Anal Human Papillomavirus (HPV) Prevalences and Factors Associated with Abnormal Anal Cytology in HIV-Infected Women in an Urban Cohort from Rio de Janeiro, Brazil

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Abstract

Identifying factors, including human papillomavirus (HPV) genotypes, associated with abnormal anal cytology in HIV-infected women have implications for anal squamous cell cancer (SCC) prevention in HIV-infected women. Anal and cervical samples were collected for cytology, and tested for high-(HR-HPV) and low-risk HPV (LR-HPV) genotypes in a cross-sectional analysis of the IPEC Women's HIV Cohort (Rio de Janeiro, Brazil). Multivariate log-binomial regression models estimated prevalence ratios for factors associated with abnormal anal cytology [$\geq$ atypical squamous cells of undetermined significance, (ASC-US)]. Characteristics of the 863 participants included: median age 42 years, 57% non-white, 79% current CD4+ T-cell count >350 cells/mm³, 53% HIV-1 viral load <50 copies/mL, median ART duration 5.8 years. Fifty-one percent of anal specimens contained ≥ 1 HR-HPV genotype; 31% had abnormal anal cytology [14% ASC-US, 11% low-grade squamous intra-epithelial lesion, (LSIL); 2% atypical squamous cells-cannot exclude high-grade SIL (ASC-H); 4% high-grade SIL/cancer (HSIL+)]. In multivariate analysis, cervical LSIL+, nadir CD4+ T-cell count ≤ 50 cells/mm³, HIV-1 viral load ≥ 50 copies/mL, and anal HPV 6, 11, 16, 18, 33, 45, 52, 56, and 58 were associated with $\geq$ anal ASC-US ($p < 0.05$). Abnormal anal cytology and HR-HPV prevalences were high. HIV-infected women with cervical LSIL+, low nadir CD4+ counts, or detectable HIV-1 viral loads should be a particular focus for enhanced anal SCC screening efforts.

Introduction

HUMAN PAPILLOMAVIRUS (HPV) INFECTION remains a significant health concern among HIV-infected women worldwide and in Brazil. The increased prevalence of HPV and invasive cervical cancer (ICC) in HIV-infected women compared to HIV-uninfected women is well-documented in the literature,^{1–3} yet data on anal HPV and anal cytologic abnormalities in this population are limited. While the prevalence of anal squamous cell carcinoma (SCC) among the general population is relatively low, HIV-infected women are up to seven times more likely to develop anal SCC compared to HIV-uninfected women.^{4–6} Additionally, recent

estimates of the incidence of anal SCC among HIV-infected women⁶ are higher than the current ICC rate among HIV-infected women⁷ (30/100,000 vs. 26/100,000 person-years, respectively), with evidence of increasing risk of anal SCC among both the aging HIV-infected male and female populations in the past two decades.^{6,8,9} In comparison to cervical cytologic abnormalities, less is known about anal HPV genotype prevalences and the timeframe of progression from HPV-induced anal pre-cancerous lesions to anal SCC among HIV-infected men and women.¹⁰

While HPV strains 16 and 18 account for more than 70% of ICC worldwide,^{11,12} the disease burden of the remaining oncogenic or high-risk HPV (HR-HPV) genotypes (31, 33, 33,

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35, 39, 45, 51, 52, 56, 58, 59, 68, 73, and 82) remains high¹¹ and varies by geographic location.¹³ Recent research suggests that HIV-infected women have an increased risk of infection with several cervical HR-HPV genotypes other than 16 and 18 compared to HIV-uninfected women.¹⁴ Cervical HPV infections often consist of multiple HPV genotypes, and may increase the risk of concomitant anal HPV infection in HIV-infected women.^{15,16} Similar to abnormal cervical pathology, the HR-HPV genotypes are linked to higher grade anal lesions and carcinoma.¹⁷

Data on factors associated with abnormal anal cytology in HIV-infected women are scarce, particularly from low and middle-income countries.^{8,18–20} Very few studies in Brazil have documented the prevalence of anal HR-HPV genotypes and abnormal anal cytology in HIV-infected women.^{18–19} To address this gap, we assessed anal HPV genotypes and factors associated with abnormal anal cytology in a cross-sectional study of a cohort of HIV-infected women in Rio de Janeiro, Brazil. These findings have implications for anal SCC prevention and screening guidelines for HIV-infected women and possibly HIV-infected men, two populations that are particularly vulnerable to anal HR-HPV infection and progression.

Methods

Data collection

We conducted a cross-sectional analysis of participants enrolled in the Women's HIV Cohort of the Evandro Chagas Clinical Research Institute (IPEC) at Fiocruz in Rio de Janeiro, Brazil (est. 1996). Cohort procedures have previously been published.²¹ Briefly, participants in the IPEC Women's HIV Cohort ≥ 18 years of age were invited by their gynecologists to participate in the IPEC Women's HIV Anal HPV and Lesions Study. Anal and cervical swab specimens were collected by on-site gynecologists between January, 2011 and August, 2013 for anal and cervical cytology, and anal HPV genotyping. Additionally, cervical HPV genotyping results were available for a separate sub-study, started in 2006, to assess trends in cervical HPV genotypes among the IPEC Women's HIV Cohort.

For HPV genotyping, anal swab specimens were collected in liquid cytology media (PreservCyt[®] solution, Hologic, USA), refrigerated, and transported to the virology lab at the Instituto de Medicina Tropical da Universidade de São Paulo, where an aliquot was removed for DNA extraction by the QIAamp DNA Mini Kit (Qiagen, Chattersworth, CA). Five μ L (corresponding to 20–500 ng of DNA) were submitted for HPV detection and genotyping using the PapilloCheck[®] HPV-Screening Test (Greiner Bio-One GmbH, Frickenhausen, Germany), a PCR-based DNA microarray system for the detection and identification of 24 HPV genotypes, including 15 HR-HPV genotypes (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, and 82), two probable high-risk HPV genotypes (53 and 66), and seven LR-HPV genotypes (6, 11, 40, 42, 43, 44, and 70).

Cervical specimens were collected every 6 months or yearly by gynecologists according to the IPEC Women's HIV Cohort protocol.²² Cervical samples were collected with a cervical brush or swab, placed in Sample Transport Medium (STM, Qiagen, Valencia, CA) and stored in frozen conservative media. DNA was extracted by phenol-chloroform standard method. Five μ L aliquots were used for HPV detection and genotyping using the Linear-Array HPV Genotyping Test (Roche Molecular Systems Inc., Alameda,

CA) which targets HPV types 6, 11, 16, 18, 26, 31, 33, 35, 39, 40, 42, 45, 51, 52, 53, 54, 55, 56, 58, 59, 61, 62, 64, 66, 67, 68, 69, 70, 71, 72, 73, 81, 82, 83, 84, IS39, and CP6180.

Anal cytology analysis was performed by Salomão Zoppi Diagnósticos in São Paulo using the Bethesda grading system: atypical squamous cells of undetermined significance (ASC-US), atypical glandular cells (AGC), low-grade squamous intraepithelial lesion (LSIL), atypical squamous cells—cannot exclude high-grade squamous intraepithelial lesion (ASC-H), high-grade squamous intraepithelial lesion (HSIL), or cancer. Cervical cytology analysis was performed at the IPEC Pathology Lab using conventional standardized methods, using the same Bethesda grading system outlined above. Participants with abnormal anal or cervical cytology results were referred for anoscopy or colposcopy (respectively), according to the IPEC Women's HIV Cohort guidelines.^{21,22} Demographic, clinical and behavioral data were collected by gynecologists using semi-structured interviews from the women at the time of specimen collection.

The IPEC Women's HIV Anal HPV and Lesions Study was approved by the Institutional Review Board of Evandro Chagas Clinical Research Institute (IPEC) at Fiocruz, Rio de Janeiro, Brazil. Written informed consent was obtained from all participants prior to enrollment and initiation of study procedures. This secondary analysis of de-identified data was exempt from review by the UCLA IRB.

Statistical analysis

Descriptive analysis of the demographic, behavioral, and clinical characteristics was performed. Single clinical data points (cervical cytology, cervical HPV genotypes, and current CD4+ T cell-count) were selected based on proximity to the date of the anal specimen collection, using 365 days as the upper-limit for inclusion. HSIL and cancer were collapsed into a single HSIL+ category for analysis. Nadir CD4+ T-cell counts were obtained from the participant's medical records, and defined as the lowest CD4+ T cell-count recorded, up to 90 days after the anal specimen collection. For the HIV-1 RNA results, a participant was categorized as having ≥ 50 copies/mL if she had at least one viral load ≥ 50 copies/mL in the 365 days prior to anal cytology. Fifty-two women had one-time “blips” (defined as an HIV-1 RNA 50–399 copies/mL, with HIV-1 RNA < 50 copies/mL before and after the blip) and were categorized in the < 50 copies/mL category. Thymidine analogue exposure was defined as exposure to zidovudine or stavudine at any time prior to the anal specimen collection. Combination antiretroviral therapy (cART) class and duration were calculated for the subset of the study population on cART ($n=744$) at the time of anal specimen collection. Additionally, anal HPV genotyping was available for 781/863 (91%) study participants. Cervical HPV genotyping was available for 518/863 (60%) women participating in a separate sub-study to assess trends in cervical HPV genotypes among the IPEC Women's HIV Cohort.

Fisher's exact test was used to assess associations between the four anal and cervical cytology categories: negative, ASC-US, LSIL, and ASC-H/HSIL/cancer. Cervical ASC-US ($n=72$) and cervical AGC ($n=5$) were combined into one category. Log-binomial regression models^{23,24} were used to estimate crude and adjusted prevalence ratios (PR) of factors associated with abnormal anal cytology (defined as cytologic

TABLE 1. CHARACTERISTICS OF IPEC WOMEN'S HIV ANAL HPV & LESIONS STUDY, RIO DE JANEIRO, BRAZIL, 2011–2013

	N = 863
Age	
Median (IQR)	42 (35–49)
< 30	107 (12.4%)
30–39	237 (27.5%)
40–49	325 (37.6%)
≥ 50	194 (22.5%)
Race	
White	358 (41.5%)
Non-white	493 (57.1%)
Unknown	12 (1.4%)
Marital status	
Married/living together	370 (42.9%)
Not currently married/ Not living together	315 (36.5%)
Never married	115 (13.3%)
Unknown	63 (7.3%)
Education	
< 11 years	533 (61.8%)
≥ 11 years	286 (33.1%)
Unknown	44 (5.1%)
Anal cytology	
Negative	596 (69.1%)
ASC-US	124 (14.4%)
AGC	0 (0%)
LSIL	97 (11.2%)
ASC-H	13 (1.5%)
HSIL +	33 (3.8%)
Cervical cytology	
Negative	670 (77.6%)
ASC-US	72 (8.4%)
AGC	5 (0.6%)
LSIL	86 (10.0%)
ASC-H	2 (0.2%)
HSIL +	14 (1.6%)
Unknown	14 (1.6%)
Lifetime sexual partners	
Median (IQR)	5.0 (3.0–10.0)
History of receptive anal intercourse	
No	511 (59.2%)
Yes	352 (40.8%)
Menopause	
No	612 (70.9%)
Yes	251 (29.1%)
Current cigarette smoker	
No	695 (80.5%)
Yes	168 (19.5%)
Current CD4+ count (cells/mm ³) ^a	
> 350	679 (78.7%)
≤ 350	169 (19.6%)
Unknown	15 (1.7%)
Nadir CD4+ count (cells/mm ³) ^b	
> 350	181 (21.0%)
201–350	280 (32.4%)
51–200	263 (30.5%)
≤ 50	128 (14.8%)
Unknown	11 (1.3%)

(continued)

TABLE 1. (CONTINUED)

	N = 863
HIV-1 viral load (copies/mL) ^c	
≥ 50	356 (41.3%)
< 50	458 (53.1%)
Unknown	49 (5.7%)
Thymidine analogue exposure	
Yes	596 (69.1%)
No	267 (30.9%)
cART anchor	
PI	422 (48.9%)
NNRTI	279 (32.3%)
Both/other	43 (5.0%)
Not on ART	119 (13.8%)
cART duration	n = 744 ^d
Median years (IQR)	5.8 (2.6–12.5)
Mean years, SD	7.3, 5.4

^aMost recent CD4+ count within ± 365 days of anal specimen collection.^bNadir CD4+ count defined as the lowest CD4 count in medical records, up to 90 days after anal specimen collection.^cA patient was categorized in ≥ 50 copies/mL if she had at least one viral load ≥ 50 copies/mL in the 365 days prior to the anal specimen collection; 52 women with one-time “blips” (50 ≤ x < 400) were categorized in viral load < 50 copies/mL.^dWomen on cART at time of anal specimen collection.

abnormalities of ASC-US or higher, reference category: negative anal cytology). Factors in the bivariate analysis with a *p* value ≤ 0.20 were included in the initial multivariate model. Backward removal of nonsignificant covariates was used to construct the final multivariate model. Age was forced into the final multivariate model, given the established connection between age and increased risk of abnormal cytology.¹⁵ Multivariate analysis was also performed for the subset of women on cART, using the same modeling approach described above. In addition, multivariate analysis was performed for the subset of women with cervical HPV genotype results, again using the same modeling approach (see Supplementary Tables S1 and S2 for multivariate analysis results for these two subsets at www.liebertpub.com/apc). Statistical analysis was performed using R version 3.01, with statistical significance defined using a two-sided $\alpha < 0.05$.

Results

Eight hundred seventy-eight women were enrolled in the IPEC Women's HIV Anal HPV and Lesions Study. Fifteen of the 878 women were excluded from analysis due to invalid anal cytology results (insufficient collection or error in processing). Of the 863 women analyzed, the median age was 42 years (IQR 35–49), 57% were non-white, 79% had current CD4+ T-cell counts > 350 cells/mm³, 15% had nadir CD4+ T-cell counts ≤ 50 cells/mm³, and 53% had HIV-1 viral loads < 50 copies/mL (Table 1). Thirty-one percent of the entire study population had abnormal anal cytology: ASC-US 14%, LSIL 11%, ASC-H 2%, or HSIL+ 4%. Among the 744 women on cART at the time of their anal specimen collection, the median cART duration was 5.8 years, with 49% on a protease inhibitor (PI), 32% on a non-nucleoside reverse transcriptase inhibitor (NNRTI), and 5% on both or another cART class.

FIG. 1. Anal and cervical HPV prevalences of IPEC Women's HIV Anal HPV and Lesions Study, Rio de Janeiro, Brazil, 2011–2013.

Figure 1 describes the anal and cervical HPV genotype prevalences among the study population. Fifty-one percent of anal specimens tested positive for ≥ 1 HR-HPV type. The three most prevalent anal HR-HPV genotypes were 16 (17%), 56 (10%), and 68 (9%). The three most prevalent anal low-risk HPV (LR-HPV) genotypes were 44 (14%), 53 (11%), and 6 (9%). Among the subset of women with cervical HPV genotype results ($n=518$), the three most prevalent cervical HR-HPV genotypes were 58 (23%), 59 (16%), and 16 (15%); the three most prevalent cervical LR-HPV genotypes were 66 (26%), 53 (17%), and 6 (7%).

Anal and cervical cytology results were available for 849 women (Table 2). Four hundred ninety-seven women (59%) had both negative anal and cervical cytology results, 11 (1%) had both anal and cervical ASC-US/AGC, 21 (3%) had both anal and cervical LSIL, and 2 (<1%) had both anal and cervical HSIL/ASC-H/cancer. The association between the anal and cervical cytology categories was statistically significant ($p<0.01$).

The crude and adjusted PRs for factors associated with abnormal anal cytology are shown in Table 3. In the initial bivariate analysis, there was a positive association for any

TABLE 2. ASSOCIATION BETWEEN ANAL AND CERVICAL CYTOLOGY AMONG IPEC WOMEN'S HIV ANAL HPV & LESIONS STUDY, RIO DE JANEIRO, 2011–2013

Cervical cytology	Anal cytology				Total
	Negative	ASC-US	LSIL	HSIL/ASC-H/cancer	
Negative	497 (58.5%)	88 (10.4%)	53 (6.2%)	32 (3.8%)	670 (78.9%)
ASC-US ^a	51 (6.0%)	11 (1.3%)	14 (1.6%)	3 (0.4%)	79 (9.3%)
LSIL	38 (4.5%)	19 (2.2%)	21 (2.5%)	8 (0.9%)	86 (10.1%)
HSIL/ASC-H/cancer	5 (0.6%)	1 (0.1%)	6 (0.7%)	2 (0.2%)	14 (1.6%)
Total	591 (69.6%)	119 (14.0%)	94 (11.1%)	45 (5.3%)	n = 849

Fisher's Exact Test ($p<0.01$).

^aThe 5 participants who had cervical AGC were included in the cervical ASC-US category.

TABLE 3. CRUDE AND ADJUSTED PREVALENCE RATIOS FOR FACTORS ASSOCIATED WITH ABNORMAL ANAL CYTOLOGY (ASC-US+) AMONG STUDY POPULATION (N=863) OF IPEC WOMEN'S HIV ANAL HPV & LESIONS STUDY, RIO DE JANEIRO, BRAZIL, 2011–2013

	<i>Crude PR</i>			<i>Adjusted PR</i>		
	<i>ASC-US +</i>			<i>ASC-US +</i>		
	<i>PR</i>	<i>95% CI</i>	<i>p Value</i>	<i>PR</i>	<i>95% CI</i>	<i>p Value</i>
Age (years)						
< 30	1.0	Reference	Ref	1.0	Reference	Ref
30–39	1.2	0.8–1.7	0.34	1.0	0.7–1.4	0.88
40–49	1.2	0.9–1.7	0.23	1.0	0.8–1.4	0.84
≥ 50	1.2	0.8–1.7	0.39	1.0	0.7–1.4	0.93
Race						
White	1.0	Reference	Ref			
Non-white	1.2	0.9–1.5	0.10			
Marriage						
Married/living together	1.0	Reference	Ref			
Not currently married/not living together	1.0	0.7–1.2	0.59			
Never married	1.0	0.7–1.4	0.96			
Education (years)						
< 11	1.0	Reference	Ref			
≥ 11	1.1	0.9–1.3	0.51			
Cervical cytology						
Negative	1.0	Reference	Ref	1.0	Reference	Ref
ASC-US/AGC	1.3	0.9–1.8	0.07	1.0	0.7–1.4	0.97
LSIL+ ^a	2.2	1.8–2.7	<0.01	1.5	1.2–2.0	<0.01
Lifetime sexual partners						
< 5	1.0	Reference	Ref			
≥ 5	1.2	0.9–1.5	0.09			
History of receptive anal intercourse						
No	1.0	Reference	Ref			
Yes	1.5	1.2–1.8	<0.01			
Menopause						
No	1.0	Reference	Ref			
Yes	0.7	0.6–0.9	<0.01			
Current smoker						
No	1.0	Reference	Ref			
Yes	0.8	0.6–1.2	0.31			
Current CD4+ (cells/mm ³)						
> 350	1.0	Reference	Ref			
≤ 350	1.6	1.3–2.0	<0.01			
Nadir CD4+ (cells/mm ³)						
> 350	1.0	Reference	Ref	1.0	Reference	Ref
201–350	1.2	0.9–1.5	0.32	1.2	1.0–1.6	0.13
51–200	1.0	0.8–1.4	0.81	1.2	0.9–1.5	0.25
≤ 50	1.4	1.1–1.9	<0.05	1.4	1.1–1.9	<0.05
HIV-1 viral load (copies/mL)						
≥ 50	1.0	Reference	Ref	1.0	Reference	Ref
< 50	0.6	0.4–0.7	<0.01	0.7	0.6–0.9	<0.01
Thymidine analogue exposure						
Yes	1.0	Reference	Ref			
No	1.0	0.8–1.3	0.82			
Anal HR-HPV genotypes						
Negative	1.0	Reference	Ref	1.0	Reference	Ref
16	2.1	1.7–2.6	<0.01	1.9	1.5–2.3	<0.01
18	2.4	1.8–3.1	<0.01	1.7	1.2–2.4	<0.05
31	1.6	1.1–2.2	<0.05			
33	2.5	1.9–3.2	<0.01	2.3	1.7–3.0	<0.01
35	2.2	1.5–3.2	<0.01			

(continued)

TABLE 3. (CONTINUED)

	Crude PR			Adjusted PR		
	ASC-US +			ASC-US +		
	PR	95% CI	p Value	PR	95% CI	p Value
39	2.2	1.7–2.8	<0.01			
45	2.0	1.4–2.8	<0.01	1.6	1.1–2.3	<0.05
51	1.9	1.4–2.5	<0.01			
52	2.0	1.5–2.7	<0.01	1.5	1.1–2.0	<0.05
56	2.0	1.6–2.6	<0.01	1.5	1.2–2.0	<0.01
58	2.4	1.9–3.1	<0.01	1.9	1.4–2.6	<0.01
59	1.7	1.3–2.4	<0.01			
68	1.7	1.3–2.2	<0.01			
73	2.3	1.6–3.4	<0.01			
82	2.0	1.4–2.9	<0.01			
Anal LR-HPV genotypes						
Negative	1.0	Reference	Ref	1.0	Reference	Ref
6	2.4	2.0–3.0	<0.01	2.2	1.8–2.7	<0.01
11	2.9	2.4–3.6	<0.01	2.3	1.7–3.1	<0.01
40	1.2	0.7–2.1	0.47			
43	1.5	0.9–2.4	0.11			
44	1.6	1.3–2.1	<0.01			
53	1.7	1.3–2.2	<0.01			
66	1.8	1.3–2.6	<0.01			
70	1.4	0.9–1.9	0.10			

^aLSIL, ASC-H, HSIL or cancer=LSIL+.

anal HR-HPV genotype with abnormal anal cytology (PR 2.6, 95% confidence interval [2.1–3.2], $p < 0.01$). Given the diversity of HR-HPV genotypes, we chose to examine the PR of the individual anal HPV genotypes. In multivariate analysis, factors associated with abnormal anal cytology included cervical LSIL+ (PR 1.5 [1.2–2.0] ref: negative cervical cytology), nadir CD4+ T-cell count ≤ 50 cells/mm³ (PR 1.4 [1.1–1.9] ref: > 350 cells/mm³), and HIV-1 viral load < 50 copies/mL (PR 0.7 [0.6–0.9] ref: ≥ 50 copies/mL). Anal HPV genotypes positively associated with abnormal anal cytology included LR-HPV 6 (PR 2.2 [1.8–2.7]) and 11 (PR 2.3 [1.7–3.1]), and HR-HPV genotypes 16, 18, 33, 45, 52, 56 and 58 (all p values < 0.05).

The crude and adjusted PRs for factors associated with abnormal anal cytology for the subset of women on ART are shown in Supplementary Table S1 (See Supplementary Table S1 at www.liebertpub.com/apc). In contrast to the entire study population (Table 3), a history of receptive anal intercourse was also significant for abnormal anal cytology (PR 1.3 [1.1–1.5], $p < 0.05$), as was anal HR-HPV genotype 51 (PR 1.5 [1.1–2.0], $p < 0.05$). However, anal HR-HPV genotypes 45 and 52 were no longer significant. Supplementary Table S2 describes the crude and adjusted PRs for factors associated with abnormal anal cytology for the subset of women with available cervical HPV genotype results (See Supplementary Table S2 at www.liebertpub.com/apc). In contrast to the entire study population (Table 3), a history of receptive anal intercourse (PR 1.4 [1.1–1.7], $p < 0.05$), anal HR-HPV 31 (PR 1.6 [1.1–2.2], $p < 0.05$), and cervical HR-HPV 52 (PR 1.7 [1.1–2.4], $p < 0.05$) were also significant for abnormal anal cytology, but anal HR-HPV genotypes 45, 52, and 56 were no longer significant.

Discussion

In this cross-sectional study of 863 HIV-infected women in urban Brazil, 51% of anal specimens tested positive for ≥ 1 HR-HPV genotype and 31% had abnormal anal cytology. The three most prevalent anal HR-HPV genotypes (in decreasing order of prevalence) were 16, 56, and 68; the three most prevalent cervical HR-HPV genotypes (in decreasing order of prevalence) were 58, 59, and 16. Using Fisher's exact test, the association between the anal and cervical cytology categories was statistically significant ($p < 0.01$). In multivariate analysis, women with cervical LSIL+, nadir CD4+ ≤ 50 cells/mm³, a detectable HIV-1 viral load, or certain anal HPV genotypes were more likely to have abnormal anal cytology.

Greater than one-half of the anal specimens tested positive for ≥ 1 HR-HPV genotype, consistent with previous studies documenting a high prevalence of anal HR-HPV among HIV-infected women.^{15,18,25} While anal HR-HPV 56 and 68 were the second and third most prevalent anal HR-HPV genotypes in our study, the prevalences for both genotypes were $< 1\%$ in a 2008 study by Gonçalves et al. assessing anogenital HPV among 138 HIV-infected women in São Paulo.¹⁸ Recent studies on anal HPV in HIV-infected women from the United States also document HPV 16 as the most prevalent anal HR-HPV genotype,^{15,16,25} although the distribution of other HR-HPV genotypes varies by study. These variations in anal HR-HPV genotype prevalences and differences compared to cervical HR-HPV genotypes among different populations illustrate the diversity of HPV genotypes by region.^{13,26} It should also be noted that 12% of our study population tested positive for anal HPV 53, which is considered a probable HR-HPV genotype.

Nearly one-third of the women had abnormal anal cytologic findings. Consistent with other studies that document an association between abnormal cervical cytology and abnormal anal cytology in HIV-infected women,^{16,25,27} we found that cervical cytology $\geq$ LSIL was positively associated with abnormal anal cytology. Given that the IPEC HIV Women's Cohort participants receive regular cervical Pap smears, the cervical cytology results of our study population might not accurately reflect those of HIV-infected women in Brazil. However, even with regular monitoring, abnormal cervical cytology was high in this study population, and women with cervical LSIL+ were more likely to have abnormal anal cytology as well. Paired with the significant association between anal cytology and cervical cytology category, these observations suggest an increased prevalence of higher grade anal lesions with the presence of higher grade cervical lesions.

We found that women with nadir CD4+ ≤ 50 cells/mm³ and HIV-1 RNA ≥ 50 copies/mL had a greater prevalence of abnormal anal cytology. Although current CD4+ T-cell count ≤ 200 cells/mm³ has been identified as a potential risk factor for abnormal anal cytology in HIV-infected women,^{19,27} low nadir CD4+ count and not current CD4+ count was significant in our multivariate model. An increased conversion from Th1 to Th2 cells in HIV-infected women with high-grade lesions, as well as a defective immune-response to the HPV oncogenes E6/E7 in T cell lines derived from HIV-infected individuals has also been documented.¹⁷ Similar to HPV-induced ICC, research suggests an association between HPV persistence and anal SCC in HIV-infected populations.^{10,17} Our findings indicate that HIV-infected women with a history of significant immune devastation might be at increased risk of abnormal anal cytology and dysplasia.

While less is understood regarding the etiology of HPV-induced anal SCC, evidence suggests an overlap with the mechanism responsible for HPV-induced ICC.^{10,17} There are differences between the HPV-mediated oncogenic processes in the two mucosae,¹⁷ as evidenced by the high disease burden of ICC among HIV-uninfected women but relatively low prevalence of anal SCC among HIV-uninfected populations.²⁸ Recent research suggests that the progression from high-grade anal intraepithelial neoplasia (HGAIn) to anal SCC is higher in immunocompromised populations compared to immunocompetent populations, likely secondary to HPV persistence.²⁹ However, a recent retrospective cohort study among both HIV-infected and HIV-uninfected MSM in Australia documented a higher rate of HGAIn spontaneous regression compared to progression when left untreated.^{10,29} These results were consistent with findings from a recent meta-analysis of cross-sectional studies, documenting substantially lower rates of progression of HGAIn to anal SCC among MSM compared to progression of high-grade cervical intraepithelial neoplasia to ICC in women.³⁰ While the incidence of anal SCC has increased in HIV-infected populations, these findings on HGAIn spontaneous regression raise the question of whether the cytologic parameters and guidelines used for ICC are appropriate for the management of abnormal anal lesions. Further research on the pathologic process of HPV-induced anal SCC, and long-term prospective studies to explore the natural history of abnormal anal lesions are needed to optimize the development of anal SCC screening protocols and guidelines for the treatment of abnormal anal lesions in HIV-infected women.³¹

Additionally we found that after adjustment, the anal HR-HPV genotypes 16, 18, 33, 45, 52, 56, and 58, and the LR-HPV genotypes 6 and 11 remained significant predictors of abnormal anal cytology. Studies document significant global variations in cervical HR-HPV genotypes among and between HIV-infected and HIV-uninfected women,^{13,14} with increasing reports of HR-HPV genotype 58 associated with cervical pre-malignant lesions in Brazil, in addition to the most commonly cited HR-HPV genotype 16 worldwide.^{32,33} While HPV genotypes 16 and 18 historically have the worst prognosis for cervical cancer, less is understood regarding the HPV genotypes linked to anal SCC. A meta-analysis assessing the prevalences of HPV in carcinoma of the vulva, vagina, and anus found that high-grade anal histologic samples from HIV-infected individuals compared to those from HIV-uninfected individuals had increased odds of HPV positivity for all HR-HPV genotypes except 16, 68, 73, and 82.³⁴ Our findings highlight the need for further investigation to identify the anal HR-HPV genotypes most commonly associated with anal pre-cancerous lesions among HIV-infected women. Additionally, for the subset of women with available cervical HPV genotyping, cervical HPV 52 was identified as an independent factor associated with abnormal anal cytology. It is unclear whether the association of cervical HPV 52 with abnormal anal cytology results from its impact on the cervical region, or through another mechanism.

Although smoking is a known risk factor for anal pre-cancerous lesions, current smoking status was not significant in our multivariate analysis.^{19,35} However, the literature documents an association between smoking and HSIL, while our study assessed potential risk factors for anal ASC-US+, which might explain the absence of a significant association in our study.

While a history of receptive anal intercourse was not significant in the multivariate analysis for the entire study population, it was positively associated with abnormal anal cytology in the subset of participants on cART, and the subset of those with available cervical HPV genotype results. Current literature on the association between a history of receptive anal intercourse and abnormal anal cytology shows inconsistent results,^{15,19,25,27} which might explain the discrepancy across population subsets in our study. Additionally, nearly half (40.8%) of the women in our study reported a history of receptive anal intercourse, comparable to reports from other studies on anal HPV and cytology among HIV-infected women in Brazil.^{18,19} There are little data on whether the rates of anal intercourse among HIV-uninfected women differ from HIV-infected women in Brazil, and whether potential differences in these rates drive higher prevalences of abnormal anal or cervical cytology among HIV-infected women. However, a recent cross-sectional study from a cervical colposcopy clinic in Vitoria, Brazil, documented that 49.1% of HIV-uninfected women with high-grade cervical dysplasia reported a history of anal intercourse, whereas only 26.5% of HIV-uninfected women with negative high-grade cervical lesions reported anal intercourse, suggesting high prevalences of anal intercourse among HIV-uninfected women as well.³⁶

Our study has strengths and limitations. The anal cytology and anal HPV genotyping results were collected in a cross-sectional design from the larger Women's HIV Cohort at IPEC. All efforts were made to collect both anal and cervical

specimens on the same day, which was achieved for 831/863 (96%) of the study population. One cervical sample from this group was invalid, leaving 830 cervical samples available for the analysis that were collected on the same day as anal specimens. However, 4% had cervical specimens that were collected as close to the anal specimen collection as possible, using 365 days as the upper limit. Among these 32 women, 13 had samples that fell outside of the 365-day marker, and were thus classified as not having a cervical sample available for the study. For the remaining 19 cervical samples, the mean difference between anal and cervical cytology collection was 32 days. Given the short interval between specimen collections, we believe this had little impact on the study results. Cervical HPV genotyping was available for 518/863 (60%); however, when we compare our sample size with that of published studies on anogenital HPV in HIV-infected women, 518 women is a large sample to report HPV-genotyping results.^{15,18,25} For the variables, lifetime sexual partners and history of receptive anal intercourse, the validity of both are limited by recall and reporter bias, respectively. Menopause was a clinical diagnosis based on ≥ 12 months of amenorrhea, not confirmed by FSH/LH testing, which could influence the analysis results.

Recent reviews of anal SCC prevention protocols in the United States, Canada, and the UK recommend targeted anal SCC screening among HIV-infected men and women, with an emphasis on those with a history of cervical or vulvar precancerous lesions, low CD4 counts, immunosuppression, and a history of receptive anal intercourse.^{37,38} While the Brazilian Ministry of Health recommends annual anal Pap smears for HIV-infected women with a history of “receptive anal sex, HPV infection or abnormal cervical or vulvar histology,”³⁹ findings from our study indicate that women with a low nadir CD4 count or detectable HIV-1 viral load might also benefit from anal cytology testing, consistent with recent recommendations in other international settings.³⁸ Given the distribution of cervical HPV prevalences among our study population, the new V503 HPV vaccine, covering HPV genotypes 6, 11, 16, 18, 31, 33, 45, 52, and 58,⁴⁰ could potentially impact the incidence of cervical HPV among HIV-infected women in Brazil. Additional studies evaluating the epidemiology of anal HR-HPV infection and disease progression may enhance our understanding of the impact of the new HPV vaccine in our and other settings. Further investigation is needed to determine the optimal anal SCC screening protocol and recommendations for HPV vaccination for this population of HIV-infected women.

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