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ORIGINAL RESEARCH

Associations of HIV RNA Viral Load, CD4 Counts, HPV Detection, and Cytology Support Co-Testing for Cervical Cancer Screening Among Women Living with HIV

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Introduction: This study addresses a research gap in cervical cancer screening for Women Living with HIV (WLHIV) in Puerto Rico, evaluating the associations between viral load (VL) thresholds, CD4 count, HPV detection, and the benefits of co-testing with cytology.

Methods: We analyzed the HIV viral load (VL), HPV infection, CD4 counts, and cervical cytology in 354 WLHIV receiving consecutive comprehensive care, including Highly Active Antiretroviral Therapy (HAART). Retrospective data were obtained from visits from 2018 to 2023. Consecutive visits with ThinPrep co-testing for cytology and HPV were evaluated. Only one observation per woman was included to maintain a cross-sectional dataset. HPV was detected using Hologic ThinPrep Pap Test and PCR-based genotyping.

Results: The prevalence of any HPV was 27% which correlated inversely with CD4 counts: 42.9% (≤200 cells/mm^3), 35.5% (201–499 cells/mm^3), and 23.2% (>500 cells/mm^3) (p=0.017). Multivariate analysis demonstrated that HPV positivity was significantly associated with the likelihood of having an abnormal Pap result (OR = 6.35; 95% CI: 3.22–12.52; p < 0.001). Cytologic abnormalities were more frequent with lower CD4 counts: 32.1% (≤200 cells/mm^3), 11.8% (201–499 cells/mm^3), and 11.2% (>500cells/mm^3) (p=0.007). HPV was detected more frequently among women with detectable VL > 201 copies/mL (35.4% vs 26.1%). Abnormal cytology occurred in 22.9% of women with VL > 201 copies/mL versus 11.4% with VL ≤ 200 copies/mL (p=0.028) and among 34.8% of women with negative HPV tests, underscoring the value of co-testing.

Conclusion: WLHIV demonstrate higher HPV prevalence and abnormal cytology rates, with persistence inversely related to CD4 counts. Viral suppression correlates with lower HPV prevalence and fewer cytologic abnormalities. Co-testing identified 34.8% of WLHIV with abnormal cytology and HPV-negative results. This study supports consideration of co-testing for cervical cancer screening in WLHIV, even among those with high ART uptake. Further prospective and multi-site studies are warranted to confirm these observations and inform policy or guideline changes.

Keywords: human immunodeficiency virus-HIV, human papilloma virus-HPV, CD4 count, viral load, cervical cancer, immunization, co-testing

Introduction

According to the American Cancer Society's Global Cancer Facts (2024), cervical cancer is becoming more common among younger women, particularly those aged 30 to 44. However, women aged 20 to 24 who were part of the initial

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group to receive the Human Papilloma Virus (HPV) vaccine have experienced an annual decrease in cervical cancer rates by 11% between 2012 and 2019. Hispanic individuals tend to have lower rates of common cancers like breast and prostate cancer compared to non-Hispanic Whites, but cervical cancer—driven by HPV—is 35% more common in Hispanic women than White women.[1] This difference highlights persistent disparities and the need for targeted prevention efforts.

Cervical cancer is most frequently diagnosed between ages 35 and 44, with an average diagnosis age of 50, and rarely develops in women younger than 20. For 2024, the American Cancer Society estimates 13,820 new cases of invasive cervical cancer in the US, with 4360 deaths. Cervical pre-cancers, however, are diagnosed far more often than invasive cancers,[2] emphasizing the importance of early detection.

Recent data from Puerto Rico indicate significant local trends. As reported by AP Ortiz et al,[3] cervical cancer incidence per 100,000 person-years rose from 9.2 to 13.0 between 2001 and 2017, especially among women younger than 35 up to 2010, and an overall increase among those aged 35–64. By comparison, US incidence across all women was 7.1 per 100,000, rising to 10 and 9 per 100,000 in Hispanic and African American women.

Human Papilloma Virus (HPV)-Related Cancers and Human Immunodeficiency Virus (HIV)

Cervical cancer is one of the most HPV-related cancers worldwide, with approximately 530,000 new cases annually. Half of these cases affect women under 50 and are concentrated in less developed regions, particularly South-Eastern Asia, Latin America, and sub-Saharan Africa. HPV infection is the main risk factor for cervical cancer, causing 570,000 cancer cases in women (8.6% of all female cancers) and 60,000 in men each year (0.8% of global cancer cases).[4]

Women living with HIV (WLHIV will be used in document) are six times more likely to develop cervical cancer compared to HIV-negative women, driven by higher prevalence and persistence of HPV, likely due to immunosuppression. Persistent HPV infection, if untreated, causes 95% of cervical cancers; progression from abnormal cells to cancer typically takes 15–20 years, but accelerates to 5–10 years in immunosuppressed women, particularly those with untreated HIV. HIV infection consequently increases the risk and mortality of cervical cancer, making it an AIDS-defining illness.[5] Data from the NHANES survey indicated 27% of US women had cervical HPV DNA, while among WLHIV, prevalence ranged from 40–70%.[6–8] WLHIV have nearly twice the risk of dying of cervical cancer compared to HIV-negative women. These risks are extreme in sub-Saharan Africa, which bears 85% of the global WLHIV-cervical cancer burden. As ART expands life expectancy among HIV-positive women, enhancement of cervical cancer screening and prevention is increasingly urgent, especially in resource-limited settings.[9–12] Our data from a retrospective analysis of consecutive visits among WLHIV adds to the known HIV-HPV interactions. The interaction between HPV and HIV presents a major public health challenge globally and regionally.

HPV and HIV Prevalence in Puerto Rico

Puerto Rico ranked 7th in HIV prevalence among adults/adolescents in 2019 and 14th in new diagnoses. By 2022, 16,568 people were living with HIV in Puerto Rico, with 9% undiagnosed. Three-quarters are age 45 or older.[13]

HPV prevalence in the general female population of Puerto Rico ranges from 29.4% to 45.5% by study, and among WLHIV, the rate is even higher. We published a 50.3% HPV prevalence from 302 consecutive women sampled between 2009 and 2010 for any HPV type; 41.1% carried low-risk types, and 29.5% high-risk types, primarily HPV 52 (8.3%) and HPV 16 (6%).[7] In analyses from both cervical and anal samples, 21% were positive at both sites, 19% had anal HPV alone, and 13% had cervical HPV alone, highlighting the multiplicity of site involvement.[8] With Puerto Rico's elevated HIV prevalence, there are increasing concerns about concurrent HIV and HPV infections and resulting cervical cancer risk.

Cervical Cancer Screening and HPV Vaccination in the U.S

Cervical cancer screening programs and the HPV vaccine have both successfully lowered the incidence of cervical cancer and precancerous lesions attributable to HPV types 16 and 18. Since the vaccine's introduction, rates of squamous cell carcinoma and adenocarcinoma have declined among young women (aged 15–20), a group seldom screened for cancer.

HPV vaccination was first recommended in 2006 for girls aged 11–12 years, and the current 9-valent vaccine (since 2015) covers more high-risk types. Routine vaccination is advised at age 11–12 (catch-up through age 26); vaccination for older adults (up to age 45) is based on shared decision-making. Coverage with 2–3 doses among girls aged 13–17 increased from 29% (2008) to 59.9%.2023,[14] The screening intervals for HIV-positive women in the US are every three years after three normal results, with WHO recommending 3–5 years using primary HPV DNA testing for WLHIV (compared to 5–10 years for the general population). These changes, plus guidance from other professional organizations, consider AIDS-related risks and co-infections.[15–17]

HIV Treatment: Viral Load, CD4, and Cervical Cancer Risk

For individuals with HIV, CD4 counts and viral load (VL) are essential markers for disease management and cancer risk stratification. A CD4 count below 200 cells/μL is considered AIDS-defining and increases susceptibility to opportunistic infections and HPV-associated cancer; ART and monitoring restore immune function, reducing HPV risk. Those with CD4 between 200 and 500 remain vulnerable to complications, requiring ongoing care, while CD4 over 500 provide more robust protection. The HIV RNA VL (copies/mL) measures disease activity. Suppressed VL (undetectable, typically <20–50 copies/mL) is the target of ART and means no sexual transmission of HIV ("U=U"). High VL (>10,000 copies/mL) signals active viral replication, faster disease progression, and greater transmissibility. Links between low CD4, high VL, and HPV positivity are well described, directing attention to tailored screening and care for WLHIV.[18,19]

Gaps and Study Rationale

There is a lack of longitudinal, clinic-based data evaluating associations between HIV control (VL/CD4), HPV detection, and abnormal cytology among Women Living with HIV (WLHIV) in Puerto Rico, a setting with distinct epidemiologic characteristics and barriers to prevention. Importantly, this study analyzes the population per patient, using each woman's most recent paired HPV/cytology and virologic data, in keeping with reviewer suggestions and contemporary guidelines. The retrospective data is based on consecutive clinic visits of a cohort of WLHIV in care, with access to ART, laboratories, and gynecologic services. Changes in ART patterns and viral suppression over time in Puerto Rico may influence HPV epidemiology, affecting results and their broader generalizability. These factors support the need for locally informed approaches to cervical cancer prevention and screening.

The primary goal of this study was to evaluate the relationship between CD4 counts, HIV RNA Viral Load (VL), Human Papillomavirus (HPV) status, and cervical cytology among women living with HIV (WLHIV) receiving comprehensive care, including Highly Active Antiretroviral Therapy (HAART). The study aimed to provide insight into immune status (CD4), the prevalence of HPV infection in the cervicovaginal area, and how viral control and immune function affect HPV persistence and cervical disease risk. We specifically assessed whether abnormal cervical results occurred in the absence of HPV detection, and with what frequency. The study also explored discrepancies in immune recovery after ART, specifically cases where patients exhibited simultaneously low CD4 counts and low HIV VL, suggesting poor immune reconstitution even after suppression of HIV replication.

Beyond simple relationship analysis, associations were determined between HPV co-infection, CD4 count, and VL in women with HIV. HPV co-infection may further compromise immunity and accelerate HIV progression. By analyzing these interactions, the research aims to guide clinical care for HIV-positive women, particularly regarding HPV-related outcomes, and to assess whether co-testing (HPV + cytology) offers improved sensitivity to premalignant lesions, an important consideration as screening recommendations evolve. Additional objectives included evaluating the impact of co-testing compared to HPV-alone testing, given current recommendations favor HPV as a primary screen, especially for high-risk women such as WLHIV.

Materials and Methods

The retrospective data were collected from consecutive clinic visits of 354 women. The women are provided comprehensive services as part of a women's HIV clinic, which follows approximately 550 women annually. The women receive Gynecologic evaluations including family planning services, HIV management, including antiviral therapy (ART), laboratory monitoring including CD4 counts, HIV RNA quantitative viral load, Hepatitis and other STI screening,

case management, and mental health interventions, among other services. Laboratories are performed every six months, and Genital tract evaluations, such as Thin prep and HPV co-testing, are performed annually as per Ryan White performance measures.[20–22]

We selected the VL threshold of HIV RNA of 200 copies or less for the main analyses, since it is also a performance measure defined by HRSA and by the ACTG Guidelines.[23] We analyzed the relationship between HIV viral load (VL), HPV infection, CD4 counts, and cervical cytology in 354 WLHIV receiving consecutive comprehensive care, including Highly Active Antiretroviral Therapy (HAART). Consecutive visits with ThinPrep co-testing for cytology and HPV were evaluated. The initial total sample included 829 women evaluated from 2018 to 2023. After excluding patients with missing data, the analysis was performed with complete data from 354 women.

Study Design and Data Collection

A descriptive analysis of a longitudinal data extraction was conducted to determine the CD4 count, Viral Load and HPV status of 829 women who are or have received care at the Maternal-Infant Studies Center (CEMI– Spanish acronym) in Puerto Rico between 2018 and early 2023.

For inclusion in the study, women were required to have CareWare (CW) data, as well as documented CD4 count, viral load, HPV and pap smear results between 2018 and early 2023, with no more than 6 months between any of these results.

Exclusion criteria included male patients, individuals younger than 9 years old, deceased individuals, those without CW data, or those whose laboratory results were collected more than 6 months apart. The 6-month interval was established to ensure that the data points analyzed were contemporaneous and representative of a specific period, thereby strengthening the validity and reliability of the findings. Additionally, only one observation per woman was included to maintain a cross-sectional dataset and avoid repeated measures in the analysis.

Patient HPV/HIV profiles were extracted from CW, an electronic health record system used by CEMI since 2012 to store patient information. Data was retrieved from CAREWare (Ryan White HIV/AIDS provider platform, used since 2012) and cross-checked for completeness and eligibility. The cervicovaginal and anal samples were collected using the ThinPrep® Pap Test, though only cervicovaginal results were analyzed for this study. All patients were tested by the same protocol. ThinPrep Genesis processor ensured sample automation and chain of custody.

ThinPrep® and HPV Genotyping Detailed Methodology (from Package Insert)

The ThinPrep® Cervicovaginal Cytology Collection Vial is an international standard for cervical sample collection and preservation, trusted by healthcare professionals worldwide. Over 1 billion ThinPrep vials have been used globally for cervical cancer screening. Only one sample is needed for cytology and molecular testing. The equipment used was a Cobas 6800 (Manufacturer – Roche Kit – HPV).

Intended use: cobas® HPV for use on the cobas® 6800/8800 Systems (cobas® HPV) is a qualitative in vitro test for the detection of Human Papillomavirus in clinician-collected cervical specimens using an endocervical brush/spatula or broom and placed in the ThinPrep® Pap Test™ PreservCyt® Solution. This test detects the high-risk HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68. cobas® HPV is indicated for use in routine cervical cancer screening as per professional medical guidelines, including triage of ASC-US cytology, co-testing (or adjunctive screen) with cytology, and HPV primary screening of women to assess the risk for cervical precancer and cancer. Patients should be followed up in accordance with professional medical guidelines, results from prior screening, medical history, and other risk factors.

Limits of Detection (LoD) at the clinical cutoff for HPV16 and HPV18 was assessed using SiHa and HeLa cell lines in the background of pooled HPV negative patient specimens. Cell lines were diluted to concentrations below, above and at the expected LoD levels. A minimum of 24 replicates were tested for each cell line level using 3 reagent lots with an equal number of runs performed on the cobas® 6800 and the cobas® 8800 Systems.

The LoD was defined as the level of HPV DNA in the sample that has positive test results at least 95% of the time with a concentration above the clinical cutoff. The LoD for SiHa and HeLa was 16 cells/mL.

The data extracted from the CareWARE data of each patient included their latest CD4 count, viral load, and most recent pap-smear results. Data was acquired for all 354 eligible patients and then cross-checked before it was considered primed for analysis.

By adhering to these rigorous data selection criteria and leveraging the capabilities of the CW system, the study aimed to ensure the inclusion of pertinent and reliable data points within the specified timeframe, ultimately enhancing the quality and validity of the research findings.

As such, the sample size comprised all eligible cases identified during the study period, reflecting the entire accessible patient population.

Target Population Description

The Maternal Infant Studies Center (CEMI) at the UPR School of Medicine, Medical Sciences Campus (UPR-MSC), is the largest and oldest outpatient clinic for women with HIV in Puerto Rico. Providing services since 1987 to over 2,500 women, the clinic currently serves about 550 women yearly. CEMI's multidisciplinary model includes medical and behavioral health, IPV screening and referrals, family planning, case management, and is supported by Ryan White Part D and A funding. Possible selection bias includes the geographic location of the clinic (in the San Juan area), but services are available to women from any part of the territory. The age of the participants (older than 40) is consistent with the aging of the population and the aging of PLHIV. The study cohort reflects the overall clinic patient profile described as follows.

A 2023 cohort review showed 426 WICY (women, infants, children, and youth) patients attended CEMI, with 96.9% HIV-positive and 3.1% HIV-indeterminate (children <2 years). Most patients were women (94.6%), and 44.8% were over age 54. Nearly all (97.0%) identified as Hispanic, and 96.7% lived below the federal poverty level, with 80.7% having government insurance, and all patients reporting stable housing. Transmission was primarily heterosexual (88.5%), followed by perinatal (8.5%), MSM (0.2%), IDU (1.9%), and transfusion (1.2%). HAART coverage was 98.5%, viral suppression was about 80%, and HPV vaccination was not part of prior care due to the older patient's age. About 98.5% of the women are receiving HAART, and the viral suppression for the years 2018–2023 was 81%, 81%, 83%, and 87%, respectively, achieving substantial improvement to 91% for the year 2024.

Data Analysis

Data were analyzed using IBM SPSS Statistics version 31.0.1.0, which was used to perform logistic regression analyses of CD4 count, viral load (VL), and HPV status, as well as chi-square tests to assess bivariate associations between categorical variables. The analysis used one observation per patient (index visit within the six-month window). Odds ratios (OR) with 95% confidence intervals (CI) are reported.

Chi-Squared Test

Chi-squared tests compared CD4 groupings (≤200, 201–499, >500) and their association with HPV diagnosis in WLHIV. Additional tests explored relations between HPV diagnosis and VL > 200 copies as detectable (<200 = undetectable). AIDS is defined as CD4 <200 cells/mm^3. All subgroup analyses include counts, proportions, and p-values, as recommended.

Ethical Considerations

All data were de-identified for privacy. The protocol received approval from the UPR-MSC IRB (Ref #2290032654) and complies with the Declaration of Helsinki and STROBE reporting guidelines for observational studies.

Results

This section presents a detailed analysis of HPV prevalence among women living with HIV (WLHIV), focusing primarily on two variables: CD4 cell count and HIV RNA Viral Load (VL). Results are reported by CD4 count categories (≤200, 201–499, and >500 cells/mm^3) and VL thresholds (≤200 and >201 copies/mL). We selected the VL threshold of

HIV RNA of 200 copies or less for the main analyses, since it is also a performance measure defined by HRSA and by the ACTG Guidelines.[23]

The guidelines and the AIDS Clinical Trials Group (ACTG) now define virologic failure as a confirmed viral load >200 copies/mL- a threshold that eliminates most cases of apparent viremia caused by viral load blips or assay variability.[24] All subgroup sample sizes (n) and corresponding confidence intervals (CI) are provided for transparency.

The overall prevalence of any HPV was 27% for our cohort. Differences were noted for VL subgroups and CD4 categories. The HPV prevalence was higher in WLHIV with lower CD4 counts. Specifically, the prevalence rates 23.2% (N=58) for CD4 >500 cells/mm^3 ($\chi^2(2)=8.10$, p=0.017), as illustrated in Figure 1. These findings demonstrate an inverse relationship between CD4 count and HPV infection risk, supporting the role of immune function in HPV persistence and related cervical disease. On a logistic regression analysis, individuals with HPV-positive results have 6.35 (p<000.1) times higher odds of having an abnormal PAP smear compared with HPV-negative individuals, after controlling for VL and CD4 count. After adjustment for CD4 count and HPV status, VL does not predict an abnormal PAP results. Similarly, CD4 count is not a significant predictor of abnormal PAP outcomes when adjusted for the other variables in the model.

When examining the association between categorized HIV viral load (≤200 vs >200 copies/mL) and HPV status, HPV detection was slightly higher among women with VL >200 copies/mL (35.4%) than among those with VL ≤ 200 copies/mL (26.1%), but this difference was not statistically significant ($\chi^2(1) = 1.79$, p = 0.18), and all test assumptions were met (no expected cell count <5). This is shown in Figure 2. The abnormal cervical cytology (ThinPrep) results were more frequent among women with higher HIV viral load: 22.9% in those with VL > 201 copies/mL compared with 11.4% in those with VL ≤ 200 copies/mL, and this association was statistically significant ($\chi^2(1) = 4.84$, p = 0.028), with all test assumptions satisfied (no expected cell count <5) as shown in Figure 3. The abnormal cervical cytology (ThinPrep) became progressively more common with decreasing CD4 counts, occurring in 32.1%, 11.8%, and 11.2% of women with CD4 ≤200, 201–499, and ≥500 cells/mm^3, respectively. The association between categorized CD4 level and PAP result (normal vs abnormal) was statistically significant ($\chi^2(2) = 9.88$, p = 0.007), and although one cell (16.7%) had an expected count <5, this remained within the acceptable threshold (<20%), so the test assumptions were considered met as shown in igure 4. Approximately 34.8% of women exhibited abnormal cervical cytology despite a negative HPV test, indicating that co-testing yields additional abnormal cases compared to HPV-only screening shown in Figure 5. Potential explanations other than false-negative results include post-menopausal atrophic vaginitis and other

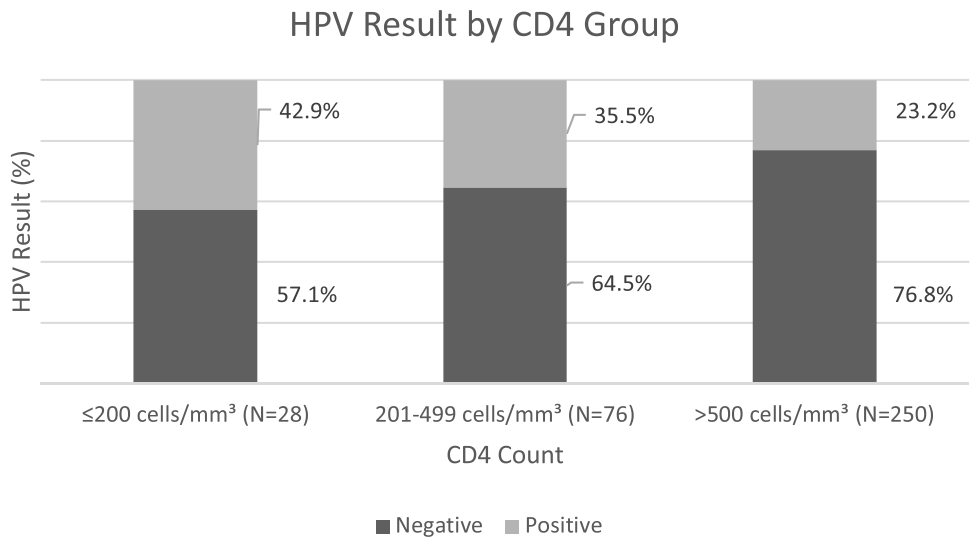

Figure 1 HPV Test Result in Women with a Positive HIV Diagnosis According to CD4 Counts. HPV prevalence was higher in WLHIV with lower CD4 counts. Specifically, the prevalence rates 23.2% (N=58) for CD4 >500 cells/mm^3 ($\chi^2(2)=8.10$, p=0.017), as illustrated in Figure 1. These findings demonstrate an inverse relationship between CD4 count and HPV infection risk, supporting the role of immune function in HPV persistence and related cervical disease.

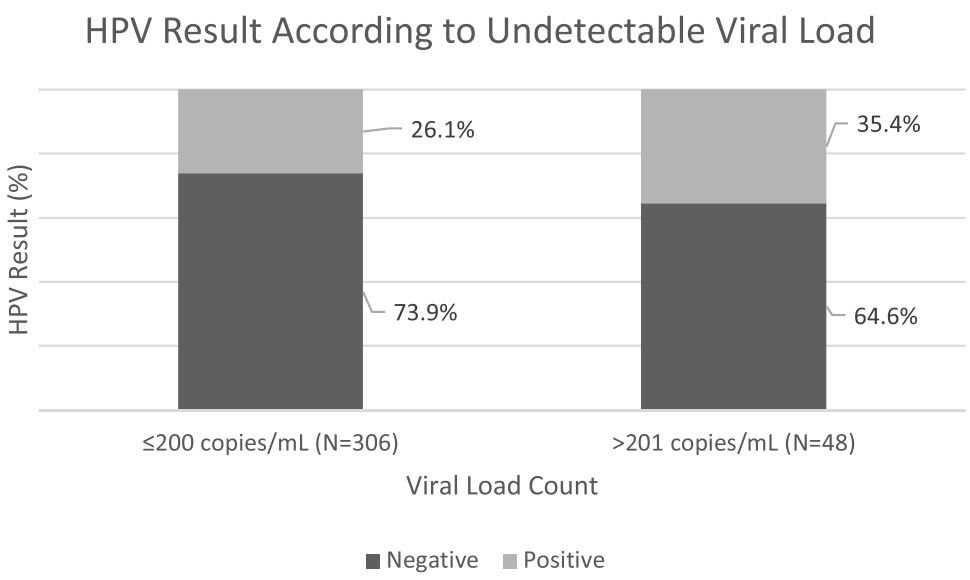

Figure 2 HPV Test Result in Women with a Positive HIV Diagnosis According To Viral Load as defined by < or> 200 copies/mL. When examining the association between categorized HIV viral load (≤200 vs >200 copies/mL) and HPV status, HPV detection was slightly higher among women with VL >200 copies/mL (35.4%) than among those with VL ≤200 copies/mL (26.1%), but this difference was not statistically significant ($\chi^2(1) = 1.79$, $p = 0.18$), and all test assumptions were met (no expected cell count <5).

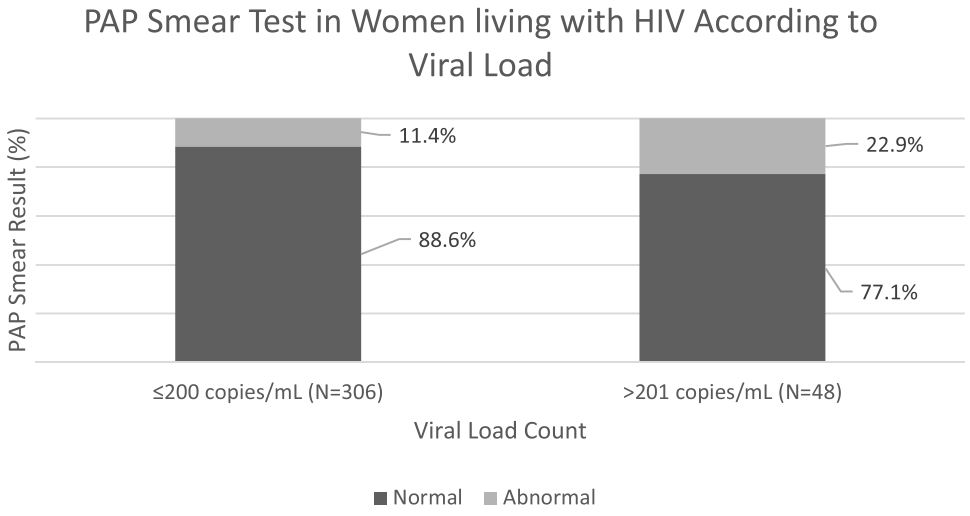

Figure 3 Cervical Cytology (Thin prep) among Women living with HIV according to Viral Load defined as ≤200 copies/mL. Abnormal cervical cytology (ThinPrep) was more frequent among women with higher HIV viral load: 22.9% in those with VL >201 copies/mL compared with 11.4% in those with VL ≤200 copies/mL, and this association was statistically significant ($\chi^2(1) = 4.84$, $p = 0.028$), with all test assumptions satisfied (no expected cell count <5).

inflammatory abnormalities. These data suggest that while HPV persistence is necessary for disease progression, cytology can detect additional cases missed by HPV testing alone.

Improvements in HPV Prevalence Over Time for WLHIV

The HPV prevalence decreased from 50.3% in 2009–2010 to 27% in this cohort from 2018 to 2023, when compared with our published HPV rates for WLHIV.[7] This decrease in HPV prevalence might reflect improved immune status due to access to HAART, increased adherence, and enhanced clinical management. The clinic's 85% viral suppression rate is likely a contributing factor. These data contextualize our findings and underscore the need for further study of co-testing and evolving ART strategies in this aging, predominantly Hispanic population with low HPV vaccination rates.

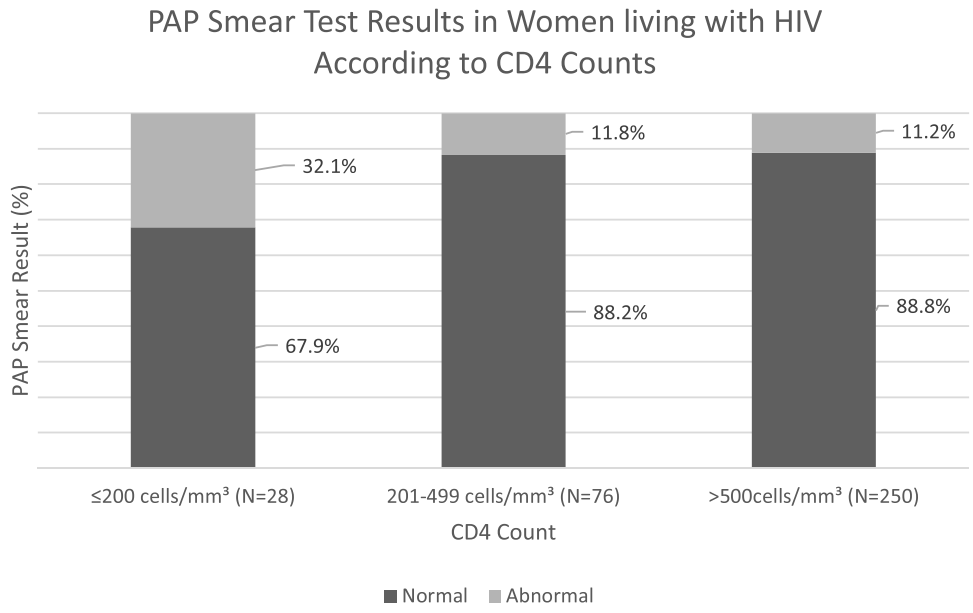

Figure 4 Cervical cytology (Thin prep) Results in Women living with HIV According to CD4 Counts. Abnormal cervical cytology (ThinPrep) became progressively more common with decreasing CD4 counts, occurring in 32.1%, 11.8%, and 11.2% of women with CD4 ≤200, 201–499, and ≥500 cells/mm^3, respectively. The association between categorized CD4 level and Pap result (normal vs abnormal) was statistically significant ($\chi^2(2) = 9.88$, $p = 0.007$), and although one cell (16.7%) had an expected count <5, this remained within the acceptable threshold (<20%), so the test assumptions were considered met..

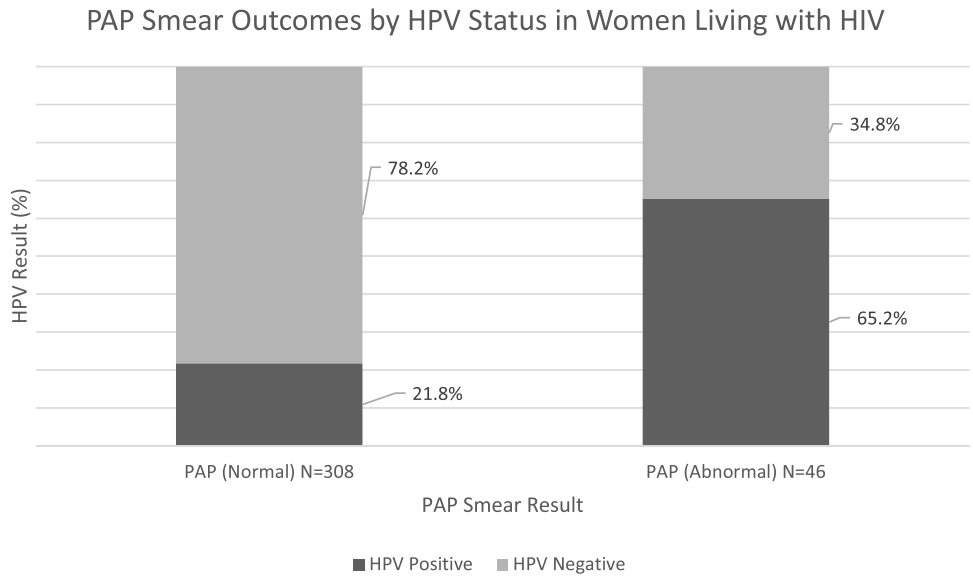

Figure 5 Cervical Cytology (Thin Prep) results by HPV Status among Women Living with HIV. Approximately 34.8% of women exhibited abnormal cervical cytology despite a negative HPV test, indicating that co-testing yields additional abnormal cases compared to HPV-only screening . Potential explanations other than false-negative results include post-menopausal atrophic vaginitis and other inflammatory abnormalities. These data suggest that while HPV persistence is necessary for disease progression, cytology can detect additional cases missed by HPV testing alone.

Discussion

These findings demonstrate a higher prevalence of HPV infection among cervical samples from women living with HIV (WLHIV), consistent with prior studies and regional reports. An inverse relationship persists between HPV manifestations and CD4 count, where lower CD4 counts are consistently associated with higher HPV prevalence (Figure 1). Importantly, even in WLHIV with higher CD4 counts (>500 cells/mm^3), HPV prevalence remains above that seen in HIV-negative populations, reflecting elevated susceptibility despite immune restoration.[16]

Greater viral suppression is associated with lower HPV prevalence, denoting improved control of infection, though this is an association rather than direct causation. Among WLHIV, abnormal cervical cytology (Pap test) in the presence of HPV appears linked to the level of viral suppression. This association underscores the interaction of HIV control, HPV persistence, and cytologic changes, though other unmeasured factors (eg, sexual behavior, smoking, contraception, parity) may also influence risk. A substantial proportion of women presented with abnormal cytology despite a negative HPV test (34.8%), which could only be detected through co-testing. Such cases may be attributed to assay sensitivity limitations (eg, false-negative HPV PCR, subbias genotype coverage), specimen adequacy, or non-HPV-related cytologic atypia (eg, inflammation, other infections, post-menopausal atrophic vaginitis), and other non-HPV-related inflammatory changes.

By examining Pap smear reports alongside HPV status, HIV RNA viral load, and CD4 counts, these results demonstrate that while persistent HPV infection is necessary for progression to cervical cancer, cytology remains valuable for early management and prevention, particularly as early detection may inform subsequent clinical management decisions. These data align with recent studies, such as Kauffman et al,[25] indicating that liquid-based cytology (LBC) might identify additional precancer or cervical cancer cases than HPV testing alone, reinforcing the added diagnostic yield of co-testing in high-risk WLHIV populations. Our population, with older age and elevated HPV prevalence, benefits from the additional information LBC provides for assessing disease evolution and response to treatment.

Recent literature, including Terrinoni et al[26] and broader integrative reviews, has highlighted the complex role of HPV across fertility, pregnancy, and cervical disease management. These findings provide locally relevant evidence supporting the benefit of co-testing in at-risk populations but also underscore the need for further evaluation before changing screening policy or clinical guidelines. The potential impacts of these findings on fertility-sparing management and pregnancy in WLHIV are also important considerations for future research.

Approximately 34.8% of patients had abnormal cervical cytology with a negative HPV result (Figure 5), highlighting co-testing's potential to detect disease missed by HPV-only screening. While co-testing appears to improve detection of premalignant lesions in WLHIV, these findings support consideration of co-testing as preferred in WLHIV until validated by larger, multi-site prospective studies.

Given that our cohort is older and lacks prior HPV vaccination, the population-level benefits of vaccination may not be realized until future cohorts are followed. Additionally, liquid-based cytology's incremental benefit must be considered alongside resources and local guideline priorities, especially in low-resource settings.

Access to HAART, high adherence, and consistent clinical care were strongly associated with lower HPV-related cancer risks and improved outcomes. When comparing our current HPV prevalence of 27% to our prior published data, we have seen a decline from a 50.3% prevalence among women sampled in 2009–2010.[7] This might likely reflect expanded access to ART, improved immune restoration, and more aggressive detection and management of premalignant cervical lesions. Clinic-based reductions may also be influenced by sample selection and changes in practice patterns.

Limitations

As a retrospective, single-site study, generalizability is limited, particularly given the cohort's older age, predominantly Hispanic, urban, and low-income profile. Additional unmeasured confounders (eg, risk behaviors, clinical history, technical differences in HPV assays) may exist; variable definitions for viral suppression and CD4 thresholds were used and should be standardized or justified in future research. Assay sensitivity and genotype coverage could explain HPV-negative cytology, and pathologic confirmation of cancer was not assessed.

Conclusions

This study identified a higher prevalence of HPV infection in cervical samples from women living with HIV (WLHIV), with an inverse relationship between HPV persistence and CD4 counts. Higher CD4 counts are consistently associated with lower HPV prevalence (Figure 1). Even among WLHIV with CD4 counts greater than 500 cells/mm^3, HPV prevalence remains significantly higher compared to HIV-negative women. Greater viral suppression correlates with lower HPV prevalence and improved immune status, as demonstrated by higher CD4 levels.

A key finding was that 34% of WLHIV exhibited abnormal cervical cytology despite negative HPV results, highlighting the diagnostic value of co-testing. These results suggest that, in populations like ours, cervical cancer screening programs should consider including co-testing strategies to optimize detection of precancerous lesions, especially where HPV vaccination rates are low, and ART coverage is high. While current guidelines emphasize HPV testing as the primary screening method, our findings support the potential benefit of co-testing to identify abnormal cervical cytology in the presence of negative HPV, rather than recommending universal adoption currently. In conclusion, the study supports consideration of co-testing for cervical cancer screening in WLHIV, especially in populations with low vaccination rates and high ART uptake. Further prospective and multi-site studies are warranted to confirm these observations and inform policy or guideline changes. The implications of co-testing for early disease detection, management, and outcomes in WLHIV should be studied in more diverse and longitudinal cohorts before making population-wide recommendations.

Use of AI Statement

AI software (Grammarly and ChatGPT) was used for English editing and Grammar.

Abbreviations

HIV, Human immunodeficiency virus; WLHIV, Women living with HIV; HPV, Human Papillomavirus; HIV RNA Viral count, HIV Ribonucleic Acid; CD4, CD4 T lymphocytes (CD4 cells) are a type of white blood cell that fights infection. CD4 cell count is an indicator of immune function in patients living with HIV; LBC, Liquid-based Cytology; AUC, area under the curve; LS, least squares; NE, not estimable.

Ethics Approval and Informed Consent

This study was approved by the UPR Medical Sciences Campus IRB.

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Disclosure

The authors declare no conflicts of interest.

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