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

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\begin{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 women 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 women.

\end{abstract}

\textbf{Key words:} human papillomavirus, human immunodeficiency virus, cervical cytology abnormalities, colposcopy, HPV genotypes

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 aspirated 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 -80°C 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 (Biomérieux, 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: TTTGTATCTGTGTTAGATACTAC and GAAAAATAAACTGTAATCATATTC.

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

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](image-url)
Table 2. Association of HPV with demographic, behavioral, and HIV variables

<table>
<thead>
<tr>
<th></th>
<th>HPV infection</th>
<th>P (OR)</th>
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<tbody>
<tr>
<td></td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>Age (yrs)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>30.8 ± 11.1</td>
<td>38.7 ± 11.3</td>
</tr>
<tr>
<td>Median</td>
<td>36 (17–71)</td>
<td>36 (18–60)</td>
</tr>
<tr>
<td>&lt; 30</td>
<td>6/35 (17.1%)</td>
<td>13/49 (26.5%)</td>
</tr>
<tr>
<td>&gt; 30</td>
<td>29/35 (82.9%)</td>
<td>36/49 (73.5%)</td>
</tr>
<tr>
<td>Origin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ethiopian</td>
<td>24/35 (68.6%)</td>
<td>39/49 (79.6%)</td>
</tr>
<tr>
<td>Age at first sexual intercourse</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>17.4 ± 2.9</td>
<td>15.5 ± 2.7</td>
</tr>
<tr>
<td>Median</td>
<td>17.5 (12-27)</td>
<td>15 (9-22)</td>
</tr>
<tr>
<td>&lt; 16 yrs</td>
<td>10/29 (34.5%)</td>
<td>34/47 (72.3%)</td>
</tr>
<tr>
<td>Lifetime no. of sex partners</td>
<td></td>
<td></td>
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<tr>
<td>&lt; 3</td>
<td>29/34 (85.3%)</td>
<td>42/49 (85.7%)</td>
</tr>
<tr>
<td>No. of pregnancies</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 3</td>
<td>23/35 (65.7%)</td>
<td>25/49 (51%)</td>
</tr>
<tr>
<td>Condom use</td>
<td>6/35 (17.1%)</td>
<td>7/49 (14.3%)</td>
</tr>
<tr>
<td>History of STIs</td>
<td>7/35 (20%)</td>
<td>9/49 (18.4%)</td>
</tr>
<tr>
<td>Drug abuse</td>
<td>1/34 (2.9%)</td>
<td>5/49 (10.2%)</td>
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<tr>
<td>Smoking</td>
<td>8/34 (23.5%)</td>
<td>5/49 (10.2%)</td>
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<tr>
<td>Duration of documented HIV</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 2 yrs</td>
<td>9/35 (25.7%)</td>
<td>24/49 (49%)</td>
</tr>
<tr>
<td>CD4+ count at diagnosis</td>
<td>10/35 (28.6%)</td>
<td>23/48 (47.9%)</td>
</tr>
<tr>
<td>CD4+ count, current</td>
<td>3/35 (8.6%)</td>
<td>11/48 (22.9%)</td>
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<tr>
<td>HIV RNA, current</td>
<td></td>
<td></td>
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<tr>
<td>Undetectable</td>
<td>27/35 (77.1%)</td>
<td>33/48 (68.8%)</td>
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<tr>
<td>HAART</td>
<td>30/35 (85.7%)</td>
<td>41/49 (83.7%)</td>
</tr>
<tr>
<td>Compliance</td>
<td>31/35 (88.6%)</td>
<td>37/45 (82.2%)</td>
</tr>
</tbody>
</table>

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 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.
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.

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CIN = cervical intraepithelial neoplasia
References

Capsule
Cap binding and immune evasion revealed by Lassa nucleoprotein structure
Lassa virus, the causative agent of Lassa fever, causes thousands of deaths annually and is a biological threat agent, for which there is no vaccine and limited therapy. The nucleoprotein (NP) of Lassa virus has essential roles in viral RNA synthesis and immune suppression, the molecular mechanisms of which are poorly understood. Qi et al. report the crystal structure of Lassa virus NP at 1.80Å resolution, which reveals amino (N)- and carboxy (C)-terminal domains with structures unlike any of the reported viral NPs. The N domain folds into a novel structure with a deep cavity for binding the m7GpppN cap structure that is required for viral RNA transcription, whereas the C domain contains 3′–5′ exononuclease activity involved in suppressing interferon induction. The authors declare that to the best of their knowledge this is the first X-ray crystal structure solved for an arenaviral NP, which reveals its unexpected functions and indicates unique mechanisms in cap binding and immune evasion. These findings provide great potential for vaccine and drug development.

Nature 2010; 468: 779
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“Human beings, who are almost unique in having the ability to learn from the experience of others, are also remarkable for their apparent disinclination to do so”
Douglas Adams (1952-2001), English writer and dramatist. He is best known as the author of The Hitchhiker’s Guide to the Galaxy, which started life in 1978 as a BBC radio comedy before developing into five books that sold over 15 million copies in his lifetime, a television series, several stage plays, comics, a computer game, and in 2005 a feature film