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ASSOCIATION OF INFECTIONS WITH HUMAN IMMUNODEFICIENCY VIRUS AND HUMAN PAPILLOMAVIRUS IN HONDURAS

ANNABELLE FERRERA, WILLEM J. G. MELCHERS, JOHAN P. VELEMA, AND MANUEL FIGUEROA

Departamento de Microbiologia, Universidad Nacional Autonoma de Honduras, Tegucigalpa, Honduras;
Department of Medical Microbiology, University of Nijmegen, Nijmegen, The Netherlands;
Department of Public Health, Erasmus University, Rotterdam, The Netherlands

Abstract. The etiologic role of the oncogenic types of human papillomavirus (HPV) in the development of cervical cancer has been widely proven. Since this cancer occurs more frequently in immunosuppressed individuals, we sought to evaluate the prevalence of HPV infection among human immunodeficiency virus (HIV)–infected and HIV-noninfected prostitutes in Tegucigalpa, Honduras. Cervical scrapes were collected from 23 HIV-seropositive and 28 HIV-seronegative prostitutes for HPV DNA detection by the polymerase chain reaction. Fifty-six percent of the HIV-seropositive women and only 18% of the seronegative women were HPV DNA positive (odds ratio = 6.0). In addition, there was a significant association between seropositivity for HIV with a history of sexually transmitted diseases ($P < 0.01$). Our data confirm the association between infections with HIV and HPV.

Human immunodeficiency virus (HIV) is the causative agent of the acquired immunodeficiency syndrome (AIDS). Infection with the virus is followed over widely varying periods of time by progressive immunodeficiency and increasing rates of infectious diseases and neoplasms.

Honduras has the highest incidence of AIDS cases in Central America and up to January 1996, a total of 5,008 cases had been recorded, 65.7% males and 34.3% females.[1] Furthermore, the heterosexual spread of HIV in this country has occurred at an alarming rate during the past four years, with the proportion of cumulative AIDS cases attributable to heterosexual contact increasing from 77% of the total number of cases in 1990 to 88% in 1995.[1] This is even higher than that observed in the United States, Europe, and other Latin American countries.[2]

Of particular clinical significance are recent reports that women with HIV have a higher rate of cervical abnormalities, including human papillomavirus (HPV) infection, abnormal Pap smears, and squamous intraepithelial lesions (SILs).[3–5] This recognition of the importance of cervical disease in HIV infection has led the Centers for Disease Control and Prevention (Atlanta, GA) to include cervical cancer as an AIDS-defining illness in women.[6]

Epidemiologic and experimental data support the etiologic role of HPV in the development of cervical cancer.[7–9] The oncogenic HPV genotypes (mainly 16 and 18) have been detected in 80–90% of cervical intraepithelial neoplasia (CIN) grade 3 lesions and invasive cervical cancers. The prevalence of particular HPV types varies between different geographic regions.[10] In addition, CIN associated with genital HPV infection occurs with increased frequency and severity among persons with immunodeficiency[11] and intragenically immunosuppressed women after kidney transplantation.[12]

A number of studies have indicated that the prevalence of HPV infection and HPV-induced epithelial abnormalities is higher in HIV-seropositive women than in HIV-seronegative women.[13–15] Schrager and others[16] in their study determined that 31% of HIV-infected women had cytologic squamous atypia, compared with only 4% of HIV-seronegative women. Feingold and others[17] extended these observations, presenting molecular evidence of HPV infection in the cervicovaginal epithelium of women with HIV infection. They found that 49% of the HIV-infected women studied had HPV infections compared with 25% of a group of non-HIV-infected women. In addition, 40% of the HIV-infected women had SILs on cervical cytology, compared with 9% of the noninfected women. Recent reports have also described increased rates of both HPV and anal dysplasia or neoplasia in HIV-positive men.[18]

Considering the high prevalence of HIV infection and taking into account that carcinoma of the cervix is the most common type of cancer among women in Honduras, it is of utmost importance to the public health interest to gain a better understanding of the association between HIV and HPV with respect to the development of cervical cancer.[19] We are also not aware of any study that has addressed this problem in the Central American region. This investigation was designed to assess the possible association between HPV and HIV among prostitutes with and without HIV infections in Tegucigalpa, Honduras.

SUBJECTS AND METHODS

Study population. Between November 1994 and February 1995, 23 HIV-seropositive and 28 HIV-seronegative prostitutes attending the Las Crucitas outpatient public health care clinic and the clinic of the Nongovermental Organization (NGO) Fight Against AIDS, both located in Tegucigalpa, Honduras were recruited for the study. These women were routinely screened for sexually transmitted diseases because of their profession and were without any evidence of gynecologic complaints. Participation in the study was voluntary and all women gave informed consent. The study was approved by the Ethical Commission of the Ministry of Health.

The Las Crucitas health care center of the Ministry of Public Health is a clinic for the control of sexually transmitted diseases where women attending supposedly at weekly intervals are routinely diagnosed and treated for syphilis, gonorrhea, and HIV. The NGO is located in the red light district of Tegucigalpa and was established in 1989 mainly to implement interventions aimed at reducing high-risk behavior in the HIV epidemic. Furthermore, medical care and advice is also provided to people already infected with the virus.

TABLE 1

Selected demographic and behavioral characteristics in the study population*

	HIV-seropositive (n = 23) Mean	HIV-seronegative (n = 28) Mean
Age (years)	33	31
No. of pregnancies	4	3
Age at first pregnancy	16	16
Age at first intercourse	15	16

* HIV = human immunodeficiency virus.

All the women in the study, who defined themselves as prostitutes, were given a physical examination, including a pelvic examination. A vaginal secretion or genital ulcer directly visualized was noted. They were interviewed regarding demographic variables and questions concerning HPV-associated risk factors, sexual history and practices, presence of sexually transmitted diseases, and use of oral contraceptives. A blood sample was obtained from all woman for detection of antibodies to HIV. No cytologic analysis was performed for these women because of logistic and financial problems.

Serologic analysis for HIV. Sera were tested for antibodies to HIV using a commercially available enzyme-linked immunoassay (HIVAB-HIV-1 EIA; Abbott Laboratories, North Chicago, IL). Positive and borderline samples were retested and confirmed by Western blot assay (Cambridge Biotechnology, Worcester, MA). A Western blot was considered positive if two of the following bands were found: p24, gp41, or gp120/160.

Detection of HPV DNA. For the analysis of HPV, cervical scrapes were taken from the transformation zone of all women with a wooden spatula and the cells were eluted in 5 ml of phosphate-buffered saline (PBS) containing 0.05% merthiolate. Cells were vortexed, centrifuged, washed, pelleted, and resuspended in 0.5 ml of PBS and stored at −20°C for further virologic studies.

Extraction of DNA from the thawed cells was performed according to the standard sodium dodecyl sulfate–proteinase K-phenol-chloroform method as previously described elsewhere.[20]

All samples were prescreened with the β-globin primers PCO3/PCO4[21] to assess sample integrity. A general primer-mediated polymerase chain reaction PCR (GP-PCR) strategy was then followed for the analysis of the samples for the presence of HPV DNA.[22] Briefly, the overall presence of HPV was assessed using a general primer set (GP5/GP6) directed against the late viral capsid proteins (L1) open reading frame, which is highly conserved among all papillomaviruses. This GP-PCR assay detects a broad range of genital HPV types as well as unidentified HPV types (HPV X). A low-stringency Southern blot analysis was performed with a cocktail probe consisting of GP-PCR products specific for HPV 6, 11, 16, 18, 31 and 33.[22] To prevent contamination, strict spatial partitioning of the different technical steps of the PCR was done and the recommendations of Kwok and Higuchi[23] were followed.

Data analysis. Data were analyzed using EpiInfo (Centers for Disease Control and Prevention, Atlanta, GA) statistical program. Statistical significance was determined by Fisher's exact test. Any P value less than 0.05 was considered significant. The odds ratio (OR) and exact 95% confidence interval (95% CI) were calculated to measure the association between HPV and HIV infection.

TABLE 2

Comparison of oral contraceptive use, STD history, and ulcers*

	HIV-positive (n = 23)	HIV-negative (n = 28)	P
Oral contraceptive	7	10	0.77
STD history	19	9	<0.01
Ulcers	3	0	<0.09

* STD = sexually transmitted disease; HIV = human immunodeficiency virus.

RESULTS

The general characteristics of the 51 prostitutes concerning age and selected behavioral variables are summarized in Table 1. The HIV-seropositive and -seronegative women enrolled in this study did not differ significantly from each other for these characteristics. The women were in their early 30s, all had had at least one pregnancy, and had experienced their first sexual intercourse and first pregnancy before the age of 20.

Differences between HIV-positive and HIV-negative prostitutes concerning use of oral contraceptives, history of sexually transmitted diseases, and presence or absence of ulcers in the genital area were further analyzed. As shown in Table 2, HIV-seropositive women reported more frequently a history of sexually transmitted diseases than HIV-negative women ($P < 0.01$). The presence of ulcers in the genital area was of a borderline significance ($P < 0.09$). There was no difference in use of oral contraceptives among HIV-seropositive and HIV-seronegative women (30% and 35%, respectively).

As seen in Table 3, a total of 18 (35%) of 51 prostitutes tested positive for HPV DNA by PCR: 13 (56.5%) of 23 of the HIV-positive women compared with only 5 (18.0%) of 28 of the HIV-negative women, giving a strong association between detection of HPV DNA in cervical scrapes and positivity for antibody to HIV in serum (OR = 6.0, 95% CI = 1.5–26.7).

DISCUSSION

Prostitutes are recognized as a high-risk group for all sexually transmitted diseases, including infections with HPV and HIV. In Honduras, the number of AIDS cases and the seroprevalence of HIV infection among prostitutes has been steadily increasing. According to Mann and others,[24] the prevalence of HIV infection among female prostitutes in San Pedro Sula, the second largest city in Honduras with the

TABLE 3

Prevalence of human papillomavirus (HPV) DNA*

	HIV-positive (n = 23)	HIV-negative (n = 28)	OR	95% CI
HPV DNA	13 (56.5%)	5 (18.0%)	6.0	1.5–26.7

* HIV = human immunodeficiency virus; OR = odds ratio; CI = confidence interval.

highest incidence of AIDS cases, has increased from 19% in 1987 to 37% in 1990.

The results of our survey indicate that women practicing prostitution and testing positive for antibodies to HIV had a six-fold increased risk for HPV infection as compared with HIV-negative prostitutes. The association between HPV infection and HIV infection found in this study parallels the results from other countries.[14, 15] Laga and others,[14] in a study among prostitutes in Kinshasa, Zaire, reported a significant association of HPV infection and CIN with HIV seropositivity. Eight (73%) of 11 seropositive women with CIN had HPV detected in that study. Vernon and others,[25] in another study among prostitutes also in Kinshasa, Zaire of HPV DNA in HIV-1-seropositive and -seronegative women, showed that detection of HPV DNA in the cervix was highly associated with HIV-1 seropositivity. They estimated a 42.8% prevalence of HPV in HIV-1-seropositive women and 13.4% in seronegative women.

In a study of mainly intravenous, hard drug-using, HIV-infected prostitutes in Amsterdam, The Netherlands, van Doornum and others[26] demonstrated that in this group HIV infection was associated with a higher prevalence of HPV infection but not with a higher rate of abnormal cervical cytology. These results are comparable with the findings of a study among prostitutes in Nairobi, Kenya, in which 37% of 147 HIV-positive women and 24% of 51 HIV-negative women were found to be positive for cervical HPV DNA.[27] Nevertheless, the 1.7-fold increase in the risk of cervical HPV infection associated with HIV infection was not statistically significant. These investigators indicated that their results were caused by the composition of their study population, which included only a small number of women with advanced HIV-related disease and by the occurrence of HPV-related disease after a long latent period. A recent study among women in Malawi reported that HIV-infected women had twice the risk of abnormal cervical cytology than uninfected ones and were more likely to have persistent HPV infections (48% versus 23%).[28]

In our study, HPV DNA was found in 56.5% of the HIV-seropositive prostitutes. A higher prevalence of HPV infection in HIV-infected women may be the first step leading to dysplastic lesions. Data from a study by Koutsky and others[29] of CIN support the hypothesis that high-grade lesions may develop early in the course of HPV infection. Since the progression to invasive disease is more aggressive in the HIV population, they need closer surveillance at clinics that provide frequent cytologic screening and appropriate counseling.

Although our study sample size was small, it included HIV-negative controls and the women were relatively homogeneous in terms of sexual activity and high exposure risk for HIV. In addition, HPV DNA was assayed with a sensitive GP-PCR, which detects a broad spectrum of genital HPVs,[22] whereas some previous studies have estimated HPV infection based on cervical cytology.[15, 16] Cytologic evidence of HPV infection is highly subjective, with koilicytotic atypia, characterized by the presence of cells with perinuclear cytoplasmic vacuolation, nuclear enlargment, and regarded as the hallmark of HPV infection, being the only pathognomonic finding. This suggest that PCR-based HPV detection methods could reduce the underestimation of the association of HPV and HIV infection found in some studies.

In conclusion, it may be stated that in this group of HIV-positive and HIV-negative prostitutes the prevalence of cervical HPV DNA was found to be associated with HIV infection.

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Authors' addresses: Annabelle Ferrera, PO Box 30078, Tegucigalpa, Honduras. Willem J. G. Melchers, Department of Medical Microbiology, University Hospital Nijmegen, 6500 HB Nijmegen, The Netherlands. Johan P. Velema, Department of Public Health, Erasmus University, PO Box 1738, 3000 DR Rotterdam, The Netherlands. Manuel Figueroa, Departamento de Microbiologia, Universidad Nacional Autonoma de Honduras, Tegucigalpa, Honduras.

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