Prevalence and genotypes of the human papillomavirus in laryngeal tissue samples of patients with laryngeal cancer from Northeastern Mexico

Prevalencia y genotipos del virus del papiloma humano en muestras de tejido laríngeo de pacientes con cáncer de laringe del noreste de México

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Abstract

Background: Laryngeal cancer represents 21.7% of malignancies of the upper aerodigestive tract. The prevalence of the Human Papillomavirus (HPV) in laryngeal cancer ranges 0 to 80%. **Methods:** We included 112 laryngeal tissue samples obtained from patients with laryngeal cancer. DNA was extracted and amplified by PCR. HPV presence and genotype were analyzed by the reverse hybridization INNO-LiPA[®] assay. Chi-square, Fisher's and unpaired Student t tests were used. **Results:** Samples from 107 male (95.5%) and 5 female patients (4.5%) were evaluated, aged 65.3±10.1 years, 108 with smoking history (96.4%), 9 with alcoholism history (8.0%), and in 96 the histological diagnosis was moderately differentiated keratinizing squamous cell carcinoma (85.7%). HPV was detected in 60 samples (53.5%), HPV-11 in 51 (45.5%), HPV-52 in 27 (24.1%), HPV-16 in 9 (8.0%), HPV-45 in 3 (2.6%), and coinfection by more than one genotype in 31 (27.6%). There was no difference between patients with and without HPV infection with respect to age, sex, tumor location and histology, smoking and alcoholism history ($p > 0.05$). **Conclusions:** The prevalence of HPV infection in laryngeal cancer was 53.5% with coinfection with more than one genotype in 27.6%. The most frequent genotype was HPV-11, an oncogenic low-risk genotype, followed by HPV-52, a high-risk genotype.

Key words: Laryngeal cancer. Human papillomavirus virus. HPV. Genotype.

Resumen

Antecedentes: El cáncer de laringe representa el 21.7% de las neoplasias malignas de vías aerodigestivas superiores. La prevalencia del virus del papiloma humano (VPH) en el cáncer de laringe oscila entre el 0 y el 80%. **Método:** Se incluyeron

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112 muestras de tejido laríngeo de pacientes con cáncer de laringe. Se amplificó el ADN y se analizó la presencia y el genotipo del VPH mediante hibridación reversa (INNO-LiPA®). Se realizaron pruebas de ji cuadrada, Fisher y t de Student no pareada. **Resultados:** Se incluyeron muestras de 107 hombres (95.5%) y 5 mujeres (4.5%), con una edad de 65.3 ± 10.1 años, con antecedente de tabaquismo 108 (96.4%), alcoholismo 9 (8.0%) y carcinoma epidermoide moderadamente diferenciado queratinizante 96 (85.7%). Se identificó VPH en 60 (53.5%), VPH-11 en 51 (45.5%), VPH-52 en 27 (24.1%), VPH-16 en 9 (8.0%), VPH-45 en 3 (2.6%) y coinfección por más de un genotipo en 31 (27.6%). No hubo diferencia entre pacientes con y sin infección por VPH en cuanto a edad, sexo, localización, diagnóstico histopatológico, tabaquismo ni alcoholismo ($p > 0.05$). **Conclusiones:** La prevalencia de infección por VPH en el cáncer de laringe fue del 53.5%, con coinfección por más de un genotipo en el 27.6%. El genotipo más frecuente fue el VPH-11, tipo de bajo riesgo, seguido por el VPH-52, de alto riesgo oncogénico.

Palabras clave: Cáncer de laringe. Virus del papiloma humano. VPH. Genotipo.

Introduction

Laryngeal cancer accounts for 21.7% of malignant neoplasms of the upper aerodigestive tract, but only for 0.8% of total malignancies¹. Laryngeal cancer has as main risk factors smoking and alcoholism. Information in international literature is scarce with regard to other possible risk factors, such as gastroesophageal reflux and human papillomavirus (HPV) infection²⁻⁴.

HPV is a double-stranded DNA virus whose tumor activity is mainly associated with oncoproteins E6 and E7. HPV genotypes are divided in those with low oncogenic risk (6, 11, 40, 42, 53, 54 and 57) and those associated with high risk (16, 18, 32, 35, 39, 45, 51, 56 and 58)⁵. HPV has been identified as the cause of different squamous cell head and neck cancers, with HPV-16 being the most commonly associated with laryngeal carcinoma⁶⁻⁹.

In the international literature, the presence HPV has been reported in laryngeal cancer at a percentage that ranges between 0 and 80%⁹⁻¹⁷. In Mexico, there are three published studies concerning HPV prevalence in larynx cancer. In the first of them, 16 of 32 patient samples were HPV-positive; the second found that only 4 of 16 studied samples had HPV infection; and in the third study, only 2 out of 45 samples were HPV-positive^{9,18,19}. In the present study, HPV prevalence and genotypes were evaluated in laryngeal tissue samples of patients with laryngeal cancer from a tertiary care medical unit of northeastern Mexico.

Method

Samples of cancer-diagnosed laryngeal tissue embedded in paraffin blocks, which were obtained by biopsy or complete resection in the otorhinolaryngology and pathological anatomy department of a tertiary

care medical unit of the city of Monterrey, located in northeastern Mexico, were collected. Samples obtained and processed in the period from 2012 to 2015, which were 112, were included. Those with extensive necrosis, histologically defined as the presence of inappropriate necrotic tissue for DNA recovery in more than 50% of the slide, samples with useless material due to inadequate histological processing due to poor fixation of the sample, cases of insufficient sample when, due to scarce sample of tissue, there was no representative neoplastic cells, and those from patients whose record was not available in the hospital archive were eliminated. Information on patient clinical characteristics, such as age, gender, history of smoking, alcohol consumption, tumor location and histopathological diagnosis was collected from medical records.

DNA extraction

Two fragments of the paraffin-embedded tissue were obtained by punch (biopsy punch). To obtain DNA, the Nucleo Spin® FFPE DNA kit was used (Macherey-Nagel, Düren, Germany). The fragments were placed in a 1.5-mL microcentrifuge tube and 400 µL of reagent was added to dissolve the paraffin; the sample was incubated for 3 minutes at 60 °C, vortexed and allowed to temper at room temperature. 100 µL of FL buffer were added and centrifuged at 11,000 x g for 1 minute, with generation of two phases: lower-aqueous (tissue and buffer) and higher-organic (paraffin). 10 µL proteinase K were added to the lower phase and mixed with pipette; then, it was incubated at room temperature for 3 hours. 100 µL of Decrosslink® buffer were added and it was incubated at 90 °C for 30 minutes to remove the material interspersed in the isolated DNA. Once the process was finished, it was left to stand at room temperature for

2 minutes. 200 μL of ethanol (96-100%) were added and vortexed (2-5 seconds), centrifuged for 30 seconds at 11,000 $\times g$ to separate the phases. The aqueous phase was pipetted and placed on the NS-FFPE column installed in a 2-mL collecting tube. The DNA present in the sample binds to the silica membrane of the column. Salts, metabolites and macromolecular cellular components were removed with two wash and centrifugation assays (400 μL buffer B5, 2,000 $\times g$ for 30 seconds and 11,000 $\times g$ for 30 seconds). The collector tube used was removed and the column was placed in a new microcentrifuge tube. 20 μL of BE buffer was placed at the center of the silica membrane and centrifuged for 30 seconds at 11,000 $\times g$, whereby a final DNA eluate was obtained (15-20 μL), whose purity and concentration were analyzed by spectrophotometry.

Viral DNA amplification

The viral genome was analyzed using the reverse linear hybridization INNO-LiPA® HPV Genotyping Extra kit (Innogenetics, Belgium). A Master Mix was prepared on ice, containing 37.7 μL of AMP MIX (biotinylated primers, deoxyribonucleotides, MgCl_2 and 0.05% NaN_3 as preservative). 2.3 μL of ENZ MIX (AmpliTaq Gold®) were added, and this mixture was then added 10 μL of the final DNA eluate. As DNA positive control, 10 μL of the control provided for the kit were used, which was positive as hybridization internal control in all experiments and corresponds to HPV-6. As DNA negative control, 10 μL of DNA-free distilled H_2O were used in all experiments

Detection and typing

- Hybridization: 10 μL of the amplified biotinylated product were used for hybridization in the strip, which were mixed with 10 μL of denaturation solution in the hybridization independent channels placed on a plastic plate for 5 minutes at room temperature. 10 μL of the biotinylated amplified product were mixed with 10 μL of denaturation solution during 5 minutes at room temperature at each independent hybridization channel of the plastic plate provided by the manufacturer. Then, 2 mL of hybridization solution were added to each channel and mixed by pipetting. One previously-marked test strip was placed at its respective channel. To finish this stage, the channels were

placed in a water bath at 49 °C with agitation (approximately 80 agitations per minute) for 60 minutes.

- Washing: after hybridization, the plastic plate was held at an angle that allowed the fluid contained in each channel to be aspirated with a pipette. Then, 2 mL of the washing solution, preheated at 37 °C, were added to each channel and agitated for 10 to 20 seconds at room temperature. This step was repeated three times, with the last one being left to incubate in a water bath at 49 °C under agitation for 30 minutes.
- Coloring development: all subsequent incubations were carried out at a temperature ranging from 20 to 25 °C in agitation. Each strip was washed twice for 1 minute using 2 mL of the rinse solution. Then, 2 mL of the conjugated were added to each channel and incubated for 30 minutes with agitation. After aspirating the fluid of each channel, each strip was washed twice using the rinse solution under agitation for 1 minute, and then the same step was carried out using 2 mL of substrate buffer. Then, the buffer was aspirated to then add 2 mL of substrate solution to each channel, leaving them in incubation for 30 minutes under agitation. This last step provided coloration of the lines that displayed hybridization. The reaction was stopped by adding 2 mL of distilled water under agitation for 3 minutes.
- Interpretation of results: the hybridization strips they have three controls, which consist of a conjugate control band, a human DNA control band and two HPV control bands. Subsequently, the reading of the number of reactive bands resulting from the hybridization process between amplification products and the immobilized probes on the strip was carried out. The band pattern of each strip was compared with the interpretation table provided by the manufacturer to determine the genotype(s) present in each sample.

Statistical analysis

For analysis of the results, absolute frequencies, percentages, means and standard deviations were used. In addition, the chi-square test, Fisher's exact probability test and Student's t-test were used for independent samples. The analysis was performed with the SPSS package v. 20.0 for Windows, and a p-value < 0.05 was considered significant.

Ethical aspects

The present study adheres to the ethical principles of the Declaration of Helsinki and its subsequent developments, and to the Principles of Good Clinical Practice. In addition, it is in accordance with the standards established in the General Statute of Health and with institutional health research regulations. The protocol was authorized by the National Commission of Scientific Research of the institution where the study was conducted with registration No. R-2014-785-055. Obtaining informed consent was not required since only patient records were reviewed.

Results

Hundred and twelve laryngeal tissue samples corresponding to 112 patients with laryngeal cancer from a tertiary care medical unit of northeastern Mexico were included. These samples corresponded to 72 slides and paraffin blocks obtained by biopsy (64.3%) and 40 slides and paraffin blocks obtained by laryngectomy (35.7%). Five patients were females (4.5%) and 107 were males (95.5%). Mean age was 65.3 ± 10.1 years. Most patients ($n = 108$) had a smoking history (96.4%), and few ($n = 9$) had a positive history of alcoholism (8.0%). Tumors most common location was glottic, in 65 patients (58%), followed by transglottic in 26 (23.2%) and supraglottic in 17 (15.2%). The most common histological diagnosis was moderately-differentiated keratinizing squamous cell carcinoma in 96 patients (85.7%), followed by well-differentiated invasive squamous cell carcinoma in 11 (9.8%), moderately-differentiated non-keratinizing squamous cell carcinoma in 3 (2.7%) and poorly-differentiated squamous cell carcinoma in 2 (1.8%) (Tables 1 and 2).

HPV infection was detected in 60 of the 112 samples (53.5%). Most common genotype was HPV-11 in 51 samples (45.5%), followed by HPV-52 in 27 (24.1%), HPV-16 in 9 (8.0%) and HPV-45 in 3 (2.6%). Coinfection with more than one genotype was detected in 31 samples (27.6%), with the most common co-infections being with HPV-11 and HPV-52 (Table 2). There was no significant difference between patients with and without HPV infection in terms of distribution by age, gender, tumor location and histopathological diagnosis, or with regard to previous history of smoking or alcoholism ($p > 0.05$) (Table 1).

Table 1. Clinical and histopathological characteristics of 112 Northeastern Mexico patients with laryngeal cancer classified according to HPV identification in laryngeal tissue

	Total (n = 112)	With HVP (n = 60)	Without HVP (n = 52)	p
Age (years)*	65.3 $\pm$ 10.1	65.5 $\pm$ 8.9	64.9 $\pm$ 11.4	0.756
Gender				0.432
Males	107 (95.5%)	58 (54.2%)	49 (45.8%)	
Females	5 (4.5%)	2 (40%)	3 (60%)	
Sample				0.022
Biopsy	72 (64.3%)	33 (45.8%)	39 (54.2%)	
Laryngectomy	40 (35.7%)	27 (67.5%)	13 (32.5%)	
Location				0.556
Glottic	65 (58.0%)	32 (49.2%)	33 (50.8%)	
Subglottic	4 (3.6%)	3 (75.0%)	1 (25.0%)	
Supraglottic	17 (15.2%)	11 (64.7%)	6 (35.3%)	
Transglottic	26 (23.2%)	14 (53.8%)	12 (46.2%)	
Histopathological diagnosis				0.406
WDISCC	11 (9.8%)	5 (45.5%)	6 (54.5%)	
MDNKSCC	96 (85.7%)	53 (55.2%)	43 (44.8%)	
MDKSCC	3 (2.7%)	2 (66.7%)	1 (33.3%)	
PDSCC	2 (1.8%)	0 (0%)	2 (100%)	
Smoking				0.365
Yes	108 (96.4%)	57 (52.8%)	51 (47.2%)	
No	4 (3.6%)	3 (75%)	1 (25%)	
Alcoholism				0.120
Yes	9 (8.0%)	7 (77.8%)	2 (22.2%)	
No	103 (92.0%)	53 (51.5%)	50 (48.5%)	

*At the time of biopsy collection or laryngectomy.

Values presented as mean $\pm$ standard deviation or absolute frequency (percentage)
 WDISCC: well-differentiated invasive squamous cell carcinoma; MDNKSCC: moderately-differentiated non-keratinizing squamous cell carcinoma; MDKSCC: moderately-differentiated keratinizing squamous cell carcinoma; PDSCC: poorly-differentiated squamous cell carcinoma.

Discussion

Laryngeal cancer accounts for 21.7% of upper aerodigestive tract malignant neoplasms, and has as main risk factors smoking and alcoholism; information in international literature is scarce with regard to HPV infection as possible risk factor¹⁻⁴. The presence of HPV in larynx cancer has been reported in 0 to 80% of cases⁹⁻¹⁷. In Mexico, there are three published studies related to the subject: in the first of them, 16 of 32 samples of patients with laryngeal cancer were positive for HPV (50%), in the second, HPV was demonstrated in 4 of 16 samples of patients with laryngeal cancer (25%), and in the third one, only in 2 of 45 samples of laryngeal cancer patients (4.4%) could HPV be detected^{9,18,19}. The present study evaluated HPV prevalence and genotypes in samples of laryngeal tissue of patients with laryngeal cancer who were

Table 2. Clinical and histopathological characteristics and HPV genotype, in 60 patients with laryngeal cancer of northeastern Mexico

Patient	Age	Gender	Sample	Localization	Histopathological diagnosis	Smoking	Alcoholism	VPH genotype(s)
1	54	F	Biopsy	Glottic	MDKSCC	Yes	No	11
2	66	M	Biopsy	Supraglottic	WDISCC	Yes	Yes	11
3	66	M	Biopsy	Glottic	MDKSCC	Yes	No	11
4	54	M	Biopsy	Glottic	MDKSCC	Yes	No	11, 16
5	64	M	Laryngectomy	Transglottic	MDKSCC	Yes	No	11
6	64	M	Biopsy	Glottic	MDKSCC	Yes	No	11, 16, 40, 82
7	70	M	Laryngectomy	Glottic	MDKSCC	Yes	No	11
8	52	M	Biopsy	Glottic	MDKSCC	Yes	No	11
9	67	M	Biopsy	Supraglottic	MDKSCC	Yes	No	11
10	67	M	Biopsy	Glottic	MDKSCC	Yes	Yes	11
11	58	M	Laryngectomy	Subglottic	MDKSCC	Yes	No	11
12	71	M	Laryngectomy	Transglottic	MDKSCC	Yes	No	11
13	59	M	Biopsy	Glottic	MDNKSCC	Yes	Yes	16
14	49	M	Laryngectomy	Transglottic	WDISCC	Yes	No	11
15	66	M	Biopsy	Glottic	MDKSCC	Yes	No	11
16	65	M	Laryngectomy	Glottic	MDKSCC	Yes	Yes	11
17	67	M	Biopsy	Supraglottic	MDKSCC	Yes	No	11
18	76	M	Biopsy	Supraglottic	MDKSCC	Yes	No	11
19	80	M	Biopsy	Glottic	MDKSCC	No	No	11
20	61	M	Laryngectomy	Subglottic	MDKSCC	Yes	Yes	52
21	53	M	Laryngectomy	Supraglottic	MDKSCC	Yes	No	16
22	61	M	Biopsy	Supraglottic	MDKSCC	Yes	No	52
23	63	M	Biopsy	Glottic	MDKSCC	Yes	No	16
24	68	M	Biopsy	Supraglottic	MDKSCC	Yes	No	11, 16
25	79	M	Biopsy	Supraglottic	MDKSCC	Yes	No	11
26	81	M	Biopsy	Glottic	MDKSCC	Yes	No	11, 52
27	67	M	Biopsy	Glottic	MDKSCC	Yes	No	11, 16
28	71	M	Laryngectomy	Transglottic	MDKSCC	Yes	No	6, 11, 52
29	80	M	Biopsy	Supraglottic	MDKSCC	Yes	No	6, 11
30	63	M	Laryngectomy	Transglottic	MDKSCC	Yes	No	11, 52
31	68	M	Laryngectomy	Glottic	MDKSCC	No	Yes	11, 45, 52
32	83	M	Biopsy	Supraglottic	MDKSCC	Yes	No	6, 11, 52
33	66	M	Biopsy	Glottic	MDKSCC	Yes	No	52
34	60	M	Laryngectomy	Transglottic	MDKSCC	Yes	No	11, 52
35	67	M	Biopsy	Glottic	WDISCC	Yes	No	45
36	69	M	Biopsy	Glottic	MDKSCC	Yes	No	6, 11, 52

(Continues)

Table 2. Clinical and histopathological characteristics and HPV genotype, in 60 patients with laryngeal cancer of northeastern Mexico (Continued)

Patient	Age	Gender	Sample	Localization	Histopathological diagnosis	Smoking	Alcoholism	VPH genotype(s)
37	60	M	Laryngectomy	Glottic	MDKSCC	Yes	No	11, 45
38	72	F	Biopsy	Glottic	WDISCC	Yes	No	11, 52
39	72	M	Laryngectomy	Glottic	MDKSCC	Yes	No	11, 52
40	51	M	Laryngectomy	Transglottic	MDKSCC	Yes	No	52
41	61	M	Biopsy	Glottic	MDKSCC	Yes	No	11, 52
42	78	M	Biopsy	Glottic	MDKSCC	Yes	Yes	11, 16
43	61	M	Laryngectomy	Transglottic	MDKSCC	Yes	No	6, 11, 52
44	56	M	Laryngectomy	Subglottic	MDKSCC	Yes	No	11, 52
45	62	M	Biopsy	Glottic	MDKSCC	Yes	No	11, 52
46	79	M	Biopsy	Glottic	MDKSCC	Yes	No	11, 52
47	84	M	Laryngectomy	Glottic	MDKSCC	Yes	No	6, 11, 52
48	55	M	Biopsy	Glottic	MDKSCC	Yes	No	6, 11, 52
49	65	M	Laryngectomy	Transglottic	MDKSCC	Yes	Yes	11, 16, 52
50	58	M	Laryngectomy	Transglottic	MDKSCC	Yes	No	11, 52
51	72	M	Laryngectomy	Transglottic	MDKSCC	No	No	11, 52
52	74	M	Laryngectomy	Transglottic	MDKSCC	Yes	No	11, 52
53	67	M	Laryngectomy	Glottic	MDKSCC	Yes	No	52
54	72	M	Laryngectomy	Supraglottic	MDKSCC	Yes	No	11
55	64	M	Laryngectomy	Glottic	MDKSCC	Yes	No	11
56	44	M	Laryngectomy	Transglottic	MDKSCC	Yes	No	11
57	62	M	Biopsy	Supraglottic	MDKSCC	Yes	No	11, 52
58	70	M	Biopsy	Glottic	MDNKSCC	Yes	No	11, 52
59	49	M	Biopsy	Glottic	MDKSCC	Yes	No	11, 52
60	72	M	Laryngectomy	Glottic	MDKSCC	Yes	No	11, 52

F: female; M: male; MDKSCC: moderately differentiated keratinizing squamous cell carcinoma; MDNKSCC: moderately differentiated non-keratinizing squamous cell carcinoma; WDISCC: well differentiated invasive squamous cell carcinoma;.

provided care in a tertiary care medical unit of north-eastern Mexico.

Patient age of presentation was around 65 years, which is consistent with descriptions in international literature, since larynx cancer occurs more commonly in the sixth decade of life^{1,2}. No cases of pediatric age patients with larynx cancer were found. To date, there are about 80 pediatric patients with larynx cancer reported since 1868, some of them related to HPV infection²⁰⁻²⁴. In this study, patients of the male gender predominated, which is consistent with previously described observations, since larynx cancer especially

affects men^{1,2}. In 2009, Joos et al.²² reported a predominance of the male gender of up to 80%; however, this relationship is different in younger population, in whom only 60% of male predominance has been reported. In recent years, a change has been observed in laryngeal cancer behavior pattern, including an increase in its frequency in young people, women and tobacco or alcohol non-consumers^{22,25}.

Up to 96.4% of patients with laryngeal cancer of the present study had a history of smoking, which is higher than the rates reported in previous studies. In the United Kingdom, there is a history of smoking in up

to 79% of larynx cancer patients²⁶. People with a smoking history have a relative risk up to 8.3-fold higher for developing larynx cancer in comparison with never smokers²⁷.

The frequency of alcoholism in the sample of this study was 8%. Alcoholism increases up to four times the risk for developing larynx cancer when alcohol consumption is ≥ 25 ounces per month in comparison with non-alcoholic people²⁸. Additionally, a positive relationship has been identified between alcohol consumption and the predominantly supraglottic location of larynx neoplasms²⁹. In the laryngeal samples with HPV infection of the present study, the most common location was glottic, followed by transglottic and supraglottic, a location order similar to that reported in a recent systematic review.¹³ The low HPV prevalence in supraglottic tumors is explainable by the fact that these neoplasms have a more important association with alcohol consumption²⁹.

In this study, HPV DNA was detected in 53% of analyzed samples. Hobbs et al.⁸ assessed the relationship between HPV and head and neck cancer, demonstrating a strong association with cancers located in the tonsils (odds ratio [OR]: 15.1; 95% Confidence Interval [CI]: 6.8-33.7), an intermediate risk for the rest of the oropharynx (OR: 4.3; 95% CI: 2.1-8.9) and a very weak risk for the oral cavity (OR: 2.0; 95% CI: 1.2-3.4) and the larynx (OR: 2.0; 95% CI: 1.0-4.2). The prevalence of HPV infection in larynx cancer is variable, ranging from 0 to 80%⁹⁻¹⁷. In Mexico, there are only three studies reporting the prevalence of HPV infection in larynx cancer: one performed on a sample of 16 patients with a prevalence of 25%, another in 32 patients with a prevalence of 50%, and another that assessed 45 patients, with a prevalence of 4.4%^{9,18,19}. Therefore, the prevalence of HPV infection in larynx cancer detected in this series is the highest reported in Mexico. It should be noted that in this study there was a large number of samples available than in previous studies conducted in Mexico^{9,18,19}. The wide variability in HPV prevalence between studies could be due to bias in sample selection, differences in the techniques used for HPV detection, different anatomical sampling sites, poor quality of samples or individual genetic, ethnic, geographic and cultural differences^{12,13}.

The most common HPV genotype in this study was 11, in 45% of the samples, followed by 52 in 24% and 16 in 8.0%. Other detected genotypes were 6, 45, 82 and 40. Genotypes 6, 11 and 40 are of low oncogenic risk, while genotypes 16, 45, 52 and 82 are of high

oncogenic risk⁵. The low prevalence of HPV-16 detected in this series is remarkable since, in other studies, it has been the genotype that is most associated with the pathogenesis of laryngeal carcinoma⁶⁻⁹. In 2016, Villagómez-Ortiz et al.¹⁹ reported the HPV-11 genotype in 2 of 45 studied samples. This genotype shows a more aggressive behavior in patients with recurrent laryngeal papillomatosis, who require a larger number of surgical resections per year³⁰. There are cases that describe the transformation of recurrent laryngeal papillomatosis into squamous cell carcinoma in the presence of infection with low-risk genotypes, such as 6 and 11³¹.

In this study, coinfection was found with more than one genotype in 31 samples (27.6%), and the most common coinfection was with HPV-11 and HPV-52, which are genotypes of low and high oncogenic risk, respectively. Coinfection with more than one genotype was reported in two of the 16 samples examined in one of the previous studies conducted in Mexico, and it was with low- and high-risk genotypes¹⁸. This suggests that, in some cases of laryngeal cancer, HPV could be involved regardless of its oncogenic risk, or that both genotype viral groups could act synergistically¹⁸. In this study, one case of coinfection with genotypes 11, 16, 40 and 82 was identified; in international literature there are only reports of two cases of laryngeal cancer related to HPV-82, one of them with coinfection with HPV-73^{32,33}.

Among the possible clinical implications of HPV infection as a causative agent of larynx cancer, the genotypes present in this malignancy would be expected to resemble those found in cervical lesions, since orogenital contamination is the transmission route proposed as the more common. Therefore, the role of the HPV vaccines currently available in the market in the prevention of head and neck cancers, including laryngeal cancer, is still unknown¹². Furthermore, HPV infection in larynx cancer opens the possibility to new therapies with HPV-infected cells by as a target¹². On the other hand, knowing the prognosis of these patients is a matter of interest, given that there are reports of a greater therapeutic response and survival in patients with oropharyngeal cancer associated with HPV infection³⁴⁻³⁶.

Conclusions

The results of the present study show a prevalence of HPV infection of 53.5% in samples of laryngeal tissue of patients with laryngeal cancer, with coinfection

with more than one genotype in 27.6% of the samples. The most common genotype was HPV-11, a low-risk type, followed by HPV-52, a high-risk oncogenic genotype, but infection with high-risk genotypes was detected in 34.8% of the samples. A positive result for HPV DNA amplified by Polymerase Chain Reaction (PCR) only demonstrates its presence and not necessarily implies its role in carcinogenesis. The conduction of epidemiological studies at a larger scale is required, not only with larger samples, but also including simultaneous assessment of histopathological, immunohistochemical findings, of in situ hybridization and DNA amplification by PCR, in order to know the true prevalence of HPV infection in laryngeal mucosa and larynx cancer. This would also allow having more solid evidence of its true oncogenic potential in the larynx, as well as its possible interaction with well-established risk factors, such as alcohol and tobacco consumption. The prognosis of patients with laryngeal cancer associated with HPV infection, as well as the long-term impact of currently available vaccines on the development of this disease, remain to be established.

Ethical disclosure

Protection of people and animals. The authors declare that no experiments were performed on humans or animals for this investigation.

Confidentiality of data. The authors declare that they have followed the protocols of their work center on the publication of patient data.

Right to privacy and informed consent. The authors declare that no patient data appear in this article.

Conflict of interests

The authors declare that they have no conflicts of interests.

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