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Association of High-Risk Human Papillomavirus with Genital Tract Mucosal Immune Factors In HIV-Infected Women

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Abstract

Problem—High-risk human papillomavirus (HR-HPV) is prevalent in HIV-infected women and may be associated with mucosal changes that promote HIV replication.

Method of Study—Innate immune molecules, antimicrobial activity, HIV RNA, and HPV DNA genotypes were measured in a cross-sectional study of 128 HIV-infected women categorized into HPV-16 (n=8), other HR-HPV (n=41), and non-HR-HPV controls (n=79).

Results—Compared to controls, HR-HPV groups had higher plasma viral loads (p=0.004), lower CD4 cells (p=0.02), more genital tract HIV RNA (p=0.03), greater number of different HPV types (p<0.001), higher cervicovaginal lavage (CVL) IL-1α (p=0.03) and human beta defensin 2 (HBD2) (p=0.049), and less anti-HIV_{Bal} activity (p=0.03). HPV-16 remained significantly associated with higher HBD2 (p=0.03), higher IL-1α (p=0.009), and lower anti-HIV_{BaL} activity (p=0.03) compared to controls after adjusting for plasma viral load and CD4 T cell count.

Conclusion—HR-HPV is associated with mucosal changes in HIV-infected women that could adversely impact genital tract health.

Keywords

defensins; cervicovaginal immunity; HIV; HPV

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Introduction

Human papillomavirus (HPV) associated cervical dysplasia and cancer are more common among HIV-infected women[1, 2]. HPV is associated with higher HIV plasma viral loads (PVL) and lower CD4 cell counts[1–3]. Moreover, recent epidemiological studies suggest that HPV may facilitate HIV acquisition and transmission. For example, a meta-analysis found that prevalent HPV, independent of type, was associated with an ~2-fold increased risk of HIV acquisition[4]. These epidemiological findings suggest that persistent HPV may promote changes in the local (genital tract) cellular or soluble mucosal environment and facilitate HIV replication. For example, biopsies from high-grade cervical intraepithelial neoplasia (CIN) lesions are often characterized by an infiltration of lymphocyte and macrophages, which are targets for HIV replication[5]. Similarly, increases in pro-inflammatory or reduction in antimicrobial mediators in the genital tract could promote HIV replication and spread. In a prior study, HIV-uninfected women with cervical intraepithelial neoplasia (CIN) displayed significantly higher levels of pro-inflammatory cytokines and lower levels of anti-inflammatory mediators and antimicrobial peptides in cervicovaginal lavage (CVL) compared to Papanicolaou (Pap)-negative controls[6].

These observations in HIV-uninfected women led to the hypothesis that changes in genital tract mucosa associated with HPV could contribute to HIV genital tract shedding in HIV-infected (HIV+) women. Therefore, to explore the link between high risk (HR)-HPV and HIV, we conducted a cross-sectional study among HIV+ women and compared the concentrations of soluble immune mediators and levels of HIV-1 RNA in CVL and plasma and antimicrobial activity of CVL in women with HR-HPV DNA genotypes compared to women with no or non-HR-HPV.

Methods

Participants and sample collection

With approval from the Einstein College of Medicine Institutional Review Board and informed consent from participants, we recruited 128 women. The majority was from the Women's Interagency HIV Study (WIHS), a previously described prospective cohort study of HIV-infected and HIV-uninfected women (122 from Bronx WIHS, 1 from Washinton, DC WIHS, and 5 from a local Bronx clinic)[7–9]. The study visit was scheduled at a time when participants were not menstruating at a median of 32 days after the most recent WIHS visit [IQR 22–43 days]. Sampling included vaginal swabs for pH and Nugent score[10], 10 mL normal saline CVL, endocervical cytobrush for immune cells, and blood for measurements of plasma immune mediators and, for subjects not previously identified as seropositive, HSV serostatus (HerpeSelect 1&2 Immunoblot IgG; Focus Diagnostics Cypress, CA). HIV PVL and CD4 count were obtained from the preceding WIHS visit. Participants with a previously abnormal Pap also underwent colposcopy and biopsy. The CVL were processed within 24 h. CVL were centrifuged at 800 × g for 10 minutes and supernatants were divided into aliquots and stored at −80°C. CVL pellets were suspended in phosphate buffered saline and stored at −80°C. Plasma was divided into aliquots and stored at −80°C.

HPV DNA determination

HPV DNA testing was performed on CVL using a well-established MY09/MY11 PCR method combined with oligonucleotide hybridization for HPV DNA type determination of PCR products [2].

Immune mediators

CVL protein concentration was measured by microBCA™ Protein Assay kit (Thermo Scientific, Rockford, IL). IL-1α, IL-1 receptor antagonist (IL-1ra), IL-1β, IL-2, IL-6, IL-8, IL-17A, IFN-α2, TNF, IFN-γ, MIP-1α, MIP-1β, and RANTES were measured in plasma and CVL by Luminex[100] (Austin, Texas) with beads from Chemicon International (Billerica, MA) and analyzed using StarStation (Applied Cytometry Systems, Sacramento, CA). Concentrations below the lower limit of detection (LLOD) were set at the midpoint between zero and the LLOD. CVL concentrations of secretory leukocyte protease inhibitor (SLPI) (R&D Systems, Minneapolis, MN), human neutrophil peptides 1–3 (HNP1–3) (Hycult Biotech, Uden, the Netherlands), elafin (Hycult Biotech, Uden, the Netherlands), IgA and IgG (Cygnus Tech, Southport, NC), lactoferrin (Calbiochem-EMD Millipore, Billerica, MA), and human beta defensins (HBD) 1, 2, and 3 (Alpha Diagnostics, San Antonio, TX) were determined by enzyme-linked immunosorbent assays (ELISA).

Antimicrobial activity of CVL

CVL antimicrobial activity against HIV-1 (BaL and IIIb), HSV-2 and *E. coli* was measured within 6 months of collection as described previously[11]. For each microbe, the results are presented as the mean percentage inhibition relative to controls.

CVL viral loads

HIV RNA levels were measured in CVL supernatants using the Abbott *m*2000 HIV-1 RealTime System (Abbott Molecular, Des Plaines, IL) with LLOD of 40 copies/ml. CMV DNA was detected in CVL cell pellets using the *artus* CMV TM PCR kit (Qiagen, Inc, Valencia, CA) on the Qiagen Rotor Gene instrument with a LLOD of 300 IU/mL. The amplification target was a 105 base pair region of the immediate-early exon 4 gene of the CMV genome. HSV was detected in CVL cell pellets using a homogenous kinetic PCR designed to amplify a conserved sequence within the polymerase gene *(pol)* of HSV-1 and HSV-2 (Emory Center For AIDS Research).

Statistical analysis

Participants were initially categorized into five groups based on a hierarchical categorization of HPV type: HPV-16, other HR-HPV (HPV-18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68), intermediate-risk-HPV (HPV-53, 82, and 26), low-risk-HPV or no HPV[12]; the latter three groups were subsequently consolidated as non-HR controls. ART adherence was ascertained by self-report and dichotomized at < vs. ≥95% taking of prescribed doses. Concentrations of mediators were $\log_{10}$ transformed where appropriate. For mediators in which >25% of samples were below the LLOD, the data were dichotomized at the LLOD. For participants with a detectable PVL, the data was dichotomized at the median. Categorical variables were compared between groups by chi-square or Fisher's exact tests.

Continuous variables were compared by t-tests or Mann-Whitney's U tests. Spearman correlation coefficients were calculated to assess associations between mediators and antimicrobial activity. Differences between anti-HIV_{IIIb} activity and anti-HIV_{BaL} activity were compared by Wilcoxon signed-rank tests. Nonparametric analysis of covariance and logistic regression models were used to compare differences in immune mediators between HR and non-HR HPV groups adjusted for PVL dichotomized at the LLOD and CD4 count. Statistical analyses were performed using SAS version 9.3 (Cary, NC) and two-tailed p values <0.05 were considered significant.

RESULTS

Participant characteristics

128 HIV+ women enrolled in the study of whom 8 (6.3%) were positive for HPV-16 DNA, 41 (32.0%) for other HR-HPV types, 14 (10.9%) for intermediate risk-HPV, and 33 (25.8%) for low-risk-HPV; 32 (25.0%) were HPV-negative. The five groups did not differ with respect to age, race, current smoking, Nugent scores, use or adherence to antiretroviral therapy (ART) or HSV serostatus (Table 1). The majority were both HSV-1 and HSV-2 seropositive. Participants with HR-HPV were more likely to have abnormal Pap results; HGSIL was identified in 50% and 12.2% of the HPV16 and other HR-HPV groups, respectively, and LGSIL or atypical cells (ASCUS) was identified in 50% and 46.3% of the HPV-16 and other HR-HPV groups, respectively. In contrast, 68.4% of the consolidated controls (intermediate risk-HPV, low-risk-HPV, or HPV-negative groups) had normal Pap results (Table 1).

HR-HPV was associated with increased mucosal, but not systemic inflammatory immune mediators

We selected a panel of cytokines/chemokines and antimicrobial peptides for measurement based on prior mucosal studies[6, 9, 13–15]. There were no differences among intermediate, low-risk, or HPV-negative groups and thus these three were consolidated into a non-HR-HPV control group (n=79) to provide greater statistical power for comparisons with the HPV-16 and other HR-HPV groups (Supplemental Table 1). There was a trend or statistically significant increase in CVL concentrations of several pro-inflammatory cytokines including IL-1α (p=0.03), IL-1ra (p=0.15), TNF (p=0.13), IL-6 (p=0.13), and IL-8 (p=0.15) in participants with HPV-16 and/or other HR-HPV compared to controls (Table 2). There was also a significant increase in HBD2 (p=0.049) in the HR-HPV groups compared to controls and trend towards increased IFN-γ in HPV-16 participants (p=0.15). HBD2 has antimicrobial properties, but is also pro-inflammatory. No statistically significant differences were detected in concentrations of plasma cytokines or chemokines (Table 3).

HPV-16 was associated with lower CVL HIV inhibitory activity

Measurements of individual immune mediators may not reflect complex interactions between these molecules. A complementary approach, therefore, is to measure the antimicrobial activity of CVL against HIV, HSV and *E. coli*. Prior studies have shown that genital tract secretions have endogenous inhibitory activity against these pathogens *in vitro*, presumably reflecting contributions from molecules expressed by host epithelial or immune

cells and vaginal microbiota[16–21]. In HIV-infected women, ART may also contribute to the antiviral activity of CVL. Overall, the median [IQR] % inhibition of CVL against HIV_{IIIb} (X4 virus) was greater than against HIV_{BaL} (R5 virus) (87 [62.5, 97.0] vs. 57 [14.2, 86.8], p< 0.001). There was significantly lower anti-HIV activity in women with HR-HPV with the least inhibition in the women with HPV-16 and with several HPV-16 participants having CVL showing enhancement (−18.2 [−125.8–57.2]) (Table 2). The inhibitory activity against HIV_{IIIB} correlated positively with the activity against BaL (r=0.66, p<0.001) and negatively with PVL (r=−0.39, p< 0.001), but did not correlate with HIV genital tract shedding. Anti-HIV_{IIIb} activity also correlated modestly with HNP1–3 (r=0.25, p=0.004) and elafin (r=0.26, p=0.004). Genital tract or plasma drug levels are not available but women in all groups reported high adherence to ART.

There were no significant differences in the percent inhibition of HSV or *E. coli* by CVL obtained from women with HPV-16 or other HR-HPV types compared to controls, although overall the activity against both pathogens was substantially lower than what has been previously seen for HIV-uninfected women[17, 22]. The anti-HSV activity correlated modestly and significantly with CVL protein (r=0.34, p<0.001), IL-6 (r=0.31, p<0.001), RANTES (r=0.31, p< 0.001), and IgA (r=0.35, p<0.001) and the anti-*E. coli* activity correlated modestly and significantly with CVL protein (r=0.41, p<0.001, RANTES (r=0.22, p=0.01) and IgA (r=0.25, p=0.005).

HR-HPV was associated with higher HIV plasma viral load (PVL) and genital tract HIV and HSV shedding

Although there were no differences in ART use or self-reported adherence (Table 1), the HR-HPV groups had higher HIV PVL (p<0.0001) and lower CD4 T cells counts (p=0.005) compared to controls. Moreover, HIV RNA was detected in the genital tract more often in women with HR-HPV (Table 1) compared to controls (9/49 vs. 4/79; p=0.03), although all of the women in the former were in the other HR-HPV group. HR-HPV (both HPV16 and other HR types) were also more likely to have HSV DNA detected in CVL (8/37 vs. 4/63 (p=0.05). However, in a model that included PVL and CD4 count, the association between HR-HPV and HIV or HSV genital tract shedding was no longer significant. HPV-16 remained significantly associated with higher HBD2 (p=0.03), higher IL-1α in (p=0.009), and lower anti-HIV_{BaL} activity (p=0.03) compared to the controls after adjusting for PVL and CD4 count.

Increase in the number of HPV types detected was associated with changes in soluble mucosal immune environment

The median [IQR] number of different HPV types detected in CVL was 1 [0, 1] in the no-HR-HPV group compared to 3 [2,4] in the other HR-HPV group and 5.5 [3, 8.5] in the HPV-16 group. To explore whether number of HPV types (regardless of oncogenicity) was also associated with changes in mucosal immune environment, we categorized participants into the following groups: 4 or more HPV types (n=26), 2–3 HPV types (n=35), 1 HPV type (n=35) and no HPV (n=32) (Table 4). Women with 4 or more HPV types had the highest PVL (p=0.01), lowest CD4 count (p=0.02), highest CVL IL-1α (p=0.01) and highest HBD2 (p=0.02). However, there were no significant differences in the anti-HIV activity of CVL

between these groups. These findings suggest that both the total number of HPV types present and the specific type, particularly HPV-16, contribute to mucosal changes.

DISCUSSION

The hypothesis that HPV is associated with changes in mucosal immunity that could contribute to HIV replication emanated from our prior study conducted among HIV-uninfected women in which we observed increases in pro-inflammatory and decreases in anti-inflammatory proteins in CVL in women with HR-HPV compared to those with a normal Pap test[6]. Results obtained here support this hypothesis. Specifically, we observed increases in pro-inflammatory cytokines as well as in HBD2 in women with HR-HPV compared to HIV-infected controls. The statistically significant increase in IL-1α and HBD2 persisted after adjusting for PVL and CD4 T cell count, particularly in women who harbored HPV-16 or multiple HPV types. Notably, HBD2 was decreased in HIV-uninfected women with HR-HPV compared to controls[6], whereas in this HIV-infected cohort, coinfection with multiple HPV types or HPV-16 was associated with increased HBD2. The biological significance of changes in HBD2 are difficult to predict because, while defensins have *in vitro* antiviral activity against HIV and HPV, these molecules are also associated with inflammation, which could promote HIV replication[23, 24]. The observation that HPV-16 was also associated with reduced CVL inhibitory activity against HIV_{BaL} suggests that the increase in HBD2 may be a biomarker of increased inflammation rather than augmented antiviral activity. Notably, higher genital tract HBD2 levels were associated with HIV seroconversion in a small study further suggesting that it may serve as a marker of inflammation[25].

HR-HPV was also associated with a decrease in anti-HIV activity of CVL, which likely reflects cumulative effects of ART, innate immune molecules and possibly the vaginal microbiome [20, 26, 27]. The differences between HR-HPV and controls observed here are unlikely to reflect ART as a high level of adherence to ART was reported by all of the participants, although we were not able to measure drug levels. The anti-HIV_{IIIb} activity correlated modestly with HNP1–3 and elafin, molecules that have anti-HIV activity *in vitro*[28–30], but did not correlate with Nugent scores and the Nugent score did not differ between groups. Notably, CVL from some of the HPV-16 infected women enhanced HIV infection *in vitro*. Enhancement has been observed in a small percentage of women in other studies. For example, in a substudy of women participating in a tenofovir preexposure prophylaxis study in the United States, 16% of the baseline CVL samples (n=61) enhanced HIV_{Bal} infection[14]. HPV DNA was not measured in that study.

The cross sectional design of this study precludes determining causality. The finding of a higher PVL and increased HIV genital tract shedding in the HR-HPV cohort and among women harboring more HPV types suggests either that impaired control of HIV (even in the setting of ART adherence) predisposes to HPV persistence and/or persistent HPV and the associated immune response contributes to HIV replication or interferes with systemic immunological control. However, the association between HR-HPV and HIV genital tract shedding did not retain significance when PVL and CD4 cell count were included in the model, suggesting that HR-HPV infection may not have a causal role. A larger, prospective

study is needed to address these questions of causality and of mechanisms at the mucosa. Additional limitations that could be addressed in future studies include the absence of vaginal microbiome data, mucosal or systemic immune cell function, and ART drug levels. Importantly, the findings support the possibility that the adverse interaction between HPV and HIV is bidirectional and suggest that screening for HPV may have implications for HIV disease in addition to its role in cervical cancer.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Table 1

Demographic and clinical data on participants

Variable	HPV Negative (n = 32)	Low-risk HPV (n=33)	Intermediate-risk HPV (n=14)	HR-HPV (excluding HPV16) (n=41)	HPV16 (n=8)	p-value
Age, (mean ± SD)	49.3 ± 8.1	50.8 ± 8.3	50.9 ± 5.4	48.7 ± 8.5	48.6 ± 9.8	0.794
Race, n (%)						0.073
White	3 (9.4%)	4 (12.1%)	3 (21.4%)	5 (12.2%)	2 (25%)	
Black	18 (56.2%)	21 (63.6%)	10 (71.5%)	31 (75.6%)	2 (25%)	
Other	11 (34.4%)	8 (24.3%)	1 (7.1%)	5 (12.2%)	4 (50%)	
Pap result, n (%)						0.016
Normal	24 (75%)	22 (66.7%)	8 (57.2%)	17 (41.5%)	2 (25%)	
ACUS/LGSIL	6 (18.8%)	11 (33.3%)	5 (35.7%)	19 (46.3%)	4 (50%)	
HGSIL	2 (6.2%)	0	1 (7.1%)	5 (12.2%)	2 (25%)	
Current smoker, n (%)	12 (37.5%)	11 (34.4%)	9 (64.3%)	19 (46.3%)	6 (75%)	0.132
Currently on ART, n (%)	27 (84.4%)	32 (97.0%)	11 (78.6%)	34 (85%)	8 (100%)	0.197
ART adherence ≥ 95%, n (%)	23 (76.7%)	29 (87.9%)	10 (71.4%)	29 (76.3%)	8 (100%)	0.352
HSV-1 seropositive, n (%)	24 (75%)	26 (81.3%)	10 (71.4%)	29 (70.7%)	5 (62.5%)	0.745
HSV-2 seropositie, n (%)	29 (90.6%)	30 (93.8%)	14 (100%)	40 (97.6%)	8 (100%)	0.657
Vaginal pH, median [IQR]	5.4 [5 – 6]	5 [4.5 – 5.5]	5.5 [5 – 5.5]	5.5 [5 – 5.5]	5.3 [4.8 – 6]	0.561
Nugent Score ≥ 7, n (%)	5/25 (20%)	11/28 (39.3%)	2/12 (16.7%)	12/34 (35.3%)	3/6 (50%)	0.309
CD4 cells/mm^3 median [IQR]	520 [383.5 – 672]	488 [401.5 – 744]	450.5 [350 – 637]	409 [267–529]	275 [225.5–464.5]	0.021
* Plasma viral load, n (%)						0.004
≤ 20 copies/ml	23 (71.9%)	19 (61.3%)	7 (50%)	15 (36.6%)	3 (37.5%)	
< 15,800 (median)	7 (21.9%)	11 (35.5%)	7 (50%)	14 (34.2%)	5 (62.5%)	

Variable	HPV Negative (n = 32)	Low-risk HPV (n=33)	Intermediate-risk HPV (n=14)	HR-HPV (excluding HPV16) (n=41)	HPV16 (n=8)	p-value
≥ 15,800 (median)	2 (6.2%)	1 (3.2%)	0	12 (29.3%)	0 (0%)	
		Genital tract viral shedding (CVL), n(%)				
HIV RNA	1 (3.1%)	2 (6.1%)	1 (7.1%)	9 (22%)	0 (0%)	0.084
HSV DNA	1 (4.6%) (n=22)	2 (7.4%) (n=27)	1 (7.1%) (n=14)	7 (22.6%) (n=31)	1 (16.7%) (n=6)	0.256
CMV DNA	1 (4.8%) (n=21)	0 (n=26)	0 (n=14)	1 (3.5%) (n=29)	1 (20%)(n=5)	0.226

* PVL was dichotomized at the median of 15,800 copies/ml.

Table 2

Immune molecules and antimicrobial activity in cervicovaginal lavage samples.

Variable (median [IQR])	Non-HR-HPV (n=79)	HR-HPV (n=41)	HPV-16 (n=8)	p-value
Protein (log10 μg/mL) mean ± SD	2.33±0.3	2.37±0.37	2.45±0.31	0.54
IL-1α (pg/mL)	62.2 [21.9–235.9]	97.2 [36.3–275]	195.7 [102.6–1234.1]	0.03
IL-1β (log10 pg/mL)	0.43±0.69	0.57±0.82	0.85±1.09	0.27
IL-1ra (pg/mL)	6026.2 [2975.2–8855.8]	6446.0 [4516.6–8319]	8644.1 [7414.1–10000]	0.15
TNF, n (%)	38 (50%)	21 (52.5%)	7 (87.5%)	0.13
IL-6, n (%)	42 (55.3%)	27 (67.5%)	7 (87.5%)	0.13
IL-2, n (%)	9 (14.8%)	8 (27.6%)	1 (20%)	0.24
IL-17, n (%)	45 (64.3%)	19 (51.4%)	6 (75%)	0.30
IFN-α2, n (%)	0 (0%)	2 (6.9%)	0 (0%)	0.19
IFN-γ (pg/mL),	0.53 [0.27–1.03]	0.71 [0.41–1.63]	1.26 [0.53–2.73]	0.15
IL-8 (log10 pg/mL)	2.45±0.75	2.34±0.75	2.91±0.8	0.15
MIP-1α n (%)	47 (61.8%)	18 (45%)	4 (50%)	0.24
MIP-1β n (%)	24 (31.6%)	9 (22.5%)	2 (25%)	0.62
RANTES, n (%)	41 (54%)	20 (50%)	5 (62.5%)	0.80
IgA (ng/mL)	1340 [411–3136]	1421 [412–4139]	3084.5 [503.5–4126.5]	0.60
IgG (ng/mL)	8262.5 [2807–16968]	11522 [4926–19125]	9509 [5008.5–41907]	0.50
Elafin (ng/mL)	103.9 [27.7–2113.4]	43.9 [17.6–190.7]	86.8 [54.6–475.7]	0.24
Lactoferrin (ng/ml)	409.5 [115–959.7]	334.1 [50–694.7]	1000.6 [145–3385.1]	0.43
SLPI (pg/mL)	59808 [11246–175179]	66402 [3750–266049]	32120 [3750–100875]	0.58
HNP1–3 (log10pg/mL)	5.07±0.56	4.86±0.69	5.05±0.85	0.22
HBD1 (pg/mL)	495.6 [167–2179.9]	503 [210.5–2317.9]	484.2 [253–654.1]	0.86
HBD2 (pg/mL)	387.6 [107.4–1610.5]	812.9 [145.5–2349.1]	1794.0 [962.6–6261.9]	0.049
HBD3, n (%)	49 (63.6%)	27 (65.9%)	4 (50%)	0.66
E. coli inhibition (%)	14 [2–32]	10 [−7–31]	−4 [−19–30.5]	0.19
HSV inhibition (%)	20 [6–44]	29 [2–45]	18 [12–26]	0.73
HIVBaL inhibition (%)	60.3 [29.5–90]	48 [9–80.5]	−18.2 [−125.8–57.2]	0.03
HIVIIIb inhibition (%)	88.25 [71–98]	84.7 [27–96.7]	57.8 [22.3–85]	0.03

CVL IL-6, IFN-α2, MIP-1α, MIP-1β, RANTES, IL-2, TNF, IL-17, and HBD-3 with > 25% of samples below the lower limit of detection (LLOD) were dichotomized at the LLOD and are presented as n (%) with detectable levels.

Table 3

Plasma immune mediators

Variable (median [IQR])	Non-HR HPV (n=76)	HR HPV (n=40)	HPV16 (n=8)	p-value
IL-2 (pg/mL)	0.57 [0.26–0.76]	0.57 [0.15–0.99]	0.87 [0.31–1.34]	0.55
IL-6 (pg/mL)	1.61 [0.67–6.32]	1.67 [0.87–3.97]	3.2 [1.66–5.74]	0.48
MIP-1β (pg/mL)	24.4 [14.5–42.4]	21.0 [16.6–35.4]	21.9 [18.3–34.5]	0.93
IL-8 (pg/mL)	17.9 [7.9–80.5]	15.5[8.3–185.4]	12.9 [5.9–72.0]	0.69
TNF (pg/mL)	6.63 [4.18–9.91]	6.3 [4.86–10.47]	7.17 [5.12–13.68]	0.57
IFN-γ (pg/mL)	2.49 [1.37–4.27]	1.83 [0.49–3.33]	2.21 [1.98–4.09]	0.12
IL-1α, n (%)	11 (14.5%)	3 (7.5%)	0 (0%)	0.45
IL-1β, n (%)	33 (43.4%)	15 (37.5%)	4 (50%)	0.73
IFN-α2, n (%)	44 (57.9%)	15 (37.5%)	4 (50%)	0.12
IL-1ra, n (%)	20 (32.8%)	9 (28.1%)	1 (20%)	0.96
RANTES, n (%)	43 (56.6%)	27 (67.5%)	6 (75%)	0.37
MIP-1α, n (%)	54 (71.1%)	22 (55%)	4 (50%)	0.14
IL-17, n (%)	51 (67.1%)	27 (67.5%)	7 (87.5%)	0.49

Plasma IL-1α, IL-1β, IFN-α2, IL-1ra, RANTES, MIP-1α, and IL-17 with > 25% of samples below the lower limit of detection (LLOD) were dichotomized at the LLOD and the n (%) of participants with detectable levels are shown.

Table 4

Clinical variables, immune molecules and antimicrobial activity in cervicovaginal lavage following categorization of participants by number of different types of HPV detected.

Variable	0 HPV types (n=32)	1 HPV types (n=35)	2–3 HPV types (n=35)	≥4 HPV types (n=26)	p-values
Pap result, n (%)					0.002
Normal	24 (75%)	24 (68.6%)	19 (54.3%)	3 (23.1%)	
ACUS/LGSIL	6 (18.8%)	9 (25.7%)	13 (37.1%)	17 (65.4%)	
HGSIL	2 (6.2%)	2 (5.7%)	3 (8.6%)	3 (11.5%)	
CD4 cells/mm^3 median [IQR]	520 [383.5 – 672]	458 [315 – 573]	473 [349 – 737]	310.5 [209 – 513]	0.02
* Plasma viral load, n (%)					0.01
≤ 20 copies/ml	23 (71.9%)	21 (61.8%)	17 (50%)	6 (23.1%)	
< 15,800 (median)	7 (21.9%)	11 (32.3%)	12 (35.3%)	14 (53.8%)	
≥ 15,800 (median)	2 (6.2%)	2 (5.9%)	5 (14.7%)	6 (23.1%)	
IL-1α (pg/mL)	64 [24.1–116.1]	61.2 [19.0 – 111.7]	68.4 [28.4 – 303.8]	218.1 [68.8 – 671.7]	0.01
IL-8 (log10 pg/mL)	2.43 ± 0.85	2.50 ± 0.72	2.29 ± 0.67	2.59 ± 0.82	0.47
IFN-γ (pg/mL),	0.77 [0.32–1.07]	0.51 [0.26–0.90]	0.64 [0.30 – 1.07]	0.78 [0.32–3.66]	0.18
SLPI (pg/mL)	102950 [15225–243259]	43399 [3750–163226]	33255 [3750–207490]	64671 [10659–221049]	0.51
HBD-2 (pg/mL)	349.2 [142–758.8]	245.7 [47.9–1237.6]	567.8 [269.6–2226]	1624.8 [166.2–3187.5]	0.02
HNP1–3 (log10pg/mL)	5.15 ± 0.49	5.07 ± 0.66	4.96 ± 0.65	4.78 ± 0.67	0.14
E. coli inhibition (%)	22 [7 – 34.5]	13 [1.5 – 31]	8 [−5 – 28]	12.5 [−9 – 32]	0.25
HSV inhibition (%)	23 [8.7–48]	15 [4.2 – 47]	22 [4 – 45]	14.5 [0 – 36]	0.49
HIVBaL inhibition (%)	57 [29–91]	58.8 [8.5 – 86.5]	59 [15 – 87]	47.7 [−37.5 – 61.5]	0.50
HIVIIIb inhibition (%)	87 [64–97]	91.7 [74 – 99]	81.9 [61 – 96.3]	83.3 [44 – 96.7]	0.31

* PVL was dichotomized at the median of 15,800 copies/ml.