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HPV infection in HIV-positive females: the need for cervical cancer screening including HPV-DNA detection despite successful HAART

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Abstract. – PURPOSE: To identify the presence of Human Papilloma Virus (HPV) infection and evaluate the role of Highly Active Antiretroviral Treatment (HAART) in patients with HIV-HPV co-infection. We also compared cytological screening results with HPV-DNA detection to implement screening programs and prevention of invasive cervical cancer (ICC) in HIV-infected females.

PATIENTS AND METHODS: We enrolled HIV-infected females presenting for routine clinical evaluation. HPV-DNA of high/intermediate and low-risk types was detected from cervical specimens by nucleic acid hybridization assay with signal-amplification. Patients were divided into two groups according to the presence of HPV co-infection (HPV+) or not (HPV-).

RESULTS: We enrolled 57 HIV-infected females. Median age was 40 (IQR 35-44) years, mean CD4 count was 547 ± 227 cells/mm^3, 45 (78.9%) had undetectable HIV-RNA and 52 (91.2%) received HAART. Globally, 19/57 (33.3%) patients were HPV-infected, 16/57 (28.1%) with high/intermediate and 3/57 (5.3%) with low-risk types. Five of the 19 (26.3%) HPV+ patients carried both types. Correlating high-risk genotype HPV-DNA detection with cytology, 17.5% of women with negative cytology, 36.4% with ASCUS (Atypical Squamous Cells of Uncertain Significance) and 83.4% of women with positive cytology (50% of LSIL: low-grade squamous intraepithelial lesion and 100% of HSIL: high grade SIL) were HPV positive. No statistical difference when comparing HPV+ and HPV-patients in age, CD4 cell count, in the proportion of previous intravenous-drug use, previous AIDS and of those receiving HAART with undetectable HIV-RNA was observed.

CONCLUSIONS: Cervical cancer screening including HPV-DNA detection should be implemented in HIV infected females across Europe, also when receiving successful HAART, to early identify the HIV patients at risk for ICC to be submitted to more frequent follow up and proper treatment.

Key Words:
HPV, Cervical cancer, HIV, HAART.

Introduction

The infection by Human Papilloma Virus (HPV) is a common sexually transmitted disease in the world[1]. National Italian cancer register data for the years 1998-2002 shows that each year about 3,500 new cases of cervical cancer are diagnosed, with an annual incidence of 10 cases/100,000 women and that about 1,000 women died from the disease over that period. Regard to the prevalence of HPV infection in Italy, available data on women aged between 17 and 70 years, which attend routine checks-ups or gynecological screening program (Pap test) show a prevalence of 7-16%[2]. In women diagnosed with abnormal cytology, the prevalence rises to 35% and 96% in the diagnosis of severe dysplasia or Cervical Intraepithelial Neoplasia[3].

The female population currently accounts for 50.2% of incident HIV infections, the majority of which live in Africa[4]. Women also account for almost 30% of new HIV infection in 2011 in Italy[5].

Studies[6] on the correlation between HIV, HPV and squamous intraepithelial lesion (SIL) have shown that HIV-positive patients have a higher prevalence of HPV infection and cervical in-

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traepithelial lesions that tend to increase in proportion to the degree of immunodeficiency. The studies carried out in the pre-HAART (Highly Active Antiretroviral Treatment) have shown that cervical cancer associated with HPV were present in numbers five times greater in women infected with HIV than those not infected[7].

The introduction of HAART has significantly changed the natural history of HIV infection with a significant reduction in the incidence of HIV-related events[8]. However, the risk of non-HIV-related morbidity and mortality, including cardiovascular and bone disease, neurocognitive impairment and malignancies, has greathy increased in recent years[9-30].

In the recent HAART era, invasive cervical cancer (ICC) incidence has declined but continues to represent a significant cause of morbidity and mortality in HIV population[31].

HPV infection is recognized as the primary cause of cervical cancer (CC) development and has been associated with other types of cancer in immunosuppressed patients[32]. HIV-infected patients are at increased risk for HPV chronic infection and development of CC that, in its invasive form (ICC), is considered an AIDS-defining illness. Data from large observational cohorts suggest that CC screening with Pap-test is poorly utilized in HIV-infected females[33].

The role of HPV-DNA detection in CC screening campaigns remains to be determined in HIV-infected patients.

The aim of our study was to evaluate the presence of HPV infection in HIV infected females using HPV-DNA detection and to compare the results with cytology. We also aimed to investigate the possible role of HAART in patients co-infected with HIV and HPV.

Patients and Methods

Population Studied

Between March 2008 and September 2009, 57 HIV-infected females taking part to a screening program run by the Unit of Infectious Diseases and the Unit of Obstetrics and Gynecology of the University of Sassari, Italy, were consecutively enrolled in the study. The study design included annual evaluation of all patients in case of a negative cytology and immediate recall for further investigation (repeat cytology, colposcopy and histology) for patients with doubtful or positive cytology. The 57 cervical cytology specimens collected were analyzed for the presence of DNA of high-risk HPV at the Section of Virology, Institute of Infectious Diseases of Sassari. At the time of the sample collection for the virological test samples, cytologic examination were also performed in all women. Pap test results were evaluated according to the Bethesda System in the Institute of Pathology[34-36] at the University of Sassari. Lesions were classified as normal cytology, atypical squamous cells of undetermined significance (ASCUS), low-grade squamous intraepithelial lesion (LSIL) and high-grade squamous intraepithelial lesion (HSIL). For all patients data regarding HIV infection such as CD4, the HIV-viremia, CDC stage, current and past therapy and the mode of transmission of HIV infection were collected. Furthermore, in the case of positive cytology, second level examinations including colposcopy and histological examination were performed.

The study was approved by the Bioethical Committee of the University of Sassari Hospital.

DNA Detection of High/Intermediate and Low-risk HPV Genotypes

Detection of high/intermediate-risk[16,18,31,33,35,39,45,51,52,56,58,59,68] and low-risk[6,11,42-44] HPV-DNA in cervical cytology specimens was performed with the Hybrid Capture II (HC II, Digene, Silver Spring, MO, USA) assay according to the manifacturer's instructions. The reaction is quantified using a luminometer, and the relative light units (RLU) emitted in time are proportional to the amount of target nucleic acid present in the original sample.

Statistical Analysis

Descriptive statistics included mean, median, standard deviation (SD), interquartile range. The Shapiro-Wilks test was used to check the normality of the distribution. Between-group differences were assessed by using Student *t* test or and Mann-Whitney U-test for continuous normally or non-normally distributed variables, as appropriate.

Categorical variables were compared using χ^2 test or by Fisher's exact test, when appropriate. A statistical significance was considered present for $p < 0.05$.

Results

We enrolled 57 HIV-infected women with a median age of 40 (35-44) years. The presumed mode of transmission of HIV infection was sexu-

al intercourse for 77.2% (44 patients) of the women and the use of intravenous drugs use (IVDU) for 22.8% (13 patients). The mean CD4 count was 547 ± 227 cells/mm^3, HIV-RNA was undetectable in 45 (78.9%). Fourteen out of 57 (24.6%) of the HIV-infected females were in the asymptomatic phase of HIV infection (CDC stage A), 24 (42.1%) were in the symptomatic phase but not AIDS (CDC stage B) and the remaining 19 (33.3%) were in the stage of overt disease (CDC stage C). As for the antiretroviral therapy, 5 (8.8%) were not treated, of these 5.3% were naive and 3.5% were previously treated with HAART; 52 (91.2%) patients were currently receiving a HAART regimen (Table I).

HPV-DNA Detection

Of the 57 cervical swabs obtained from 57 HIV-infected women studied, 19 (33.33%) tested positive on HC II, of which 3 (14%) only for low-risk HPV and 16 (28.1%) only for high/intermediate risk HPV types; among the 19 HPV+, 5 (8.5%) were positive for both subtypes (Figure 1).

Cytology was negative in 40 (70.2%) of cases, 11 (19.3%) samples were defined as ASCUS, 2 (3.5%) as LSIL, 4 (7.0%) as HSIL.

HPV-DNA and Cytology Results Comparison

Correlating high risk genotype HPV-DNA detection with cytology, 7/40 (17.5%) women with negative cytology, 4/11 (36.7%) with ASCUS and 5/6 (83.3%) women with positive cytology, in particular 1/2 (50%) with LSIL and 4/4 (100%) with HSIL, were HPV-DNA positive, as shown in Figure 2.

Subdividing the patients according to age groups, 7/57 (12.3%) were aged ≤ 30 years, 22/57 (38.6%) between 31 and 40 years, 26/57 (45.6%) between 41 and 50 years and 2 (3.5%) were over 50 years old. The high/intermediate-risk HPV-DNA detection was positive in 4/7 (57.1%) patients under the age of 30 years, 4/22 (18.2%) patients aged between 30 and 40 years and 8/26 (30.8%) patients aged between 41 and 50 years and 0/2 of the patients older than 50 years.

Of the patients with HPV + for high/intermediate risk 13/16 (81.2%) had HIV-RNA negative. No patient between HPV + high/intermediate risk had less than 200 CD4 cells/mm^3.

No statistical difference was observed in age, current CD4 cell count and CD4 cell nadir when comparing HPV infected (HPV+) and HPV non-infected (HPV-) patients (Table I).

HPV+ were more frequently previous IVDU (36.8%) than HPV- (15.8%) and more likely to be HBV or HCV co-infected, but not significantly (Table I).

The proportion of patients with previous AIDS (CDC stage C) was higher in HPV+ (36.8%) than in HPV- (31.6%), but not significantly (Table I).

Table I. Demographic and clinical characteristics of the entire cohort of 57 HIV infected females and of the patients grouped according to the positivity (HPV positive) or negativity (HPV negative) of HPV DNA detection in cervical samples.

Parameter	All cohort (n = 57)	HPV positive (n = 19)	HPV negative (n = 38)	p
Age (years)	40 (35-44)	41 (30-44)	40 (37-43)	0.524
IVDU	13 (22.8%)	7 (36.9%)	6 (15.8%)	0.074
CDC stage C	19 (33.3%)	7 (36.9%)	12 (31.6%)	0.691
Current CD4 cell count (cells/mm^3)	547 ± 227	512 ± 232*	565 ± 225	0.408
Nadir CD4 cell count (cells/mm^3)	226 ± 139	269 ± 179	207 ± 117	0.244
Undetectable HIV-RNA	45 (78.9%)	15 (78.9%)	30 (78.9%)	1.000
HBV infection	8 (14.0%)	5 (26.3%)	3 (7.8%)	0.072
HCV infection	15 (26.3%)	8 (42.1%)	7 (18.4%)	0.055
Current HAART	52 (91.2%)	17 (89.4%)	35 (92.1%)	0.741
HAART duration (months)	54 (22-120)	24 (11-27)	70 (24-120)	0.166
PI duration (months)	24 (16-48)	25.5 (17.5-28)	22 (16-60)	0.957
NNRTI duration (months)	46 ± 35	28 ± 36	48 ± 36	0.462
NRTI duration (months)	54 (22-120)	24 (11-27)	70 (24-120)	0.166
Naive to antiretrovirals	3 (5.2%)	0 (0.0%)	3 (7.9%)	0.288
HAART experienced not receiving therapy	2 (3.5%)	2 (10.6%)	0 (0.0%)	0.107

Data are expressed as number (%), mean ± standard deviation or median (interquartile range). IVDU: intravenous drug users; CDC: Center for Disease Control; HBV: hepatitis B virus HCV: hepatitis C virus; HAART: highly active antiretroviral therapy; PI: protease inhibitors; NNRTI: nonnucleoside reverse trasncriptase inhibitors; NRTI: nucleoside reverse transcriptase inhibitors.

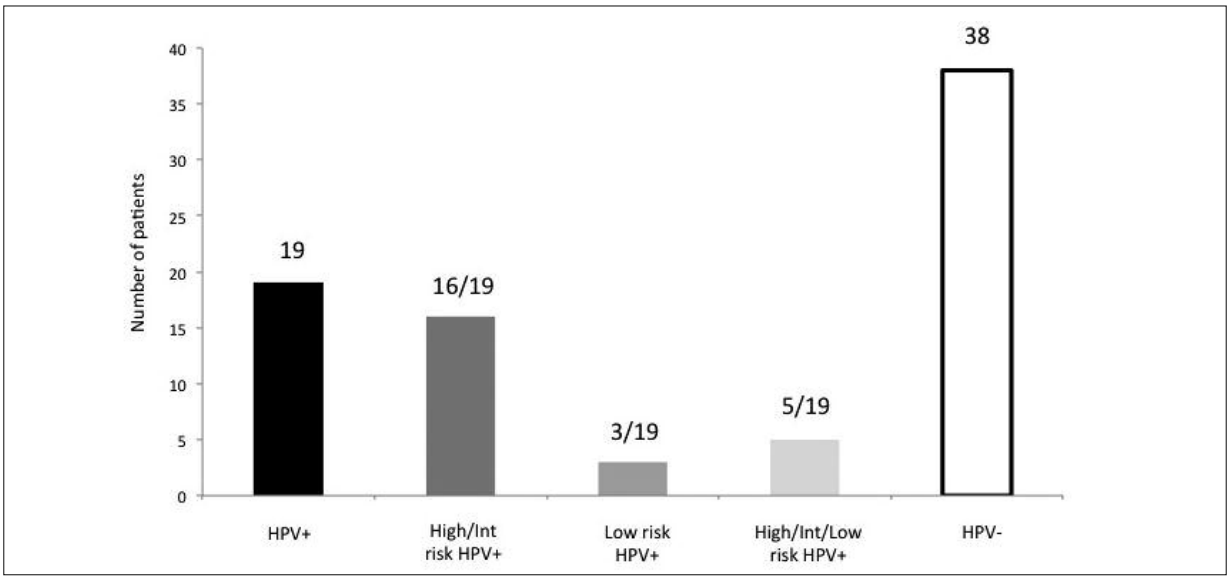

Figure 1. Number and percentage of patients with HPV infection, HPV infection with high/intermediate (high/int) and low-risk types among 57 HIV-infected females.

No statistical difference was observed in the proportion of HPV+ receiving HAART with undetectable HIV-RNA in respect of HPV-. The duration of HAART, PI, NNRTI and NRTI was similar among the two groups (Table I).

Discussion

Our data, according with literature[37], showed an higher prevalence of HPV infection in HIV-seropositive (33.3%), similar with other high risk populations[38], compared with HIV-seronegative women in the general Italian population.

HPV infections were caused by high and intermediate risk genotypes in 28.1% of cases, as noted in other studies[39]. When we compared the cytology with HPV-DNA detection, we detected HPV-DNA in 100% of patients with HSIL, 50% in LSIL, 36.4% in ASCUS and in 17.5% of patients with normal cytology. These data suggest a subclinical persistence of HPV infection in pa-

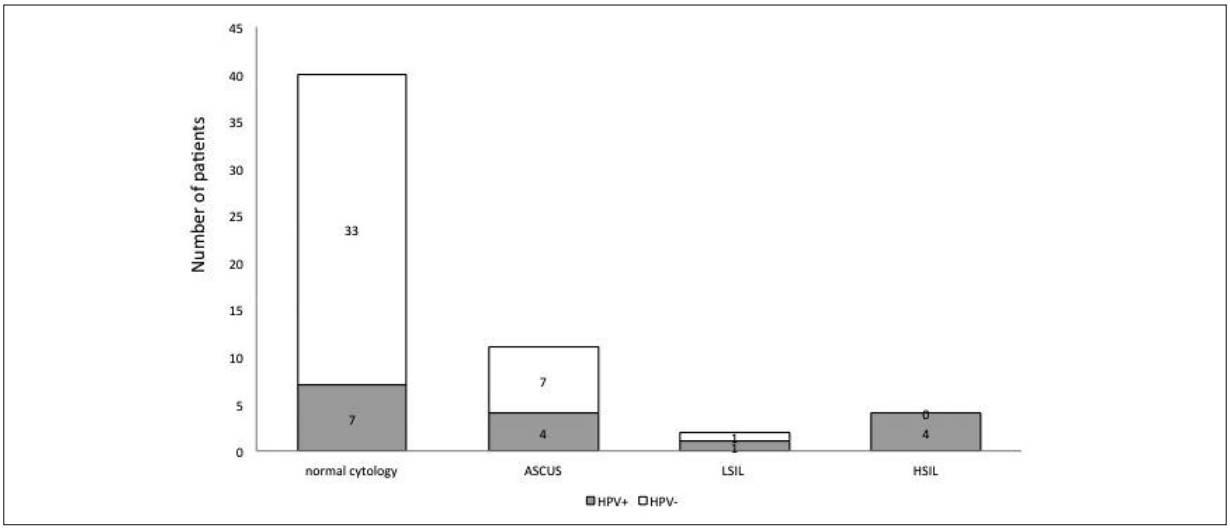

Figure 2. Proportion of high risk HPV genotypes according to cytologic results. ASCUS: atypical squamous cells of undetermined significance; LSIL: low-grade squamous intraepithelial lesion; HSIL: high-grade squamous intraepithelial lesion.

tients with HIV infection. Cervical cytology was characterized by a low sensitivity in detecting low-grade cytological abnormalities and ASCUS requiring repeat testing or analysis with other diagnostic tests, as observed in European studies[39].

In our series, in addition, the low sensitivity was not due to problems of reproducibility between laboratories and different operators having been performed at a single reference laboratory. It should be noted, therefore, the need to combine HPV-DNA detection with traditional cytological screening.

European HIV guidelines suggest, in the case of normal cytology in sexually active women, to repeat PAP-test after 1-3 years and HPV-DNA detection is not considered[40]. In contrast, in HIV negative setting the American Cancer Society, American Society for Colposcopy and Cervical Pathology, and American Society for Clinical Pathology Screening Guidelines for the Prevention and Early Detection of Cervical Cancer suggest in the case of positive HPV DNA to repeat both with cytology and HPV test after one year and if positive for high risk genotypes (16 and/or 18) to perform colposcopy in women aged > 30 years[41].

HPV-DNA testing has allowed us to identify 7 HIV infected patients with HPV infection among those negative at cytologic evaluation thus avoiding a delay in detecting the disease, in these cases. Therefore, our data strongly support the use of both cytology and HPV-DNA test for the screening of HPV related lesions. Although US guidelines suggest only FDA approved tests, some European countries may not be able to implement such strategies in the current setting of reduced resources. Therefore, further studies are needed to identify new and less expensive HPV-DNA detection methods for screening campaigns in all European countries.

Regarding demographic characteristics we found an initial reduction of the prevalence of HPV infection from 57.1% positivity in patients younger than 30 years to 18% of those aged between 30 and 40 years and an increase (28.6%) in patients older than 40 years. This result is in contrast with the observed prevalence by age group in the general Italian population in which positivity for HPV-DNA is 15% in women younger than 30 years, around 10% in women between the ages of 30 and 40 years and around 5% in women over the age of 40 years[2].

Furthermore, we did not observe a significant difference regarding age in HPV+ patients compared to HPV-. Taken together, these results confirm the high persistence of HPV infection in all age groups suggesting the need for a specific screening campaign for HIV-infected females regardless of the age of the patient.

With regard to the immuno-virological parameters, no statistically significant differences were identified between HPV+ patients compared with HPV- in terms of CD4 cell count and plasma HIV-RNA.

The percentage of patients receiving HAART regimens with complete suppression of viral load did not differ between HPV+ and HPV-.

These data suggest that the immune recovery and the complete suppression of HIV viral load, secondary to HAART, are not sufficient to reduce the prevalence of HPV infection in our cases, as noted in another recent Italian study[42].

Furthermore, the introduction of HAART did not lead to viral clearance in a study performed on samples of cervical cells[43].

The role of HAART in modifying the natural history of progression of HPV-related lesions is still debated. A study in the early HAART era have shown a non statistically significant reduction in the occurrence of SIL compared with untreated women[44]. Recent reports, on the other hand, have suggested a minimum impact of HAART on the incidence of cervical lesions[45-47]. The percentage of patients with a diagnosis of AIDS was similar in patients HPV+ and HPV- suggesting that the degree of immunosuppression is not associated with an increased persistence of HPV infection. Data from the literature are controversial regarding this issue. A study in Finland of 153 HIV-positive women showed that the risk of CIN was not associated with low CD4 count and that the prevalence of SIL was not associated with the degree of immunosuppression in a work of 298 HIV-positive patients screened cytology and colposcopy[48].

The increase in survival of patients undergoing HAART regimens exposes, in relation to the lack of impact on the prevalence and evolutionary HPV lesions, to an increased risk of developing ICC. In a study conducted in Italy on 132 HIV infected patients the interval of time between the diagnosis of HIV infection and that the ICC was more than 10 years in about half of patients This finding suggests a failure of secondary prevention in reducing the progression of lesions from HPV. The lack of screening campaigns or poor adherence of patients to the proposed program has been identified as the main cause of progression to ICC[49].

The reasons for these observations are cultural and social, and should be carefully considered in order to improve the counseling and the access to gynecological care.

Taken together our results suggest continuing screening in all age groups also in the presence of fully suppressive HAART.

HPV vaccination with either quadrivalent or bivalent vaccine has proven safe and effective in the general population[50]. However, the safety and efficacy has not been demonstrated so far in immunocompromised individuals.

Considered the high prevalence of HPV infection, in HIV positive females there is a rationale to consider the vaccination. Nevertheless, the clinical benefits in patients infected with HIV should be further clarified. Several studies have shown that the distribution of HPV types in women with HIV infection with CIN 2-3 and ICC, may be different from that of HIV negative with lower percentages of HPV 16. HIV infected women harbor more frequently multiple HPV infections which have been associated with worst cytological lesions[42].

A recent research[51] conducted in Northern Sardinia has shown an high prevalence of genotype 51 (37.9%) and a lower than expected prevalence of 18 genotype (5.2%).

The HPV vaccines have no therapeutic effect and do not prevent the development of CIN in women already infected with HPV. The maximum efficiency is found, therefore, with the administration before being infected with HPV. The efficacy and immunogenicity of HPV vaccines in HIV-infected children are currently under study. The quadrivalent HPV vaccine has been shown to be safe and has generated a significant antibody response in 126 HIV-infected children[52] and studies in HIV-infected women are currently ongoing.

The prophylactic vaccines primarily stimulate the humoral immune response. It is not known, therefore, whether the depletion of CD4 cells can be associated with a reduced ability to develop and maintain an adequate antibody response, as already noted for other bacterial and viral diseases.

It would, thus, be useful to perform specific clinical trials designed to evaluate the effectiveness of HPV vaccination in different age groups and immunocompromised patients with HIV infection. Our findings showed an higher prevalence (33.3%) of HPV infection in HIV-infected females when compared with the Italian population (7-16%). Most infections were caused by high/intermediate risk HPV types. Successful HAART does not seem to reduce the prevalence of HPV infection in our cases. The high prevalence of HPV infection in all age groups and the limited impact of HAART, make it necessary to implement campaigns for screening and prevention of ICC in patients with HIV infection. Waiting for data on safety and efficacy of HPV vaccines in women with HIV infection, the proper management of HPV infection lies in the accurate early diagnosis and follow-up. Our results emphasize that the use of sensitive virological tests for high-risk HPV DNA detection represents a fundamental part of the screening for the prevention of cervical cancer in women with HIV infection, as it allows to identify a significant proportion of patients at risk of neoplastic evolution which would be negative for screening based solely on cytology. The combination of cytological and virological screening, thus, appears to be particularly important in HIV-infected women because it allows an early identification of HPV infection and, consequently, a more accurate monitoring of lesions.

Conclusions

Cervical cancer screening including HPV-DNA detection should be implemented in HIV patients across Europe, also when receiving successful HAART, to early identify the HIV patients at risk for ICC to be submitted to more frequent follow up and proper treatment.

Acknowledgements

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Conflict of Interest

The Authors declare that there are no conflicts of interest.

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