Oral HPV infection and persistence in patients with head and neck cancer

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Objective. To investigate the presence and persistence of human papillomavirus (HPV) infection in the oral mucosa of patients with head and neck squamous cell carcinoma (HNSCC), and its correlation with prognosis.

Study design. HPV infection was characterized in tumors and pre and posttreatment oral scrapings in 51 patients with HNSCC and matched controls using the SPF10 LiPA Extra assay. p16INK4A immunostain and in situ hybridization for high-risk HPV genotypes recognized transcriptionally active infection in tumor samples. The risk of infection was compared in patients and controls. The association of pretreatment HPV status with recurrence and survival and with posttreatment HPV persistence was assessed.

Results. Oral HPV infection risk was significantly higher in patients with HNSCC than in controls ($P < .001$). Oral HPV infection was associated with infection in the first posttreatment scrapings ($P = .015$), but did not affect recurrence or prognosis.

Conclusion. Oral HPV infection is frequent in patients with HNSCC and has no prognostic implications, suggesting that posttreatment polymerase chain reaction monitoring on oral cells is not effective to monitor patient recurrence risk.
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The human papillomavirus (HPV) is involved in the genesis of tumors of the upper digestive tract, particularly of squamous cell carcinomas (SCCs) arising in the tonsils and the oropharyngeal region.[1,2] Despite changes in behavioral exposure to traditional risk factors for head and neck SCC (HNSCC),[3] the worldwide incidence of oropharyngeal SCC has steadily increased over recent years, which has been attributed largely to the increasing number of HPV-associated tumors.[4] The identification of HPV in oropharyngeal carcinomas has prognostic significance, with longer survival and higher rate of response to therapy in cases positive for HPV.[5-7]

The natural history of HPV infection has been extensively detailed in the uterine cervix,[8,9] whereas less data is available on the different phases of HPV infection and oncogenesis in the head and neck. In the female genital tract, HPV persistence in the cervical mucosa is the strongest risk factor for high-grade intraepithelial and invasive SCC,[10,11] and increases the risk of tumor recurrence.[12,13] HPV infection and persistence is easily monitored on cervical exfoliated cells, which can be used for the molecular identification and typing of HPV.[14,15] It has been suggested that oral exfoliated cells could be similarly used to monitor HPV infection in the oral cavity and the risk of oropharyngeal SCC.[16] Several authors[17-20] reported that HPV infection is more common in oral mucosa cells of patients with HNSCC as compared with controls, although scarce information is available on the persistence of viral infection after tumor treatment[21] and on its correlation with tumor recurrence.[22]

Defining the role of HPV infection in head and neck cancerogenesis is further complicated by a wide range of analytical methods available for virus detection, each with different sensitivity and specificity and different targets. In particular, when compared with the gold standard of viral oncogene transcription,[23] widely

Statement of Clinical Relevance

The study sustains the hypothesis that incidental oral human papillomavirus infection and persistence are frequently observed in head and neck carcinoma and bear no prognostic implications, whereas oncogenic infection is rare and limited to a subset of oropharyngeal cancers.

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used consensus polymerase chain reaction (PCR)-based methods are not sufficiently specific, while p16INK4A (p16) immunostain and in situ hybridization (ISH) have low sensitivity.[23] Determination of p16 expression and ISH are nonetheless currently the methods of choice in most laboratories to identify HPV-associated oropharyngeal SCC,[20,21,23] but cannot easily be applied to oral cytology monitoring, where HPV DNA amplification is the most convenient method to assess the presence of the virus.[20,21]

We have recently demonstrated with a highly sensitive commercial PCR and reverse hybridization-based assay the presence of several low-risk (LR) and high-risk (HR) HPV genotypes in over 90% of paired HNSCC biopsies and oral mucosa samples, with an excellent agreement for HPV infection and genotype characterization between cancer and cytologic samples.[24] In the present study, we investigated the persistence of HPV infections in the oral cavity of patients with HPV-positive and HPV-negative HNSCC after cancer treatment by means of repeated oral mucosa scrapings during patient follow-up, to shed light on the natural history of HPV infection in the upper digestive tract and on its correlation with disease recurrence and survival.

MATERIALS AND METHODS

Patients and sampling

The study series consists of 51 consecutive patients presenting to our center between 2008 and 2011, who underwent an endoscopic biopsy for SCC of the mouth, tongue, oropharynx, larynx, or hypopharynx. Only patients with biopsy and cytologic samples adequate for the study were included.[24] The mean age of patients was 62.2 (standard deviation, SD 11.5) years and the male to female ratio was 43:8 (Table I). Inclusion criteria were the first presentation of a previously untreated invasive SCC, and intention to referring to our center for further therapy and follow-up. After biopsy, 23 (45%) patients were treated with surgery alone, 5 (9.8%) only with radiotherapy, 4 (7.8%), 4 (7.8%), and 1 (1.9%), respectively, with chemo and radiotherapy, surgery and radiotherapy, and surgery and chemotherapy, and 14 (27.4%) patients with surgery followed by chemo and radiotherapy (Table I). Biopsy samples were fixed in formalin and processed routinely for histopathologic study. The first cytologic sample (t0) was obtained from each patient 3-8 days after endoscopy via a gentle scraping of the mucosa of the cheeks with a plastic spatula. Follow-up cytologic samples were obtained in the same way during planned control visits to the outpatient clinic at 6 and 12 months after hospital discharge, and at any subsequent control visit until June 2012. Posttreatment scrapings were classified as t1-t5 according to the number of scrapings obtained from each patient. The scraped cells were suspended immediately in ThinPrep-Preserv-Cyt solution (Cytec Corporation, Marlborough, MA) and stored at 4 °C. All follow-up biopsies performed in the head and neck region and metastatic sites from the enrolled patients were included in the study. The negative control group consisted of patients who were seen at the otolaryngology clinic for benign conditions and who agreed to a buccal scrape. For each enrolled patient, 1 control was chosen after matching by age (within 5-year categories) and sex among the control group. The mean age of the control subjects was 61.6 (SD 11.1) years. The protocol was reviewed and approved by the Institutional Ethical Review Board and is in compliance with the Helsinki Declaration. Each subject enrolled in the project signed a detailed informed consent form.

HPV DNA detection and typing

DNA extraction from buccal scrapes was performed within 1 week of sampling by lysis and digestion with proteinase K. Briefly, pelleted cells from 1.5 mL of Preserv-Cyt solution were washed in phosphate buffered saline (PBS) and resuspended in 100 μL of lysis solution (KCL 50 mM, Tris−HCl 10 mM (pH 8.3), MgCl 2 2.5 mM, Tween 20 0.45%, NP40 0.45%, proteinase K 500 mg/mL) at 56 °C for 1 h. Following heat inactivation of proteinase K, 10 μL of the solution was used for PCR amplification of the HPV sequences from the L1 region using SPF10 primers in a final reaction volume of 50 μL for 40 cycles.

For DNA isolation from the formalin-fixed paraffin-embedded biopsies, 3-5 10-μm-thick sections were incubated in 200 μL of lysis solution (1 mg/mL proteinase K in Tris 50 mM, pH 8.0, ethylenediaminetetraacetic acid (EDTA) 1 mM, Tween 20 0.45%, and octylphenoxypolyethoxyethanol (IGEPAL) CA-630 0.45%) for 16-24 h at 56 °C. Proteinase K was inactivated with heat and the lysates were centrifuged to eliminate wax, purified with spin columns (Quiagen, Crawley, UK), and resuspended in 100 μL of TRIS-EDTA 0.1%. Ten microliters of the solution were used for the reaction. Positive and negative controls were introduced in each set of 12 reactions; these included DNA from SiHa and HeLa cell lines at a specified number of HPV copies and blank reagents throughout all steps of the procedure. Concurrent amplification of human *HLA-DPB1* gene was included in the assay as an internal control for DNA adequacy. HPV type-specific sequences were detected by the line probe INNO-LiPA HPV genotyping assay version Extra (Innogenetics NV, Ghent, Belgium) according to the manufacturer's instructions. The Extra version of the assay allows the simultaneous and separate detection of 18 HR (16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73, and 82), 7 LR (6, 11, 40, 43, 44, 54, and 70), and