Int. J. Cancer: **50,** 45–48 (1992)

Publication of the International Union Against Cancer
Publication de l'Union Internationale Contre le Cancer

GENITAL PAPILLOMAVIRUS INFECTION AND CERVICAL DYSPLASIA—OPPORTUNISTIC COMPLICATIONS OF HIV INFECTION

Marie LAGA[1,5], Joseph P. ICENOGLE[1], Richard MARSELLA[2], Abib Thiam MANOKA[3], Nzilambi NZILA[3], Robert W. RYDER[1,3], Sten H. VERMUND[4], William L. HEYWARD[1,3], Ann NELSON[2] and William C. REEVES[1,6]

[1]*Center for Infectious Diseases, Centers for Disease Control, Atlanta, GA;* [2]*Armed Forces Institute of Pathology, Washington, DC;* [3]*Projet SIDA, Department of Public Health, Kinshasa, Zaire; and* [4]*Epidemiology Branch, Division of AIDS, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, USA.*

Certain human genital papillomaviruses (HPV) are strongly associated with cervical dysplasia and cancer. Evidence is accumulating that HPV infection and ano-genital cancers are more common in patients with the acquired immunodeficiency syndrome. The objective of our study was to evaluate the extent to which HPV infection and associated cervical disease constitute opportunistic complications of human immunodeficiency virus (HIV) infection in a population of sexually promiscuous, HIV-infected women in Kinshasa, Zaire. In 1989 we obtained Pap smears and cervicovaginal lavage specimens for HPV DNA testing from 47 HIV-seropositive and 48 HIV-seronegative prostitutes who were part of a cohort under observation since 1988. Thirty-eight percent of the HIV-seropositive and 8% of the seronegative women (odds ratio = 6.8; $p = 0.001$) had HPV DNA detected by either ViraType, a dot-blot assay which detects specific genital HPV types, or low-stringency Southern blot, which detects all HPV types. Eighty-two women (86%) had an interpretable Pap smear; 11 of 41 (27%) HIV-seropositive women and one of 41 (3%) seronegative women had cervical intra-epithelial neoplasia (CIN) (odds ratio = 14.7; $p = 0.002$). HIV seropositivity, HPV infection and CIN were highly associated. Eight (73%) of 11 seropositive women with CIN had HPV detected. Both HPV infection and cervical cancer may emerge as opportunistic complications of HIV infection in populations in which HIV, HPV and cervical cancer are common.

Cervical cancer is one of the most common cancers throughout the developing world and in lower socio-economic groups in the United States (Peto, 1986). Age-specific incidence rates in these populations are highest among young women and often exceed one per 1,000 in 25- to 45-year-olds (Waterhouse *et al.,* 1982). A strong association between infection with human papillomavirus (HPV) types 16 and 18 and development of cervical cancer has been documented (Reeves *et al.,* 1989*a*,*b*; Koutsky *et al.,* 1988). Other risk factors associated with cervical cancer include diet (Schneider and Shah, 1989), smoking (Herrero *et al.,* 1989), pregnancy (Brinton *et al.,* 1989), use of hormonal contraceptives (Hildesheim *et al.,* 1990) and infection with other sexually transmitted agents (Herrero *et al.,* 1990).

Evidence suggests that HPV, cervical intra-epithelial neoplasia (CIN), and cervical cancer may be opportunistic complications of human immunodeficiency virus (HIV) infection. A series of publications have reported that women with HIV infection have an increased prevalence of HPV and CIN (Bradbeer, 1987; Spurrett *et al.,* 1988; Maiman *et al.,* 1988; Provencher *et al.,* 1988; Schrager *et al.,* 1989; Byrne *et al.,* 1989; CDC, 1990; Maiman *et al.,* 1990; Feingold *et al.,* 1990). However, these preliminary studies have been based on selected patients attending clinics specializing in HIV, sexually transmitted diseases or other diseases. Most of the studies have been uncontrolled or have enrolled HIV-seronegative "controls" who were not comparable to HIV-seropositive cases in terms of sexual behavior. Lack of appropriate controls precludes estimating strengths of association and limits the ability to draw conclusions from most of these studies. For these reasons, we evaluated the associations between HPV infection and cervical disease, and HIV infection among prostitutes in Kinshasa, Zaire.

MATERIAL AND METHODS

Study population

Subjects were from a cohort of prostitutes who resided in Kinshasa, Zaire and were enrolled in the Project SIDA Women's Health Center (Nzila *et al.,* 1991). This Health Center is located in Matonge, the center for transportation and night-life in Kinshasa, Zaire's capital city of 4 million inhabitants. The Health Center was established in 1988 to evaluate the prevalence, incidence and associated risk factors of HIV infection and other sexually transmitted diseases in female prostitutes and implement interventions aimed at reducing high-risk behavior. One thousand two hundred women from throughout the Matonge district, who defined themselves as prostitutes, were initially interviewed and examined for sexually transmitted diseases and HIV serology. Six hundred fifty (450 HIV-seronegative and 150 HIV-seropositive) women from the original group have been followed for a mean duration of 18 months and comprise the Health Center cohort. Women in the cohort have been examined monthly for sexually transmitted diseases and signs of AIDS (WHO clinical definition) and have received free health care. In August 1989 we enrolled 47 HIV-seropositive and 48-seronegative women in order of their arrival for routine monthly appointments. In addition to conducting the routine health examinations and obtaining specimens for ongoing sexually transmitted disease studies, health center staff collected specimens for cervical cytopathology and HPV DNA detection.

HIV serology

Sera for HIV serology were tested twice in an enzyme-linked immunosorbent assay (ELISA) (Organon Vironostica, Geneva, Switzerland). Sera positive in ELISA were confirmed by Western blot (DuPont de Nemours, Wilmington, DE). A Western blot was considered positive if 2 of the following bands were found: p 24, gp 41, or gp 120/160.

Cervical cytopathology

The endocervix was sampled with a cytobrush and the exocervix with an Ayre spatula. Each specimen was smeared on a slide, fixed, and stained by standard methods. Cervical cytopathology was read and interpreted at the Armed Forces Institute of Pathology, Washington, DC. Cytopathologic interpretation was accomplished without knowledge of subjects' HPV or HIV status.

[5]Current address: Institute of Tropical Medicine, Antwerp, Belgium.

[6]To whom correspondence and reprint requests should be sent at the Viral Exanthems and Herpesvirus Branch, G-18, Division of Viral and Rickettsial Diseases, Centers for Disease Control, Atlanta, GA 30333.

Received: June 17, 1991 and in revised form July 24, 1991.

HPV DNA detection

Specimens for HPV DNA testing were collected by cervicovaginal lavage with normal saline (Vermund *et al.,* 1989). All specimens were tested for HPV types 6 and 11, 16 and 18, 31, 33 and 35 DNA by ViraType kits (Life Technologies, Gaithersburg, MD) according to manufacturer's instructions. In addition, total DNA was extracted from specimens, digested with *Pst* I, and blotted according to Southern (1975). HPV DNA on blots was detected by low-stringency hybridization (Tm-30 C) using a mixture of ^{32}P-labelled HPV types 6, 11, 16, 18, 31, 33, and 35 DNA probes (Oncor, Gaithersburg, MD). The Southern blot assay under low-stringency conditions should detect all HPV types, not just those represented by the probes. All laboratory testing and interpretation was done "blind" with respect to clinical/epidemiologic data and previous laboratory test results.

Statistical methods

Statistical significance was determined by Chi-square or Fisher's exact tests. The odds ratio (OR) and 95% confidence interval (95% CI) were calculated to measure the association between HPV infection/disease and HIV infection (Fleiss, 1981).

RESULTS

Table I summarizes selected demographic and behavioral variables and prevalence of sexually transmitted diseases at time of enrollment of the study population. The 95 subjects enrolled in our study did not differ in these characteristics from the overall Health Center cohort. HIV-seropositive and seronegative women enrolled in this study did not differ significantly from each other in these characteristics.

Overall, 23% of the population had HPV DNA detected by either ViraType or Southern blotting. HPV DNA detection rates were significantly higher in HIV-seropositive women (38%) than in those who were seronegative (8%) (Table II). Thirteen women had HPV DNA detected by ViraType. Two samples reacted with the 6/11 probe, 8 with the 16/18, and 9 with the 31/33/35; 7 samples reacted with more than one probe. An additional 9 women were infected with other HPV types detected only by Southern blot. All specimens with HPV DNA detected by the ViraType dot-blot assay were also positive by Southern blotting and gave restriction patterns consistent with the ViraType results (2 HIV-seropositive, ViraType-positive women had insufficient material to test by Southern blotting).

TABLE I – SELECTED DEMOGRAPHIC/BEHAVIORAL CHARACTERISTICS, AND PREVALENCE OF SEXUALLY TRANSMITTED DISEASES IN 95 PROSTITUTES, KINSHASA, ZAIRE

Characteristic	HIV-seropositive n = 47		HIV-seronegative n = 48	
	Mean	SD	Mean	SD
Age (yrs)	26.7	(6.9)	26.2	(8.4)
Number of pregnancies	2.8	(1.9)	3.0	(2.9)
Age at first intercourse	14.8	(2.4)	15.0	(1.8)
Number of sexual partners per week	10.0	(9.5)	10.4	(14)
Experience as prostitute (months)	60.7	(49)	50.7	(68)

Prevalence at enrollment			*p* value
N. gonorrhoeae	26%	15%	0.2
C. trachomatis	9%	6%	0.7
T. vaginalis	25%	10%	0.07
Genital ulcers	10%	4%	0.2

SD, standard deviation.

TABLE II – GENITAL HPV INFECTION AND CERVICAL DYSPLASIA IN KINSHASA PROSTITUTES

Assay	HIV-seropositive number positive/ number tested (%)		HIV-seronegative number positive/ number tested (%)		OR 95% CI
HPV-positive Any assay	18/47	(38)	4/48	(8)	6.8 (1.9–26.8) $p = 0.001$
ViraType[1]	12/47	(26)	1/48	(2)	16.1 (2.0–97.5) $p = 0.002$
Cervical dysplasia[2]	11/41	(27)	1/41	(3)	14.7 (1.8–95.3) $p = 0.002$

[1]All specimens positive in the ViraType assay were also positive by Southern blot, except in the case of HIV-seropositive, Vira-Type-positive women who did not have sufficient material for testing by Southern blot.–[2]Eighty-two women had interpretable Pap smears.

TABLE III – ASSOCIATION OF CERVICAL CYTOPATHOLOGY WITH HPV DNA IN HIV-SEROPOSITIVE AND -SERONEGATIVE KINSHASA PROSTITUTES

Subjects	Cytologic diagnosis		
	CIN	No CIN	Total
HIV-positive			
HPV-positive	8	9	17
HPV-negative	3	21	24
HIV-negative			
HPV-positive	0	4	4
HPV-negative	1	36	37

We obtained Pap smears from all subjects; 13 were inadequate for interpretation. Six inadequate Pap smears were from HIV-seropositive and 7 from seronegative subjects. Smears from 12 women showed dysplastic changes (indicating cervical intra-epithelial neoplasia, or CIN): 9 were CIN-I, 1 was CIN-II, and 2 were CIN-III. HIV infection, HPV infection and CIN were highly associated (Tables II, III). Eleven (27%) of 41 women with HIV antibody had CIN compared to only 1 (3%) of 41 seronegative subjects (odds ratio = 14.7; Fisher's exact test $p = 0.002$). Eight (73%) of 11 HIV-positive women who had CIN also had HPV DNA detected compared to 9 (30%) of 30 with no CIN (odds ratio = 6.2; Fisher's exact test $p = 0.02$).

HPV-positive women were slightly younger, had less experience as prostitutes, and had slightly fewer sexual partners per week (Table IV). However, with this small sample size, no statistically significant associations between HPV infection, HIV infection, or CIN and these demographic and behavioral factors could be detected.

DISCUSSION

Our study clearly documents a significant association between HPV infection and CIN with HIV seropositivity in prostitutes in Zaire. Only 7% of HIV-seropositive prostitutes had signs and symptoms suggestive of clinical AIDS (WHO clinical definition). Since the sample size was small and most women were asymptomatic, we could not analyze the occurrence of HPV according to degree of immunosuppression. This study in Zaire differs from most previous studies because it enrolled well-matched HIV-negative controls, and the sample population was representative of inner city prostitutes. We also assayed HPV by sensitive and specific biochemical methods whereas most previous studies have estimated HPV infection based on cervical cytology.

Similar associations of CIN with HIV infection have been reported in the following locations: London, where 6 of 19 (32%) HIV-seropositive women attending a clinic for sexually transmitted disease had CIN (no controls were enrolled) (Byrne *et al.,* 1989); Miami, where 24% of HIV-seropositive and 1% of HIV-seronegative patients had CIN (Provencher *et*

TABLE IV – COMPARISON OF SELECTED EPIDEMIOLOGIC VARIABLES IN 22 HPV-POSITIVE AND 73 HPV-NEGATIVE KINSHASA PROSTITUTES

Characteristics	HPV-Positive Mean SD	HPV-Negative Mean SD	*p* value
Age	24.1 (4.8)	26.8 (8.0)	0.3
Experience as prostitute (months)	43.1 (37.9)	56.8 (62.1)	0.4
Number of sexual partners per week	5.9 (3.7)	10.8 (12.7)	0.2
Age at first intercourse	15.2 (1.5)	14.9 (2.1)	0.6

SD, standard deviation.

al., 1988); and New York, where 31% of HIV-seropositive and 4% of seronegative women had cervical or vaginal CIN (Schrager *et al.,* 1989). Another study in New York (Maiman *et al.,* 1990) found that 19% of women with biopsy-proven cervical cancer were HIV-seropositive and had more aggressive disease than those who were seronegative. The study also reported that HIV infection was common in women with abnormal Pap smears and, again, seropositive women had more severe premalignant disease. None of these studies measured HPV DNA; they estimated HPV infection based on cervical cytology and found that 18% to 26% of HIV-seropositive and 2% to 4% of seronegative women had changes consistent with HPV infection. Cytologic evidence of HPV infection is highly subjective, with koilocytotic atypia being the only pathognomonic finding on Pap smears. HPV infection which does not result in "disease" is routinely classified as negative in Pap smear reports.

A study from New York (Feingold *et al.,* 1990) evaluated 67 women undergoing routine gynecologic examinations and demonstrated significant associations between HIV and HPV (identified by Southern blot assays of cervico-vaginal lavages), and cervical dysplasia. Eleven of 22 women with symptomatic HIV infections had CIN, and 10 of these 11 women were infected with HPV. Only 6 of 45 (13%) asymptomatic HIV-seropositive or HIV-seronegative women had such lesions. A continuation of the study with 96 women demonstrated the highest risk for squamous intra-epithelial lesions among those women with symptomatic HIV infection (Vermund *et al.,* 1991). Taken in aggregate, our findings in Africa and those from other geographic locations indicate that women with HIV infection have an increased risk of HPV detection and CIN and should be followed closely because the natural history of progression to cancer may be greatly accelerated. It will be important to design appropriate studies to determine if this merely reflects lack of immunologic control over HPV replication, due to HIV-associated immunosuppression, or if it reflects molecular interaction between the viruses.

Our study also suggests that HPV types not detected by the usual probes are common in Kinshasa and perhaps in other African populations. Twelve women had HPV detected with commercially available probes. Ten others had virus detected only by low-stringency Southern blotting. Restriction digest patterns (*Pst* I) consistent with types 45, 52 and 56 were seen. This finding is important both for epidemiologic studies of cervical cancer and for application of HPV DNA testing for patient evaluation or use in screening programs. The contribution of infection with HPV 45, 52 and 56 to cervical disease rates is presently unknown. The high level of infection supports a significant contribution for these types to overall cervical disease rates, even if the oncogenic potential of these types is lower than that of HPV 16, 18, 31, 33 or 35.

Prior to the AIDS epidemic, Central Africa had one of the world's highest cervical cancer rates; age-adjusted, annual incidence was estimated at 23.3 per 100,000 (Parkin *et al.,* 1988). Among women of childbearing age, annual cervical cancer risk would be expected to be about one per 1,000. Currently about 8% of women aged 18–44 years in this region are HIV-seropositive (Chin, 1990) and, according to our data, would be expected to have at least a 10-fold excess risk of HPV infection and cervical dysplasia. It could be inferred from these numbers that the current excess cervical cancer rate due to HIV infection is about 0.8 per 1,000. Such an increase in cervical cancer would have a significant impact on the health care systems in Central African countries. Adequate cervical cancer control programs do not exist in this area. If both HPV infection and cervical cancer emerge as opportunistic complications of HIV infection, mortality rates among young women may increase substantially. In addition, HIV-seropositive, sexually active women could act as foci for spreading HPV (and its associated diseases) in the general population. Since the mechanisms of HPV transmission are poorly understood (Jenison *et al.,* 1990), we cannot assume that the practises which control HIV transmission will also control HPV.

ACKNOWLEDGEMENTS

We are indebted to Ms. F. Behets and Mr C. Edidi, of Projet SIDA, and Ms. S.-B. Buckner of AFIP for technical and consultative assistance. We thank Ms. E. Lane, Ms. D. Miller and Ms. R.A. Tucker of the Centers for Disease Control for performing HPV assays. This study was partly funded by the American Foundation for AIDS Research, the European Economic Community and the Rockefeller Foundation. Use of trade names or commercial sources is for identification only and does not imply endorsement by the Public Health Service or the US Department of Health and Human Services.

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