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Innovations in Salivary Diagnostics

Oral HPV: An Overview of the Infection and its Role in the Development of Oral Cancer

7400 Flying Cloud Drive / Eden Prairie, MN 55347
855-ORALDNA (672-5362) / Fax: 952-942-0703 / OralDNA.com

What is oral HPV?

Oral HPV is a human papillomavirus (HPV) infection affecting regions of the oro-respiratory tract, which includes the entire oropharyngeal complex, tongue, tonsils, and buccal and lingual mucosa.

The virus is composed of a double-stranded DNA genome enveloped in a protein capsid. The capsid proteins enable the virus to infect epithelium in a variety of sites, especially the skin and mucosa of the genital and oro-respiratory tracts.

There are more than 100 different HPV types. Each type is defined first by differences in the sequence of its DNA and second by its tendency or risk of being associated with malignant tumors. Low-risk HPV types such as 6 and 11 typically induce benign lesions, ie, warts or papillomas. High-risk oncogenic types such as 16, 18, 31, 33, and 35 are defined by their strong epidemiologic association with cervical cancer.¹ Types 16 and 18 are also strongly associated with squamous cell carcinomas of the head and neck region.² Moreover, HPV types 16 and 18 are known to cause cells to undergo malignant transformation in culture.³ In both research and clinical settings, however, time and other factors are required for HPV to trigger malignant transformation of cells. Hence, persistent HPV infection with a high-risk oncogenic type can lead to both cervical and oral cancers.

HPV is one of the most common sexually transmitted infections in the United States.⁴⁻⁶ According to the National Institute of Allergy and Infectious Diseases, a branch of the Centers for Disease Control, at least 20 million people in the U.S. are infected with HPV, and there are approximately 5.5 million new cases per year.⁷ Most infections will resolve spontaneously and not lead to cervical or oral cancer. However, HPV infection is considered the cause of almost all cases of cervical cancer and 60% to 70% of newly diagnosed oropharyngeal cancers.⁸

How HPV infection leads to oral cancer

HPV first takes control of the cell's mechanisms for growth and differentiation. Long-standing and continued expression of the viral genes causes destabilization of the host cell's genome, leading to permanent deregulation of host cellular proliferation. This step is often referred to as cellular immortalization. The subsequent steps that lead to cancer are evidenced in clinical samples by histological progression and, at the molecular level, by integration of the HPV genome into the host cell's chromosomes and the amplification of various cellular oncogenes.⁹⁻¹¹ In other words, the HPV virus must be present in the host cell, and once there, its viral genetic material in effect "takes control" over the host cell, leading to a cascade of events we see as the clinical manifestation of a tumor.

Oral HPV frequency

Although the frequency of oral HPV is unknown, data show that about 26% of all head and neck squamous cell carcinomas are associated with HPV.² Those not associated with HPV are likely to be associated with tobacco use and/or alcohol abuse.

Prevalence of HPV-positive oral cancer appears to vary by tissue site and geography (Table 1). Although the numbers vary by study, it is clear that HPV is associated more frequently with oropharyngeal cancers (oropharynx and tonsil) than with oral cavity or larynx cancers.^{2,12}

The importance of early detection

Squamous cancers of the oral cavity can be slow-growing or highly aggressive. At the same time, these cancers are insidious and typically present at an advanced stage. The 5-year survival drops from 83% for cancers diagnosed at a local stage to 54% and 32% for cancers diagnosed at regional and

Table 1. HPV Prevalence in Oral Cancer²

| Tissue site | Worldwide Prevalence | | North America Prevalence | |
|-------------|----------------------|------------------|--------------------------|------------------|
| | No. of Cases | % (95% CI) | No. of Cases | % (95% CI) |
| Oral cavity | 2642 | 23.5 (21.9-25.1) | 577 | 16.1 (13.2-19.4) |
| Oropharynx | 969 | 35.6 (32.6-38.7) | 285 | 47.0 (41.1-53.0) |
| Larynx | 1435 | 24.0 (21.8-26.3) | 297 | 13.8 (10.1-18.3) |

CI, confidence interval. Oral cavity includes tongue, gums, floor of mouth, and palate. Oropharynx includes vallecula, walls of the oropharynx, and tonsils. Larynx includes postcricoid region, hypopharyngeal region of the aryepiglottic fold, posterior wall of the hypopharynx, and the larynx.

distant stages, respectively.¹³ These data suggest that a focus on early detection could improve survival rates.

The dental professional's role in oral cancer detection

Dental professionals are on the front lines of oral cancer detection. The various screening options can easily be incorporated into the dental practice and only take a few minutes to complete. Regular screening can identify patients who may have oral cancer, as well as those who are at higher risk of developing it. Patients at higher risk may benefit from more frequent or extensive evaluation in order to detect cancer in the early stage. Patient education regarding the risk factors and signs of oral cancer is also important.

Screening may include:

- Patient history – tobacco and alcohol use, previous diagnosis of oral HPV infection, high-risk sexual behaviors, etc.
- Physical examination – inspection and/or palpation of the face, eyes, nose, ears, oral cavity, lips, buccal mucosa, tongue (including the base), floor of the mouth, hard and soft palate, oropharynx, tonsils, posterior pharyngeal wall, neck, hypopharynx, and larynx¹⁴
- Salivary HPV test

Oral cancer in its earliest stages has always been difficult to discern from healthy normal tissue. By the time the lesion is visually or symptomatically apparent, it is often “too late” and requires surgical removal. There are several modalities available to assist in evaluating suspicious lesions.

Toluidine blue dye can be applied to the lesion; areas that stain darker are more likely to be precancerous or malignant.

Innovative devices that shine light of a specific wavelength onto the oral mucosa are new tools for dental professionals. Unlike normal mucosa, viral-infected tissue fluoresces when exposed to certain light frequencies that are near the ultraviolet region of the spectrum. After considering the size of such lesions, the presence or absence of hardening of the surrounding tissue, and other patient risk factors, the clinician may elect to watch and wait for resolution or elect to perform a biopsy.

Brush biopsy devices employ tiny wire brushes that scrape off the top layers of skin of suspected lesions, the cells of which are analyzed in a laboratory and described on a pathology report.

The value of testing for oral HPV

Although HPV infections can resolve spontaneously, persistent infection with an oncogenic type is a significant risk factor for oropharyngeal cancer. Thus, a patient who tests positive for an oncogenic HPV type should undergo a thorough oral cancer screening by the dental professional on a regular basis. Additionally, the dentist may want to consider referring the patient to an ear, nose, and throat specialist for co-management. Outward signs of oral HPV may manifest themselves as pre-cancerous, cancerous, or benign lesions of the oral epithelium.

In addition to oral cancer risk assessment, HPV testing can help the physician assess prognosis in patients with cancer of the tongue, tonsil, posterior pharyngeal wall, or soft palate.⁸ Patients who test positive are more likely to respond favorably to treatment and survive longer than those who test negative.

Finally, HPV testing can be used in patients with a cancer of unknown primary origin.⁸ A positive test result is a good indicator of a primary tumor in the tonsil or base of the tongue. Thus, radiation therapy can be limited to these regions.⁸

Ways to detect oral HPV

Testing options include HPV detection and genotyping using polymerase chain reaction (PCR), HPV in situ hybridization (ISH), and p16 immunohistochemistry (IHC). p16, a cellular protein expressed in cells that have a higher growth rate, may be a surrogate marker for HPV-infected tissue. Tumor tissue is required for the latter 2 options, but PCR can be performed on tumor tissue or saliva.

PCR is a sensitive and specific molecular technique in which a particular segment of DNA is amplified to a detectable concentration. Electrophoresis of the amplified DNA is used to determine the presence or absence of HPV. When HPV is present, enzymes that recognize select DNA sequences are used to fragment the amplified DNA. The pattern of this fragmented DNA is compared to the known pattern of the various HPV types, thereby determining the HPV type(s) in the saliva sample.

Salivary diagnostics

Although the most commonly used laboratory diagnostic procedures are performed using blood samples, saliva is being used more often. Collection is non-invasive, and saliva requires little to no processing after collection. It has proven to be a useful medium in which to measure a wide range of hormones, pharmaceuticals,

and antibodies. Saliva has also proven to be a convenient source of human, bacterial, and viral DNA. Thus, it may be the sample of choice for many diagnostic tests.

The OraRisk[®] HPV test by OraIDNA[®] Labs is a salivary diagnostic test. The test uses PCR to detect the presence of HPV DNA in a few drops of saliva. Completely non-invasive, the OraRisk HPV test is easy and comfortable for the patient and clinician. The patient vigorously swishes and gargles with a saline solution for 30 seconds and then expectorates into a funneled collection tube. The funnel is removed, and the collection tube is sealed with a cap. The sample is then labeled, placed in the provided specimen transport bag, and shipped via FedEx[®] standard overnight (at no extra cost) to OraIDNA Labs for PCR analysis. An e-mail notification is sent to the clinician when the comprehensive, HIPAA-compliant report is available.

Patient education and communication

Sharing the laboratory report with the patient can be an effective way to communicate test results and help the patient understand them. The OraRisk HPV report specifies the presence or absence of HPV infection and the type of HPV, if present. It includes comments regarding the significance of results (eg, implications regarding the HPV type and risk of cancer). It also suggests future steps that can be taken by the doctor and patient. Thus, sharing the report can serve as an introduction to a discussion about the treatment and/or monitoring plans. This approach may result in better patient compliance.

Summary

Human papillomavirus is a common infectious disease that affects both genital and oral health. Substantial molecular evidence indicates that persistent HPV infection, especially with type 16, plays a significant role in the pathogenesis of oropharyngeal squamous cell carcinoma.

Dental professionals are on the front lines of oral cancer screening and detection. Screening techniques can be easily incorporated into routine patient visits. Since persistent infection with oncogenic types of HPV is a significant risk factor for oral cancer, along with tobacco use and alcohol abuse, it makes sense to add salivary HPV testing to traditional screening techniques.

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