Differences in human papillomavirus–positive and –negative head and neck cancers in Belgium: an 8-year retrospective, comparative study

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Objectives. This study investigated the prevalence of human papillomavirus (HPV)-related oropharyngeal squamous cell carcinoma (OPSCC) and compared patient profiles and outcomes between HPV-positive and HPV-negative groups.
Study Design. This retrospective study included all patients treated for OPSCC in the University Hospitals of Leuven between 2004 and 2012. Paraffin-embedded tumor tissue was available for all patients. Patient characteristics, treatment, and follow-up data were retrieved from medical files. HPV status was determined by immunohistochemical staining for the p16 epitope.
Results. Among 94 patients, the prevalence of HPV-positive OPSCC was 22.34%. Compared with HPV-negative tumors, HPV-positive tumors were correlated with less smoking and alcohol consumption, tonsillar sublocalization ($P < .05$), and younger age. HPV-positive OPSCC was associated with better overall survival (62.2%) compared with HPV-negative OPSCC (42.5%; $P = .0588$).
Conclusions. Among patients with OPSCC, those with HPV exhibited profiles different from those without HPV. HPV-positive OPSCC was associated with better overall survival compared with HPV-negative OPSCC. HPV-positive OPSCC prevalence increased over time. (Oral Surg Oral Med Oral Pathol Oral Radiol 2016;121:456-460)

EPIDEMIOLOGY

Each year, more than 550,000 newly diagnosed cases of head and neck tumors are reported. They comprise the sixth most commonly diagnosed cancer worldwide. In the last 2 decades, there was a slight decrease in the incidence of head and neck malignancies. However, in some subgroups, particularly among tumors of the oropharynx, the incidence increased markedly, by 2% to 3% annually, in the United States.[1] Oropharyngeal tumors comprise 24% of head and neck tumors. Of these, approximately 89% involve squamous cell carcinoma (SCC).[1]

In Belgium, oropharyngeal carcinomas comprise 24% of all head and neck cancers. In 2012, 699 new diagnoses of oropharyngeal carcinoma were reported, which is a 40% increase compared with 2004.[2] The Belgian Cancer Foundation showed that although the number of registered oropharyngeal malignancies has increased, the overall incidence of head and neck cancer has remained stable.[2]

The development of oropharyngeal squamous cell carcinoma (OPSCC) has been attributed to abuse of tobacco and alcohol. However, in recent decades, sufficient evidence has supported the hypothesis that infection with human papillomavirus (HPV), mainly subtype 16, was responsible for the increasing incidence of OPSCC.[3] A rapid increase in HPV-related oropharyngeal cancers has been reported predominantly in economically developed countries. Also, HPV-positive OPSCC was strongly associated with a good therapeutic response and improved overall survival.[4-6]

HPV prevalence

Of all head and neck tumors, 23% to 35% are considered HPV positive. The vast majority of these tumors are localized in the oropharynx. The prevalence of HPV-positive OPSCC ranges from 20% to 90%, depending on the study. HPV-positive OPSCC lesions

Statement of Clinical Relevance

Human papillomavirus-positive status is associated with a specific patient profile and has been proved to be associated with better overall survival. Furthermore, there is a rise in the prevalence of human papillomavirus–positive oral squamous cell carcinoma over time, emphasizing the clinical value of our findings.

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are most frequently located at the level of the base of the tongue and the tonsils. HPV-positive SCC lesions have also been reported, but less commonly, at the level of the oral cavity and the larynx.[7]

Patients with HPV-positive oropharyngeal tumors are predominantly nonsmokers and nondrinkers. On average, they are younger and have a better chance of survival compared with patients with HPV-negative OPSCC.[8] These findings were recently confirmed in phase III trials conducted by the Radiation Therapy Oncology Group and the Trans-Tasman Radiation Oncology Group.[9]

The prevalence of HPV-positive oropharyngeal tumors differs substantially in different geographic regions. In the United States, 40% to 80% of oropharyngeal tumors were HPV positive. In Europe, the prevalence varied; for example, in Sweden, 90% of oropharyngeal tumors were HPV positive. However, among countries with higher tobacco exposures, such as The Netherlands (23.3%-29%), France (17.2%), and Germany (16.7%-62.9%), a lower prevalence of HPV-positive oropharyngeal tumors was reported.[10] A recent report showed that the prevalence of HPV-positive oropharyngeal tumors in Flanders and Belgium was 24.78%.[11]

From 1988 to 2004, the incidence of HPV-positive OPSCC increased by 225%. By 2020, the incidence of HPV-associated OPSCC is predicted to exceed that of HPV-positive invasive cervical cancer.[12]

HPV status determination

It is highly recommended that patients be tested for HPV status when staging oropharyngeal tumors. Several techniques are available to determine HPV status. For example, the presence of the p16 epitope can be determined with immunohistochemical staining. Other techniques include in situ hybridization assays for detecting HPV DNA; real-time polymerase chain reaction (PCR) assays for determining the viral load or for detecting HPV E6/E7 messenger RNA; and immunoassays with specific antibodies directed against HPV epitopes, such as E6 and E7, for detecting viral proteins.[13,14]

p16 immunohistochemical staining has been reported to be 100% sensitive in screening for transcriptionally active infections of HPV in carcinomas, although the specificity was only 79%.[15] A recent study showed that p16 overexpression correlates better to HPV results when staining of tumor cells exceeds 70%, rather than with lower percentages positivity.[16] Independent of treatment modality, patients with OPSCC associated with p16 overexpression had a better prognosis and a better clinical outcome compared with those with p16-negative OPSCC. p16 immunohistochemical staining is generally a feasible, routine laboratory technique, and its cost was estimated to be twofold to 16-fold lower than that of other HPV-specific tests.[16] Therefore, in this study, we performed p16 immunohistochemistry (IHC) to determine the prevalence, the patient profile, and the outcome of HPV-positive OPSCC compared with HPV-negative OPSCC.

MATERIALS AND METHODS

Patients

All patients diagnosed with OPSCC between 2004 and 2012 were included in the study. The primary inclusion criterion was availability of formalin-fixed, paraffin-embedded tumor tissue. For each patient, we collected the following data: identification number, date of birth, gender, tobacco and alcohol use (as noted in the medical records), oropharyngeal subsite, tumor-node-metastasis (TNM) class, and tumor stage (based on American Joint Commission on Cancer [AJCC] *Cancer Staging Manual*, 7th edition), type of therapy, start and end dates of therapy, dates of local or regional relapses, date of distant recurrence, date of death, and date of last follow-up.

Tumor samples and laboratory studies

For all patients, formalin-fixed, paraffin-embedded tissue was used to determine HPV status. HPV testing was performed with p16 IHC on 5-μm tissue sections. A DAKO PT link module and a DAKO autostainer were used with an automated protocol for deparaffinization, antigen retrieval, and IHC. A purified mouse antihuman p16 antibody (G175-405, BD Pharmigen) was used for p16 IHC. Sections were scored as HPV-positive when a clear, p16 immunoreactive signal was detected in at least 50% of cells.[8]

Statistical analysis

A Cox proportional hazards model was used to assess differences in survival rates. A Fisher's exact test was used to evaluate the differences between the HPV-positive and HPV-negative groups in variables associated with OPSCC occurrence. The data that showed the evolution of p16-related cancers over time were fit with a generalized linear model for binary data with a logit link. Overall survival was defined as the time interval between the start of treatment and death. Overall survival analysis was performed with Cox's F test.

RESULTS

Patient characteristics, prevalence of p16 overexpression, and incidence trends

The patient and tumor characteristics of the 94 included patients diagnosed during 2004-2012 in the University Hospitals of Leuven are shown in Table I. The average