Squamous intraepithelial lesions (SILs) and HPV associated changes in HIV infected women or at risk of HIV

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Summary

Objectives: The study concerns the prevalence of squamous intraepithelial lesions (SILs) and the specific cervical cytopathological features of a group of HIV-positive and a group of HIV-negative women recruited in a multicentric cohort study. The assessment of HPV-DNA genotypes was carried out in both groups.

Method: 459 women, 266 HIV-positive and 193 HIV-negative women at risk were examined in an Italian multi-institutional study involving 14 gynaecological centres.

Results: In our samples, the risk of SILs was 29.4% for HIV-positive women and 10% for HIV-negative women (O.R. = 3.90, C.I. 95%: 2.20-6.98) while HPV-DNA-PCR genotypes had a high prevalence in both groups of HIV-positive and HIV-negative women. Cytopathological features of SILs in HIV-positive women were: a higher number of koilocytes and a more marked atypia of high grade neoplastic cells.

Conclusions: A higher prevalence of SILs as well as a specific cervical cytopathology might suggest HIV infection.

Introduction

Early epidemiological studies show that the main risk factors in cervical lesions (low and high grade SILs) are early sexual activity, multiple sexual partners, sexually transmitted diseases, smoking, and impaired immunological response [1]. There is now increasing evidence that the human papillomavirus (HPV) is a sex-related factor associated with cervical cancer and that high-risk HPV genotypes play an important role in the pathogenesis of pre-invasive and invasive cervical lesions [2-5].

Several studies show that genital warts, low-grade SILs, high-grade SILs and cervical cancer are more prevalent and severe in HIV-infected women [6-12] and that HIV-induced immunosuppression is responsible for an increase of the risk of HPV infection, suggesting that the entire spectrum of cervical lesions and cancer may represent an opportunistic complication of HIV infection [7, 13-16]. Strong associations between HIV infection, high-risk HPV genotypes and the development of squamous intraepithelial lesions (SILs) and cancer were found [5, 7, 12, 16-20], leading the Centers for Disease Control (CDC) to add invasive cervical cancer to the list of AIDS-defining diseases in 1993 [21].

HIV infection enhances the pathological effect of HPV on the cervical mucosa through a mechanism of reduction of immunological control over HPV replication at a local level [22-25]. It has also been shown that HIV affects local cervical immunity, reducing the quantity of Langherhans's cells and their capacity of functioning effectively [26]. Another possible mechanism might be a direct interaction between HPV and HIV [9, 13, 27-28].

The recent increase in human immunodeficiency virus (HIV) infection through heterosexual contacts and the growing number of women involved have boosted interest in this topic [8-12, 22].

To further investigate these issues, we have carried out a multicentric cohort study to assess the risk of development of HPV lesions and the evolution of SILs in HIV positive women and in a parallel cohort of HIV-negative women with similar life styles.

We analyzed the complex interaction over time of HIV, HPV and SILs in two groups of HIV-positive and HIV-negative women matched for similar behaviour, and compared the two groups as far as the development of HPV, SIL, cervical cytopathological features and distribution of HPV genotypes were concerned.

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Materials and Methods

Population studied

Study participants were young HIV+ or HIV- women at risk of HIV infection (intravenous drug users (IDUs), sexual partners of IDUs, sexual partners of men at risk of or with HIV infection) attending a network of 14 Italian centres dealing with HIV patients between 1996 and 1998. Details of the study protocol have been reported in previous papers [11-12].

Each woman was interviewed by a trained medical research assistant; a recruitment form was filled up with information on their socio-economic status, clinical, gynaecological, sexual history, HIV and HPV risk factors, and history of STDs.

Informed consent for enrolment in the study was obtained before the interview. If not already reported as HIV-positive, all study participants were tested for HIV-antibodies at their centre at least 6 months before enrolment. HIV antibody status was determined by enzyme-linked immunoabsorbent assay (Elisa-Dupont; Genetic System, Seattle, Washington, USA) and confirmed by Western Blot (Bio-Rad Laboratories, Hercules, California, USA).

Sample collection

Two cervical smears were collected from all participants: the first cervical smear for Pap testing was obtained using an Ayre's spatula (ectocervix) and a cytobrush (endocervix), following detailed written instructions; while the second was taken only from the ectocervix (Ayre's spatula) and investigated for molecular biology testing (HPV-DNA-PCR). Both specimens were smeared and fixed with cytofix. All the women enrolled were submitted to a gynecological examination, a colposcopy and one or more punch biopsies if necessary.

Cervical cytology

The cervical smears, containing both ecto and endocervical material, were stained according to the Papanicolaou technique and examined at the Cytopathology Unit of the National Institute of Health in Rome by two cytopathologists, blinded to the clinical and the HIV serological status of the participants. A random sample of smears within normal limits and flogistic smears together with all cases positive for ASCUS, AGUS, low-grade SIL, high-grade SIL and invasive cancer were checked and confirmed by an independent external senior pathologist. Results of the cervical smears were classified according to the 1993 version of the Bethesda classification system [29].

HPV detection by PCR and viral typing

The virological examination was carried out by the laboratory of Virology of the L. Spallanzani Hospital in Rome.

Cervical cells were scraped from a glass slide and digested with 100 microl of lysis buffer (50mM KC1, 10mM TRIS-HC1, pH 8.3, 1.5mM MgC12, 0.45% NP40, 0.45% Tween 20) containing 100 mg/ml of proteinase K (1 h at 56 °C and 10 min at 99 °C). The quality of each cell lysate was assessed by PCR-positivity for mitochondrial DNA sequences, according to Stevenson *et al.* [30]. All samples, cell lysates and DNA were tested for the presence of HPV DNA sequences, using the degenerate primers, MY09 and MY11, for the L1 region. All HPV-DNA positive samples were investigated for HPV genotyping.

The recognition of HPV genotypes (high-risk, low-risk and undetermined type) was performed by restriction-fragment-length-polymorphism (RFLP) analysis [31, 32].

The following HPV genotypes were considered high risk: 16, 18, 31, 33, 35, 53, 58.

Statistical analysis

The main demographic, behavioural and clinical features together with the risk factors of both HIV-positive and HIV-negative women were compared. Risk factors for SILs were then evaluated by comparing the frequency distribution of demographic data, behavioural data and previous STDs among women with and without SILs. Odds ratio (OR) and 95% confidence intervals were calculated to evaluate the association between specific variables of SILs.

Results

Main features of the study population

Between June 1995 and June 1998, a total of 459 women was enrolled: 266 were HIV-positive (58%) and 193 were HIV-negative (42%). The median age of participants was 29.0 years (range 17-44); 450 women (98%) were Caucasian and 9 (2%) were black African women; 189 women (41%) were injecting drug users, 34 women (7%) were sexual partners of an IDU male, 27 women (6%) were sexual partners of an HIV-infected male and 209 women (46%) reported unprotected sexual intercourse with occasional partners.

Detailed behavioural and clinical characteristics and risk factors of the study participants according to HIV status are shown in Table 1. The greater differences were

Table 1. — *Associated behavioural risk characteristics in the 459 enrolled women.*

	HIV+ N=266 (%)	HIV- N=193 (%)	
Age in years:			
Median	30	27	
<= 30	120 (45.1)	127 (65.8)	p<0.0001
> 30	146 (54.8)	66 (34.2)	
Never been cigarette smokers	237 (89.1)	140 (72.5)	p<0.0001
Never been drug users	125 (47.0)	64 (33.2)	
Age at first intercourse:			
Median	16	17	
<= 16	147 (55.3)	85 (44.0)	p<0.02
> 16	119 (44.7)	108 (56.0)	
Sexual partners:			
Only one	30 (11.4)	34 (17.6)	
2-4 partners	86 (32.6)	77 (39.9)	p<0.03
5-9 partners	59 (22.3)	36 (18.7)	
> 9 partners	89 (33.7)	46 (23.8)	
Contraceptives			
None	29 (10.9)	40 (20.7)	
Condoms	181 (68.0)	80 (41.4)	
Oral contraceptives	131 (49.2)	103 (53.3)	p<0.01
IUD	37 (13.9)	8 (4.1)	
C.I.	72 (27.0)	41 (21.2)	
Diaphragm	7 (2.6)	0 (–)	
Parity			
0	137 (51.5)	128 (66.3)	
1	84 (31.6)	47 (24.4)	p<0.01
> 1	45 (16.9)	18 (9.3)	
Previous STDs			
Herpes genitalis	23 (8.6)	2 (1.0)	
Genital warts	98 (36.8)	33 (17.1)	p<0.0001
Other STDs	47 (17.7)	20 (10.4)	

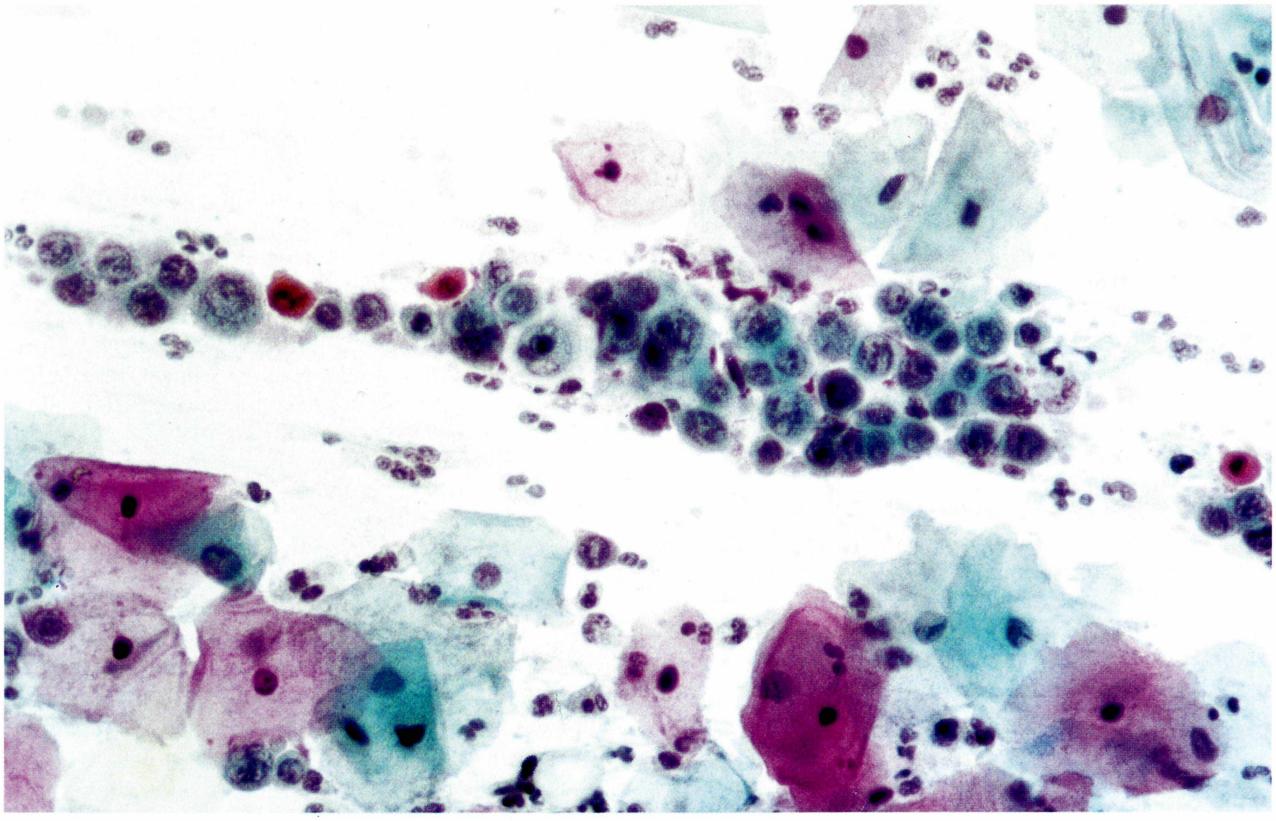

Figure 1. — Cervical smear of a HIV-positive woman with carcinoma in situ (40x).

noticed with respect to the age of enrolled women, their smoking habits, number of partners, parity, use of oral contraceptives and history of genital warts, herpes genitalis and other STDs (Table 1).

Cytopathological results

At the cytological examination, squamous intraepithelial lesions (SILs) of various degrees were more prevalent in HIV+ (29.4%) than in HIV- women (10%) (OR=3.90, CI 95%: 2.20-6.98). Most of the SILs were classified as low grade SIL both in HIV positive and in HIV negative women (58, 74% of SILs in HIV-positive women and 13, 68% of SILs in HIV-negative women).

In addition, five cases of ASCUS and one case of AGUS were present in the HIV+ subjects and only one AGUS in the HIV-women. No cases of microinvasive or invasive cervical cancer were found in either group of women (Table 2).

In the HIV-positive women, the HPV cellular changes involved several cells, and wide-spread clumpings of koilocytes were detected all over the smear; whenever HSILs were present, the abnormal cells showed more marked atypias (Fig. 1).

The age of HIV-negative women was lower when no lesions or only lesions of low grade were detected, while it was higher (though not in a statistically significant way) when high grade lesions were found (Table 1). In HIV-positive subjects with HSILs, the age was lower

Table 2. — *Distribution of SILs in the smears from the participants according to HIV status.*

Cervical cytology classification	HIV-positive N=266 (%)	HIV-negative N=193 (%)
No evidence of SILs	182 (68.4)	173 (89.5)
Low grade SILs	58 (21.8)	13 (6.6)
High grade SILs	20 (7.6)	6 (3.4)
ASCUS	5 (1.8)	1 (0.5)
AGUS	1 (0.4)	

O.R. = 3.90 (95% C.I. 2.20 - 6.98)
Relative risk 1.57 (95% C.I. 1.36 - 1.81)*

* ASCUS and AGUS excluded in this statistical analysis.

Table 3. — *Association between the presence of HPV-related cellular changes and DNA-HPV-PCR in 228 HIV-positive and 168 HIV-negative women.*

	HIV-positive			HIV-negative		
	SIL with koilocytosis	SIL without koilocytosis	No SIL	SIL with koilocytosis	SIL without koilocytosis	No SIL
HPV-PCR Positive	32 (84.2%)	22 (64.7%)	35 (22.6%)	11 (78.7%)	–	31 (20.5%)
HPV-PCR Negative	6 (15.8%)	12 (35.3%)	120 (77.4%)	3 (21.3%)	3 (100%)	120 (79.5%)
Total	38 (100%)	34 (100%)	155 (100%)	14 (100%)	3 (100%)	151 (100%)

O.R. = 12.3 (4.6<O.R.>35)

O.R. = 14.5 (3.5<O.R.>70.5)

O.R. = 13.84
5.80<O.R.>30.18

Table 4. — *Distribution of HPV genotype (high risk, low risk, undetermined risk) in 89 HIV-positive and 42 HIV-negative women.*

	High risk	Low risk	U.T.*	Not done	Total
HIV+	43 (48.5%)	11 (12%)	4 (4.5%)	31 (35%)	89
HIV-	22 (52%)	2 (5%)	8 (19%)	10 (24%)	42
Total	65 (50%)	13 (10%)	12 (9%)	41 (31%)	131

* Undetermined type

suggesting perhaps that precancerous lesions progress more rapidly in HIV-positive subjects.

HPV-DNA PCR investigation

Of the 459 samples collected, only 395 were suitable for PCR analysis (227 from HIV-positive women and 168 from HIV-negative women), because the DNA HPV PCR of 61 slides was non-competent.

Table 3 shows the distribution of HPV-DNA among the study samples according to cytopathological diagnosis and HIV status. The presence of HPV-DNA was strongly associated with the presence of koilocytes in the smears of both HIV-positive and HIV-negative women.

The positive samples for HPV-DNA PCR, classified as high-risk, low-risk and undetermined type in HIV+ and HIV- women, showed a high prevalence of high risk genotypes 16, 18, 31 and 33, in both HIV+ (62%) and HIV-women (59%) (Table 4).

Discussion

The strong link between sexual activity and cervical cancer leads to the hypothesis that a transmissible agent is involved in the pathogenesis of cervical cancer. Current research indicates that human papillomaviruses play an important role in the carcinogenic process although the mechanism by which they exert their effect has not been fully explained yet. The co-existence of HIV and HPV infections is of special interest in the cervical carcinogenic process and might suggest that HIV infection tends to alter the natural history of HPV cervical infection by increasing the risk of SILs [5, 6, 8-12, 18, 22].

In our study, carried out on a mixed cohort population of infected and uninfected women, HIV-positive women were more prone to developing SIL in comparison with HIV-negative women with similar life styles; this is in accordance with other authors [6, 9, 14, 18, 20]. In our samples, the risk of SILs was 29.4% for HIV-positive women and 10% for HIV-negative women. The relative risk was 1.57 (95% C.I. 1.36-1.81). HIV infection induces both a lack of immunological control over HPV replication at the cervical level and an impairment of local immunity. HIV infection is also likely to enhance SIL development by a mechanism of immunodepression that induces HPV replication and expression at a local level [22-26].

In our study population, most cervical lesions were HPV-DNA PCR positive in both HIV+ and HIV- women, and both groups had a high prevalence of high risk genotypes; while SILs were more frequent in HIV-women with undetermined HPV types than in HIV+ women.

HPV-DNA was detected more often among HIV+ than among HIV- women, but whenever SILs were present, the difference was not statistically significant. Women with high or low risk HPV genotypes both had an increased risk of SILs compared with the HIV-negative women.

The high prevalence of HPV, associated with a high prevalence of SILs in HIV-positive women, may be due both to a greater exposure to common risk factors and to the effect of HIV on local immunity [23-25]. Many authors have suggested that the progression of SILs in HIV-positive women is greatly accelerated in comparison to HIV-negative women. The age distribution of SILs of different severity observed in this cross-sectional study seems to confirm this.

The highest koilocyte number concentrations per smear were observed in HIV-positive subjects. This result confirms that HIV infection tends to alter the effect of HPV on cervical squamous cells, increasing the intracellular replication of the virus, and may therefore be connected to the increased HPV viral load described by Feingold [6].

The TAT protein produced by HIV-infected cells might be internalised in the epithelial cells of the cervix and may interact with the Long Control Region (LCR) of the papillomavirus, enhancing its transforming capacity [27, 28]. This assumption is corroborated by the peculiarity of the HPV-associated cellular changes like koilocytes and cellular abnormalities.

In conclusion the high prevalence and cytopathological features of SIL lesions and the higher number of koilocytes could be suggestive of HIV infection, however we plan to re-examine further developments of HPV lesions and SILs in the follow-up phase of our cohort study.

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