Chapter 7: Human Papillomavirus and Cancer of the Upper Aerodigestive Tract

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We discuss current evidence of the role of human papillomavirus (HPV) in some cancers of the upper aerodigestive tract, including the oral cavity, the pharynx, and the larynx. Cancers of the oral cavity and the pharynx are associated mainly with tobacco and alcohol exposure, but there is evidence from case series, from case–control studies, and from cohort studies that HPV plays a role in a fraction of these cancers, particularly cancer in the oropharynx and tonsil. The HPV type most commonly associated with cancers in these locations is HPV 16. Laboratory evidence indicates that the virus is integrated and that HPV oncoproteins are transcriptionally active in these tumors. Many aspects of the association remain to be investigated, including the epidemiology and natural history of HPV infection in the mouth, the role of cofactors, and the potential use of HPV testing and vaccines in the prevention of these tumors. An analogous role for the virus at other anatomic sites in the upper aerodigestive tract such as the larynx is less clear. The relationship between HPV infection and laryngeal cancer is of particular interest, given that recurrent respiratory papillomatosis is clearly caused by benign proliferative growths induced by HPV 6 or 11 infection of the laryngeal epithelium. Although HPV genomic DNA has been detected in a proportion of laryngeal cancers and despite the many efforts made during the last 15 years, there is not yet compelling evidence that HPV plays a substantial role in laryngeal cancer. [J Natl Cancer Inst Monogr 2003;31:47–51]

Cancer of the Oral Cavity and Pharynx

Magnitude of the Problem

Cancers of the oral cavity and pharynx constitute a worldwide public health problem of a magnitude larger than often recognized. Worldwide estimates for 2005, assuming constant incidence rates, predict more than 300,000 new cases in males (one-third with pharyngeal cancer) and 130,000 in females (one-fifth with pharyngeal cancer), with 160,000 and 68,000 deaths, respectively, a combined burden close to that of cervical cancer (1). Some areas traditionally report a high incidence of these cancers (e.g., in France and in South India), but recent increases have been reported in other areas (e.g., in Eastern Europe and in Japan) (2). If a substantial number of these cancers are caused by human papillomavirus (HPV), vaccines may play an important role in their prevention.

Risk Factors for Cancer of the Oral Cavity and Pharynx

Etiologic agents of these cancers are tobacco smoking or chewing and alcohol drinking, with a demonstrated synergistic effect and attributable fraction close to 90%. Diets that are low in certain micronutrients and some aspects of oral hygiene are likely cofactors. However, contrary to HPV in cervical cancer, known risk factors are not present in many cases, indicating other possible etiologic pathways. Only a fraction of smokers and drinkers develop cancer, suggesting cofactors that could include HPV or other infectious agents.

HPV in the Oral Cavity and Pharynx

There are serious gaps of knowledge about the prevalence, the determinants, and the natural history of HPV infection in the epithelium of the oral cavity and pharynx. It is unclear whether productive HPV infection occurs in the oral cavity and why the tonsil appears to be infected preferentially. The epithelium of the deep tonsillar crypts, in close contact with lymphoid tissue, could be more susceptible to HPV infection or transformation. At least one study (3) indicates that oral sex may be involved in the transmission of HPV to the oral cavity, but other mechanisms are possible, including mother to child and fomites. Reported prevalence of HPV in the normal oral cavity varies widely, with both genital and cutaneous HPV types reported. HPV-associated lesions of the oral cavity include oral papillomas (associated with HPV6 and HPV11), focal epithelial hyperplasia (HPV13 and HPV32), and erythroplakia (HPV16). The role of HPV in other oral lesions is unclear [reviewed in (4)]. In leukoplakias and erythroplakias, which are recognized precursors of oral cancer, HPV has been reported inconsistently, and a clear continuum of HPV-associated lesions considered to be cancer precursors has not been defined in the oral cavity. Similar to the situation in anogenital sites, human immunodeficiency virus (HIV) carriers appear to have more frequent infections and a wider variety of HPV types, in addition to an increased frequency of HPV-associated oral lesions (5).

HPV in Cancer of the Oral Cavity and Pharynx

Multiple case series have been reported, with the prevalence of HPV DNA detection in cancers ranging from 0% to 100%, depending on the population, the combination of topographies, the type of specimen, and the DNA detection method [reviewed in (6,7)]. Most case series report HPV16 as the predominant type and a much higher prevalence in tonsillar and oropharyngeal cancers than in other locations. Most studies have concentrated on mucosal HPV types, and skin HPV types have not been studied as extensively. In a reported series of 253 case patients (8), HPV was found in 25% of the tumors, with HPV16 present in 90% of the positive tumors. HPV positivity was much more common in cancers of oropharyngeal location (57%). These researchers (8) also investigated the genomic integration of the virus, detecting HPV by in situ hybridization within the nuclei of neoplastic cells in preinvasive, invasive, and metastatic disease.
Southern blot patterns were also consistent with integration, although these investigators did not conduct formal confirmation of integration. HPV-positive tumors of the oropharynx were more likely to occur in nondrinkers and in nonsmokers, to have a basaloid histology, and not to have TP53 mutations. In addition, HPV-positive tumors seemed to have a better prognosis. Other investigators (9) have demonstrated transcription of E6 and E7 in a majority of HPV16-positive tumors and in association with a poorly keratinized histology. The presence of HPV DNA and messenger RNA (mRNA) has been demonstrated in neoplastic cells of the oral cavity and tonsil (10), and a recent study (11) documented for the first time that viral loads by quantitative real-time polymerase chain reaction (PCR) in microdissected HPV16-positive tonsillar tumors were higher than in whole-sample DNA preparations, indicating that HPV DNA is in the tumor and not in the stroma or other cells. The same paper (11) showed that viral loads in these tumors were comparable to viral loads seen in cervical carcinomas. Strong laboratory evidence for an active role of HPV in oral cancer was obtained from a study showing that transcriptionally active, integrated HPV16 DNA persisted in an oral carcinoma cell line having features indistinguishable from those of the primary tumor (10).

In case–control studies examining HPV DNA and cancers of the oral cavity, the search for HPV DNA was done by PCR in exfoliated buccal cells. Schwartz et al. (12), in a study of 284 case patients and 477 control subjects, did not find any association with HPV detection in exfoliated oral cells (odds ratio [OR] for any type of HPV = 0.9; 95% confidence interval [CI] = 0.5 to 1.6). However, oral rinses in that study were collected for an average of 9 months after the diagnosis and treatment of the case patients. Schwartz et al. found a much higher HPV DNA detection in the tumor tissue of the cancers of the oropharynx and tonsil than at other sites. In their study, HPV16 was predominant, with almost 65% of the HPV-positive case patients. In the study by Smith et al. (13), HPV DNA positivity in exfoliated cells from the case patients was 15%, and it was less than 5% in the control subjects, with an adjusted OR of 3.7 (95% CI = 1.5 to 9.3). In our multicentric case–control study, including more than 500 case patients and control subjects, HPV detection in exfoliated cells was close to 5%, without a significant difference between case patients and control subjects (Herrero R: unpublished data). In an effort to study the markers of past exposure to HPV, investigators have compared the prevalence of antibodies against the capsid protein of HPV16 in case patients and control subjects. Schwartz et al. (12) reported that antibodies against L1 were associated with a twofold increase in the risk of oral cancer, a figure that increased to almost sevenfold when the analysis was restricted to HPV16-positive tumors. They also reported an interaction with smoking. In a prospective seroepidemiologic study, Mork et al. (14) detected antibodies against HPV16 L1 twice more often in the subjects who later developed oral or pharyngeal cancer. The risk was higher for cancers of the oropharynx, tonsil, and base of the tongue.

Additional evidence is provided by the detection of antibodies against E6 and E7 proteins in a larger fraction of patients with head and neck tumors (12%) compared with the control subjects (3.5%). The fraction was even larger (37%) in patients with tumors containing HPV16 DNA (15). These antibodies are supposed to represent an immune response to E6 and E7 proteins expressed in the tumors, supporting a biologic role of HPV. Moreover, Capone et al. (16) detected circulating HPV16 DNA in the serum of the subjects with head and neck tumors who had detectable HPV16 DNA in their primary tumors, suggesting the hematogenous spread of cancer cells.

The possible role of HPV is also suggested by a large prospective study of more than 300,000 patients from the United States with HIV infection (17), in which a threefold excess of tonsillar cancer was observed in HIV-positive men, together with excesses for all of the other anogenital sites. However, no trends were observed with advancing immunosuppression, suggesting that this higher risk may be related to higher exposure to the virus. This evidence could explain, at least partially, the recent increase in tonsillar cancer reported in the United States (18).

Limitations of Reported Studies

One of the limitations of the case–control studies has been the definition of the proper sample for HPV testing of case patients and control subjects. Most of the studies have relied on exfoliated cells obtained by brushing the oral cavity, but the extent to which this specimen represents infection of the oral cavity, particularly the tonsil and the pharynx, is unknown. Focal HPV infections may be missed, resulting in the underestimation of HPV prevalence in the relevant anatomic location. This limitation may be particularly serious for anatomic sites like the tonsils, which have recently been found to harbor infectious agents like human herpesvirus 6 in epithelial cells (19). However, Klussmann et al. (11) found no HPV in a small group of tonsillectomy control subjects. Contrary to the situation in cervical cancer, relying on exfoliated cells in oral cancers appears to seriously underestimate the prevalence of HPV. In both the study by Schwartz et al. (12) and our study, HPV was undetectable in exfoliated cells of more than 90% of the case patients harboring HPV DNA in their biopsy specimens. Serologic studies represent systemic exposure and are unable to rule out infection in other sites. Case–control studies nested in cohorts and based on specimens collected years before usually have a limited ability to adjust for confounding factors. This limitation is important because sexual behavior and HPV infection are associated with smoking and alcohol consumption, the major risk factors for oral cancer.

Summary

Extensive epidemiologic and laboratory evidence is accumulating in support of the notion that HPV16 and maybe other HPV types play an etiologic role in a fraction of oropharyngeal and tonsillar cancers and possibly in a smaller fraction of cancers of the oral cavity. Evidence includes case series, case–control and prospective studies, and exposure assessment by the detection of HPV DNA in the tumors, different markers of immune response to the virus, and circulating HPV DNA. Laboratory evidence indicates that HPV is integrated and that HPV oncogenes are transcriptionally active. However, the estimated proportion of case patients with an HPV-related etiology is probably not synonymous to PCR-detectable HPV DNA presence. Two recent studies (20,21) indicated that HPV E6/E7 transcripts are less commonly found in these tumors than the DNA of the respective HPV type. Moreover, the HPV DNA presence and p53 mutations show overlap, whereas this is not (or extremely rarely) the case for HPV mRNA versus p53 mutation. The tonsil has squamous epithelium on the surface and reticulated epithelium in the...
crypts and may undergo metaplastic processes. A model has been proposed in which a variable fraction of cancers of the tonsil and oropharynx would be caused by HPV (22). These cases would arise after a pathway independent of smoking and alcohol consumption and would be characterized by a basaloid histology and by presenting without TP53 mutations. A parallel with the dual nature of other anogenital cancers (vulvar, vaginal, penile, and anal) is likely, with a fraction of the tumors in the subjects with specific risk factor profiles presenting as HPV related. The magnitude of this fraction in different populations would depend on the prevalence of HPV infection and competing risk factors.

**Directions for Future Research**

Many aspects of the association of HPV infection with cancers of the oral cavity and pharynx remain to be investigated. Large case–control studies focusing on tonsillar and oropharyngeal cancers and including a series of biomarkers are needed to better define the role of HPV and its interaction with smoking and other risk factors. For the case patients, HPV detection in their biopsy specimens should be the norm, but innovative ways to determine HPV in the control subjects are needed (e.g., the collection of oropharyngeal or tonsillar cells during intubation from patients undergoing elective surgery, the collection of transepithelial tonsillar biopsy specimens, or the use of tonsillectomy or autopsy control subjects to investigate the presence and activity of HPV in normal tonsils). Alternatively, studies will have to rely on serologic markers of past exposure. Prospective studies with adequate risk factor information and biomarkers are also warranted (e.g., the International Agency for Research on Cancer’s European Prospective Investigations of Cancer study). Additional areas include the definition of the proportion of HPV-related case patients presenting with a basaloid histology and their survival advantage, the transmission and natural history of HPV in the oral cavity, the role of HPV in sites other than the oropharynx and the tonsil, the role of other infectious agents (e.g., Epstein-Barr virus [EBV], herpes simplex virus, and other viruses) and their interactions, the contribution of specific HPV variants, other yet unexplored HPV types, human leukocyte antigen haplotypes, and immune markers in the oral cavity. Finally, research will be required to determine the use of HPV testing of the oral cavity for the prevention or the assessment of prognosis. The impact of HPV vaccination on the incidence of these cancers will provide the final answer.

**Cancer of the Larynx**

Worldwide estimates for 2005 predict more than 160,000 new cases of laryngeal cancer among males and 22,000 cases among women, with 89,000 and 12,000 deaths, respectively (1). Similar to cancers of the oral cavity and pharynx, most of the laryngeal cancers can be attributed to alcohol and tobacco use, with joint exposures having a multiplicative effect on risk (23). Other possible risk factors may include deficiencies in dietary micronutrients, workplace exposures, and gastroesophageal reflux disease (24). The laryngeal epithelium is susceptible to HPV infection and is capable of sustaining viral replication, since it is the most common site of involvement in juvenile- and adult-onset laryngeal papillomatosis. This disease is characterized by multiple, recurrent benign papillomatous growths initially arising most often in the vocal cords, with subsequent spread to other areas of the respiratory tract. Although most of the larynx is covered by “respiratory” epithelium (pseudostratified, ciliated, and columnar epithelium), the true vocal folds or vocal cords and the lingual surface of the epiglottis are covered by stratified squamous epithelium. Clinically, papillomas tend to arise from the junction of squamous and respiratory (ciliated) epithelium and areas of iatrogenically induced squamous metaplasia (25,26). The incidence of recurrent respiratory papillomatosis is estimated to be 0.3–1.0 per 100,000 children in the United States, with approximately 80–1500 incident cases occurring in the United States each year (27).

HPV6 or HPV11 (and rarely other low- and high-risk types) is detected and transcriptionally active in the majority of cases of laryngeal papillomatosis (28–30). The prevalence of laryngeal infection in individuals without papilloma is unknown; however, low-risk HPV11 has been found in the normal larynx at autopsy (31). Laryngeal papillomatosis has a bimodal age distribution, with peaks before 5 years and between 20 and 30 years of age, indicating transmission during passage through an HPV-infected birth canal and through sexual contact. In rare instances, papillomas do become malignant, particularly in subjects with longstanding disease, radiation therapy, and tobacco exposure. The majority of these tumors are positive for HPV6 or HPV11 alone, constituting the only clearly established setting in which low-risk HPV6 play a role in a human malignancy. HPV6 or HPV11 infection is necessary, but not sufficient, for the development of respiratory papillomatosis, and cofactors remain to be elucidated.

**HPV in Laryngeal Cancer**

Most malignancies in the larynx are squamous cell carcinomas (>90%). Similar to oral and pharyngeal cancers, multiple case series have reported prevalences of HPV DNA in laryngeal cancer ranging from 0% to 100% (32). The proportion of laryngeal cancers in which the detected HPV may play an etiologic role is currently unclear, because the majority of studies did not use more specific confirmatory methods for HPV detection. As an example, HPV that was detected in four of 45 laryngeal tumors by PCR was limited to the superficial and intermediate epithelial layers when examined by in situ hybridization and was not present in the invasive neoplastic cords (33), suggesting that secondary superinfection was not pathophysiologically related to the tumor. Other studies (34,35) have provided an indication of clonal relationships (high copy number) with HPV16 and HPV30. In a detailed analysis of 25 fresh-frozen laryngeal cancer biopsy specimens (36), HPV DNA was detected in 13 tumors by PCR and typed as HPV16 in seven case patients, as HPV type 45 in one case patient, and as HPV6 in five case patients. In the same study, viral DNA was detected by PCR and in situ hybridization in normal tumor margins in all HPV6-positive samples and in five of the seven HPV16-positive samples, and patterns consistent with episomal and integrated virus were found in normal margins by gel electrophoresis. HPV16 E6 transcripts were detected in three tumor specimens, but transcripts were also detected in the surrounding margins from two samples, arguing against the specificity of HPV to the tumor cells, although other possible explanations not inconsistent with a pathophysiologic role for virus are conceivable (37). The presence of HPV in laryngeal dysplasias would support a role for the virus in laryngeal malignancies, but viral DNA is rarely detected in...
such lesions (38,39). Moreover, data from the cell cultures derived from laryngeal cancers have shown detection of HPV with viral loads insufficient to represent a clonal relationship between the virus and the tumor (40). Although a 2.4-fold (95% CI = 1 to 5.6) increase in the risk of laryngeal cancer among HPV16 L1-seropositive individuals was reported in a nested case–control study after adjustment for tobacco exposure, no adjustment for alcohol consumption could be made (14). In the same study, the risk of oropharyngeal cancer was 14.4 (95% CI = 3.6 to 58.1).

There are no large case–case studies comparing the risk factors or other characteristics of HPV-positive and HPV-negative tumors, and current literature is conflicting about the role of HPV in verrucous carcinoma of the larynx, a histopathologic variant of squamous cell carcinoma previously considered to be HPV16 associated (41–43).

**Summary and Directions for Future Research**

It is becoming increasingly clear that squamous carcinomas of the upper aerodigestive tract are etiologically heterogeneous cancers, often with unique risk factors (e.g., EBV in nasopharyngeal carcinomas). Even within an anatomic site, there is etiologic heterogeneity, as is the case for oropharyngeal cancers. There are considerable data to support the conclusion that HPV infects the laryngeal epithelium and causes benign papillomas, and that malignant degeneration of HPV6- and HPV11-induced papillomas does occur as a rare complication of a rare disease. The genome of high-risk HPV types has been detected in a papillomas does occur as a rare complication variant of squamous cell carcinoma previously considered to be expected of an HPV-associated tumor. Further case series are needed to look for the full signature of the virus, including viral load per tumor cell genome, viral transcripts, and localization of the virus to the tumor cell nucleus by *in situ* hybridization. The characteristics of these confirmed HPV-positive cases should be compared with those of HPV-negative laryngeal cancers with regard to risk factor profiles (including sexual behavior, tobacco use, and alcohol consumption) in a case–case study analyzed in a manner similar to that of a case–control study. This comparison should include careful classification of tumor histology, with particular attention to the differentiation, keratinization, baso-loid, and verrucous features, by pathologists masked to HPV status. If these data are compelling, then case–control studies will need to be performed to better define the role of HPV and its interaction with smoking and other risk factors of laryngeal cancer.

**References**


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