Original
article

HIV and human papillomavirus as independent risk factors for cervical neoplasia in women with high or low numbers of sex partners

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Objective: To explore whether HIV types 1 and 2 and CD4 cell count affect cervical neoplasia independent of human papillomavirus (HPV) in women with high or low numbers of sexual partners residing in Abidjan, Côte d'Ivoire.

Methods: The study population and methods are described in the companion paper. Additional methods include a Papanicolaou smear for cytological diagnosis and statistical analysis.

Results: In maternal women, both HIV-1 and high risk HPV were significant independent risk factors for squamous intraepithelial lesions (SIL) (adjusted odds ratio (OR) 11.0 (95% CI 1.1–112) and 5.4 (1.5–18.8), respectively). Only high levels of HPV DNA in the lavage were associated with SIL (OR 13.2 (3.6–47.8)) in the maternal group. In female sex workers, high risk HPV was significantly associated with SIL (OR 23.7 (4.4–126)); HIV seropositivity was not. Any positive level (high or low amounts) of HPV DNA was significantly associated with SIL in sex workers (ORs 15.9 (3.3–76) and 12.7 (3.6–44), respectively). There was no association of SIL with CD4 cell counts $\leq 500 \times 10^6/l$ in HIV seropositive women from either group.

Conclusion: HPV or HIV-1 infection independently affect cervical neoplasia in women with low numbers of sex partners.

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Keywords: HIV; human papillomavirus; cervical neoplasia

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Introduction

Increased rates of preinvasive cervical neoplasia have been noted repeatedly in women infected with HIV compared with uninfected controls.^{1,2} Most studies linking HIV infection to human papillomavirus (HPV) and cervical neoplasia have focused on populations of women with high risk sexual behaviours. Since sexual practices are strongly associated with both cervical disease and HIV transmission, it is difficult to determine if the association is due to shared risk factors or to a biological effect.³ In our companion paper, we observed different associations between HIV and HPV that depended on numbers of sex partners and CD4 cell count.⁴ Here we examine whether HIV and HPV can be independent risk factors for cervical neoplasia.

Methods

STUDY POPULATIONS AND METHODS

The study populations and methods were described in the companion article.⁴

CERVICAL CYTOPATHOLOGY

Papanicolaou smear results were reported by using the Bethesda system.⁵ For data analysis, low grade and high grade squamous intraepithelial lesions (SIL) were grouped as SIL. Inflammatory and reactive changes were classified with normal smears. Unsatisfactory samples were excluded from the analysis.

DATA ANALYSIS

For evaluation of the association between HIV and SIL, women were classified into groups

according to their serostatus (negative, HIV-1, HIV-2, or HIV-D). The odds of SIL were estimated for each HIV exposure group relative to HIV negative women. A sample was considered HPV positive when the relative light unit (RLU) ratio (RLU of sample/mean RLU of three positive controls (PC)) was ≥ 1 .⁶ To determine if SIL was associated with HPV viral load, we grouped samples with positive high risk results as either 1–20 RLU/PC or >20 RLU/PC.

Results

ABNORMAL CERVICAL CYTOLOGY RATES

In the maternal group, 10/56 (18%, $p < 0.001$) HIV-1, 4/55 (7%, $p = 0.02$) HIV-2, 3/21 (14%, $p < 0.001$) HIV-D, and 1/119 (0.08%, reference) seronegative women had SIL. By contrast, SIL rates among sex workers did not differ significantly. Among sex workers, 11/109 (10%, $p = 0.14$) HIV-1, 5/63 HIV-D (8%, $p = 0.4$), and 3/68 (4%, reference) seronegative women had SIL. Abnormal Pap smears interpreted as atypical squamous cells of undetermined significance (ASCUS) were not significantly different between HIV seropositive and HIV seronegative women in either group (data not shown).

ASSOCIATION OF HIV SEROSTATUS AND HPV WITH SIL AND ASCUS

The association of HIV serostatus or HPV with SIL is shown in table 1. In the maternal women, both HIV-1 and high risk HPV DNA detection were significantly and independently

Table 1 Association of SIL* with HIV types and HPV in maternal women and female sex workers

	Maternal women		Female sex workers	
	Freq (%)	OR† (95% CI)	Freq (%)	OR† (95% CI)
HIV-1	10/48 (21)	11.0 (1.1–112.0)	11/91 (12)	0.6 (0.1–5.0)
HIV-2	4/51 (8)	3.9 (0.4–40.0)	0	—
HIV-D	3/17 (18)	7.1 (0.6–9.4)	5/57 (9)	0.4 (0.1–1.4)
HIV-neg	1/107 (1)	Reference	3/60 (5)	Reference
High risk HPV	14/62 (23)	5.4 (1.5–18.8)	16/64 (25)	23.7 (4.4–126.0)
Low risk HPV	0/14 (0)	—	1/15 (7)	4.4 (0.3–57.0)
Negative HPV	4/146 (3)	Reference	3/155 (2)	Reference

*SIL versus normal (ASCUS excluded).
†OR were adjusted for HIV type, HPV, age, number of lifetime sex partners (maternal group), smoking (sex workers), and education.
— No SIL present for reference or variable value.

Table 2 Association of SIL with high risk HPV DNA level

HPV DNA (RLU ratio)	Maternal women		Female sex workers	
	OR* (95% CI)	p Value	OR* (95% CI)	p Value
1–20	1.4 (0.2–8.3)	0.7	12.7 (3.6–44.0)	0.0001
>20	13.2 (3.6–47.8)	0.0001	15.9 (3.3–76.0)	0.0005

*Controlling for HIV-1, HIV-2, HIV-D.

associated with SIL. In female sex workers, only high risk HPV detection was significantly associated with SIL. In the maternal women, ASCUS was not significantly associated with any of the variables examined (data not shown). In the female sex workers, high risk HPV detection was significantly associated with ASCUS (OR 2.9 (1.1–7.3)).

LOW CD4 COUNTS AND SIL

To determine if low CD4 counts were associated with SIL, we did a stratified univariate analysis of HIV-1 seropositive women. Among HIV-1 seropositive maternal women, there was no association of SIL with CD4 counts $\leq 500 \times 10^6/l$ (OR 2.5 (0.4–16)). Similarly, no association of SIL with CD4 cell counts $\leq 500 \times 10^6/l$ was detected among the HIV-1 seropositive sex workers (OR 2.5 (0.5–18)).

HIGH RISK HPV DNA LEVELS

We determined if the level of high risk HPV DNA had an effect on SIL (table 2). Only high levels of HPV DNA were found to be significantly associated with SIL in maternal women (OR 13.2 (3.6–47.8)). Among sex workers, both low (1–20) and high (>20) levels of HPV DNA were significantly associated with SIL (ORs 12.7 (3.6–44), 15.9 (3.3–76) respectively).

Discussion

We examined whether HIV serostatus and HPV could be independent risk factors for cervical neoplasia in women with high or low numbers of sexual partners. We found that HIV-1 could be an independent risk factor for SIL in the maternal women but not in the sex workers. Why HIV-1 was a risk factor in the maternal women but not in the sex workers is not clear. Smith *et al*⁷ studied a high risk population and found that in the absence of immunosuppression there was no difference in the rate of SIL or HPV between HIV seropositive and seronegative women. Only one other study has examined the relation between HIV

infection and cervical disease in a low risk population.⁸ These investigators observed a significant relation between HIV infection and cervical disease but did not measure HPV or any other risk factor associated with cervical disease. HIV serostatus in the maternal women could be a surrogate marker for a factor(s) of cervical disease that we did not measure.

We did not find HIV-2 seropositivity in maternal women to be associated with SIL in multivariate analysis. One other group has studied HIV-2 and cervical neoplasia in high risk African women and found that SIL was associated with HIV-2 infection.^{9 10} In their analysis, ORs were adjusted for number of sex partners per week and study site, but not for HPV infection. Even though HIV-1 and HIV-2 are both sexually transmitted, the epidemiology of HIV-2 is different. Slower heterosexual spread, lower infectivity and lower HIV-2 viral load compared with HIV-1¹⁰ may be related to the lack of a measurable effect of HIV-2 on cervical neoplasia in our study.

The strong association between SIL and high risk HPV DNA in both groups was expected. It was surprising, however, to find that SIL was associated with different amounts HPV DNA detected in these two groups. The amount of HPV DNA may vary depending on a number of factors specific to the population such as sexual behaviour,⁴ or the specific HPV type(s) that predominate in a population.¹¹ This indicates the difficulty in using HPV quantitation for distinguishing clinically significant HPV infections.

Most studies attribute the increased rates of HPV associated cervical disease in HIV infected women to immunosuppression. In the results reported here, immunosuppression as measured by CD4 cell counts of $\leq 500 \times 10^6/l$ was not associated with SIL in either group. Admittedly, very few women had very low CD4 cell counts, and this may be one explanation for the relatively low rates of SIL observed in both groups. However, if we analysed SIL among only the HIV-1 seropositive women with CD4 $\leq 500 \times 10^6/l$, low CD4 counts did not account for all the SIL detected in HIV-1 seropositive women from either group. It may be that in the absence of significant immunosuppression, HIV alters pathogenesis of HPV associated cervical disease.¹²

The observation that HIV-1 seropositivity is an independent risk factor for SIL in women with low numbers of sexual partners needs to be confirmed. Our study is limited by differences inherent in these two groups and factors we did not measure, such as diet and nutritional status. Despite these limitations, it is intriguing to once again observe that HIV-1 may directly or indirectly alter pathogenesis of cervical disease.

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All study participants were volunteers who gave informed consent. Complete study protocols were approved by the

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Contributors: SD Vernon, ER Unger, and WC Reeves conceived the study and together with SZ Wiktor and AE Greenberg, implemented the study design and carried it out. ST Severin and PD Ghys ran the clinics and cared for the women. IR Horowitz provided gynaecological training. ER Unger participated in pathology assessments. L Miller provided laboratory expertise and performed HPV tests. MA Piper performed statistical analysis. SD Vernon and ER Unger co-wrote the paper.

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