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Association of HIV status with infection by multiple HPV types

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Abstract

OBJECTIVES To identify the clinical and demographic characteristics of HIV-positive and HIV-negative women infected by multiple HPV types.

METHODS 1399 women participated in the study (240 HIV-positive and 1159 HIV-negative women). Samples were provided for Pap tests and for HPV detection and typing by PCR. Data were collected on HPV infection, frequency of multiple infection, and HPV type distribution. Odds ratios were reported from logistic regression models.

RESULTS Compared with HIV-negative women, HIV-positive women had higher frequencies of cervical abnormality (30% *vs*. 20.8%), higher HPV prevalence (68.3% *vs*. 51.3%) and were more commonly infected with multiple HPV types (78.7% *vs*. 44.3%). HPV-16 was the most common type detected in the study population, with other types showing variable associations with HIV status. Positive associations were observed between infection by multiple HPV types and HIV status, cervical abnormality and having had more than three pregnancies. The odds of multiple infection by HPV types were higher in HIV-positive women who used an intrauterine device, who had a history of abortions and who had HIV viral loads >100 000 copies/ml, whilst the odds were lower in women with >500 CD4 cells/mm^3.

CONCLUSIONS HIV immunosuppression favours infection by multiple high-risk HPV types, mainly in women affected by low-grade squamous intraepithelial lesions. Antiretroviral therapy had no effect on infection by multiple HPV types. Risk factors related to progressive damage to the cervix were positively associated with infection by multiple HPV types in women living with HIV.

keywords human papillomavirus, human immunodeficiency virus, multiple infection, epidemiology, risk factor

Introduction

High-risk (HR) human papillomavirus (HPV) types are highly prevalent sexually transmitted diseases (STDs) in women living with human immunodeficiency virus (HIV) [1]. HIV favours HPV persistence and modulates the expression of the HPV *E6* and *E7* genes, which are responsible for oncogenic transformation [2, 3]. HPV persistence is favoured as a result of HIV-induced impairment of host immune responses, which limits the host's ability to control infection and permits infection by multiple HPV types. Infection by multiple HR-HPV types has been observed in a significant proportion of HIV-positive women [4].

Persistent infection by HR-HPV constitutes a risk factor directly associated with cervical cancer (CC) [5, 6]. HIV-associated immunosuppression is a key factor contributing to increased morbidity and mortality of this neoplasia [7]. The risk of developing lesions arising from HPV infection is significantly increased in HIV-positive women, with CC incidence approximately 20-fold higher than in HIV-negative women [8].

The clinical importance of infection by multiple HR-HPV types remains controversial. An intraepithelial lesion

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has traditionally been attributed to infection by a single HPV type [9] Nevertheless, studies have revealed high frequency of multiple HPV infections in women with cervical lesions (low-grade squamous intraepithelial lesions (LSILs) or more serious lesions); and attributing an etiological relationship to a single genotype is difficult [9]. *In vitro* studies of the biology of multiple HPV infection have demonstrated a single cell's ability to be simultaneously infected by more than one HPV type; however, studies of multiple HPV infection in women with lesions have produced inconclusive results [9–11].

Studies of HIV-positive women have shown conflicting results regarding the prevalence of simultaneous infection by multiple HPV genotypes and their contribution to the risk of developing CC. Some studies have suggested that HPV types have different behaviours in immunosuppressed women (including HIV-positive women), thereby accelerating the progression of premalignant lesions to CC. By contrast, other studies have suggested that the biology of HPV infection is not influenced by HIV status [3, 12, 13]. Whilst HPV's natural history is clearly altered in HIV-positive women, more studies are needed to understand the risk factors for simultaneous infection by more than one HPV genotype and the populations at risk of multiple infection [8].

This study was thus aimed at understanding the determinants of infection by multiple HPV types in Colombian women with and without HIV infection. The information provided by this study should contribute towards improved understanding the factors involved in development of cervical neoplastic lesions and may help in developing effective interventions and prevention programs for CC. These are aimed at increasing the quality of life and life expectancy for the high-risk group of women living with HIV.

Materials and methods

Study population and ethical approval

This was a cross-sectional observational study; convenience sampling was used to enroll HIV-positive and HIV-negative women. Eligibility criteria included: (i) known HIV status reported by participating institutions with permission of each woman, (ii) clinically stable, (iii) not hospitalised at the time of their inclusion in this study, and (iv) agreed to participate in the study through signed informed consent. Women with alterations in the urogenital tract that affected the ability to obtain a sample were excluded.

All women included in this study were from Bogotá, Colombia's capital city. The HIV-positive women were recruited at the Asistencia Científica de Alta Complejidad S.A.S. clinic, which specializes in treating this infection, whilst the HIV-negative women were attending three hospitals' sexual and reproductive health programs (Hospital de Bosa, Hospital de Fontibón and Hospital Engativá). Additional characteristics regarding the population and study design have been reported previously [14].

All protocols were approved by the ethics committee of each participating hospital and by the Fundación Instituto de Inmunología de Colombia's ethics committee. All women included in the study were informed of its purpose and procedures and voluntarily accepted an invitation to participate by signing an informed consent form. A structured questionnaire was used to compile sociodemographic information and data regarding participants' sexual behaviours.

This study included tests for the identification of cervical changes (Papanicolau test) and detection of six HR-HPV types (molecular detection); as a cross-sectional study, both tests were conducted during a single visit. However, all results obtained (Pap test and molecular detection of HPV) were sent to each institution included in the study and interpreted by each institution's expert gynecologists, who defined the follow-up schemes following established recommendations according to the cervical cancer detection and control program of Colombia's health security system.

Collecting, processing and detection of HPV DNA

The women were received by the head nurse responsible for each institution's promotion and prevention (P&P) program. Informed consent forms were signed and demographic data collected, then samples were obtained. Pap tests were conducted first according to Colombian technical standards and the Bethesda system was used for reporting cytological findings. The second sample provided was used for the molecular detection of HPV. A cervical mucosa scrape was obtained using a Cytobrush®, preserved and transported in 95% ethanol.

Total DNA was recovered by vigorous vortexing and extracted using a QuickExtract kit following the manufacturer's instructions. PCR was used for all molecular analyses. All samples were subjected to a first amplification specific for a segment of the human *β-globin* gene (GH20/PC04 primers) to verify DNA quality [15]. Samples showing positive amplification of *β-globin* were then subjected to generic HPV identification PCRs using three sets of primers (GP5+/6+, MY09/11 and pU1M/2R). Amplification was simultaneous and independent for all infected women, as using more than one set of generic detection primers ensures the robustness and sensitivity of epidemiological studies [15].

PCR type-specific identification assays were then carried out on all samples from HPV-positive women (positivity being defined as amplification by any of the generic primer sets). The methodological design included the detection of six genotypes (HPV-16, −18, −31, −33, −45 and −58) believed to be responsible for up to 85% of CC cases. These genotypes have been shown to have a wide distribution in Colombian populations [16, 17]. Independent PCR reactions were carried out with primers specific for each HPV genotype using previously described amplification conditions [15, 17].

Statistical analysis

Quantitative variables were included in statistical analyses, along with their respective means and standard deviations (SD). Variables such as number of lifetime sexual partners and number of full-term pregnancies were treated as categorical variables and summarised using percentages and 95% confidence intervals (95% CI). Chi^2 or Fisher's exact tests were used to assess differences between proportions.

Associations between combinations of pairs of HPV types were analysed according to HIV status. Association strength was measured using odds ratios (ORs) adjusted for confounding variables (age, age at first sexual intercourse, pregnancies, history of other STDs, number of lifetime sexual partners, contraceptive methods used, history of abortions and smoking status).

Ordinal logistic regression models were used to estimating adjusted ORs, considering the number of infecting HPV types as the dependent variable (ranked 0–4, with 4 referring to infection by 4 or more genotypes). Clinical variables (CD4 cell count, HIV viral load and antiretroviral therapy (ART)) were added to the model describing HIV-positive women. HIV status, age, age at first sexual intercourse, total number of pregnancies, history of STDs, number of sexual partners, contraceptive method used, history of abortions and smoking status were considered independent variables. An additional model was constructed taking only HIV-positive women into account, to evaluate whether further independent variables (CD4 cell count in cells/mm^3, HIV viral load in copies/ml and ART use) were associated with the dependent variable. STATA12® software was used for statistical analyses, with statistical significance assumed at an alpha-value of 0.05.

Results

The study population consisted of 240 HIV-positive women and 1114 HIV-negative women who were eligible and agreed to participate in the study between February 2007 and February 2013. The average age of women enrolled in the study was 37.5 years (SD: 10.6 years) and the average age at first sexual relationship was 17.7 years (SD: 3.4 years). The sociodemographic and clinical characteristics of study participants were stratified according to HIV status and are shown in Table 1.

Pap smears and molecular detection test results showed that 70 HIV-positive women (29.1%; 95% CI 23.4–35.3%) had abnormal cytological findings using the Bethesda system (Table 1) and 164 HIV-positive women (68.3%; 95% CI: 62.0–74.1%) tested positive for HPV. Of the 164 HPV positive-women, 129 (78.7%; 95% CI: 71.5–84.6%) were simultaneously infected by more than one HPV type (defined as multiple infection) (Table 1). Analysis of type-specific distribution revealed that HPV-16 had highest prevalence in the study population, followed by HPV-31 and -18; these prevalence figures showed statistically significant differences ($P = 0.001$) (Figure 1a).

Compared with HIV-positive women, fewer HIV-negative women had cellular abnormalities and HPV infection. In total, 227 HIV-negative women (20.4%; 95% CI 18.0–22.8%) had some degree of cervical abnormality by Pap test, 486 were HPV positive (43.6%; 95% CI 39.0–44.9%) and 250 (51.4%; 95% CI 44.3–53.1%) were simultaneously infected by more than one HPV type (Table 1). In HIV-positive women, HPV-16 had the highest prevalence but the distribution of the remaining HPV genotypes differed from that in HIV-negative women. HPV-58 was the second most-prevalent type followed by HPV-31; these prevalence figures were statistically different from one another ($P = 0.039$). The distributions revealed that HPV-45 had the lowest prevalence in the women studied here ($P = 0.009$) (Figure 1b).

In HIV-positive women, HPV prevalence was higher in those having an abnormal Pap test result (76.4%) than in those without cervical lesions (64.9%) ($P = 0.001$). A similar effect was observed in HIV-negative women, where HPV was detected more often in women with some sort of cervical abnormality (52.8% *vs.* 41.2%; $P = 0.043$).

The number of genotypes involved in multiple infections was determined (simultaneous infection by 2, 3, 4 or more HPV genotypes) in women having some degree of cervical lesion (Figure 2). In HIV-positive women, infection by an increased number of HPV types was associated with an increase in lesion severity (mainly LSIL) ($P = 0.046$) (Figure 2a); this contrasted with the HIV-negative group, where women with a cytological finding of atypical squamous cells of undetermined significance

Table 1 Demographic (A) and Clinic (B) characteristics of the HIV-positive (n = 240) and HIV-negative women (n = 1114) enrolled in the study

A. Demographic variable	HIV-positive (n = 240)	HIV-negative (n = 1114)
Age, years*	37.3 [20–73] SD = 10.6	37.5 [20–76] SD = 10.6
	n (%)	n (%)
Ethnicity		
Indigenous	3 (1.2)	2 (0.2)
Mestizo	232 (96.7)	1083 (97.2)
Afrocolombian	5 (2.1)	29 (2.6)
Age at first Intercourse, years		
<15	86 (35.9)	257 (23.1)
16–19	120 (50.0)	592 (53.1)
>19	34 (14.1)	265 (23.8)
Pregnancies		
None	17 (7.1)	57 (5.1)
1–3	174 (72.5)	751 (67.4)
>4	49 (20.4)	306 (27.5)
History of other STD		
No	152 (63.3)	629 (56.5)
Yes	88 (36.7)	485 (43.5)
Lifetime number of sexual partners		
1–2	100 (41.7)	753 (67.6)
>3	140 (58.3)	361 (32.4)
Contraceptive method		
None	100 (41.6)	636 (57.1)
Intrauterine device	20 (8.4)	196 (17.6)
Hormonal	12 (5.0)	176 (15.8)
Condom	108 (45.0)	106 (9.5)
Abortions		
No	134 (55.8)	703 (63.1)
Yes	106 (44.2)	411 (36.9)
Smoking status		
No	197 (83.0)	750 (67.3)
Yes	43 (17.9)	364 (32.7)

B. Clinical variable	HIV-positive (n = 240)	HIV-negative (n = 1114)
	n (%)	n (%)
Cytological findings		
Normal	170 (70.9)	887 (79.6)
ASCUS	26 (10.8)	121 (10.8)
LEIBG	40 (16.7)	93 (8.4)
LEIAG	4 (1.6)	13 (1.2)
HPV infection		
Negative	76 (31.7)	628 (56.4)
Positive	164 (68.3)	486 (43.6)
HPV infection status†		
Single Infection	35 (21.3)	236 (48.6)
Multiple Infection	129 (78.7)	250 (51.4)
CD4 cell/mm^3 count		
<200	89 (37.1)	–
200–349	59 (24.6)	–
350–500	53 (22.1)	–
>500	39 (16.3)	–
HIV viral load copies/ml		
<1000	182 (75.8)	–
1000–4000	22 (9.2)	–
4000–99 999	22 (9.2)	–
>100 000	14 (5.8)	–
ART use		
With treatment	220 (91.7)	–
Without treatment	20 (8.3)	–

ASCUS, atypical squamous cells of undetermined significance; LSIL, low-grade squamous intraepithelial lesion; HSIL, high-grade squamous intraepithelial lesion; ART, antiretroviral therapy. SD, standard deviation; STD, sexually-transmitted diseases.
*Mean [Range].
†Denominator for this category was 164 HIV-positive and 486 HIV-negative women.

(ASCUS) had higher multiple infection frequency (P = 0.007) (Figure 2b).

Considering that multiple infections occurred with high frequency in the study population, the frequency of simultaneous infection by 2, 3 and >3 HPV genotypes was evaluated separately (Figure 1). In HIV-positive women, HPV-16, -18 and -31 were the most prevalent types in all multiple infection scenarios (Figure 1a and Table S1).

In HIV-negative women, HPV-16 had the highest prevalence (6.7%) in simultaneous infections with only 2 genotypes, whilst HPV-45 had the highest prevalence (4.2%) in multiple infections by 3 or >3 types (2.0%) (Figure 1b and Table S1).

Crude and adjusted ORs were calculated to evaluate the association between pairs of HR-HPV types according to HIV status. Positive associations were observed for the HPV-16 and HPV-31 combination in HIV-positive women. This contrasted with HIV-negative women, where positive associations were found for all genotypes evaluated; these associations were strongest between HPV-16 and -18, HPV-45 and -18, and HPV-45 and -58 (Figure 3).

Ordinal regression analysis was used to establish whether an association was present between risk factors

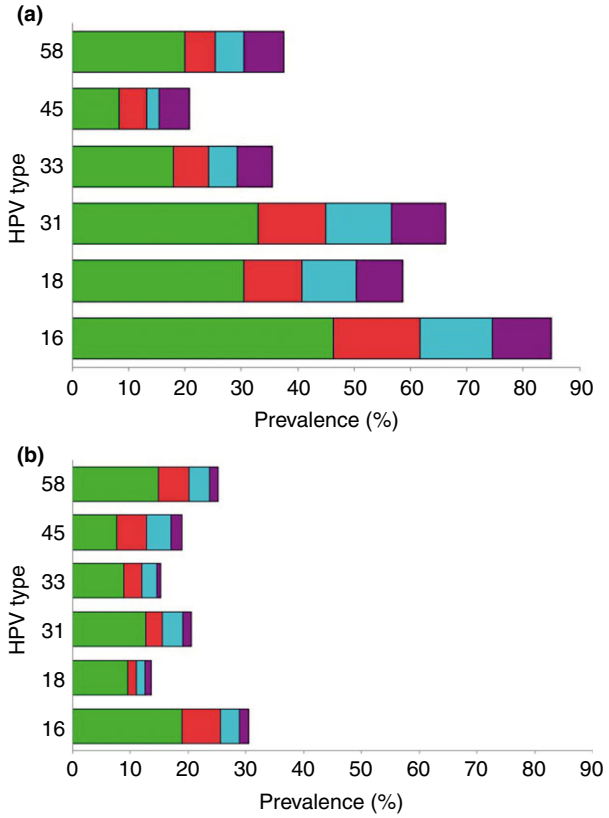

Figure 1 Relative frequency and distribution of multiple infection events for six high-risk HPV types according to HIV status (HIV-positive *vs*. HIV-negative patients). Green highlights each HPV type's relative frequency, red shows frequency of simultaneous detection of another genotype in the presence of each genotype, blue shows frequency of multiple infection with three genotypes for each genotype and purple shows the frequency of multiple infection with four or more genotypes for each genotype. [Colour figure can be viewed at wileyonlinelibrary.com].

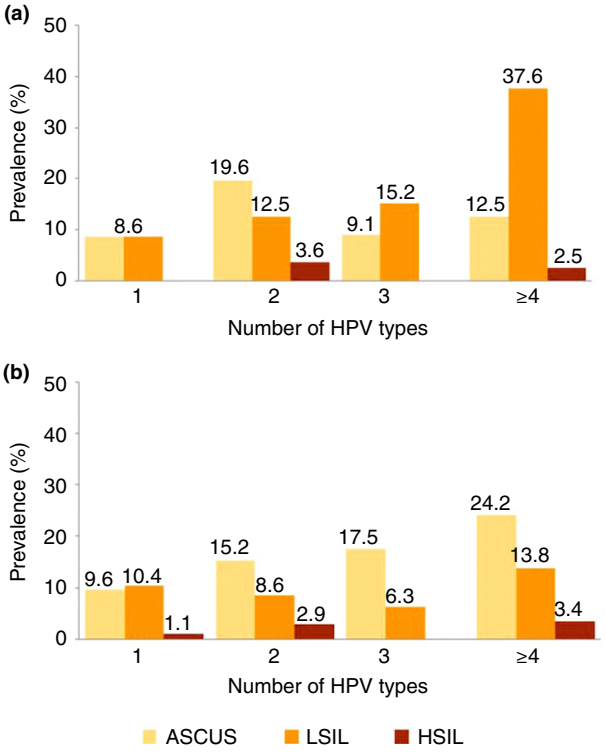

Figure 2 Distribution of the number of HPV genotypes in HIV-positive and HIV-negative women according to cytological findings. [Colour figure can be viewed at wileyonlinelibrary.com].

and the number of HPV types in multiple infections. Several risk factors showed significant associations. Women aged 35–49 years old were less likely to be infected by multiple HPV types (adjusted OR: 0.67; 95% CI 0.52–0.87). By contrast, positive associations were observed for risk factors such as three previous pregnancies (adjusted OR 1.72; 95% CI 1.01–2.92) and HIV-positive status (adjusted OR 3.27: 95% CI 2.34–4.57) (Table 2).

In a similar analysis involving only HIV-positive women other risk factors were identified associated with the number of HPV types in multiple infections. Positive associations were observed for women who had one to three pregnancies (adjusted OR 8.55; 95% CI 1.29–56.35) or more than four pregnancies (adjusted OR 3.65: 95% CI 1.38–9.66), used an intrauterine device (adjusted OR 5.98; 95% CI 1.15–36.19) or had a history of abortions (adjusted OR 2.63; 95% CI 1.17–5.89). The clinical characteristics (determined exclusively in HIV-positive women) showed that a viral load >100 000 copies/ml (adjusted OR 7.76; 95% CI 1.64–63.19) was strongly associated with multiple infection; by contrast, women with CD4 cell counts >500 cells/mm^3 were at lower risk of multiple infection (adjusted OR 0.38; 95% CI 0.13–0.98) (Table 3).

The association between cytological findings and the number of HPV types was evaluated in the study population. The results highlighted that women with ASCUS (adjusted OR 1.96; 95% CI 1.41–2.73), LSIL cytological findings (adjusted OR 1.71; 95% CI 1.20–2.44) or high-grade squamous intraepithelial lesion (HSIL) cytological findings (adjusted OR 2.43; 95% CI 1.03–5.72) had a higher probability of being infected by multiple HPV types (Table 4A). This positive association was maintained in women with LSIL cytological findings (adjusted OR 2.97; 95% CI 1.29–6.83) in the group of women living with HIV (Table 4B).

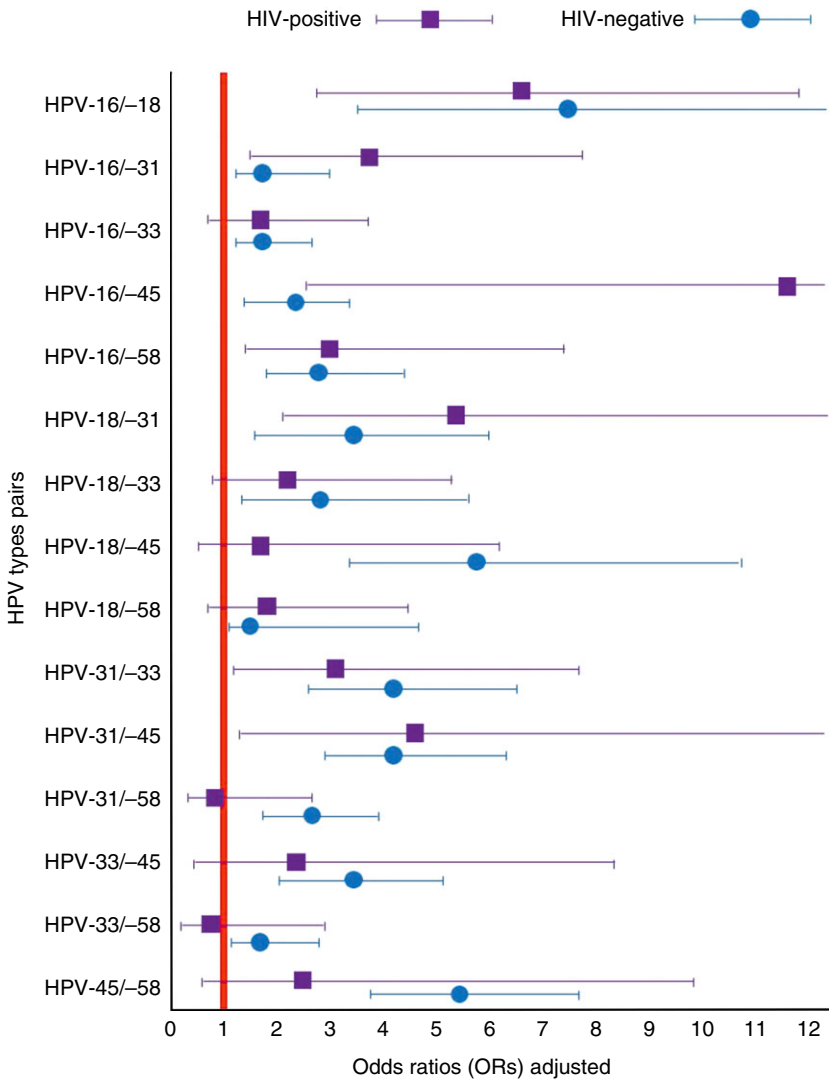

Figure 3 Number of infection pairs and odds ratios (ORs) according to pairwise combinations of specific HPV types and HIV status. [Colour figure can be viewed at wileyonlinelibrary.com].

Discussion

This study was intended to help understand the factors and determinants involved in simultaneous infection by more than one HPV genotype in women living with HIV, as these viruses share the same transmission route. We found that HPV infection and infection by multiple HPV types was more common in HIV-positive women (Table 1 and Figure 1a). This was expected since HIV-associated immunosuppression leads to low pathogen clearance due to reduced efficiency of cell-mediated, systemic and local immunity, thereby influencing the persistence of more than one viral genotype in the cervical epithelium [12, 18].

The high frequency of infection by multiple HPV types reported here agrees with previous work in other regions with high HIV prevalence [8], bearing in mind that both infections are sexually transmitted. HPV infection is favoured by HIV-immune responses generated during seroconversion as well as HIV-associated immunosuppression, which contributes to reactivation of latent stage HPV infections and to persistence of multiple cervical HPV types [4, 6].

HPV-16 had the highest prevalence in the study population (regardless of HIV status) (Figure 1); nevertheless, the results regarding this HPV type's contribution to CC development in HIV-positive women have been contradictory. The Women's Interagency HIV Study (WIHS) found that this HPV type had lower prevalence and weak association with development of high-grade cervical abnormalities in HIV-positive women (compared with

Table 2 Risk factors regarding the women included in this study (n = 1354) associated with the amount of HPV types

	OR*	95% CI
HIV status		
Negative	Reference	
Positive	**3.27**	**2.34–4.57**
Age		
20–34	Reference	
35–49	**0.67**	**0.52–0.87**
>49	1.33	0.92–1.93
Age at first intercourse		
<15	Reference	
16–19	0.88	0.68–1.14
>19	1.06	0.75–1.43
Pregnancies		
None	Reference	
1–3	**1.72**	**1.01–2.92**
More of 4	1.29	0.97–1.71
History of other STD		
No	Reference	
Yes	1.14	0.87–1.41
Lifetime number of sexual partners		
1–2	Reference	
More of 3	1.19	0.94–1.50
Contraceptive method		
None	Reference	
Intrauterine device	1.03	0.69–1.55
Hormonal	0.82	0.54–1.24
Condom	1.07	0.77–1.49
Abortions		
No	Reference	
Yes	1.21	0.95–1.54
Smoking status		
No	Reference	
Yes	0.87	0.69–1.10

Values in bold = $P < 0.05$. CI, confidence interval; STD, sexually-transmitted diseases; ART, antiretroviral therapy.
*OR adjusted for HIV status, age, age at first intercourse, pregnancies, history of other sexually- transmitted diseases, the number of lifetime sexual partners, contraceptive methods used, history of abortions and smoking status.

Table 3 Multivariable ordinal logistic regression with all factors included in the model in HIV-positive women (n = 240)

	OR*	95% CI
Age		
20–33	Reference	
34–49	0.91	0.41–2.04
>49	2.24	0.63–7.90
Age at first intercourse		
<15	Reference	
16–19	0.86	0.40–1.83
>19	0.67	0.20–2.17
Pregnancies		
None	Reference	
1–3	**8.55**	**1.29–56.35**
More of 4	**3.65**	**1.38–9.66**
History of other STD		
No	Reference	
Yes	1.04	0.49–2.20
Lifetime number of sexual partners		
1–2	Reference	
More of 3	1.67	0.74–3.61
Contraceptive method		
None	Reference	
Intrauterine device	**5.98**	**1.15–36.19**
Hormonal	0.72	0.14–3.61
Condom	1.78	0.81–3.91
Abortions		
No	Reference	
Yes	**2.63**	**1.17–5.89**
Smoking status		
No	Reference	
Yes	1.68	0.68–4.14
CD4 cell/mm^3 count		
<200	Reference	
200–349	1.13	0.44–2.89
350–500	0.79	0.32–1.98
>500	**0.38**	**0.13–0.98**
HIV viral load copies/ml		
<1000	Reference	
1000–4000	1.38	0.39–4.89
4000–99 999	5.83	0.25–9.33
>100 000	**7.76**	**1.64–63.19**
ART use		
With treatment	Reference	
Without treatment	1.88	0.47–7.54

Values in bold = $P < 0.05$. CI, confidence interval; STD, sexually-transmitted diseases; ART, antiretroviral therapy.
*OR adjusted for age, age at first intercourse, pregnancies, history of other sexually- transmitted diseases, the number of lifetime sexual partners, contraceptive methods used, history of abortions and smoking status, CD4-count, HIV-viral-load and antiretroviral therapy (ART).

immunocompetent ones) [19]. By contrast, studies in countries with heterogeneous populations have indicated that HPV-16 continues to have the highest prevalence (regardless of HIV status) and contributes towards development of cervical lesions in the population living with HIV [2, 20, 21].

The results of several studies have shown that non-HPV-16 types have high frequencies in the HIV-positive population and could play an important role in high-grade cervical lesion development. Bearing in mind that non-HPV-16 genotypes are less efficient at evading the immune system, the impact of HIV-related immunosuppression favours the persistence of these less common HPV types in the host and reduces clearance rates [3, 11, 22]. This suggests the need to include new genotypes in vaccination schemes, which would represent an

Table 4 Multivariable ordinal logistic regression of cytological findings associated with the amount of HPV types

	A. All Women (n = 1354)		B. HIV-positive (n = 240)	
	OR*	95% CI	OR*	95% CI
Cytological findings				
Normal	Reference		Reference	
ASCUS	**1.96**	**1.41–2.73**	1.61	0.69–4.16
LSIL	**1.71**	**1.20–2.44**	**2.97**	**1.29–6.83**
HSIL	**2.43**	**1.03–5.72**	1.54	0.23–5.95

CI, confidence interval; ASCUS, atypical squamous cells of undetermined significance; LSIL, low-grade squamous intraepithelial lesion; HSIL, high-grade squamous intraepithelial lesion.
*OR adjusted for HIV status, age, age at first intercourse, pregnancies, history of other sexually- transmitted diseases, the number of lifetime sexual partners, contraceptive methods used, history of abortions and smoking status.

appropriate strategy for reducing mortality caused by this neoplasm in HIV-positive women.

The present study demonstrated that the risk of acquiring multiple HPV infections was lower in women aged 35–49 years (Table 2), in agreement with previous descriptions of the natural history of infection by this virus [23, 24]. Number of births/parity was found to be positively associated with HPV multiple infection. The magnitude of this association was higher in HIV-positive women, which could be explained by the hormonal changes occurring with each pregnancy, favoring efficient viral replication [25].

The HPV life cycle in the host begins with its success in reaching the stratified squamous epithelium's basal cells, requiring a mechanical rupture to facilitate access [26, 27]. The results presented here showed positive associations between infection by multiple HPV types and use of an intrauterine device as well as a history of abortion in HIV-positive women (Table 3). Both of these factors result in epithelial inflammation, oxidative stress and cell damage, thereby facilitating mechanical rupture [28, 29] and providing HPV access to basal cells. The results suggested that exposure to these risk factors favoured the acquisition of multiple HPV types in HIV-positive women.

HPV penetration of the epithelium is favored by the expression of HIV proteins, leading to alteration in the cervix's normal architecture [18, 27]. It has been shown *in vitro* that expression of the HIV proteins Tat and gp120 increases transcription of genes encoding the HPV E6 and E7 proteins [24, 30]. A positive association between HIV viral loads greater than >100 000 copies/ml and infection by multiple HPV types was found here (Table 3), suggesting that HIV replication facilitates the coexistence and replication of multiples HPV types in cervical tissues.

The results also revealed a protective effect against multiple infection events in women having more than 500 CD4 cells/mm^3, indicating that the immune response is the key factor regarding HPV infection clearance. Immunity mediated by these cells favours efficient HPV clearance, limiting the pathogenesis of these infections [2, 31].

ART increases life expectancy in HIV-positive women since its consistent use leads to higher CD4 counts and lower viral loads; however, its usefulness in preventing CC development is still controversial. ART seems to favor elimination of HPV infection and also reduces the chance of acquiring new infections; however, ART does not seem to be able to completely restore immunity, as shown by the high HPV infection frequency in HIV-positive women. This suggests that ART has minimal effect on the natural history of CC infection and its development [2]. Our results indicated that using ART did not appear to have any additional effect on CD4 cell count and/or viral load; and no association between ART and HPV infection dynamics was observed in the HIV positive women in this study.

Strong associations between the HR-HPV pairs evaluated were observed in HIV-negative women, regardless of their phylogenetic relationships (Figure 3) [15]. There were fewer such associations between combinations of HPV genotypes observed in HIV-positive women; however, some were conserved including the association between HPV-16 and -31, suggesting that simultaneous infection by more than one HR-HPV genotype might occur differently in an immunosuppressed population [3]. Previous reports of HIV-positive women have indicated that whilst this infection facilitated HPV acquisition, specific HPV types might favor infection by additional HPV types, thereby promoting viral integration and cervical carcinogenesis [3].

The association between HPV multiple infection and low-grade cervical lesions occurred regardless of HIV status (Figure 2). Regarding HR-HPV multiple infection dynamics, two stages of the viral cycle (episomal replication and integration) were found to simultaneously promote the expression of proteins whose interactions might inhibit oncogenesis. Such negative regulation has been described for the E2-HPV-16 protein which blocks E6/E7-HPV-16/18 transcription, reducing integration and the appearance of high-grade cervical lesions [32, 33].

Nevertheless, a lack of follow-up of individuals with multiple HPV infections has limited a detailed relationship being established between multiple infections, HIV

status and the development of cervical lesions with poor prognosis. Prospective studies are needed to address these issues in detail. These would enable characterisation of the chronology of infection events and the natural history of cervical disease in HIV-positive women.

Conclusion

The results of this study should contribute towards understanding the natural history of CC development in HIV-positive women. The results indicated that HIV influences multiple HPV infection behaviour and dynamics. Further knowledge is required regarding the factors involved in the complex interactions between these two viruses and how their relationship influences the progression of pre-neoplastic lesions. Factors such as using ART appear to have no significant effect on the acquisition of multiple HPV types. Understanding the link between these viruses will provide better information for CC P&P programs regarding this population group and aid evaluation of the impact and effectiveness of prophylactic HPV vaccines currently being marketed.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Percentage of multiple infection events for six high-risk HPV types according to HIV status.

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