PAN AMERICAN JOURNAL OF PUBLIC HEALTH

Original research

High frequency of HPV high-risk preventable genotypes in Ecuadorian women with invasive cervical cancer

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Suggested citation García Muentes GD, Abdul Latif F, Beltrán Rodríguez CC, Medina González S, Ruiz Cabezas JC. High frequency of HPV high-risk preventable genotypes in Ecuadorian women with invasive cervical cancer. Rev Panam Salud Publica. 2024;48:e128. https://doi.org/10.26633/RPSP.2024.128

ABSTRACT

Objective. To determine the distribution of human papillomavirus (HPV) genotypes in invasive cervical cancer samples from Ecuadorian women who attended the Cancer Institute (Sociedad de Lucha Contra el Cáncer – SOLCA).

Methods. Archived formalin-fixed paraffin-embedded (FFPE) cervical cancer tissue samples collected during 2017–2021 were deparaffinized, and nucleic acid extraction and purification was performed using silica columns. The obtained nucleic acids were analyzed using INNO-LiPA® HPV Genotyping Extra II per the manufacturer's specifications. Data were retrieved from records, and HPV genotypes were determined from the FFPE samples.

Results. The study included samples from 190 women diagnosed with invasive cervical cancer, with a median age of 52.78 years. Squamous cell carcinoma accounted for 78.94% of the cases, while 21.05% had adenocarcinoma. Among the 190 samples, 80.53% tested positive for HPV DNA, while 19.47% were negative. The most common genotypes detected were HPV 16 (64.05%), 18 (16.99%), and 58 (6.54%). HPV infection frequency was higher in samples from patients with elementary level education ($p < 0.05$).

Conclusions. This study provides valuable insights into the distribution of HPV genotypes in invasive cervical cancer samples from Ecuadorian women. The results indicate an elevated presence of HPV 16, HPV 18, and HPV 58, which are vaccine-preventable genotypes.

Keywords Papillomavirus infections; uterine cervical neoplasms; Ecuador.

Persistent infection by human papillomavirus (HPV) is the primary etiologic factor for developing cervical cancer and other types of cancer including vaginal, anal, penile, and head and neck cancers (1, 2).

Currently, more than 230 HPV genotypes have been identified, from which the International Agency for Research on Cancer (IARC) has recognized 12 genotypes as carcinogenic in humans: HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59 (3). Among these carcinogenic genotypes HPV 16 and HPV 18 are the most prevalent, causing about 70% of all invasive cervical cancers (4).

Globally, cervical cancer ranks fourth in incidence among women, and it is commonly diagnosed in low- and middle-income countries in Africa and Latin America, settings with serious limitations in access to early cancer detection and health services in general (5).

In Ecuador, cervical cancer is the second leading cause of female cancer, with an age-standardized incidence rate of 17.5 per 100 000 women and a mortality rate of 8.2 per 100 000 women (6).

However, there is a lack of research on HPV genotype distribution in advanced cervical cancer samples in Ecuador, and

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the limited studies available have shown varying frequencies of HPV 58, which differs from the distribution observed in other countries in the region and should be interpreted with caution due to small sample size (7, 8). Understanding the local prevalence of HPV genotypes in cervical cancer samples is crucial for effective prevention and control strategies, as it can have implications for screening, diagnosis, and treatment strategies. Thus, there is a need for accurate and up-to-date information on HPV genotype distribution in Ecuador.

Considering these gaps in knowledge, this study aimed to provide additional local information on HPV genotype distribution in invasive cervical cancer samples from women attending an oncological hospital in Ecuador.

MATERIALS AND METHODS

Study design

This retrospective cross-sectional study utilized 190 archival formalin-fixed paraffin-embedded (FFPE) invasive cervical cancer tissue samples. The samples were obtained from the Pathology Department of Sociedad de Lucha Contra el Cáncer (SOLCA) between 2017 and 2021. SOLCA is a private nonprofit healthcare organization located in the city of Guayaquil, Ecuador, founded in 1951, with a comprehensive approach to cancer care from prevention to palliative care. Guayaquil has a high incidence rate of cervical cancer, and SOLCA is a major center for diagnosis and treatment.

Ethical approval. The Ethics Committee of the Kennedy Hospital Group approved the study under the number CEISH-22-0014 on 6 June 2022.

Case selection. The primary database for case extraction was the SOLCA Guayaquil hospital-based cancer registry. Cases were identified according to the ICD-10 code C53, including patients with histopathological diagnosis of cervical cancer of any type. The corresponding FFPE samples were retrieved from the archives of the Pathology Department and processed at the Laboratory of Molecular Biology for HPV detection. Only samples obtained as pre-treatment biopsies or resection tissue samples were included, following SOLCA's routine protocols for diagnostics and handling. The study included patients aged 18 or older diagnosed with confirmed invasive cervical cancer at SOLCA between 2017 and 2021.

Exclusion criteria. Cases were excluded if FFPE tissues could not be identified in the archive of the Pathology Department or if the available tissue samples were of low quality, preventing appropriate HPV status assessment (e.g., blocks in poor condition, too thin, or judged as poorly preserved). Additionally, specimens that histologically did not confirm the presence of invasive cancer, including cervical cancer precursors like carcinoma in situ, were excluded.

Sample size estimation. Based on the estimated incidence of cervical cancer in 2018, according to IARC (1 612 cases), a minimum of 70 invasive cervical cancer samples were estimated to achieve a 5% precision level. A total of 200 samples from the study period were identified, and after removing duplicates and applying the inclusion/exclusion criteria, 190 FFPE samples were included in the study.

Data collection. Epidemiological and clinical data were retrieved from clinical records and hospital databases. The frequency and distribution of HPV genotypes were determined based on the analysis of the FFPE samples.

Statistical analysis

Given the observational nature of this study, descriptive statistics were used to summarize the distribution of HPV genotypes in the sample. Observed frequencies and relative frequencies (percentages) were calculated for categorical variables, such as the prevalence of specific HPV genotypes.

To assess HPV prevalence, the numerator for the calculation was the subset of patients diagnosed with invasive cervical cancer attributable to HPV. This was determined based on the positivity of HPV DNA in the study period. The denominator was the total number of patients diagnosed with cervical cancer tested for HPV by the central lab during the study period.

The estimated HPV prevalence or attributable fractions were described using frequencies and related percentages. The HPV genotypes detected were described for the overall sample, accounting for potential coinfections.

Sample processing

After tissue recovery and deparaffinization, the samples were digested using tissue lysis buffer and proteinase k and then processed for viral nucleic acid extraction and purification using silica columns.

HPV genotypes were identified using the Fujirebio INNO-LiPA® HPV Genotyping Extra II. During the workflow, a conventional PCR using the SPF10 set of primers was performed in Applied Biosystems®/GeneAmp® 9700 thermal cyclers, following the correct temperature profile.

Immediately after hybridization, the PCR product was denatured and hybridized with specific probes for the detection of HPV genotypes 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68; 26, 53, 66, 70, 73, 82; 6, 11, 40, 42, 43, 44, 54, 61, 62, 67, 81, 83, and 89, using the Fujirebio TENDIGO® hybridizer, according to the manufacturer's indications.

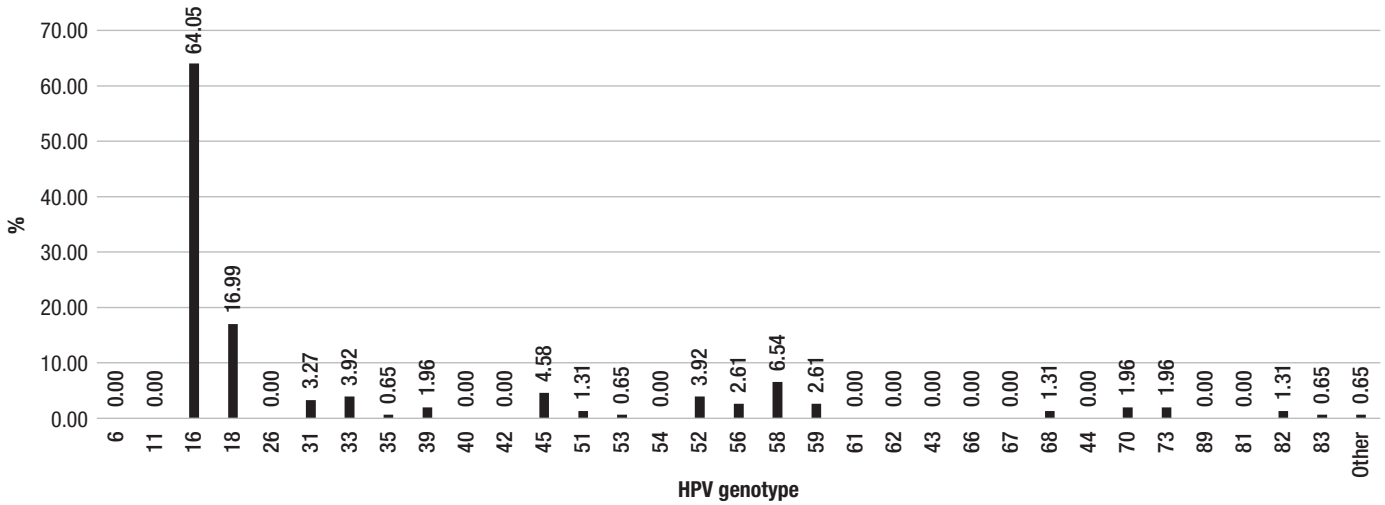
RESULTS

This study included 190 women diagnosed with invasive cervical cancer with a mean age of 52.78 years. Of these, 150 (78.94%) had squamous cell carcinoma, while 40 (21.05%) had adenocarcinoma.

Regarding HPV distribution, 153 samples (80.53%) tested positive for HPV DNA, while 37 (19.47%) were negative. High-risk carcinogenic genotypes, including HPV 16, HPV 18, and HPV 58, as recognized by IARC, showed the highest relative frequencies (Figure 1). HPV 16 and 18 were the most frequent genotypes in squamous cell carcinoma and adenocarcinoma histological types.

Among the samples, 127 (66.84%) tested positive for a single HPV genotype. The most prevalent genotypes were HPV 16, found in 79 samples (41.58%), HPV 18 in 20 samples (10.53%), HPV 58 in 6 samples (3.16%), and HPV 45 in 5 samples (2.63%). One sample tested positive for a genotype not detectable by the testing methodology and was classified as "other" (0.53%). Altogether, genotypes different from HPV 16 and HPV 18 were found in 27 cases (14.21%).

FIGURE 1. Frequency of different human papillomavirus genotypes



Source: Prepared by the authors based on the study data.

In 26 cases (13.68%), multiple genotypes were detected. The most frequent combinations included HPV 16/HPV 58 in 6 samples (3.16%), HPV 16/HPV 18 in 3 samples (1.58%), and HPV 16/HPV 33 in 2 samples (1.05%). All combinations involved at least one high-risk genotype.

Regarding sociodemographic and clinical characteristics, the majority of women included in the study had a low socioeconomic status (155, 81.58%), with elementary-level education being the most common (139, 73.16%), followed by high school (51, 26.84%). The most prevalent occupation was domestic chores (161, 84.74%).

The frequency of HPV detection was higher in patients with a lower level of education, with 117 testing positive for HPV (61.58%), compared to patients with higher education, where 36 tested positive (18.95%) ($p < 0.05$ by chi-square test). No statistically significant associations were observed between HPV presence and other characteristics such as age, socioeconomic status, occupation, and histopathology (Table 1).

A total of 146 cases (76.84%) had unavailable or lack of screening information before cervical cancer diagnosis. Among the remaining patients, a Pap smear was performed less than three years before diagnosis in 30 cases (15.79%), more than three years prior in 11 cases (5.79%), and more than 10 years prior in 1 patient (0.53%).

The most common clinical manifestations reported were bloody discharge in 98 patients (51.58%) and pelvic pain in 63 cases (33.16%).

Data on the International Federation of Gynecology and Obstetrics stage (FIGO stage) were available for 148 cases (77.89%), while 42 patients (22.11%) had no recoverable data. Among the cases with available information, 24 (12.63%) were classified as FIGO stage I (tumor confined to the cervix), 82 (43.16%) as FIGO stage II (tumor invades beyond the uterus but has not extended to the lower third of the vagina or pelvic wall), 36 (18.95%) as FIGO stage III (tumor involves the lower third of the vagina, extends to the pelvic wall, or causes hydronephrosis), and 6 (3.16%) as FIGO stage IV (carcinoma has spread beyond the pelvis, to the bladder or rectum).

DISCUSSION

Persistent infection by HPV high-risk genotypes is necessary for further cervical cancer development (9). Cervical cancer is the fourth most common cancer among women globally and occupies the second place behind breast cancer in places with a lower development index (9, 10). In Ecuador it is the second most common cancer in women, and despite the current cervical cancer prevention strategy recommending conventional cytology for women aged 21–67 years, it is reported that only 58% of women have been screened (11).

The lack of a structured program for prevention, low investment in preventive care, and sociocultural problems have been proposed as the cause of this low coverage (11). These same factors can contribute to underestimating the prevalence of HPV infection and the distribution of its genotypes.

To our knowledge this is the first time a study aims to determine the distribution of HPV genotypes in invasive cervical cancer samples in Ecuador. Most of the included samples had little recoverable data on the patient's clinical condition or demographic characteristics, which represents the major limitation of the study.

The most frequently detected genotypes were HPV 16 and HPV 18, included in all the available vaccines, and HPV 58, included in the nonavalent vaccine.

Together, the frequency of other high-risk genotypes including HPV 31, 33, 35, 39, 45, 52, 58, and 59 reached 31.37%, which is considerably higher than the frequency obtained for HPV 18 alone and almost half of the frequency for HPV 16. Most of these genotypes, specifically HPV 31, 33, 45, and 58, correspond to the second most common types associated with cervical cancer globally (12).

TABLE 1. Frequency analysis according to sample characteristics

Variable	HPV+	HPV–	Total	*p* value X^2 test
Education level				
Elementary	117	22	139	0.036*
High school/tertiary	36	15	51	
Total	153	37	190	
Socioeconomic status				
Low	123	32	155	0.391
Middle	30	5	35	
Total	153	37	190	
Occupation				
Domestic chores	132	29	161	0.231
Other	21	8	29	
Total	153	37	190	
Age				
40–59	79	16	95	0.270
18–39	29	12	41	
60–79	40	7	47	
≥80	5	2	7	
Total	153	37	190	
Histopathology				
Adenocarcinoma	28	12	40	0.058
Squamous cell carcinoma	125	25	150	
Total	153	37	190	

Note: *Statistically significant ($p < 0.05$), chi-square test.
Source: Prepared by the authors based on the study data.

Considering the obtained genotype distribution, any of the currently available vaccines may be useful in preventing more than 60% of cervical cancer cases, while almost 30% of the cases related to other types justify the need for broader genotype coverage, especially for HPV 58.

The infection prevalence was higher in women with elementary level education, a result that should be interpreted with caution due to the sociocultural complexity of the analyzed sample. The majority of the sample included women with a lower level of education and lower socioeconomic status, which accounts for most of the patients attended at a nonprofit healthcare institution, and so further and more complex studies are needed to clarify.

The major contribution of this study is to provide a valuable understanding of HPV genotype distribution in invasive cervical cancer samples from Ecuadorian women and indicate an elevated presence of HPV 16, HPV 18, and HPV 58, which are vaccine-preventable types.

Author contributions. CCBR, GMGD, and JCRC conceived the original idea; CCBR planned the experiments; GMGD collected and analyzed the data and interpreted the results; GMGD, FAL, and SMG wrote the paper; CCBR, FAL, SMG, and JCRC reviewed the paper. All authors reviewed and approved the final version.

Acknowledgments. The authors thank Dr. Rina Quinto Briones, Chief of the Preventive Medicine Department at SOLCA Hospital, for facilitating data collection.

Conflict of interest. This study was funded by MSD Colombia, a subsidiary of Merck & Co., Inc., Rahway, NJ, United States of America.

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Manuscript submitted on 23 May 2024. Revised version accepted for publication on 25 September 2024.

Frecuencia elevada de genotipos de VPH de alto riesgo prevenibles en mujeres ecuatorianas con cáncer cervicouterino invasivo

RESUMEN

Objetivo. Determinar la distribución de los genotipos del virus del papiloma humano (VPH) en muestras de cáncer cervicouterino invasivo de mujeres ecuatorianas que acudieron a la Sociedad de Lucha contra el Cáncer (SOLCA).

Métodos. Se desparafinizaron muestras tisulares archivadas de cáncer cervicouterino fijadas en formol e incluidas en parafina (FFPE) recolectadas durante el período 2017-2021; y se realizó una extracción y purificación de ácidos nucleicos mediante columnas de sílice. Los ácidos nucleicos obtenidos se analizaron mediante el INNO-LiPA® HPV Genotyping Extra II, según las especificaciones del fabricante. Los datos se extrajeron de los registros y los genotipos del VPH se determinaron a partir de las muestras FFPE.

Resultados. El estudio incluyó muestras de 190 mujeres con un diagnóstico de cáncer cervicouterino invasivo, de una edad media de 52,78 años. El carcinoma de células escamosas representó el 78,94% de los casos, mientras que el adenocarcinoma supuso el 21,05%. De las 190 muestras, el 80,53% presentaron un resultado positivo para ADN del VPH, mientras que en el 19,47% el resultado fue negativo. Los genotipos más frecuentes detectados fueron los VPH 16 (64,05%), 18 (16,99%) y 58 (6,54%). La frecuencia de la infección por el VPH fue mayor en las muestras de pacientes con un nivel educativo básico ($p < 0,05$).

Conclusiones. Este estudio proporciona información valiosa sobre la distribución de los genotipos del VPH en muestras de cáncer cervicouterino invasivo de mujeres ecuatorianas. Los resultados indican una presencia elevada de VPH 16, VPH 18 y VPH 58, que son genotipos prevenibles mediante vacunación.

Palabras clave Infecciones por papillomavirus; neoplasias del cuello uterino; Ecuador.

Alta frequência de genótipos de alto risco de HPV preveníveis em mulheres equatorianas com câncer invasivo do colo do útero

RESUMO

Objetivo. Determinar a distribuição de genótipos de papilomavírus humano (HPV) em amostras de câncer invasivo do colo do útero em mulheres equatorianas atendidas no Instituto de Câncer (Sociedad de Lucha Contra el Cáncer – SOLCA).

Métodos. Amostras arquivadas de tecido de câncer do colo do útero fixadas em formalina e embebidas em parafina (FFPE, na sigla em inglês) coletadas entre 2017 e 2021 foram submetidas a desparafinização, com posterior extração e purificação de ácidos nucleicos usando colunas de sílica. Os ácidos nucleicos obtidos foram analisados usando INNO-LiPA® HPV Genotyping Extra II segundo as especificações do fabricante. Extraíram-se dados dos prontuários, e os genótipos de HPV foram determinados a partir das amostras de FFPE.

Resultados. O estudo incluiu amostras de 190 mulheres diagnosticadas com câncer invasivo do colo do útero, com idade mediana de 52,78 anos. O carcinoma de células escamosas foi responsável por 78,94% dos casos, e 21,05% tinham adenocarcinoma. Das 190 amostras, 80,53% tiveram resultado positivo para DNA de HPV e 19,47%, resultado negativo. Os genótipos mais frequentemente detectados foram HPV 16 (64,05%), 18 (16,99%) e 58 (6,54%). A frequência de infecção por HPV foi maior nas amostras de pacientes com nível de escolaridade fundamental ($p < 0,05$).

Conclusões. Este estudo fornece informações valiosas sobre a distribuição dos genótipos de HPV em amostras de câncer invasivo do colo do útero de mulheres equatorianas. Os resultados indicam presença elevada de HPV 16, HPV 18 e HPV 58, genótipos preveníveis por vacinação.

Palavras-chave Infecções por papillomavirus; neoplasias do colo do útero; Equador.