# Increased Frequency of Genital Human Papillomavirus Infection in Human Immunodeficiency Virus-Seropositive Taiwanese Women

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***Background and Purpose:*** Human papillomavirus (HPV) infection is associated with increased incidence and severity of HPV-related cervical dysplasia and cervical cancer in women with human immunodeficiency virus (HIV) infection. This study examined the incidence of genital HPV infection in HIV-infected Taiwanese women and its relationship with cervical neoplasia.

***Methods:*** This hospital-based, case-control study enrolled 31 consecutive HIV-seropositive women and 124 age-matched women who were free from HIV infection. Polymerase chain reaction (PCR) was used to distinguish high-risk (types 16, 18, 31, 33, 52 and 58) and low-risk HPV (types 6 and 11). The occurrence of genital HPV infection was compared between women with and without HIV infection. In addition, CD4 lymphocyte counts were determined by flow cytometry and Papanicolaou test was done in women with HIV infection.

***Results:*** HPV and Papanicolaou test were done soon after the diagnosis of HIV infection. HIV seropositive women had a significantly greater high-risk HPV infection rate (48.4%; 15/31) than women without HIV infection (20.2%; 25/124; odds ratio, 3.71; $p = 0.001$). However, the prevalence of cervical intraepithelial neoplasia was similar between women with and without HIV infection. The CD4 lymphocyte counts in HIV-seropositive women were similar between those with and without genital HPV infection.

***Conclusions:*** The risk of genital HPV infection was significantly increased in HIV-infected women. Due to the association between high-risk HPV infection and the development of cervical dysplasia and cervical cancer, regular follow-up of Papanicolaou test is necessary in these women.

**Key words:** Cervical intraepithelial neoplasia; CD4 lymphocyte count; HIV; Papillomavirus, human

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The immunocompromised status in human immunodeficiency virus (HIV)-infected women is associated with a high risk of various infectious diseases, including fungal infection and other sexually transmitted diseases.[1–3] Previous reports suggested a higher rate of cervical dysplasia and an increased prevalence of genital human papillomavirus (HPV) infection in HIV-infected women.[4–6] HPV infection has been found to have a strong association with the occurrence of cervical intraepithelial neoplasia (CIN) and cervical carcinoma.[7,8]

As a result, invasive cervical cancer was designated as an acquired immunodeficiency syndrome (AIDS)-defining disorder by the US Centers for Disease Control and Prevention since 1993.[7] Moderate to severe cervical dysplasia was also reported as part of the course or as a complication after HIV infection.[9,10]

According to a report published by the Department of Public Health of the Executive Yuan in 2002, cervical cancer is the leading lethal gynecological disease in Taiwanese women. In addition, the prevalence rate of HIV infection was around 3 cases per 100,000 Taiwanese women according to the data reported by Center for Disease Control Taiwan, ROC in 2003. However, information on the incidence of HPV, CIN and vaginal infection in HIV-infected Taiwanese women was not included in these reports. This study examined the prevalence of HPV and the relationship with cervical neoplasia in HIV-infected women.

## Methods

Thirty one HIV-seropositive women who received medical treatment and consulted the gynecologic

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clinic of National Taiwan University Hospital were enrolled in this study. All of them had enzyme-linked immunosorbent assay (ELISA) and Western immunoblotting evidence of HIV infection.

The Papanicolaou test and specimen collection for HPV survey were all performed by the same investigator. Papanicolaou smear was done with a Cytobrush and an Ayres spatula from the cervix. The same cytopathologist, who was blinded to the participants' HIV serostatus, examined the cervical smear according to predefined criteria (an expansion of the Bethesda scoring system for cervical cytologic findings).[11] CIN was further confirmed by colposcopic examination and pathologic results. The control group consisted of 124 age-matched women. Randomly selected to provide age-matched women from 1431 women who underwent genital HPV examination and Papanicolaou test from February 2000 to February 2002. Approval for this study was obtained from the institutional review board of our hospital.

Cervical and vaginal secretions as well as the exfoliated cells were collected using swabs, and were then washed in a tube containing phosphate-buffered saline solution. This suspension was then divided into aliquots and frozen at -70°C before testing. Among 31 HIV-infected women, 4 did not have available data for CD4 lymphocyte counting due to inadequate blood and tissue collection.

## Detection and typing of HPV genome

The genotyping of HPV was done by polymerase chain reaction (PCR) with L1 consensus primers (MY09 and MY11; Roche Molecular Systems, Branchburg, NJ, USA). Gamma-interferon gene served as the internal control. Various HPV types in the amplified products were identified after hybridization with a generic probe (MY09 and MY11) designed to recognize most of the HPV types.[12] Samples that were positive with the generic probe mix but negative with all type-specific probes were categorized as "untypable" HPV. An HPV risk category was assigned to each sample according to the PCR determination of HPV types. Type 6 and 11 were classified as low risk, whereas type 16,18,31,33,52 and 58 were classified as high risk. The type-specific probes used included the sense primer pU-1M, 5'-TGTCAAAAACCGTTGTGTCC-3', sense primer pU-31B, 5'-TGCTAATTCGGTGCTACCTG-3' and antisense primer pU-2R, 5'-GAGCTGTCGCTTAAT TGCTC-3'. The pU-31B/pU-2R primers were used to amplify the E6/E7 region of HPV 6 and 11, while pU-1M/pU-2R was used to amplify the E6/E7 region of HPV 16, 18, 31, 33, 52b and 58.[13]

Selected specimens were further tested with a hybridization assay for HPV. Genomic DNA (100 ng) from each sample was amplified using PCR in a final volume of 20 μL containing 2 mM $MgCl_2$, 0.2 mM of each dNTP, 50 mM KCl, 10 mM Tris-HCl (pH 8.3) and 0.2 μM of each primer. These DNA solutions were incubated for 10 minutes at 94°C and chilled quickly for DNA denaturation before 30 cycles of amplification using a DNA thermal cycler (Perkin-Elmer, Norwalk, CT, USA).

Each cycle consisted of 30 seconds at 95°C, 30 seconds at 55°C and 1 minute at 72°C for MY09/MY11, and 1 minute at 94°C, 2 minutes for 55°C and 2 minutes at 72°C for pU-1M/pU-2R and pU-31B/pU-2R. The PCR product with or without restriction enzyme digestion *Pst*I was electrophoresed on a gel containing 1.5% agarose (MY09/MY11, expected size about 450 bp) or 4.5% Metaphore agarose (FMC Bioproducts, Rockland, ME, USA) [pU-1M/pU-2R and pU-31B/pU-2R, expected size 220-270 bp]. Each PCR included a positive and negative control gene (gamma-interferon) to evaluate sample variation. Each sample was tested twice. HPV type was identified by the enzyme-digestion pattern of each HPV. Sequential low- and high-stringency washes allowed detection and identification of a large spectrum of HPV types on the basis of the sizes of the hybridizing bands. A single experienced observer made Southern blot interpretations. When hybridization data were insufficient to identify a specific type of HPV, the virus was classified as "uncharacterized".

For purposes of classification, HPV risk categories were assigned according to the results of PCR testing, and women with multiple HPV types were classified according to the highest risk type assigned by PCR testing. Specimens that showed negative results by PCR for HPV and positive results for the control were classified as "no HPV present".

## $CD4^+$ lymphocyte count

The $CD4^+$ lymphocytes in peripheral blood were counted using direct immunofluorescent monoclonal antibodies (Coulter and Becton and Dickinson Co., San Jose, CA, USA) and flow cytometry (Coulter EPICS Profile, Hialeah, FL, USA).[14] Briefly, 1 mL of aspirated blood was collected in a tube containing heparin, and was processed within 30 minutes. Twenty μL of OptiClone CD3-FITC/CD4-PE (Immunotech Co., Marseilles, France) was pipetted to the bottom of the tube, and 100 μL of anticoagulated blood was added to the tubes. After a complete vortex, the tubes were incubated for 15 minutes at room temperature in the dark. The red blood cells were lysed with Optilyse lysing solution, and the cell preparations were then analyzed by flow cytometry to obtain the CD4 lymphocyte counts.

### Statistical analysis

Statistical analysis was performed using Statistical Package for the Social Sciences (SPSS) version 10 (SPSS Inc., Chicago, IL, USA). Comparison of the 2 groups was done using Student's *t* test for quantitative parameters, and chi-squared test for qualitative parameters. A probability value less than 0.05 was considered statistically significant.

## Results

The ages of 31 HIV-seropositive women ranged from 23 to 71 years, with a mean value of 39.2 years. Among them, 6 (19.4%) were less than 29 years, 20 (64.5%) were between 30 and 49 years, and 5 (16.1%) were more than 50 years old. Fifteen (48.4%) out of 31 women had high-risk HPV infection, and 4 (12.9%) had low-risk HPV infection. Seven (22.6%) women were found to have CIN after Papanicolaou test (Table 1).

Among 124 age-matched non-HIV women, 6 (4.8%) had low-risk HPV, 25 (20.2%) had high-risk HPV, and 20 (15.3%) had CIN. Women with HIV infection had a higher risk of developing HPV [odds ratio (OR), 3.07; $p = 0.006$] and high-risk HPV infections (OR, 3.07; $p = 0.001$) compared to those without HIV infection. The prevalence of low-risk HPV infection and CIN was also higher in women with, than in those without HIV infection, but this difference was not significant (Table 2).

CD4 lymphocyte count in HIV-seropositive women was not correlated with HPV infection or the presence of CIN (Table 3).

**Table 1.** Clinical characteristics of 31 human immunodeficiency virus (HIV)-seropositive women and 124 age-matched HIV-seronegative women.

| | HIV-positive (n = 31) | HIV-negative (n = 124) |
|---|---|---|
| Age (years)* | 39.2 ± 1.8 | 39.2 ± 1.8 |
| < 30 | 6 (19.4%) | 24 (19.4%) |
| 30-49 | 20 (64.5%) | 80 (64.5%) |
| ≥ 50 | 5 (16.1%) | 20 (16.1%) |
| Gravida* | 2.0 ± 0.3 | NA |
| Parity* | 1.5 ± 0.3 | NA |
| > 1 sexual partner | 14 (45.2%) | NA |
| HPV infection | 15 (48.4%) | 29 (23.4%) |
| Low-risk types | 4 (12.9%) | 6 (4.8%) |
| High-risk types | 15 (48.4%) | 25 (20.2%) |
| CIN | 7 (22.6%) | 20 (16.1%) |
| HSIL | 3 (9.7%) | 12 (9.7%) |
| LSIL | 4 (12.9%) | 8 (6.4%) |

* Mean ± standard error.
HPV = human papillomavirus; CIN = cervical intraepithelial neoplasia; HSIL = high-grade squamous intraepithelial lesion; LSIL = low-grade squamous intraepithelial lesion; NA = not available.

**Table 3.** CD4 lymphocyte counts in human immunodeficiency virus (HIV)-infected women in relation to human papillomavirus (HPV) infection and cervical intraepithelial neoplasia (CIN).

| | Case no. | CD4 counts (per μL)* | *p* value |
|---|---|---|---|
| HPV infection | | | |
| Yes | 14 | 227.6 ± 53.9 | 0.89 |
| No | 13 | 213.9 ± 59.9 | |
| CIN | | | |
| HSIL | 3 | 391.3 ± 98.0 | 0.32 |
| LSIL | 4 | 221.8 ± 120.3 | |
| No | 20 | 195.4 ± 44.7 | |

* Mean ± standard error.
HSIL = high-grade squamous intraepithelial lesion, LSIL = low-grade squamous intraepithelial lesion.

## Discussion

This study found a significantly higher rate of HPV infection in HIV-seropositive women in comparison with age-matched controls. A previous study by Duerr et al found that squamous intraepithelial neoplasia was more common in HIV-infected women, and was most commonly associated with high- and intermediate-risk HPV types.[4] However, their study did not control the age before data analysis. Zietkowiak et al found that the presence of HPV infection decreased with age, because of differences in pattern of sexual behavior and hormonal status.[15]

This study found a high prevalence rate (16.1%) of CIN in the control group. A previous volunteer-based cohort study found that at least 1 HPV type was positive in 16.0% of women whereas 6.3% had CIN.[16] Since HPV test is not reimbursed by health insurance in Taiwan, it is usually given only to women with suspected cervical lesion, those with a previous CIN history and those who had multiple sexual partners. In this study, therefore, the occurrence of CIN was

**Table 2.** Human papilloma virus (HPV) and cervical intraepithelial neoplasia (CIN) status in women with and without human immunodeficiency virus (HIV) infection.

| | HIV-positive (n = 31) | HIV-negative (n = 124) | OR | 95% CI | *p* value |
|---|---|---|---|---|---|
| HPV infection | 15 (48.4%) | 29 (23.4%) | 3.07 | 1.36-6.96 | 0.006 |
| Low-risk HPV | 4 (12.9%) | 6 (4.8%) | 2.91 | 0.77-11.04 | 0.102 |
| High-risk HPV | 15 (48.4%) | 25 (20.2%) | 3.71 | 1.62-8.51 | 0.001 |
| CIN | 7 (22.6%) | 20 (15.3%) | 1.52 | 0.58-4.00 | 0.397 |

OR = odds ratio; CI = confidence interval.

relatively higher in the control group. By contrast, Papanicolaou test found CIN in only 228 (1.4%) out of 15,998 exams in 1998 and 85 (0.7%) out of 11,338 exams in 1999 in our hospital.

The observation that women with HIV infection had a higher rate of high-risk HPV infection could be attributable to multiple factors, including compromised immunity and complex sexual behavior of the patient or her sexual partner. Mucosal immunity impairment may also play a role in the development of genital HPV infection. The shift from the helper T cell type 1 (Th1) to type 2 (Th2) immune response, which determines the downregulation of cell-mediated immunity, may explain the loss of immunologic control of HPV and its further oncologic complications.[17]

Close correlation between high-risk HPV infection and the occurrence of CIN or invasive cervical cancer has been well-documented in HIV-infected women.[18] In this study, however, we did not find a positive relationship between CIN occurrence and HIV infection. This might have been due to the small sample size in this study. Unlike previous studies,[9,19,20] we did not find a correlation between low CD4 lymphocyte counts of women who suffered from cervical dysplasia and HPV infection. This may also be due to the relatively small cohort in this study. Taylor et al reported that the degree of increase in immunosuppression, as measured by the nadir of a patient's CD4 lymphocyte count, was the strongest predictor of genital dysplasia in HIV-infected women.[21] Since serial CD4 lymphocyte counts were not measured in this study, we were unable to confirm these findings. The lack of serial testing of CD4 counts might also explain the insignificant difference between low CD4 lymphocyte counts and cervical dysplasia in this study.

This is the first report to delineate the characteristics of genital HPV infection in HIV-infected Taiwanese women. Although the case number was limited, the results indicate that there is a higher risk of HPV infection in women with pre-existing HIV infection. Because of the strong association among CIN, cervical cancer and HPV infection, frequent and regular surveillance with Papanicolaou test in women with HIV infection is indicated.

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