Human papillomavirus (HPV) type distribution in cervical carcinoma, low-grade, and high-grade squamous intraepithelial lesions in Venezuelan women

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ABSTRACT

Objective. Cervical cancer is an important cause of mortality among women in developing countries, especially in the Latin America and Caribbean (LAC) region. Infection with high-risk (HR) human papillomavirus (HPV) has been identified as the primary cause of cervical cancer. The aim of this study was to determine the frequency of HR-HPV genotypes in low-grade and high-grade squamous intraepithelial lesions (LSIL, HSIL) and cervical carcinoma (CC) among Venezuelan women.

Materials and methods. Subjects with histopathological diagnosis of LSIL, HSIL, and CC (LSIL = 200; HSIL = 100; CC = 150) were enrolled in the study after obtaining informed consent. Biopsy samples of these subjects were analyzed to determine the lesion type. HPV detection and typing was done using polymerase chain reaction (PCR) and reverse hybridization. HPV type specific prevalence was determined in subjects with single and multiple infections.

Results. HPV DNA was detected in 68%, 95%, and 98.7% of LSIL, HSIL, and CC cases, respectively. HR-HPV and low-risk oncogenic HPV (LR-HPV) was observed in 66.9%/11.8% of LSIL cases, 87.3%/3.2% of HSIL cases, and 91.2%/0.7% of CC cases. HPV types -16/-18 (65%) were the most common high-risk HPV types observed, followed by types -52, -33, -45, and -31.

Conclusion. Cervical cancer burden in Venezuelan women is substantial. HPV types -16/-18 were the most common types prevalent among Venezuelan women followed by types -52, -33, -45, and -31 (prevalence, ~90.1%). The results of this study provide baseline information on the HPV type distribution, which may facilitate the development of a cervical cancer prevention and control program in Venezuela.

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Introduction

Cancer of the cervix is the second most common cancer among women worldwide. Globally, approximately 529,000 new cases and 274,000 cervical cancer-related deaths were reported in 2008 [1]. Previous studies have reported that 83% of all cervical cancer cases recorded worldwide are observed in the developing countries; separate modeling studies have projected that this percentage is most likely to increase to 90% by 2020 [2].

In the Latin America and Caribbean (LAC) region, cervical cancer is the second most common type of cancer [3]. The average age-standardized incidence of cervical cancer in the LAC region is 29.2 cases per 100,000 women (range, 8.8–87.3 cases per 100,000). Previous studies have not yielded sufficiently precise incidence and mortality estimates of cervical cancer disease in most countries in the LAC region. This is primarily due to underreporting of new cases/deaths and inadequate screening coverage associated with cervical cancer in the rural areas [4]. In Venezuela, cervical cancer is the second most common cancer among women after breast cancer. Recent available estimates indicate that, in 2008, the age-standardized incidence of cervical cancer in Venezuela was 31.4 per 100,000 women and the age-standardized mortality due to cervical cancer was 14.4 cases per 100,000 women [1].

Previously published studies have established a causal relationship between human papillomavirus (HPV) infection and cervical cancer lesions; the prevalence of HPV in cervical cancer cases has been found to be as high as 99.7% [5]. HPV-16 and HPV-18 are the two most

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prevalent types associated with cervical cancer and persistent infection with these two HPV types is associated with the progression of HPV infection to cervical cancer [6,7]. HPV-16 and HPV-18 cause 70% of all invasive cervical cancer (ICC) cases, 41%–67% of high-grade squamous intraepithelial lesions (HSIL), and 16%–32% of low-grade squamous intraepithelial lesions (LSIL) [8]. Other common oncogenic HPV types (-52, -31, -33, and -45) account for 30% of cervical cancer cases. HPV-45 is the third most prevalent type after HPV-16 and HPV-18 and is usually associated with adenocarcinoma (ADC) and ICC [9–11].

Data on HPV prevalence in Venezuela is limited [12]. This is attributed primarily to the absence of HPV specific surveillance systems and/or specific epidemiology studies, which may have resulted in incomplete information in the Yearbooks of Epidemiology and Vital Statistics of the Venezuelan Ministry of Health (MPPS). A few research groups in Venezuela have conducted studies to determine the prevalence of HPV infection. However, these studies were either conducted in asymptomatic women of Venezuela [13] or in very limited numbers of cervical carcinoma (CC) cases [14].

HPV vaccination offers protection against specific HPV types, and the subsequent development of pre-malignant and malignant cervical cancer lesions. It has been indicated that implementation of a prophylactic vaccination program in 12-year-old girls as part of current screening programs in the LAC region is expected to reduce the number of cases of cervical intraepithelial neoplasia (CIN) I/II/III lesions, cervical cancer, and associated deaths. HPV vaccines which target HPV-16 and HPV-18 are being considered as one of the most cost-effective interventions for cervical cancer control, particularly in developing countries worldwide where more adequate infrastructure is available to support vaccination programs when compared to screening programs. The two prophylactic HPV vaccines Gardasil® (Merck & Co., Inc., Whitehouse Station, NJ, USA) and Cervarix® (GlaxoSmithKline Biologicals, Wavre, Belgium) are licensed for use in most LAC countries [15].

The Pan American Health Organization (PAHO) strongly supports introduction and implementation of HPV vaccination through an evidence-based decision making process in the LAC region [16]. Policy makers rely on data on disease prevalence and incidence for decisions on prioritizing vaccines for national immunization programs. Considering that HPV prevalence information in countries of the LAC region, especially Venezuela, is not clearly established, it is essential to make available baseline data from the region.

The aim of this study was to determine the prevalence of high-risk HPV types among Venezuelan women diagnosed with pre-cancerous LSIL, HSIL, and CC.

Materials and methods

Case selection

This observational study, conducted in 2009, retrospectively identified subjects with a primary histopathological diagnosis of LSIL, HSIL, and CC in Venezuela between December 2002 and March 2009. This study was conducted in accordance with the principles of Good Clinical Practice, the Declaration of Helsinki, and relevant local regulations. All study related procedures were approved by the appropriate Ethics Committees.

All subjects signed a surgical procedure informed consent for sample collection and studies to be performed, after which they were enrolled. The selection criteria for a case was based on the availability of the following—a sample record in the pathology department of Padre Machado Hospital, a report with histological diagnosis, cervical biopsy specimen, and a diagnostic confirmation of the histological lesion type. Biopsies were selected consecutively starting from the first subject meeting the selection criteria until the target enrolment was reached for each group. The biopsies were stored in paraffin blocks at the Pathological Anatomy Laboratory of Padre Machado Hospital in Caracas, Venezuela.

After the completion of selection of the cases and diagnostic confirmation of the histology, the biopsies were sent in series and periodically to the Molecular Genetics Laboratory of the Oncology and Hematology Institute, MPPS, Caracas, Venezuela, where HPV detection and typing was done. The biopsy samples were analyzed to determine the lesion type according to Richard homologated to Bethesda 2001 (CIN I = LSIL; CIN II; or CIN III = HSIL) among subjects with HSIL and for subjects with CC (SCC [squamous cell carcinoma], ADC, or ASC [adenosquamous carcinoma]).

HPV detection and genotyping

The biopsy samples were processed for HPV detection and typing using polymerase chain reaction (PCR) and reverse hybridization. After histological confirmation, six sections of formalin-fixed, paraffin-embedded cervical tissue were sliced from each block and placed in sterile 1.5 mL Eppendorf™ tubes for the extraction of genomic DNA. DNA was obtained from these cervical sections by treating it with 100 μl of lysis buffer (Tris–HCl, 10 mM; pH 8.0 + 0.1% sarcosine) and proteinase K 1000 μg/mL. After overnight incubation, proteinase K was inactivated at 95 °C for 5 min, followed by phenol–chloroform–isoamyl alcohol extraction and ethanol precipitation. The pellet was suspended in 50 μl of nuclease-free distilled water and was stored at −20 °C until used for molecular processing.

The quality and integrity of the DNA obtained was verified by PCR amplification of a 268 bp fragment of the β-globin gene using GH20 and PC04 primers, as previously described [17]. For HPV detection, amplification of the L1 fragment was performed using GP5+ and biotinylated GP6+ primers as previously described [18]. Detection of 28 HPV types (-6, -11, -16, -18, -26, -31, -33, -35, -39, -40, -43, -44, -45, -51, -52, -53, -54, -56, -58, -59, -66, -68, -69, -70, -71, -73, -74, -82) was achieved using an INNO-LiPA Genotyping Extra Amp kit (Innogenetics®, Belgium).

The 28 HPV types are classified into two categories based on the oncogenic risk associated with the HPV type. These are high-risk oncogenic HPV (HR-HPV) and low-risk oncogenic HPV (LR-HPV). HR-HPV constitutes 15 HPV types (-16, -18, -31, -33, -35, -39, -45, -51, -52, -56, -58, -59, -68, -69, -70, -73, -74, -82) was achieved using an INNO-LiPA Genotyping Extra Amp kit (Innogenetics®, Belgium).

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HPV-type specific prevalence was determined for the subjects diagnosed with LSIL, HSIL, and CC. Common HPV types among subjects with single and multiple infections were reported. The value of each HPV type detected in mixed infections was assigned as proportional fractions based on their distribution in single infections, using the procedure described previously by Muñoz et al. [19] and Murillo et al. [20]. Prevalence ratios for the common HPV types were calculated using the same methodology as previously described [11]. The differences among the variables were analyzed with the non-parametric Chi-square test and Monte Carlo correction was performed for the confidence intervals. The statistical analyses were performed using SPSS 15.0 software.

Results

Description of the cases studied

A total of 200 subjects with a confirmed diagnosis of LSIL (mean age, 37.2 ± 9.49 years [range, 18–69 years]); 100 subjects with a confirmed...
observed among the Venezuelan women. The overall prevalence of HPV types -52, -33, -45, and -31 were the other most common types analyzed as a group. The prevalence of the other common HPV types most frequently found, followed by type 18, similar to the cases subjects, and 9.7% (15/150) of subjects with CC. Regarding the HPV was detected in 5.8% (12/200) of LSIL subjects, 17.2% (17/100) of HSIL subjects, 37.4% (37/100) of subjects with HSIL, and 55.3% (83/150) of prevalent type and was detected in 20.8% (42/200) of subjects with LSIL, 5.8% (42/731) showing the clonal nature of CC cases (Table 3). It was difficult to define the specific HPV type responsible for the different lesions in co-infection/multiple infection. Nevertheless, CC cases were mostly associated with a single type HPV (HPV-16, -18, -52, -31, -33, and -45) in 73% of the HPV positive cases (108/148); but the remaining 27% of HPV positive cases (40/148) were associated with co-infections containing also the most common oncogenic HPV types (-16, -18, -31, -33, -45, and -52).

Prevalence of HPV types observed in different cervical lesions

In order to determine the prevalence of each HPV type, multiple infections have been handled differently in several studies. Most estimations assume a unique multiple-infection several times for infections with unde

<table>
<thead>
<tr>
<th>Histological type</th>
<th>LSIIL(^a) (N = 200)</th>
<th>HSIL(^b) (N = 100)</th>
<th>CC(^c) (N = 150)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPV-(^d)</td>
<td>64</td>
<td>32.0</td>
<td>5</td>
</tr>
<tr>
<td>HPV+</td>
<td>136</td>
<td>68.0</td>
<td>95</td>
</tr>
<tr>
<td>Infections with HR-HPV(^e)</td>
<td>91</td>
<td>66.9</td>
<td>83</td>
</tr>
<tr>
<td>Infections with undeﬁned-risk HPV</td>
<td>29</td>
<td>21.3</td>
<td>9</td>
</tr>
<tr>
<td>Infections with LR-HPV(^f)</td>
<td>16</td>
<td>11.8</td>
<td>3</td>
</tr>
</tbody>
</table>

\(a\) Low-grade squamous intraepithelial lesion.
\(b\) High-grade squamous intraepithelial lesion.
\(c\) Cervical carcinoma.
\(d\) High-risk oncogenic HPV.
\(e\) Low-risk oncogenic HPV.

Discussion

This study is the first comprehensive study conducted in Venezuela that investigated the distribution of HPV types in LSIL, HSIL, and CC lesions. Previously published studies have established a causal relationship between HPV infection and cervical cancer lesions [5].

In Venezuela, previous studies have established that the most common HPV types circulating among women are the HR-HPV types associated with LSIL and HSIL, which are critical to the development of CIN and HSIL, the precursors of cervical cancer [6,10,14,21]. In the present study, infection with HR-HPV types was observed in a large percentage of subjects (66.9% of subjects with LSIL, 87.3% of subjects with HSIL, and 91.2% of subjects with CC). As observed in this study, HPV-16 and HPV-18 were identified as the most common HPV types prevalent among Venezuelan women and the overall prevalence of these two HPV types was 65%. This prevalence rate is in agreement with the prevalence rates observed in other developing regions like Central and South America (65.0% of all CC cases) [20,22] and in sub-Saharan Africa (64% of all CC cases). These observations are also in line with published literature which establishes that the prevalence of these two HPV types in developing countries is 60–65%, which is lower than that in developed nations (70%–75%) [23]. The other common HPV types observed among Venezuelan women in this study were HPV types -52, -33, -45, and -31, which is consistent with global epidemiology data for HPV type specific prevalence in CC (overall prevalence, ~90.1%) [8].

Published literature reports HPV-45 to be an important oncogenic type, which is usually indicated in ICC cases and is responsible for the progression from infection to malignancy [11,20]. The role of HPV-45 along with HPV-18 is implicated in cervical ADC [24,25]. Data from this study also provided evidence of the association between HPV-45 and CC (6.3% [10/150]). HPV-45 was significantly over-presented in CC cases in Venezuelan women when compared to the other HR-HPV types,
suggesting differences in propensity of specific HPV types to progress from HSIL to CC. The high pathogenicity of HPV-45 observed in this study re-affirms its importance as an important etiological agent in CC.

Cytology-based screening programs (using Papanicolaou [Pap] smears) have enabled the early detection of cervical lesion, thereby facilitating the decline of cervical cancer incidence rates, in regions where they have been successfully implemented. Developed countries in particular have benefitted from this approach; however, the same level of success has not been replicated in developing countries where it is difficult to implement screening programs due to their low-resource settings [26,27]. Among the LAC countries, a publicly funded screening program has been in existence in Mexico for 20 years but has had little impact on cervical cancer related incidence and mortality rates. In Cuba, no significant decline in incidence and mortality was reported following the introduction of a similar screening program in 1968. However, in Chile and Costa Rica, a reduction in cervical cancer mortality rates has been observed, although the impact has been less than expected and much has been discussed about whether this decrease is due to the actual impact of screening or to other socio-economic factors [27]. In countries like Colombia, Brazil, Peru, and Venezuela, the cervical cancer incidence and mortality rates have remained high and stable. This is because screening in these countries is opportunistic [28,29]. Since the implementation of screening programs in similar settings such as Mexico and Cuba have met with limited success, integrated programs which offer both novel technologies for HPV testing and vaccination should be considered; in these circumstances, this can be the most compelling strategy for maximal impact on the burden of cervical cancer in the LAC region.

Primary prevention of cervical cancer can be achieved by introduction of efficacious HPV vaccines. HPV vaccines with the ability to provide cross-protection against other oncogenic HPV types (-52, -33, -45, and -31) will

### Table 2
Comparison of HPV type distribution in cervical carcinoma and HSIL lesions.

<table>
<thead>
<tr>
<th>HPV type</th>
<th>CC (N = 150)</th>
<th>HSIL (N = 100)</th>
<th>CC/HSIL (prevalence ratio)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>% HPV positive</td>
<td>n</td>
</tr>
<tr>
<td>16</td>
<td>83</td>
<td>55.3</td>
<td>37</td>
</tr>
<tr>
<td>18</td>
<td>15</td>
<td>9.7</td>
<td>17</td>
</tr>
<tr>
<td>33</td>
<td>10</td>
<td>6.3</td>
<td>12</td>
</tr>
<tr>
<td>45</td>
<td>10</td>
<td>6.3</td>
<td>3</td>
</tr>
<tr>
<td>31</td>
<td>8</td>
<td>5.0</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>137</td>
<td>90.1</td>
<td>77</td>
</tr>
</tbody>
</table>

a Cervical carcinoma.
b High-grade squamous intraepithelial lesion.

c Cervical carcinoma.
d Not containing HPV-16 or -18.
e Not containing HPV -16, -18, -31, -33, -45, or -52.

### Table 3
Frequency of co-infections by type of lesion.

<table>
<thead>
<tr>
<th>Histological type</th>
<th>LSIL</th>
<th>HSIL</th>
<th>CC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>%</td>
<td>n</td>
</tr>
<tr>
<td>HPV negative</td>
<td>64</td>
<td>32.0</td>
<td>5</td>
</tr>
<tr>
<td>Single infections</td>
<td>78</td>
<td>39.0</td>
<td>53</td>
</tr>
<tr>
<td>Co-infections with HPV-16 or -18</td>
<td>25</td>
<td>12.5</td>
<td>25</td>
</tr>
<tr>
<td>Co-infections with HPV-33, -31, -45, or -52d</td>
<td>14</td>
<td>7.0</td>
<td>11</td>
</tr>
<tr>
<td>Co-infections with other typese</td>
<td>19</td>
<td>9.5</td>
<td>6</td>
</tr>
<tr>
<td>Total</td>
<td>200</td>
<td>100.0</td>
<td>100</td>
</tr>
</tbody>
</table>

a Low-grade intraepithelial squamous lesion.
b High-grade intraepithelial squamous lesion.
c Cervical carcinoma.
d Not containing HPV-16 or -18.
e Not containing HPV -16, -18, -31, -33, -45, or -52.
potentially offer additional benefits especially in the relatively large proportion of women that are infected with multiple HPV types. Data from this study indicated that the percentage of Venezuelan women with multiple HPV infection is reasonably high (42.6% [58/136] of HPV positive subjects with LSIL, 44.2% [42/95] of HPV positive subjects with HSIL, and 27% [40/148] of HPV positive subjects with CC); implementation of HPV vaccination can be beneficial in this setting.

Integration of HPV vaccination, however, requires a thorough analysis of the affordability of the vaccine and operational feasibility of attaining significant vaccine coverage in line with national health priorities of the specific country in question. It has been demonstrated that cervical cancer vaccines are cost-effective even at high prices per-dose [30].

In conclusion, this study provides baseline information on the distribution of HPV types among Venezuelan women. High frequency of circulating HPV-16, HPV-18, and other HPV types (-52,-33, -45, and -31) among women with single infection and co-infection/multiple infection in Venezuela reported in this study supports the need for the implementation of an HPV surveillance and control program in Venezuela, which involves primary prevention by vaccination and secondary prevention by the introduction of novel screening techniques and improvement of existing screening programs. Taking into consideration the high disease burden of cervical cancer in the LAC region, introduction of HPV vaccines will facilitate improvement in the health and the quality of life of women.

Conflict of interest
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