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Risk factors for cervical presence of human papillomavirus DNA among women at risk for HIV infection

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SUMMARY

Risk factors for cervical infection with human papillomavirus (HPV) were assessed among 236 Italian women at risk for human immunodeficiency virus (HIV) infection (intravenous drug users (IVDU) or sexual partners of males at risk for HIV infection). All study participants underwent a structured interview, determination of HIV serostatus and detection of HPV cervical infection by means of polymerase chain reaction (PCR). Overall, the cervical presence of HPV DNA was ascertained in 86 of these 236 women (36·4%), while squamous intraepithelial lesions (SIL) were diagnosed in 57 (24·1%). HPV-infected and non-infected women did not differ in age, education and cigarette smoking. A statistically significant trend in the risk of HPV infection with increasing number of lifetime sexual partners was noted ($P = 0{\cdot}01$), but such trend was attenuated in multivariate analysis (multiple logistic regression (MLR) odds ratio (OR) for $\geqslant 20$ partners *vs* 1 = 1·6, 95% confidence intervals (CI): 0·4–5·9). A nearly threefold higher risk of HPV cervical infection emerged among IVDU women (MLR–OR: 2·7, 95% CI: 1·4–5·0), and this difference was not influenced by HIV serostatus. The prevalence of HIV infection was higher among HPV-positive than HPV-negative women (62·8% and 54·0%, respectively) (MLR–OR = 1·9, 95% CI: 0·9–3·8), and the proportion of women with less than 200 CD4+ cells/mm^3 was slightly and not significantly higher among HPV-positive (47·1%) than negative women (37·2%).

INTRODUCTION

Recent epidemiologic investigations using polymerase chain reaction (PCR) techniques have confirmed the role of some types of human papillomaviruses (HPV), chiefly 16 and 18, in the aetiology of squamous intraepithelial lesions (SIL) and cervical cancer [1, 2]. HPV presence largely accounts for the long known association of various indicators of sexual activity

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(e.g. high number of sexual partners, early age at first intercourse) and cervical cancer risk [1, 2]).

Among women seropositive for human immunodeficiency virus (HIV) antibodies, an increased prevalence of HPV DNA in the uterine cervix has been attributed to the immunosuppressive role of HIV, although the relationships between these two viral infections are still not fully understood [3]. The association between HIV and HPV infections may be confounded by sexual promiscuity, and there is some debate on which HPV types are able to produce SIL and cancer of the cervix in immunodepressed women [4].

To contribute to such debate, we analysed data concerning Italian women at risk for HIV infection enrolled in a prospective study on cervical HPV infection.

METHODS

Study population

The study group was 236 sexually active women at risk for HIV infection who attended, between June 1994 and July 1995, a network of 16 clinical centres in Italy. Details of the study methods have been previously reported [5]. Briefly, these women had either made use of intravenous drugs (and they were classified, in this study, as intravenous drug users (IVDU)) or were sexual partners of IVDU or of other men with or at risk for, HIV infection (and they were classified as heterosexuals). Trained interviewers used a standard questionnaire to elicit information on: sociodemographic characteristics, smoking and sexual habits (i.e. age at first intercourse and lifetime number of sexual partners), reproductive history (including use of various contraceptive methods), and history of gynaecological and sexually transmitted diseases. All women underwent HIV serological testing (and Western blot confirmation if needed), gynaecological examination and a cervico-vaginal smear. Further information on clinical and immunological status of HIV-positive participants were also collected.

HPV analysis

Cervico-vaginal samples were obtained from each woman using Cytobrush from the endocervix. Samples smeared on glass slides were fixed with Cytofix(R) (Cellpath). Cell smears were scraped from the glass slides and digested with 100 μl of lysis buffer (50 mM KCl, 10 mM Tris-HCl, pH 8·3, 1·5 mM MgC12, 0·45% NP40, 0·45% Twen 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 human mitochondrial DNA sequences, according to Stevenson and colleagues [6]. When mitochondrial/PCR was negative, the amplification was repeated, using as template DNA extracted from cell lysate by phenol-chloroform method. In cell lysates or DNA samples, the presence of HPV DNA was assessed by PCR using degenerate primers, MY09 and MY11, for the L1 region [7, 8], as previously described [9]. The efficiency of our HPV detection systems was assessed by determining the presence of HPV DNA in serial dilutions of Hela cells (this cell line contains 10–50 HPV DNA copies/cell) additioned with aliquots of HPV-negative endocervical material of an healthy woman. The end-point PCR positivity for HPV DNA was obtained in samples containing three Hela cells with a range of HPV detection between 30 and 150 HPV copies.

To type HPV, an aliquot of MY09/MY11 PCR product of positive specimens was digested with *Bam*HI, *Ddel*, *Hae*III, *Pst*I, *Rsa*l and *Sau*3aI. Digested products were electrophoretically separated on 8% acrylamide gel in the presence of Φx DNA cut *Hae*III as size marker. The HPV types were identified by means of the fragment length polymorphism (RFLP) analysis, and they were grouped into high (i.e. 16, 18, 31, 33, 35, 53, 58), low (i.e. 11, 44, 46, 54, 59, 66) or undetermined (i.e. CP6108 and CP8304) oncogenic risk types [10].

Statistical methods

HPV-positive and HPV-negative women were compared with respect to their socio-demographic, behavioural, immunological characteristics and HIV-status by means of odds ratios (ORs) and corresponding 95% confidence intervals (CIs) [11]. CD4+ cell count was assessed within 3 months of DNA HPV measurement. Unconditional multiple logistic regression (MLR) analysis was performed in order to assess the independent role played by each factor identified as relevant in the univariate analysis [11].

RESULTS

Of the 236 women included into the study, 86 (36·4%) turned out to be positive for the PCR determination of cervical HPV DNA. SIL was diagnosed in 57 of these 236 women (24·1%): cervical HPV DNA was

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Table 1. *Distribution of 236 women according to cervical-HPV-DNA status and selected characteristics. Italy, 1994–6*

	HPV positive ($n = 86$)‡	HPV negative ($n = 150$)‡	OR (95% CI)*	MLR–OR (95% CI)†
Age (yr)				
17–29	47 (55·3)	69 (46·3)	1·0	1·0
30–45	38 (44·7)	80 (53·7)	0·70 (0·39–1·23)	0·80 (0·45–1·41)
Education (yr)				
1–8	28 (32·6)	53 (35·8)	1·0	1·0
⩾ 9	58 (67·4)	95 (64·2)	1·16 (0·63–2·11)	1·24 (0·66–2·31)
Number of cigarettes/day				
0	7 (8·1)	26 (17·3)	1·0	1·0
1–19	51 (59·3)	86 (57·3)	2·20 (0·83–6·04)	1·15 (0·41–3·27)
⩾ 20	28 (32·6)	38 (25·3)	2·74 (0·95–8·10)	1·14 (0·35–3·70)
Age at first intercourse (yr)				
⩾ 17	35 (40·7)	77 (51·3)	1·0	1·0
⩽ 16	51 (59·3)	73 (48·7)	1·54 (0·87–2·72)	1·36 (0·74–2·52)
Lifetime number of sexual partners				
1	5 (5·8)	18 (12·0)	1·0	1·0
2–4	25 (29·1)	60 (40·0)	1·50 (0·45–5·22)	1·07 (0·33–3·44)
5–9	21 (24·4)	28 (18·7)	2·70 (0·77–9·96)	1·38 (0·39–4·90)
10–19	16 (18·6)	23 (15·3)	2·50 (0·68–9·67)	1·45 (0·39–5·39)
⩾ 20	19 (22·1)	21 (14·0)	3·26 (0·89–12·5)	1·58 (0·42–5·92)
Lifetime condom use				
Never	35 (40·7)	65 (43·3)	1·0	1·0
Ever	51 (54·3)	85 (56·7)	1·11 (0·63–1·98)	1·30 (0·71–2·36)
Lifetime use of oral contraceptives				
Never	47 (54·6)	78 (52·0)	1·0	1·0
Ever	39 (45·3)	72 (48·0)	0·90 (0·51–1·58)	0·86 (0·49–1·52)
Parity				
0	60 (69·8)	87 (58·0)	1·0	1·0
1	13 (15·1)	44 (29·3)	0·43 (0·20–0·91)	0·43 (0·20–0·93)
> 1	13 (15·1)	19 (12·7)	0·99 (0·42–2·31)	1·36 (0·56–3·31)
HIV exposure category				
Heterosexuals	29 (33·7)	94 (62·7)	1·0	1·0
IVDU	57 (66·3)	56 (37·3)	3·30 (1·82–5·99)	2·68 (1·43–5·03)
HIV infection				
No	32 (37·2)	69 (46·0)	1·0	1·0
Yes	54 (62·8)	81 (54·0)	1·44 (0·81–2·57)	1·89 (0·93–3·82)
No. of CD4+ cells/mm^3§				
⩾ 200	27 (52·9)	49 (62·8)	1·0	1·0
⩽ 199	24 (47·1)	29 (37·2)	1·50 (0·69–3·28)	1·47 (0·68–3·18)

* Crude odds ratios and 95% confidence intervals (CI).

† Multiple logistic regression (MLR) OR and 95% CI, adjusted for age, number of cigarettes per day, number of lifetime partners and HIV exposure category.

‡ In some items, the sum does not add up to the total because of missing values.

§ It includes only 135 HIV infected women.

found in 72% of women with SIL and in 25% of those without SIL.

HPV-positive women appeared to be younger (median age, 29 years) and better educated (median number of years of education, 13) than HPV-negative women, but the difference did not attain statistical significance (Table 1). The apparent higher proportion of heavy smokers (20 or more cigarettes per day) among HPV-infected women (32·6% against 25·3% among HPV-negative ones) was accounted for by confounding factors (MLR–OR; 1·1, 95% CI: 0·4–3·7). A significant trend of increasing risk with increasing number of lifetime sexual partners was found (x^2 for trend = 6·26, $P = 0·01$), but such

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Table 2. *Ods ratios* for the presence of cervical HPV DNA according to HIV infection and HIV-exposure category*

	HIV infection		
	Negative	Positive	All
HIV exposure			
IVDU−	1	2·0 (0·82–4·86)	1
IVDU+	3·68 (1·43–9·49)	4·39 (1·83–10·53)	2·68 (1·47–4·86)
All	1 (0·81–2·89)	1·52	

* Multiple logistic regression (MLR) OR and 95% CI, adjusted for age, number of cigarettes per day, number of lifetime partners and, when appropriate, HIV exposure category or HIV infection.

difference was attenuated in multivariate analysis (MLR–OR for women with 20 or more partners Vs 1: 1·6, 95% CI: 0·4–5·9). HPV infection was not associated with the use of condom, or oral contraceptives nor by parity (Table 1).

After allowance for confounding factors, HPV-positive women were nearly twofold likelier to be HIV seropositive than HPV negative ones (62·8% and 54·0%, respectively, were positive for HIV antibodies) (MLR–OR: 1·9, 95% CI: 0·9–3·8). HIV exposure category was a significant determinant of HPV DNA presence: IVDU women had a nearly threefold higher risk of being infected with HPV, as compared to heterosexual women (MLR–OR: 2·7, 95% CI: 1·4–5·0) (Table 1). The proportion of women with less than 200 CD4+ cells/mm^3 was slightly and not significantly higher among HPV-positive (47·1%) than negative ones (37·2%) (MLR–OR: 1·5, 95% CI: 0·7–3·2) ones (Table 1).

Among the 135 HIV-infected women, the combined effect of being IVDU and of HIV infection on the risk of cervical HPV infection was also assessed (Table 2). IVDU women were at significantly increased risk of showing cervical HPV infection even if they were not seropositive for HIV infection (MLR–OR = 3·7, 95% CI: 1·4–9·5). The joint effect of being IVDU and HIV seropositive was, however, substantially less than multiplicative (Table 2).

Risk factors for the cervical presence of HPV DNA were also assessed according to high and low-risk HPV subtypes. Women under 30 years of age had a twofold higher risk of being infected with the high-risk subtypes (OR = 2·0, 95% CI: 1·1–3·9) than older women, while they were at a non-significantly reduced risk of infection with the low-risk subtypes (OR = 0·6, 95% CI: 0·3–1·4). No differences were found with respect to the other factors under investigation, including HIV serostatus, HIV-exposure category and the number of CD4+ cells (data not shown in tables).

DISCUSSION

In the last few years, several studies, based on molecular methods, have reported an increased frequency of ano-genital HPV infection among HIV-infected women [12–16]. Although the results from these investigations consistently indicate a positive association between HIV and HPV infections, some uncertainty remains. Most notably, it is not clear whether the increase in ano-genital HPV infection observed in HIV-positive women is a direct result of HIV-induced immunosuppression or it reflects an increased exposure to HPV due to high risk sexual behaviour.

In agreement with the results from a hybridization technique study conducted among Italian women not at risk for HIV infection [17], in this study 36% of women at risk for HIV infection were positive for cervical HPV DNA. Being IVDU turned out to be the strongest determinant of cervical HPV infection, since IVDU women had a nearly threefold higher risk of being HPV positive than heterosexuals. Sexual promiscuity of IVDU women, among whom a high prevalence of prostitution and a very low frequency of condom use has been reported [18, 19], is likely to account for high risk of HPV and, to some extent, even of HIV exposure. This finding was in accordance with data from Italy showing that IVDU women with AIDS had a risk of invasive cervical cancer (ICC) twice as higher than among heterosexuals [20].

Also in agreement with previous reports [12–16], women with cervical HPV infection were more likely to be infected with HIV than HPV-negative women. The magnitude of the increased OR (OR = 1·9) was similar to that reported by Sun and colleagues [15], but, due to the smaller number of women included in this study, the difference was not significant.

As concerns the role of immunosuppression, it should be noted that some studies have found an increasing frequency of cervical HPV-infection with decreasing levels of CD4+ cell count [10, 14, 21, 22]. A particularly increased effect of immunosuppression on clinically expressed, compared to latent, HPV infections has been observed [15]. In this investigation,

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a reduced number of CD4+ cells/mm^3 was marginally associated with a higher prevalence of HPV infection.

Women with cervical HPV infection were more likely to have a younger age at first intercourse, and to have reported a higher number of lifetime sexual partners than HPV-negative ones. Such differences were attenuated by multiple logistic regression analysis, but the small number of cases prevents further considerations.

In conclusion, our study highlighted the high prevalence of HPV cervical infection in women at high risk for, or with, HIV infection, particularly among IVDU. High prevalence of HPV cervical infection even in women who lacked evidence of SIL suggests that they are, anyhow, at increased risk of cervical neoplasia and should be monitored carefully.

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