Abstract. Background/Aim: The causal relation between human papillomavirus (HPV) infection and squamous cell carcinoma (SCC) of the larynx has not been yet clarified. The aim of the present study was to investigate HPV infection in 54 SCC of the larynx and correlate it with patients’ epidemiological and clinicopathological data. Materials and Methods: Fifty-four biopsies were collected from patients with laryngeal SCC and tested for HPV DNA. Local recurrence analysis was performed at the 2-year follow-up. Results: HPV DNA was detected in 18.5% (10/54) of laryngeal SCC; infection from high risk (hr) HPV and low risk (lr) HPV types was found in 16.7% (9/54) and 1.8% (1/54) of the samples, respectively. HPV 16 was the commonest type detected in 7.5% (4/54). The presence of HPV DNA was significantly associated with the absence of tobacco use (p=0.001) and poorly differentiated tumors (p=0.003). Conclusion: This study confirms the prevalence of HPV infection among patients with SCC of the larynx.

Head and neck squamous cell carcinomas (HNSCC) include malignancies occurring in the oral cavity, pharynx and larynx. Human papillomavirus (HPV) has been established as a potential risk factor for the development of a subset of HNSCC, particularly those of the oropharynx (1, 2).

Squamous cell carcinoma (SCC) of the larynx is the sixth most common cancer worldwide (3), including cancers arising in the supraglottic, glottic and hypoglottic areas. Besides the unquestionable association of SCC of the larynx with well-established risk factors, such as smoking and alcohol abuse, in a minority of cases, laryngeal cancer occurs in non-smokers and non-drinkers (4) indicating that other cofactors may be implicated to laryngeal carcinogenesis.

HPV infection of the laryngeal epithelium is feasible, mainly in the special microenvironment of the glottic region, due to the squamocolumnar junction in the ventricle, which is similar to the transformation zone of the cervix (5, 6). Although the most clinically significant manifestation of laryngeal HPV infection is recurrent respiratory papillomatosis, HPV DNA of high-risk (hr) genotypes has also been observed in 24% of SCC of the larynx (7-9). HPV 16 has been found to be the most prevalent genotype in laryngeal cancer (5, 10, 11) and has been suggested to play a role in the malignant transformation of the laryngeal mucosa (5, 12, 13). Nevertheless, the association of HPV with the development of SCC of the larynx is still unclear due to the large discrepancy in the prevalence rates of HPV reported among studies, ranging from 3 to 60% (13-15).

The aim of the present study was to investigate HPV infection in a series of 54 SCC of the larynx and correlate it with patients’ epidemiological and clinicopathological data.

Materials and Methods

Patients and tissues. Tissue samples were obtained from 54 biopsies of untreated laryngeal tumors during first examination or surgery, at the ENT (Ear, Nose and Throat) Department of the Regional Anticancer Oncology Hospital “Metaxas”, Piraeus, Greece, between April 2011 and September 2012. A part of the tissue sample was preserved in Thin-Prep (PreservCyt Solution; Hologic, Crawley, UK) and sent to the Department of the Virology of Regional Anticancer Oncology Hospital “St. Savvas”, Athens, Greece for HPV detection. All samples were subsequently diagnosed as SCC of the larynx.
Tumors were staged according to the American Joint Committee on Cancer staging criteria (AJCC, 1997) and graded as moderately and poorly differentiated. Local recurrence analysis was performed at the 2-year follow-up. Ethical approval was granted by the ethics committee of Regional Anticancer Oncology Hospital of Piraeus “Metaxas” and all patients provided written, informed consent.

**Extraction of nucleic acids.** Total nucleic acid was extracted using the QIAamp® DNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer’s instruction. DNA quality test was carried out using the Human Globin, Beta, Primer set kit (Maxim Biotech, Inc., South San Francisco, CA, USA).

**HPV detection and genotyping.** The PapilloCheck HPV genotyping assay (Greiner Bio-One GmbH, Frickenhausen, Germany) was used. This technology is based on a DNA chip for the type-specific identification of 24 types of HPV (high-risk: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, 82; probable high-risk: 53 and 66; and low-risk: 6, 11, 40, 42, 43, 44/55, 70). E1-based polymerase chain reaction (PCR) was performed according to the manufacturer’s guidelines. For each sample, we mixed 19.8 μl PapilloCheck MasterMix, 0.2 μl HotStarTaq plus DNA polymerase (5 U/μl) and 5 μl DNA from the tissue sample. Hybridization followed by mixing 30 μl of the PapilloCheck hybridization buffer in a fresh reaction tube with 5 μl of the PCR product at room temperature and transferring 25 μl of the hybridization mix into each compartment of the chip. We incubated the chip for 15 min at room temperature in a humid atmosphere. The chip was washed in 3 washing solutions, centrifuged for 3 min at 5,000 rpm and scanned on the CheckScanner™ (Greiner Bio-One GmbH, Frickenhausen, Germany).

**Statistical analysis.** Data were analyzed using the SAS v9.0. (SAS Institute Inc., North Carolina, USA) software. Absolute frequencies were used to present HPV positivity. Chi-squared tests were performed to assess statistical significance of any differences in prevalence. 2×2 contingency tables and Fisher’s exact test was performed to estimate p-values. p-Values of less than 0.05 were considered statistically significant.

**Results**

**HPV detection.** A total of 54 SCC of the larynx were tested for the presence of HPV DNA. The patients’ age ranged from 21 to 88 years (mean age=62.6). Thirty-eight patients were male and 16 were female. HPV DNA was detected in 18.5% (10/54) of SCC of the larynx; infection from hrHPV and lrHPV types was found in 16.7% (9/54) and 1.8% (1/54) of the samples, respectively. HPV 16 was the most common type detected in 7.4% (4/54) of SCC of the larynx, followed by HPV 18 and HPV 51 in 3.7% (2/54) each and HPV 33 and HPV 6 in 1.8% (1/54) each.

Sixty percent (6/10) of HPV DNA-positive tumors were located in the glottis, 30% (3/10) in the supraglottis and 10% (1/10) in the diaphragmatic area of the larynx. Sixty percent (6/10) of HPV DNA-positive tumors were characterized as poorly-differentiated, 20% (2/10) as moderately-differentiated and 20% (2/10) were well-differentiated tumors.

The presence of HPV DNA was significantly associated with the absence of tobacco use (46.7% vs. 7.7%, p=0.001) and poorly differentiated tumors (46.2% vs. 9.8%, p=0.003). No significant associations were observed between HPV infection and gender, age, alcohol consumption, tumor stage or lesion localization (Table I).

**Tumor recurrence.** At the 2-year follow-up, 16 (29.6%) of the group of 54 patients with SCC of the larynx suffered local recurrence. Only 1 (10%) out of 10 HPV DNA-positive patients developed local recurrence, while 15 (34.1%) out of 44 HPV DNA-negative patients had local recurrence. Although patients harboring an HPV infection were considerably less than those being negative for HPV infection in terms of local recurrence, this difference was not statistically significant (p=0.132).

**Discussion**

The causal relation between HPV infection and oropharyngeal cancer has been confirmed through multiple studies worldwide (7, 16-19). On the contrary, a similar link has not yet been clarified in cancers of the oral cavity and larynx. This is not only due to the wide range of HPV prevalence in oral and laryngeal cancers reported by several studies, but also to the limited published data regarding the association of HPV status with clinical outcome (8).
The results of the present study showed that 18.5% of the cases of SCC of the larynx were HPV DNA-positive. Limited data have been published regarding HPV infection in Greek patients with SCC of the larynx. A study conducted in Greece showed that HPV DNA was present in 10% of SCC of the larynx (20), similarly to other reports worldwide (6, 15, 21, 22). On the contrary, a higher frequency of HPV infection has been observed in another Greek study where 40% of laryngeal cancers were HPV DNA-positive (23) which is in agreement with other studies (24-26).

In the literature, the different frequencies of HPV infection in laryngeal cancer are possibly attributed to the differences in the analyzed population, the sample size, the sample type and, mainly, the HPV detection methods used (8, 10, 26).

A recent meta-analysis of 55 studies on HPV infection and laryngeal cancer reported that the HPV prevalence was higher in studies using PCR-based assays (29.5%) than that determined by non-PCR-based assays (20.4%) (5). In addition, it has been stated that, in contrast to the genotype-specific PCR or other conventional methods, more recent techniques, such as DNA chips, may reveal lower detection rates of HPV DNA due to their high accuracy (6). In the current study, a HPV DNA chip was used, which is a relatively recent and high throughput, state-of-the-art technology (6, 27, 28).

HPV 16 was the most common genotype detected in SCC of the larynx, which is in accordance with what is seen not only in laryngeal cancers harboring HPV infection but also in the majority of HPV-associated head and neck cancers (5, 21, 29).

It is important to note that the lrHPV 6 was found in one case of SCC of the larynx, similar to other reports (7, 8, 21, 22, 30). It has been postulated that the lrHPVs 6 and 11 have a predilection to the larynx as it is well-known that they play a significant role in the malignant conversion of recurrent respiratory papillomatosis (31-33). Therefore, it is possible that the lrHPV 6 may be implicated in the development of SCC of the larynx (22, 34).

An interesting aspect of this series is that HPV infection was significantly more common in non-smokers than in smokers. However, other studies did not demonstrate a relationship between HPV infection and tobacco use (22, 26, 35-38), while others suggested a synergic relation between smoking and HPV infection, possibly contributing to the cervical transition zone (7, 8). In this series, HPV DNA was more frequently detected in glottic than in supraglottic cancers, however, tumors located in the glottis comprised the majority of the samples used. Accordingly, previous studies have reported higher rates of HPV infection in glottic than in supraglottic tumors (6, 26, 42).

Relatively few studies have investigated the influence of HPV on the clinical outcome of patients with laryngeal cancer (10, 23, 24, 36, 40). The results of the present study indicated that HPV DNA-positive SCCs of the larynx were considerably less prone to the development of local recurrence than HPV DNA-negative cancers, but this finding did not reach statistical significance. Similarly, other studies did not report significant differences between patients with HPV-positive and HPV-negative tumors regarding local recurrence (40, 43).

In conclusion, this study showed that 18.5% of SCC of the larynx harbored HPV infection. However, the relatively few cases of laryngeal cancer can only provide a rough estimate of the importance of HPV infection in the population of this series. Future analysis and follow-up of more patients with SCC of the larynx are required to better-assess the possible implication of HPV in laryngeal carcinogenesis.

Conflicts of Interest

The Authors declare that there are no conflicts of interest.

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References


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