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Human papillomavirus genotypes in women with invasive cervical cancer with and without human immunodeficiency virus infection in Botswana

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Abstract

Cervical cancer remains a significant cause of morbidity and mortality in women worldwide and is the leading cause of cancer-related death in Botswana. It is well established that women with HIV have a higher risk of persistent HPV infection leading to cervical cancer. We assessed HPV prevalence and genotype distribution in 126 tissue specimens from confirmed invasive cervical cancer cases using Abbott real-time PCR assay. Overall, 88(69.8%) women were HIV-infected. Fifty-seven (64.8%) of the HIV-infected women had a baseline $CD4^+$ count ≥ 350 cells/μl, and 82(93.2%) were on antiretroviral therapy at the time of cervical cancer diagnosis. The median age of HIV-infected patients was signficantly younger than that of HIV-uninfected patients ($p < 0.001$). HPV DNA was detected in all of 126(100%) of tissues analysed in our study. The HPV genotypes identfied included the HPV-16 (75.4%), HPV-18 (28.6%) and *other* high-risk (hr) HPV genotypes

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(16.7%). HIV infection was positively associated with the presence of the HPV-16 genotype (p=0.036), but not with HPV-18 or with *other* high-risk (hr)-HPV genotypes. Thirty-three percent of the patients had multiple hr-HPV genotypes, with higher rates in HIV-infected women. These results highlight the importance and potential impact of large-scale HPV vaccination programs covering HPV-16 and HPV-18 genotypes in countries like Botswana with high burden of HIV infection.

Keywords

Botswana; invasive cervical cancer; HIV; HPV-16; HPV-18; multiple infections

Introduction

Cervical cancer is the fourth most common malignancy in women globally with an estimated 570,000 cases and 311,000 deaths in 2018. It is currently the leading cause of cancer-related death in women in sub-Saharan Africa (SSA).[1] Human papillomavirus (HPV) infection plays a crucial role in the development of cervical cancer.[2] To date, over 100 HPV genotypes have been identified and within the 40 HPV genotypes that can infect the genital mucosa,[3] strains have been classified as high-risk (hr) or low-risk (lr) depending on their association with the development of cancer.[4] Although hr-HPV genotypes include HPV-16, HPV-18, HPV-31, HPV-33, HPV-35, HPV-39, HPV-45, HPV-51, HPV-52, HPV-56, HPV-58, HPV-59 and HPV-68,[5,6] HPV-16 and HPV-18 genotypes alone are responsible for approximately 70% of cervical cancer cases worldwide.[4,7–9]

Globally, an estimated 36.9 million people are infected with human immunodeficiency virus (HIV).[10] Approximately 75% of women infected with HIV live in SSA.[10] As a result of successful HIV treatment and control efforts, patients with HIV are living longer than ever before. This increased life expectancy has led to an increased risk for developing noncommunicable diseases, including cancer.[11–13] Women infected with HIV are at increased risk for HPV infection and persistence, with subsequent risk of cellular transformation and progression to invasive cervical cancer.[14] Furthermore, HIV infection leads to a decline in both the number and function of cluster of differentiation 4 ($CD4^+$) T-cells, which can lead to a high rate of persistent HPV infection and reduces the chance of spontaneous clearance of HPV.[14] Although previous studies have explored the frequency and distribution of the hr-HPV genotypes in HIV-infected women across Africa, understanding local patterns of HPV variability according to HIV status is a critical step in determining the potential impact of HPV vaccine programs most often target HPV-16 and HPV-18 genotypes in countries with high HIV burden.[14,15]

In Botswana, cervical cancer is the leading cause of cancer death. More than two-thirds of cases are found in HIV-infected women.[16] One study in Botswana examined the association of HPV genotypes with precancerous cervical intraepithelial neoplasia in women with HIV infection and found that the rate of coinfection with HPV was as high as 92% and that 61% of women carried multiple HPV genotypes[17] Another study from Botswana showed that in a cohort of women with HIV infection, the rate of coinfection with HPV for subjects with or

without cervical lesions was 68% and that 71% of women carried multiple HPV genotypes.[18] Additional studies have identified differences in the distribution of HPV genotypes between invasive cervical cancer patients with and without HIV in Botswana.[19,20]

However, data from these studies were not able to assess HPV prevalence from populations that had long-term access to cost-free antiretroviral treatment (ART). In our study, we determined the association between hr-HPV genotypes, demographics, and clinical characteristics in a group of women diagnosed with invasive cervical cancer with and without HIV infection in Botswana between 2013 and 2016, more than a decade after ARTs had become readily available to the population.

Materials and Methods

Study design and population

This retrospective, cross-sectional study used residual formalin-fixed paraffin-embedded tissues from 126 patients diagnosed with invasive cervical cancer who attended public health facilities for diagnostic and treatment services in Botswana between 2013 and 2016. Cervical biopsy specimens were obtained as part of routine investigative procedures (patients referred for a colposcopy and cervical biopsy and managed with loop excision and electrical procedure) at the two participating hospitals (Princess Marina and Nyangabwe Hospital) and then embedded in paraffin. Sections of the tissue blocks were analyzed by a pathologist for the histopathological diagnosis of invasive cervical cancer. Cervical cancer was staged clinically according to the International Federation of Gynecology and Obstetrics (FIGO) staging system for cervical cancer using clinical exam, chest X-ray and abdominal ultrasound. All the study patients were black Botswana women and all cervical cancer diagnoses were made at the National Health Laboratory (NHL) in Gaborone, the public health reference laboratory of Botswana. Demographics, clinical data, HIV status, histopathology results and year of diagnosis were obtained from patient medical records (Integrated Patient Management System, IPMS) through a centralized electronic system accessible at Princess Marina Hospital in Gaborone, Botswana.

Histological diagnosis

Histopathological diagnosis of invasive cervical cancer was determined by light microscopy examination of hematoxylin- and eosin-stained sections of cervical specimens. Samples with a confirmed histopathological diagnosis of invasive cervical cancer underwent HPV DNA genotyping using the same tissue blocks from the confirmatory diagnosis. If the presence of invasive cervical cancer was not confirmed, no further study procedures were performed on tissue blocks and they were excluded from subsequent analysis. Thus, all cases included in our study were confirmed to be invasive cervical cancer.

Tissue sectioning

The paraffin-embedded tissue blocks were chilled on ice before sectioning. Tissue sections were cut with a semiautomatic precision microtome (Slee CUT 5062). The microtome manufacturer's instructions were followed when setting the clearance angle. The first 20-μm tissue section for each block was discarded to ensure consecutive cleaner sections were

collected. Strict aseptic techniques were employed when handling tissue blocks and samples were transferred with sterile instruments. The microtome stage and blade were cleaned with xylene followed by absolute alcohol and DNA/RNA Away solution after each tissue block section. To further monitor for cross-contamination between samples during sectioning, blank paraffin control blocks were cut after every 10th sample and analyzed for HPV DNA presence.

DNA extraction

Tissue sections of 20-μm thicknesses were then incubated in xylene within 24 hr after arrival in the laboratory and left overnight to ensure complete removal of paraffin wax, based on a previously published protocol.[21] All 126 specimens had adequate tissue volume for optimal nucleic acid extraction (DNA), and tissues remained intact after sectioning. Tissue adequacy refers to the amount of epithelium with which histological evaluation of invasive cervical cancer can be rendered accurately for diagnosis. After tissue rehydration, samples were centrifuged at 3,500 rpm, enzymatically digested with proteinase K and transferred into an Abbott Real-Time hr-HPV assay (Abbott GmbH & Co. KG, Wiesbaden, Germany) reaction vessel for DNA isolation using the mSample Preparation System DNA kit with the Abbott m2000sp instrument according to the manufacturer's instructions.[22]

Abbott hr-HPV genotyping

Subsequent hr-HPV genotyping was performed using the Abbott Real-Time hr-HPV assay according to the manufacturer's instructions (Abbott GmbH & Co. KG, Wiesbaden, Germany).[22] The Abbott Real Time hr-HPV assay qualitatively detects DNA from 14 different hr-HPV genotypes using a modified GP5+/6+ primer mix consisting of three forward primers and two reverse primers that target the conserved L1 region of HPV and an internal control primer pair that targets a human β-globin sequence. The assay provides specific probes that are differentially labeled to qualitatively detect HPV-16 and HPV-18, and an evaluation of the human beta-globin internal control. Probes for hr-HPV genotypes HPV-31, HPV-33, HPV-35, HPV-39, HPV-45, HPV-51, HPV-52, HPV-56, HPV-58, HPV-59, HPV-66 and HPV-68 are labeled with the same dye, and the presence of any one or combination of these genotypes is reported as "*other* hr-HPV" detected.[22] Ten known hr-HPV-positive and 10 hr-HPV-negative control samples were genotyped according to a previously published protocol to validate the assay.[21]

Statistical analysis

Stratified by HIV-infection status, the prevalence of hr-HPV genotypes was calculated as the number of observed hr-HPV genotype divided by the total number of invasive cervical cancer patients in each group. Categorical demographic and clinical factors were compared between HIV-infected and HIV-uninfected patients using Chi-squared tests. Data cleaning and statistical analyses were performed using SAS version 9.4 (SAS Institute Inc., Cary, NC). Variables with p values of ≤0.050 were considered statistically significant.

Ethics statement and consent process

The Institutional Review Board (IRB) at the University of Botswana, the Human Resource Development Council (HRDC) at the Botswana Ministry of Health and Wellness and the University of Pennsylvania's IRB approved our study. This research was granted exemption for written informed consent from the participants. All specimens retrieved were de-identified. The study was conducted in accordance with the Declaration of Helsinki.

Data availability

The data that support the findings of our study are available from the corresponding author upon reasonable request.

Results

Demographic and clinical characteristics by HIV status

Clinical and demographic characteristics of study participants are summarized in Table 1. All available tissue samples from women with a confirmed diagnosis of invasive cervical cancer were included. Of 126 specimens, 88 (69.8%) were from HIV-infected women and 38 (30.2%) were from HIV-uninfected women. The HIV-infected patients were younger than their HIV-uninfected counterparts (average age of 45 *vs.* 61 years, respectively, $p < 0.001$; Table 1). Among HIV-uninfected patients, 19 (50.0%) had cancer Stage I or II disease, and 19 (50.0%) had cancer Stage III or IV disease. Fifty-eight (65.9%) HIV-infected patients had cancer Stage I or II disease, and 30 (34.1%) had cancer Stage III or IV disease. Disease stage was not significantly different between the HIV-infected and HIV-uninfected patients ($p = 0.093$). The median baseline $CD4^+$ T-cell count at the time of cancer diagnosis among HIV-infected women was 487 cells/μl (Q1, Q3 = 325, 600). Fifty-seven (64.8%) HIV-infected women had a baseline $CD4^+$ T-cell count ≥350 cells/μl, 23 (26.1%) had a nadir $CD4^+$ ≥350 cells/μl and 82 (93.2%) were known to be taking ART when they were diagnosed with cancer, with 40 (45.5%) having been on ART >60 months.

HPV genotyping and prevalence

All samples studied were positive for hr-HPV (HPV-16 and/or HPV-18 and/or *other* hr-HPV). Table 2 shows the prevalence of single and multiple hr-HPV infections according to the HIV status. The HPV-16 genotype was detected in 95 (75.4%) samples (alone or in combination), and the HPV-18 genotype was detected in 36 (28.6%) samples (alone or in combination). Eighteen (14.3%) biopsy specimens were positive for both HPV-16 and HPV-18. Twenty-one (16.7%) patients had hr-HPV genotypes *other* than HPV-16 and HPV-18. HIV-infected samples showed a significantly higher pooled prevalence of HPV-16 overall (alone or in combination; $n = 71$, 80.7%) compared to HIV-uninfected samples ($n = 24$, 63.2%; $p = 0.036$; Table 2). This trend was not observed for single-strain infection with HPV-16 alone ($p = 0.160$). The lower likelihood of women with HIV to harbor infection with only *other* hr-HPV genotypes was found to be borderline significant ($p = 0.056$). Infection with multiple HPV genotypes was detected in 29 (37.7%) HIV-infected women compared to 12 (31.6%) HIV-uninfected women ($p = 0.629$).

A subanalysis was conducted for HIV-infected subjects stratified by $CD4^+$ count at cancer diagnosis and hr-HPV genotype distribution (Table 3). Data shown represent the 81 patients and excludes seven who did not have CD4+ count recorded. No difference by $CD4^+$ count was found. HPV-16 was found in 87.5% of the patients with CD4 <350 counts/μl and 79.0% patients with CD4 ≥350 counts/μl (p-value = 0.366).

Discussion

This retrospective cross-sectional study provides valuable information regarding the distribution of hr-HPV genotypes in HIV-infected and HIV-uninfected women with invasive cervical cancer in Botswana. The most common hr-HPV genotypes, regardless of HIV status, were HPV-16 (75.4%) and HPV-18 (28.6%), while the *other* hr-HPV genotypes accounted for only 16.7%. The high prevalence of HPV-16 and HPV-18 reported in the present study was expected given that these genotypes are the two most common hr-HPV genotypes across the world.[4,23] HPV-16 was the most common hr-HPV genotype in both HIV-infected and HIV-uninfected groups. This finding is in agreement with previous reports, [18–20] including a systematic review by Clifford *et al.*[15] that analyzed studies of HIV-infected patients with invasive cervical cancer in 12 African countries. Interestingly, we found that in our cohort of women with invasive cervical cancer, HPV-16 was positively associated with HIV infection, whereas the HPV-18 genotype was not. Conversely, women without HIV infection were borderline more likely to carry only *other* hr-HPV strains without HPV-16 or HPV-18 present. These results would suggest a closer relationship between HIV and HPV-16 coinfection than previously reported. This finding can be contrasted to those reported by Clifford *et al.*,[15] who found the opposite to be true from HPV-16 and *other* hr-HPV. No literature currently available can explain why HIV-infected women might be more susceptible to HPV-16 genotype. It is possible that despite the oncogenic potential of the other hr-HPV genotypes, they are not able to evade the immune system as efficiently as HPV-16 and HPV-18 except in HIV-infected individuals who are immunocompromised. However, it has been reported that HPV-16 is less associated with $CD4^+$ count than other genotypes, suggesting the possibility of superior immune evasion that may result in higher prevalence despite well-controlled HIV infection.[24] Discrepancies between our results and that of Clifford *et al.*[15] may be attributed to the excellent management of HIV in Botswana, as the high rate of $CD4^+$ cell counts (baseline $CD4^+$ count ≥350 cells/μl; Table 1) in our cohort suggests, or the relatively higher access to screening for cervical cancer as the age at diagnosis suggests. In general, our findings may be attributed to the relatively high number of women living with well-managed HIV in Botswana compared to the other SSA nations, as demonstrated by high median $CD4^+$ counts and months on ART in the study cohort. Moreover, our findings may also be attributed to relatively higher access to cervical cancer diagnosis, particularly in urban areas of Botswana. This is also supported by the higher rate of early cancer stages (I and II) in HIV-infected patients in Botswana (Table 1).

Although the mechanisms underlying the interaction between HIV and HPV-16 are not yet clearly understood, our data underscore the impact of HPV-16 as the primary causal agent of cervical cancer development in HIV-infected women in Botswana. We further investigated the prevalence of HPV-16 and HPV-18 together and found no significant association with HIV status (Table 2). Similar to our findings, De Vuyst *et al.*[25] examined cervical

intraepithelial neoplasia 2 and 3 lesions specimens from women living in Kenya and South Africa, and found that the prevalence of HPV-16 or HPV-18 in combination was similar in HIV-infected and HIV-uninfected patients. Conversely, *other* hr-HPV types (alone or in combination) had a lower prevalence in our study (16.7%) compared to a previous study (47.8%) by Ermel *et al.*[19] One reason for this discrepancy may be that our study focused exclusively on hr-HPV genotypes, whereas Ermel *et al.*[19] focused on both hr-HPV and lr-HPV genotypes.

Regardless of their HIV status, 32.5% of the women with invasive cervical cancer had multiple hr-HPV infections (thus also including HPV-16 and HPV-18). All 126 samples (100%) in our study tested positive for at least one hr-HPV genotype. This high prevalence differs from a previous study in Botswana that demonstrated a 64% prevalence of hr-HPV genotypes,[18] although the study focused exclusively on women with HIV that had abnormal cytology in only 68.5% of cases.[18] The prevalence of hr-HPV genotypes in our study is similar to that reported by Ermel *et al.*[18] and Ramogola-Masire *et al.*,[17] corresponding to 95.6 and 92%, respectively, for patients from Botswana. The current study, as well as that by Ermel *et al.,*[19] focused entirely on patients with invasive cervical cancers, unlike the study from Ramogola-Masire *et al.*[17] that focused on HIV-infected women with premalignant cervical intraepithelial neoplasia 2 and 3 lesions. Our findings corroborate these studies and highlight the high burden of infection with hr-HPV genotypes associated with cervical cancer in Botswana.

Our study did not detect a statistical difference in carriage of multiple hr-HPV genotypes between HIV-infected (37.7%) and HIV-uninfected (31.6%) women. This finding reflecting similar data reported by Ermel *et al.*[19] However, others have previously documented a positive association and this topic is currently debated in the literature.[26] An increased rate of infection with multiple hr-HPV genotypes in HIV-infected women was seen in the Clifford meta-analysis (27.8% *vs.* 15.9%).[15] Other comparable studies using polymerase chain reaction (PCR)-based detection of HPV among HIV-infected women have reported a prevalence of coinfection with multiple HPV genotypes ranging from 12% to 79%.[27–32] Infection with multiple HPV genotypes has been associated with a higher rate of persistent HPV infection compared to infection with a single genotype[29,30] but has not been associated with increased development of cervical preinvasive lesions or with invasive cancer.[33,34] However, for those with established cancer, multistrain HPV infection has been associated with poor treatment response and reduced survival outcomes.[30] Increased access to ART and better surveillance associated with the systematic use of ART[35] may play a role in the notable lack of increase in multiple infections in HIV-infected women.

The current study is not without limitations. Our sample size of 126 subjects may have limited our ability to adequately power the study. The Real-Time PCR approach adopted is not able to identify the specific genotypes in samples that tested positive for genotypes other than HPV-16 and HPV-18, resulting in the inclusion of the category of "*other* hr-HPV" in the analysis. We did not use laser capture microdissection to isolate the areas of invasive cervical cancer, with a possible but low risk of detection of HPV also from adjacent/contiguous areas of the cervix. Despite these limitations, the current study provides valuable contributions to the literature on hr-HPV prevalence in invasive cervical cancer patients in

SSA. Importantly, our findings highlight the need for large-scale HPV vaccination programs in Botswana. Available vaccines in Botswana cover HPV-16 and HPV-18, which are the most commonly identified HPV genotypes in cervical cancer in Botswana. However, the prevalence of genotypes other than HPV-16 and HPV-18 identified in invasive cervical cancer patients establishes a need for vaccines that cover *other* hr-HPV genotypes as HPV-31, HPV-33, HPV-45, HPV-52 and HPV-58.[18,23]

Conclusions

In conclusion, HPV-16 is a clear priority target for cervical cancer prevention in HIV-positive patients in Botswana. HIV may influence the distribution of some HPV genotypes given the significant increase in prevalence of HPV-16 among HIV-infected patients observed in our study. Although HPV-16 and HPV-18 are most frequently associated with cervical cancers in Botswana, *other* hr-HPV genotypes also contribute to the development of cervical cancer, and more than one-third of all of the patients we studied harbored multiple hr-HPV genotypes with higher rates in HIV infected women. Additional research is needed to better understand the role of infection with multiple HPV genotypes in the development and prognosis of invasive cervical cancer in HIV-infected women. Finally, in a setting in which two-thirds of cervical cancer arises in HIV-positive women, the causal genotypes were confirmed to be broadly similar to previous findings, namely with HPV-16 and HPV-18 accounting for a large majority of cases, irrespective of HIV status. This reassures public health officials that vaccines targeting HPV-16 and HPV-18 are equally relevant in the setting of high HIV prevalence in Botswana.

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Abbreviations:

ART	antiretroviral treatment
CD4	cluster of differentiation 4
DNA	deoxyribonucleic acid

HIV	human immunodeficiency virus
HPV	human papillomavirus
hr	high-risk
IRB	Institutional Review Board
lr	low-risk
PCR	polymerase chain reaction
RNA	ribonucleic acid
SSA	sub-Saharan Africa

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What's new?

Because cervical cancer is the leading cause of cancer-related death in many countries, a better understanding of the complex biology of oncogenic HPV genotypes is crucial. This includes quantifying HPV diversity of individual cervical lesions, evaluating vaccine efficacy, designing novel detection assays, and evaluating the impact of co-infection with HIV. In this study, the authors found that HIV-infected women in Botswana have a higher prevalence of HPV-16, compared to HIV-uninfected women. These results reinforce the potential impact of large-scale HPV vaccination programs in countries like Botswana with a high burden of HIV infection.

Table 1.

Demographic and clinical characteristics of invasive cervical cancer patients stratified by HIV status ($n = 126$)

Characteristic	HIV-uninfected $n = 38$	HIV-infected $n = 88$	p-value[1]
Age in years			
<50	4 (10.5%)	57 (64.8%)	<0.0001
≥50	34 (89.5%)	31 (35.2%)	
Age in years, median (IQR)	61 (55–68)	45 (39–52)	<0.0001[2]
Cancer stage			
Stage I, II	19 (50.0%)	58 (65.9%)	0.093
Stage III, IV	19 (50.0%)	30 (34.1%)	
Nadir CD4 (cells/μl)			
≥350	N/A	23 (26.1%)	N/A
<350	N/A	62 (70.5%)	
Missing data	N/A	3 (3.4%)	
Baseline CD4 (cells/μl)			
≥350	N/A	57 (64.8%)	N/A
<350	N/A	24 (27.3%)	
Missing data	N/A	7 (7.9%)	
Baseline CD4 (cells/μl), median (IQR)	N/A	487 (325–600)	N/A
Months on ART			
≤60 months	N/A	42 (47.7%)	N/A
>60 months	N/A	40 (45.5%)	
Missing data	N/A	6 (6.8%)	

[1] Based on Chi-squared test when data counts were sufficient for statistical analysis.

[2] Based on Mann–Whitney test.

Abbreviations: IQR, interquartile range; ART, antiretroviral therapy; N/A, not applicable.

Table 2.

Distribution of hr-HPV genotypes in invasive cervical cancer patients stratified by HIV status ($n = 126$)

Characteristic	HIV-uninfected $n = 38$	HIV-infected $n = 88$	p-value[1]
HPV genotypes			
HPV-16 alone	13 (34.2%)	42 (47.7%)	N/A[2]
HPV-16 and HPV-18	6 (15.8%)	12 (13.6%)	
HPV-16 and *other* hr-HPV	3 (7.9%)	11 (12.5%)	
HPV-16, HPV-18 and *other* hr-HPV	2 (5.3%)	6 (6.8%)	
HPV-18 alone	3 (7.9%)	6 (6.8%)	
HPV-18 and *other* hr-HPV	1 (2.6%)	0 (0%)	
Other hr-HPV (non-16 or non-18)	10 (26.3%)	11 (12.5%)	
HPV genotypes (HPV-16 alone or multiple)			
HPV-16 alone	13 (34.2%)	42 (47.7%)	0.160
Others	25 (65.8%)	46 (52.3%)	
HPV genotypes (non-16/non-18 or multiple)			
hr-HPV (non-16 or non-18)	10 (26.3%)	11 (12.5%)	0.056
Others	28 (73.7%)	77 (87.5%)	
HPV genotypes (alone or multiple)			
HPV-16 or HPV-18 alone	16 (42.1%)	48 (54.5%)	0.629
Multiple hr-HPV genotypes	12 (31.6%)	29 (37.7%)	
HPV genotypes (HPV-16 or HPV-18 present)			
HPV-16 present	24 (63.2%)	71 (80.7%)	0.036
HPV-18 present	12 (31.6%)	24 (27.3%)	0.623

[1] Based on Chi-squared test.

[2] Statistics were not calculated due to insufficient data counts.

Table 3.

hr-HPV genotypes stratified by immunocompetency status based on CD4+ T cell counts at baseline among HIV-infected patients (n = 81)

Characteristic	<350 cells/μl n = 24	≥350 cells/μl n = 57	p-value[1]
HPV genotypes			
HPV-16 alone	13 (54.2%)	25 (43.9%)	N/A[2]
HPV-16 and HPV-18	3 (12.5%)	9 (15.8%)	
HPV-16 and *other* hr-HPV	2 (8.3%)	8 (14.0%)	
HPV-16, HPV-18 and *other* hr-HPV	3 (12.5%)	3 (5.3%)	
HPV-18 alone	1 (4.2%)	4 (7.0%)	
HPV-18 and *other* hr-HPV	0	0	
Other hr-HPV	2 (8.3%)	8 (14.0%)	
HPV genotypes (HPV-16 alone or multiple)			
HPV-16 alone	13 (54.2%)	25 (43.9%)	0.396
Others	11 (45.8%)	32 (56.1%)	
HPV genotypes (alone or multiple)			
HPV-16 or HPV-18 alone	14 (58.3%)	29 (50.9%)	0.723
Multiple hr-HPV genotypes	8 (33.3%)	20 (35.1%)	
HPV-16 genotype			
HPV-16 present	21 (87.5%)	45 (79.0%)	0.366
HPV-18 genotype			
HPV-18 present	7 (29.2%)	16 (28.1%)	0.920

[1] Based on Chi-squared test.

[2] Statistics were not calculated due to insufficient data counts.