Research Article

Cancer Prevention Research

Detection and Genotyping of HPV DNA in a Group of Unvaccinated Young Women from Colombia: Baseline Measures Prior to Future Monitoring Program

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Abstract

In 2012, Colombia launched human papillomavirus (HPV) vaccination program for girls ages 9 to 12, and in 2013, the target age was expanded to 9 to 17 years. Monitoring the changes of HPV infection prevalence among young women has been proposed as an endpoint for early assessment of HPV vaccination programs. However, the data on HPV prevalence in young ages are very limited. The purpose of this study was to determine the prevalence of HPV infection and the distribution of genotypes in a group of nonvaccinated women ages 18 to 25 years old in three Colombian cities as baseline for the monitoring of the HPV national vaccination program. A total of 1,782 sexually active women were included. Cervical smear samples were collected to perform the Pap smear and HPV DNA detection using a Linear Array HPV assay. Of the 1,782 specimens analyzed, 60.3% were positive for any HPV type; 42.2% were positive for high-risk HPV (HR-HVP) types, and 44.4% for low-risk HPV (LR-HPV) types. Multiple and single infections were identified in 37.1% and 23.2% of samples, respectively. HR-HPV types -16, -52, and -51 were the most predominant with proportions of 11.3%, 7.92%, and 7.9%, correspondingly. The prevalence for HR-HPV 16/18 was 14.4%. HR-HPV prevalence in women with abnormal cytology (75.16%) was higher than in women with normal cytology (38.6%). In conclusion, a high prevalence of HR-HPV was observed among younger women. This HPV type-specific prevalence baseline may be used to monitor postvaccination longitudinal changes and to determine its impact on HPV-related disease incidence in Colombia population. *Cancer Prev Res; 11(9); 581–92.* *©2018 AACR.*

Introduction

Following the establishment of persistent HPV infection as a causal agent of cervical cancer, HPV prophylactic vaccination has been proposed as one of the most important strategies to effectively reduce the burden of this type of cancer worldwide. Three prophylactic human papillomavirus (HPV) vaccines have been shown to be highly effective to prevent the development of precancerous lesions caused by high-risk HPV (HR-HPV) included in the vaccine, principally HPV 16 and 18 types (1–7). As a result, The World Health Organization (WHO) recommends the inclusion of HPV vaccine in national immunization programs having as priority the population consisting of girls ages 9 to 14 years, prior to becoming sexually active and as secondary target females ages $\geq$15 years or males (only when feasible, cost-effective, and does not divert resources from vaccination of the primary population; refs. 8–11).

Aiming at preventing cervical cancer and genital warts, in 2012, quadrivalent HPV vaccination was included in the Colombian National Immunization Program for girls ages 9 to 12 years. In 2013, the target age was expanded to 9 to 17 years old and the original 0-2-6-month immunization schedule was modified to an alternative one of 0-6-60 months. The latter modification was based on

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Note: Supplementary data for this article are available at Cancer Prevention Research Online (http://cancerprevres.aacrjournals.org/).

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published evidence showing that the immunogenicity of 2-dose prime boost schedule administered with a 6-month interval was not inferior to that observed in the 3 dose prime-prime-boost schedule (12–14).

With the introduction of HPV vaccination, a change in the dynamic of HPV transmission is expected. Therefore, it is imperative to implement HPV monitoring programs that allow the evaluation of the changes in the prevalence of HPV infection (particularly for HR-HPV types) as well as epidemiologic changes in disease patterns associated with HPV-associated disease (15).

Although in Colombia, information regarding the prevalence of HPV infection in women with normal cytology, preneoplastic lesions, and cervical cancer exists (16), this information for the age group 18 to 25 years is limited, which constitutes a drawback for comparing the prevalence of HPV infection and disease between unvaccinated and vaccinated girls.

Several studies have shown that HPV is often acquired shortly after the first sexual intercourse and that the highest prevalence of HPV infection is found in women $\leq$25 years old (17). In this context, the evaluation of overall HPV prevalence has been proposed as a short-term biological endpoint for determining the impact of HPV vaccination programs (18). Therefore, the goal of this study was to determine the baseline prevalence of HPV infection and their distribution among women ages 18 to 25 years, not vaccinated for HPV in three Colombian cities: Soacha, Girardot, and Manizales. The results of this study are expected to be used as the baseline for the monitoring of future changes in overall and type-specific HPV prevalence after HPV vaccination.

Materials and Methods

Study population

This study was conducted in three different Colombian cities: Soacha, Girardot, and Manizales. Girardot and Soacha were selected because the team has an ongoing HPV screening project, which made it easier to understand the purpose of this study. Manizales was selected because this city has a well-structured population-based cancer registry (19), allowing long term to evaluate the effectiveness of the HPV vaccine program in this population. Because the age range in this study includes women younger than 21, women were sampled not only at primary health care facilities for Pap smear screening but also at universities (6 in Manizales and 2 in Girardot and Soacha each) and technical institutions (4 in Manizales and 1 in Girardot in Soacha each) in order to also include those women aged 18–25 years that did not visit the already mentioned primary health care facilities. In Colombia, cervical cancer screening starts at 21 years old or 3 years after the onset of sexual activity. Usually asymptomatic or nonpregnant women from 18 to 25 years do not attend health centers for screening. The women were invited through different open communications strategies like local radio broadcast announcements, written communication, poster and flyers, or personal invitation by classroom established at local health centers and higher education institutes.

From May 2014 to February 2015, sexually active women between 18 and 25 years old from these cities were requested to participate in this study. Women were eligible if they had never been vaccinated against HPV and did not suffer from mental impairment. Exclusion criteria included women who were pregnant, had undergone hysterectomy, or if they never had sexual intercourse. Women who voluntary accepted to participate signed an informed consent form and answered a questionnaire including basic demographics and determinants of HPV infection (i.e., age, education degree, marital status, age at sexual debut, number of sexual partners, use of contraceptive methods, age at first gravidity and number of children). Thereafter, each woman underwent gynecologic examination, and cervical samples were taken to perform the Pap smear and the HPV DNA detection and genotyping.

Sample collection and Pap smear testing

Cervical samples were obtained from both the endocervix and the exocervix by using a Cervex-Brush (Rovers Medical Devices) that was introduced into the cervical canal and rotated 360 degrees 5 times. The sample obtained was spread on the slide using conventional technique and then the brush-head was detached into a vial with 20 mL of PreservCyt transport medium (Roche Diagnostics, GmbH) for HPV testing. Cervical samples were stored at -20°C until use. The cytologic slides were then referred to COLCAN, a certificate clinical laboratory offering Pap smear reading services. The slides were stained with Papanicolaou stain, evaluated for a pathologist-supervised cytotechnologist, and classified according to The Bethesda System 2014. For quality control, an expert pathologist again evaluated the positive cervical smears and 10% of negative cervical smears.

DNA extraction from cervical samples

One aliquot of 1 mL PreservCyt media was centrifuged at 6,000 rpm for 10 minutes to pellet the cervical exfoliated cells. After removing the supernatant, DNA extraction was performed using the Qia-cube with the AmpliLute Liquid Media Extraction Kit according to the manufacturer's instructions (Roche Diagnostics). DNA was eluted in 100 mL of elution buffer.

HPV genotyping

The HPV detection and genotyping was assessed by Linear Array HPV Genotyping Tests (Roche Diagnostics), which detects 13 HR-HPV and 24 low-risk HPV (LR-HPV) types. HPV 52 is not determined directly by a type-specific probe but rather by a probe that cross -hybridizes with HPV 33, 35, 52, and 58. According to the manufacturer's

protocol, samples that are negative for HPV 33, 35, and 58 individually, but positive for the cross-reactive probe, are classified as HPV 52 positive. Samples that are positive for HPV 33, 35, and/or 58 individually, as well as the cross-reactive probe, have an unclear HPV 52 status. For our study, these samples were considered negative for HPV 52.

Fifty microliters of purified DNA was transferred to a tube with 50 μL of HPV LINEAR ARRAY Master Mix, and the mixture was amplified by PCR using a MJ Research PTC-200 Peltier Thermal Cycler. Controls for contamination and assay sensitivity were included in each 96-well assay. Negative samples were included in each assay to demonstrate the specificity of the test. Hybridization steps up to color development were performed by the Roche HPV LA Detection Kit using a ProfiBlot T48 instrument (Tecan Group). Results were visually interpreted. In addition to HPV type-specific prevalence, the combined prevalence of different clusters of HPV types was also estimated. The groups were based on the risk for cervical cancer (HR-HPV included HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68 and low-risk types included HPV 6, 11, 26, 40, 42, 53, 54, 55, 61, 62, 64, 66, 67, 69, 70–73, 81–84, IS39, and 89). Groups based on types targeted by HPV vaccines are as follows: quadrivalent Gardasil (HPV 6, 11, 16, 18) or nonavalent vaccine (HPV 6, 11, 16, 18, 31, 33, 45, 52, 58), and other nonvaccine types are as follows: HPV 35, 39, 51, 56, 59, and 68. To determine the factor associated with HPV infection, we established three HPV subgroups: HPV 16/18 (subgroup A), other HR-HPVs included in the nonavalent vaccine different to HPV 16 and 18 (subgroup B) and other HR-HPVs not included in the vaccines (subgroup C).

Statistical analysis

Prevalence of type-specific HPV was estimated as the proportion of participants who tested DNA positive for a given DNA HPV type. Additionally, the cytological reports were stratified by HPV genotyping results. Comparisons of HPV types' prevalence between cities and Pap smear results were done using χ^2 statistic. Adjusted prevalence estimates and 95% confidence intervals were calculated for prespecified HPV type subgroups according to well-known HPV infection determinants using robust Poisson regression methods. Any difference across the strata of these variables was considered statistically significant at $P < 0.05$. Statistical analysis was performed with STATA 11.2 and SPSS 19 software.

Results

Study population

Of the 2,002 women enrolled, 220 were excluded, because in 125 women, the samples were not suitable for HPV typing and in 95, the samples were B-globin negative. Of the 1,782 samples included, 738 samples were from Girardot, 951 from Manizales, and 93 from Soacha. Because from the aforementioned 1,782 samples, 122 samples had no cytologic report and one had a cytology interpreted as atypical glandular cells, the analysis for HPV prevalence by cytology was made in only 1,659 samples.

Characteristics of the study population

Mean age of participants was 21.4 years. A total of 45.3% of participants had completed secondary school. More than half of the participants (71.0 %) had their first intercourse between 14 and 17 years old and only 7.3% before age 14. Sixty-one percent of women were using hormonal contraceptive methods, whereas 11.8% reported no use of any contraceptive method. A total of 62.6% women enrolled had between two and five regular sexual partners and 10.9% had more than five sexual partners. A total of 22.1% reported to have casual sexual partners (Table 1).

HPV prevalence

Of the 1,782 specimens analyzed, 60.3% women were positive for any HPV type, 42.2% were positive for HR-HPV types, and 44.4% for LR-HPV types (Fig. 1A). No differences were observed across the participant cities. Among HPV-positive women, multiple and single infections were identified in 61.5% and 38.5% of samples, respectively. The maximum number of coinfection by LR-HPV and HR-HPV types in an individual was 11 (Fig. 1B).

The combined prevalence for HPV 16 and 18 types was 14.4%, whereas the prevalence of any HR-HPV types included in the nonavalent vaccines (HR-HPV 16, 18, 31, 33, 45, 52, and 58) was 30.75%. For this latter group a statistically significant lower prevalence was observed in Soacha when compared to Girardot and Manizales ($P = 0.019$) (Fig. 2A). However, the difference of prevalence between cities for HPV types 16 and 18 (included in the tetravalent vaccine) was not statistically significant ($P > 0.05$). HR-HPV types -16, -52, and -51 were the most predominant types with prevalences of 11.3%, 7.92%, and 7.9%, respectively, whereas the prevalence of HPV-18 was 3.93%, ranking ninth out of 13 HR-HPV types included in the analysis (Fig. 2A). Regarding LR-HPV types, the most frequent types were HPV 53 (7.01 %) followed by HPV CP6108 (6.3%) and HPV 61 (6.0%). HPV 6 and 11 were identified in 2.92% and 0.4% of the specimens, respectively. The LR-HPV analysis by city showed a higher prevalence in Girardot, although no statistical significant differences were observed (Fig. 2B).

HR-HPV infection prevalence according to socio-demographic and behavioral sexual characteristics

The multivariate model showed that there was a trend for increasing HPV prevalence with each year of age up to women ages 20 to 21 years for all three groups, which was followed by a gradual decline through 25 years.

Table 1. Characteristics of the study population

Characteristics	Girardot % ($n = 738$)	Manizales % ($n = 951$)	Soacha % ($n = 93$)	Total % ($N = 1,782$)
Age (years)				
18–21	47.8 (353)	54.4 (517)	58.0 (54)	51.8 (924)
22–25	52.2 (385)	45.6 (434)	42.0 (39)	48.1 (858)
Education				
Elementary or none	15.0 (117)	11.4 (108)	8.6 (8)	13.1 (233)
Secondary	47.9 (353)	44.2 (421)	35.5 (33)	45.3 (807)
Technical	20.5 (152)	25.9 (246)	43.0 (40)	24.6 (438)
University	14.5 (107)	17.1 (163)	12.9 (12)	15.8 (282)
Without date	1.2 (9)	1.4 (13)	0	1.2 (22)
Contraception				
Hormonal	49.3 (364)	71.5 (680)	46.3 (43)	61.0 (1,087)
Barrier	35.6 (263)	17.2 (63)	37.6 (35)	25.9 (461)
Surgery	0 (0)	2.4 (23)	0	1.3 (23)
No family planning	14.9 (110)	8.9 (85)	16.1 (15)	11.8 (210)
Without date	0.2 (1)	0	0	0.05 (1)
Life sexual partners				
1	24.4 (180)	23.7 (226)	38.7 (36)	24.8 (442)
2–5	61.4 (453)	64.1 (609)	57.0 (53)	62.6 (1,115)
>5	11.8 (87)	10.9 (104)	3.2 (3)	10.9 (194)
Without date	2.4 (18)	1.3 (12)	1.1 (1)	1.7 (31)
Casual sexual partners ever				
Yes	19.3 (142)	25.0 (238)	16.1 (15)	22.1 (395)
No	79.9 (590)	74.7 (710)	82.8 (77)	77.3 (1,377)
Without date	0.8 (6)	0.3 (3)	1.1 (1)	0.6 (10)
Sexual onset (years old)				
<14	6.4 (47)	8.3 (79)	4.3 (4)	7.3 (130)
14–17	69.9 (516)	71.6 (681)	73.1 (68)	71.0 (1,265)
>17	23.2 (171)	19.9 (189)	22.6 (21)	21.4 (381)
Without date	0.5 (4)	0.2 (2)	0	0.3 (6)
Number of gestations				
None	47.4 (350)	53.7 (511)	37.6 (35)	50.3 (896)
1	30.9 (228)	32.8 (312)	29.0 (27)	31.8 (567)
2	13.9 (103)	9.6 (91)	23.7 (22)	12.1 (216)
>3	7.6 (56)	3.9 (37)	9.7 (9)	5.7 (102)
Without date	0.2 (1)	0	0	0.6 (1)

According to behavioral sexual characteristics, this analysis showed that women who reported having had casual sexual partners had a higher HPV prevalence in all prespecified HPV subgroups ($P < 0.05$). Moreover, it was observed that an upward trend of HPV prevalence with increasing number of lifetime sexual partners and age of sexual onset with subgroup B and C but not subgroup A. Aside from the inverse association of subgroup B with number of pregnancies, no other statistical significant associations were observed in the analysis (Table 2).

HPV prevalence by Pap smear result

The Pap test result was available for 1,659 of 1,782 cervical smears tested for HPV, where 91.0% (1,510) of participants had a normal cytology and 57.0% (861/1,510) of them had an infection by any type of HPV and 38.6% (583/1,510) had HR-HPV infections. HPV 16 and 18 were detected in 9.9% and 3.4% of women, respectively, as shown in Fig. 3. The HR-HPV 16/18 types were detected in 12.9%, whereas the HR-HPV types included in the nonavalent vaccine were detected in 27.9% of samples. A total of 21.5% were found to have other nonvaccine HR-HPV infection.

From 149 women (8.97 %) with abnormal cytology, 103 (6.2%) were reported as ASC-US, 3 (0.2%) as ASC-H, 40 (2.4%) as L-SIL, and 3 with H-SIL (0.2%). The prevalence of any HPV in women with cervical cytologic abnormalities was 87.9% (131/149), whereas for HR-HPV, it was 75.16% (112/149). For ASC-US, 84.5% had an infection for any type of HPV. The prevalence of HPV 16 and 18 was 18.5% and 5.8%, respectively. The HR-HPV 16/18 types were detected in 22.3%, whereas HR-HPV types included in the nonavalent vaccine were detected in 56.3% of samples. The HPV 16/18 infection varied little with the severity of cytologic abnormality: 33.3% for ASC-H, 37.5% for L-SIL, and 33.3% for H-SIL, whereas HR-HPV types included in the nonavalent vaccine were detected in 100.0%, 57.5%, and 33.3%, respectively. For other HR-HPV types not included in the vaccine, the prevalence was 60.0% and 66.7% to L-SIL and H-SIL, respectively. It should be noted that only 3 women were diagnosed with ASC-H and H-SIL. In addition, 61.7% of women with cervical cytologic abnormalities had coinfections with different HR-HPV types, whereas 26.2% only had a single infection and a higher proportion of these coinfections were associated with the HR-HPV

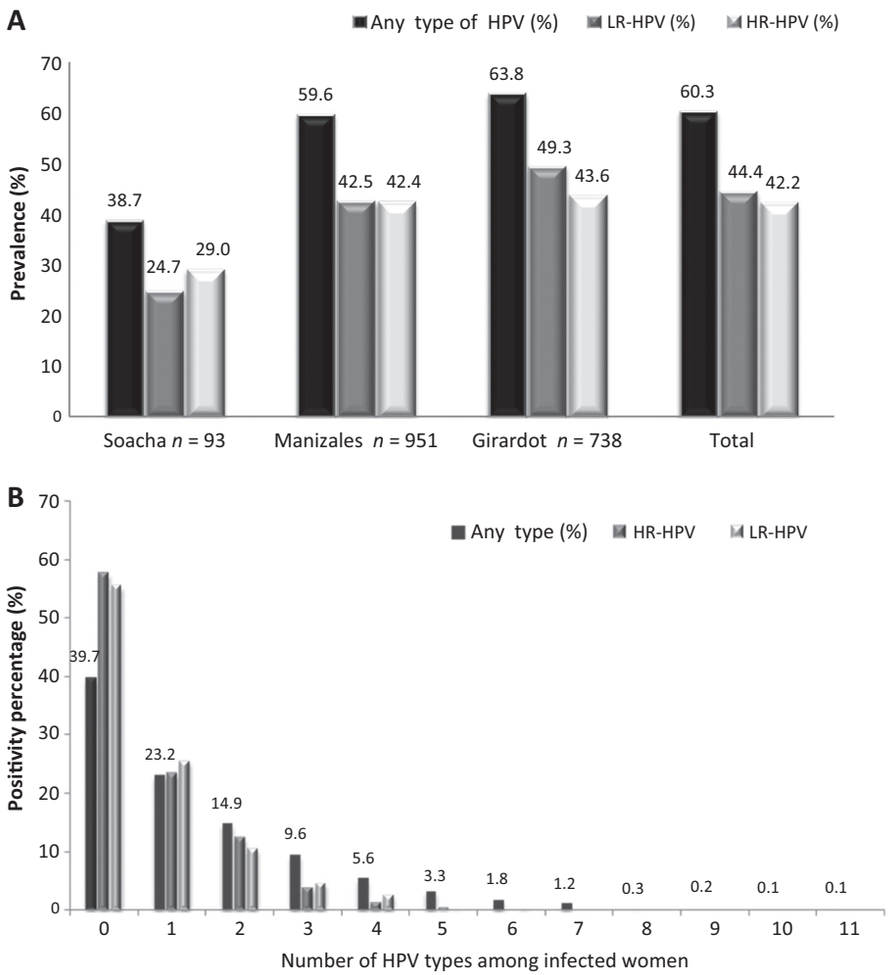

Figure 1.
A, Overall HPV prevalence by municipality.
B, Frequency of multiple HPV infections.

types included in nonavalent vaccine (Supplementary Fig. S1).

Discussion

In Colombia, the recent introduction of the HPV vaccine program for girls ages up to 17 years represents a new opportunity for the prevention of cervical cancer. Nevertheless, as in any new strategy for primary prevention, the assessment of its impact is required.

It has been established that a reduction in the prevalence of vaccine types 6, 11, 16, and 18 in young women could measure the initial impact of HPV vaccine program. Yet, prior to initiating comprehensive monitoring of changes in HPV prevalence, it is necessary to measure the baseline prevalence of HPV infections in the age group of interest in order to assess any change while comparing unvaccinated and vaccinated women (15). In Colombia, there are few data on the frequency of HPV infections in sexually active young women. This study reports the HPV prevalence and genotype distribution among sexually active young women not exposed to the National Immunization Program in three cities of Colombia.

The results show that HPV infection is very common among sexually active young women. A total of 60.3% of participants were HPV positive, which confirms that HPV is highly endemic in our population (20). This prevalence is greater to the prevalence reported in other regions like Brazil, United States, and Europe, which has ranged from 25% to 49.9% among sexually active women (15, 21–26). However, the high prevalence of HPV observed here is in line with other studies in Sweden and Africa where prevalences close to 70% among nonvaccinated young women has been reported (27, 28).

Our prevalence of 42.2% for HR-HPV among young women is consistent with previous studies, reporting HR-HPV prevalence ranging from 15.3% to 54.5% among women under 25 years old (15, 21–29). In addition, the peak of HPV infection in our population was observed among women ages 20 to 21 years old. These findings are similar with other reports where the highest prevalence is achieved between 19 and 21 years (15, 18, 27, 28).

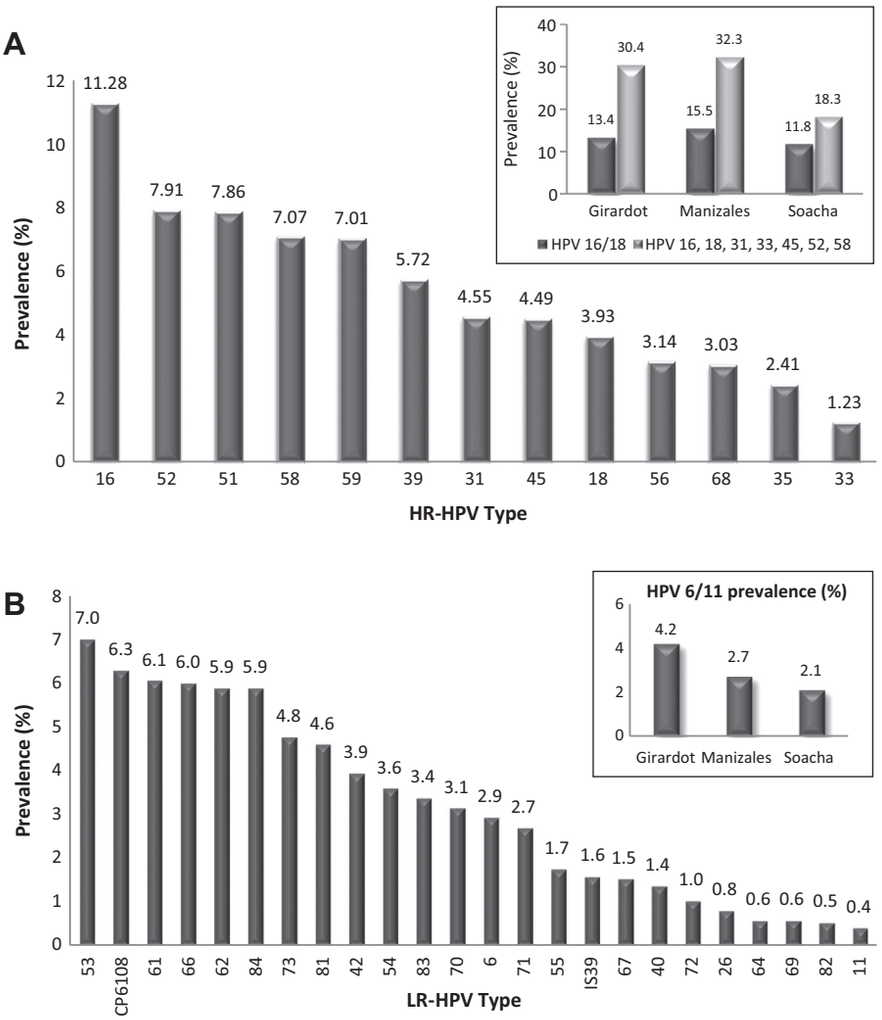

Figure 2.
A, Prevalence of high-risk HPV infections.
B, Prevalence of LR-HPV.

Nevertheless, in other regions, the peak is observed between 21 and 23 years (29, 30). These variations could be explained by differences in the onset of sexual activity of the populations. The median age at the first sexual encounter in our study was 15.6 years, similar to reported by Watson-Jones in an African population, in which the median age was 16 years and the peak at 19 to 21 years of age (28) and different to the reported in the studies in France and Italy, in which the median age at the first sexual encounter was 16.5 years old (29, 30). Similar to other studies of the same age group, the number of sexual partners and the age of the first sexual intercourse were confirmed as determinant of HPV infection in our population (24, 30). However, it was only significant for other HR-HPV different to HPV 16 and 18. Another important risk factor for any HR-HPV infection in our population was a history of having casual sexual partners. These observations confirm on one hand that women under 25 years old represent the most relevant group for detecting an early impact of HPV vaccination on the prevalence of HPV (15) and that the most suitable age for HPV vaccinations is the period preceding sexual activity.

The distribution of HPV genotypes varies greatly worldwide likely in relation to the geographical complex and biological interplay between different HPV types and host immunogenetics factors (31). In this study, the prevalence dates and distribution of most common genotypes found in our population are consistent with the types found worldwide showing a low regional variation (32). The most common HPV types reported in women with normal cytology were HPV 16 (3.2%), HPV 18 (1.4%), HPV 52, (0·9%), HPV 31 (0·8%), HPV 58 (0·7%), and HPV 51 (0·6%), which contribute about 50% of all HPV infections (32). In our population, the most prevalent genotype detected was HPV 16, followed by HPV 52, 51, 58, 59, and 39. HPV 16 was the most common genotype, being present in 9.9% among women with normal cytology and 11.28% in all women, whereas the second most frequent type was HPV 52, being present in 7.1% among women with normal cytology and 7.91% of HPV DNA positive

Table 2. Adjusted estimates of HR-HPV infection prevalence according to socio-demographic and behavioral sexual characteristics

	HPV 16/18		HPV 31/33/45/52/58		Other HR-HPV not included in available vaccines	
Characteristics	**Prevalence (95% CI)**	***P***	**Prevalence (95% CI)**	***P***	**Prevalence (95% CI)**	***P***
Age (years)						
18–19	15.69 (12.05–19.32)		22.39 (17.95–26.82)		27.91 (22.80–33.02)	
20–21	16.37 (13.07–19.66)		24.71 (20.74–28.68)		25.44 (21.28–29.60)	
22–23	13.16 (9.97–16.35)		21.66 (17.65–25.67)		21.44 (17.46–25.42)	
24–25	12.39 (8.77–16.00)	0.06	18.18 (13.94–22.42)	0.01[a]	18.78 (14.55–23.00)	<0.01[a]
Education level						
None	9.07 (−8.20–26.35)		0		19.88 (−3.84–43.59)	
Elementary	13.73 (8.92–18.55)		23.21 (14.66–31.77)		21.73 (15.41–28.04)	
Secondary	16.32 (13.70–18.93)		23.56 (18.48–28.63)		24.74 (21.36–28.11)	
Technical	12.54 (9.36–15.72)		21.13 (18.02–24.24)		23.17 (18.92–27.41)	
University	13.33 (9.37–17.29)		19.24 (15.33–23.15)		21.36 (16.31–26.41)	
Oher	57.41 (−3.90–118.72)	0.3	0	0.53	81.67 (−61.94–225.29)	0.51
Age first sexual intercourse (y)						
<14	14.28 (7.85–20.71)		10.92 (5.63–16.21)		16.67 (10.17–23.17)	
14–17	14.91 (12.92–16.89)		22.66 (19.96–25.36)		22.78 (20.12–25.44)	
>18	13.37 (9.54–17.20)	0.54	24.25 (19.12–29.39)	0.01[a]	28.65 (23.03–34.28)	<0.01[a]
Number of life sexual partners						
1	12.71 (9.27–16.15)		15.35 (11.72–18.98)		15.81 (12.25–19.37)	
2–4	15.56 (13.41–17.70)		23.51 (20.72–26.30)		25.73 (22.72–28.74)	
5[b]	12.85 (8.06–17.63)	0.63	28.39 (21.33–35.46)	<0.01[a]	28.49 (21.28–35.70)	<0.01[a]
Casual sexual partners ever						
Yes	18.87 (14.65–23.08)		26.44 (21.70–31.19)		28.42 (23.34–33.49)	
No	13.33 (11.49–15.16)	0.01	20.59 (18.14–23.04)	0.03	21.96 (19.37–24.56)	0.04
Pregnancies						
None	14.15 (10.00–18.31)		27.68 (17.63–37.74)		22.1 (17.24–26.96)	
1	14.91 (9.27–20.55)		19.57 (13.67–25.48)		25.35 (18.41–32.28)	
2	14.69 (6.55–22.82)		15.18 (8.90–21.45)		28 (17.34–38.66)	
>3	16.84 (5.93–27.74)	0.84	12.37 (5.32–19.43)	0.01[a]	18.55 (8.19–28.91)	0.78
Contraception						
Hormonal	15.04 (12.86–17.22)		22.52 (19.77–25.27)		23.97 (21.03–26.92)	
Barrier	12.99 (9.86–16.11)		22.23 (18.19–26.26)		23.12 (18.99–27.25)	
Surgery	22.32 (3.97–40.68)		35.11 (13.00–57.22)		32.29 (9.98–54.60)	
Natural	59.46 (−45.47–164.39)		51.71 (−4.34–107.77)		49.77 (−40.87–140.41)	
None	14.21 (9.40–19.02)	0.38	16.87 (11.60–22.15)	0.21	20.06 (14.30–25.82)	0.2
Age first childbirth						
<14	10.0 (0.1–98.5)		64.61 (−20.48–149.70)		0	—
14–17	13.41 (7.70–19.11)		28.9 (15.81–41.98)		21.03 (14.80–27.25)	
18[b]	11.4 (6.66–16.13)		25.09 (14.04–36.15)		21.81 (15.71–27.90)	
No childbirth	16.66 (11.13–22.18)	0.41	19.28 (14.53–24.03)	0.1	25.31 (19.27–31.35)	0.72

[a]P_{trend}.
[b]Poisson regression post estimates with robust error variance.

women. The high prevalence of this genotype has been reported also in others populations including Eastern Africa, Japan, and Eastern Asia among women with normal cytology and even in women with abnormal cytology. However, previous studies in Colombia have shown HPV 58 as the second most prevalent type in young women in cervical samples, but in urine samples, HPV 52 was the second type (20). These differences might be explained by differences in the method used to detect HPV DNA. Although HPV 18 is the second type more reported after HPV 16 in other populations, in our population, HPV 18 was observed in ninth position; however, their frequency is still significant (3.93%). Near one third of HPV samples exhibited HPV 16/18 infection like in the other population (21). Prevalence has been shown to be highly variable by geographical region and age depending on sexual behavior, host control of the virus, and widespread use of high-quality cervical cancer screening.

On the other hand, a lower prevalence of HR-HPV types included in the nonavalent vaccine was observed in Soacha compared with the other two cities; this difference could be due to a statistical effect given by the size of the sample in Soacha. This sample has a much lower power to detect prevalence below 7%, and it is likely that this effect underestimates the low frequencies of other HR-HPVs. This is supported by the similarity in the HPV 16 prevalence in three cities, compared to a low frequency observed for other types.

In addition, differences in the number of sexual partners, age of onset of relationships, and pregnancies of this population may also influence such dissimilarity; these women were more stable sexual partners.

In this study, although a high prevalence of HR-HPV infections was observed, a low frequency of cervical cytologic abnormalities was found. These results may reflect the high rate of transient infections resulting from productive infections in this age group, many of which

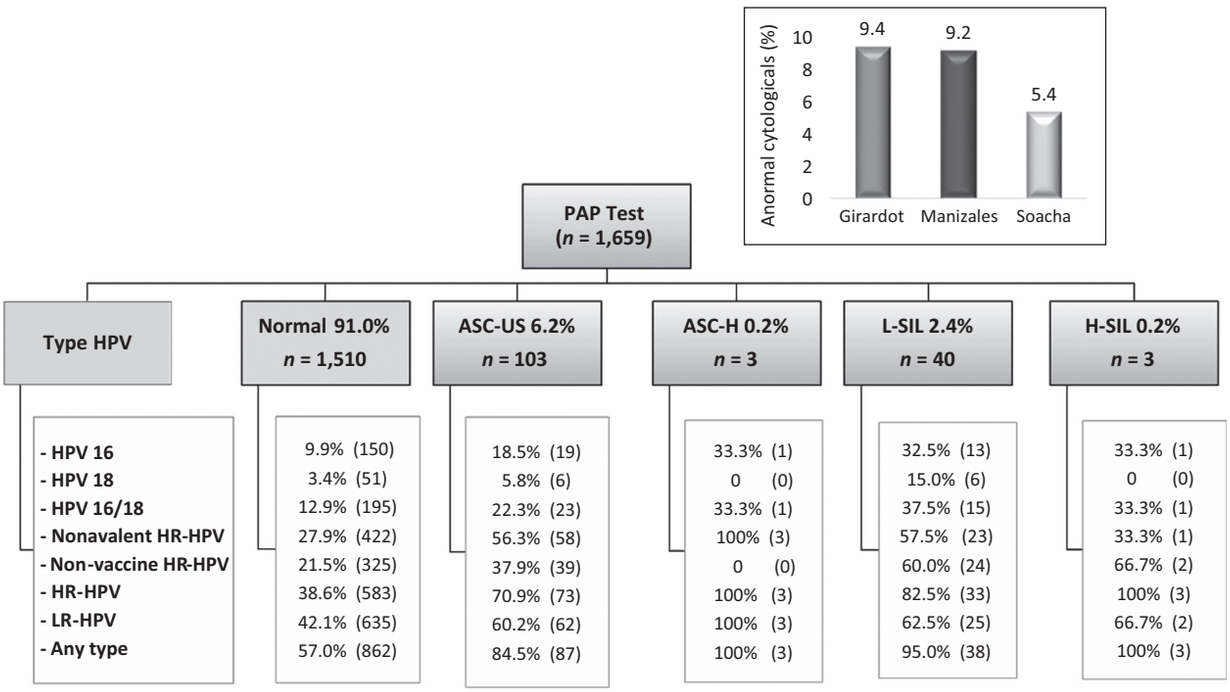

Figure 3.
HPV prevalence by cytology.

tend to regress over time (21). Approximately 88% of the women with a positive Pap test (including ASC-US) were infected with any HPV and about 75% were infected with an HR-HPV type. These results point out the association between HPV infection and cytologic abnormalities and at the same time, underline the central role of genotypes 16 and 18 as well as other HR-HPV types as determinants of cytologic abnormalities.

Some studies have reported an association between multiple HPV infections and a higher risk of cytologic abnormalities and cervical neoplasia. However, the biological effect of multiple infections on cervical diseases has not yet been established (21, 33–36). Despite a low frequency of abnormal cytology in this study, we observed a significant association between cytologic abnormalities and multiple infections mainly by other HR-HPVs different to HPV 16 and 18. It is necessary to carefully address the analysis of coinfections in postvaccinated women due to the potential viral replacement that could exist (15).

The possibility of cross-protection against other HPV types is an extremely important question, because it could increase the fraction of cervical cancers prevented (37). To date, several evidences have reported cross-protection of the bivalent and tetravalent vaccine against HPV 31, 33, and 45 types (38–40). These studies have shown that the efficacy in the prevention of 6-month persistent infection in HPV-naïve women vaccinated with current vaccines does not exceed 30% against nonvaccine genotypes, namely HPV 31, 33, 45, 52, and 58 (38, 41–43). In addition, in a previous study, we found a possible induction of cross-protection for HPV 31, 45, and 58 in a group of Colombian women vaccinated with the quadrivalent vaccine. However, the rapid decrease in seroprevalence against these types suggested that protection does not last for a long period after immunization (44) Therefore, studies concerning the prevalence and risk of nonvaccine HPV type are important to better evaluate the effectiveness of current HPV vaccines. Moreover, it will be useful to initiate vaccine programs with second-generation vaccines, such as the nonavalent vaccine to achieve greater coverage for other types of HPV frequently detected in our population and responsible for 90% of cervical cancers.

The main limitation of our study is that unlike other surveillance studies of HPV prevalence that are population-based studies (19, 22), our study is not population based, and therefore, we cannot claim that our study population represents the population of young women from the 3 cities. The recruitment strategy in universities and technical centers may have induced a selection bias, but the comparison of the prevalence of HPV in our study population with the prevalence of HR-HPV in a study conducted in five cities of Colombia, where a prevalence between 49%–61% and 52% in women under 24 years was observed (45), indicates that the prevalence of HPV observed in this study

could be a reflection of HPV infections in this part of the Colombian population. However, these results should be interpreted with caution.

In conclusion, our findings provide first information about the genotype distribution among young women in Colombia. It is important to have a baseline of HPV prevalence among unvaccinated young women in a Colombian region in order to monitor the impact of vaccination.

The large sample size could enable precise estimates of both increases and decreases in HPV type-specific prevalence. Moreover, these results would help to plan an appropriate strategy for monitoring of HPV vaccine.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: D. Puerto, D. Garcia, R.H. Murillo, N. Muñoz, G.A. Hernandez, C. Wiesner, A.L. Combita

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Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): D. Puerto, V. Reyes, D. Garcia, A.L. Combita

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): V. Reyes, L. Buitrago, D. Garcia, R.H. Murillo, N. Muñoz, G.A. Hernandez, A.L. Combita

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