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REVIEW ARTICLE

DETECTION OF HPV IN ORAL CANCER

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ABSTRACT:
The connection between Human Papilloma Virus (HPV) and Head and Neck Squamous Cell Carcinoma (HNSCC) has been well established in this decade. This challenging virus should be detected as early as possible especially by dentist in order to increase the prognosis of the oral or oropharyngeal cancer. Given that , interest is growing worldwide in the potential for use of HPV diagnostics in HNSCC cancer prevention programs. The currently available tests likely are too expensive and technologically demanding for widespread use. Wide knowledge and usage of the diagnostic test will reduce the incidence of HPV infection thereby increase the prognosis of oral cancer caused by Human papilloma virus. This review article discusses the various diagnostic methods to detect the HPV infections in Oral Cancer patients.
Keywords: HPV; oral cancer; HNSCC

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INTRODUCTION

Human Papilloma Virus (HPV) is the name of a group of viruses that include more than 80 different types associated with a variety of epidermal warts, skin lesions and also skin cancer. Low-risk HPV subtypes (eg. Type 6, 11) are associated with more benign skin lesions such as papillomas whereas High-risk subtypes (eg. Type 16, 18) can cause neoplasia (abnormal cell growth) or dysplasias and are associated with the development of cervical and anal cancers. HPV is one of the most common sexually transmitted diseases (STD) in the world.

While there is no pharmacologic cure for HPV, the infection can induce an antibody mediated immune response, which is thought to clear the virus from the body. However, HPV can also effectively evade the body's immune system, residing dormantly (latent infection) inside certain cells. It is estimated that there are one million women in the U.S. with HPV-related dysplasia, 55,000 with in-situ carcinomas, and 15,000 with cervical cancer.

The connection between HPV and Head and Neck Squamous Cell Carcinoma (HNSCC) has been well established in this decade. This challenging virus should be detected as early as possible especially by dentist in order to increase the prognosis of the oral or oropharyngeal cancer.[1]

FREQUENCY OF ORAL HPV INFECTIONS

In a 2003 multinational study conducted by the International Agency for Research on Cancer, only 18% of oropharyngeal tumors were HPV-positive, indicating that this proportion likely varies by geography. A 2005 study based on the meta-analysis of several studies estimated that HPV is

associated with approximately 26% of all head and neck squamous cell carcinomas. The data linking HPV to oropharyngeal cancers is even stronger, with various published series showing detection of HPV in 50% or more of cases. Regardless of the study population, HPV-16 accounts for the majority—90% to 95%—of HPV-positive tumors, whereas other high-risk types (-31, -33, and -35) account for the minority. [2]

RISK OF HEAD AND NECK SQUAMOUS CELL CARCINOMA

For pharyngeal cancer, risk increased with increasing alcohol consumption (OR 5.1 for ≥ 25 vs < 3 drinks/week) and smoking (OR 6.9 for ≥ 45 pack year vs never smoked) among HPV-16seronegative subjects but not among HPV-16 seropositive subjects. However, among light drinkers or never smokers, HPV-16 seropositivity was associated with a 30-fold increased risk of pharyngeal cancer. Alcohol or tobacco use does not further increase risk of HPV-16- associated pharyngeal cancer.

There is additional evidence that supports considering HPV- 16-positive HNSCCs and HPV-16-negative HNSCCs as distinct cancers because the risk factor profiles differ. HPV-associated cancers represent a distinct clinic pathological entity, which is generally characterized by a younger age at onset, basaloid or warty histopathology, association with sexual behavior, and better prognosis, when compared with their HPV-negative counterparts.

Two recent case reports described HIV-infected patients who first developed HPV-related anal squamous cell carcinoma (SCCA) and later developed oral SCCA. Both patients were responding to antiretroviral therapy with undetectable viral loads when they developed oral cancer. This further supports the need for careful oral and anal screening for HIV-infected patients.

A study from the Centers for Disease Control and Prevention identified and reviewed 44,160 (US, 1998-2003) cases of potentially HPV-associated cancers of the oro pharynx and oral cavity and found out that Tonsil was the most common site (43.6%), Base of tongue was second (38.4%), Other sites (18.0%). [3]

PATHOGENESIS OF HPV

The initial effect of HPV infection is to take control of the cell's mechanisms for growth and differentiation. Longstanding and continued expression of the viral genes, in particular the so-called E6 and E7 genes, causes destabilization of the cell's genome that in turn makes permanent deregulation of the cellular proliferation. This step is often referred to as cellular immortalization. The subsequent steps that lead to cancer are evidenced in clinical samples as histologic progression and, at the molecular level, integration of the HPV genome into the cell's chromosomes and the amplification of various cellular oncogenes. 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.

A study done by **Dr. No-Hee Park** showed that the mouth was, at the cellular level, structurally very similar to the vagina and cervix. Both organs have the same type of epithelial cells that are the target of HPV 16 and HPV 18. The majority of oral cancers are cancers of epithelial cells, primarily squamous cell carcinomas, not unlike the cancers that affect the cervix. **Dr. Park's** study also showed that smoking and drinking alcohol help promote HPV invasion. Combine tobacco and alcohol with HPV, and the epithelial cells in the mouth, and you may have the formula for the development of an oral cancer. A recent study conducted by **Dr. Maura Gillison** at the Johns Hopkins Oncology Center furthered the premise that HPV is linked with certain types of oral cancer. [4] In 25% of 253 patients diagnosed with head and neck cancers, the tissue taken from tumors was HPV positive and HPV 16 was present in 90% of these positive HPV tissues.

This information helps to confirm that there is a strong link between HPV 16 and oral cancer. 25% of those diagnosed with oral cancer are non-smokers while the other 75% of those diagnosed have used tobacco in some form during their lifetimes. The research into the relationship of HPV and oral malignancies may give us clues as to the origin of cancer in those 25% of diagnosed individuals who did not smoke.

HPV DETECTION IN ORAL CANCER

As reviewed by **Kreimer AR et al** HPV DNA has been found by polymerase chain reaction (PCR) in HNSCC arising from various anatomic sites. [5] Various studies, mainly involving HPV16, have shown that viral DNA is diffusely present in neoplastic cells throughout the tumor when detected by in situ hybridization (ISH), indicating clonality **(Gillison M et al 2000).** [4] Demonstrated retention of viral DNA upon growth of tumor cells in culture, as shown for some oral cavity and oropharyngeal cancer cell lines, provides further evidence for viral

clonality. Detection of HPV DNA in HNSCC by PCR alone is, however, insufficient to prove causality. Expression of HPV E6/E7 region mRNA is far less common in HNSCC than viral DNA detection by PCR**.** [6] HPV E6/E7 oncogene expression is considered necessary for carcinogenesis, and therefore its absence points to absence of causality. Indeed, HPV DNA-positive tumors without E6/E7 mRNA are similar to HPV DNA-negative tumors in terms of p53 mutation status and number of chromosomal imbalances at 3p, 9p and 17p. Viral physical status can be heterogeneous within one tumor, with parts harboring only episomal DNA and other parts only integrated viral DNA**.** [7]

Genetic alterations in HPV-positive vs HPV-negative tumors HPV E6/E7 mRNA expressing tumors have genetic alterations distinct from HPV E6/E7 mRNA-negative HNSCC that are indicative of viral oncoprotein function. Deregulated HPV E6/E7 activity in proliferating cells results in increased expression of p16INK4A triggered by the E7-mediated induction of the histone demethylase, KDM6B. P16 expression is considered a marker for cervical pre(malignant) lesions harboring transforming HPV infections. Indeed, HNSCCs displaying viral E6/E7 expression generally display diffuse p16INK4A immune staining**.**[8] However, a subset of HPV DNA and mRNA-negative HNSCCs show diffuse p16INK4A staining, indicating expression is not specific for HPV activity. Indeed, HPV-positive HNSCCs are less likely to contain inactivating p53 mutations than HPV-negative tumors. [9] HPV E6/E7 mRNA-positive HNSCCs are characterized by distinct microarray expression profiles[10][11] and exome sequencing studies have revealed that HPV-positive HNSCCs have fewer somatic mutations in coding regions than HPV negative tumors.

SALIVARY DIAGNOSTICS

Saliva has also proven to be a convenient source of human, bacterial, and viral DNA. Thus, it is an ideal source of diagnostic information, and serves as the basis for salivary diagnostic tests. One such salivary diagnostic test is the OraRisksm HPV test by OralDNA® Labs. The OraRisksm HPV test identifies the presence of HPV DNA in a patient's saliva sample using a laboratory process called polymerase chain reaction (PCR). In the case of the OraRisksm HPV test, the laboratory can positively identify oral HPV through the analysis of a few drops of saliva, and comparing the DNA contained in that sample to the DNA of specific types of oral HPV. If there is a match, then the person is positive for that particular type of HPV. It is completely non-invasive, the OraRisksm HPV test is easy and comfortable for the patient and clinician.

Recently, **Chuang et al.** have associated the presence of HPV-16 DNA in surveillance salivary rinses with a significant risk for recurrence in HNSCC.[12] They hypothesized that the improved prognosis of many patients with HPV-related oropharyngeal carcinoma is due to the temporary nature of the infection, and therefore in the absence of lesions no HPV genomic DNA is detected in oral smear**.**

The detection of HPV genomic DNA was performed using the Pap type kit (Progenie-Molecular S.L, Valencia. Spain). In brief, a polymerase chain reaction (PCR) targeting the L1 region of the viral genome, harbouring an internal control to avoid false negatives, was carried out. Subsequently, and in HPV+ samples, typing was performed using a restriction fragment length polymorphism (RFLP).

HIGH-RISK HPV DETECTION BY HC2 ASSAY

High-risk (HR) HPV DNA testing was performed using the HC2 assay method (Digene Corp.) and rapid capture system (RCS) according to the manufacturer's protocol. The HR HC2 signal amplification assay contains a cocktail of probes complementary to 13 HR HPV types including 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68.

HPV GENOTYPING TEST

HPV genotyping steps were carried out on all HC2 HR-HPV positive specimens using the Linear Array HPV Genotyping Test and Linear Array Detection Kit (Roche Molecular Systems Inc., Pleasanton, CA) according to the manufacturer's instructions. The Roche Linear Array HPV Genotyping test (Roche Diagnostics, Branchburg, NJ) was used for the detection of 37 low and high-risk HPV types, according to manufacturer's instructions. Briefly, it utilizes bio tinylated PCR of the HPV L1 region and reverse blotting to multiple HPV genotypes.

HPV types were determined by lining up them manufacturer's HPV reference guide with the genotyping strip. A low- and high-copy b-globin internal control is included in each run to assess the quality of DNA sample. All experiments included an HPV positive control, an HPV negative control, and ano-template control. Cases that were HPV positive were repeated, without the presence of a positive control, to verify results and exclude the risk of contamination**.**

THE INFLUENCE OF TUMOUR LOCATION AND METHODS USED FOR HPV PREVALENCE ANALYSIS

A serious problem in HPV research is that there is no consensus as to how to identify HNSCC caused by HPV. This may undermine the true importance of HPV for HNSCC patients. There are many assay variables that differ between studies, making it difficult to compare various studies and to generalize from the results. The frequencies of HPV-positive tumours in HNSCC show considerable variation in published studies.[13] Some studies report frequencies of 0% in oral and laryngeal carcinomas, whereas others report up to 93% in oro pharyngeal carcinomas**.** [14]

In a meta-analysis by Termine et al, the pooled prevalence of HPV DNA in the overall samples was 34.5%, in oral cavity SCC (OSCC) it was 38.1%, and in non tumour site-specific HNSCC it was 24.1%. Regarding the detection methods, PCR-based studies report a higher prevalence rate than for in situ hybridization (ISH)-based rates (34.8 vs 32.9%) especially in the OSCC subgroup (OSCC PCR-based: 39.9%).[15] In a meta-analysis, **Hobbs et al**. found that the association between HPV16 and cancer was the strongest for the pharyngeal tonsils (OR: 15.1), intermediate for the oro pharynx (OR: 4.3), and weakest for the oral cavity (OR: 2.0) and the larynx (OR: 2.0). Besides the influence of tumour site and technical aspects on outcome of the analyses, different results may be obtained when using fresh frozen or formalin-fixed paraffin-embedded material.

There may also be a problem associated with the type of assay used and the criterion for positivity. In 2009, more than 90 studies of HPV in HNSCC were published, and only selected studies from this period with comprehensive materials or remarkable HPV prevalence are included. Many different assays for HPV detection are available, each with its own analytical sensitivity. There are the PCR-based assay systems, often linked to a specific genotyping system. Amplicor35 and SPF1036, as well as various type-specific PCR methods, are very sensitive systems, whereas GP5+/GP6+-PCR37 and PGMY38 are somewhat less sensitive in detecting HPV. In addition, there are methods based on DNA hybridization with specifically labelled RNA probes ISH that can be applied to detect HPV on histological sections. [16]

In a recently published study by Shi et al., the prognostic value of HPV16 E6 mRNA was compared with that found using ISH and p16 immuno staining in human oro pharyngeal SCC (31). HPV16 E6 mRNA was positive in 73 (66%) of 111 samples; ISH was positive in 62 of 106 samples (58%), with 86% concordance. p16 was over expressed in 72 samples (65%), and strongly associated with HPV16 status by either method. Classification of HPV positivity by HPV16 E6 mRNA, HPV16 ISH or p16 IHC was all associated with better disease-free survival. However, the latter two assays were technically easier to perform**.** [17] Winder et al compared the MY09/ 11 and GP5+/GP6+ primer sets with a GP5+/GP6+ nested PCR, showing that the older and commonly used MY09/ 11 and GP5+/GP6+ primer sets may not be sufficient for primary HPV detection on non-cervical clinical samples, and that negative results with primary PGMY PCR screening should be considered for GP5+/GP6+ nested PCR.

Another approach for detecting clinically relevant HPV infection is by a combination of p16 immuno histochemistry and GP5+/6+ PCR. This can be used for high throughput analysis of paraffin-embedded material and has been used in several studies. HPV in saliva and oral exfoliated cells has been detected in some recent studies, but the sensitivity and specificity for HPV-related HNSCC are too low and the role of HPV detection in saliva and oral exfoliated cells seems uncertain. [18] In general, it is recommended that at least two standardized and recognized methods should be used to confirm the diagnosis of clinically relevant HPV-positive HNSCC. [19]

CONCLUSION

HPV has clearly been shown to be the cause Head and Neck cancers. Given that, interest is growing worldwide in the potential for use of HPV diagnostics in HNSCC cancer prevention programs. The currently available tests likely are too expensive and technologically demanding for widespread use. Wide knowledge and usage of the diagnostic test will reduce the incidence of HPV infection thereby increase the prognosis of oral cancer caused by Human papilloma virus.

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