Role of Human Papilloma Virus in Oral Squamous Cell Carcinoma: Review Article

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ABSTRACT
Oral cancer etiology is multifactorial and the main risk factors are tobacco chewing and smoking along with alcohol consumption. 10% to 20% of population do not have any habits and are still diagnosed with oral cancer. This suggest the role of other factors, including viruses like Human papilloma virus (HPV) in oral carcinogenesis. Human papilloma virus is considered as a prime suspect in the etiology of oral squamous cell carcinoma (OSCC) due to their ability to immortalize oral keratinocytes and bring about transformation of epithelial cells. Molecular classification of tumors thus provides important new information which allows better estimate of prognosis and use of targeted therapy.

Keywords: Human Papilloma Virus, Oral Cancer, Oral Squamous Cell Carcinoma, Polymerase Chain Reaction

INTRODUCTION
Oral cancer (OC) is the sixth most common cancer in world and ranks third among developing countries and eighth among developed countries. Oral cancer etiology is multifactorial and the main risk factors are tobacco chewing and smoking along with alcohol consumption. Literature shows that about 10% to 20% of patients with oral cancer are non-tobacco users and non-drinkers suggesting that other factors, including viruses, may have implications in oral carcinogenesis.¹

Human papilloma virus (HPV) is considered as a prime suspect in the etiology of oral squamous cell carcinoma (OSCC) because of their ability to immortalize oral keratinocytes by bringing transformation of epithelial cells.

HISTORY
Ernest Ayre, Canadiana cytologist in 1951 described and demonstrated squamous epithelial cells with a ‘perinuclear halo’ in smears from the uterus. He proposed these squamous cells with ‘halo’ as ‘precancer cells’ which could be due to long standing infection, inflammation or virus. In 1956 Koss and Durfee named these cells with perinuclear clearing surrounded by a thin rim of cytoplasm as “koilocytes”. Greek word ‘koilos’ means ‘hollow cell’.² Syrjanen and cols. described a relationship of HPV and oral cancer in 1983. Role of HPV in oral carcinogenesis can be attributed to:

1. Established etiological role of HPV in cervical Squamous cell carcinoma;
2. Epithelial tropism of HPV;
3. Detection of HPV genotypes in cases of OSCC.
4. Similarity between oral and genital epithelia.³

HUMAN PAPILLOMAVIRUS
The HPV is a non-enveloped DNA viruses with a diameter of 52–55 nm belonging to the Papillomava viridiae family. More than 200 types of HPV have been recognized on the basis of genotypic variations in the DNA base sequences in the E6 and E7 region. High risk includes HPV-16, 18, 31, 33, 35, 45, 51, 52, 56, 58, 59 and low risk are HPV 6, 11, 42, 43, 44.⁴

STRUCTURE
The HPV genome contains a double-stranded DNA molecule that is bound to cellular histones and contained in a protein capsid. The HPV-DNA genome consists of eight open reading frames (ORFs) which is further divided into three functional parts: the early (E) region, the late (L) region and a long control region (LCR).⁵,⁶

TRANSMISSION OF HPV INFECTION
HPV transmission to the oral cavity could be due to sexual activity or transmission to the neonate during its passage through an infected birth canal of the mother.⁷ HPV transmission usually does not occur through direct contact, normal intact skin or mucosa. Oral mucosa is constantly exposed to infections and trauma which results in an abraded surface making it susceptible for HPV to gain entry into the basal cells.⁸ Usually the immune system clears HPV naturally within 2 years (about 90%), but the ones that persist can cause serious diseases.⁹ HPV needs terminally differentiated epithelial cells like the squamous cells for its replication. HPVs do not kill the infected basal epithelial cells but as the basal cells divide and progress into squamous cells, HPV is carried within them.⁹

HOST CELL ENTRY OF HPV
HPV enters the host cell by binding to cell surface receptors. The virions bind initially to the basement membrane before entering the basal keratinocyte cell surface. Glycosaminoglycans (GAGs), especially heparan sulfate found in the extracellular matrix (ECM) and on the surface of most cells, were suggested as initial attachment receptors for HPV. A secondary receptor or co-receptor like α6β1-integrin is also involved in the internalization of HPV. HPVs are internalized via a clathrin-dependent endocytic mechanism. Few HPVs use alternative uptake pathways to enter cells, like caveolae-dependent route or the involvement of tetraspanin-enriched domains as a platform for viral uptake.¹⁰

Once HPV infects the host tissue, its genome is integrated into the host genome and two products are formed – ‘E6 protein’ and...
that forms a complex leading to the degradation of p53 gene thereby inhibiting apoptosis, and ‘E7 protein’ that disturbs the retinoblastoma tumor suppressor gene which finally leads to the increase of DNA synthesis.11

**HPV DETECTION METHODS**

The diagnosis of HPV infection can be made through cytology and biopsy. The cytological features of the HPV infection includes major and minor criteria.

**A. Major criteria:** Koilocytes, perinuclear cytoplasmic halos and nuclear dysplasia.

**B. Minor criteria:** Dyskeratocytes, atypical immature metaplasia, macrocyte and binucleation.

The methods used to detect the HPV DNA in lesions vary based on sensitivity and specificity.

**Low Sensitivity methods:** Immunohistochemistry and in situ hybridization - hybridization allows virus detection when present in more than 10 copies of the viral DNA per cell;

**Moderate Sensitivity methods:** Southern blot, dot blot and reverse dot hybridizations, hybridizations - as these methods detects viruses only when it is present in 1 to 10 copies of the viral DNA per cell;

**High Sensitivity methods:** PCR, because it detects the virus in less than 1 copy of the viral DNA per cell.12 Over the years, Polymerase Chain Reaction (PCR) has emerged as the “gold standard” for identification of HPV types.

**HPV IN ORAL SQUAMOUS CELL CARCINOMA**

Not much data is available regarding the incidence of HPV-induced oral cancers in the Indian scenario. In India, the prevalence of HPV varies based on differences in geographical regions. Patel et al. reported that HPV was not found in the population of Western India.1 South Indian studies have shown high HPV prevalence in their population which was 74% in the study by Balaram et-al13 and 48.3% by Elango et-al.14 Many western researchers have said that HPV induced OSCC is more common in Oropharynx specially tonsillar fossa. Indian studies have an entirely different picture. Koppi et-al15 found that maximum prevalence of HPV was in oral cavity. Nagpal et-al16 showed maximum positivity of HPV in the mandible region followed by buccal mucosa. A multicentre case-control study was done on oral cavity and oro-pharynx carcinomas in 9 countries.17 It was observed that 70% of the tumor harboured HPV DNA. HPV16, commonly observed in genital cancers was also the most common type found in oral tumours. The study finally concluded that HPV appears to play an aetiological role in many cancers of the oropharynx and possibly a small subgroup of cancers of the oral cavity.17 In contradiction, Tsuchiya et al.18 was unable to find any association between HPV 16 and OSCC in his study. Recently a strong correlation was showed between HPV16 positivity and OSCC in a systematic review conducted from 1996 to 2010 by Syrjänen et al.19

HPV have been detected in a variable proportion of HNSCC from 10% to 100%. This variation in the prevalence of HPV detection rate could be due to differences in anatomic locations of tumors or the techniques used in detecting HPV-DNA.20 In a Meta-analysis during (1980-1998)21 it was observed that the likelihood of detecting HPV in normal oral mucosa was 10.0% which was significantly less than that of OSCC which is 46.5%. In another meta-analysis during (1988–2007)22 investigating HPV infection in OSCC and head and neck squamous cell carcinoma, the prevalence of HPV DNA in OSCC was 38.1%. Saghravanian et al. found no significant differences between HPV positive and negative groups in relation to the variables like age, gender, tumors site, tumor size, tumor grading and also the recurrence rate.23 In gender wise distribution of HPV positive OSCC cases, Werness et al.24 found statistical correlation with male predominance. Priya Koppi et al.25 also found male predominance in their study. But in another study by Agrawal et al there was no correlation with gender.26 Study by Agrawal et al.27 showed no significant correlation with sitewise distribution of OSCC cases amongst HPV16 positive cases which is in accordance with the findings of Cruz et al.28 Study by Dhanapal et al.29 showed that HPV associated oral cancer cases were predominantly from buccal mucosa.

The relation of habit and demographics in HPV induced oral carcinogenesis is controversial. Some authors state that the presence of HPV has no relation to the habits, site of the tumor, age and gender and thus HPV can be considered as an independent factors in the pathogenesis of cancer. While other studies show tobacco have an additive effect and alcohol consumption, a synergistic effect with HPV positive cancers.29 In addition, patients with HPV-associated OSCC are often nonsmokers and nondrinkers and on average 5 years younger.30 D’Souza et al. found no correlation between HPV positive OSCC and tobacco or alcohol consumption, but a strong association was found between sexual behavior and risk of HPV infection.31 Even though patients with HPV in OPSCC are not chronic smokers, smoking while exposed to HPV can lead to the retention of HPV in the oropharynx (because of its local immune- suppressing effects) and develop HPV related oropharyngeal cancer later in life.32

HPV 16 association in OSCC with clinical staging was studied by several investigators. Mellin et al.28 found significant correlation between TNM staging and HPV positivity. Agrawal et al.30 in his study on the association of HPV 16 with TNM staging found positivity of about 51.15% in Stage I, 28.5% in Stage II, and 14.28% in Stage III. Saghravanian et al.31 in his study concluded that stage and lymph nodal stage was significantly higher in the HPV positive group compared to the HPV negative group. HPV-associated OSCC tend to be poorly differentiated, often basoloid in histology, and frequently present at an advanced stage.26 Agrawal et al in his study on HPV 16 positivity and degree of differentiation observed that 71.44% belonged to well differentiation, 14.28% to moderate differentiation and 14.28% to poorly differentiation.23 Study by Sharma et al showed that moderately differentiated squamous cell carcinoma was most prevalent (81%) followed by well differentiated (16.7%) and poorly differentiated squamous cell carcinoma.5 Dhanapal et al could not find any association between the presence of HPV and the grade of the tumor.27

Review of literature showed that, the presence of HPV in head and neck squamous cell carcinoma constitutes a positive prognostic marker of disease. The favorable outcome of HPV-induced oropharyngeal cancers might be attributable to the absence of field cancerization or enhanced radiation sensitivity.20
But in a study by Saghravanian, the disease free survival and overall survival rates were lower for the HPV positive group when compared to the HPV negative group.23 Despite improvements over the last decade, five year survival rates for head and neck squamous cell carcinoma still remains at 50%. Detection of HPV in head and neck squamous cell carcinoma have therapeutic implications in treatment or targeted intervention. In addition, the presence of HPV in these tumors represents a public health issue, underscoring the need for comprehensive HPV vaccination programs.

**CONCLUSION**

The molecular pathway used by human papilloma virus to trigger malignant transformation of tissue is different from that of other well known risk factors, i.e. smoking and alcohol, associated with squamous cell carcinoma. Thus it is important to distinguish HPV-associated HNSCC from tobacco/alcohol-associated HNSCC. Molecular classification of tumors provides important new information that will allow a better estimate of prognosis and may well influence treatment decisions and also can be used for targeted therapy. In the future, antiviral pharmaceutical approaches and therapeutic vaccination may allow effective, nontoxic therapy. The clinico-pathological correlation and prognosis of HPV associated OSCC is still under controversy. More studies involving different population is required to know the exact mechanism of tumors which could finally help in modifying the treatment plan.

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