

Human papillomavirus (HPV) detection and Papanicolaou cytology in low-resource women in Posadas city, Misiones, Argentina

INES BADANO^{1*}; RENE W. PEDROZO²; LAURA S. RUIZ DIAZ²; JUAN A. GALUPPO¹;
MARIA A. PICCONI³; RODOLFO H. CAMPOS⁴; DOMINGO J. LIOTTA¹

¹Laboratorio de Biología Molecular Aplicada. Facultad de Ciencias Exactas, Químicas y Naturales. Universidad Nacional de Misiones. Av. Mariano Moreno 1375, 3300 Posadas, Misiones; ²Ministerio de Salud Pública de la Provincia de Misiones, Tucumán 2184, 3300 Posadas, Misiones; ³Servicio Virus Oncogénicos, Departamento de Virología, INEI-ANLIS "Dr. Malbrán", Av. Velez Sarsfield 563, 1282 Buenos Aires; ⁴Cátedra de Virología. Facultad de Farmacia y Bioquímica. Universidad de Buenos Aires. Junín 954. 4to piso, 1113 Buenos Aires. Argentina.
*Correspondence. E-mail: inesbadano@yahoo.com.ar

ABSTRACT

The objective of this study was to determine the prevalence of HPV infection and cervical lesions present in women who attended a health center in a low-resource area of the city of Posadas, Misiones, Argentina. Cervical cell samples (n = 163) were processed for Papanicolaou cytology and HPV-PCR tests. Socio-cultural risk factors were estimated using the odds ratio (OR, CI 95 %). Cervical lesions were detected in 14.7 % of women. The general prevalence of HPV infection was of 38 %. The most common types among the total population were HPV-16 (9.8 %) and HPV-33 (9.3 %). HPV-16 was detected in association with 29.2 % and 6.5 % of women with and without cervical lesions, respectively, the OR being 5.3 (1.8-15.8). Risk factors for HPV-16 infection were a smoking habit and a history of previous sexually-transmitted diseases. These data are important for the implementation of prevention programs, including an appropriate introduction of vaccination and the baseline for virological surveillance in the vaccine era.

Key words: *Human papillomavirus* (HPV); screening; cervical cancer

RESUMEN

Detección del virus papiloma humano (HPV) y citología de Papanicolaou en mujeres de bajos recursos de la ciudad de Posadas, Misiones, Argentina. El objetivo de este estudio fue determinar la prevalencia de la infección por HPV y de lesiones cervicales en mujeres asistidas en un centro de salud situado en un área de bajos recursos de la ciudad de Posadas, Misiones, Argentina. Las muestras (n = 163) fueron examinadas mediante las pruebas de Papanicolaou y de PCR para HPV. Los factores socio-culturales de riesgo fueron identificados mediante el cálculo de la *odds ratio* (OR, IC 95 %). Se detectaron lesiones cervicales en el 14,7 % de las mujeres. La prevalencia de infección por HPV fue de 38 %. Los tipos más frecuentes en la población total fueron HPV-16 (9,8 %) y HPV-33 (9,3 %). El HPV-16 se detectó asociado al 29,2 % y al 6,5 % de las mujeres con lesiones del cuello uterino y sin ellas, respectivamente, con un OR de 5,3 (1,8-15,8). Los factores de riesgo para la infección por HPV-16 fueron el hábito de fumar y el antecedente de enfermedades de transmisión sexual. Estos datos son importantes para la ejecución de los programas de prevención, incluyendo una introducción adecuada de la vacunación y la línea de base para la vigilancia virológica en la era de la vacuna.

Palabras clave: Virus papiloma humano; tamizaje; cáncer cervical

The relationship between *Human papillomavirus* (HPV) genital infections and the development of cervical cancer is firmly established. Currently, more than sixteen HPV types are considered high-risk types for cervical cancer (*Papillomaviridae* family, *Alpha Papillomavirus* genus, species A6, A7 and A9) (6).

Cervical cancer is the second most frequent cancer in women in Argentina. Current estimates indicate that 3,996 women are diagnosed with cervical cancer and that 1,809 die every year (15). The Province of Misiones is considered a region with high mortality rates for cervical carcinoma (15.5/100,000) compared to urban areas of

the country such as Buenos Aires city (6.3/100,000) (2). Epidemiological studies performed locally in women with normal cytology have shown HPV prevalence of 43 % in a white urban population and of 64 % in an indigenous Guaraní population (13). Therefore, screening for cervical cancer remains an important health concern throughout the region; the efforts must ensure that all women have access to preventive services including educational health, Papanicolaou (Pap) screening and HPV vaccines.

The present study was conducted at Nueva Esperanza, a recently established neighborhood in Posadas city, as part of a relocalization program associated with the con-

struction of the Yacyreta Dam. In this neighborhood live more than 1,000 low income families originally settled on the coast of the Paraná River until 2001. Although Nueva Esperanza has a peripheral health center, the nearest public hospital is located 10 km away; this distance and the cost of public transportation limit the population's access to regular gynecological care. The objective of this study was to determine the prevalence of HPV infection and cervical lesions present in women who attended a health center in a low-resource area of the city of Posadas, Misiones, Argentina.

The project was carried out during 2005-2006 at the community health center. The inclusion criteria limited the study to sexually active women. Nueva Esperanza neighborhood contains approximately 6,000 people. Data about sexually active women were unknown and therefore was calculated indirectly using local references (<http://www.indec.gov.ar/>). We estimated that 1,620 women over 15 years old were under this condition (27 % of the total population). A total of 170 participants who gave their informed consent were included in the study. A standardized questionnaire was given to each participant to determine economic status, educational level, smoking habits, sexual behavior (first sexual intercourse age and number of lifetime sexual partners), parity, and history of sexually transmitted diseases (STDs). All procedures were performed in accordance with the Helsinki Declaration and the study was approved by the *Comité de Bioética, Departamento de Docencia e Investigación del Hospital Escuela de Agudos Dr. Ramón Madariaga* (Posadas, Argentina). Samples consisting of endo-ectocervical cells were collected with wooden spatulas for Pap smears and with cytobrushes for HPV-DNA analysis.

The Pap smears were classified as: 1) negative for intraepithelial lesion or malignancy (NILM); 2) low-grade squamous intraepithelial lesion (L-SIL); 3) high-grade squamous intraepithelial lesion (H-SIL); and 4) invasive squamous cell carcinoma (ISCC). If cervical lesions were detected, the patient was referred to Hospital Dr. Ramón Madariaga for a complementary biopsy and eventual treatment.

For HPV DNA analysis, genomic DNA was extracted from cervical cells using standard methods. Sample quality was tested by PCR targeted to 268 base pairs (bp) of the human beta-globin gene using primers GH20 (5'-GAAGAGCCAAGGACAGGTAC-3') and PG04 (5'-CAACTTCATCCACGTTCCACC-3'). HPV was detected with MY09 and MY11 consensus primers (3). HPV typing was based on the E6-Nested Multiplex PCR protocol (E6-NMPX) (11). Samples that failed to be typed by E6-NMPX were subjected to RFLP analysis (3).

The association between socio-cultural risk factors and cervical lesion grade, HPV or HPV-16 infection was assessed by univariate analysis (odds ratios; 95 % confidence intervals). The statistical analysis was also adjusted by age range, including for this purpose only women older than 30 years old (≥ 30 y). This decision was based on

the assumption that women ≥ 30 y have been reported to be at a higher risk for acquiring persistent and clinically important infections and therefore may represent a better group for association analysis (9). The distributions of HPV infection were compared by the chi-square test (χ^2).

Of the 170 samples included in the study, 7 were discarded due to degradation of the material. The remaining 163 participants represented 10% of the sexually active women of Nueva Esperanza neighborhood. The median sample age was 31 years old (range 18-62). According to our survey, the population was categorized as having a low socio-economic level, with 96.3 % (157/163) of women having incomes compatible with poverty and 73.0 % (119/163) being unemployed. One-third of the population studied had not finished elementary school (51/163). We believe that this social context will likely affect their ability to access the health system. Remarkably, Pap smears were not available at the health center before this work and this study represents the first Pap attention for 35.0 % (57/163) of the women studied.

Pap smears showed NILM with inflammatory processes in 85.3 % (139/163) of the women studied and cervical lesions in 14.7 % (24/163). Cervical lesions reported were: twenty L-SIL (12.3 %), three H-SIL (1.8 %) and one ISCC (0.6 %). Participants with H-SIL and ISCC were surgically treated.

The general prevalence of HPV infection was 38 % (62/163). After stratifying by Pap cytology, HPV was found in 33.1 % (46/139) of NILM subjects, in 100 % (4/4) of women with H-SIL/ISCC, and in only 60.0 % (12/20) of women with L-SIL. The latter result could be attributable to a cytological overdiagnosis.

In order to establish regional comparisons for HPV prevalence in NILM women, we elaborated Table 1, which includes data available in other Argentinean studies (1, 4, 5, 7, 8, 10, 12, 14, 15). The analysis of these data indicates that: (i) the value obtained in this work was not significantly different from that of a previous study performed locally by our group ($\chi^2 p > 0.05$), but was significantly higher than those reported for other cities in the Argentinean littoral region like Concordia (Entre Ríos) and Corrientes (Corrientes) ($\chi^2 p < 0.05$); (ii) the prevalence throughout the country is not homogeneous; (iii) the highest values of HPV prevalence are reported in the aboriginal populations of the country, including the Guarani communities of Misiones.

The prospect of prophylactic vaccines reinforces the need for more knowledge regarding regional prevalence of high-risk HPV types. In this study, a total of 14 different viral types were identified. Mixed infections were presented in 19.4 % of positive women. HPV-16 was the most frequent viral type among the total population with ten monoinfected patients and six patients with mixed infections; giving a total prevalence of 9.8 % (16/163). Moreover, HPV-16 infection was higher in women with cervical lesions than in the NILM, with 29.2 % (7/24) and

Table 1. Prevalence of HPV infection in Argentina. Analysis of selected studies.

Location/Population	HPV	Source
Concordia/Urban	16.6 % (166/987)	(8)
Corrientes/Urban	17.6 % (9/53)	(5)
Ushuaia/Urban	26.4 % (23/87)	(11)
Rosario/Urban	43.0 % (40/93)	(4)
Misiones/Urban	43.0 % (92/214)	(13)
La Plata/Urban	46.0 % (70/152)	(1)
Formosa/Indigenous	43.6 % (58/133)	(Deluca G, personal communication)
Jujuy/Indigenous	51.8 % (56/108) ⁽¹⁾	(9)
Misiones/Indigenous	64.2 % (133/207) ⁽¹⁾	(15)
Misiones/Urban	33.1 % (46/139)	Present Study
IARC Reference Data	20.1 % (17.7-22.7)	(16)

⁽¹⁾ Statistically significant differences compared to the present study ($\chi^2 p < 0.05$)

Table 2. HPV type distribution stratified by Pap cytology.

HPV type	Normal cytology		Cervical Lesions			
	n=139		n=24		n=163	
	n	%	n	%	n	%
High-Risk						
HPV-16	6	4.3	4	16.7	10	6.1
HPV-33	10	7.2	1	4.2	11	6.8
HPV-56	5	3.6	1	4.2	6	3.8
HPV-52	6	4.3	-	-	6	3.8
HPV-31	2	1.5	1	4.2	3	1.8
HPV-59	1	0.7	-	-	1	0.6
HPV-58	-	-	1	4.2	1	0.6
HPV-82	-	-	1	4.2	1	0.6
HPV-70	1	0.7	-	-	1	0.6
Subtotal High-Risk	31	22.3	9	37.5	40	24.5
Low-Risk						
HPV-6/11	2	1.5	1	4.2	3	1.8
HPV-40	1	0.7	-	-	1	0.6
Subtotal Low-Risk	3	2.2	1	4.2	4	2.4
Mixed Infections						
HPV-16 + HPV-6/11	1	0.7	1	4.2	2	1.2
HPV-16 + HPV-56	1	0.7	-	-	1	0.6
HPV-16 + HPV-33	1	0.7	1	4.2	2	1.2
HPV-16 + HPV-52	-	-	1	4.2	1	0.6
HPV-31 + HPV-56	2	1.5	-	-	2	1.2
HPV-31 + HPV-45	1	0.7	-	-	1	0.6
HPV-59 + HPV-58	1	0.7	-	-	1	0.6
HPV-45 + HPV-52	-	-	1	4.2	1	0.6
HPV-18 + HPV-59 + HPV-58	1	0.7	-	-	1	0.6
Subtotal Mixed Infections	8	5.7	4	16.7	12	7.4
Undetermined	4	2.9	2	8.0	6	3.8
Negative	93	66.9	8	33.3	101	61.9
Total	139	100	24	100	163	100

6.5 % (9/139), respectively ($\chi^2 p < 0.05$), and the associated risk for infection with HPV-16 and the development of cervical lesions in women ≥ 30 y showed an OR of 11.8 (1.8-79.7). These results are in agreement with the Argentinean reference values for HPV-16 prevalence and are consistent with the well established role of HPV-16 on cervical cancer development (9, 15). It is interesting to note that the second most frequent type was HPV-33 with 9.3 % (13/163) between mono and co-infections, which was higher than the reference value for Argentina of 1.4 % (15). HPV type distributions are detailed in Table 2.

Given that HPV infection is transmitted mainly by sexual intercourse, certain patterns of sexual behavior may be associated with an increased risk for HPV acquisition. According to our survey, HPV infection was associated with the number of sex partners (three or more) in women ≥ 30 y. Particularly, HPV-16 infection was associated with the history of previous STDs and a smoking habit. Details are shown in Table 3.

The Screening Guidelines of the American College of Obstetricians and Gynecologists indicate that if HPV testing is used in combination with Pap smears, women who are negative for both tests should return to

be controlled in three years. This strategy is meant to minimize unnecessary follow-up visits and invasive procedures without compromising the detection of disease, making this scheme more appropriate to communities with limited access to public health services like Nueva Esperanza population. The National Cervical Cancer Prevention Program of Argentina is starting a project in Jujuy province, introducing the HPV test as primary screening (Picconi MA, personal communication). Therefore, the availability of new screening tools to detect precancerous lesions provides great opportunities for cervical cancer prevention.

Despite all its limitations, this study aims to contribute to public health by providing epidemiological information about this community and implementing Pap screening. These data can also be useful for the implementation of prevention programs, including an appropriate introduction of vaccination and the baseline for virological surveillance in the vaccine era.

Conflict of Interest: The authors have no conflicts of interest to declare

Table 3. Evaluation of risk factors for HPV infection and the development of cervical lesions in the population studied.

	HPV		OR	HPV-16		OR	Normal	Cervical	OR
	(+)	(-)	(CI 95%)	(+)	(-)	(CI 95%)	cytology	lesions	(CI 95%)
Age at first intercourse									
≥ 19	8	14	1	2	20	1	21	1	1
≤ 18	54	86	1.1 (0.4-2.8)	15	125	1.2 (0.2-5.6)	117	23	4.1 (0.5-32.2)
			1.0 (0.4-2.9) ⁽²⁾			0.4 (0.1-2.5) ⁽²⁾			3.7 (0.4-30.6) ⁽²⁾
Lifetime no. of Sex Partners									
1-2	37	73	1	9	101	1	96	14	1
≥ 3	25	25	2.0 (1.0-3.9)	8	42	2.1 (0.8-5.9)	40	10	1.7 (0.7-4.2)
			4.2 (1.6-10.8) ^(1,2)			4.0 (0.6-25.4) ⁽²⁾			2.4 (0.7-8.0) ⁽²⁾
Parity									
1-2	14	25	1	5	34	1 31	8	1	
≥ 3	48	76	1.1 (0.5-2.4)	12	112	0.7 (0.2-2.2)	108	16	0.6 (0.2-1.5)
			0.9 (0.3-3.1) ⁽²⁾			0.7 (0.1-6.6) ⁽²⁾			1.0 (0.2-4.8) ⁽²⁾
History of STIs									
No	55	90	1	12	133	1 125	20	1	
Yes	7	9	1.3 (0.4-3.6)	5	11	5.0 (1.5-16.9) ^(1, 2)	12	4	2.1 (0.6-7.1)
			0.9 (0.2-3.9) ⁽²⁾			7.8 (1.1-54.8) ^(1, 2)			3.7 (0.8-17.4) ⁽²⁾
Smoking									
No	47	78	1	11	114	1 105	20	1	
Yes	15	18	1.4 (0.6-3.0)	6	27	2.3 (0.8-6.8)	29	4	0.7 (0.2-2.3)
			1.4 (0.5-3.8) ⁽²⁾			6.6 (1.0-42.6) ^(1, 2)			1.2 (0.3-4.7) ⁽²⁾
Pap Smears									
Normal	46	93	1	10	129	1			
Cervical Lesions	16	8	4.0 (1.6-10.1) ⁽¹⁾	7	17	5.3 (1.8-15.8)			
			8.4 (2.1-33.3) ^(1, 2)			11.8 (1.8-79.7) ^(1, 2)			

⁽¹⁾ Statistically significant differences; ⁽²⁾Odds ratios adjusted by age (≥ 30 y).

Acknowledgements: This research was supported by grants of the Agencia Nacional de Promoción Científica y Tecnológica (PICT Red 311/02) and the Comité Ejecutivo de Desarrollo e Innovación Tecnológica (Misiones). The funding agencies were not involved in the study design, data collection, analysis, paper writing or submission. The authors would like to thank the social workers and healthcare professionals at "Nueva Esperanza". This work is dedicated to the memory of Sergio Tonon.

REFERENCES

1. Abba MC, Gomez MA, Golijow CD. Human papillomavirus genotype distribution in cervical infections among woman in La Plata, Argentina. *Rev Argent Microbiol* 2003; 35: 74-9.
2. Arrossi S. Proyecto para el mejoramiento del programa nacional de prevención del cáncer de cuello uterino en Argentina: informe final: diagnóstico de situación del Programa Nacional y Programas Provinciales/ Silvina Arrossi; con colaboración de Melisa Paolino – 1ed.-Buenos Aires: Organización Panamericana de la Salud-OPS 2008. 163p; ISBN 978-950-710-114-4
3. Bernard HU, Chan SY, Manos MM, Ong CK, Villa LL, Delius H, Peyton CL, Bauer HM, Wheeler CM. Identification and assessment of known and novel human papillomaviruses by polymerase chain reaction amplification, restriction fragment length polymorphisms, nucleotide sequence, and phylogenetic algorithms. *J Infect Dis* 1994; 170: 1077-85.
4. Chouhy D, Gil LB, Nocito AL, Wojdyla D, Ornella L, Cittadini J, Gardiol D, Giri AA. Development and evaluation of a colorimetric PCR system for the detection and typing of human papillomaviruses. *Int J Mol Med* 2006; 18: 995-1003.
5. Deluca GD, Marin HM, Schelover E, Chamorro EM, Vicente L, Albhorn M, Alonso JM. Chlamydia trachomatis and papillomavirus infection in women with cytohistological abnormalities in uterine cervix. *Medicina (B Aires)* 2006; 66: 303-6.
6. de Villiers EM, Fauquet C, Broker TR, Bernard HU, zur Hausen H. Classification of papillomaviruses. *Virology* 2004; 324: 17-27.
7. Matos E, Loria D, Amestoy GM, Herrera L, Prince MA, Moreno J, Krunfly C, van den Brule AJ, Meijer CJ, Munoz N, Herrero R. Prevalence of human papillomavirus infection among women in Concordia, Argentina: a population-based study. *Sex Transm Dis* 2003; 30: 593-9.
8. Picconi MA, Gronda J, Alonio LV, Villa LL, Sichero L, Miranda S, Barcena M, Teyssie A. Human papilloma virus in Quechua women from Jujuy with high frequency of cervical cancer: viral types and HPV-16 variants. *Medicina (B Aires)* 2002; 62: 209-20.
9. Schiffman M, Glass AG, Wentzensen N, Rush BB, Castle PE, Scott DR, Buckland J, Sherman ME, Rydzak G, Kirk P, Lorincz AT, Wacholder S, Burk RD. A long-term prospective study of type-specific human papillomavirus infection and risk of cervical neoplasia among 20,000 women in the Portland Kaiser cohort study. *Cancer Epidemiol Biomarkers Prev* 2011; 20:1398-409.
10. Sijvarger CC, Gonzalez JV, Prieto A, Messmer AG, Mallimaci MC, Alonio VL, Teyssie AR, Picconi MA. Cervical infection epidemiology of human papillomavirus in Ushuaia, Argentina. *Rev Argent Microbiol* 2006; 38: 19-24.
11. Sotlar K, Diemer D, Dethleffs A, Hack Y, Stubner A, Vollmer N, Menton S, Menton M, Dietz K, Wallwiener D, Kandolf R, Bultmann B. Detection and typing of human papillomavirus by E6 nested multiplex PCR. *J Clin Microbiol* 2004; 42: 3176-84.
12. Tonon SA, Picconi MA, Zinovich JB, Liotta DJ, Bos PD, Galuppo JA, Alonio LV, Ferreras JA, Teyssie AR. Human papillomavirus cervical infection and associated risk factors in a region of Argentina with a high incidence of cervical carcinoma. *Infect Dis Obstet Gynecol* 1999; 7: 237-43.
13. Tonon SA, Picconi MA, Zinovich JB, Nardari W, Mampaey M, Galuppo JA, Bos PD, Badano I, Di Lello F, Basiletti J, Gonzalez JV, Alonio LV, Teyssie AR. Prevalence of cervical infection by Human papillomavirus (HPV) in the Caucasian and Guarani populations residing in the province of Misiones, Argentina. *Rev Argent Microbiol* 2003; 35: 205-13.
14. Tonon SA, Picconi MA, Zinovich JB, Nardari W, Mampaey M, Badano I, Di LF, Galuppo JA, Alonio LV, Teyssie AR. Human papillomavirus cervical infection in Guarani Indians from the rainforest of Misiones, Argentina. *Int J Infect Dis* 2004; 8: 13-9.
15. WHO/ICO Information Centre on HPV and Cervical Cancer (HPV Information Centre). Human Papillomavirus and Related Cancers in Argentina. Summary Report 2010. [Online] http://apps.who.int/hpvcentre/statistics/dynamic/ico/country_pdf/ARG.pdf?CFID=4207009&CFTOKEN=73053825. [Accessed August 29, 2010].