Laryngeal Cancer, HPV DNA vs E6/E7 mRNA Test: A Systematic Review

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Summary: Objective. The reported range of involvement of human papillomavirus (HPV) in laryngeal squamous cell carcinoma (SCC) is wide because of the methods used to detect HPV.

Data Sources. A computerized Medline study was carried out using the following as key words: "Papillomavirus Infections"[Mesh] and "Laryngeal Neoplasms"[Mesh].

Materials and Methods. Studies that were included were written in English and reported results of HPV DNA with RNA in laryngeal SCC.

Results. There were six reported HPV mRNA extraction. Among these studies, Lewis et al reported that out of the 31 cases analyzed, only 2 were HPV DNA+ and of these only 1 was mRNA HPV+ (3%). Halec et al reported 102 cases of which 32 were HPV DNA+ cases and of which only 6 were mRNA+ (5%). Chernock et al reported 76 cases of which 13 were HPV DNA+ cases and of which 4 were mRNA+ (5%). Masand et al reported 8 cases of which 1 was HPV DNA+ case and none was mRNA+. Gheit et al reported 43 cases of which 4 were HPV DNA+ cases and of which 2 were mRNA+ (4%). Castellsagné et al reported 1042 cases of which 59 were HPV DNA+ case and of which 51 were mRNA+ (4.8%)

Conclusions. When determining the role of HPV in laryngeal SCC, evidence of HPV DNA warrants further examination for E6/E7 mRNA as simple assays such as p16 are nonspecific in laryngeal SCC. Further studies of HPV and its role in laryngeal SCC are warranted.

Key Words: Human papillomavirus–Laryngeal squamous cell carcinoma–HPV DNA–E6/E7 mRNA–p16.

INTRODUCTION

Tobacco smoking and alcohol consumption are considered the major risk factors of laryngeal cancer.[1] Nowadays, the role of Human papillomavirus (HPV) DNA is widely recognized in head and neck cancer[2–7]; however, consistent biological evidence for viral involvement in laryngeal squamous cell carcinoma (SCC) is still lacking.

Currently, of 51 mucosal HPV types known today,[8] 12 are classified as carcinogenic or high-risk (HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59), 8 as probably carcinogenic (HPV26, 53, 66, 67, 68, 70, 73, and 82), and 31 as types of low/undetermined carcinogenicity (IARC, 2011).

Most of the previous studies have investigated HPV in tumors using DNA-based polymerase chain reaction (PCR),[7] reverse transcriptase-polymerase chain reaction (RT-PCR), quantitative real-time PCR, and DNA-based *in situ* hybridization (ISH).[9–11]

Ang et al demonstrated that a low level of HPV DNA and the absence of viral transcription have a limited biological value and could indicate that HPV does not play a causative role in malignant transformation.[9] HPV DNA has been found in benign and normal tissue of the larynx[10,11], supporting the theory that HPV DNA presence alone cannot demonstrate causality. Therefore, it is becoming increasingly acknowledged that HPV can be present without necessarily being biologically active, such that the mere presence of the virus (as detected by these methods) may not indicate biological/clinical significance.

E6 and E7, the two transforming proteins encoded by HPV, operate through their associations with the tumor suppressor proteins p53 and Rb in oncogenesis.[12] *The presence of virus in a transcriptionally active form, as indicated by the expression of high-risk HPV proteins E6 and E7, has been identified as the gold standard biomarker for oncogenic HPV. The presence of HPV DNA or p16 overexpression do not provide sufficient evidence that HPV has contributed to the oncogenic process and viral RNA detection becomes particularly important.*[13–15] Obviously, other oncogenic factors may have acted simultaneously with HPV since laryngeal SCC is a multifactor tumor.

The concept for causal involvement of mucosal HPV in the pathogenesis of SCC includes the following:

(a) presence of at least one viral genome copy per tumor cell
(b) active transcription of the viral oncogenes E6 and E7
(c) interaction of the viral oncoproteins with central cellular regulator proteins, such as p16.[16,17]

Our study has revealed that HPV DNA, E6/E7 RNA, and p16 positivity vary widely in laryngeal SCC. Current manuscripts are still simply reporting the presence of HPV-16 as evidence for oncogenicity in laryngeal SCC and as we want to point out, this is insufficient.

For this reason we compared the data obtained from the literature including the mRNA of viral proteins vs HPV DNA test.

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TABLE 1.
Six studies that reported HPV DNA and mRNA extraction

	Cases	HPV DNA+	HPV RNA+
Lewis et al	31	2 (6%)	1 (3%)
Halec et al	102	32 (31%)	6 (5%)
Chernock et al	76	13 (17%)	4 (5%)
Masand et al	8	1 (12%)	0 (0%)
Gheit et al	43	4 (9%)	2 (4%)
Castellsagué et al	1042	59 (5.6%)	51 (4.8%)
Total	1302	111	64

MATERIALS AND METHODS

Study selection

In January 2016, a computerized Medline study was carried out through the use of PubMed at the US National Library of Medicine using the following key words: "Papillomavirus Infections"[Mesh] and "Laryngeal Neoplasms"[Mesh].

The initial research produced a total of 300 results. The obtained abstracts and titles were analyzed and inclusion criteria were

- cohorts of patients with laryngeal cancer and which were evaluated for HPV positivity applying DNA-based PCR, RT-PCR, quantitative real-time PCR, or DNA-based ISH simultaneously with RNA-based PCR
- English language.

The exclusion criteria included

- articles that did not clearly describe analysis procedures for papillomavirus
- review articles.

The consensus after review by all authors isolated 21 of the 300 studies that satisfied inclusion criteria.

Data analysis

The authors extracted the following data from the articles: the first author, year of publication, the country where the research was carried out, the number of cases, the preservation method of the sample (fresh, frozen, or paraffin-embedded), HPV primers, HPV genotypes, and sample adequacy for HPV analysis.

Cases in articles describing tumors which involved other extralaryngeal sites such as the hypopharynx were excluded from the data analysis.

The classification of secondary sites (supraglottis, glottis, hypoglottis) was not possible because these data were often not available in the manuscripts.

RESULTS

Of the 21 articles revised, only 6 reported DNA-based PCR or DNA-based ISH combined with mRNA.

Among these studies, Lewis et al reported 2 of 31 HPV DNA+ cases (6%), of which only 1 was mRNA HPV+ (3%).[18] Halec et al reported 32 of 102 HPV DNA+ cases (31%), of which only 6 were mRNA+ (5%).[19] Chernock et al reported 76 cases, of which 13 were HPV DNA+ (17%) and 4 were mRNA+ (5%).[20] Masand et al reported 1 of 8 HPV DNA+ case (12%), of which none was mRNA+.[21] Gheit et al reported 4 of 43 HPV DNA + cases (9%), of which 2 were mRNA+ (4%).[22] Castellsagué et al reported 59 of 1042 HPV DNA+ cases (5.6%), of which 51 were mRNA+ (4.8%)[23] (Table 1).

DISCUSSION

In the literature, HPV infection in laryngeal SCC has been reported with a wide range of results (0%–60%). This is due to various sampling procedures (biopsy or scraping, formalin fixation, and paraffin-embedding or tissue freezing), HPV DNA and HPV mRNA analysis procedures such as Southern blot, PCR/RT-PCR or ISH, the primers used (MY9/MY11, GP5/GP6, or specific genotype primers), and the serotype identification tests used (linear essays, direct sequencing reverse dot blot analysis, etc).

Data in the literature are, therefore, often difficult to compare because a standard procedure for HPV DNA and mRNA analysis and identification does not exist.

Assessment of the etiologic involvement of HPVs in laryngeal SCC is challenged by its multifactorial etiology largely attributed to tobacco and alcohol use.[3–5] Furthermore, the presence of HPV DNA in laryngeal SCC is not sufficient to prove viral causation as it might just reflect a transient infection unrelated to the carcinogenic process.[7,8] The identification of the transcripts of the viral oncogenes E6/E7, through mRNA techniques,[11] is widely accepted as the present gold-standard test to strongly suspect the oncogenic role of HPV in the tumor.[24] E6 and E7 are two primary oncoproteins of high-risk HPV types.

E6 is capable of inducing degradation of the oncosuppressor p53 protein. p53 is a transcription factor which induces cell-cycle arrest or apoptosis in response to cellular stress or DNA damage.

However, E7 protein inactivates another oncosuppressor protein Rb. The role played by pRb is to prevent progression to the S

TABLE 2.
Five studies that reported the comparison HPV DNA vs E6/E7 mRNA vs p16 test

	Halec et al	Chernock et al	Ndiaye et al's review	Bishop et al's review	Castellsagué et al
No. of cases evaluated	102	76	2739	64	1042
HPV DNA	34.7%	17.1%	22.1%,	1.5%	5.6%
mRNA E6/E7	6.5%	5.2%	8.6%,	1.5%	4.8%
p16	3.2%	27.6%	19.1%	12.5%	5.6%