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CLINICAL INVESTIGATION **Head and Neck Cancer**

ATYPICAL CLINICAL BEHAVIOR OF p16-CONFIRMED HPV-RELATED OROPHARYNGEAL SQUAMOUS CELL CARCINOMA TREATED WITH RADICAL RADIOTHERAPY

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Purpose: To report atypical clinical behavior observed in human papillomavirus (HPV)–related oropharyngeal carcinoma (OPC) treated with radiotherapy.

Methods and Materials: A retrospective cohort study was conducted for all newly diagnosed OPC cases treated with radiotherapy on July 1, 2003 to April 30, 2009. HPV positivity was determined by p16 immunostaining in tumors. The incidence of additional malignancies and the pattern of distant metastases (DMs) were compared between the HPV-positive (HPV+) and HPV-negative (HPV–) cohorts.

Results: HPV status was evaluated in 318 of 613 consecutive OPC cases (52%), showing 236 HPV+ and 82 HPV– patients. Compared with HPV–, HPV+ cases were less likely to have additional malignancies (prior: 11% vs. 20%, $p = 0.038$; synchronous: 1% vs. 9%, $p = 0.001$; metachronous: 6% vs. 16%, $p = 0.003$). Whereas the majority (10 of 12) of HPV– additional head-and-neck (HN) mucosal malignancies were in the oral cavity, there was none (0 of 7) in the HPV+ cohort ($p < 0.001$). HPV+ synchronous HN second primaries (SPs) were in the supraglottis, post-cricoid, and nasopharynx; metachronous HN SPs were in the glottis, supraglottis, and ethmoid plus glottis/post-cricoid region. All SPs that could be tested were HPV+. There was no difference in DM rate (10% vs. 15%, $p = 0.272$), but HPV+ DMs were more likely to involve multiple organs (46% vs. 0%, $p = 0.005$) and unusual sites.

Conclusions: This study reports atypical clinical behavior seen in HPV+ OPC, including multicentric lesions in HN mucosa and DM to multiple organs and unusual sites. The frequency of these events is low, but they may have clinical implications. The routine assessment of HPV status for all OPC is warranted. © 2012 Elsevier Inc.

Human papillomavirus, Oropharyngeal carcinoma, Clinical characteristics, Second primary, Distant metastasis.

INTRODUCTION

Oropharyngeal squamous cell carcinoma (SCC) is traditionally attributed to excessive tobacco use and alcohol consumption (1). However, an increasing proportion of oropharyngeal carcinoma (OPC) occurs in patients without a significant smoking and drinking history, suggesting the presence of other causative factors not linked to exposure to alcohol or tobacco. The involvement of human papillomavirus (HPV) in OPC was proposed as early as the mid 1980s (2, 3). A direct role for HPV infection in the development of OPC was suggested in 1990 by Ishibashi *et al.*, (4) who detected HPV-16 DNA in a tonsillar SCC primary and two lymph node metastases. Since the late 1990s, many epidemiologic and molecular studies have confirmed the etiologic role of HPV in the development of OPC (5–7).

HPV-positive (HPV+) OPC has been shown to carry distinctive molecular signatures (8, 9), a different genomic profile (10, 11), different histopathologic features (12, 13), and a more favorable outcome compared with HPV-negative (HPV–) OPC (14–16). These epidemiologic and

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molecular data seem to show consistent differences between HPV+ and HPV– OPC. This suggests that HPV+ OPC is a distinct molecular and clinical entity (17, 18). However, the biology of HPV infection, as well as the mechanism of viral transformation for HPV+ OPC, has not yet been clearly elucidated.

Because HPV+ OPCs exhibit a distinct molecular signature and genomic profile (10), it is plausible that the clinical behavior of such lesions would be different from HPV– tumors. To date, the majority of studies regarding HPV+ OPC have focused on the influence of HPV status on treatment outcome. Recently a few case reports described synchronous multifocal HPV-related neoplasms in HPV+ OPC (19, 20), but no cohort study has been conducted. Anecdotally, we have observed occasional unusual clinical behavior in HPV+ OPC compared with the more conventional smoking- and/or drinking-related OPC. This includes multiple HPV-related second primaries (SPs) in the head-and-neck (HN) mucosa outside of the oropharynx and an unusual pattern of distant metastases (DMs). To further appreciate and document these atypical clinical behaviors, a cohort study was conducted to compare the frequency of prior malignancies and synchronous and metachronous SPs, as well as the pattern of DM, in HPV+ and HPV– OPC cohorts.

METHODS AND MATERIALS

After institutional research ethics board approval, a retrospective chart review was conducted for all newly diagnosed OPC patients referred for radical treatment between July 2003 and April 2009. All patients included in this analysis were treated curatively with primary radiotherapy with or without concurrent chemotherapy. Each patient was assessed by an HN multidisciplinary team, and treatment decisions were based on standardized policies and individualized according to patient tumor extent, comorbidities, and performance status. HPV status was unknown at the time of treatment decision. Patients underwent routine patient care and documentation. Standard staging studies included computed tomography (CT) of HN, as well as the chest, with or without magnetic resonance imaging (MRI) (typically, if tongue base involvement was suspected). Panendoscopy with directed biopsies of candidate primary sites was mainly used for patients with an initial "unknown primary" presentation. Positron emission tomography–CT was not routinely available in our geographic jurisdiction (Ontario, Canada) during the study period.

OPC patients with an available initial biopsy tissue block were included in the study and underwent assessment for HPV status in the tumor. HPV status was ascertained by $p16^{INK4A}$ (p16) immunohistochemistry staining on all available tissue blocks, which has been reported to be a reliable biomarker for tumors harboring clinically and oncogenetically relevant HPV infection (21). As we previously described, p16 over-expression was strongly correlated with HPV-16 status, defined by either HPV-16 *in situ* hybridization or E6 messenger RNA, at a rate of 92% and 86%, respectively (16). Strongly or diffusely positive p16 staining was classified as HPV+, whereas weak or absent p16 staining was classified as HPV–. All scoring was conducted by pathologists without knowledge of the patients' clinical characteristics or outcomes.

Information regarding patient, disease, treatment characteristics, and outcome was collected prospectively via an institutional Head and Neck Cancer Anthology of Outcomes (HN-Anth) System (22). Data from the provincial population-based Ontario Cancer Registry was linked to the HN-Anth system to update survival outcomes. All patients were staged by the TNM Staging System (2002, sixth edition) of the International Union Against Cancer and American Joint Committee on Cancer (23, 24). Significant tobacco exposure was defined as more than 10 pack-years smoking in a patient's lifetime. Significant alcohol consumption was defined as regularly drinking of 1 standard drink or more daily currently or in the past. Complete response was defined as complete disappearance of all clinically detectable disease at 3 months after radiotherapy. The length of follow-up was calculated from the date of diagnosis to the last follow-up before May 2010.

The frequency and location of prior malignancies and synchronous and metachronous SPs, as well as the pattern of DM, were compared between the HPV+ and HPV– cohorts. In this study SP was defined based on the criteria of Warren and Gates (25) used in our center as well as by other institutions (26): an additional malignancy should have different histology or arise from a different anatomic site at least 2 cm separated from the original OPC. Prior malignancy was defined as any other cancer diagnosis occurring before the OPC diagnosis. A synchronous SP was defined as any additional cancer diagnosis occurring simultaneously with the OPC diagnosis or during its treatment; a metachronous SP referred to any other cancer diagnosed after completion of OPC treatment up to May 2010. Metachronous SPs in the lung were all solitary lesions and diagnosed by thoracic specialists.

Statistical analysis

Descriptive statistics were used to report the frequency and location of additional malignancies, as well as the pattern of DM. The chi-square test was used to evaluate the differences in categorical variables between the HPV+ and HPV– cohorts. The Student *t* test was used to compare the differences between means of variables for the HPV+ and HPV– cohorts. An α level of 0.05 indicated statistical significance.

RESULTS

Patient characteristics

Among the 613 consecutive OPC patients treated during the study period, HPV status was evaluated in 318 cases (52%). Of these, 236 (74%) were HPV+ and 82 (26%) were HPV–. The clinical characteristics of the 318 cases with HPV status evaluated were not statistically significantly different from those for whom HPV status was not tested, except that the former had a longer follow-up (3.3 vs. 2.5 years, $p < 0.001$) (Table 1). When we compared the characteristics of the HPV+ and HPV– cohorts, HPV+ OPC patients tended to be younger (57 vs. 66 years old, $p < 0.001$), less likely to have a significant history of tobacco use (>10 pack-years) (47% vs. 94%, $p < 0.001$) and alcohol consumption ($\geq$1 glass per day) (32% vs. 65%, $p < 0.001$), less likely to be N0 category (11% vs. 24%, $p = 0.004$), more likely to present with stage III–IV disease (94% vs. 80%, $p < 0.001$), and more likely to have tumors originating from the tonsil/base of the tongue (93% vs. 72%, $p < 0.001$). In addition, in keeping with our preference for combined-modality therapy in