

**HUMAN PAPILLOMAVIRUS INFECTION IN HUMAN IMMUNODEFICIENCY  
VIRUS POSITIVE WOMEN UNDER ROUTINE PAP SMEAR**

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**ABSTRACT**

The present study aimed to investigate HPV prevalence associated with cervical cytology and to evaluate data from 140 HIV positive women routinely attended at an out-patient public gynecological service in Rio de Janeiro state. The CD4 cell counts and HIV-1 RNA levels were determined using standardized protocols. HPV status and HPV 16 detection were ascertained respectively by My09/11 consensus primers and type specific primers. Colpocytology was performed at the first or subsequent visit to the service. Among women, 98.6% of them were under antiviral treatment, 92.1% had over 200 CD4 cells/mm<sup>3</sup>, 62.9% had detectable HIV RNA, 60.7% were HPV infected and 21.2 % harbored the HPV type 16. Normal/inflammatory results were prevalent (57.1%) and 7.9% of the patients had HSIL. Studying demographic data and HIV markers that could affect HPV infection, we found that time elapsed since positive diagnosis of less than four years was a significant factor. First sexual intercourse beginning earlier than 17 years, people younger than 30 years and white ethnicity were also linked to HPV infection. Alcohol use was marginally related to HPV prevalence. Patients with severe immune depression were strongly predisposed to HPV infection (from 90.9% of patients carrying less than 200 cells/mm<sup>3</sup> to 45.5% in those with CD4 cell level above 500 cells/mm<sup>3</sup>) and the development of cervical lesions. There was no relation between HIV immune status and HPV type 16, but HIV seropositive women with abnormal cytology were three times more likely to harbor this HPV type. The high HPV prevalence as opposed to absence of cancer cases was probably due to the moderate immune status of the women studied added to routine cancer prevention exams.

## INTRODUCTION

HIV-HPV co-infection has specific aspects that merit investigation in the light of public health perspectives. Several epidemiological reports have pointed out that human papillomavirus (HPV) co-infects human immunodeficiency virus (HIV) seropositive women in a higher prevalence (more frequently) than seronegative women (Feingold et al. 1990, Maiman et al. 1990, Palefsky et al. 1999, Hankins et al. 2000, Fruchter et al. 1996). Besides the majority of demographic and risk factors that are common to both infections, immunologic status added an additional risk to HPV infection, and led to progression to severe lesions (Mougin et al. 2001). Moscicki et al. (2004) also verified that the time span to HPV elimination in HIV seronegative teenagers was shorter than in seropositive ones.

HPV are small DNA viruses that infect human epithelia. Molecular studies have already identified more than 100 different types. The most important types are classified in the mucosa HPV group and present a high prevalence worldwide, such as HPV 16, 18, 31, 33, 35, 45, 51 and 58. In this way, HIV infected women tend to acquire high risk HPV types in single or in multiple infections (Levi et al. 2002). Among high risk types linked to squamous intraepithelial lesions (SIL), HPV 16 is the most common type in HIV seronegative people, and it is associated with the majority of high grade lesions and carcinoma. Nevertheless, HPV 16 prevalence in HIV seropositive women, despite having increased in comparison to healthy women, occurs at lower prevalence when compared to other oncogenic HPV types. This suggests that it has less association with immune status than other HPV types (Strickler et al. 2003). Although unequivocal association between HPV infection and immune status has been well established, the relationship between specific types and immunosuppression grade is complex and presents a diverse profile

according to different risk factors for HIV infection, duration of HIV infection and antiretroviral therapy (Palefsky & Holly 2003).

Antiretroviral treatment is a controversial factor in reducing genital lesions linked to HPV infection. While some authors described an inverse correlation between these lesions and CD4 counts, other investigations did not confirm these findings in patients with recovered immune status promoted by anti-HIV therapy (Palefsky 2006, Lillo et al. 2001, Heard et al. 2002).

The aim of this study was to determine demographic and health status pertaining to HPV/HIV co-infection amongst women attended at an out-patient gynecological service of a public hospital in Rio de Janeiro State.

## **MATERIALS AND METHODS**

### **Subjects and specimens.**

A cross-sectional study population included cervical samples from 140 HIV infected women collected for HPV detection between 2003 and 2005. Patients were referred to the Cervical Pathology Service of the Hospital dos Servidores de Estado (HSE), Rio de Janeiro, Brazil, according to the routine schedule of HIV cervical screening. Colpocytology was performed at the first or subsequent visit to the Service. Biopsies were performed for women with abnormal cervical cytology. Cervical lesions were classified as Normal, HPV infection (koilocytosis), atypical squamous cells of undetermined significance (ASCUS), low grade squamous intraepithelial lesions (LSIL), or high grade squamous intraepithelial lesions (HSIL) (Solomon et al. 2002). The Ethics Committee of the College of Medicine at the University approved the protocols for collection and for informed consent. Demographic, reproductive history and lifestyle information were

obtained through a structured questionnaire. Final diagnoses were the result of cytological/histological examination.

**Clinical laboratory data.**

The CD4 counts were determined in real time by flow cytometric immunophenotyping using standardized protocols (Health Ministry 2008). All of the assays were done in a laboratory participating in the National STD and AIDS Program. Plasma HIV-1 RNA levels were measured in virology quality assurance–certified laboratories according to the same Program with the nucleic acid sequence–based amplification technique (NASBA- Nuclisens HIV-QT, Biomerieux); the lower threshold of detection was 80 copies/ml (Health Ministry 2008).

**DNA extraction and PCR procedure.**

Samples were incubated for 4 hours at 50°C in digestion buffer (10 mM Trishydrochloric acid pH 8.3, 1 mM EDTA pH 8.0, 0.5% Tween 20, proteinase K; final concentration of 400 µg/ml). Later, they were extracted with phenol-chloroform-isoamyl alcohol (25:24:1). DNA was precipitated with one-tenth volume of 0.3 M sodium acetate and three volumes of 100% ice-cold ethanol, washed with 70% ethanol, air-dried and suspended in 50 µl of sterile water.

MY09/11 consensus primers, which amplify 450-bp (base pair) DNA sequences within the L1 region of HPV, were used to detect generic HPV DNA (Manos et al. 1989). Amplification was carried out in 50 µl of reaction mixture (1 X polymerase chain reaction [PCR] buffer, 200 mM dNTPs, 1.5 mM MgCl<sub>2</sub>, 50 pmol of each primer, 0.25 U unit of Taq polymerase [Invitrogen Brazil, São Paulo, SP, Brazil] and 5 µl of sample) with 35 cycles of amplification. Each cycle included a denaturation step at 94° C for 1 minute, an annealing step at 55° C for 2 minutes, and a chain elongation step at 72° C for 2 minutes using DNA

Thermal Cycler (Perkin Elmer, Cetus). The beta-actin primers Ac1 and Ac2 (0.1 pmol each), which amplify a 330-bp region of the human DNA, were used as a sample internal control (Gall et al. 1993). Negative controls for background contamination did not add to the DNA template. HPV typing was done by PCR using primers from the E6 gene DNA sequences of HPV 16 (Young et al. 1989). These primers yielded one 132-bp fragment. The PCR product was analyzed on a 1.3% agarose gel with ethidium bromide staining for visualization of DNA under ultraviolet light, and molecular weight was determined by comparison with a 100-bp DNA ladder.

**Statistical analysis.**

Statistical significance of results has been analyzed by using the  $X^2$  test for heterogeneity with Yates continuity correction, and the Fisher exact test when appropriate. The significance level of tests (p) was set at 0.05.

**RESULTS**

The patients were aged 14-59 years (mean 32.2 years), with 58.6% of them younger than 30. Most of them had family income lower than two minimum salaries (78%) and had only elementary school education (66.2%). Over half of the sample was non-white (51.4%). All the patients had an active sexual life and 92.0% of them had more than one lifetime sexual partner. Sexual life began before 17 years for 70% of those. Most patients had two children (64.3%) and 49.3% of them reported at least one abortion episode. Of the total, 26.6% were alcohol users, 26.4% were smokers and 11.7% were illicit drug users. In addition to HIV/HPV co-infection, 36.5% of the women were suffering from other STD. Herpetic genital lesions were found in 23.6% of the patients.

Length of time since diagnosis of less than four years was reported by 56.4% of the patients, with mean length 4.2 years. Nearly all patients (98.6%) were under antiretroviral treatment. Regarding the immune status of the sample, 92.1% of the women had over 200 CD4 cells/mm<sup>3</sup> and 47.1% had below 350 CD4 cells/mm<sup>3</sup>. RNA viral load was detected in 62.9% of the women (Table 1). Cytology tests showed that normal/inflammatory results were prevalent (57.1%) and 7.9% of the sample had HSIL. No cancer cases were found. However, 60.7% of the people were HPV infected and 21.2 % of them harbored HPV type 16 (Table 1).

In determining demographic data and HIV markers that could affect HPV infection, time elapsed since positive diagnosis of less than four years was strongly correlated with HPV infection (OR = 2.70, p=0.004). However, the presence of cervical lesions showed no correlation (data not shown). Amid lifestyle variables, first sexual intercourse below 17 years (OR=2.49, p= 0.01) was associated with an increased risk for HPV infection. Among the demographic variables, people younger than 30 years (OR=3.14, p = 0.02) and white ethnicity (OR=2.04, p = 0.03) were linked to HPV infection. Alcohol use (OR=2.13, p=0.05) was marginally related to HPV prevalence. The remaining data did not reach statistical significance (Table 2).

The relationship between immune status and cervical lesions in the HIV positive sample can be observed in Table 3. Patients with severe immunodepression (less than 200 CD4 cells/mm<sup>3</sup>) showed a nearly seven greater chance of presenting cervical lesions than people who reported immune status above this score (OR = 6.88, p = 0.008) (Table 3).

**Table 1.** Health status results of the HIV seropositive subjects. Cervical Pathology Service, HSE/RJ, 2003-2005.

Variable	N (%)
<b>HIV diagnosis time length</b>	
≤ 4 years	79(56.4)
>4 years	61(43.6)
<b>Antiretroviral therapy</b>	
Yes	138(98.6)
No	2(1.4)
<b>CD4/ mm<sup>3</sup> count</b>	
0-200	11(7.9)
201-350	63 (45.0)
351-500	33 (23.5)
>500	33 (23.5)
<b>Viral load</b>	
Undetectable	52(37.1)
≥ 80 copies/ml	88(62.9)
<b>Cytology</b>	
Normal/inflamatory	80 (57.1)
HPV	13 (9.3)
ASCUS	7 (5.1)
LSIL	29 (20.6)
HSIL	11 (7.9)
<b>HPV detection</b>	
Yes	85(60.7)
No	55(39,3)
<b>HPV 16 detection</b>	
Yes	18 (21.2)
No	67(78.8)

**Table 2.** HPV infection versus baseline characteristics in HIV seropositive women. Cervical Pathology Service, HSE/RJ, 2003-2005.

Variable	HPV+ No (%)	HPV - No (%)	OR (95 % CI)	P value
HIV diagnosis time length			2.69(1.26-5.75)	0.004
≤ 4 years	56 (70.9)	23 (29.1)		
>4 years	29 (47.5)	32 (52.5)		
Age of sexual debut			2.49 (1.19-5.23)	0.01
≤17 years	66(67.3)	32(32.7)		
> 17years	19(45.2)	23(54.8)		
Age			3.14(1.49-6.59)	0.02
≤ 30 years	44 (75.9)	14 (24.1)		
> 30 years	41 (50.0)	41 (50.0)		
Ethnicity			2.04 (1.01-4.00)	0.03
White	47 (69.1)	21 (30.9)		
Non white	38 (52.8)	34 (47.2)		
Alcohol user			2.13 (0.93-4.86)	0.05
Yes	27 (73.0)	10 (27.0)		
No	57 (52.8)	45 (44.1)		
Family income			1.62 (0.66-3.96)	0.19
0-2 minimum salary	60 (63.8)	34 (36.2)		
> minimum salary	13 (52.0)	12 (48.0)		
Number of sexual partners			1.9 (0.56-6.73)	0.22
>1	78 (61.9)	48(38.1)		
1	5(45.5)	6 (54.5)		
Drug user			0.63 (0.22-1.80)	0.27
Yes	8(50.0)	8(50.0)		
No	74 (61.2)	47 (38.8)		
Genital herpes outbreak			1.46(0.46-4.56)	0.35
Yes	17 (51.5)	16 (48.5)		
No	8 (42.1)	11 (57.9)		
Antiretroviral therapy			-	-
Yes	83(60.1)	55(39.9)		
No	2(100.0)	0		
Abortion episodes			0.84 (0.45-1.77)	0.44
Yes	41 (59.4)	28 (40.6)		
No	44(62.0)	27 (38.0)		
Smoker			0.93 (0.43-2.00)	0.50
Yes	22 (59.5)	15 (41.5)		
No	63 (61.2)	40 (38.8)		
Viral load			2.05 (0.5-8.85)	0.62
0	43(58.0)	4(42.0)		
>0	42(63.6)	8(36.4)		
Parity			1.01 (0.52-1.99)	0.95
> 3 children	39 (59.1)	27 (40.9)		
≤ 3 children	44 (58.7)	31 (41.3)		
Education level			1.40(0.70-2.78)	0.33
Illiterate, incomplete elementary school	39 (65.0)	21(35.0)		
Complete elementary school or above	45 (57.0)	34(43.0)		

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OR – Odd ratio CI – Confidence Interval

**Table 3.** Citology results according to immune status of HIV seropositive women. Cervical Pathology Service, HSE/RJ, 2003-2005.

CD4 count	ASCUS, HPV, LSIL, HSIL		Normal, inflammatory	OR (95 % CI)	P value
	No (%)				
0-200/mm <sup>3</sup>	9 (81.8)		2 (18.2)	6.88 (1.42-3.15)	0.008
201-350/mm <sup>3</sup>	30 (47.6)		33 (52,4)	1.59 (0.62-4.14)	0.29
351-500 /mm <sup>3</sup>	9 (27.3)		24 (72.7)	0.66 (0.20-2.10)	0.43
> 500/mm <sup>3</sup>	12 (36.4)		21 (63.6)	1.00	

OR – Odd ratio CI – Confidence Interval

The HPV prevalence also varied according to CD4 count. It has changed from 90.9% for patients carrying less than 200 cells/mm<sup>3</sup> to 45.5% in women with CD4 level over 500 cells/mm<sup>3</sup> (chi-square=0.02). In contrast, we did not find any relation between HIV immune status and HPV type 16 (Table 4).

**Table 4.** HPV prevalence according to immune status of HIV seropositive women. Cervical Pathology Service, HSE/RJ, 2003-2005.

CD4 count	HPV+	HPV-	OR (95 % CI)	P value	HPV16+	HPV 16-	OR (95 % CI)	P value
	No (%)	No (%)				No (%)		
0-200/mm <sup>3</sup>	10 (90.9)	1 (9.1)	12 (1.28-280.1)	0.02	2 (20)	8 (80)	2.00 (0.23-20.27)	0.78
201-350/mm <sup>3</sup>	41 (65.1)	22 (34.9)	0.45 (0.17-1.45)	0.06	9 (22)	32 (78)	1.78 (0.40-7.83)	0.60
351-500 /mm <sup>3</sup>	19 (57.6)	14 (42.4)	0.61 (0.21-1.81)	0.32	2 (10.5)	17 (89.5)	4.25 (0.55-39.7)	0.23
> 500/mm <sup>3</sup>	15 (45.5)	18 (54.5)	1.00		5 (33.3)	10 (66.7)	1.00	

OR – Odd ratio CI – Confidence Interval

HPV infection was also strongly associated with altered cytology (OR = 5.44, p = 0.0). In spite of HPV 16 prevalence not being affected by immune status, HIV seropositive women with abnormal cytology were three times more likely to harbor HPV 16 than those who did not present cervical abnormalities (OR = 3.1, p = 0.05) (Table 5).

**Table 5.** Distribution of HPV and HPV 16 according to cytology results of the HIV seropositive women Cervical Pathology Service, HSE/RJ, 2003-2005.

Cytology	HPV+ No(%)	HPV - No(%)	OR (95 % CI)	P value	HPV 16+ N (%)	HPV16 – N (%)	OR (IC 95%)	P value
Normal, inflammatory	36(45)	44(55)			4 (11.4)	31 (88.6)		
ASCUS, HPV, LSIL, HSIL	49(81.7)	11(18.3)	5.44(2.47- 10,97)	0<0.001	14 (28.6)	35 (71.4)	3.1 (0.92-0.41)	0.05

OR – Odd ratio CI – Confidence Interval

## DISCUSSION

In order to elucidate the high HPV prevalence detected in the study population (60.7%), variables related to viral infection were investigated (Table 2). Time elapsed since HIV diagnosis of less than four years was strongly associated with HPV infection. Despite this variable having been implicated in HIV serostatus self-disclosure (Pettrak et al. 2001), it is difficult to determine the steps of this association, since we do not know if HPV was acquired before or after HIV infection. If we consider that HPV infection is a common genital virus among sexually active women, then this virus might have been acquired early in their sexual life and, thus, before HIV infection. It is possible that the time of knowledge about HIV is part of the set of risk behavior. Unprotected sexual intercourse increases the risk of acquiring sexually transmitted diseases, and the lack of condom use could be an important role in the transmission of both infections (Epstein 2005). On the other hand,

clinic attendance after HIV diagnosis intervenes in decreasing risk behaviors. Another idea attached to the described variable is the delayed timespan to HPV clearance from HIV positive hosts (Moscicki et al. 2004). A woman could harbor HPV until four years after known HIV diagnosis. After this period, the use of condoms could significantly reduce the risk for HPV infection and/or re-infection.

HPV incidence in the general population below 30 years suggests transient infection in seronegative populations (Moscicki et al. 1998). Since HPV is acquired early after sexual debut (Ley et al. 1991), younger age and age of the sexual life beginning are factors closely related to HPV acquisition. In our study, both variables (age and early first sexual intercourse) were strongly associated with HPV infection, events that could indicate a preceding HPV infection in these women.

Since most of the people were non-white, white ethnicity was a measure of increasing risk for HPV infection. This finding was an expected occurrence in this work. Facing the small size of our sample as well as the cross sectional design of this study, this data should be looked at with caution.

Khan et al. (2002), studying mediators to association between HPV infection and age of first sexual intercourse, included alcohol use as an important mediator factor. Besides, in a selected sample of HPV positives affected by HSIL, a high rate of alcohol and drug use were found in the HIV seropositive patients, compared to the HIV negative patients. Our data points out the alcohol drinkers were more prevalent among HPV infected women. It is possible that this represents one side of important stressful life events experienced by the people analyzed. Psychological factors have also been associated with SIL and women living with HIV (Pereira et al. 2003). For example, they can refrain from

self-care behaviors. However, it is important to state that the small size of our sample limits the generalization of these study findings.

These analyzed women presented an impressive herpetic ulcer incidence. To investigate if this STD could increase the risk for HPV cervical lesions, the subjects carrying these ulcers were examined for HPV, abnormal cytology and CD4 count. However, we did not find any association with the variables. According to LeGoff et al. (2007), HSV-2 ulcers are independent of the immune level, in spite of the close transmission characteristics present in both viruses.

The sample was characterized by antiretroviral treatment (99%) and serological status above the critical 200 CD4 cells/ml (92.1%). These conditions turn HIV infection into a chronic disease and could alter the development of opportunistic HPV-related cervical diseases. However, the effects of antiretroviral therapy are complex and not always associated with HPV clearance or cervical lesion decrease. Otherwise, there is a clear relationship between low CD4 cell counts and HPV infection (Mougin et al. 2001, Palefsky 2006). According to the established concept, our results showed a significant association between the prevalence of cervical cytological abnormalities and severe or moderate immune depression ( $<500/\text{mm}^3$ ). However, above this count cervical lesions were no longer dependent on immune status (Table 3). Cardillo et al. (2001) assert that grade of immunosuppression affects the development of cervical lesions, but once they have already been established, lesions are not affected by CD4 cell levels. However, other studies have pointed out that if severe immunodepression is a risk factor for cervical lesions, antiviral therapy leads to immune restoration and decreasing severity of cervical lesions (Delmas et al. 2000). HIV positive women have significantly higher rates of cervical lesions than HIV negative women (Ferenczy et al. 2003). We found 11 (7.9%) of HSIL cases. This rate is

high compared to that of HIV seronegative women (1.1%) (Kitchener et al. 2006). In addition, the high rate of low risk cervical lesions, in opposition to much less rate of HSIL, found in this study could be due to antiretroviral treatment as well as to routine cervical prevention exams and treatment of other genital co-infections associated with the risk of progression to severe cervical lesions.

The high HPV incidence found in normal/inflammatory cytology (45%) agrees with other Brazilian findings on HIV positive women (Levi et al. 2002, Gonçalves et al. 1999, Cerqueira et al. 2006). However, HPV infection was strongly associated with cytology changes (Table 4). HPV prevalence was high (60.7%) in these people, associated with a marked result: patients with CD4 cells below 500 cells/mm<sup>3</sup> were strongly susceptible to this virus, independently of cervical abnormalities (OR 6.88). In a large HIV cohort study, Hankins et al. (1999) detected 67.2% of HPV prevalence. A CD4 cell count below 200 cells/mm<sup>3</sup> was an independent predictor for HPV infection in these women.

One of the targets of our work was evaluate if the HPV 16 was dependent on immune status. Garbuglia et al. (2007), in a sample of 544 women, detected 9.1% of HPV 16. Strickly et al. (2003) found that the prevalence of HPV16 is more weakly associated to immune status in HIV-seropositive women than that of other HPV types, suggesting that HPV16 may be better at avoiding the effects of immune surveillance. HPV 16, like other types, is increasing while the CD4 cell count is falling, but the increasing is less than the other types (Burk et al. 2001). This relative independence of the immune status could be associated to high prevalence of the infection in immunocompetent women (Sun et al. 1997). Our findings are according to these authors. Infection by the HPV type 16 was not affected by immune status. Although the type 16 prevalence was significant in patients with abnormal cytology (OR = 3.10; p = 0.05), it was not affected by CD4 cell count rate.

In summary, the significant results regarding co-factors should be interpreted with caution since they were obtained from a small sample. Time elapsed since HIV diagnosis was a HIV specific marker that hardly affected HPV prevalence. We described a high HPV prevalence in this sample, but a low incidence of high grade cervical lesions in subjects with CD4 cell counts above 350/mm<sup>3</sup> cells. This is probably due to antiviral treatment added to routine cancer prevention exams as well as treatment of other genital co-infections and psychological support.

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