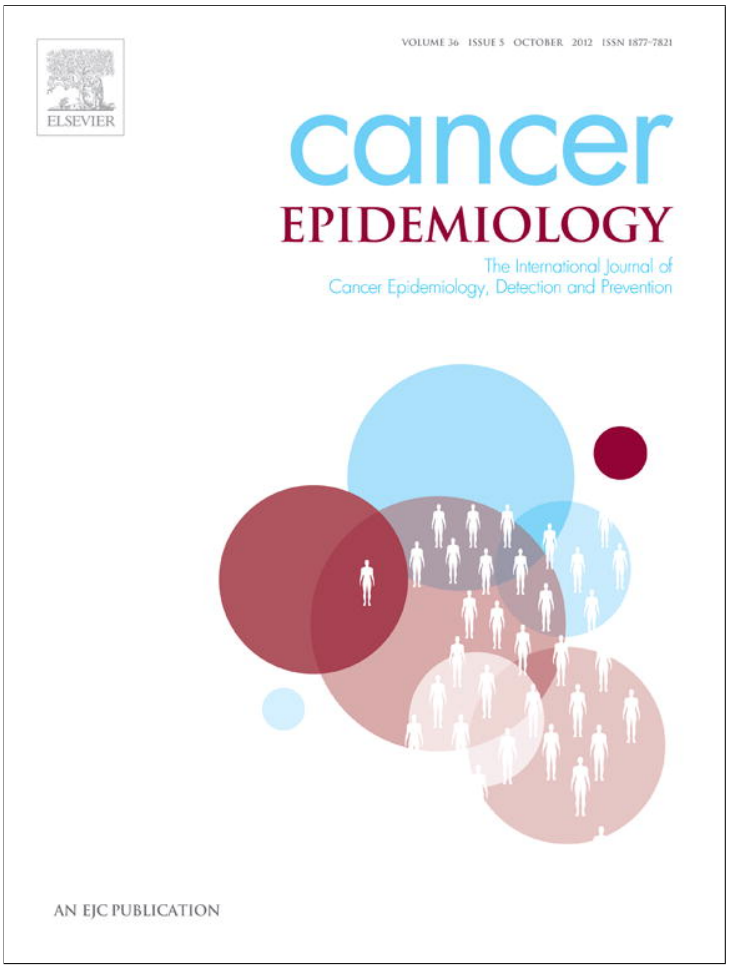

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Human papillomavirus in invasive cervical cancer and cervical intraepithelial neoplasia 2 and 3 in Venezuela: A cross-sectional study☆

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ABSTRACT

Background: This study investigated the distribution of human papillomavirus (HPV) types in invasive cervical cancer (ICC), cervical intraepithelial neoplasia 2 (CIN2) and cervical intraepithelial neoplasia 3 (CIN3) in Venezuela.
Methods: Paraffin-embedded samples from 329 women from 29 medical centers of the 24 states of Venezuela were analyzed to determine the distribution of HPV types for ICC, CIN2, and CIN3, the prevalence of single and multiple infection, and the association of HPV types with severity of lesion, comparing CIN2 versus CIN3+ (CIN3 and ICC). The samples were analyzed with the polymerase chain reaction (PCR) followed by reverse hybridization for the identification of HPV types.
Results: HPV was identified in 95/96 ICC specimens (98.9%), in 142/149 CIN3 (95.3%) and in 78/84 CIN2 samples (92.8%). The most common types for ICC and CIN3 were: HPV16, 18, 31, and 33, and for CIN2 were HPV16, 31, 51, 52, and 18. HPV single infection was found in 82.1% of ICC cases, in 79.4% of CIN2 cases, and in 77.4% of CIN3 cases. HPV16 was identified as a single infection more frequently in women with CIN3+ than in those with CIN2 (68.6% versus 46.7%, $P = 0.002$), and HPV16 or HPV18 types were more prevalent in CIN3+ than in CIN2 (73.4% versus 50%, $P = 0.0006$).
Conclusion: this is the first study of the distribution of HPV types in ICC, CIN2, and CIN3 conducted throughout the territory of Venezuela. HPV16 and HPV18 were the most frequent HPV types identified in single and multiple infections in both ICC and CIN3 groups, and are associated with severity of lesion. The knowledge of the distribution of HPV types would allow organization of an HPV-DNA-based screening test, and consideration of the implementation of prophylactic vaccination in Venezuela.

1. Introduction

Invasive cervical cancer (ICC) is the first cause of morbidity and mortality from cancer in Venezuelan women [1], and is an important cause of cancer-related death worldwide [2].

Persistent infection with high-risk human papillomavirus (HPV) is considered the most important causal factor for the development of ICC and cervical intraepithelial neoplasia grades 2 (CIN2) and 3 (CIN3) [2].

Although a local study has been reported [3], to date the distribution of HPV types involved in ICC and premalignant lesions in all regions of Venezuela is unknown.

The crude incidence rate of ICC in Venezuela is 31.4 per 100,000 women/year, similar to that reported for Latin America (33.5 per 100,000 women/year [4]), but higher than the rate for South America and globally (24.1 and 15.3 per 100,000 women/year, respectively [5]). In 2008, 3785 new cases of ICC were diagnosed in Venezuela, and of these, 1764 were in women under 45 years of age. By 2025, approximately 2381 ICC cases could be registered in women who are now at vaccination age [6]. There are no data available for Venezuela in relation to the distribution of HPV types in women with normal cytology, premalignant lesions and ICC.

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There are available two prophylactic HPV vaccines with high efficacy for preventing diseases related to infections with HPV16, 18, 6 and 11 (quadrivalent vaccine) and HPV16, and 18 (bivalent vaccine) [7]; these vaccines are not yet approved in Venezuela.

The knowlegde of the prevalence of HPV types in ICC and premalignant lesions in Venezuela will be useful in considering the introduction of prophylactic vaccines, and it would be helpful to plan the use of HPV testing in the screening programs. The objective of this study is to analyze the distribution of HPV types in Venezuela in patients with ICC and premalignant lesions.

2. Methods

2.1. Study design

The sample size was estimated at 8–10% of the incidence of ICC in 2008 (3785 cases), being proportional to the population density of each region from the latest population census [8], and was stratified as follows: one third for ICC (squamous-cell cancer and adenocarcinoma) and two thirds for premalignant disease (CIN2 and CIN3).

After approval by the respective independent commissions of bioethics at Luis Razetti Institute of Oncology and Venezuelan Scientific Research Institute (IVIC), 344 paraffin-embedded histological samples were selected randomly from all cases available, obtained from the files of 29 medical centers in Venezuela, and grouped into five geographic zones (Table 1). Of the 344 samples, 15 were excluded because of misdiagnosis (CIN1) after histological confirmation by the central panel of pathologists. We studied 329 samples of patients aged between 18 and 89 years (mean age 39 years), diagnosed with FIGO (International Federation of Gynecology and Obstetrics) stage-I ICC (squamous-cell carcinoma and adenocarcinoma), CIN2, and CIN3, between 2001 and 2011.

We evaluated the absolute and relative prevalence of HPV types and the proportion of single and multiple infections for ICC, CIN2, and CIN3. Since CIN2 has a higher regression rate [9], and is the least reproducible histopathological lesion [10], this category is considered an equivocal diagnosis of premalignant lesions and is probably associated with low-severity lesions; we performed an analysis to compare the prevalence of HPV types in single infections for CIN2 versus CIN3+ (CIN3 and ICC) to evaluate the association of HPV types with lesion severity.

2.2. HPV genotyping

We included 329 samples, evaluated by a central panel of pathologists, to confirm the histological diagnosis. Subsequently, we made three sections of each paraffin block of 1 mm thick (approximately 25 mg) using disposable material for each sample. The first section was used for histopathological evaluation and the second section was used for biological processing; the third section was kept in reserve.

The specimens were deparaffinized and DNA was obtained with proteinase K and subsequently genotyped by INNO-LiPA HPV Genotyping Extra Amp System (Innogenetics®, Belgium), which determines 28 HPV types (HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 69–71, 73, 74 and 82 considered to be high-risk oncogenic, and HPV6, 11, 40, 43, 44, 54, 61 and 70 considered low risk). HPV26, 53 and 66 are classified as potentially high risk. This system allows the detection of HPV DNA, amplifying small fragments of viral DNA by the polymerase chain reaction (PCR) followed by a reverse hybridization that identifies the HPV types. Appropriate negative controls were included.

2.3. Statistical analysis

The absolute and relative prevalence of HPV types and the proportion of single and multiple infections were calculated for ICC, CIN2, and CIN3. Statistical analysis to evaluate differences was performed using Pearson's chi-square test ($P < 0.005$).

3. Results

Details of the population studied are shown in Table 1. In total, 329 paraffin-embeded samples were studied, and HPV DNA was identified in 95 of 96 ICC samples (98.9%), in 142 of 149 CIN3, (95.3%) and in 78 of 84 CIN2 samples (92.8%). The results were tabulated by considering three groups: 95 cases of ICC, including 93 cases of squamous-cell carcinoma (97.9%) and two cases of adenocarcinoma (2.1%), 78 cases of CIN2 and 142 cases of CIN3.

3.1. Distribution of HPV types according to histological lesion

The HPV type distribution for ICC is shown in Table 2, highlighting the presence of HPV16 in 68.4% and HPV18 in 11.5% of the cases. The identification of HPV type was not possible in one case (undetermined), and HPV44 (low risk) was detected in one case as a single infection in this group.

For CIN2 the distribution resulted as follows: HPV16 44.8%, HPV31 10.25%, and HPV18 6.4% (Table 2). In six cases (7.6%) HPV

Table 1
Distribution of the samples by geographical zones and ages by histological cases.

	ICC ($n=95$)	CIN3 ($n=142$)	CIN2 ($n=78$)
Mean age (range)	44.5 (21–87)	37.67 (18–89)	36.9 (19–57)
Geographic zones			
1 Capital district, Miranda, and Vargas	23	46	18
2 Mérida, Táchira, Trujillo, Barinas, and Zulia	23	16	26
3 Lara, Falcón, Yaracuy, and Portuguesa	18	19	13
4 Aragua, Carabobo, Cojedes, Guárico, and Apure	14	26	16
5 Anzoátegui, Monagas, Sucre, Nueva Esparta, Bolívar, Amazonas, and Delta Amacuro	17	35	5

Table 2
HPV type prevalence in women with invasive cervical cancer (ICC), cervical intraepithelial neoplasia 3 (CIN3) and CIN 2 in single and multiple infections. Some women can be counted more than once because of multiple infections.

HPV type	Invasive cervical cancer ($n=95$)n (%)	CIN 3 ($n=142$)n (%)	CIN 2 ($n=78$)n (%)
16	65 (68.42)	90 (63.3)	35 (44.8)
18	11 (11.5)	14 (9.8)	5 (6.4)
31	6 (6.3)	10 (7.04)	8 (10.25)
33	6 (6.3)	10 (7.04)	5 (6.4)
45	6 (6.3)	6 (4.2)	5 (6.4)
58	3 (3.15)	7 (4.9)	5 (6.4)
52	2 (2.1)	7 (4.9)	6 (7.6)
51	1 (1.05)	5 (3.5)	7 (8.9)
44	2 (2.1)	7 (4.9)	0
40	0	3 (2.1)	3 (3.8)
66	1 (1.05)	4 (2.8)	1 (1.2)
6	2 (2.1)	2 (1.4)	1 (1.2)
73	1 (1.05)	2 (1.4)	2 (2.5)
82	0	2 (1.4)	2 (2.5)
26	1 (1.05)	2 (1.4)	0
35	1 (1.05)	1 (0.7)	1 (1.2)
53	1 (1.05)	0	2 (2.5)
56	0	2 (1.4)	1 (1.2)
54	1 (1.05)	0	1 (1.2)
Undetermined	1 (1.05)	2 (1.4)	6 (7.6)

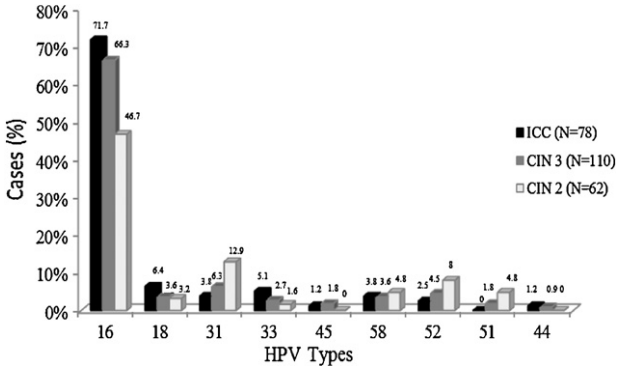

Fig. 1. Ten most frequent human papillomavirus (HPV) type prevalence in single infections in invasive cervical cancer (ICC), cervical intraepithelial neoplasia 3 (CIN3) and CIN2.

type was undetermined. For CIN3 HPV16 was identified in 63.3% and HPV18 in 9.8% (Table 2). In two cases (1.4%) HPV type was undetermined.

HPV single infection was found in 78 of 95 ICC samples (82.1%), in 62 of 78 CIN2 cases (79.4%), and in 110 of 142 CIN3 cases (77.4%).

The most common HPV types in single infections are shown in Fig. 1. Within the 78 ICC single-infection cases, HPV16 was identified in 56 (71.7%), HPV16 and 18 were identified in 61 (78.1%), and – taking into account HPV16, 18, 33, 31, 45, 52, and 58 together – the proportion rises to 94.6%. In the two adenocarcinoma samples HPV16 and HPV18 were identified as single infections in each case.

HPV16 and 18 were identified in the 31 samples (50%) of CIN2 and 77 samples (70%) of the CIN3 cases considered. Two cases of low-risk HPV single infection were found in the CIN2 group and one case in the CIN3 group (Fig. 1).

HPV16 was the most prevalent type in ICC, CIN2, and CIN3 in all geographic zones. In all multiple infections (two or more HPV types) in the three groups at least one high-risk HPV type was always detected.

3.2. HPV type distribution and severity of lesion

In the analysis of the relationship between HPV type and severity of lesion, HPV16 was identified as a single infection more frequently in women with CIN3+ than with CIN2 (68.6% versus 46.7%, $P = 0.002$). Single infection – with HPV16 or HPV18 – was more prevalent in CIN3+ (73.4%) than in CIN2 (50%), $P = 0.0006$. HPV18 as single infection was identified in 3.2% of CIN2 cases and in 4.8% of CIN3+ cases; this difference was not statistically significant ($P = 0.8$). Single infections with HPV types different from HPV16 and 18 (31, 33, 45, 52, 58) were identified in 27.4% and 18.1% of CIN2 and CIN3+ respectively; this difference was not statistically significant ($P = 0.1$).

4. Discussion

In this paper we present data on the distribution of HPV types in ICC, CIN2, and CIN3 in Venezuela. In the ICC group, 79.9% of cases are attributable to HPV16 and 18, and the frequencies of other HPV types are similar to those found in Central and South America [11].

For the current series, the third place of frequency is occupied by HPV31, 33 and 45; in other published series it is considered that these types show a frequency similar to each other, ranging between 2% and 7% [11,12]. In a study performed in a single center in Caracas (150 cases of ICC), the most frequent types were HPV16 (55.3%) and 18 (9.7%), followed by HPV52 (7.5%) [3]. Although a significant variability in the third most frequent HPV type has been described in the subcontinent, it is considered that the third most common type in Central and South America could be HPV31 or HPV45 [11]; in the series mentioned, and the current study, the frequency of HPV52 as a single infection does not exceed 3%. The incidence of adenocarcinoma in this series was lower than that in other series, at around 5% [12]; despite this limitation, we observed the same pattern described by other series in which HPV16, 18, and 45 could be associated with 74–94% of adenocarcinomas [6,12].

In the CIN2 and CIN3 groups, HPV16 and 18 were identified in 44.5% and 63.3% of the cases, respectively. Despite the variability in the frequency of HPV types that occupy third to tenth places, probably related to geographical location and the genotyping method used, it is frequently found that HPV31, 33, 52, 45 and 58 in CIN2 and CIN3 could approach, or equal, the frequency of HPV18.

HPV45, which along with HPV31 and HPV33 ranked third in frequency in the ICC group (6.3%), falls to the fourth place (6.4%) in the CIN2 and to the fifth place (4.22%) in CIN3 group. Since HPV45 was not very frequently found in this study and, in many cases, was found as a co-infection with HPV16 or 18, it was not possible to evaluate the role of single infection with HPV45 and relate it to the age of the patient. It was considered that ICC related to HPV16, 18 and 45 occurs more frequently in younger women (around 43 years) when compared with cases of ICC due to HPV31 or 33, with mean ages of 64 and 59 years, respectively [13].

HPV16 and 18 are the predominant types in single and multiple infections in ICC and CIN3 [12], and these types are more frequent in CIN3+ than in CIN2, probably due to their oncogenic potential. High-risk or probable high-risk HPV types were identified in 99% of the single infections in ICC.

Since CIN2 is now considered to be associated with low-grade lesions [9], and CIN3 is a good marker for ICC risk, comparison between CIN2 and CIN3+ allows evaluation of the relationship between HPV type and severity of lesions. The identification of HPV16 and HPV16 or 18, as a single infection, was significantly more prevalent in the CIN3+ group than in the CIN2 group, which links the infection with these types with a more severe lesion. This result is in agreement with previous studies [14] where HPV16 – as a single infection or in combination – was more prevalent in the CIN3+ group than in the CIN2 group (54.9% versus 37%, $P < 0.001$).

In conclusion, despite the limitations of this study given a limited total sample and few cases of adenocarcinoma, it is shown that the distribution of HPV types in Venezuela is similar to that reported in other series, with the presence of HPV16 and 18 as the predominant types in ICC and CIN3.

Knowledge of the distribution of HPV types in Venezuela allows the prediction that the impact of prophylactic vaccines on ICC prevention would be near 70%. Taking into account the partial cross-protection described against HPV31, 33 and 45 [15], which were the most prevalent types identified in this study after HPV16 and 18, the benefit could be greater. Additionally, a similar reduction in the incidence of CIN2, CIN3 and cervical excisional procedures would be expected [16]. Besides, the high prevalence of HPV16 and 18 in more severe lesions would allow us to take into account the introduction in our country of HPV testing for screening.

Finally, the data obtained allow us to consider that the prophylactic vaccination of girls and women in Venezuela, together with the strengthening of screening programs and early treatment of premalignant lesions, will significantly reduce the incidence, morbidity and mortality attributable to invasive cervical cancer.

Role of funding sources

This study was supported by an independent grant from Merck & Co, Inc. Studies Program for Researchers. The sponsors reviewed

and approved the study design but they did not participate in sample collection, analysis or interpretation of data, or in the final report. The writing committee had access to all the results of the study, performed the final report, and was responsible for the decision to submit the paper for publication. All the authors reviewed and approved the manuscript.

Conflict of interest statement

JSL, PC, FM and HA have received payments for lectures from Merck, Sharp & Dohme. The other authors declared no conflict of interest.

Appendix A

For the Venezuelan Independent Working Group on HPV: Bolívar, Amazonas and Delta Amacuro: José Merheb (Los Aceiticos Health Care Centre, Ciudad Bolívar); Ezzat Saab and Henri Fernández (Ruiz y Páez University Hospital); Anzoátegui: Luis Bracamonte and Jorge Amundaray (Luis Razetti University Hospital, Barcelona); Apure: Aura Urdaneta-López (Pablo Acosta Ortiz Hospital, San Fernando de Apure); Aragua: Nora López and Jordan Barráez (Aragua State Health Program, Maracay); Barinas: Ana Isabel Simoza (León Fortoul Saavedra Health Care Centre, Barinas); Carabobo and Cojedes: Rodolfo Pérez-Aguirre (Miguel Pérez Carreño Institute of Oncology, Valencia); Guárico: Rosa Hernández (Screening Program in Cervical Cancer Altagracia de Orituco) and Aarón Cohen (University Hospital, Caracas); Lara: Francisco Menolascino-Bratta (Antonio María Pineda University Hospital, Barquisimeto) and Carolina Martínez (Society Against Cancer of Venezuela, Lara); Mérida: Antonio Villavicencio-Moreno (Los Andes University Hospital, Mérida); Monagas: Alejandra Rodríguez and Hernán Salazar-Román (Manuel Nuñez Tovar Hospital, Maturín); Portuguesa: Pedro Escalona-Brito (José Gregorio Hernández Maternity Hospital, Acarigua) and Eustiquio Salazar (JM Casal Ramos Hospital, Araure); Nueva Esparta and Sucre: Juan Carlos Cedeño-Guerrero (Antonio Patricio de Alcalá University Hospital, Cumaná); Táchira: José López-Saavedra (Central Hospital, San Cristóbal); Trujillo: Francisco Llavaneras (José Gregorio Hernández Hospital, Trujillo); Vargas: Irene Ágreda (Health Care Centre, La Guaira); Zulia: Felipe Díaz-Araujo and Mariela Fox (Chiquinquirá Hospital, Maracaibo).

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