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Factors associated with increased prevalence of human papillomavirus infection in a cohort of HIV-infected Brazilian women

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Summary
Objectives: Human papillomavirus (HPV) infection is a major risk factor for cervical disease. Using baseline data from the HIV-infected cohort of Evandro Chagas Clinical Research Institute at Fiocruz, Rio de Janeiro, Brazil, factors associated with an increased prevalence of HPV were assessed.
Methods: Samples from 634 HIV-infected women were tested for the presence of HPV infection using hybrid capture II and polymerase chain reaction. Prevalence ratios (PR) were estimated using Poisson regression analysis with robust variance.
Results: The overall prevalence of HPV infection was 48%, of which 94% were infected with a high-risk HPV. In multivariate analysis, factors independently associated with infection with high-risk HPV type were: younger age (<30 years of age; PR 1.5, 95% confidence interval (CI) 1.1—2.1), current or prior drug use (PR 1.3, 95% CI 1.0—1.6), self-reported history of HPV infection (PR 1.2, 95% CI 0.96—1.6), condom use in the last sexual intercourse (PR 1.3, 95% CI 1.1—1.7), and nadir CD4+ T-cell count <100 cells/mm^3 (PR 1.6, 95% CI 1.2—2.1).

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Conclusions: The estimated prevalence of high-risk HPV-infection among HIV-infected women from Rio de Janeiro, Brazil, was high. Close monitoring of HPV-related effects is warranted in all HIV-infected women, in particular those of younger age and advanced immunosuppression.

Introduction

Human papillomavirus (HPV) infection has been consistently identified as a major risk factor for cervical squamous intraepithelial lesions and invasive cervical cancer.[1] In HIV-infected women, HPV infection and associated cervical diseases are more prevalent.[2–4] One explanation for this finding is HIV-induced immunosuppression, which may potentiate HPV replication and progression to cervical squamous intraepithelial lesions.[2,5] HIV-infected women are at increased risk of acquiring HPV infection, show higher HPV prevalence at subsequent time points, and persistently shed virus.[6,7] Finally, the HPV types most frequently associated with cervical cancer, such as HPV 16, are also more prevalent among HIV-infected women.[6]

In many developing countries, cervical cancer is the most common form of cancer in women.[8] In Brazil, cervical cancer is the third most common form of cancer and the fourth cause of death by cancer.[9] Limited cytological screening contributes to the high incidence of cervical cancer. In Brazil, life expectancy of HIV-infected women has increased with highly active antiretroviral treatment (HAART) availability.[10] We can speculate that in settings with limited cytological screening, HIV-infected women may potentially be at greater risk of developing cervical cancers from persistent cervical squamous intraepithelial lesions. Actually, published data has shown no clear impact of HAART on the incidence of cervical cancers.[11,12]

Therefore, the accurate assessment of the burden of HIV/HPV co-infection is of great importance. Data on HPV infection among HIV-infected women in Brazil are limited. To our knowledge, few studies have assessed prevalence and risk factors for HPV among HIV-infected Brazilian women. This study evaluated the prevalence of HPV infection among HIV-infected women in Rio de Janeiro, Brazil. Factors associated with prevalence of high-risk HPV were explored using baseline data obtained from the HIV-infected cohort of Evandro Chagas Clinical Research Institute at Fiocruz.[13] In addition we assessed the distribution of specific HPV types in a subsample of the women.

Methods

The cohort of HIV-infected women at the Evandro Chagas Clinical Research Institute at Fiocruz is a prospective open cohort followed in a clinical research hospital in Rio de Janeiro, Brazil. The focus of the cohort is to study the natural history of HIV infection in women, particularly in women with HIV/HPV co-infection. The cross-sectional analysis was performed using baseline demographic, behavioral, and epidemiological data. The baseline HPV infection status was established from specimens collected at enrollment. Informed consent was obtained from all women; the study was approved by the ethics review board of the institution.

Cohort procedures have been described elsewhere.[13] Briefly, during the initial pelvic examination, samples were collected in Digene HPV Hybrid Capture Universal Collection Medium (UCM®) to test for the presence of lower genital tract infections. Samples for HPV detection were collected, frozen, and sent to Digene do Brasil (Digene, Inc.).

For all samples, HPV detection and quantification were assessed using the hybrid capture II method (HC II), a non-isotopic, signal amplification technique, based on the hybridization of biotinylated RNA probes to the HPV DNA of the samples. The RNA—DNA hybrids were immobilized in microplate wells using an antibody adsorbed to the well surface. A cocktail of probes allowed characterization of the HPV positive samples into low-risk HPV types (group A, including HPV types 6, 11, 42, 43, and 44) and high-risk HPV types (group B, including HPV types 16, 18, 31, 33, 35, 39, 45, 51, 53, 56, 58, 59, and 68). HPV viral load results were calculated using a ratio of relative light units (RLU). The RLU ratio was defined as the signal generated from the test sample in the presence of the calibrator compared to the signal generated by 1 pg/ml of the HPV positive calibrator (PC). Samples that generated a signal greater than 1 RLU/PC were considered 'positive' for the respective HPV probe mix.

In addition to HC II, cervical samples from the last 112 women enrolled in the cohort were classified according to HPV type by polymerase chain reaction analysis (PCR). DNA was extracted using the proteinase K—phenol—chloroform standard method. Five μl of DNA plus 45 μl of deionized and distilled water (ddH_2O) were added to the linear array HPV Master Mix (Roche). The mixture contained PCR reagents and biotinylated primers spanning a 450-bp fragment from HPV L1 gene from 37 HPV genotypes. Human beta-globin biotinylated primers were also included to control specimen collection, DNA extraction, and PCR inhibition. Amplification took place in a gold-plated 9600 thermocycler (Applied Biosystems) and cycled according to the procedures listed in the package insert. PCR products were denatured and submitted to hybridization to immobilized oligonucleotide probes specific for each HPV type, plus control probes for beta-globin amplification. Following the hybridization and washing steps, the bound amplicons were identified based on color development via conventional streptavidin—horseradish peroxidase chemistry. The HPV genotype assessment was performed by comparing each strip signal to a reference guide provided in the kit. Samples that displayed an HPV-positive signal but not a beta-globin signal were excluded from the analysis, while those with no HPV or beta-globin signal were considered inadequate and not scored.

CD4+ T-cell count (Becton Dickinson FACScan) results were obtained from the participant's medical record; two different assessments of immunosuppression were considered. The nadir CD4+ T-cell count was defined as the lowest level of immunosuppression reported by the patient since HIV diagnosis but prior to entry into the cohort. The baseline CD4+ T-cell count was defined as the reported level of immunosuppression within 90 days of entry in the cohort.

The outcome of interest was infection with a high-risk HPV type, which was determined using the HC II. Since the prevalence of high-risk HPV infection was elevated, logistic regression analysis was not used for the estimation of the odds ratio.[14] A Poisson regression model with robust variance was used to estimate prevalence ratios for the independent factors associated with high-risk HPV infection.[14–16] Bivariate analysis was performed to assess the association of variables with high-risk HPV infection. With two exceptions, all factors independently associated with the outcome in the bivariate analysis, at the significance level of 0.15, composed the initial Poisson multivariate model (full model). The exceptions were 'baseline CD4+ T-cell count' and 'number of sexual partners within the past year', which were not included in the initial Poisson, although the statistical threshold was met. The rationale for these exceptions is related to the biological significance of these variables. With respect to the immunodeficiency level of the patient, the nadir CD4+ T-cell count best represents the level of immunosuppression reached by the patient, as it is not modified by the antiretroviral treatment.

Upon entry into the cohort, 53% of the women were already receiving antiretrovirals (ARV) and this might have affected their baseline CD4+ T-cell count. Therefore, baseline CD4+ T-cell count was not included in the initial multivariate model. With respect to the 'number of sexual partners within the past year', we considered that the 'total number of lifetime sexual partners' was more realistically related to the risk of exposure to HPV, as this infection is usually acquired near the sexual debut. As a result, 'number of sexual partners within the past year' was not included in the multivariate model. Finally, the variable codifying the use of HAART was forced into the initial Poisson model. The use of a backwards elimination procedure, with a 5% type I error acceptance threshold, guided the removal of covariates until a more parsimonious model was reached. Interactions between covariates were also explored. The statistical package STATA 7.0 was used for all analyses.

Results

A total of 634 women were enrolled into the cohort from May 1996 through December 2006. Demographic, behavioral, and epidemiological data for the cohort are presented in Table 1. At enrollment, the majority of the women were older than 30 years of age (71%, median age 36 years, interquartile range 29—43 years, mean age 36.3 years, standard deviation 9.6 years) and unmarried/not living with partner (63%). Most patients were non-white (53%), had received over 8 years of formal education (41%), and were unemployed (55%). For those who were employed, the majority earned less than US$ 300 per month (58%). Approximately 30% of the women reported having used drugs at least once (marijuana, snorted cocaine, crack, or solvents) and the majority were not habitual or previous cigarette smokers (50% were non-smokers and 76% were not current smokers). Sexual debut occurred at $\leq$17 years of age for 54% of the participants; the number of sexual partners within the past year and lifetime was '1' for 65% and 'up to 4' for 53% of the women, respectively. Approximately 20% of the participants reported a history of HPV infection; 52% reported condom use in the last sexual intercourse. At the time of enrollment into the cohort, 49% of the women had shown signs of advanced immunosuppression: nadir CD4+ T-cell count was $<$100 cells/mm^3 for 22% and between 100 and 200 cells/mm^3 for 49%, respectively. At enrollment, 53% of the participants had already received ARV for at least 60 days, of whom 60% were receiving HAART. The median time under HAART before enrollment was 213 days (interquartile range 113—432 days).

HC II was performed on all 634 samples, which revealed an overall prevalence of HPV infection of 48%. Of the 306 HPV-positive women, 287 (94%) were infected with at least one high-risk HPV type, while 19 (6%) had only low-risk HPV types. The overall prevalence of high-risk HPV types in this cohort was 45% (287 out of 634). Figure 1 shows the prevalence of HPV types for the subset of 112 participants for whom PCR genotyping analysis was available. Among these women, 87% had more than one HPV type detected. The HPV types most frequently detected were 68 (47%), 58 (41%), and 39 (24%). The HPV types most often associated with cervical squamous intraepithelial lesions and invasive cervical cancer, HPV types 16 and 18, were detected in 23% and 13% of the samples, respectively. HPV types 6 and 11 were each detected in less than 2% of the women. Also within this subset of women, 14% were infected with only one type while 16% were infected with two, 21% with three, 19% with four, 11% with five, and 12% with more than five types.

Table 2 shows the results from the bivariate analysis using the Poisson regression model for the estimation of the prevalence ratios (PR). Several demographic, behavioral, and epidemiological characteristics were independently associated with infection by a high-risk HPV type. An inverse relationship was found between high-risk HPV infection and age: women $<$30 years of age had a prevalence of high-risk HPV almost 50% higher than women over 40 years of age (PR 1.5, 95% confidence interval (CI) 1.2—1.9 for age $<$30 years). Being married/living with partner (PR 1.2, 95% CI 1.0—1.5), reported drug use at any point in life (PR 1.2, 95% CI 1.0—1.4), and earlier sexual debut ($\leq$17 years of age; PR 1.2, 95% CI 1.0—1.4) were significantly associated with high-risk HPV infection. The number of sexual partners in the previous year (PR 1.5, 95% CI 1.1—2.1 for '2 or more partners' compared to 'none') and total lifetime sexual partners (PR 1.2, 95% CI 0.97—1.4) were also significantly associated with high-risk HPV infection. As expected, self-reported history of prior HPV infection was associated with detection of a current infection (PR 1.3, 95% CI 1.1—1.5). Condom use in the last sexual intercourse was significantly associated with high-risk HPV infection (PR 1.3, 95% CI 1.1—1.7). In the bivariate analysis, the variable most significantly associated with high-risk HPV infection was the nadir CD4+ T-cell count (PR 1.6, 95% CI 1.3—2.0 for CD4+ T-cell count $<$100 and PR 1.4, 95% CI 1.1—1.8 for CD4+ T-cell count 100—200). Immunosuppression at baseline, defined as a CD4+ T-cell count $<$200 cells/mm^3, was also significantly associated with high-risk HPV infection (PR 1.6, 95% CI 1.2—2.1).

Figure 2 shows that the overall HPV viral load increased, as reflected by higher RLU ratios, as the baseline CD4+ T-cell count decreased (p = 0.002). No significant associations were found between HPV infection and race/ethnicity, schooling, income, smoking (current or ever), number of pregnancies, and self-reported history of STDs (except HPV).

The result of multivariate modeling is shown in Table 3. As previously stated, with two exceptions, the covariates

Table 1 Demographic, behavioral, and epidemiological data for the 634 women enrolled in the HIV-infected cohort of Evandro Chagas Clinical Research Institute at the Oswaldo Cruz Foundation, Rio de Janeiro, Brazil

Characteristic	*n* (%)
Age (years)	
<30	181 (28.5)
30–40	267 (42.1)
>40	186 (29.3)
Race/ethnicity	
White	295 (46.5)
Non-white	339 (53.5)
Married/living with partner	
Yes	237 (37.4)
No	397 (62.6)
Schooling (years of formal education)	
<5	150 (23.9)
5–8	220 (35.1)
>8	257 (41.0)
Monthly income (US Dollars)[a]	
<75	93 (14.7)
75–300	275 (43.4)
>300	266 (42.0)
Employment status	
Employed (formal or informal)	283 (44.7)
Unemployed	350 (55.3)
Ever smoked	
No	230 (50.5)
Yes	225 (49.5)
Current smoker	
No	343 (75.6)
Yes	111 (24.4)
Ever used drugs	
No	440 (69.5)
Yes	193 (30.5)
Age at first sexual intercourse (years)	
>17	290 (45.7)
≤17	344 (54.3)
Number of sexual partners in the previous year	
None	146 (23.0)
1	414 (65.3)
2 or more	74 (11.7)
Number of lifetime sexual partners	
Up to 4	334 (52.7)
5 or more	300 (47.3)
Number of pregnancies	
None	54 (8.5)
1–3	376 (59.4)
4 or more	203 (32.1)
Self-reported history of STD (except HPV)	
No	387 (61.6)
Yes	241 (38.4)
Self-reported history of HPV	
No	349 (80.2)
Yes	86 (19.8)
Condom use in the last sexual intercourse	
No	276 (47.9)
Yes	300 (52.1)
Nadir CD4+ T-cell count (cells/mm^3)	
<100	105 (22.0)
100–200	234 (49.2)
≥200	137 (28.8)
Baseline CD4+ T-cell count (cells/mm^3)	
<200	129 (25.8)
200–500	234 (46.8)
≥500	137 (27.4)
Use of ARV therapy for at least two months before enrollment	
No	295 (46.8)
Yes	336 (53.2)
Use of HAART among those in use of ARV	
No	136 (40.5)
Yes	200 (59.5)

Percentages are given within the valid responses of each variable.
STD, sexually transmitted disease; HPV, human papillomavirus; ARV, antiretroviral; HAART, highly active antiretroviral therapy.
[a] Conversion: US$ 1 = R$ 2.

significantly associated with high-risk HPV infection in the bivariate analysis, assuming a threshold of 0.15, were introduced into the final model. The variable codifying the use of HAART was forced into the initial model. The final multivariate model, comprised of five variables, highlights the independent predictors of high-risk HPV infection. Age (<30 years of age) was associated with a 52% higher prevalence of infection when compared to women ≥40 years of age (PR 1.5, 95% CI 1.1–2.1). A higher prevalence of high-risk HPV infection was associated with 'reported use of drugs at least once' (PR 1.3, 95% CI 1.0–1.6) and 'reported history of HPV infection' (PR 1.2, 95% CI 0.96–1.6). 'Reported history of HPV infection' was kept in the final multivariate model because of its biological plausibility and borderline statistical significance. Condom use in the last sexual intercourse was significantly associated with high-risk HPV infection (PR 1.3, 95% CI 1.1–1.7). Finally, the degree of immunosuppression prior to enrollment within the cohort was the factor most significantly associated with high-risk HPV infection. Women having a nadir CD4+ T-cell count lower than 100 cells/mm^3 had a prevalence of infection 56% higher than those who were not immunosuppressed (PR 1.6, 95% CI 1.2–2.1). A nadir CD4+ T-cell count of 100–200 cells/mm^3 was associated with a prevalence of infection 34% higher compared to women who were not immunosuppressed (PR 1.3, 95% CI 1.2–2.0).

Discussion

With the introduction and availability of HAART, the spectrum of disease in the AIDS epidemic has been shifting. It is estimated that up to 40% of HIV-infected individuals may develop a neoplastic lesion, including cervical cancer.[17] The

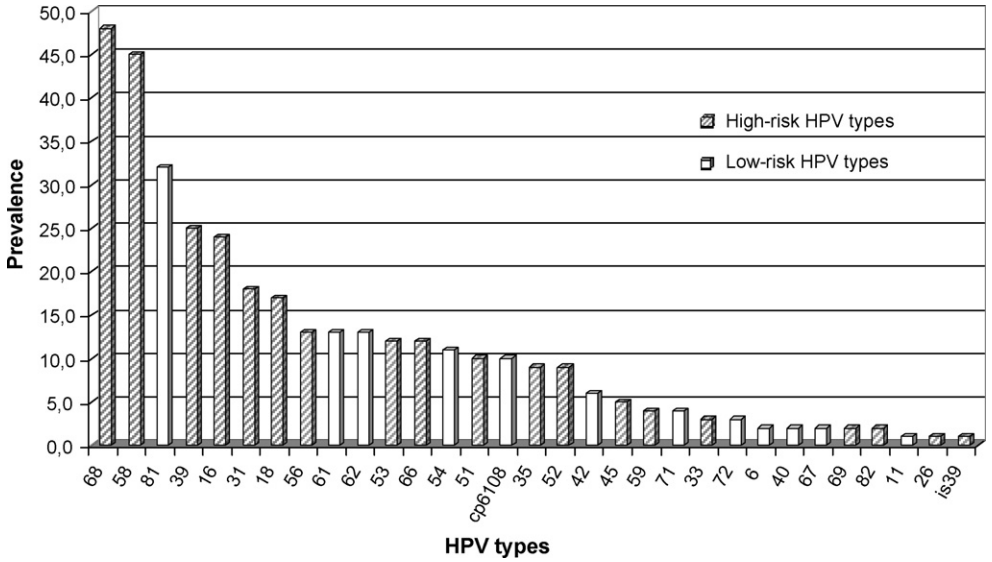

Figure 1 Prevalence of HPV type for 112 HPV infected women enrolled in the HIV-infected cohort of Evandro Chagas Clinical Research Institute at Oswaldo Cruz Foundation, Rio de Janeiro, Brazil.

relationship between cervical neoplasia, the end stage of HPV-associated cervical intraepithelial lesions, and HIV infection was identified early in the epidemic as an important gender-specific problem.[18] In this study we have assessed the prevalence of HPV infection among the HIV-infected women of the Evandro Chagas Clinical Research Institute cohort. Risk factors for HPV infection were statistically assessed to better characterize the population at greater risk of HPV infection and, possibly, HPV derived complications.

We detected a high prevalence of HPV infection (48%) in this cohort. This finding is in accordance with studies conducted in other parts of the world using the same HPV detection methodology.[19–21] A higher prevalence of HPV infection has been detected in Brazil and in other countries using laboratory assays with increased sensitivity, such as PCR.[22–27] In Brazil, the prevalence of HPV among women of unknown HIV status has been shown to be substantially lower (14%[28]) than that measured in this population.

We have also detected a high overall prevalence of high-risk HPV types (45%), and a high prevalence of high-risk HPV types among HPV-infected women (94%). In addition, increased HPV viral load levels were directly related to lower baseline CD4+ T-cell counts (Figure 2). These findings are in agreement with several studies in HIV-infected women that detected a higher prevalence of high-risk HPV types and of HPV co-infections, and an increased HPV viral load in immunosuppressed women.[2,6,23,25,29–31] It remains unclear whether the increased prevalence of infection and the increased number of HPV types detected reflect recent exposure or reactivation of prior HPV infection due to HIV induced immunosuppression.[5] Our data suggest that HIV-induced immunosuppression increases the likelihood of detecting an HPV genital infection. Other studies suggest that different mechanisms may be involved, such as higher shedding of viral particles caused by cellular changes secondary to the HIV/HPV interaction,[32] and higher persistence of HPV infection among HIV-infected women facilitating diagnosis.[27,31,32] Also, higher HPV viral load among individuals with lower CD4+ T-cell counts may facilitate HPV detection in this population.[5]

Of the studies conducted in Brazil in HIV-infected women, few have evaluated the association between covariates and the prevalence of HPV infection. The knowledge of risk factors for HPV infection among HIV-infected women identifies women at particularly high risk of HPV infection and cervical neoplasia, who should be targeted by cervical cancer prevention programs. This is particularly important in places where access to cytological screening and gynecological care is limited. Factors statistically associated with a higher prevalence of high-risk HPV infection are described below. Some of these factors have been found to be associated with other sexually transmitted diseases.[13]

The prevalence of genital HPV infection has typically been shown to decrease with age.[33,34] Consistent with these data, in our study, young age was found to be a significant risk factor for high-risk HPV infection. It has been shown that cervical HPV infection is acquired early after sexual debut. HPV prevalence peaks in the late teens/early adulthood and then declines.[35] Although the mechanism of the age-related decline of HPV prevalence is not well understood, it may reflect acquisition of cell-mediated immunity.

Total number of lifetime sexual partners was significantly associated with high-risk HPV infection. This suggests that the detection of HPV in HIV-infected women is a reflection of either reactivation or persistence of pre-existing HPV infection rather than recent HPV acquisition. Other reports have associated genital HPV with the total number of lifetime sexual partners.[33,36,37] Interestingly, in the Women's Interagency HIV Study (WIHS) and the HIV Epidemiology Research Study (HERS), the number of male sexual partners within the past 6 months was not associated with the prevalence of HPV infection.[25] In the WIHS cohort, 22% of sexually inactive HIV-positive women with a CD4+ T-cell count of <200 cells/mm^3 had at least one incident HPV detection.[38] This is consistent with the idea that a substantial proportion of HPV infection detected in older women may reflect reactivation of an old HPV infection rather than a new infection.[38]

Table 2 Prevalence ratios of bivariate analysis of covariates for high-risk HPV[a] for the 634 women enrolled in the HIV-infected cohort of Evandro Chagas Clinical Research Institute at the Oswaldo Cruz Foundation, Rio de Janeiro, Brazil

Variable	PR	95% CI
Age (years)		
<30	1.47	1.16–1.86[d]
30–40	1.29	1.02–1.62[d]
>40	1.00	
Race/ethnicity		
White	1.00	
Non-white	1.01	0.85–1.19
Married/living with partner		
Yes	1.21	1.00–1.45[d]
No	1.00	
Schooling (years of formal education)		
<5	0.95	0.76–1.19
5–8	1.03	0.85–1.25
>8	1.00	
Monthly income (US Dollars)[b]		
<75	0.94	0.71–1.25
75–300	1.13	0.94–1.35
>300	1.00	
Ever smoked		
No	1.00	
Yes	1.02	0.84–1.24
Current smoker		
No	1.00	
Yes	1.02	0.81–1.27
Ever used drugs		
No	1.00	
Yes	1.19	1.00–1.42[c]
Age at first sexual intercourse (years)		
≤17	1.19	1.00–1.42[c]
>17	1.00	
Number of sexual partners in the previous year		
None	1.00	
1	1.26	0.99–1.59[c]
2 or more	1.50	1.10–2.04[d]
Number of lifetime sexual partners		
Up to 4	1.00	
5 or more	1.15	0.97–1.37[c]
Number of pregnancies		
None	1.18	0.89–1.57
1–3	1.04	0.86–1.24
4 or more	1.00	
Self-reported history of STD (except HPV)		
No	1.00	
Yes	1.07	0.90–1.28
Self-reported history of HPV		
No	1.00	
Yes	1.27	1.06–1.53[d]
Condom use in the last sexual intercourse		
No	1.00	
Yes	1.34	1.09–1.65[d]
Nadir CD4+ T-cell count (cells/mm^3) ever before enrollment		
<100	1.59	1.28–1.98[d]
100–200	1.42	1.11–1.81[d]
≥200	1.00	
Baseline CD4+ T-cell count (cells/mm^3) within 90 days of enrollment		
<200	1.58	1.20–2.07[d]
200–500	1.13	0.86–1.49
≥500	1.00	
Use of ARV therapy for at least two months before enrollment		
No	1.00	
Yes	1.10	0.92–1.31
Use of HAART among those in use of ARV		
No	1.00	
Yes	1.14	0.89–1.45

PR, prevalence ratio; CI, confidence interval; STD, sexually transmitted disease; HPV, human papillomavirus; ARV, antiretroviral; HAART, highly active antiretroviral therapy.
[a]Hybrid capture II results.
[b]Conversion: US$ 1 = R$ 2.
[c]*p*-Value less than 0.15.
[d]*p*-Value less than 0.05.

A self-reported history of HPV infection was also an independent predictor of HPV infection among study participants. This finding is consistent with the reports from the WIHS cohort.[25] A history of previous exposure to HPV, as an independent risk factor for HPV infection, suggests that HIV infection can act as a modulating factor of the natural history of HPV infection, which can lead to a longer persistence and the potential development of HPV-related cervical lesions. HPV infection was also more prevalent among women who reported drug use at least once in their life. This finding has been reported in other studies.[30,39]

In this study, use of condom in the last sexual intercourse was a predictor of HPV infection. Although condom use is an effective barrier against genital HIV transmission, the protective effect of condom use for other sexually transmitted infections, including HPV, is ambiguous.[40–42] A paradoxical effect has been reported stating that condom use increases risk of HPV infection.[40,43,44] This is likely a result of an increased probability of infection among partners with whom condoms are used. That is, people tend to use condoms with partners that they consider to be 'high risk' (e.g., new partners, casual partners, sex workers), but not with partners they consider 'safe' (e.g., long-term, regular partners or spouses).

In 1991, Brazil made available ARV drugs for HIV/AIDS patients, and since 1996, HAART has been universally available in the country. In our cohort, at the time of enrollment, 53% of the women were already using ARV, of whom almost 60% were receiving HAART. It is possible that by the time of cohort entry, some level of immune restoration in response to ARV had already occurred in a fraction of the women. As a result, we chose to exclude the baseline CD4+ T-cell counts in the multivariate modeling. In our cohort, the nadir CD4+ T-

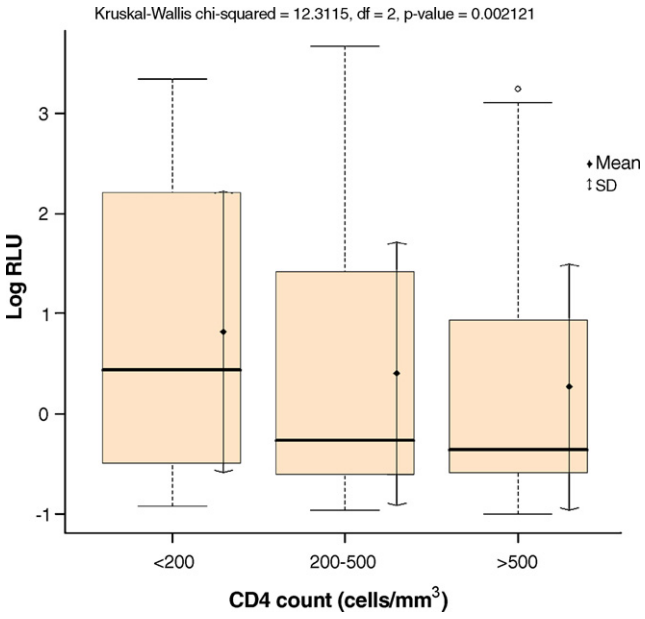

Figure 2 HPV viral load as a function of immunosuppression as defined by the baseline CD4+ T-cell count. (The baseline CD4+ T-cell count was used instead of the nadir CD4+ T-cell count since the objective is to correlate the level of immunosuppression at enrollment with the CD4+ T-cell count at that time.).

cell count best reflects the level of immunodeficiency reached by these women. Nadir CD4+ T-cell count was the strongest independent risk factor for HPV infection in the bivariate and multivariate analyses (Tables 2 and 3, respectively). This result is consistent with the role of the immune response in controlling HPV infection. After controlling other factors, HPV prevalence was 56% and 34% higher among those women with nadir CD4+ T-cell counts less than 100 cells/mm^3 and between 100 and 200 cells/mm^3, respectively (compared to women with higher CD4+ T-cell counts). This result suggests that as the CD4+ T-cell count declines, vigilant follow-up of the anogenital tract, particularly with cervical cytological screening, is warranted.

In this analysis, use of ARV or HAART was not shown to be associated with a protective effect for HPV infection. An immunological mechanism may be invoked in order to interpret this finding. CD4+ T-cell count reconstitution as a function of HAART does not necessarily reflect HPV-specific immune reconstitution. However, although a protective effect of HAART on cervical HPV infection has not been shown, a higher rate of regression of high-grade cervical intraepithelial neoplasia (CIN) among women on HAART compared to those not on HAART has been observed.[3]

In the multivariate analysis five factors remained independently associated with HPV infection: (a) younger age, (b) drug use ever, (c) self-reported history of HPV infection, (d) condom use in the last sexual intercourse, and (e) immunosuppression. Of note, the most significant factor associated with HPV infection was advanced immunosuppression as measured by the nadir CD4+ T-cell count. Our results support the association of immunosuppression as an important determinant of HPV infection. Perhaps HIV acts as a modulating factor of the natural history of HPV infection, leading to a longer persistence and greater production of virus particles[31] (and thus allowing detection). Actually the longer persistence and greater production of virus might lead to greater infectivity. Yet this effect would appear to persist well beyond the period of low CD4+ T-cell count, when HIV is controlled and CD4+ T-cell counts rise, and HPV is still detected. This may contribute to the higher risk of development of HPV related cervical lesions in the HIV-infected subgroup.

Of note, the use of newly available preventive measures, specifically the HPV vaccines targeting types 16 and 18, may help mitigate the impact of HIV on cervical cancer in developing countries. In settings where cytologic screening remains insufficient, prevention of HPV infection might be the best strategy. HPV prevalence studies such as this one are fundamental for planning HPV immunization programs for HIV-infected women. The relatively low prevalence of HPV types 16 and 18, the higher prevalence of other high-risk HPV types, and the frequent detection of multiple HPV types in our study should be carefully taken into account when considering immunization strategies for HIV-infected women in our setting. Currently, HPV types 58, 68, and 39 are not included in the tetravalent vaccine and there are very limited data available considering cross-protection to these types in immunized subjects.[45] HPV 58 is highly prevalent in South American women, independent of cytological classification or HIV status.[46–48] While the oncogenic potential of HPV 39 is well-established, that of HPV 68 remains undetermined, being both included in the high-risk group and belonging to the species 7 of the genus alpha-papillomavirus, together with HPV 18.[49] HPV 68 appears to be more prevalent in Brazilian HIV-infected women than in other populations, including HIV-infected women from other countries. Our results suggest that the investigation of prevalence and clearance of HPV types 58, 68, and 39 is of importance in vaccine trials among Brazilian HIV-infected women. Increased HPV prevention strategies are needed particularly in the HIV infected population, where the incidence of HPV-related lesions may increase due to the extended survival of HIV patients under HAART treatment.

This study has some limitations particularly as regards the fact that the cross-sectional analysis of the cohort only allows detection of factors associated with prevalence of HPV infection. Currently, a longitudinal study of the cohort is underway to evaluate if the HPV infections here described are transitory or persistent, bringing further consistency to these results. Also, HPV typing results were restricted to a subset of patients and studies with larger series of patients with HPV typing results are needed.

In summary, the high prevalence of HPV infection, particularly of high-risk HPV types, in HIV-infected women, is worrisome given the still limited access to cytological screening and gynecological care. Widespread availability of HAART adds further complexity to the HIV–HPV co-infection problem. Increased patient survival rates may allow for cervical lesions to further evolve, resulting in an increased risk of developing a neoplasia. Current data on the effect of HAART on the natural history of CIN are mixed, but it seems clear that if there are beneficial effects, these are relatively modest. There is still much to be learned with respect to the impact of HAART on HPV infection and associated cervical diseases. HIV-infected

Table 3 Prevalence ratios (PR) in multivariate analysis for factors independently associated with high-risk HPV for the 634 women enrolled in the HIV-infected cohort of Evandro Chagas Clinical Research Institute at the Oswaldo Cruz Foundation, Rio de Janeiro, Brazil. Parameters of the Poisson regression model were estimated by maximum likelihood

Variable	Initial model[a]		Final model	
	PR	95% CI	PR	95% CI
Age (years)				
<30	1.47	0.99–2.17[c]	1.52	1.08–2.12[c]
30–40	1.11	0.77–1.60		0.86–1.65
>40	1.00		1.19	
Married/living with partner				
Yes	1.05	0.78–1.41		
No	1.00			
Ever used drugs				
No	1.00		1.00	
Yes	1.20	0.92–1.58	1.28	1.02–1.60[c]
Age at first sexual intercourse (years)				
≤17	1.15	0.85–1.55		
>17	1.00			
Number of lifetime sexual partners				
Up to 4	1.00			
5 or more	1.23	0.92–1.63		
Self reported history of HPV				
No	1.00		1.00	
Yes	1.16	0.85–1.59	1.23	0.96–1.59
Condom use in the last sexual intercourse				
No	1.00		1.00	
Yes	1.18	0.89–1.57	1.34	1.06–1.70[c]
Nadir CD4+ T-cell count (cells/mm^3)				
<100	1.46	1.05–2.02[c]	1.56	1.18–2.06[d]
100–200	1.45	1.05–2.00[c]	1.34	1.16–1.96[d]
≥200	1.00		1.00	
Use of highly active antiretroviral therapy[b]				
No	1.00			
Yes	1.09	0.82–1.44		

PR, prevalence ratio; CI, confidence interval; HPV, human papillomavirus.
[a]Covariates entering initial full model were selected with a 0.15 cutoff value in bivariate analysis. Exceptions are 'number of partners in the previous year' and 'baseline CD4+ T-cell count' which were not included. 'Use of highly active antiretroviral therapy' was forced into initial model. Variable removal was guided by effect size and statistical significance using the 0.05 threshold.
[b]For those in use of ARV therapy for at least 2 months prior to enrollment.
[c]*p*-Value less than 0.05.
[d]*p*-Value less than 0.01.

women should be among those prioritized in screening programs. Focus should rest on the youngest, with advanced immunosuppression, and history of HPV and of drug use. It is urgent that public health officials be aware of the potential impact of the HIV epidemic on cervical cancer.

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