Vaginal microbiota associated with oncogenic HPV in a cohort of HPV-vaccinated women living with HIV

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Abstract

Background: Women living with HIV (WLWH) experience higher rates of human papillomavirus (HPV) infection and cervical cancer than women without HIV. Changes in the vaginal microbiome have been implicated in HPV-related disease processes such as persistence of high-risk HPV infection but this has not been well defined in a population living with HIV.

Methods: Four hundred and 20 girls and WLWH, age ≥9, across 14 clinical sites in Canada were enrolled to receive three doses of quadrivalent HPV vaccine for assessment of vaccine immunogenicity. Blood, cervical cytology, and cervico-vaginal swabs were collected. Cervico-vaginal samples were tested for HPV DNA and underwent microbiota sequencing.

Results: Principal component analysis (PCA) and hierarchical clustering generated community state types (CSTs). Relationships between taxa and CSTs with HPV infection were examined using mixed-effects logistic regressions, Poisson regressions, or generalized linear mixed-effects models, as appropriate. Three hundred and fifty-six cervico-vaginal microbiota samples from 172 women were sequenced. Human papillomavirus DNA was detected in 211 (59%) samples; 110 (31%) contained oncogenic HPV. Sixty-five samples (18%) were taken concurrently with incident oncogenic HPV infection and 56 (16%) were collected from women with concurrent persistent oncogenic HPV infection.

Conclusions: No significant associations between taxa, CST, or microbial diversity and HPV-related outcomes were found. However, we observed weak associations between a dysbiotic microbiome and specific species, including Gardnerella, Porphyromonas, and Prevotella species, with incident HPV infection.

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**Background**

There are higher rates of both incident and persistent human papillomavirus (HPV) infections in women living with HIV (WLWH) compared to women without HIV. Women living with HIV also thereby experience increased rates of cervical intraepithelial neoplasia (CIN), faster progression of CIN, and increased rates of cervical cancer. Changes in the vaginal microbiome have been associated with infections and disease states such as HPV, HIV, bacterial vaginosis, and cervical cancer. The healthy vaginal microbiome is typically dominated by a Lactobacillus species, most commonly L. crispatus or L. gasseri, which creates an acidic environment and keeps the growth of other bacterial species at bay. Increased diversity and the dominance of anaerobic bacteria including Gardnerella, Prevotella, and Atopobium increase the vaginal pH due to the loss of the Lactobacillus predominance. The lactic acid produced by lactobacilli has a role in inhibition of pro-inflammatory cytokines, increased degradation of pathogens through autophagy, and inactivation of pathogens such as HIV. Thus, the higher pH seen in some vaginal microbiomes creates a permissible environment for deleterious processes such as increased survival of cell-associated HIV in leukocytes and increased inflammatory cytokines which can disrupt the vaginal epithelium.

The role of the vaginal microbiome with respect to HPV infection in women with and without HIV is not well understood. Studies in women without HIV have shown increased biological diversity and a greater proportion of community state type (CST) III (L. iners dominated – considered an intermediary state type) and CST IV-B (low Lactobacillus) microbiota contributing to HPV infection. In fact, the clearance of high-risk HPV (HR-HPV) in individuals without HIV has been associated with a specific increase in L. crispatus, a decrease in dysbiosis, and a decrease in inflammatory cytokines, compared to those with persistent HR-HPV. However, the literature is conflicting in that a lack of association between HPV persistence and cervical microbiota has also been reported.

The causal relationship between persistent HPV infection and cervical cancer is well established. The prevalence of HPV is doubled in WLWH compared to women without HIV at approximately 50%, while the rate of persistent HPV is 3–6 fold higher among WLWH at approximately 20–24%. This highlights the importance of understanding factors contributing to HPV persistence in WLWH.

Alterations in the vaginal microbiome in the setting of both prevalent HPV infection and CIN include a decreased abundance of L. crispatus and a predominance of species such as L. iners, Atopobium vaginae, Gardnerella vaginalis, and Mycoplasma. However, the contribution of the cervico-vaginal microbiome to the incidence and persistence of HPV infection is still unclear, particularly in the presence of HIV co-infection. The objective of this analysis was to assess the relationship between the vaginal microbiota and HPV-related outcomes, including incidence and persistence, in WLWH. We hypothesized that WLWH with oncogenic HPV infection would be more likely to have non-Lactobacillus dominated microbiota than WLWH without oncogenic HPV infection.

**Methods**

As part of an HPV vaccine study, 420 girls and WLWH aged nine and over from 14 clinical sites across Canada were enrolled to receive three doses of quadrivalent HPV vaccine at months 0, 2, and 6. Study visits took place at months –3, 0, 2, 6, 12, 18, 24, and annually thereafter, until a maximum of 8 years of follow up. Blood samples for serology and cervical cytology samples (ThinPrep liquid based cytology) were collected throughout the study with HPV DNA testing on samples collected at months –3, 0, 6, 12, 18, 24, and annually thereafter. An aliquot of the cytology samples in PreservCyt underwent HPV DNA testing. Extracted DNA was tested with the Linear array (Roche Diagnostic, Laval, QC, Canada) for the detection of 36 genotypes of HPV and for β-globin to determine the adequacy of the sample. Weak positive HPV controls were included in each amplification run. Samples negative for HPV DNA and β-globin were considered inadequate. The HPV types detected have previously been published. Cervico-vaginal swab samples were collected by physicians during genital examination, prior to pap collection, at up to three visits between years three and eight of the study. Physicians rolled sterile flocked swabs against the lateral vaginal wall three times.

Total genomic DNA was purified from cervico-vaginal swabs using the MagMAX Total Nucleic Acid Isolation Kit (Applied Biosystems, Life Technologies, Burlington, ON, Canada). Extraction negative controls including only kit reagents were included to monitor for contaminants. cpn60 barcode PCR and sequencing library preparation was performed as described in detail elsewhere.

No template
controls were included with each batch of PCR reactions. Indexed amplicon libraries from samples and all negative controls were pooled and sequenced on the MiSeq platform (500 cycles, with 400 cycles for read 1; only read 1 used in downstream analysis).

Amplification primer sequences were removed using CUTADAPT. Quality trimming was then performed using TRIMMOMATIC with a quality cut-off of 30 and minimum length of 150. Quality trimmed reads were loaded into QIIME2 for sequence variant calling and read frequency calculation with DADA2 and a truncation length of 150. Quality trimmed reads were loaded into TRIMMOMATIC with a quality cut-off of 30 and minimum length of 150. Quality trimming was then performed using CUTADAPT. Variant sequences were aligned with the cpnDB_nr reference database (version 20190305, downloaded from www.cpndb.ca) using WATERED-BLAST for taxonomic identification. In instances where sequence variants had the same best database reference, they were grouped together into nearest neighbour ‘species’ by summing their total read counts within samples. The nearest neighbour taxonomic labels were used in this analysis.

We used compositional data analysis methods including centre log-ratio transformation of data in ALDEx2 and then visualised communities with principal component analysis (PCA) and hierarchical clustering to generate the CSTs. Human papillomavirus variables we investigated included any HPV positivity, incident oncogenic HPV infection (HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, and 82; defined as detection of a new HPV genotype not detected in the prior visit), persistent oncogenic HPV infection (defined as the same HPV genotype detected in ≥2 consecutive samples taken ≥6 months apart), clearance of any oncogenic HPV (defined as presence of a type in one visit followed by absence at the subsequent visit), HIV viral load (suppressed at <50 copies/mL vs. not suppressed), and the total number of oncogenic HPV types. The relationship between taxa abundance or CSTs and incident or persistent oncogenic HPV infection and HIV viral load was investigated with mixed-effects logistic regressions. The relationship between taxon relative abundance or CSTs with number of oncogenic HPV types. The relationship between taxa abundance or CSTs with number of oncogenic HPV types. The relationship between taxa abundance or CSTs with number of oncogenic HPV types.

Analyses were restricted to cervico-vaginal microbiota samples that had at least 1000 quality-filtered sequence reads, of which there were a total of 356 samples from 172 women (28% women had one sample sequenced, 37% had two samples, and 35% had three samples). Sample were taken between 3 and 8 years post-HPV vaccination (8% at 3 years, 12% at 4 years, 17% at 5 years, 28% at 6 years, 21% at 7 years, and 14% at 8 years). The presence of HPV DNA was detected in 211 samples (59% of samples) from 122 women (Table 2). One hundred and 10 samples (31%) from 73 women had detectable oncogenic HPV. Incident oncogenic HPV infection was found in 65 samples (18%) from 59 women, persistent oncogenic HPV infection was found in 56 samples (16%) from 32 women, while antiretroviral therapy was variable, including integrase inhibitor-based regimens in 39%, protease inhibitor (PI)-based regimens in 34.9%, and non-nucleoside reverse transcriptase inhibitor (NNRTI)-based regimens in 24.4% of study participants.

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Results

The demographics of the study population eligible for this sub-analysis are shown in Table 1. The median age at study baseline was 37.8 years (interquartile range [IQR]: 31.9–44.3, range = 13.6–58.6), and most women received all three doses of quadrivalent HPV vaccine (94.2%). The median baseline CD4+ T-cell count was 489 cells/mm³ (IQR: 370–675), and a modest majority had an HIV viral load under 50 copies/mL (64.5%). The baseline

Table 1. Participant characteristics at baseline (n = 172).

| Characteristic                          | n (%) or median (IQR) |
|----------------------------------------|-----------------------|
| **Age at vaccination, years**          | 37.8 (31.9–44.3)      |
| **Age at vaccination, categorical**    |                      |
| 14–19                                  | 6 (3.5%)              |
| 20–24                                  | 10 (5.8%)             |
| 25–29                                  | 17 (9.9%)             |
| 30–34                                  | 28 (16.3%)            |
| 35–39                                  | 37 (21.5%)            |
| 40–44                                  | 33 (19.2%)            |
| 45+                                    | 40 (23.3%)            |
| **Ethnicity**                          |                       |
| African/Black/Caribbean                | 66 (38%)              |
| White                                  | 63 (37%)              |
| Indigenous                             | 30 (17%)              |
| Other                                  | 12 (7%)               |
| **Number of HPV vaccine doses**        |                       |
| 1                                      | 4 (2.3%)              |
| 2                                      | 5 (2.9%)              |
| 3                                      | 162 (94.2%)           |
| Missing                                | 1 (0.6%)              |
| **Baseline HIV viral load**            |                       |
| ≥50 copies/mL                          | 55 (32.0%)            |
| <50 copies/mL                          | 111 (64.5%)           |
| Missing                                | 13 (7.6%)             |
| **Baseline antiretroviral therapy**    |                       |
| Integrase inhibitor-based              | 67 (39.0%)            |
| PI Based-based                         | 60 (34.9%)            |
| NNRTI based-based                      | 42 (24.4%)            |
| NRTI only                              | 1 (0.6%)              |
| None                                   | 1 (0.6%)              |
| Missing                                | 1 (0.6%)              |
| **Baseline CD4 count, cells/mm³**      |                       |
| 489 (370–675)                          |                       |
| CD4 nadir, cells/mm³                   | 230 (120–360)         |
clearance of any oncogenic HPV was found in 24 samples (6.7%) from 24 women.

Principal component analysis resulted in three principal component axes that explained 42% of the variance in taxon relative abundance (Figure 1; PC1 = 26%, PC2 = 9%, PC3 = 7%). Higher scores on PCA axis 1 (PC1) indicated greater relative abundance of Clostridiales sp., Megasphaera genomosp type 1, Prevotella timonensis, Prevotella buccalis, Porphyromonas uenonis, Prevotella amnii, and Dialister pneumosintes. Lower scores on PC1 indicated a greater relative abundance of Lactobacillus crispatus, L. iners, and L. jensenii. Higher scores on PCA axis 2 (PC2) indicated a greater relative abundance of L. crispatus, while lower scores indicated a greater relative abundance of L. iners and Gardnerella vaginalis.

Hierarchical clustering analysis on the Euclidean distances in relative abundance had relatively good support for six clusters. Both silhouette width and Pearson-Gamma indicated that six clusters had the most support (Figures 1 and 2). The six clusters are as follows: (i) CST IVA with a mixture of profiles with diverse dominant bacterial types, but very little Lactobacillus or Megasphaera, (ii) CST IVC has communities with high relative abundance of Gardnerella vaginalis, and G. swidsinskii, (iii) CST IVD.1 contains communities with high relative abundance of Megasphaera, Clostridiales sp., Prevotella spp., Dialister pneumosintes and Porphyromonas uenonis, (iv) CST IVD.2 contains very little Megasphaera, with appreciable abundance of Clostridiales sp., Prevotella spp., and Porphyromonas uenonis, (v) CST III/V with high relative abundance of L. iners, and/or L. jensenii, (vi) CST I with communities dominated mainly by L. crispatus.

A number of weak and non-significant associations to specific taxa were found. Incident oncogenic HPV infection was non-significantly increased with greater relative abundance of Gardnerella swidsinskii (OR = 1.10, 95%CI = 0.98–1.22, p = .08) and decreased with greater relative abundance of L. crispatus (OR = 0.91, 95%CI = 0.84–1.01, p = .09). Greater total number of oncogenic HPV types were associated with greater relative abundance of Porphyromonas uenonis (p = .09) and Prevotella timonensis (p = .02). No taxa were significantly associated with HPV persistence or clearance.

**Table 2.** HPV results for samples sequenced.

| HPV result                  | n (%) |
|-----------------------------|-------|
| Incident oncogenic HPV       | 65 (18) |
| Persistent oncogenic HPV     | 56 (16) |
| Any oncogenic HPV            | 110 (31) |
| Any HPV                      | 211 (59) |

**Figure 1.** Hierarchical clustering results with PCA axes 1 and 2. The ellipses indicate the clusters and they extend to 1SD in both directions. Grey = CST IVD.1, orange = CST IVA, green = CST IVC, blue = CST IVD.2, light blue = CST III + V (mixed lactobacilli), and pink = CST I.
Investigations at the level of CST suggested that individuals with incident oncogenic HPV infection, a higher number of oncogenic HPV types, and a higher number of total HPV types were more likely to be classified into CST IVD.2 (Porphyromonas, Clostridiales, and Prevotella), but this did not remain significant after controlling for repeated measures. There were no significant relationships between any of the HPV variables and diversity either as Shannon’s diversity index (H) (Figure 3), or the number of detected species.

Discussion

In our study of HPV-vaccinated WLWH, we detected incident oncogenic HPV DNA in 31% of cervico-vaginal swabs and persistent oncogenic HPV DNA in 16% of cervico-vaginal swabs. We did not find any strongly significant associations between taxa, CST, or microbial diversity and HPV variables including incident or persistent oncogenic HPV infection, especially given the large number of tests conducted. However, the trends we found were consistent with expectations based on the current understanding of the roles of the specific taxa investigated and were consistent with the associations found in some other studies. For example, we found a non-significant association of decreased incident oncogenic HPV infection with greater relative abundance of L. crispatus. Prior evidence has shown that D-lactate produced by L. crispatus increases the viscosity of the cervico-vaginal mucus, thereby enhancing its viral particle trapping potential; such enhancement could represent a mechanism behind the reduction in incident oncogenic HPV infection we observed with increasing relative abundance of these bacteria.42 Additionally, the associations between oncogenic HPV infection and greater relative abundance of Gardnerella and Prevotella were not unexpected, as it is known that some of
these species exhibit sialidase activity, which can negatively impact the cervical mucosa and reduce viral trapping via mucin degradation, potentially facilitating HPV infection. *Gardnerella* species have further been implicated in disrupting vaginal epithelial cytoskeleton proteins, causing damage and desquamation which may facilitate HPV entry into its target basal epithelial cells. The lack of associations between bacterial taxa and HPV persistence is consistent with recent findings from a Norwegian study.

This cohort included some WLWH who did not have a suppressed HIV viral load at baseline (32%). However, this rate of HIV viral load suppression is consistent with other studies among WLWH in Canada and therefore appears to be representative of the Canadian context. A small proportion of participants included in this analysis acquired HIV through perinatal infection (*n* = 10, 5.8%), and therefore may have differing risks for HPV acquisition. The lack of strong associations between vaginal microbiota and HPV infection outcomes in this analysis may have been due to the small overall number of HPV infection outcomes, particularly given that this cohort of WLWH had been previously vaccinated against HPV. Future studies with larger sample sizes are needed to fully elucidate the role of the vaginal microbiome in HPV infection and disease within WLWH. Such improvements in understanding are critically important, as they could lead to interventions to reduce the high burden of HPV among WLWH, and ultimately contribute to the global elimination of cervical cancer.

**Conclusions**

This analysis supports previous associations of dysbiotic microbiota and specific bacterial taxa, including *Gardnerella*, *Porphyromonas*, and *Prevotella* species, with incident HPV infection. The lack of association between dysbiosis and HPV persistence may be related to low numbers of events in this cohort of HPV-vaccinated WLWH.
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Declaration of conflicting interests

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Ethics approval

Ethical approval for central study coordination was obtained from the University of British Columbia Clinical Research Ethics Board (approval H08-00997) and all recruiting clinical sites received research ethics approval locally.

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