Chlamydia trachomatis as a probable cofactor in human papillomavirus infection in aboriginal women from northeastern Argentina

ABSTRACT

Objectives: High-risk types of human papillomavirus (HPV) are strongly associated with cervical cancer (CC), and *Chlamydia trachomatis* (CT), the most frequent sexually transmitted bacterial infection (STBI) worldwide, seems to be a risk factor for HPV infection and for CC. It is also known that both agents are more prevalent in vulnerable communities where lack of adequate primary health care is a cause for concern. The aim of this work was to determine the impact of CT and HPV infections in women belonging to an isolated aboriginal population (Pilaga community) from a poor region in Northern Argentina (province of Formosa). For this purpose, a cross-sectional study was performed in all sexually active Pilaga women, who attended a local community-based gynecological health screening project. The polymerase chain reaction (PCR) method on a cervical brush specimen was used to detect both agents. **Results:** A total of 227 women (20% of the total female population of the Pilaga community) were studied and the overall prevalence was 26.4% for CT, 46.7% for HPV and 16.3% for concurrent infection. CT infection was higher in HPV DNA positive (34.2%) than in HPV DNA negative women (19%; OR: 2.22/95% CI = 1.16-4.28 / p = 0.009) and the most prevalent HPV types were HPV-16 (19.4%), 6 and 18 (5.3%), 58 (3.5%) and 33 (3.1%). **Conclusions:** The prevalence of CT and HPV observed in Pilaga women are among the worst registered in Latin America. Also, data collected suggest that chlamydial infection may play an important role in the natural history of HPV infection. On this respect, we propose that the association between these two agents seems to be more related to a mutual potentiation than to the fact that they share a common route of transmission.

Keywords: *Chlamydia trachomatis*; papillomavirus infections; indigenous population; uterine cervical diseases.

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INTRODUCTION

Chlamydia trachomatis (CT) is the most frequent sexually transmitted bacterial infection (STBI) worldwide, with an estimated 89 million new cases per year.[1,2] Up to 70% of such infections are asymptomatic and the detection of cases has an important epidemiological implication, because the main consequences (infertility, ectopic pregnancy, pelvic inflammatory disease and perinatal infections) of symptomatic and asymptomatic infections are often identical.[2,3] Although many authors refer to an association between CT infection and dysplastic or neoplastic cervical atypia, epidemiological data have not yet provided consistent evidence about its real implication in cervical cancer (CC) development.[4-6] However, the asymptomatic nature of CT infection, its long-term persistence in the absence of treatment, and its capability of inducing metaplasia and chronic inflammation are well-defined characteristics to strongly consider this bacteria as a potential cofactor for high-risk persistent HPV infection (the main proven cause of CC).[7-9] HPV genital infection is highly frequent worldwide and its prevalence in the general population ranges from 2-44% depending on the region of the world.[10] However, it is known that most HPV infections are transient and only a small proportion of women with high-risk persistent HPV infection will develop CC.[11-13] Therefore, numerous cofactors that might promote virus persistence or enhance its oncogenicity have been studied in recent decades to better understand the oncogenic process.[14,15] CT is one of these cofactors.[4,8,9]

The annual mortality rate for CC in Argentina is 7.8/100,000 women, but this rate is three times higher in the northeastern region

of the country,[16] where a wide spectrum of Indian and isolated white communities live in unfavorable socioeconomic and health conditions.

The aim of this study was to determine the prevalence of CT and HPV infections among vulnerable populations of native Indians belonging to the Pilaga community in northern Argentina.

METHODS

Study population

This study was nested in a community-based gynecological health screening project for the Pilaga aboriginal people of the central region of Formosa province (northern Argentina). Two hundred and twenty-seven women aged 13-68 years (mean age 30 years), corresponding to 20% of all sexually active women of the Pilaga Indians in Argentina, were recruited from different aboriginal settlements between March 2007 and March 2009. A standardized questionnaire was used to interview the subjects regarding their clinical history, sexual behavior, cultural habits and socio-economic and living conditions. After providing informed consent, all women were examined by a gynecologist.

The study was reviewed and approved by the Ethics Committee of the Institute of Regional Medicine of Northeast National University, Argentina.

Sample collection and DNA extraction

Cervical exfoliated cells were collected from all women by sampling the ecto- and endocervix using a wooden spatula for PAP smears and a cytobrush for HPV and CT DNA detection. Cervical cells from the latter were eluted in sterile phosphate-buffered saline (PBS) contained in a tube, and then transported on ice to the processing laboratory. Upon arrival to the lab and after checking the sample container's integrity, the cells of each sample were pelleted by centrifugation and kept frozen at -70°C until processing.

DNA was obtained by treating pellets with 400-700 μL of homogenization solution (2% cetyltrimethylammonium bromide-CTAB, 1.4 M NaCl, 0.2% β-mercaptoethanol, 20 mM EDTA, 100 mM Tris-HCl pH 5.0), extracted with chloroform isoamyl alcohol method, then precipitated with absolute ethanol and resuspended in 50-100 μL of sterile double-distilled water. All samples were checked to assess DNA quality and integrity by amplifying a known region of the human β-globin gene (268 bp). The samples negative for the β-globin test were discarded.

Cytological analysis

PAP smears were processed and analyzed according to the 2001 Bethesda Classification System. The final diagnosis was based on the worst morphological picture.

CT and HPV DNA amplification

CT DNA was detected in cervical samples by polymerase chain reaction (PCR) using the KL1/KL2 primers, as previously described.[17] This primer pair amplifies a 241 bp fragment of the chlamydial multicopy, and a genetically conserved, cryptic plasmid. PCR was performed in a final volume of 20 μL using 3 μL of DNA sample; 10 mM Tris-HCl pH = 8.3, 50 mM KCl, 3.5 mM $MgCl_2$, 5 nmol of each deoxyribonucleoside triphosphate (dNTP), 20 pmol of each primer, 1.25 U Taq DNA polymerase (Go-Taq® DNA polymerase, Promega, USA). Each amplification reaction was carried out as follows: an initial cycle at 93°C for 3 min; 35 cycles of 1 min at 93°C, 1 min at 64°C and 1 min at 72°C and a final extension at 72°C for 5 min. Finally, the PCR products were electrophoresed on a 2% agarose gel, stained with ethidium bromide (EB) and photographed under UV light.

HPV DNA amplification and typing were conducted using the widely known MY09/11 PCR (450 bp PCR product) followed by the restriction fragment-length polymorphism (RFLP) technique, as previously described.[18,19] Briefly, the products of MY09/11 PCR were first electrophoresed on a 2% agarose gel, stained with EB and visualized under UV light. Then, the amplicons of positive samples were digested by seven restriction enzymes (*BamHI*, *DdeI*, *HaeIII*, *HinfI*, *PstI*, *RsaI* and *Sau3AI*) in individual microtubes. After that, the RFLP product of each sample was electrophoresed on a 3% agarose gel, stained with EB, and photographed under UV light. Each restriction pattern was compared with published data to identify the genotype(s) involved.

Statistical analysis

Continuous variables were categorized, and the risk associated with CT positivity was calculated for each category of a variable, adjusted by age. Univariate analysis was performed for all variables, and multivariate logistic regression analysis was performed in order to identify the independent variables that influence the relative risk of CT positivity (95% CI). The association between CT and HPV infections was evaluated in all women regardless of their cytological diagnosis. Data were analyzed using the Epi-Info™ 3.5.1 Statistical Program (Centers for Disease Control and Prevention, Atlanta, GA) and statistical significance was determined by Fisher's exact test, and p-values ≤ 0.05 were considered significant.

RESULTS

The overall prevalence was 26.4% for CT, 46.7% for HPV and 16.3% for concurrent infection. The mean crude prevalence did not significantly differ from the age-adjusted one. The mean frequency of infection, for both agents considered, increased with the severity of cervical lesions; however, this

was more notorious for HPV infection and particularly for high-risk HPV types. The HPV prevalence estimates were 27.8% in women with normal pap smear, 46.2% in women with reactive cellular changes (RCC), 87.5% in patients with low-grade squamous intraepithelial lesion (L-SIL), and 100% in patients with high-grade squamous intraepithelial lesion (H-SIL) and CC (p < 0.05) (Table 1).

The prevalence of CT infection was significantly higher among women with positive HPV DNA (37/106 = 34.9%) than among those with negative HPV DNA (23/121 = 19%; OR: 2.28, 95% CI = 1.20 - 4.38, p = 0.007). This association remained significant when high- and low-risk HPV types were considered separately, as well as infections with one or multi-

ple viral types (Table 2). Nevertheless, there was no clear association between age, age at first intercourse, number of lifetime sexual partners or parity and CT infection. Also, we could not evaluate the impact of tobacco or the use of oral contraceptive (OC) because only a minor proportion of women referred to a smoking habit (3.1%) and less than 6% of women had used OC at some point in life. The socio-economic evaluation of the living conditions in the Pilaga community showed that the majority of participants had completed elementary school education (76.8%), but only 27.5% of women had a formal or informal occupation at the moment of the study, 98.3% had no sanitary latrines in their houses and only 5% had well water for drinking and cleaning.

Table 1. Chlamydia trachomatis *and human papillomavirus infections stratified by grade of cervical status among sexually active women of the Pilaga community*

Cytology	n (%)	HPV positive (%)	p	High-risk HPV positive (%)	p	CT positive (%)	p
Negative for SIL or malignancy	36 (15.8)	10 (27.8)		7 (19.4)		8 (22.2)	
RCC	173 (76.2)	80 (46.2)		50 (28.9)		47 (27.2)	
L-SIL	16 (7)	14 (87.5)	0.015	12 (75.0)	0.010	5 (31.3)	0.968
H-SIL	1 (0.4)	1 (100)		1 (100)		0 (0)	
CC	1 (0.4)	1 (100)		1 (100)		0 (0)	
Total	227 (100)	106 (46.7)		71 (31.3)		60 (26.4)	

SIL, squamous intraepithelial lesion; RCC, reactive cellular changes; L-SIL, low-grade squamous intraepithelial lesion; H-SIL, high-grade squamous intraepithelial lesion; ISC, *in situ* carcinoma.

Table 2. *Association between* Chlamydia trachomatis *and human papillomavirus infections in cervical samples*

	Total of women studied (n = 227)		Women with CT DNA positive (n = 60)		OR (95% CI)
	n	%	n	%	
HPV-	121	53.3	23	19	1[a]
HPV+	106	46.7	37	34.9	2.28 (1.20 - 4.38) p = 0.007
HPV+ according to risk group					
High-risk types	71	31.3	24	33.8	2.18 (1.06 - 4.49) p = 0.021
Low-risk types	23	10.1	9	39.1	2.74 (0.95 - 7.83) p = 0.033
Single HPV infections	78	34.4	26	33.3	2.13 (1.05 - 4.32) p = 0.022
Multiple HPV infections	28	12.3	11	39.3	2.76 (1.04 - 7.28) p = 0.021

[a] Reference group.
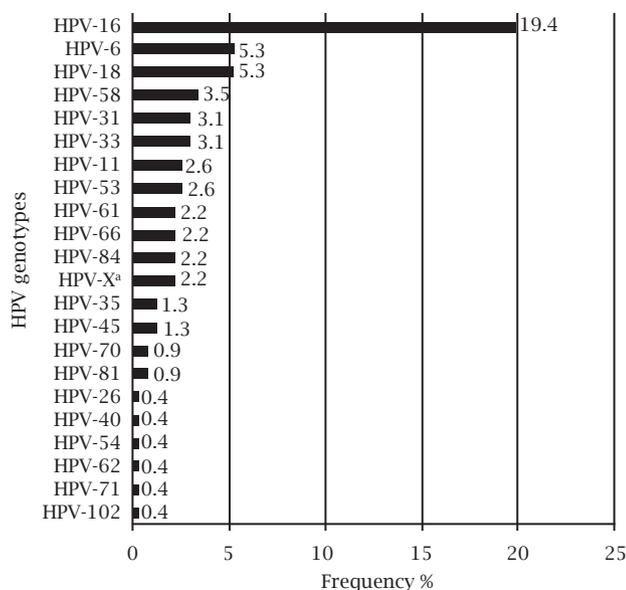
Figure 1: Prevalence of individual HPV types in cervical scrapes among Pilaga women detected by broad-spectrum polymerase chain reaction (MY 09/11 PCR) and a restriction fragment-length polymorphism assay (RFLP).

[a]HPV genotypes that could not be defined by the RFLP technique.

The individual prevalence for the 21 different detected HPV types is presented in Figure 1. Among the high-risk types, HPV-16 was clearly the most prevalent (44/227; 19.4%), followed by HPV-18 (12/227; 5.3%), -58 (8/227; 3.5%), and -31 and -33 (7/227; 3.1%); while among low-risk type, HPV-6 was the most prevalent (12/227; 5.3%), followed by HPV-11 (6/227; 2.3%), and -61 and -84 (5/227; 2.2%). Taking into account all the types found in this series, the most frequent were, in order, HPV-16, 18, 6, 58 and 33. In addition, 28 infections by multiple HPV types were observed, corresponding to 12.3% of the total women studied.

According to the data obtained, the only independent variable associated with CT-DNA positivity was concurrent HPV infection.

DISCUSSION

Despite the numerous logistical and cultural barriers that hindered this work in the Pilaga population, we were able to recruit and study 20% of the sexually active women of the community.

At large, developing countries seem to have higher prevalence of CT infections.[20] These nations have values that range from 5% to 22% in the general population and can reach peaks as high as 41% among female adolescents.[1,21-24] On the other hand, in developed countries, the prevalence levels range from less than 2% to 8-10% in general population and from less than 5% to 20% among female adoles-

cents.[1,25] In Argentina, many prevalence studies of CT infection have been conducted among women, but these studies have two important shortcomings: (I) most of them used non-standardized populations, different analytical methods to detect CT or only considered symptomatic cases; and (II) there is scarce epidemiological information about populations from northern Argentina, the region with the highest reported clinical diagnosis of STBI of the country. The available data show that prevalence ranges from 1.8% to 13% in the general population and from 20% to 47% in women with gynecological alterations.[20,26-28] The overall prevalence of CT found in our study (26.4%) is clearly the highest reported in Argentina for the general population and is very close to the worst prevalence rates worldwide.

For HPV, the worldwide infection prevalence ranges from 2-44% in the general population. Once again, developing countries usually have the highest prevalence rates.[10] A recent worldwide meta-analysis on global HPV prevalence demonstrated an infection rate of 10.4% for the world and 12.9% for Latin America,[29] and HPV-16 was the most common type in both normal and pathological populations. In our study, the overall prevalence of HPV was very high (46.7%), similar to the values found in developing countries with serious problems with implementing and maintaining a sustainable gynecological health policy. Also, it is comparable with the results obtained by other authors in other indigenous populations of northern Argentina (prevalence ranging from 52% to 60%), but clearly exceeds the mean frequency for populations from the central and southern regions of the country and for Latin American women.[10,29,30] In relation to the genotypes found, HPV-16 clearly stands out among the 21 different types identified in this work, which coincides with worldwide data and with previous studies on different populations in Argentina.[31-34] In addition, the four most prevalent HPV types among Pilaga women (HPV-16, 18, 6 and -1) represent 70% of all infections detected. These are very important baseline data to take into account for future post-vaccination epidemiological surveillance.

It is well-known that CT infection may play a major role in the etiology of cervical intraepithelial neoplasia by facilitating high-risk HPV entrance and persistence. Probably, this is due to the chronic inflammation induced by the bacteria, and to the resistance to cell apoptosis that persistent CT infections appear to confer. In this respect, our data highlight a moderate but significant association between concurrent CT and HPV infection. However, due to the cross-sectional design of this study, it was not possible to determine the primary infection in patients with concurrent CT and HPV infections. CT and HPV DNA detection by PCR-based assay only indicates an acute or chronic infection in the first case and a transient or persistent HPV infection. Nonetheless, the association between these two agents seems to be more related to a mutual potentiation

than to the fact that they share a common route of transmission.[35] In fact, controversial and discordant information exists on this topic, and since the role of CT in the natural history of HPV infection is not sufficiently clear, this particular issue merits further study.

CONCLUSIONS

The overall prevalence of CT and HPV infections found in this study is very high, even greater than other Latin American prevalences. The epidemiological data obtained in this work referred to a specific population, but numerous small communities exist in a similar situation in northern Argentina. One could then suppose that similar infection rates prevail requiring effective and sustainable health policies to be implemented. In the case of HPV, this is very important in order to prevent CC development. For CT, its control is essential for preventing pelvic inflammatory disease and infertility. Our data may help physicians to prevent diseases in their gynecological practices, particularly when dealing with vulnerable populations.

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