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CHLAMYDIA TRACHOMATIS AND GENITAL HUMAN PAPILLOMAVIRUS INFECTIONS IN FEMALE UNIVERSITY STUDENTS IN HONDURAS

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Abstract. Sexually transmitted infections are a serious health problem in Honduras. Human papillomavirus (HPV) and *Chlamydia trachomatis* are major causes of sexually transmitted diseases. To determine the prevalence of *C. trachomatis* and HPV in young women, 100 female university students in Honduras were assayed for the presence of these pathogens. Twenty-eight percent were positive for HPV and 6% were positive for *C. trachomatis*. These results show that genital HPV and *C. trachomatis* infections are very common among sexually active young women in Honduras. It is vital to promote extensive public awareness campaigns among sexually active women concerning preventive measures of these diseases.

INTRODUCTION

Carcinoma of the cervix is one of the most common types of cancer in the developing world and the leading cause of death from cancer among women. Worldwide death rates of 500,000 per year have been reported, of which 80% occurs in developing countries. In Central and South America, the incidence rate is approximately five times as high as in western Europe. Honduras is no exception, with a hospital cervical cancer registry of 833 cases in 2001.[1]

Infections with human papillomavirus (HPV) are prevalent in the general population, ranging from 20% to 46% in young women in different countries. Epidemiologic studies indicate that 50% of women contract a genital HPV infection within two years of becoming sexually active.[2,3] The lifetime risk of a genital HPV infection is estimated to be 80%, but only a small number of these women will develop cervical cancer.[4]

Chlamydia trachomatis is the most common sexually transmitted bacterial infection in the world and sexually active young persons are at highest risk.[5,6] Little is known about the prevalence of these infectious agents in Honduras. Therefore, the absence of this information limits the ability of public health officials to design effective prevention strategies. To address this deficit, the present study was conducted to determine the prevalence of *C. trachomatis* and HPV infections in young Honduran women attending the Universidad Nacional Autónoma de Honduras in Tegucigalpa, the capital of Honduras.

MATERIALS AND METHODS

We conducted a cross-sectional study to investigate the prevalence of HPV and *C. trachomatis* in 100 female students between 18 and 35 years of age from the Health Program at the Universidad Nacional Autónoma de Honduras. Subjects were selected consecutively from this pool on a totally volunteer first-come, first-serve basis. After providing written informed consent, all women in the study received a physical examination, including a pelvic examination by a gynecologist. A standard validated questionnaire was used to interview the subjects regarding their medical history, use of contraceptives and sexual behavior, smoking habits and other HPV- and *Chlamydia*-associated risk factors. Two smears were made, one for cytologic examination and the other for direct detection of *C. trachomatis* by immunofluorescence. Two additional scrapes were collected in transport medium to assay for HPV by extraction of DNA and a polymerase chain reaction (PCR) and for *C. trachomatis* by an enzyme-linked immunoassay. The samples were immediately transported on ice to the molecular biology laboratory at the Universidad Nacional Autonoma de Honduras. The study was reviewed and approved by the Ethical Committee of The School of Medicine of the Universidad Nacional Autónoma de Honduras.

A direct immunofluorescence antigen detection assay (Bio-Rad Laboratories, Hercules, CA) was conducted on endocervical specimens to detect the 15 serovars of *C. trachomatis*. Endocervical specimens were also tested by a solid-phase enzyme immunoassay (Chlamydiazyme; Abbott Laboratories, Chicago, IL) for chlamydial antigen. For the analysis of HPV, cervical scrapes were taken from the transformation zone with an Ayre wooden spatula and the cells eluted in 5 mL of phosphate-buffered saline containing 0.05% thimerosal and processed according to the method of Boom and others as previously described.[7]

All samples were prescreened with β-globin primers PCO3/PCO5 [8] to assess sample integrity. Broad-spectrum HPV DNA amplification was conducted using a short-fragment PCR, which can detect at least 43 different HPV types.[9] A single-reaction reverse hybridization line probe assay (LiPA) capable of simultaneous detection and identification of 25 different HPV types was used to test the HPV-positive samples.[10]

Data were analyzed using Epi-Info statistical program (Centers for Disease Control and Prevention, Atlanta, GA). Statistical significance was determined by Fisher's exact test. Any P value ≤ 0.05 was considered significant. To increase the positive predictive value of *C. trachomatis* results, only women with positive results in both the EIA and the direct immunoflurescence test were considered.

RESULTS

We enrolled 110 female university students attending the Health Program at the Universidad Nacional Autónoma de

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Honduras for various reasons (mainly for advice on contraceptive use, reproductive tract problems, or routine physical examinations). One hundred agreed to participate in the study. The participants ranged in age from 18 to 35 years (mean = 24 years). The average number of lifetime sexual partners was two, and the average age at first intercourse was 21 years. All women had normal results on cytologic smears.

Twenty-eight (28%) of the students were positive for HPV. The results are summarized in Figure 1. Six of the 28 women positive for HPV were infected with a single HPV type. Overall, 19 different genotypes were detected, and HPV-11 and HPV-51 were the most common types. The majority (89%) of the HPV-positive university students were positive for HPV-16 and related types (31, 33, 35, 52, and 58), and 46% were positive for HPV-18 and related types (45, and 59).

In one woman, only the conjugate control line of the HPV-LiPA strip was colored, and no reaction with the HPV type-specific probes was observed. This suggests the presence of an HPV type that was not included in the detection strip (HPV X). We observed a high prevalence of cervical scrapes containing multiple HPV types. Of the 100 women tested, 22% contained more than one HPV type: six women had two different HPV types, eight had three types, four had four types, and four had five or more types.

Of the 100 women tested, 6% were infected with *C. trachomatis*, as indicated by positive enzyme immunoassay and immunofluoresnce test results. None of the positive women had a history of *C. trachomatis* infection. At the time the sample was taken, only 2% had leukorrhea. Four women were positive for both HPV and *C. trachomatis*. A statistically significant association was observed between detection of HPV DNA in cervical scrapes and positivity for *C. trachomatis* antigen (odds ratio = 5.83, 95% confidence interval = 0.84–49.51, $P = 0.05$).

We observed that single female students (58%) were more likely to be infected with HPV than married women (42%). Conversely, for women infected with both HPV and *C. trachomatis*, married women were more likely to be infected (75%). Those with a positive result for HPV DNA (57%) or *C. trachomatis* (50%) were significantly younger (< 25 years of age) and had sexual intercourse for the first time at a younger age (< 25 years of age) (89% and 83%, respectively) than those with a negative result (Table 1). Other variables such as menarche, contraceptive use, pregnancies, abortions, smoking, and a family history of cancer did not show statistically significant differences and were not tabulated.

DISCUSSION

This study reports prevalence of *C. trachomatis* and HPV in female Honduran university students. *Chlamydia trachomatis* was detected in 6% of the students and HPV in 28%. Of the 28 women positive for HPV, 22 contained more than one HPV type. The significance of multiple infections is not clear. Previous studies found that infection with multiple HPV genotypes increases the likelihood of persistent HPV infection in healthy young women,[11] and might also contribute to the development or progression of cervical dysplasia,[12] but more studies are needed to clarify the importance of multiple HPV infections.

In agreement with the results of previous studies, we found that the prevalence of HPV in women less than 25 years of age was significantly higher than in older women.[13] Positivity for *C. trachomatis* was also higher in students less than 25 years than in older women. When the epidemiologic data of the university students were analyzed, we observed that the percentage of women infected with *C. trachomatis* decreases with age. Since this is a sexually transmitted infection, it is possible that women more than 25 years old are involved in more stable relationships and less exposed to this infection.

None of the women had a history of *C. trachomatis* infection. At the time when the sample was taken, only 2% had leukorrhea, a finding that must be considered when docu-

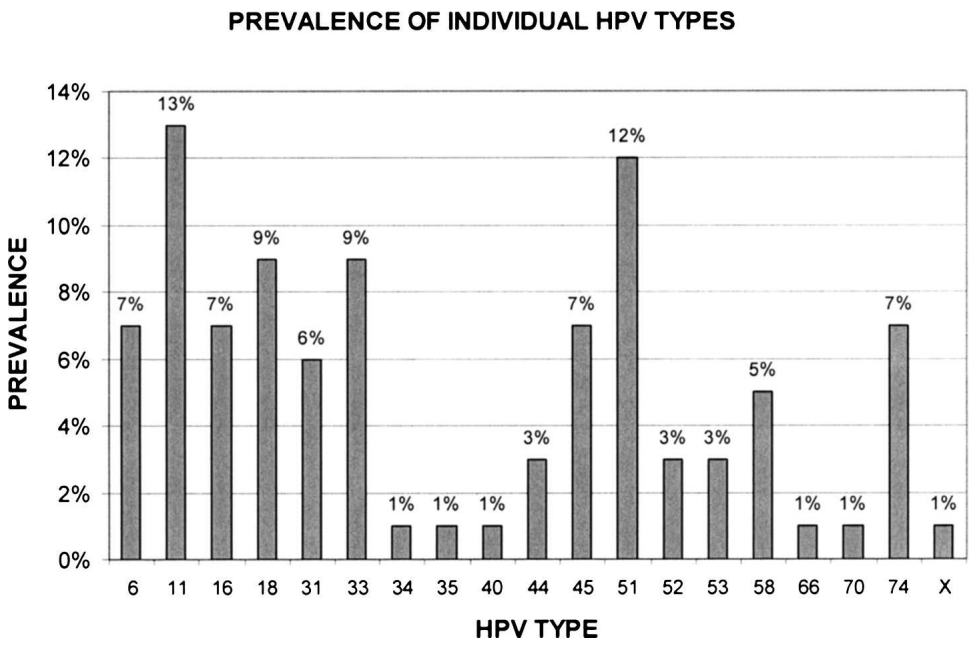

FIGURE 1. Prevalence of individual human papillomavirus (HPV) types in cervical scrapes of Honduran university students detected by a short fragment polymerase chain reaction and a single-reaction reverse hybridization line probe assay.

TABLE 1

Risk factors associated with human papillomavirus (HPV) and *Chlamydia trachomatis*–positive infections in Honduran university students

Characteristic	HPV DNA positive, no. (%)	*C. trachomatis* antigen positive, no. (%)	HPV DNA and *C. trachomatis* antigen positive, no. (%)
Age, years			
≤ 25	16 (57)	3 (50)	2 (50)
26–30	7 (25)	2 (33)	2 (50)
≥ 31	5 (18)	1 (17)	
Marital status			
Single	16 (58)	3 (50)	1 (25)
Married	12 (42)	3 (50)	3 (75)
Age at first intercourse, years			
< 25	25 (89)	5 (83)	3 (75)
≥ 25	3 (11)	1 (17)	1 (25)

menting the presence of asymptomatic infections with *C. trachomatis*. This is relevant since this silent infection can lead to sever complications and also represents a potential source of infection for their partners.

Infection with HPV is common, especially in young, sexually active women worldwide. However, not every women infected with HPV develops cervical cancer. Clearly, additional virologic, environmental, immunologic or genetic factors must be implicated in the pathogenesis of cervical cancer.

Ferrera and others[14] found that 39% of Honduran women with negative cytologic test results for cervical cancer were positive for HPV. In addition to the strong association between HPV and cervical cancer, dose-response relationships were observed for education level, age at the time of sexual intercourse for the first time, and exposure to wood smoke, levels of antibodies to *C. trachomatis* were higher in cases of cervical intraepithelial neoplasia grade III and invasive cancer than in the corresponding controls, although this difference was not statistically significant.

Four percent of the university students were positive both for *C. trachomatis* and HPV. Although this finding was statistically significant, the population was too small to make a clear-cut association between these two pathogens. Many studies have consistently indicated a potential etiologic role for infection with *C. trachomatis* as a cofactor of HPV in the development of cervical cancer.[15–18] If *C. trachomatis* modulates the immune response of the host, it should be considered an important cofactor in HPV-induced carcinogenesis.

In conclusion, we found a prevalence of 6% for *C. trachomatis* and 28% for HPV in Honduran female university students. The incidence of sexually transmitted infections continues to be a major health problem in Honduras. Thus, identifying young persons infected with *C. trachomatis* and HPV is of paramount importance in preventing cervical diseases, especially cervical cancer. This will also help in determining the prevalence of sexually transmitted infections and improving prevention strategies.

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