Received: 24 June 2016 | Accepted: 24 January 2017

DOI 10.1002/jmv.24805

RESEARCH ARTICLE

WILEY JOURNAL OF MEDICAL VIROLOGY

Prevalence of Human Papillomavirus infection among Chilean women from 2012 to 2016

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Here, we evaluated the prevalence of Human Papillomavirus (HPV) in two groups of Chilean women. The first group consisted of 3235 women aged 18-64 years attended in six primary care centers of Santiago. The second group consisted of 456 women 18-85 aged who consulted the Gynaecology Department of the Reference Hospital of Santiago. Samples were collected from October 2012 to February 2016. Cervical swabs were analyzed both HPV genotyping by PCR and Reverse Line Blot, and cervical cytology by Pap testing. Results showed a prevalence of 12.0% HPV positive, 10.3% high-risk (HR) HPV types positive, 3.9% low-risk (LR) HPV types positive, and 1.0% Pap positive in group 1. The most frequent types were 16, 66, and 59, with a prevalence of 3.0%, 1.6%, and 1.5%, respectively. The prevalence were 71.9% HPV positive, 67.3% HR-HPV types positive, 13.6% LR-HPV types positive, and 62.5% Pap positive in group 2. The most frequent types were 16, 31, and 58, with prevalence of 33.6%, 10.5%, and 7.0%, respectively. Among infected women with HPV: 7.6% were infected with HPV16 or HPV18, 3.0% with HPV31, HPV33 or HPV45, and 6.7% with any other HR-HPV. These findings show great difference in HPV prevalence and types between primary care and reference center, and provide useful epidemiological information to assess the impact of HPV vaccination in the future.

KEYWORDS
Chilean women, genotypes, human papillomavirus, prevalence

1 | INTRODUCTION

Infection with Human Papillomavirus is the main cause of cervical squamous intraepithelial lesions and invasive cervical cancer.[1] Persistent infection with HR-HPV is the requirement for cellular transformation of the cervical epithelium. HPV16 and HPV18 types cause about 70% of precancerous lesions and cervical cancer.[2]

Cervical cancer is the fourth most frequent cancer women in the world. It was estimated 527 624 new cases and 265 672 deaths in 2012.[3] South America has some of the highest cervical cancer incidence and mortality rates in the world.[4] In Chile, cervical cancer is the fifth most frequent cancer among women and the second most frequent cancer among women between 15 and 44 years-old. Annually, it is estimated that 1450 new cases are diagnosed with cervical cancer and 734 die from the disease.[3,5] The current Chilean mortality rate of 6-7.5/100 000, which is approximately twice the value observed in developed countries.[3,6] These data highlight the need to improve the effectiveness and equity of the Chilean Cervical Cancer Prevention Program.

The knowledge of the prevalence of HPV genotypes in different populations is a need to provide information to enable the impact of key changes in prevention and control of the diseases caused by HPV. HPV16 and HPV18 are the two most prevalent HR-HPV types in the world. However, there are significant variations in the frequency of viral types in diverse geographic regions. Some genotypes are specifically common in different continents (eg, HPV45 and HPV33 in Africa; HPV33 and HPV31 in Europe; HPV31, HPV33, and HPV45 in America, and HPV58 and HPV52 in Asia).[7]

The introduction of HPV vaccine has been a fundamental strategy within national cancer control programs. Since 2007, vaccination programs have been implemented in many countries in the world. The two worldwide HPV vaccines have used HPV16 and HPV18 antigens,

and the quadrivalent vaccine also has added HPV6 and HPV11 antigens. A national vaccination program in girls between 9 and 11 years-old has been implemented in Chile with quadrivalent vaccine since 2014.

This article reports a large pre-vaccine prevalence of HPV genotypes in sexually active Chilean women recruited from two groups: 1. women recruited via primary health care centers and 2. women who referred the gynaecology department of the Reference Hospital. These epidemiological findings could be useful to establish the baseline for surveillance and to assess the impact of the vaccination program in Chile.

2 | MATERIALS AND METHODS

2.1 | Study population and sample collection

We performed this study from October 2012 to February 2016 in two groups of women in Chile (n = 3691). The volunteers of the first group (n = 3235) were recruited via primary health care centers in the Metropolitan area of Chile. This survey was carried out from March 2014 to February 2016 from the Santiagós north side and central Metropolitan Health Service. Six public health care centers (three from the north side and three from central side) participated in this research. During this study, health care centers developed all clinical protocols according to guidelines of the Chilean cervical cancer prevention program. Women aged 18-64 years, with a mean age of of 40.0 ± 11.0 years-old, were admitted to this study. Pregnant, hysterectomized or virgins patients were discarded from the research. The second group (n = 456) consisted of women 18-85 aged, with a mean age of 36.0 ± 10.8 years, who consulted the gynaecology department of the West side Reference Hospital of Metropolitan Health Service (San Juan de Dios Hospital) from Santiago. Data were collected from October 2012 to March 2015. Eligible women who agreed to participate signed an informed consent form to enter the study. The study protocol was approved by the Ethics Committee of the Servicio de Salud Metropolitano Central.

Cervical exfoliated cell samples were obtained by a gynaecologist or a midwife according to the routine procedures used in the primary health care centers or hospital. For each patient, two separate cervical exfoliated cell specimens were collected independently for HPV genotyping assay and cytological diagnosis.

Pap smears were processed and analyzed by experienced cytopathologists. Bethesda classification was used to classify the Pap analysis. Samples with an invalid cytological result were discarded from this study. Participants with atypical results by cytology were followed up, according to the guidelines of the Chilean Cancer Program.

2.2 | Detection and typing of HPV

Detection of HPV DNA was performed on exfoliated cell samples at the Seccion Virus Oncogenicos of the Instituto de Salud Publica de Chile. The extraction of cellular DNA was carried out from samples using a commercial and automated assay (NucliSENS® easyMAG®, cat 280140, bioMérieux, France). Four different internal controls were used (without cells, K-562 HPV non-infected cells, SiHa HPV16 infected cells, and HeLa HPV18 infected cells) to verify the extraction and amplification methods.

The amplification of DNA was carried out with Brilliant II SYBR® Green QPCR Master Mix (Agilent Technologies, cat 600828, La Jolla, CA). We amplified a genomic fragment of 450 bp of HPV L1 gene with primers PGMY 09/11.[8] Another internal control fragment of 141 bp of albumin gene was amplified with primers ALB-Fw and ALB-Rv.[9] PCR assays were performed using 100 ng DNA samples and 0.5 nM each primer with 20 µL reaction mixture containing 1x Brilliant II SYBR® Green QPCR Master Mix. DNA amplification were carried out in a thermocycler Stratagene M×3000P (Agilent Technologies) by a thermal reaction with three steps. First, a hot start step of 10 min at 95°C; second, 45 cycles of amplification step (10 s at 95°C, 10 s at 56°C, and 60 s at 72°C); and third, a controlled denaturation gradient from 65 to 95°C. Purity of amplicons was confirmed by detection of a single melting point at 78-79°C and 86°C with HPV and albumin amplification, respectively.

Genotyping of HPV was carried out by a conventional PCR followed with Reverse Line Blot (PCR-RLB). Amplification reactions were performed with PGMY09 and biotin-labeled PGMY11 generic HPV primers to amplify a fragment of 450 bp in the L1 viral gene. PCR positive samples were typed by RLB assay using 33 type-specific probes for HPV6, 11, 16, 18, 26, 31, 33, 34, 35, 39, 40, 42, 43, 44, 45, 51, 52, 53, 54, 55, 56, 57, 58, 59, 66, 68, 69, 70, 73, 82, 83, and 84.[10] Positive reactions were detected revealed by a chemiluminescence reaction using Amersham™ ECL™ Detection Reagents according to manufacturer recommendations (GE Healthcare, Little Chalfont, UK).

2.3 | Statistical analysis

The frequency of each HR-HPV and LR-HPV type was calculated as HR-HPV-positive and LR-HPV-positive samples, respectively. All statistical analyses were performed using Prism 6.04 software (GraphPad Software, Inc., San Diego, CA).

3 | RESULTS

3.1 | Frequency of HPV types in women attending primary care centers and gynaecology hospital service

This HPV epidemiological surveillance was conducted in 3235 women attending six primary care health centers from Santiago city during 2 years. Three hundred eighty seven HPV positive samples were detected by molecular biology methods. Overall, HPV prevalence was found to be 12.0% (387/3235) in this population (Table 1). It was detected 10.8% samples with normal cytology and simultaneously infected with HPV. Likewise, 53.5%, 77.3%, and 20.0% of atypical squamous cells of undetermined significance (ASC-US), low grade squamous intraepithelial lesion (L-SIL), and high grade squamous intraepithelial lesion (H-SIL) were HPV positive, respectively. Single

TABLE 1 Overall HPV prevalence, type distribution and multiplicity of infection, and cytological findings among 3235 Chilean women checked for cervical cancer screening in Chile, 2014-2016

	Cytology *n* (%)				Total *n* (%)		
HPV type	Nomal	ASC-US	L-SIL	H-SIL	Single	Multiple	Total
Samples *n*	3160	43	22	10			3235
HPV+	342 (10.8)	23 (53.5)	17 (77.3)	5 (50.0)	243 (7.5)	144 (4.5)	387 (12.0)
HR-HPV+	291 (9.2)	22 (51.2)	16 (72.7)	5 (50.0)	199 (6.1)	135 (4.2)	334 (10.3)
LR-HPV+	110 (3.4)	9 (20.9)	5 (22.7)	2 (20.0)	44 (14)	82 (2.5)	126 (3.9)
HR infections							
16	83	6	6	2	62	35	97 (3.0)
18	17	3	1	0	10	11	21 (0.6)
31	28	5	1	1	15	20	35 (1.1)
33	9	1	0	1	7	4	11 (0.3)
35	5	1	0	0	0	6	6 (0.2)
39	23	2	0	0	10	15	25 (0.8)
45	14	2	1	0	6	11	17 (0.5)
51	42	3	2	0	11	36	47 (1.4)
52	39	3	0	0	18	24	42 (1.3)
56	14	1	4	0	8	11	19 (0.6)
58	23	2	4	0	10	19	29 (0.9)
59	44	5	1	0	11	39	50 (1.5)
66	46	2	3	0	22	29	51 (1.6)
68	6	0	0	0	3	3	6 (0.2)
73	14	1	0	1	4	12	16 (0.5)
82	7	0	0	0	2	5	7 (0.2)
LR infections							
6	14	2	3	0	9	10	19 (0.6)
11	5	0	0	0	1	4	5 (0.2)
26	2	1	0	1	0	4	4 (0.1)
32	0	1	0	0	0	1	1 (0.0)
34	4	0	0	0	1	3	4 (0.1)
40	5	0	0	0	1	4	5 (0.2)
42	28	1	1	1	4	27	31 (1.0)
44	12	0	0	0	5	7	12 (0.4)
48	0	0	1	0	0	1	1 (0.0)
53	19	0	0	0	7	12	19 (0.6)
54	16	2	0	0	2	16	18 (0.6)
55	8	0	0	0	2	6	8 (0.2)
57	2	0	0	0	0	2	2 (0.1)
69	3	0	0	0	1	2	3 (0.1)
70	6	0	0	0	3	3	6 (0.2)
72	1	0	0	0	0	1	1 (0.0)
83	4	2	1	0	3	4	7 (0.2)
84	12	1	0	0	7	6	13 (0.4)

and multiple HPV infections were detected in 7.5% and 4.5% of women, respectively (Table 1). The most prevalent HPV types were HPV16 (3.0%), HPV66 (1.6%), and HPV59 (1.5%). The most prevalent LR-HPV types were HPV42 (1.0%), HPV6 (0.6%), HPV53 (0.6%), and HPV54 (0.6%). Frequencies of HR-HPV and LR-HPV types were 10.3% and 3.9% among the 387 positive samples, respectively (Table 1). A single infection with HPV16 and HPV33 was confirmed in two and one H-SIL cases, respectively. Multiple infections with HPV26/HPV31 and HPV42/HPV73 were detected in two additional H-SIL cases.

TABLE 2 Overall HPV prevalence, type distribution and multiplicity of infection, and cytological findings among 456 Chilean women referring the gynaecology department in Chile, 2012-2015

	Cytology *n* (%)				Total *n* (%)		
HPV type	**Nomal**	**ASC-US**	**L-SIL**	**H-SIL**	**Single**	**Multiple**	**Total**
Samples *n*	39	132	128	157			456
HPV+	24 (61.5)	87 (65.9)	98 (76.6)	119 (75.8)	214 (46.9)	114 (25.0)	328 (71.9)
HR-HPV+	22 (56.4)	83 (62.9)	89 (69.5)	113 (72.0)	196 (43.0)	111 (24.3)	307 (67.3)
LR-HPV+	4 (10.2)	18 (13.6)	24 (18.7)	16 (10.2)	18 (3.9)	44 (9.6)	62 (13.6)
HR infections							
16	12	39	35	67	100	53	153 (33.6)
18	2	5	5	6	9	9	18 (3.9)
31	4	15	11	18	25	23	48 (10.5)
33	2	2	4	7	6	9	15 (3.3)
35	1	2	2	5	2	8	10 (2.2)
39	1	9	7	4	6	15	21 (4.6)
45	0	3	2	1	2	4	6 (1.3)
51	0	4	13	4	2	19	21 (4.6)
52	0	7	7	8	10	12	22 (4.8)
56	0	6	13	4	7	16	23 (5.0)
58	1	12	8	11	18	14	32 (7.0)
59	0	7	11	2	2	18	20 (4.4)
66	0	7	4	6	3	14	17 (3.7)
68	1	0	2	1	3	1	4 (0.9)
73	0	1	2	3	0	6	6 (1.3)
82	0	1	2	1	0	4	4 (0.9)
LR infections							
6	3	3	5	1	4	8	12 (2.6)
11	0	1	2	0	1	2	3 (0.6)
26	1	1	0	0	0	2	2 (0.4)
32	0	0	0	0	0	0	0
34	0	0	0	0	0	0	0
40	0	0	1	1	0	2	2 (0.4)
42	0	4	8	4	5	11	16 (3.5)
44	0	0	1	2	0	3	3 (0.6)
48	0	0	0	0	0	0	0
53	0	3	2	3	2	6	8 (1.8)
54	0	6	0	2	1	7	8 (1.8)
55	0	0	1	0	0	1	1 (0.2)
57	0	0	0	1	1	0	1 (0.2)
69	0	1	2	0	2	1	3 (0.6)
70	1	0	0	2	1	2	3 (0.6)
72	0	1	0	0	1	0	1 (0.2)
81	0	0	1	0	1	0	1 (0.2)
83	0	0	1	1	0	2	2 (0.4)
84	0	1	4	2	0	7	7 (1.5)

In addition, 456 women consulting the gynaecology department were evaluated from 2012 to 2015. It was detected 328 HPV positive cases, showing a high prevalence of HPV (71.9%) (Table 2). Single and multiple HPV infections were detected in 46.9% and 25.0% women attending tertiary care, respectively (Table 2). It was detected 61.5% samples with normal cytology and simultaneously infected with HPV. Similarly, 65.9%, 76.6%, and 75.8% of ASC-US, L-SIL, and H-SIL were HPV positive, respectively. The most prevalent HPV types were HPV16

TABLE 3 High-risk HPV infections in 3691 Chilean women

HR-HPV type	Primary health care centers	Gynaecology department	Total	Ratio primary/ gynaecology
HPV16	97	153	250	0.6
HPV18	21	18	39	1.2
HPV31	35	48	83	0.7
HPV33	11	15	26	0.7
HPV35	6	10	16	0.6
HPV39	25	21	46	1.2
HPV45	17	6	23	2.8
HPV51	47	21	68	2.2
HPV52	42	22	64	1.9
HPV56	19	23	42	0.8
HPV58	29	32	61	0.9
HPV59	50	20	70	2.5
HPV66	51	17	68	3.0
HPV68	6	4	10	1.5
HPV73	16	6	22	2.7
HPV82	7	4	11	1.8

(33.6%), HPV31 (10.5%), and HPV58 (7.0%). The most frequent viruses among LR-HPV types were HPV42 (3.5%), HPV6 (2.6%), HPV53 (1.8%), and HPV54 (1.8%). The frequencies of HR-HPV genotypes and LR-HPV genotypes were 67.3% and 13.6%, respectively. It was detected 157 H-SIL cases primarily associated with HPV16 ($n = 67$), HPV31 (16), HPV58 (7), HPV18 (5), HPV33 (4), and HPV52 (4) types.

Overall, the frequency of single infection (primary care 62.8% [243/387] and 65.2% tertiary care [214/328]) and multiple infections (primary care 37.2% [144/387], and 34.8% tertiary care [114/328]) were similarly detected between both populations (Tables 1 and 2).

3.2 | HR-HPV prevalence and genotypes distribution

HPV16 was the most frequent type found, with a total prevalence of 6.8% (250/3691) in both populations: 2.6% (97/3691) in women attended in health care and 4.1% (153/3691) in women consulted the Gynaecology Hospital Department. Moreover, the frequency of other HR-HPV types showed significant differences between both studied populations (Table 3). Here, the most prevalent genotypes were HPV31 2.2% (83/3,691), HPV59 1.9% (70/3,691), HPV51 1.8% (68/3,691), and HPV66 1.8% (68/3,691) types. The smallest Primary/Gynaecology ratio (0.6) was detected with HPV16 and HPV35. This value indicates a higher frequency of these viruses among women, who consulted the reference or gynaecology department. Instead, the biggest ratios (3.0) were detected with HPV66. This finding would indicate that an increased frequency of this virus was present among women attending primary care.

3.3 | HPV type distribution according to cytology

The results of molecular biology detection and cervical cytology were analyzed in all samples to study the correlation between HR-HPV detection and Pap testing. HR-HPV types were grouped into three categories: vaccine HPV16 and HPV18 types (group 1); non-vaccine HR-HPV types with suggested cross-protection HPV31, HPV33, and HPV45 (group 2); and other HR-HPV non-vaccine types without cross-protection (group 3). The distribution of three arbitrary group of HR-HPV by cytological findings was determined (Table 4). Normal cytology was observed in 313 (9.8%) HR-HPV infected cases: 112 (3.5%) cases were infected with HPV16 or HPV18, 49 (1.5%) women were infected with HPV31 or HPV33 or HPV45, and 152 (4.8%) cases were infected with different HR-HPV from HPV16, HPV18, HPV31, HPV33, or HPV45 (Table 4). Abnormal cytology was observed in 328 (51.2%) HPV infected cases. Single HPV16 or HPV18 infections were detected in 152 (30.9%) and 17 (3.4%) cases with abnormal cytology, respectively. Multiple HPV16 and HPV18 infections were detected in 3 (0.6%) cases with abnormal cytology. Consequently, the overall prevalence of HPV16 and HPV18 was 35.0% (172/492) among women with abnormal cytology. HPV31, HPV33, or HPV45 infected cases were detected in 61 (12.4%) women with abnormal cytology. Finally, any other HR-HPV infections were detected in 95 (19.3%) cases with abnormal cytology. Finally, six ICC were detected: three

TABLE 4 Association between cervical cytological results and HR-HPV type in 3691 Chilean women

	Cytology n (%)					
HR-HPV type	Normal	ASC-US	L-SIL	H-SIL	ICC	Total
Samples *n*	3199	175	150	161	6	3691
HPV-	2833	65	35	43	0	2976
Any HR-HPV	313 (9.8)	105 (60.0)	105 (70.0)	112 (69.6)	6 (100)	641 (17.4)
HPV16	93 (2.9)	43 (24.6)	41 (27.3)	64 (39.8)	4 (66.7)	245 (6.6)
HPV18	17 (0.5)	6 (3.4)	6(4.0)	5(3.1)	0 (0.0)	34 (0.9)
HPV16 and HPV18	2 (0.0)	2 (1.1)	0(0.0)	1(0.6)	0 (0.0)	5 (0.1)
HPV31, HPV33, or HPV45	49(1.5)	25 (14.3)	13 (8.7)	23 (14.3)	0 (0.0)	110 (3.0)
Any HR-HPV without HPV 16,18,31,33,45	152 (4.8)	29 (16.6)	45 (30.0)	19 (11.8)	2 (33.3)	247 (6.7)

Normal, negative for intraepithelial lesion or malignancy; ASC-US, atypical squamous cells of undeterminated significance; L-SIL, low grade squamous intraepithelial lesion; H-SIL, high grade squamous intraepithelial lesion; ICC, invasive cervical cancer.

single HPV16 infected, one single HPV52 infected, one single HPV56 infected, and one multiple HPV16 and HPV66 infected.

4 | DISCUSSION

In this article, we presented a 12.0% HPV prevalence result among women who visited primary health care centers. Consequently, this HPV prevalence was in accordance with the 10.7% and 11.8% positive rate reported in other previous studies among Chilean women.[11,12] Furthermore, this HPV prevalence is alike to those described by other Latin American countries among women from similar populations: Mexico, 8.6-14.5%[12–14]; Brazil, 10.5-12.3%[13–16]; Argentina, 16.6%[15,17]; Peru, 12.6%[16,18]; Colombia 14.6%.[17,19] In contrast, the Chilean prevalence of infection is higher than in many regions of Europe[18–22]and Asia.[21–24]

Epidemiological data on LR-HPV prevalence in Chile showed consistent findings with previous studies in other Latin American countries. Here, it was found that HPV42 was the most common type of LR-HPV, both among women attending health care centers and gynaecological services. Furthermore, HPV6 and HPV53 were the second and third common types, respectively. In addition, HPV11 was the seventh and eighth LR-HPV prevalent type among women from primary care centers and the department of gynaecology, respectively. Previously, low prevalence of HPV6 and HPV11 were similarly reported among Chilean women attended in primary care.[11,23] Here, the prevalence of HPV6 and HPV11 were 0.6% and 0.2%, respectively. Frequencies of 0.2% and 0.4% with HPV6 and HPV11 were previously detected in Chile.[11,23] This situation seems to be different in North America and other continents, where HPV6 and HPV11 prevalence fluctuates between 0.5-2.9% and 0.8-5.9%, respectively.[21,24,25] However, the prevalence of HPV6 and HPV11 infection among Chilean women is alike to those described among women from similar populations in different Latin American countries: Mexico, 0.4-1.9% HPV6 and 0.2-1.0% HPV11[13,25,26] Brazil, 0.5-2.1% HPV6 and 1.6-2.3% HPV11[27,28] Argentina, 0.0% HPV6 and 0.4% HPV11.[15,17] Also, it was reported a prevalence of 0.4% and 0.3% for HPV6 and HPV11 in Spain, respectively.[29]

In addition, we detected unique findings about HR-HPV epidemiology in Chile. Here, HPV16 was the most common type among Chilean women attended either primary or tertiary level. This finding is comparable with HPV16 prevalence in most other regions of the world. However, HPV18 prevalence was very low in the same group. This viral type showed the ninth and eighth HR-HPV prevalence among women attending primary health centers and gynaecological service, respectively. This finding is not consistent with results found in other Latin American countries.[21,24] Furthermore, HPV59, HPV66, and HPV51 were the second, third, and fourth common types, respectively. The HPV66 type is one of the most prevalent HR-HPV among women attending primary health centers in Santiago which was an unexpected result and different from that the one previously reported in Chile.[11,23] We hypothetize that this previous study probably underestimated HPV66 detection because generic primers GP5+/6+ PCR and HPV probes cocktails coupled to an ELISA were used to detect and typing simultaneously HPV56, HPV66, and other HPV.[11,23] It was described that PGMY09/11 primers are more sensitive than GP5+/6+ primers to detect a wider range of HPV types in cervical samples, especially with regard to multiply infected samples.[30,31] The HPV66 frequency found in this study was higher than those described in the world.[3,7,24] However, HPV66 positive rate was similar to the frequency detected in some new studies with normal cytology cases.[29,32] Similarly, HPV16 was the most prevalent type in women who visited the gynaecology department. Furthermore, HPV31, HPV58, and HPV56 were respectively the second, third, and fourth common types in this population (Table 2). Overall, HPV16 is the most prevalent viral types in Chilean women. HPV31, HPV59, HPV51, and HPV66 were the second, third, and fourth common types, respectively (Table 3).

HPV16, 18, 31, 33, and 35 are the most prevalent HPV types in cervical cancer worldwide.[33] HPV16, 18, and 31 were reported the three most common type in H-SIL and ICC in Latin America.[34] In our study, high-risk HPV16 was the most prevalent genotype in H-SIL among women who visited the primary public care centers (Table 1). However, high-risk HPV16, 31, and 58 were the most prevalent genotypes in H-SIL among women who visited the gynaecology department (Table 2). Women attending the gyneacology unit are most probably referred due to abnormal cytology or symptoms. Consequently, they are a higher risk group for cervical pre-cancer and cancer. This explains the high HR-HPV prevalence among them. Overall, the most prevalent HR-HPV types were HPV16, 31, and 58 in H-SIL in Chilean women. Instead, HPV18 was found the ninth prevalent HR-HPV in H-SIL. Recently, Mexico reported a frequency of HPV18 lower than the rest of the world. In addition, a high prevalence of HPV58 and HPV52 was detected in ICC cases.[26] Moreover, HPV66 and 16 were the most prevalent HPV types among abnormal cytology in Argentina.[35] These findings suggest that HPV18 in H-SIL among Chilean women is less frequent as described in others countries from the region. These findings would indicate the different prevalence of HPV types in Latin America countries. Also, they demonstrate the need to carry out new research in women with abnormal cytology.

As far as we know, there are two effective vaccines to prevent HPV infections associated with cervical cancer: the bivalent vaccine, which targets HPV16 and HPV18, and the quadrivalent vaccine, which in addition targets HPV6 and HPV11. The immunogenicity of HPV vaccines for the non-vaccine HPV types (HPV31, HPV33, HPV35, HPV52, and HPV45) was evaluated.[36] Neutralizing antibodies against HPV31, HPV52, HPV33, and HPV45 in vaccinated girls (aged 12-15 years) were significantly higher with bivalent than quadrivalent HPV vaccine.[36] Moreover, it was detected a positive association between neutralizing antibodies to non-vaccine HPV types and efficacy against persistent infection and CIN 2 or worse.[36] Another study showed that the bivalent vaccine had greater efficacy than the quadrivalent vaccine against persistent infection with HPV31 and HPV45.[37] Also, it was reported the bivalent vaccine induced higher titer of HPV31 and HPV45 cross-neutralizing antibodies than the quadrivalent vaccine.[38] However, follow-up studies suggested that these antibodies may decrease over time.[37] Currently, it is unclear the meaning or impact of

different levels of antibodies detected between the two vaccines. A correlation between these antibodies levels and effective protection is unknown.[39,40] A follow-up study suggested that quadrivalent vaccine would have efficacy against the onset of CIN 1-3 or adenocarcinoma in situ associated with 10 non-vaccine HPV types.[41] However, cross-protective efficacy was only significant for HPV31.[37]

Since 2014, Chile has been implemented HPV vaccination program with quadrivalent vaccine in girls between 9 and 11 years-old. However, it has not been shown some cross-protection by this vaccine against other non-vaccine HPV types, that is, HPV39, HPV51, HPV56, HPV58, HPV59, and HPV66. Consequently, in Chile where HPV39, HPV51, HPV52, HPV56, HPV58, HPV59, and HPV66 were more prevalent than HPV18, the current vaccines could not be enough in terms of efficacious protection. The use of vaccines designed only against some HPV types could have a significant effect on the selective pressure of other circulating HPV.[42] In countries with HPV vaccination programs with coverage greater than 50%, a significant decrease in the circulation of HPV16 and HPV18 has been observed among girls (by 64% in girls 13-19 years of age). In addition, among non-vaccine HPV types, only significant decreases in HPV31, 33, and 45 were detected in these populations. This finding suggests cross-protection with these non-vaccine HPV types. However, HPV vaccines would not affect the prevalence of other HR-HPV types, for example, HPV52 and 58, which did not suggest cross-protection against these types.[37] Here, we found that 7.6% (284/3691) of women were infected with HPV16 and/or 18 (Table 4). Consequently, it is possible to hypothesize that this population could have been protected by immunization with quadrivalent vaccine. The 3.0% (110/3691) rate of women that were infected with HPV31, HPV33, and/or HPV45, could have been protected by cross-protection with quadrivalent vaccine. However, 6.7% (247/3,691) of women that were infected with non-vaccine HPV types 35, 39, 51, 52, 56, 58, 59, 66, 68, 73, or 82 would have not showed cross-protection or group protection. Consequently, quadrivalent vaccine could not be enough to prevent the HPV infection in these women. Recently, a new nonavalent vaccine that incorporates five additional HR-HPV (HPV31, 33, 45, 52, and 58) has been developed.[43,44] If this vaccine had been used in these Chilean women, it would have hypothetically protected an additional 1.5% (57/3691). Therefore, it would be desirable to evaluate the impact on public health and resource savings that can achieve by introducing the nonavalent vaccine in Chile.

The scope of our findings is limited because we could not provide results of colposcopy among both studied female groups. These confirmatory results would be useful to analyze the association of the HR-HPV types with cytology in their samples. Moreover, our report represents a first analysis of HPV prevalence in Chilean women from two levels (primary and tertiary) of health care and therefore, they should be interpreted cautiously. Further studies with a larger number of cases and other geographic locations are required. Additionally, surveillance studies based on vaccinated Chilean girls could be very helpful to understand cross-protection with non-vaccine types.

In conclusion, our research shows important epidemiological information on the pre-vaccine prevalence of HPV types among Chilean sexually active women. Our findings might be useful to assess the effect of HPV vaccination programs in Chile during the next years.

ACKNOWLEDGMENT

The authors gratefully acknowledge the comments and suggestions provided by Dr. Alexis Aceituno of the Agencia Nacional de Medicamentos (ANAMED) at the Instituto de Salud Pública de Chile.

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How to cite this article: Vergara N, Espinoza G, Balanda M, et al. Prevalence of Human Papillomavirus infection among Chilean women from 2012 to 2016. *J Med Virol*. 2017;89:1646–1653. https://doi.org/10.1002/jmv.24805