behaviors, such as sex between men and men [14]. The risk of oropharyngeal mucosa infection due to HPV and associated complications is known to be higher for PWH than for the general population [15], but few data are available on the current situation in our setting.

The main objectives of this study of PWH participating in a program for the screening, diagnosis, treatment, and prophylaxis of anal and cervical dysplastic lesions were to determine the prevalence and incidence of HPV-related dysplasia and the clearance and acquisition rates of high-risk HPV (HR-HPV) in the oropharyngeal and anal mucosae of PWH, and in the genital mucosa of women with HIV (WWHIV), and to evaluate factors related to HR-HPV infection in oropharyngeal mucosa at 12 months.

Material and methods

This observational, prospective study consecutively enrolled 276 PWH from the infectious diseases unit of the Virgen de las Nieves University Hospital of Granada (HUVN), Jaén Hospital Complex, and San Cecilio Clinical Hospital of Granada between December 2022 and April 2023. Inclusion criteria were age at least 18 years, current participation in the anal and/or genital cancer screening program of the HUVN, and the signing of informed consent. Data gathering strictly complied with national data protection legislation (Organic law 3/2018, of December 5, on Personal Data Protection), and the study was approved by the Clinical Research Ethics Committee of the hospital on 1 April 2022 (#OROHPV, 0098-N-22).

At the baseline visit (V1), data were gathered on sex; age; nationality; number of different sexual partners during the previous 12 months; number of total sexual partners since first sexual intercourse; time in months since first sexual intercourse; percentage utilization of prophylactics during oral, anal, and/or genital sex; employment status (active or retired); schooling (illiterate, primary, secondary, or university); smoker or ex-smoker (packs/year); alcohol consumption [standard drink units (SDU)]; injection drug user (IDU) or ex-IDU, risk for HIV acquisition (MSM, heterosexual, or IDU); HPV vaccination status, including completion or not of vaccination schedule, time since last dose, and vaccine type; months since HIV diagnosis; stage by Centers of Disease Control and Prevention (CDC) classification; AIDS diagnosis; ART status; months of ART; number of ARTs before V1 virological failure (two consecutive determinations of HIV plasmatic viral load >50 copies/ml); polypharmacy; presence/history of other infections [chronic liver disease or past infection by hepatitis B virus (HBV), chronic liver disease, past/treated/cured hepatitis C virus (HCV) infection, and syphilis]; HBV vaccination status; presence and history of other STIs in anal, oropharyngeal, and female genital mucosa; the presence and history of condylomas at any site and their treatment; and the history and grade of anal, cervical, and/or oropharyngeal dysplasia.

At V1 and at 12 months (V2), data were collected on HIV-related analytical variables, including $CD4^+$ nadir; $CD4^+$ and $CD8^+$ cell counts, $CD4^+/CD8^+$ ratio, and viral load of HIV [HIV-1 quantification was performed by rt-PCR using the Cobas HIV assay on the Cobas 6800 platform (Roche Diagnostics, Basel, Switzerland)]. Anal canal and female genital mucosa samples were obtained by self-sampling with a pair of cotton swabs impregnated with physiological saline, which were used for HPV detection and genotyping by PCR using a Linear Array HPV Genotyping Test in a thermocycler (GeneAmp PCR System 9700, Applied Biosystems, Roche, Basel, Switzerland). Cytology studies were performed after immersion in liquid medium (ThinMayer Liquid) and analysis by thin-layer technique (Processor Thin Prep 2000, Hologic). These studies were conducted in the HUVN pathology laboratory by the same pathologist

(J.L.H.). Oropharyngeal mucosa samples were studied by PCR in the HUVN Microbiology Department for the presence of HPV and other STIs (*Neisseria gonorrhoeae, Chlamydia trachomatis, Trichomonas vaginalis*).

When anal cytology results were abnormal and/or HPV infection was detected in female genital mucosa, rectal inspection and high-resolution anoscopy (HRA) were performed, using a Carl Zeiss 150 fc© colposcope (Carl Zeiss, Oberkochen, Germany). In this procedure, introduction of the transparent disposable anoscope was followed by the instillation of 5 ml acetic acid and its removal after around 3 min for inspection of the mucosa; next, 5% Lugol's iodine was instilled for 1 min, and the mucosa was again inspected through the anoscope. Samples were only taken from aceto-white Lugol's iodine-negative lesions. Biopsies were performed by using an endoscopic retrograde cholangiopancreatography (ERCP) catheter. At V1 and V2, women with abnormal cytology or genital HPV infection were referred to the Gynecology Department for evaluation. All patients underwent oropharyngeal mucosa examination before samples were taken for HPV PCR. Participants with HPV-positive PCR at oropharyngeal level or the presence of symptoms (e.g. voice changes or visible lesions) were referred to the Otorhinolaryngology Department for examination and any necessary treatment.

Cytology results were categorized using the Bethesda classification as squamous intraepithelial lesions, subdivided between low-grade (LSIL) and high-grade (HSIL) lesions, or as undetermined lesions (ASCUS) or undetermined lesions that cannot rule out high-grade (ASCUS-H). Following the Reagan classification system, ASCUS, LSIL, or HSIL lesions were considered as dysplasia, defining ASCUS and LSIL as low-grade and HSIL as high-grade dysplasia [16].

Histology results were categorized using the Lower Anogenital Squamous Terminology (LAST) Standardization Project for HPV, classifying lesions as LSIL (AIN1/condyloma), HSIL (AIN2, AIN3, C. *in situ*), or invasive carcinoma, anal squamous cell carcinoma (ASCC) [17].

Genotypes 16,18, 26, 31, 33, 35, 39, 45, 51–53, 56, 58, 59, 66, 68, 73, and 82 were considered high-risk (HR-HPV). Genotypes 6, 11, 34, 40, 42–44, 54, 55, 57, 61, 70–72, 81, 83, 84, and 89 were considered low-risk (LR-HPV). Genotypes 39, 45, 59, and 68 were classified as subspecies of HPV 18 and genotypes 31, 33, 35, 52, 58, and 67 as subspecies of HPV 16 [18].

Definition of variables

Clearance of HR-HPV infection was defined by a negative cotton swab at 12 months in participants with positive swab at baseline [19].

Acquisition of HR-HPV infection was defined by a positive cotton swab at 12 months in participants with negative swab at baseline [19].

Statistical analysis

Sample size was calculated using statistical software Ene 2.0 published by Ene-CTM in 2005. A sample size of at least 123 PWH was estimated for an expected 25% frequency in the population and based on previously published data with 5% precision and 95% confidence interval [20].

In a descriptive analysis, means, standard deviations, medians, and percentiles were calculated for quantitative variables and absolute and relative frequencies for qualitative variables. The prevalence of HPV infection was calculated with a 95% confidence interval. The normality of variable distribution was checked using the Kolmogorov–Smirnov test. In bivariate analyses of factors related to HPV clearance in oropharyngeal mucosa, the Student's *t* test for independent samples was used for normally distributed quantitative variables and the Mann–Whitney *U* test those with nonnormal distribution. The Wilcoxon test was used for related quantitative variables. Qualitative variables were analyzed with the Pearson's chi-square test or, when application criteria were not met, Fisher's test. Finally, multivariate analysis was performed by stepwise logistic regression, entering variables that were significant in bivariate analyses or considered relevant factors in the literature (age, sex, number of sexual partners during the previous 12 months, smoking, ART, time since HIV diagnosis, total ART time, current $CD4^+$ cell count (cell/μl) and $CD4^+/CD8^+$ ratio, and HPV vaccination). A significance level of 0.05 was considered in all tests. IBM SPSS 21.0 was used for these statistical analyses.

Results

Cohort description

Epidemiological characteristics

The study enrolled 276 PWH with a mean age of 45.3 years; 79% were men and 88% held Spanish nationality. The median number of sexual partners during the previous 12 months was 1 [interquartile range (IQR): 1–1] and the median number since first sexual intercourse was 5 (IQR: 3–13); 1.5% used a condom during oral sex, 35.8% during vaginal sex, and 37.5% during anal sex; 36.4% were active smokers; 33.7% had received the HPV vaccine and 30.1% had completed the vaccination schedule; 10.5% had received the tetravalent vaccine and 23.9% the nonavalent vaccine; 1.8% had chronic HBV infection and 5.4% had HCV infection; 67% had received the HBV vaccine; 3.3% presented with active syphilis and 2.9% with anogenital condylomatosis.

Table 1 lists the remaining epidemiological characteristics of the cohort.

Variables related to HIV infection

HIV had been acquired sexually by 93.9% (78.3% were MSM). The median interval since HIV diagnosis was 23 years, 24.3% were in AIDS stage at the diagnosis, and all participants had received ART for a mean of 20.2 years. Table 2 exhibits results for the remaining HIV-related variables.

Table 1. Epidemiological characteristics of the cohort.

	N = 276
Age [mean (years) (±SD)]	45.3 (10.8)
Sex [*n* (%)]	
Male	218 (79)
Female	58 (21)
Spanish nationality [*n* (%)]	243 (88)
Median NP12m (IQR)	1 (1–1)
Median NPT (IQR)	5 (3–13)
Median years since first sexual relations (IQR)	29 (31–44.5)
Use of condoms for oral sex [*n* (%)]	4 (1.5)
Use of condoms for vaginal sex [*n* (%)]	96 (35.8)
Use of condoms for anal sex [*n* (%)]	12 (37.5)
Employment [*n* (%)]	
Active	246 (89.1)
Retired	30 (10.9)
Schooling [*n* (%)]	
Illiterate	13 (4.7)
Primary	59 (21.4)
Secondary	80 (29)
University	124 (44.9)
Smoker, n (%)	100 (36.4)
Ex-smoker, n (%)	63 (22.8)
Median packs/year [median (IQR)]	20.25 (0.75 – 36)
Alcohol [*n* (%)]	38 (13.8)
SDUs [median (IQR)]	0 (0 – 0)
IDU [*n* (%)]	2 (0.7)
Ex-IDU [*n* (%)]	17 (6.2)
Polypharmacy [*n* (%)]	23 (8.3)
HPV vaccination [*n* (%)]	
Vaccinated	93 (33.7)
Complete vaccination	83 (30.1)
Tetravalent vaccine	29 (10.5)
Nonavalent vaccine	66 (23.9)
Chronic HBV infection [*n* (%)]	5 (1.8)
Positive HBV core antibody [*n* (%)	35 (12.7)
Active chronic HCV infection [*n* (%)]	15 (5.4)
Cured HCV infection [*n* (%)]	24 (8.7)
Syphilis at baseline [*n* (%)]	9 (3.3)
History of syphilis [*n* (%)]	116 (42)
Vaccinated against HBV [*n* (%)]	185 (67)
Other STIs at baseline [*n* (%)]	20 (7.2)
History of other STIs [*n* (%)]	95 (34.4)
Condylomas at baseline [*n* (%)]	8 (2.9)
History of genital condylomas [*n* (%)]	82 (29.7)
History of condyloma treatments [*n* (%)]	
Imiquimod	34 (44.7)
Surgery	14 (18.4)
Cryotherapy	12 (15.8)
No treatment	16 (21.1)
History of condyloma relapse [*n* (%)]	22 (27.2)

Ex-IDU, ex–injection drug user; HBV, hepatitis B virus; HCV, hepatitis C virus; HPV, human papillomavirus; IDU, injection drug user; IQR, interquartile range; NP12m, number of sexual partners during previous 12 months; NPT, total number of sexual partners since first sexual relations; SDUs, standard drink units; STI, sexually transmitted infection.

Table 2. HIV-related variables.

	N = 276
HIV infection acquisition [*n* (%)]	
MSM	276 (78.3)
Heterosexual	43 (15.6)
IDU	13 (4.7)
Other	4 (1.4)
Median age since HIV infection diagnosis (IQR)	23 (17–29)
AIDS (A3, B3, C) [*n* (%)]	67 (24.3)
$CD4^+$ at diagnosis (cells/μl) [mean (±SD)]	398.8 (278.4)
Cd4 nadir (cells/μl) [mean (±SD)]	335.5 (231.5)
$CD8^+$ at diagnosis (cells/μl) [mean (±SD)]	1023.8 (477.2)
VL at diagnosis, $\log_{10}$ [mean (±SD)]	5.5 (6.1)
Current $CD4^+$ (cells/μl) [mean (±SD)]	725.2 (300.2)
Current $CD8^+$ (cells/μl) [mean (±SD)]	867.5 (417.1)
Current $CD4^+/CD8^+$ ratio [mean (±SD)]	0.96 (0.5)
Current VL, $\log_{10}$ [mean (±SD)]	2.5 (3.7)
Current HIV-RNA VL undetectable, $\log_{10}$ [*n* (%)]	239 (85.2)
Naive [*n* (%)]	0 (0)
Years with ART since initiation [median (IQR)]	20.2 (13.1–23.4)
Median ART lines since initiation [median (IQR)]	5.5 (3–7)
Current ART [*n* (%)]	276 (100)
Virological failure [*n* (%)]	2 (0.7)

ART, antiretroviral therapy; $CD4^+$, $CD4^+$ lymphocytes; $CD4^+/CD8^+$, $CD4^+/CD8^+$ ratio; $CD8^+$, $CD8^+$ lymphocytes; IQR, interquartile range; PDA, parenteral drug addiction; SD, standard difference; VL, viral load.

History of oropharyngeal, genital, and anal dysplasia

One patient (0.3%) had a history of HPV-related head and neck squamous cell carcinoma 5 years earlier; 36.2% of the WWHIV had a history of genital dysplasia (13.8% CIN1 and 22.4% CIN 2/3) but none a history of cervical squamous cell carcinoma; 84.7% of the PWH had history of anal dysplasia [63.8% LSIL (AIN1), 19.6% HSIL (AIN2/3), and 1.4% a history of anal squamous cell carcinoma] (Table S1, http://links.lww.com/QAD/D414).

HPV infection and oropharyngeal, anal, and female genital dysplasia at baseline

Human papilloma virus infection and oropharyngeal dysplasia

Among the 276 PWH, 32 (11.6%) had HPV infection in oropharyngeal mucosa, by high-risk genotypes in 22 (7.9%) and low-risk genotypes in 14 (5.1%), observing co-infection in 4 (1.4%). The most frequently isolated genotypes at oropharyngeal mucosa were high-risk genotype 16 (2.2%) and low-risk genotype 6 (1.1%). Examination by the otorhinolaryngologist revealed no visible lesions. No patient had dysplasia in oropharyngeal mucosa; 15 (5.4%) had an STI other than HPV in the oropharynx, which was *Neisseria gonorrhoeae* in 13 (86.7%). Table 3 displays results for remaining variables.

Human papilloma virus infection and female genital dysplasia

Among the 58 WWHIV, 27 (46.6%) had genital HPV infection, by high-risk genotypes in 12 (44.4%) and low-risk

Table 3. Human papilloma virus infection, other sexually transmitted infections, and oropharyngeal dysplasia at baseline.

	N = 276
HPV-positive PCR in oropharyngeal region [*n* (%)]	32 (11.6)
High-risk HPV [*n* (%)]	22 (7.9)
Number of high-risk HPV genotypes [median (IQR)]	0 (0–0)
Low-risk HPV [*n* (%)]	14 (5.1)
Number of low-risk HPV genotypes [median (IQR)]	0 (0–0)
Mixed HPV (high- and low-risk) [*n* (%)]	4 (1.4)
HPV 6 [*n* (%)]	3 (1.1)
HPV 11 [*n* (%)]	1 (0.4)
HPV 16 [*n* (%)]	6 (2.2)
HPV 18 [*n* (%)]	2 (0.7)
HPV 31 [*n* (%)]	1 (0.4)
HPV 33 [*n* (%)]	1 (0.4)
HPV 35 [*n* (%)]	1 (0.4)
HPV 39 [*n* (%)]	2 (0.7)
HPV 40 [*n* (%)]	2 (0.7)
HPV 42 [*n* (%)]	1 (0.4)
HPV 43 [*n* (%)]	1 (0.4)
HPV 44 [*n* (%)]	4 (1.4)
HPV 52 [*n* (%)]	1 (0.4)
HPV 53 [*n* (%)]	1 (0.4)
HPV 54 [*n* (%)]	1 (0.4)
HPV 56 [*n* (%)]	2 (0.7)
HPV 59 [*n* (%)]	3 (1.1)
HPV 61 [*n* (%)]	2 (0.7)
HPV 66 [*n* (%)]	2 (0.7)
HPV 68 [*n* (%)]	2 (0.7)
HPV 69 [*n* (%)]	2 (0.7)
HPV 70 [*n* (%)]	2 (0.7)
HPV-related oropharyngeal dysplasia [*n* (%)]	0 (0)
Positive PCR for other STIs [*n* (%)]	15 (5.4)
Neisseria gonorrhoeae	13 (86.7)
Chlamydia trachomatis	2 (13.3)

HPV, human papilloma virus; IQR, interquartile range.

genotypes in 18 (66.6%), observing co-infection in 8 (29.6%); the genotype was not identified in 5 (18.5%). Three (5.2%) had concomitant oropharyngeal and genital HPV infection, and 23 (39.7%) had concomitant anal and genital HPV infection. The most frquently isolated genotypes in genital mucosa were the following low-risk genotypes 62/81 (9.3%) and 42 (7.4%), and high-risk genotype 16 (11.1%). Cervical cytology was normal in 50 (89.3%) of the WWHIV. Examination by the gynecologist showed that one (1.7%) had CIN1 at baseline. Table S2, http://links.lww.com/QAD/D414 exhibits results for the remaining variables.

Human papilloma virus infection and anal dysplasia

Among the 276 PWH, 213 (79.8%) had HPV infection in anal mucosa [43 (74.1%) of WWHIV], by high-risk genotypes in 165 (63.2%) [28 (48.3%) of WWHIV] and low-risk genotypes in 157 (60.2%) [28 (48.3%) of WWHIV], observing co-infection in 112 (42.9%) [14 (24.1%) of WWHIV]; the genotype was not identified in 3 (1.1%). Twenty-one (8%) had concomitant oropharyngeal and anal HPV infection. The most frequently isolated genotypes at anal level were low-risk genotypes 44/55 (16.9%) and 62/81 (19.5%), and high-risk genotypes 16 (12.3%) and 68 (13.4%).

The anal cytology result was normal in 114 (41.3%), LSIL in 99 (35.9%), and HSIL in 8 (2.9%). HRA results showed that 79 (29.9%) had LSIL (AIN1) and 4 (1.5%) had HSIL (AIN2/AIN3). Table S3, http://links.lww.com/QAD/D414 lists results for the remaining variables.

Incidence of dysplasia and human papilloma virus clearance and acquisition rates in the three mucosae

At 12 months, the incidence of oropharyngeal mucosa dysplasia was zero, with HR-HPV clearance and acquisition rates of 5.5 and 4.4%, respectively.

The incidence of anal HSIL was 1811.6 cases × 100 000 people-year, with HR-HPV clearance and acquisition rates of 16.2 and 25.6%, respectively; 6.2% had concomitant infection in oropharyngeal and anal mucosa.

The incidence of CIN2/CIN3 or cervical cancer was zero, while the incidence of CIN1 was 3488.3 per 100 000 women/year, with HR-HPV clearance and acquisition rates of 11.3 and 7.5%, respectively; 1.8% had concomitant HPV infection in oropharyngeal and genital mucosa.

Factors related to high-risk human papilloma virus infection at 12 months of follow-up in oropharyngeal mucosa

In bivariate analyses, RNA-HIV viral load < 50 cop/ml was a protective factor against oropharyngeal HPV infection in PWH (97.2 vs. 87%; $P = 0.042$), while infection by HR-HPV genotype (6 vs. 30.4%, $P = 0.001$) and the number of genotypes [0 (IQR: 0–0) vs. 0 (0–0.5); $P = 0.0001$] at V1 (12 months earlier) were risk factors. In multivariate analysis, only an undetectable viral load retained a statistically significant association (hazard ratio = 0.184; 95% CI 0.035–0.959). Table 4 displays results of the remaining analyses.

Discussion

Participants in this study were mainly MSM and predominantly held Spanish nationality. Their utilization of a condom was low in any type of sexual activity, more than one-third were active smokers, and more than one-third were vaccinated against HPV. All were under ART and had an excellent virological and immunological status, and less than 1% were in virological failure. Regarding a history of dysplasia in these PWH, head and neck squamous cell carcinoma was recorded in 0.3%, anal HSIL in 19.6%, and anal squamous cell carcinoma in 1.4%, whereas 22.4% of the WWHIV had a history of CIN 2/3 and 0% a history of cervical squamous cell carcinoma.

There was a low prevalence of oropharyngeal HPV infection in our cohort of PWH, below the prevalence of anal or cervical HPV infections, and the most frequently isolated genotype was genotype 16, usually in monoinfection. A

Table 4. Factors related to oropharyngeal human papilloma virus infection at 12 months.[a]

	Negative for oropharyngeal HPV ($n = 252$)	Positive for oropharyngeal HPV ($n = 23$)	P*	HR (95% CI)
Baseline visit				
Age [mean (±SD)]	45.1 (±10.91)	46.74 (±9.94)	0.498	1.022 (0.963–1.086)
Male	198 (78.6)	19 (82.6)	0.793	0.892 (0.154–5.164)
Retired	27 (10.7)	3 (13)	0.726	
Schooling [*n* (%)]			0.706	
Illiterate	11 (4.4)	2 (8.7)	0.706	
Primary	55 (21.8)	4 (17.4)	0.706	
Secondary	71 (28.2)	8 (34.8)	0.706	
University	115 (45.6)	9 (39.1)	0.706	
Spanish nationality [*n* (%)]	222 (88.1)	21 (91.3)	0.711	
Median NPT (IQR)	50 (15–200)	100 (25 – 457)	0.080	
Median (years) since first SR (IQR)	26 (18–35)	29.5 (20 – 39.5)	0.214	
Use of condom, oral sex [*n* (%)]	4 (1.6)	0 (0)	1	
Use of condom, vaginal sex (%)	11 (37.9)	1 (33.3)	1	
Use of condom, anal sex (%)	90 (36.9)	6 (26.1)	0.302	
Smoker [*n* (%)]	88 (35.1)	11 (47.8)	0.223	2.082 (0.748–5.795)
Ex-smoker [*n* (%)]	60 (23.8)	3 (13)	0.240	
Median packs/year (IQR)	3 (0–13)	11.25 (0–27)	0.116	
Ex-IDU [*n* (%)]	14 (5.6)	3 (13)	0.161	
HIV acquisition [*n* (%)]				
MSM	196 (78.7)	19 (82.6)	0.967	
Heterosexual	40 (16.1)	3 (13)	0.967	
IDU	12 (4.8)	1 (4.3)	0.967	
HPV vaccination [*n* (%)]	88 (34.9)	5 (21.7)	0.201	0.410 (0.092–1.828)
Median HIV diagnosis (years) (IQR)	10.9 (5.8–18.8)	13.8 (7 -17)	0.646	0.994 (0.980–1.009)
AIDS (stage A3, B3, C) [*n* (%)]	61 (24.2)	5 (21.7)	1	
CD4 nadir, (cells/μl) [mean (±SD)]	333.7 (2227.8)	367.6 (268.9)	0.512	
Median (years) with ART (IQR)	9 (4.8–12.4)	10.3 (6.9–14.3)	0.749	1.008 (0.993–1.024)
Median ART lines (IQR)	3 (2–5)	3 (1–4.8)	0.482	
Oropharyngeal HPV infection				
HR-HPV [*n* (%)]	15 (6)	7 (30.4)	0.001	0.955 (0.029–31.456)
LR-HPV [n (%)	12 (4.8)	2 (8.7)	0.33	1.387 (0.026–72.860)
Median HR-HPVs (IQR)	0 (0–0)	0 (0–0.5)	0.0001	4.901 (0.217–110.559)
Median LR-HPVs (IQR)	0 (0–0)	0 (0–0)	0.076	0.402 (0.006–28.548)
At 12 months				
Median NP12m (IQR)	1 (1–4)	1 (1–8.5)	0.289	1.002 (0.986–1.017)
New STI [*n* (%)]	34 (13.5)	4 (17.4)	0.54	
STI in oropharynx [*n* (%)]	8 (3.2)	0 (0)	1	
STI in anal canal [*n* (%)]	9 (3.5)	1 (4.3)	0.379	
Female genital STI [*n* (%)]	1 (1.9)	0 (0)	0.379	
Male urethral STI [*n* (%)]	2 (1)	1 (5.3)	0.379	
Anogenital condylomas [*n* (%)]	4 (1.6)	1 (4.3)	0.357	
Positive genital HPV PCR [*n* (%)]	18 (32)	2 (50)	0.607	
Positive anal HPV PCR [*n* (%)]	196 (80)	16 (76.2)	0.777	
$CD4^+$ (cells/μl) [mean (±SD)]	786.35 (312.48)	778.35 (333.64)	0.914	0.999 (0.998–1.001)
$CD4^+/CD8^+$ ratio [mean (±SD)]	1.008 (0.53)	0.95 (0.46)	0.502	1.090 (0.370–3.212)
HIV-RNA VL <50 copies/ml [*n* (%)]	245 (97.2)	20 (87)	0.042	0.184 (0.035–0.959)

ART, antiretroviral therapy; $CD4^+$, $CD4^+$ lymphocytes; $CD4^+/CD8^+$ ratio; ex-PDA, ex– injection drug user; HPV, human papillomavirus; IDU, injection drug user; NP12m, number of sexual partners during previous 12 months; NPT, number of total sexual partners since first sexual relations; SDUs, standard drink units; SR, sexual relations; STI, sexually transmitted infection; VL, viral load.
[a]Bivariate and multivariate analyses.
*$P < 0.05$ (significant).

cross-sectional Spanish study of 103 MSM with HIV and similar clinical/epidemiological characteristics to the present participants also described a very low prevalence of oropharyngeal mucosal HPV infection (14%), most often by HPV 16 [21].

Around one-third of the present WWHIV had genital HPV infection by a combination of high-risk and low-risk genotypes, with slightly less than half being infected by high-risk genotypes, most frequently HPV 16. A meta-analysis of 34 articles. which included 10 336 WWHIV from Europe, reported that the prevalence of HR-HPV ranged between 30.5 and 33.9%, mostly from cervical samples. According to the authors, scant and controversial data were available on HPV-related ano-genital cancer in European WWHIV, especially on anal cancer. They noted that HPV DNA testing was not a routine practice in HPV-related cancer screening and called for its incorporation in screening guidelines with the appropriate indications. They also encountered little

information on HPV vaccination rates, which appeared to be low in these women [22]. A prospective study in Brazil [23] of 115 WWHIV under ART and 139 women from the general population found a higher rate of cervical dysplasia ($P = 0.04$) among the former but similar between-group rates of cervical (44 vs. 37.4%; $P = 0.25$) and oropharyngeal (14.8 vs. 9.4%; $P = 0.25$) HPV infection. These findings are comparable to the cervical and oropharyngeal infection rates in the present WWHIV, who had a predominance of high-risk genotypes in anal HIV infections, most frequently genotypes 16 and 68, with just under half being simultaneously infected with HR-HPV and LR-HPV genotypes. A recent meta-analysis of 34 studies ($n = 16\,164$) described genotype 16 as the most frequent HR-HPV genotype, mainly in MSM [24].

Among the different mucosae studied, the highest prevalence and grades of HPV-related dysplasia at baseline were in anal mucosa samples, one-third of which were diagnosed by HRA with LSIL and less than 2% with HSIL. No patients presented with oropharyngeal dysplasia, and only 1.7% of the women were diagnosed with CIN1. A systematic review of 64 studies ($n = 29\,900$ men) described HSIL+ detection in 7.5–54.5% of HIV-positive MSM, finding HIV to be a significant predictive factor for HSIL+ (hazard ratio 1.54, 95% CI 1.36–1.73) [25]. Another systematic review of 36 studies ($n = 13\,427$ women) compared the prevalence of anal HR-HPV as a function of HIV infection, the presence of cervical HR-HPV, cervical cytology results, and age. It found a close correlation in WWHIV between cervical and anal HPV infections and between anal HSIL and cervical cytology/histopathology [from 7% (105/1421) to 25% (25/101); 3–6, 2–5–5–3, $P < 0.0001$) [26].

The incidence of HSIL was higher in anal mucosa (1811.6 cases × 100 000 people-year) than in cervical or oropharyngeal mucosa. No woman had CIN2/3, and no patients had any HPV-related lesion in oropharyngeal mucosa at 12 months. Clearance and acquisition rates of HR-HPV were highest in anal mucosa, followed by genital and oropharyngeal mucosae, which may in part explain the higher incidence of high-grade dysplasia in the anal canal. A prospective study in Bangkok (Thailand) of 89 MSM and 4 transexual women with acute HIV acquisition and a mean age of 26 years reported that the incidence of anal HSIL was 19.7 per 100 people-year and that infection by HPV genotypes 16/18/45, syphilis, and a $CD4^+$ cell count less than 350 cells/μl were risk factors [27]. The much higher incidence than in the present study may be attributable to epidemiological and immunological differences between the populations. Cervical cancer was the most frequent AIDS-defining neoplasm in WWHIV before ART, but its incidence markedly declined after the introduction of ART, screening programs, and HPV vaccination, although it remains high in some parts of the world, such as Southern and Eastern Africa [28].

Less research has been published on the natural history of oropharyngeal HPV in comparison to anogenital HPV. The risk and incidence of HPV-related head and neck cancer are higher in PWH than in the general population, in part because of the greater risk of HPV infection in this mucosa. In general, HPV infection is known to disappear more rapidly in oropharyngeal than anal mucosae, and oropharyngeal HPV infection has only been intermittently detected in PWH, which has been related to the practice of sexual abstinence. These observations suggest that oropharyngeal HPV can be variably expressed or re-expressed from a previous latent state, as in the case of anogenital HPV [29].

According to statistical analyses of the results obtained at the 12-month follow-up visit, the sole factor protecting PWH against the presence of oropharyngeal HPV infection was an undetectable HIV load. HPV clearance is favored by an improved virological and immunological status, underscoring the critical importance of ART [30]. A low $CD4^+$ cell count (<350 cells/μl) and high HIV viral load (>50 000 copies/ml) in anal mucosa have been associated, alongside other factors, with a greater risk of oncogenic HPV infection in MSM and WWHIV [31,32] and of precancerous lesions and cervical cancer in WWHIV [33]. In order to reduce this risk, multiple authors have recommended ART for immune reconstitution and virological suppression [33].

In conclusion, the prevalence and incidence of HPV infection and HSIL in PWH are highest in the anal canal and lowest in the oropharynx. The protective effect of ART against HPV infection and its complications appears to depend on good adherence to this treatment and its effectiveness to suppress the HIV viral load.

Study limitations include those related to the sensitivity of the HPV detection and cytological tests used and its single-center design. Study strengths include its prospective design (minimizing biases and data losses) and large sample size. To our best knowledge, it is the first study to describe both the prevalence and incidence of HPV-related dysplasia in oropharyngeal, cervical, and anal mucosae from PWH.

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C.-G.: investigation, formal analysis. I C-G writing (review). C.H.-T., J.R.-G., and J. L-H.: resources and investigation. I.C.-G., R.M., M. O., J.L.-H., C. G.-M., and L.M.: supervision, visualization, and validation. I C-G. and RM.: software. I C-G: follow-up/scheduling of participants and data custody. All authors have read and agreed to the published version of the manuscript.

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Conflicts of interest

There are no conflicts of interest.

References

1. Obel N, Omland LH, Kronborg G, Larsen CS, Pedersen C, Pedersen G, *et al*. **Impact of non-HIV and HIV risk factors on survival in HIV-infected patients on HAART: a population-based nationwide cohort study.** *PLoS One* 2011; **6**:e22698.
2. Wandeler G, Johnson LF, Egger M. **Trends in life expectancy of HIV-positive adults on antiretroviral therapy across the globe: comparisons with general population.** *Curr Opin HIV AIDS* 2016; **11**:492–500.
3. Walensky RP, Paltiel AD, Losina E, Mercincavage LM, Schackman BR, Sax PE, *et al*. **The survival benefits of AIDS treatment in the United States.** *J Infect Dis* 2006; **194**:11–19.
4. Deeks SG, Lewin SR, Havlir DV. **The end of AIDS: HIV infection as a chronic disease.** *Lancet* 2013; **382**:1525–1533.
5. Zhabokritsky A, Klein M, Loutfy M, Guaraldi G, Andany N, *et al*. **Non-AIDS-defining comorbidities impact health related quality of life among older adults living with HIV.** *Front Med (Lausanne)* 2024; **11**:1380731.
6. Batterham RL, Bedimo RJ, Diaz RS, Guaraldi G, Lo J, *et al*. **Cardiometabolic health in people with HIV: expert consensus review.** *J Antimicrob Chemother* 2024; **79**:1218–1233.
7. Oyouni AAA. **Human papillomavirus in cancer: infection, disease transmission, and progress in vaccines.** *J Infect Public Health* 2023; **16**:626–663.
8. Deshmukh AA, Damgacioglu H, Georges D, Sonawane K, Clifford GM. **Human papillomavirus-associated anal cancer incidence and burden among US men, according to sexual orientation, human immunodeficiency virus status, and age.** *Clin Infect Dis* 2023; **77**:419–424.
9. Mathoma A, Sartorius B, Mahomed S. **The trends and risk factors of AIDS-defining cancers and non-AIDS-defining cancers in adults living with and without HIV: a narrative review.** *er Epidemiol* 2024; **2024**:7588928.
10. Kelly H, Weiss HA, Benavente Y, de Sanjose S, Mayaud P, ART and HPV Review Group. **Association of antiretroviral therapy with high-risk human papillomavirus, cervical intraepithelial neoplasia, and invasive cervical cancer in women living with HIV: a systematic review and meta-analysis.** *Lancet HIV* 2018; **5**:e45–e58.
11. Hernández-Ramírez RU, Shiels MS, Dubrow R, Engels EA. **Cancer risk in HIV-infected people in the USA from 1996 to 2012: a population-based, registry-linkage study.** *Lancet HIV* 2017; **4**:e495–e504.
12. Cameron JE, Hagensee M. **HPV-associated oropharyngeal cancer in the HIV/AIDS patient.** *Cancer Treat Res* 2019; **177**:131–181.
13. Silverberg MJ, Lau B, Justice AC, Engels E, Gill MJ, Goedert JJ, *et al*., North American AIDS Cohort Collaboration on Research and Design (NA-ACCORD) of IeDEA. **Risk of anal cancer in HIV-infected and HIV-uninfected individuals in North America.** *Clin Infect Dis* 2012; **54**:1026–1034.
14. Hidalgo-Tenorio C, Gil-Anguita C, Ramírez-Taboada J, Esquivias J, López-Ruz MA, *et al*. **Risk factors for infection by oncogenic human papillomaviruses in HIV-positive MSM patients in the ART era (2010-2016).** *Medicine (Baltimore)* 2017; **96**:e8109.
15. Mazul AL, Hartman C, Kramer J, White DL, Royse K, Raychaudhury S, *et al*. **Incidence and survival for oropharynx and nonoropharynx head and neck cancers among veterans living with HIV.** *Cancer Med* 2020; **9**:9326–9335.
16. Wilbur DC, Nayar R. **Bethesda 2014: improving on a paradigm shift.** *Cytopathology* 2015; **26**:339–342.
17. Darragh TM, Colgan TJ, Cox JT, Heller DS, Henry MR, Luff RD, *et al*., Members of LAST Project Work Groups. **The Lower Anogenital Squamous Terminology Standardization Project for HPV-Associated Lesions: background and consensus recommendations from the College of American Pathologists and the American Society for Colposcopy and Cervical Pathology.** *J Low Genit Tract Dis* 2012; **16**:205–242.
18. Muñoz N, Bosch FX, de Sanjose S, Herrero R, Castellsagué X, Shah KV, *et al*., International Agency for Research on Cancer Multicenter Cervical Cancer Study Group. **Epidemiologic classification of human papillomavirus types associated with cervical cancer.** *N Engl J Med* 2003; **348**:518–527.
19. Taylor S, Bunge E, Bakker M, Castellsagué X. **The incidence, clearance and persistence of noncervical human papillomavirus infections: a systematic review of the literature.** *BMC Infect Dis* 2016; **14**:293.
20. Mistry HB, Lebelo RL, Matshonyonge F, Nchabeleng M, Mathebula M, Bogers JP, Wood NH. **Oral and oropharyngeal high-risk HPV prevalence, HIV status, and risk behaviours in a cohort of South African men who have sex with men.** *AIMS Public Health* 2021; **9**:129–141.
21. Callejo A, Del Mar Molina M, Dinares MC, Hernández-Losa J, Planas B, Garcia J, *et al*. **Exploratory study of an oral screening dysplasia program for HIV-infected men who have sex with men.** *AIDS* 2022; **36**:1383–1391.
22. Krankowska D, Mazzitelli M, Ucak HA, Orviz E, Karakoc HN, Mortimer H, *et al*. **Screening and prevention of HPV-related anogenital cancers in women living with HIV in Europe: results from a systematic review.** *HIV Med* 2024; **25**:769–793.
23. Suehiro TT, Damke GMZF, Damke E, de Azevedo Ramos PLR, de Andrade Pereira Silva M, Pelloso SM, *et al*. **Cervical and oral human papillomavirus infection in women living with human immunodeficiency virus (HIV) and matched HIV-negative controls in Brazil.** *Infect Agent Cancer* 2020; **15**:31.
24. Wei F, Goodman MT, Xia N, Zhang J, Giuliano AR, D'Souza G, *et al*. **Incidence and clearance of anal human papillomavirus infection in 16 164 individuals, according to human immunodeficiency virus status, sex, and male sexuality: an international pooled analysis of 34 longitudinal studies.** *Clin Infect Dis* 2023; **76**:e692–e701.
25. 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.
26. Lin C, Slama J, Gonzalez P, Goodman MT, Xia N, Kreimer AR, *et al*. **Cervical determinants of anal HPV infection and high-grade anal lesions in women: a collaborative pooled analysis.** *Lancet Infect Dis* 2019; **19**:880–891.
27. Thitipatarakorn S, Teeratakulpisarn N, Nonenoy S, Klinsukontakul A, Suriwong S, Makphol J, *et al*. **Prevalence and incidence of anal high-grade squamous intraepithelial lesions in a cohort of cisgender men and transgender women who have sex with men diagnosed and treated during acute HIV acquisition in Bangkok, Thailand.** *J Int AIDS Soc* 2024; **27**:e26242.
28. Stelzle D, Tanaka LF, Lee KK, Ibrahim Khalil A, Baussano I, Shah ASV, *et al*. **Estimates of the global burden of cervical cancer associated with HIV.** *Lancet Glob Health* 2021; **9**:e161–e169.
29. Beachler DC, D'Souza G. **Oral human papillomavirus infection and head and neck cancers in HIV-infected individuals.** *Curr Opin Oncol* 2013; **25**:503–510.
30. Beachler DC, Weber KM, Margolick JB, Strickler HD, Cranston RD, Burk RD, *et al*. **Risk factors for oral HPV infection among a high prevalence population of HIV-positive and at-risk HIV-negative adults.** *Cancer Epidemiol Biomarkers Prev* 2012; **2**:122–133.

31. Nagata N, Watanabe K, Nishijima T, Tadokoro K, Watanabe K, Shimbo T. **Prevalence of anal human papillomavirus infection and risk factors among HIV-positive patients in Tokyo, Japan.** *PLoS One* 2015; **10**:e0137434.
32. Heard I, Poizot-Martin I, Potard V, Etienney I, Crenn-Hebert C, Moore C, *et al*., ANRS-C017 VIHGY Study Group. **Prevalence of and risk factors for anal oncogenic human papillomavirus infection among HIV-infected women in France in the combination antiretroviral therapy era.** *J Infect Dis* 2016; **213**:1455–1461.
33. Liu G, Sharma M, Tan N, Barnabas RV. **HIV-positive women have higher risk of human papilloma virus infection, precancerous lesions, and cervical cancer.** *AIDS* 2018; **32**:795–808.