The Prevalence of Human Papillomavirus and Cervical Cytology Abnormalities in Women Infected with Human Immunodeficiency Virus in Southern Israel

Lilach Leibenson MD[1], Shirly Banani[2], Avraham Borer MD[2], Mihai Meirovitz MD[1], Yonat Shemer Avni PhD[3], Doron Singer MSc[3], Francisc Schlaeffer MD[2], Max Leibenson BSc MD[4], Tali Silberstein MD[1], Arnon Wiznitzer MD[1] and Klaris Riesenberg MD[2]

[1]Division of Obstetrics and Gynecology, [2]Department of Infectious Diseases, [3]Laboratory of Virology, and [4]Department of Orthopedic Surgery, Soroka University Medical Center and Faculty of Health Sciences, Ben-Gurion University of the Negev, Beer Sheva, Israel

ABSTRACT: **Background:** Concomitant human immunodeficiency virus (HIV) and human papillomavirus (HPV) infection increases both HPV persistence and the risk of invasive cervical cancer. An estimation of HPV prevalence among HIV-positive women in Israel would contribute to improving care for this population and preventing morbidity and mortality related to cervical cancer.

Objectives: To determine the prevalence of HPV infection and cervical cytology abnormalities, and to assess the possible influence of HIV infection on HPV carriage in HIV-positive women attending the Infectious Disease Clinic at Soroka University Medical Center.

Methods: The study population included 84 HIV-seropositive women. They were examined by a gynecologist and screened for HPV genotyping, and Pap smears were obtained for cervical cytology. Demographic, behavioral, and HIV infection variables were also recorded and analyzed.

Results: Forty-nine (58.3%) of the study participants were HPV-positive; 34 of them had oncogenic genotypes. Young age (< 16 years) at first sexual intercourse was the only variable significantly associated with HPV infection ($P < 0.05$). Abnormal cervical cytology was present in 17 women (20.3%); 21 women were referred to colposcopy, which was abnormal in 9 (10.7%).

Conclusions: The prevalence of HPV carriage among HIV-positive woman in our study was slightly higher than published elsewhere. The prevalence of pathological cervical cytology was much higher than in the general population. An extremely high prevalence of pathological colposcopies requiring further treatment was found. Screening for HPV and premalignant changes in the uterine cervix is highly recommended in the HIV-seropositive population. We suggest that colposcopy be considered part of the routine workup in HIV-seropositive woman.

IMAJ 2011; 13: 34–38

KEY WORDS: human papillomavirus, human immunodeficiency virus, cervical cytology abnormalities, colposcopy, HPV genotypes

HIV = human immunodeficiency virus
HPV = human papillomavirus

Infection with human papillomavirus is the most common sexually transmitted infection in the United States today [1-3]. There are more than 100 different genotypes of HPV. Some genotypes are considered high risk (oncogenic) because they may be associated with invasive cancers of cervix, vulva, penis, anus, and others. Among them are genotypes 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68 and 82 [1-3].

Being never or rarely screened for cervical cancer is the single most important determinant associated with invasive cervical cancer [1,4]. Additional factors increase both HPV persistence and the risk of invasive cervical cancer, such as immunosuppression from any cause, including human immunodeficiency virus infection, smoking, co-infection with other sexually transmitted infections, parity, prolonged use of oral contraceptives, and nutrition [1,4].

The incidence of cervical intraepithelial neoplasia, as confirmed by colposcopy, is about five times higher among HIV-positive than HIV-negative women. Invasive cervical cancer is about three times more frequent in HIV-positive women in New York City [5]. HPV is 6–18 times more likely to persist in HIV-positive women than in HIV-negative women. HIV seropositivity and lower $CD4^+$ count are the strongest predisposing factors for the persistence of HPV DNA [6]. Highly active antiretroviral therapy led to a dramatic decrease in morbidity and mortality among HIV patients. Nevertheless, the incidence of invasive cervical cancer appears to remain unaffected [7,8].

The prevalence of HPV infection and cervical dysplasia in HIV-positive women in Israel is not known. An estimation of this prevalence would allow improved care for this population and would help to prevent morbidity and mortality related to cervical cancer. The objectives of this study were to determine the prevalence of HPV infection and cervical cytology abnormalities, and to assess the possible influence of HIV infection parameters on HPV carriage in HIV-positive women attending the Infectious Disease Clinic at the Soroka University Medical Center in Beer Sheva, southern Israel.

PATIENTS AND METHODS

The study group comprised 84 consecutive HIV-seropositive women aged 17 years and older who were examined by a gynecologist (principal author) and screened for HPV genotyping and cervical cytology. All participants provided written informed consent and were interviewed for demographic data and sexual behavior. The study received ethical approval from the Local Research Ethics Committee.

The medical records of participants were reviewed to verify the duration of HIV infection and of HAART, and to determine CD4+ count at the time of diagnosis. Most recent CD4+ count and HIV viral load were also recorded.

Pap smears were screened by a senior cytopathologist. The cytological findings were reported as either negative, atypical squamous cells of unknown significance, low-grade squamous intraepithelial lesion, or high-grade squamous intraepithelial lesion.

HPV DETECTION

- ***Specimen collection and storage:*** Cervical specimens were obtained using PreservCyt solution (Cytyc Corporation, Marlborough, MA, USA). The specimen was centrifuged at 2500 rpm, and the supernatant was aspirated until 1 ml of pellet and supernatant were left. Cells were resuspended and the sample was frozen at -80ºC until further processing.
- ***Extraction of nucleic acid:*** Total nucleic acids were extracted from the 1 ml sample and eluted with 55 µl of extraction buffer, according to the manufacturer's instructions, using NucliSens EasyMag extraction kit and instrument (Biomerieux, France).
- ***HPV genotyping:*** In order to identify HPV DNA and perform HPV genotyping, the extracted sample was amplified by nested polymerase chain reaction, and amplicons of positive samples were sent for sequencing (MBC, HyLab, Rehovot, Israel). The first PCR was performed by utilizing 5 µl of extracted nucleic acid in a total reaction volume of 25 µl, using the below listed forward and reverse primers: CGTCCMARRGGAWACTGATC and GCMCAGGGWCATAAYAATGG. The nested PCR was accomplished by utilizing 5 µl of the amplified DNA from the first PCR in a total reaction volume of 50 µl, using the below listed forward and reverse primers: TTTGTTACTGTGGTAGATACTAC and GAAAAATAAACTGTAAATCATATTC.

All reactions were carried out using Readymix PCR master mix (Thermo Scientific, Germany).

HAART = highly active antiretroviral therapy
PCR = polymerase chain reaction

STATISTICAL ANALYSIS

Sample size (confidence interval 95%, power 80%) was calculated using Epi Info Version 4.3.4 software, CDC. For categorical variables, proportions were compared using Fisher's exact test or chi-square test whenever appropriate. Continuous variables were analyzed with Student's *t*-test or the Wilcoxon rank sum test, depending on the validity of the normality assumption. The Kruskal-Wallis test was used for comparisons of differences in medians. A two-tailed *P* value of < 0.05 was considered significant. All analyses were performed using SPSS version 15 (SPSS Inc., Chicago, Il, USA).

RESULTS

Demographic and behavioral characteristics of the study population are shown in Table 1. At enrollment in the study, the mean duration of HIV infection and of HAART was 53.09 months and 37 months, respectively. Mean CD4+ count was 461.10 cells/ml (315.35 cells/ml at the time of the diagnosis). In 60 patients (72.3 %) the HIV load was below detection limits (< 50 copies/ml).

Forty-nine (58.3%) of the study participants were HPV-positive. In 43 (87.8%) of them the genotype was identified. Of

Table 1. Demographic and behavioral characteristics of the study population

Age (yrs)	
Mean	39.4
Median	36
Range	17–71
Marital status	
Married	28/82 (34.1%)
Single	27/82 (32.9%)
Widowed	15/82 (18.3%)
Divorced	12/82 (14.6%)
Origin	
Ethiopia	63/84 (75.0%)
Other	21/84 (25.0%)
Age at first sexual intercourse (yrs)	
Mean	16.29
Range	9-27
Lifetime number of sex partners	
Mean	3.06
1–5 partners	71/81 (87.7%)
6–10 partners	2/81 (2.5%)
10	8/81 (9.9%)
Condom use	13/81 (16%)
History of STI	16/81 (19.8%)
Alcohol use	2/83 (2.4%)
Drug abuse	6/83 (7.2%)
Smoking	13/83 (15.7%)
Prostitution	6/83 (7.2%)
Per capita income (NIS)	
Mean	1631

STI = sexually transmitted infection, NIS = new Israeli shekel

Table 2. Association of HPV with demographic, behavioral, and HIV variables

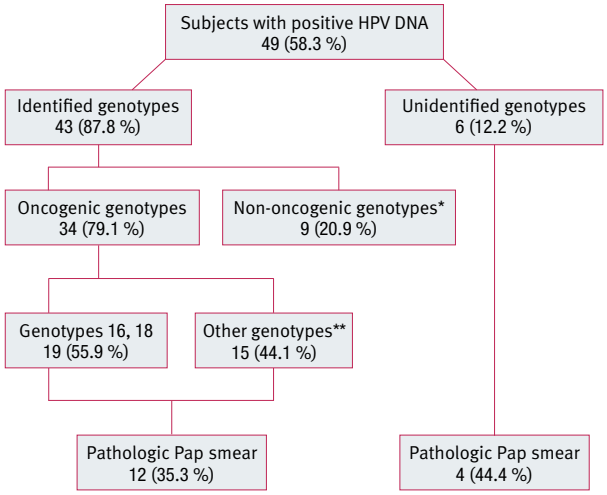
	HPV infection		*P* (OR)
	Negative	Positive	
Age (yrs)			
Mean	39.8 ± 11.1	38.7 ± 11.3	
Median	36 (17–71)	36 (18–65)	0.656
< 30	6/35 (17.1%)	13/49 (26.5 %)	
> 30	29/35 (82.9%)	36/49 (73.5%)	0.228
Origin			
Ethiopian	24/35 (68.6%)	39/49 (79.6%)	0.185
Age at first sexual intercourse			
Mean	17.4 ± 2.9	15.5 ± 2.7	0.006
Median	17.5 (12-27)	15 (9-22)	
< 16 yrs	10/29 (34.5%)	34/47 (72.3%)	0.001 (1.8-13.4)
Lifetime no. of sex partners			
< 3	29/34 (85.3%)	42/49 (85.7%)	0.598
No. of pregnancies			
< 3	23/35 (65.7%)	25/49 (51%)	0.132
Condom use	6/35 (17.1%)	7/49 (14.3%)	0.475
History of STIs	7/35 (20%)	9/49 (18.4%)	0.533
Drug abuse	1/34 (2.9%)	5/49 (10.2%)	0.209
Smoking	8/34 (23.5%)	5/49 (10.2%)	0.092
Duration of documented HIV			
< 2 yrs	9/35 (25.7%)	24/49 (49%)	0.26
CD4⁺ count at diagnosis			
< 200	10/35 (28.6%)	23/48 (47.9%)	0.06 (0.9–5.8)
CD4⁺ count, current			
< 200	3/35 (8.6%)	11/48 (22.9%)	0.074
HIV RNA, current			
Undetectable	27/35 (77.1%)	33/48 (68.8%)	0.277
HAART	30/35 (85.7%)	41/49 (83.7%)	0.525
Compliance	31/35 (88.6%)	37/45 (82.2%)	0.321

those, the genotype was oncogenic in 34 (79.1%). Association of HPV genotypes with Pap smear results is shown in Figure 1. Of the 49 HPV-positive women 13 (26.5%) were found to be concomitantly infected with two different genotypes.

Association of HPV with demographic, behavioral and HIV variables is shown in Table 2. Young age (< 16 years) at first sexual intercourse was the only variable significantly associated with HPV infection ($P < 0.05$). Duration of documented HIV infection, CD4⁺ count, HIV viral load, HAART treatment and compliance with treatment did not have a statistically significant effect on HPV prevalence.

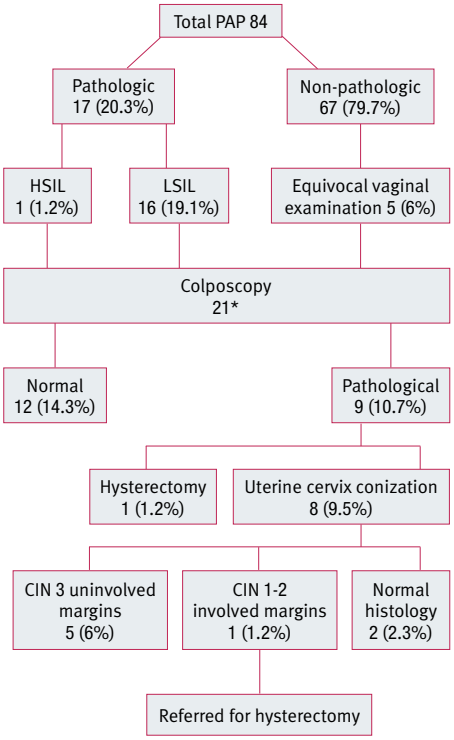
The correlation of pathological Pap smear with colposcopic and histological findings is presented in Figure 2. Abnormal cervical cytology was present in 17 women (20.3%), of whom 16 (19.1%) were HPV-positive. Colposcopy was performed in 21 women, 16 of them due to pathological Pap smear results, and the remaining 5 because of equivocal vaginal examination. Colposcopy findings were abnormal in 9 women (10.7%), one of whom (1.2%) was diagnosed with stage Ib2 cervical cancer (HPV 45 genotype) and underwent hysterectomy.

Figure 1. Association of HPV genotypes with Pap smear results



*66, 72, 81
**33, 45, 82

Figure 2. Correlation of pathological Pap smear with colposcopic and histological findings



*One patient with LSIL refused colposcopy

Cervical conization was performed in 8 women (9.5%). In one case (1.2%) CIN 1-2 with marginal involvement (HPV 45 genotype) was detected and the patient was referred for hysterectomy. In 5 women (6%) CIN 3 with uninvolved margins was detected (one with non-oncogenic HPV 81 genotype).

While a pathological cervical cytology was found in 16 of the 49 HPV-positive women (32.7%), abnormal Pap smear was detected in only one (low-grade squamous intraepithelial lesion, CIN 3) of 35 HPV-negative women (2.8%) ($P < 0.001$).

DISCUSSION

To the best of our knowledge this is the first study conducted in Israel to evaluate the prevalence of HPV infection and cervical dysplasia in HIV-positive females. There are no published studies addressing the prevalence of HPV infection in the general population in Israel. One study found that 44% of 84 women referred to colposcopy were positive for high-risk HPV infection [9]. However, since the population of that study was pre-selected, it would be hard to extrapolate from the data to the general population in Israel.

Although the rates of high-grade squamous intraepithelial lesions and of low-grade squamous intraepithelial lesions in Israel (0.69% and 0.29%, respectively) are comparable with those around the world, the incidence of cervical cancer (5/100,000) is among the lowest [10].

As known from previous studies, there is a correlation between low socioeconomic status and HPV infection in HIV-infected women [11]. Among the 84 women enrolled in the present study, 54 (66%) were unmarried and 64 (75.3%) were immigrants from Ethiopia.

Several studies indicate that high plasma HIV level and low CD4+ count are strong predictors of HPV-associated cervical disease [12-15]. Our data, as shown in Table 2, though not statistically significant, confirm the correlation between low CD4+ count and HPV-associated cervical disease in HIV-infected women. We did not find any correlation between the prevalence of HPV infection and duration of HIV carriage, HAART, HIV RNA levels, and extent of compliance.

In our study, 12 of 34 women (35.3%) with oncogenic HPV genotype, and 4 of 9 (44.4%) with non-oncogenic HPV genotype had cervical cytological abnormalities. A similar observation was made among HIV-positive women in the Bahamas [11]. This finding implies that infection with low-risk HPV genotypes may play a role in the increased rate of cervical cytological abnormalities in immunocompromised patients.

Infection with multiple HPV genotypes is common in HIV-infected women and stands at 11.9% in an extensive meta-analysis [16]. In our study we found that over a quarter (26.5%) of the women were infected with two genotypes.

CIN = cervical intraepithelial neoplasia

In the general population, younger age, unmarried status, and multiple sexual partners are independent risk factors for HPV acquisition [2]. Among HIV-positive women, young age (< 16) at first sexual intercourse was found to be an additional risk factor for concomitant HPV infection [11], as was confirmed in our study. We were not able to identify other behavioral risk factors for HPV acquisition in HIV-positive women, which may be due to the limited size of the study population.

The prevalence of HPV carriage in our study was 58.3%, which appears to be higher than in other published studies (33–47%) [11]. As previously reported [16,17], we also found a high prevalence (79.1%) of oncogenic genotypes, including 16 and 18 in HPV-positive women.

In immunocompetent women, HPV prevalence is at its peak in those under the age of 35 [18]. We found no significant difference in prevalence of HPV carriage between women aged above or below 30. This may be explained by the inability of HIV-positive women to clear HPV [19].

The prevalence of pathological cervical cytology in our study population was much higher than in the general population of Israel [10]. As in many other studies that evaluated HIV-HPV co-infection [6,20,21] we also found a high prevalence of pathological cervical cytology (20.3%).

The present study demonstrated an extremely high prevalence of pathological colposcopies (10.7%), indicating the need for further treatment. Screening for HPV and premalignant changes of the uterine cervix is highly recommended in HIV-seropositive women. A very high prevalence of pathological colposcopies (10.7%), eventually requiring cone biopsies and surgical procedures, may suggest considering colposcopy as part of the routine workup in this population.

It is well established that prophylactic HPV vaccines can reduce morbidity and mortality associated with cervical cancer and other HPV-associated diseases [22,23]. Thus, HPV vaccination should be considered in all HIV-infected women. However, due to the paucity of data regarding efficacy and safety of HPV vaccine in HIV-infected individuals [24], there is still no unequivocal recommendation to vaccinate all HIV-infected women.

There was no control group in our study, since its proclaimed purpose was to evaluate the prevalence of HPV and cervical cytology abnormalities in a specific population of women infected with HIV in southern Israel. In view of the results of the present study, as well as the known low incidence of cervical cancer in Jewish women [25], we plan a larger, country-wide study that would evaluate the prevalence of HPV and cervical cytology abnormalities in the general HIV population of Israel.

Corresponding author:
Dr. L. Leibenson
Division of Obstetrics and Gynecology, Soroka University Medical Center, Beer Sheva 84101, Israel. **Phone:** (972-8) 640-0700, **Fax:** (972-8) 648-2059
email: lilachmaxleibenson@gmail.com

References

1. Human Papillomavirus: HPV information clinicians. Atlanta, GA: Centers for Disease Control and Prevention, April 2007.
2. Dunne EF, Unger ER, Sternberg M, et al. Prevalence of HPV infection among females in the United States. *JAMA* 2007; 297(8): 813-19.
3. Ault KA. Epidemiology and natural history of human papillomavirus infections in the female genital tract [Review]. *Infect Dis Obstet Gynecol* 2006; Suppl: 40470.
4. Khan K, Curtis CR, Ekwueme DU, et al. Preventing cervical cancer: overviews of the National Breast and Cervical Cancer Early Detection Program and 2 US immunization programs. *Cancer* 2008; 113(10 Suppl): 3004-12.
5. Ellerbrock TV, Chiasson MA, Bush TJ, et al. Incidence of cervical squamous intraepithelial lesions in HIV-infected women. *JAMA* 2000; 283(8): 1031-7.
6. Jay N, Moscicki AB. Human papillomavirus infection in women with HIV disease: prevalence, risk, and management [Review]. *AIDS Read* 2000; 10(11): 659-68.
7. Lewden C, Chene G, Morlat P, et al. HIV-infected adults with a CD4 cell count greater than 500 cells/mm³ on long-term combination antiretroviral therapy reach same mortality rates as the general population. *J Acquir Immune Defic Syndr* 2007; 46(1): 72-7.
8. Patel P, Hanson DL, Sullivan PS, et al. Incidence of types of cancer among HIV-infected persons compared with the general population in the United States, 1992-2003. *Ann Intern Med* 2008; 148(10): 728-36.
9. Grisaru D, Avidor B, Niv J, et al. Pilot study of prevalence of high-risk human papillomavirus genotypes in Israeli Jewish women referred for colposcopic examination. *J Clin Microbiol.* 2008; 46(5): 1602-5.
10. Sadan O, Schejter E, Ginath S, et al. Premalignant lesions of the uterine cervix in a large cohort of Israeli Jewish women. *Arch Gynecol Obstet* 2004; 269: 188-91.
11. Dames DN, Ragin C, Griffith-Bowe A, Gomez P, Butler R. The prevalence of cervical cytology abnormalities and human papillomavirus in women infected with the human immunodeficiency virus. *Infect Agent Cancer* 2009; 4(Suppl 1): S8.
12. Harris TG, Burk RD, Palefsky JM, et al. Incidence of cervical intraepithelial lesions associated with HIV serostatus, CD4 cell counts and human papillomavirus test results. *JAMA* 2005; 293: 1471-6.
13. Mogtomo ML, Malieugoue LC, Djiepgang C, Wankam M, Moune A, Ngane AN. Incidence of cervical disease associated to HPV in human immunodeficiency infected women under highly active antiretroviral therapy. *Infect Agent Cancer* 2009; 4: 9.
14. Heard I, Tassie JM, Schmitz V, Mandelbrot L, Kazatchkine MD, Orth G. Increased risk of cervical disease among human immunodeficiency virus-infected women with severe immunosuppression and high human papillomavirus load. *Obstet Gynecol* 2000; 96(3): 403-9.
15. Ahdieh L, Klein RS, Burk R, et al. Prevalence, incidence, and genotype-specific persistence of human papillomavirus in human immunodeficiency virus (HIV)-positive and HIV-negative women. *J Infect Dis* 2001; 184(6): 682-90.
16. Clifford GM, Goncalves MA, Franceschi S. Human papillomavirus genogenotypes among women infected with HIV: a meta-analysis. *AIDS* 2006; 20(18): 2337-44.
17. Ng'andwe C, Lowe JJ, Richards PJ, Hause L, Wood C, Angeletti PC. The distribution of sexually-transmitted Human Papillomaviruses in HIV positive and negative patients in Zambia, Africa. *BMC Infect Dis* 2007; 7: 77.
18. de Sanjosé S, Diaz M, Castellsagué X, et al. Worldwide prevalence and genotype distribution of cervical human papillomavirus DNA in women with normal cytology: a meta-analysis [Review]. *Lancet Infect Dis* 2007; 7(7): 453-9.
19. Koshiol JE, Schroeder JC, Jamieson DJ, et al. Time to clearance of human papillomavirus infection by genotype and human immunodeficiency virus serostatus. *Int J Cancer* 2006; 119(7): 1623-9.
20. Kitchener H, Nelson L, Adams J, et al. Colposcopy is not necessary to assess the risk to the cervix in HIV-positive women: an international cohort study of cervical pathology in HIV-1 positive women. *Int J Cancer* 2007; 121(11): 2484-91.
21. Moodley JR, Hoffman M, Carrara H, et al. HIV and pre-neoplastic and neoplastic lesions of the cervix in South Africa: a case-control study. *BMC Cancer* 2006; 6: 135.
22. Bornstein J. Human papillomavirus vaccine: the beginning of the end for cervical cancer [Review]. *IMAJ Isr Med Assoc J* 2007; 9(3):156-8.
23. Menczer J. The human papillomavirus vaccine and its relevance in Israel. *IMAJ Isr Med Assoc J* 2007; 9(7): 546-9.
24. Luque AE, Hitti J, Mwachari C, et al. Prevalence of human papillomavirus genotypes in HIV-1-infected women in Seattle, USA and Nairobi, Kenya: results from the Women's HIV Interdisciplinary Network (WHIN). *Int J Infect Dis* 2010;14(9):e810-4.
25. Menczer J. The low incidence of cervical cancer in Jewish women: has the puzzle finally been solved? *IMAJ Isr Med Assoc J* 2003; 5(2): 120-3.