

# Human Papillomavirus Subtype 16 and the Pathologic Characteristics of Laryngeal Cancer

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## Abstract

**Objective.** Laryngeal cancer is the most common type of cancer in the head and neck. Human papillomavirus (HPV) represents a group of >150 related viruses. Infection with certain types of HPV can cause some types of cancer. This study aimed to evaluate the sociodemographic and histopathologic characters of squamous cell carcinoma of the larynx and its relationship to HPV subtype 16 (HPV-16).

**Study design.** Cross-sectional.

**Setting.** Tertiary university hospitals at 5 districts in Egypt (Minia, Cairo, Giza, Qalubia, and Bani Suief).

**Subjects and Methods.** This cross-sectional study was conducted on 50 adult patients with laryngeal cancer who were admitted at 5 tertiary care hospitals in Egypt from January 2014 through December 2014. All patients were subjected to a comprehensive preoperative assessment, histopathologic assessments of tumor biopsies, and immunohistochemical staining for HPV-16.

**Results.** HPV-16 immunostaining was positive in 9 patients (18%). A significant correlation between HPV-16 immunoreactivity and tumor grade ( $P < .001$ ) was detected, with no significant correlation between HPV-16 immunoreactivity and other clinical and pathologic variables.

**Conclusion.** The frequency of HPV-16 in laryngeal carcinoma is 18%, and there is significant correlation between HPV-16 and tumor grade.

## Keywords

squamous cell carcinoma, larynx, human papilloma virus

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The role of viruses in laryngeal carcinoma has been carefully studied, but conflicting data have been obtained according to the population and methodology used.<sup>1</sup> Although human papillomavirus (HPV) was first identified in the larynx 20 years ago, the extent of its presence in the epithelium in the normal population is unclear. HPV is clearly the etiologic agent of benign head and neck lesions, particularly laryngeal papilloma—the most common benign tumor in the larynx, which is associated with a small risk of malignant transformation.<sup>2</sup>

The search for alternate risk factors linked to the development of laryngeal cancer, particularly in patients who do not smoke or drink alcohol, has led to the hypothesis that HPV may play a pivotal role. Epidemiologic studies, though they are not conclusive, strongly implicate HPV in the etiology of a subset of laryngeal carcinomas, and recent molecular evidence supports this hypothesis.<sup>3</sup>

The potential oncogenic roles of HPV subtype 16 (HPV-16) in the development of laryngeal cancer remain the subject of debate. This study aimed to assess the presence of HPV-16 in patients with squamous cell carcinoma (SCC) of the larynx and to identify correlations with other sociodemographic and histopathologic characters of the disease in Egypt.

## Methods

This cross-sectional study was conducted on 50 patients with laryngeal cancer who were admitted to the otolaryngology–head and neck surgery departments of Minia University Hospital, 6th

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October University Hospital, Ain Shams University Hospital, Kasr-Al Aini University Hospital, and the Cancer Institute at Minia between January 2014 and December 2014. We included in the study only patients with confirmed histopathologic diagnosis of SCC of the larynx. We excluded the following patients from the study: (1) patients with negative histopathologic evidence of laryngeal cancer, even with clinical signs suspicious of malignancy, and (2) patients with laryngeal cancer other than SCC. This study was approved by the Research Ethics Committee of the Faculty of Medicine, Minia University. Informed consent was obtained from each enrolled patient.

All 50 patients were subjected to an assessment protocol that included a detailed medical history. Intensity of smoking was assessed according to a smoking index adapted by the National Cancer Institute, which was calculated by multiplying the number of packs of cigarettes smoked per day by the number of years that a person has smoked. Intensity of alcohol consumption was assessed according to the National Institute on Alcohol Abuse and Alcoholism.<sup>4</sup> Patients underwent a comprehensive general and otorhinolaryngeal examination that included an indirect endoscopic laryngeal examination. Radiologic evaluations by computerized tomography and clinical and endoscopic examinations were used to stage the tumor and assess its extent according to the TNM staging system.

Biopsy specimens were obtained from each patient and divided into 2 sections: the first was subjected to hematoxylin and eosin staining, and the other was used for immunohistochemical staining for HPV-16. Broder's grading system was used for the histologic grading of laryngeal tumors, which is a traditional pathologic tool with established prognostic value.<sup>5</sup> The immunohistochemical staining level was assessed and scored by 2 independent pathologists. The expression of HPV-16 was defined as positive if (1) distinct nuclear immunoreactivity was detected in laryngeal tumor cells, (2) cytoplasmic and nuclear staining was present, (3) staining was moderate to strong (it should be diffuse), and (4) staining was present in at least 50% of tumor cells.

## Results

This prospective study was conducted on 50 patients with laryngeal cancer. The age of the patients ranged from 38 to 70 years old, with a mean age of 57.06 years, including 48 (96%) men and 2 (4%) women. Additionally, 37 (74%) patients were heavy smokers (>20 cigarettes/day), and 13 (26%) were nonsmokers; 10 patients (20%) reported moderate alcohol consumption.

The compartmental classification of laryngeal cancer in our patients was as follows: 30 (60%) patients had glottic laryngeal cancer; 11 (22%), supraglottic laryngeal cancer; and 9 (18%), transglottic laryngeal cancer. According to the TNM classification, 3 (6%) patients were in stage I, 35 (70%) in stage II, 6 (12%) in stage III, and 6 (12%) in stage IV. According to Broder's grading system, we classified 26 (52%) patients as grade I, 14 (28%) as grade II, and 10 (20%) as grade III.

**Table 1.** Correlation between HPV-16 Positivity and the Age and Sex of Patients (N = 50).

Variable: Subtype	HPV-16		P Value
	Negative	Positive	
Age, y			.525
<58	18	5	
≥58	23	4	
Sex			.499
Male	39	9	
Female	2	0	

Abbreviation: HPV-16, human papillomavirus subtype 16.

**Table 2.** Correlation of HPV-16 Positivity with Patient Smoking and Alcohol Consumption (N = 50).

Variable: Subtype	HPV-16		P Value
	Negative	Positive	
Smoking index			.261
Heavy smokers	29	8	
Nonsmokers	12	1	
Alcohol consumption			.854
Moderate	8	2	
None	33	7	

Abbreviation: HPV-16, human papillomavirus subtype 16.

Among all study patients, 9 (18%) had positive HPV-16 immunostaining. The correlation between HPV-16 positivity and the age of the affected patients is shown in **Table 1**. There was no statistically significant correlation between the age of patients and HPV-16 positivity. Additionally, there was no significant correlation between patient sex and HPV-16 positivity among our study patients. Patient smoking index and the amount of alcohol consumption was not significantly correlated with HPV-16 positivity (**Table 2**).

**Table 3** shows correlations between the compartmental classification of laryngeal cancer and HPV-16 positivity. There was no significant correlation between the site of a laryngeal tumor and HPV-16 positivity. **Table 3** shows the correlations between the TNM stage and HPV-16 positivity. Our data revealed no significant correlation between the TNM stage and HPV-16 positivity. However, when we attempted to find correlations between Broder's tumor grade and HPV-16 positivity, a significantly greater number of patients with HPV-16-positive laryngeal cancer had grade III than either grade I or II. These data revealed a significant correlation between tumor grade and HPV-16 positivity ( $P < .001$ ).

## Discussion

Cancer of the larynx represents an important oncologic entity. It accounts for 30% to 40% of malignant head and

**Table 3.** Correlation of HPV-16 Positivity with Tumor Site, Stage, and Grade (N = 50).

Variable: Subtype	HPV-16		P Value
	Negative	Positive	
Tumor site			.172
Glottic	25	3	
Supraglottic	9	2	
Transglottic	7	4	
Tumor stage			.622
I	2	1	
II	30	5	
III	5	1	
IV	4	2	
Tumor grade			<.001
1	23	3	
2	14	0	
3	4	6	

Abbreviation: HPV-16, human papillomavirus subtype 16.

neck tumors and 1% to 2.5% of malignant neoplasms in humans. Squamous cell origin accounts for 95% to 98% of these tumors in terms of histopathology.<sup>6</sup>

Many types of HPV have been documented. Some types of HPV can affect the lining of the larynx and cause small wartlike growths termed *papillomas*. These growths frequently recur after they are removed. Papillomas may even occur in children and are known as *juvenile-onset recurrent respiratory papillomatosis*. The HPV strains that cause respiratory papillomatosis differ from those that have been linked to laryngeal SCC. HPV was detected in ~25% of laryngeal cancers by Bosch et al<sup>7</sup>; however, they were not able to determine how the presence of HPV is linked to the prevention and treatment of laryngeal cancer.

Torrente et al<sup>8</sup> studied associations between HPV infection in different laryngeal diseases and the potential to cause oncogenicity, using several methods of detection. They detected HPV DNA in benign lesions (eg, papillomatosis), indolent lesions (eg, verrucous carcinoma), and malignant laryngeal lesions (eg, SCC). The high-risk types, HPV-16 and HPV-18, are more frequently present in neoplastic lesions, especially SCC. Although a broad range of prevalence rates has been noted in individual studies, a meta-analysis found that ~25% of laryngeal SCC cases harbored HPV infections, with a common involvement of the high-risk HPV-16 and, to a lesser extent, HPV-18,<sup>8</sup> and this was the reason why we chose HPV-16 in the current study. Lie et al<sup>9</sup> reported a significantly higher risk of laryngeal SCC that was associated with HPV infection, and HPV-16 was the most frequently type observed. The preliminary findings suggest that these high-risk HPV infections appear to be biologically relevant to laryngeal carcinogenesis, undergo viral DNA integration in the cancer genome, and drive increased expression of the p16 protein.<sup>10</sup> Despite this

knowledge, the clinical significance of these infections and their implications for disease prevention and treatment remain unclear and warrant further investigation.<sup>11</sup>

In a more recent study, the overall prevalence of HPV was ~32%, with frequencies of ~30% and ~12% for high-risk HPV-16 and HPV-18 and low-risk HPV-6 and HPV-11, respectively.<sup>12</sup> However, Roshan et al<sup>13</sup> reported no significant correlation between infection with HPV-16 or HPV-18 and the occurrence of laryngeal cancer.

In the study, 18% of the patients were HPV-16 positive; these findings are consistent with the majority of published data. Our data showed a consistent distribution of HPV-16 in different medical centers, in accord with the results of Lie et al,<sup>9</sup> who reported that an association of HPV-16 positivity and laryngeal cancer was not influenced by geographic region, which raises questions about other routes for viral transmission.

In this study, we used immunohistochemical staining as a means to detect HPV-16. Lacchetti et al<sup>14</sup> reported that DNA-based amplification from polymerase chain reaction, DNA in situ hybridization, and immunohistochemical staining have the same sensitivity for detecting HPV-16. Maier et al<sup>15</sup> analyzed tumor specimens from 223 patients with SCC of the oral cavity, oropharynx, hypopharynx, and larynx for HPV DNA expression. The prevalence of HPV genotype 16 DNA in that study population was 17.5%, and the prevalence of HPV-16 was highest in tonsillar carcinoma (37.5%) and lowest in laryngeal cancer (2.8%). Those authors observed a significantly higher incidence of cervical lymph node metastases in patients with HPV-16-positive tonsillar carcinoma when compared with patients with HPV-negative tumors.

In the current study, there was no correlation between HPV-16 positivity and smoking or alcohol consumption. Tobacco use and alcohol consumption were significantly lower in patients with HPV-positive tumors in the study of Laskaris et al.<sup>16</sup> Our study shows that HPV-16 immunoreactivity was not correlated with age, sex, and tumor site or tumor stage. Some studies have reported a significantly higher prevalence of HPV-16 infection in younger patients with laryngeal cancer.<sup>15</sup>

In our study, HPV-16 positivity was correlated with tumor grade. Laskaris et al<sup>16</sup> reported a similar correlation. Besides anatomic type, radiotherapy, and surgical margin status, age and pathologic stage are independent factors affecting postoperative tumor-free survival, and the recurrence rate is high and the prognosis poor in patients with poorly differentiated carcinoma.<sup>17</sup>

There is an improved prognosis for patients with HPV-positive head and neck SCC relative to HPV-negative head and neck SCC. Fakhry et al<sup>18</sup> prospectively evaluated the association of tumor HPV status with therapeutic response and survival among 96 patients with stage III or IV head and neck SCC of the oropharynx or larynx. According to their results, patients with HPV-positive tumors had higher response rates after induction chemotherapy and after chemoradiation treatment, and patients with HPV-positive tumors had improved overall survival, lower risk of progression, and lower risk of death from any cause.

The better response to chemotherapy and radiation observed for HPV-positive versus HPV-negative tumors, as observed in multiple studies,<sup>19,20</sup> may be due to the presence of an intact p53-mediated apoptotic response to chemotherapy-induced stress in the HPV-positive tumors.<sup>21</sup> Additionally, in patients who were treated with the intent of organ preservation therapy for locally advanced laryngeal or hypopharyngeal cancers, HPV-16-positive patients exhibited no observed treatment failures.<sup>22</sup> Therefore, the biologic basis for the improved survival among HPV-positive patients warrants further study.

The current results agree with Hernandez et al regarding age, sex, stage, and site of laryngeal tumor but disagree regarding tumor grade.<sup>23</sup>

## Conclusions

The present study was carried out in 5 tertiary care hospitals. The following conclusions can be reliably reached by means of this study:

- The frequency of HPV-16-positive laryngeal cancer is ~18% in multicenter assessments of institutions throughout Egypt.
- The positivity of HPV-16 was not correlated with age, sex, smoking, alcohol consumption, and tumor site and/or tumor stage.
- The high-grade SCC of laryngeal cancer was correlated with HPV-16 positivity.

## Author Contributions

**Mohammed Abdel Motaal Gomaa**, design of the study and idea of the research, selection of cases, operative management of patients, editing of the paper and final approval; **Khalid Elsayed El Gindy**, design of the paper and idea of the research, selection of cases, operative management of patients, editing of the paper; **Usama Galal Abdel Nabi**, final approval of the work, operative management of patients, share in drafting of the paper; **Haitham M. Mohammed**, laryngoscopic evaluation of cases, drafting of the paper; **Nisreen A. Abdel Twab**, pathologic assessment of cases, analysis and interpretation of the work, sharing in writing of paper; **Rehab Mahmoud**, pathologic assessment of cases, analysis and interpretation of the work, sharing in writing of paper; **Khalid M. Mohiy**, data analysis, statistics of the work, share in writing of the paper.

## Disclosures

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