

# Influence Of Human Papillomavirus Expression in Laryngeal Carcinoma

Original  
Article

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## ABSTRACT

**Background:** Squamous cell carcinoma of the larynx represents 4.5% of all malignancies. There are known risk factors for laryngeal malignancy. One of them is human papillomavirus affection of the larynx. The risk of laryngeal carcinoma has been increased by up to 19-fold risk in people with evidence of human papillomavirus (HPV) infection in the larynx.

**Aim of the Study:** To detect the presence of HPV in patients with SCC of the larynx and to study its relation to tumour grading and lymph node invasion.

**Patients and Methods:** A Cross-sectional analytic study where detection of HPV 16 in 150 surgical formalin-fixed, paraffin-embedded (FFPE) laryngeal tissue specimens from 150 SCC lesions of patients who underwent surgical treatment at our institute. Detection of HPV is done using the qualitative method of DNA extraction and gel electrophoresis in our institute's medical biochemistry lab. All patients had no other apparent HPV-related infection at the time of examination, whether dermatological or genital.

**Results:** Examination of the specimens revealed that 6.7% had positive HPV 16 (n = 10), and there was a significant difference in the rate of lymph node invasion between the two groups. HPV-positive cases had a significantly ( $p = 0.048$ ) higher percentage of positive LNs (40% (n = 4/10)) than HPV-negative cases (28.6% (n = 40/140)). HPV-negative cases had a significantly ( $p < 0.001$ ) higher percentage of grade-I cancer (18.6% (n = 26)) than HPV-positive cases (0%). Unlikely, there was an insignificant difference ( $p = 0.210$ ) for grade-II cases (Positive cases (70% (n = 7) and negative cases 64.3% (n = 90)). Contrarily, HPV-positive cases had a significantly ( $p < 0.038$ ) higher percentage of grade-III/IV cancer (30% (n = 3)) compared with HPV-negative cases (17.3% (n = 24)).

**Conclusion:** HPV affects the grading of carcinoma as HPV-positive cases had a significantly higher percentage of grade-III/IV cancer compared with HPV-negative cases. Also, HPV associated with lymphadenopathy as HPV-positive cases had a significantly higher percentage of positive LNs than HPV-negative cases.

**Key Words:** Grading, human papillomavirus (HPV), laryngeal carcinoma, lymphadenopathy.

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## INTRODUCTION

Laryngeal cancer accounts for approximately 4.5% of all malignancies<sup>[1]</sup>, with squamous cell carcinoma (SCC) being the most common type of primary malignant tumour in the larynx<sup>[2]</sup>. Known risk factors for laryngeal malignancy include smoking and alcohol consumption. However, cases of malignancy arising in the absence of these factors suggest that additional cofactors may contribute to laryngeal cancer carcinogenesis. Notably, individuals with human papillomavirus (HPV) infection in the larynx face a significantly increased risk (up to 19-fold) of developing laryngeal carcinoma<sup>[3]</sup>.

HPV types affecting mucosal tissues are divided into

high-risk and low-risk groups, with around 15 types classified as high-risk. HPV-16, responsible for approximately 90% of HPV-positive head and neck SCC cases, is recognized as a human carcinogen due to the oncoproteins encoded in its DNA<sup>[4]</sup>. HPV is a small, non-enveloped icosahedral DNA virus from the Papillomaviridae family<sup>[5]</sup>. The virus's outer coat is subdivided into three regions: the early (E) region, which includes precursor proteins E1, E2, E4, E5, E6, and E7, and the late (L) region, which codes for the major and minor viral capsid proteins, L1 and L2<sup>[6]</sup>.

Four HPV vaccines are available, all produced using recombinant DNA technology. These vaccines consist of purified L1 protein that self-assembles into type-specific empty viral shells<sup>[7]</sup>.

HPV vaccination has proven highly effective against oral HPV-16/18 infections, reducing the incidence and mortality rates of related head and neck cancers<sup>[8]</sup>. It also helps prevent cervical, head, and neck cancers caused by HPV<sup>[9]</sup>. Additionally, studies have shown that the vaccine reduces recurrences of HPV-associated neoplasia or papilloma after surgical removal<sup>[10]</sup>.

In the Egyptian population, a single study examining oesophageal cancers with HPV expression found that HPV was detected in 54% of tumours and 24% of normal tissues<sup>[11]</sup>. To our knowledge, only one study has explored the prevalence of HPV in Egyptian laryngeal carcinomas and benign laryngeal lesions. This study revealed that 3.6% of malignant laryngeal lesions were HPV-positive, while all benign laryngeal lesion specimens were negative for HPV DNA<sup>[12]</sup>.

### AIM OF THE WORK

This study aims to detect the presence of HPV in patients with larynx SCC and investigate its relationship with tumour grading and lymph node invasion.

### PATIENTS AND METHODS

This study included 150 patients with laryngeal cancer who underwent surgical treatment in the ORL department between May 2021 and December 2022. The study was approved by the scientific committees of the Otorhinolaryngology and Medical Biochemistry departments and the Faculty of Medicine at Cairo University. Written informed consent was obtained from all participants.

Inclusion criteria were patients pathologically diagnosed with SCC of the larynx, those treated surgically (via total or partial laryngectomy), and patients without other apparent HPV-related infections (dermatological, oral, or genital) at the time of examination. Exclusion criteria included patients with laryngeal malignancies other than SCC, those with benign laryngeal lesions, and those with other HPV infections in the skin, oral, or genital regions.

HPV detection was conducted on 150 formalin-fixed, paraffin-embedded (FFPE) laryngeal tissue specimens using a qualitative method (positive or negative) based on DNA extraction and gel electrophoresis at the Medical Biochemistry Department, Kasr Al Aini. For DNA extraction, 150 tissue sections (10 µm thick) were transferred to Eppendorf vials after deep sectioning into the paraffin blocks. Total DNA was extracted, with sections deparaffinized using xylene and then washed twice with ethanol. The air-dried pellets were incubated with proteinase K and ATL lysis buffer for 10 hours at 56°C. DNA was then purified and eluted.

DNA amplification was performed using a thermal cycler with MY09 forward primer and MY11 reverse primer, following the protocol: initial denaturation at 95°C for 3 minutes; 35 cycles of denaturation at 95°C for 30

seconds, annealing at 55°C for 30 seconds, and extension at 72°C for 45 seconds; with a final extension at 72°C for 5 minutes. The products were loaded on 1% agarose gel, stained with ethidium bromide, and separated by electrophoresis.

Each PCR run included a positive and negative control (H<sub>2</sub>O). As an internal control for DNA extraction, samples were subjected to PCR for human β-globin gene amplification using PC03 and PC04 primers, targeting a 110 base pair sequence. Reagent concentrations for the β-globin PCR matched those used for HPV PCR, and all patients tested positive in the β-globin PCR.

The data were coded and analyzed using IBM-SPSS version 24. Descriptive statistics were calculated, including mean, standard deviation, median, range, frequencies, and percentages. The Chi-square/Fisher's exact test was used to compare frequency distributions between groups, and the Shapiro-Wilk test assessed data normality. Student's t-test or Mann-Whitney U test was used to compare means/medians of continuous variables (parametric and non-parametric). Statistical significance was set at  $p < 0.05$  \* IBM SPSS. Statistical Package for Social Science. Ver.24. Standard version. Copyright © SPSS Inc., 2012-2016. NY, USA. 2016.

### RESULTS

The basic demographic characteristics of the studied cases show the patients' age ranged from 43 to 73 years old, with a mean of  $56.9 \pm 7.2$  and a median of 65 years, and most of the cases were males. About 7% of cases were positive for HPV (n = 10), and LN was positive in about 29.3% of the sample (n = 44). Distribution of the sample according to disease grade was as follows: about 17% (n = 26) had grade-I, about two-thirds (n = 97) had grade-II, 16.7% (n = 25) had grade-III and only 1.3% (n = 2) had grade IV. Regarding the ratio of positive LN to the total examined LNs, the proportion ranged between 4% and 100%, with a median of 22% and a mean of  $21.7\% \pm 10\%$  (Table 1).

**Table 1:** Socio-demographic and Clinical characteristics of the studied Cases

Variable	Category	n = 150
Age in years	• Mean ± SD	56.95 ± 7.2
	• Median (Range)	65 (43 - 73)
Sex	• Male	144 (96%)
	• Female	6 (4%)
HPV	Negative	140 (93.3%)
	Positive	10 (6.7%)
LN	Negative	106 (70.7%)
	Positive	44 (29.3%)
% of Positive LN	Mean ± SD	21.7% ± 10%
	Median (Range)	20% (4% - 100%)
Grade	I	26 (17.3%)
	II	97 (64.7%)
	III	25 (16.7%)
	IV	2 (1.3%)

Regarding the sex of the patients, the male/female ratio was 80% in positive HPV cases, which was lower than 97% in negative cases, and this was statistically significant ( $p = 0.048$ ). On the other hand, Positive HPV cases were insignificantly older ( $59.7 \pm 6.6$  years) than negative cases ( $56.8 \pm 7.2$  years) (Table 2).

**Table 2:** Baseline Demographic Differences according to HPV Infection

	Negative HPV (n=140)	Positive HPV (n=10)	P-value
Age in years	56.75 $\pm$ 7.2	59.70 $\pm$ 6.6	= 0.102*
Sex			
• Female	4 (2.9%)	2 (20%)	= 0.048**
• Male	136 (97.1%)	8 (80%)	

There was a significant difference in cancer grades between the two groups. HPV-negative cases had a significantly ( $p < 0.001$ ) higher percentage of grade-I cancer (18.6% (n = 26)) than HPV-positive cases (0%). Unlikely, there was an insignificant difference ( $p = 0.210$ ) for grade-II cases (Positive cases (70% (n = 7) and negative cases 64.3% (n = 90)). Contrarily, HPV-positive cases had a significantly ( $p < 0.038$ ) higher percentage of grade-III/IV cancer (30% (n = 3)) compared with HPV-negative cases (17.3% (n = 24) (Table 3).

**Table 3:** Relationship between HPV infection and Cancer Larynx

	HPV Negative (n=140)	HPV Positive (n=10)	P-value
Cancer Grade			
Grade-I	26 (18.6%)	0 (0%)	< 0.001**
Grade-II	90 (64.3%)	7 (70%)	= 0.210*
Grade-III/IV	24 (17.3%)	3 (30%)	= 0.038*

There was a significant difference in the rate of lymphadenopathy between the two groups. HPV-positive cases had a significantly ( $p = 0.048$ ) higher percentage of positive LNs (40% (n = 4)) than HPV-negative cases (28.6% (n = 40)). However, HPV-positive cases had a significantly ( $p = 0.010$ ) higher median percentage of LN positivity (38% (7 – 100%)) compared with HPV-negative cases (16% (4 – 80%)) (Table 4).

**Table 4:** Relationship between HPV infection and Lymphadenopathy

	HPV Negative (n=140)	HPV Positive (n=10)	P-value
LN			
Negative	100 (71.4%)	6 (60%)	= 0.048*
Positive	40 (28.6%)	4 (40%)	
% of Positive LN			
Mean $\pm$ SD	19.3% $\pm$ 15.6%	45.5% $\pm$ 40.3%	= 0.010**
Median (Range)	16% (4% - 80%)	38% (7%-100%)	

## DISCUSSION

In our study, the prevalence of HPV was found to be 6.7%. Compared to other studies using similar techniques, Brandwein *et al.*<sup>[13]</sup> reported an HPV prevalence of 8% (3 out of 40 patients) in their study on HPV association with laryngeal carcinoma. Similarly, Guan *et al.*<sup>[14]</sup> found a prevalence of 19.4% (6 out of 31 patients) in their study on HPV infection in laryngeal squamous cell carcinoma and precancerous lesions. Dahm *et al.*<sup>[15]</sup> observed a prevalence of 10.6% (9 out of 85 patients) in their study on survival rates in hypopharyngeal and laryngeal squamous cell carcinomas. Davidson *et al.*<sup>[16]</sup> reported an HPV prevalence of 13.1% (426 out of 3238 patients) in their study on the impact of HPV status on the prognostic potential of the larynx cancer staging system, while Hughes *et al.*<sup>[17]</sup> found a prevalence of 13% (12 out of 94 patients) in their study on HPV-associated squamous cell carcinoma of the larynx or hypopharynx.

Mineta *et al.*<sup>[18]</sup>, using fresh tissue instead of formalin-fixed paraffin-embedded tissue, examined laryngeal carcinoma by PCR for HPV-16 and HPV-18, detecting HPV-16 in 31% (8 out of 26 patients) and HPV-18 in 8% (2 out of 26 patients). In another study by Morshed K<sup>[19]</sup>, HPV detection using monoclonal anti-human papillomavirus antibodies and PCR was performed on 130 specimens. HPV was identified in 35.5% of patients with laryngeal squamous cell carcinoma using PCR/D Enzyme Immune Assay and 27.7% using immunohistochemistry (IHC).

Several studies also confirmed HPV detection by assessing p16 protein and HPV E6/E7 mRNA expression. For instance, Shaughnessy *et al.*<sup>[20]</sup> tested 38 laryngeal cancers for HPV and p16, finding that 13 (28%) were positive for either HPV or p16. In a study by Dogantemur *et al.*<sup>[21]</sup>, HPV was detected in 11 (12.2%) of 90 cases, while p16 was positive in 18 (20%) of the cases, with statistically significant results.

Regarding the association between HPV and lymphadenopathy, our study found a significant difference between HPV-positive and HPV-negative cases. HPV-positive cases had a higher percentage of positive lymph nodes (28.6% vs 40%,  $p = 0.048$ ), consistent with findings by Panuganti *et al.*<sup>[22]</sup>, who reported that patients with HPV-positive laryngeal cancer were more likely to present with nodal disease (42.6% vs 33.0%,  $P < .001$ ).

Regarding cancer grading, our study revealed a significant difference between the two groups, with HPV-positive cases showing a higher percentage of grade-III/IV cancers (30%) compared to HPV-negative cases (17.3%), with a p-value of 0.038. This aligns with the study by Goma *et al.*<sup>[23]</sup>, which found HPV-16 positivity in 18% (9 out of 50 patients) and reported a significant correlation between HPV-16 and tumour grade ( $P < .001$ ).

Based on our findings and existing literature, HPV plays a role in the pathogenesis of laryngeal carcinoma.

Detection of HPV in surgical specimens varies depending on the technique used and the number of patients studied. This variation can be attributed to several factors, including geographical differences, the prevalence of smoking and alcohol use in the studied populations, sample selection, and the molecular techniques used for HPV detection.

### CONCLUSION

Our study shows that HPV affects the grading of carcinoma, as positive cases had a significantly higher percentage of high-grade (grade-III/IV) cancer compared with negative cases.

Also, HPV-positive cases were associated with lymphadenopathy, as positive cases had a significantly higher percentage of positive LNs than negative cases.

### CONFLICT OF INTERESTS

There are no conflicts of interest.

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