The Role of Human Papilloma virus in Oral Lesions: A Review

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Abstract

Human Papilloma Virus (HPV) is members of papilloma virus genus of the family papoviridae. They selectively infect the epithelium of the skin & mucous membrane. They are of two types high risk and low risk, depending on that they infect skin, mucosa, and genitalis. These infections may produce warts or be associated with a variety of benign & malignant neoplasia. HPV can also act as causative oncogenic agent for inducing carcinoma in non smokers. There are different methods of detection of HPV infection which are histology, cytology, PCR, In-situ hybridization.

Key Words: HPV, Oral papillary lesion, oral carcinoma

Introduction

Papillomaviruses are small, non-enveloped, epitheliotropic, double-stranded DNA viruses that infect mucosal and cutaneous epithelia in a wide variety of higher vertebrates in a species-specific manner and induce cellular proliferation. Only bovine papillomaviruses (BPVs) 1 and 2 are known to infect mesenchymal tissues and to show cross species transmission. More than 100 types of human papillomaviruses (HPVs) have been identified. Many types of HPV have been found in oropharyngeal cancers, which gives rise to the nomenclature of high and low-risk HPVs. A number of HPVs have been found to be present in skin cancers in patients who have epidermodysplasia verruciformis (EV); these types are also found in both non-melanoma skin cancers and normal skin. All papilloma viruses
share a common genetic structure that is distinct from that of polyoma viruses. A double-stranded circular DNA genome encodes approximately eight open-reading frames (ORFs). Similarly, all papilloma viruses have a non-enveloped icosahedral capsid. The advent of molecular cloning of HPV genomes in the early 1980s provided the first opportunity to study individual viral genes. However, only in the late 1990s did propagation of viruses in organotypic cultures make the first attempts at viral genomics possible. The availability of complete and partial genomic sequences from a wide variety of HPV types has enabled the establishment of a new taxonomic structure and has provided a window to study the co-evolution of papilloma viruses with their primate hosts. Early evidence suggests that HPV types, as defined by DNA sequencing, also remain serologically distinct. The oral lesions caused by HPV vary from verruca vulgaris to molluscum contagiosum & the subtypes of HPV associated with benign lesions are 1, 2, 4, 6, 7, 11, and 13. Based on their oncogenic potential, HPV types have been divided into high risk (HR-HPV) and low risk (LR-HPV) groups. High risk HPVs are associated with an elevated risk of development of cancer and are often referred to as ‘cancer associated’ or ‘oncogenic’ types. Examples of high risk HPVs include: HPV 16, 18, 31, 33, 35, 45, 51, 52, 56, 58, 59, 68, 73 and 82. HPV 16 and 18 types in particular are responsible for the development of cervical carcinomas. Some high risk HPVs are also associated with anogenital and oropharyngeal cancers. Low risks HPVs on the other hand are associated with benign wart-like epithelial lesions. These HPV types include HPV 6, 11, 42, 43 and 44. The HPV associated cancer with oral lesions are detected by serological tests (PCR & ELISA), neutralization assay. Certain vaccines are used for the prevention of HPV infection. The purpose of this review is to discuss/highlight the molecular, clinical and histopathological aspects of oral lesions caused by HPV. Dental practitioners have major role in identifying the oral lesions caused by HPV.

**Structure of the HPV**

**Viral components and physical properties**

Papillomaviruses are small, non-enveloped, icosahedral DNA viruses that have a diameter of 52–55 nm. The viral particles consist of a single double-stranded DNA molecule of about 8000 base-pairs (bp) that is bound to cellular histones and contained in a protein capsid composed of 72 pentameric capsomers. The capsid contains two structural proteins — late L1 (55 kDa in size; 80% of total viral protein) and L2 (70 kDa) — which are both virally encoded. Virus-like particles (VLPs) can be produced by the expression of L1, alone or in combination with L2, in mammalian or non-mammalian expression systems.

**Viral genome, Function of Viral Protein**

The genomes of all HPV types contain approximately eight ORFs that are all transcribed from a single DNA strand. The ORF can be divided into three functional parts: the early (E) region that encodes proteins (E1–E7) necessary for viral replication; the late (L) region that encodes the structural proteins (L1–L2) that are
required for virion assembly; and a largely non-coding part that is referred to as the long control region (LCR), which contains cis elements that are necessary for the replication and transcription of viral DNA. The viral E proteins are transcribed from the early promoter (e.g. P97 in HPV 31) where as the L proteins are transcribed principally from the late promoter (P742 in HPV 31). The E1 and E2 proteins of HPV act as factors that recognize the origin of replication. E2 protein is also the main regulator of viral gene transcription. E4, despite its name, is believed to be involved in the late stages of the life cycle of the virus E5 protein interacts with the receptors of growth factors and stimulates cellular proliferation and inhibits apoptosis and E5 may function during both early and late phases. The E6 and E7 proteins target a number of negative regulators of the cell cycle, primarily p105 Rb and p53, respectively. During the viral life cycle, E6 and E7 facilitate stable maintenance of viral episomes and stimulate differentiating cells to re-enter the S phase. E6 induces DNA synthesis, prevents cell differentiation, interacts with tumour suppressor proteins and repair factors; and E7 induces cell proliferation and interacts with negative regulators of cell cycle and tumour suppressor proteins and E6 and E7 act as oncogenes which are causally associated with carcinogenesis. L1 is a major capsid protein which interacts with cell receptors whereas L2 is a minor capsid protein which interacts with DNA. It facilitates virion assembly. The L1 and L2 proteins assemble in capsomers, which form icosahedral capsid around the viral genome during the generation of progeny virions (Figure-2).^4

**Transmission of HPV Infection**

The common mode of transmission and acquisition of HPV is by horizontal transmission consequent to sexual activity. Occasionally, HPV may be transmitted through modes other than sexual activity. These routes include vertical transmission (mother to child), fomites and skin contact. Multiple pathways for HPV transmission to the oral cavity can exist. These include sexual transmission, autoinfection and rarely through perinatal transmission of the neonate during its passage through an infected birth canal of the mother. Oral sex is a well recognized mode of transmission of HPV to the oral cavity. ^5,6

**Viral entry**

Papillomaviruses gain access to keratinocyte stem cells through small wounds, known as micro traumas, in the skin or mucosal surface. Interactions between L1 and sulphated sugars on the cell surface promote initial attachment of the virus. The virus is then able to get inside from the cell surface via interaction with a specific receptor, likely via the alpha-6 beta-4 integrin, and transported to membrane-enclosed vesicles called endosomes. The capsid protein L2 disrupts the membrane of the endosome, allowing the viral genome to escape and traffic, along with L2, to the cell nucleus.
The virus and pathogenesis of the disease

The viral life cycle is linked to the differentiation of the infected epithelial cell. The life cycle is thought to be initiated by the infection of basal epithelial cells, presumably at sites of injury. Basal cells comprise the proliferating cellular component of stratified epithelia, in which the viral genome is established when a low copy number, nuclear plasmid and early genes are expressed preferentially although at low levels (Fig-3). The ability of HPVs to establish their genome in basal cells relies upon the $E1$, $E2$ ,$E6$ and in some cases $E7$ genes. Normally, when basal cells undergo cell division, the daughter cell that loses contact with the basement membrane and migrates into the supra basal compartment withdraws from the cell cycle and initiates a programme of terminal differentiation. However, in HPV-positive human keratinocytes and epithelial cells, the supra basal cells fail to withdraw from the cell cycle and continue to support DNA synthesis and express markers for cell proliferation (Fig- 4). HPV 16 $E7$ has been shown to be necessary and sufficient to induce supra basal DNA synthesis. In addition, the E5 onco protein contributes quantitatively to this property both in HPV 16 and HPV18.

HPV & oral neoplasm

Most HPVs induce benign hyperplasia or neoplasia of the epithelium. The genotypes that induce malignant transformation of keratinocytes with genesis of squamous cell carcinomas are referred to as mucosatropic oncogenic HPVs. Where as oncogenes may be the consequence of oncogene activation by transactivation, it has been demonstrated that E6 binds to the p53 tumor-suppressor protein and activates its degradation by the ubiquitin pathways. E7 binds the Rb tumor suppressor, releasing E2F, a transcription factor that activates cell cycling. Basic cell biology studies have shown, however, that E6 and E7 sequences transfected into human keratinocytes induces transformation into papillomas (HPV 6, 11) or carcinomas (HPV 16, 18). E6 and E7 proteins play an important role in increased cell proliferation and extended cell survival in HPV associated malignancies by altering the cell cycle regulatory factors. When integration of the viral genome into the host cell genome takes place, key functions of tumour suppressor genes such as p53 and pRb are rendered useless. This leads to abnormalities in apoptosis, DNA repair mechanisms, cell cycle regulation and finally to cellular immortalization, thus inducing and maintaining a malignant cell phenotype (Fig -5).

The footprints of the virus are detected by the sensitive method of polymerase chain reaction (PCR), and RNA transcripts can be detected by reverse transcriptase PCR. Only rarely do immunohistochemical markers for HPV capsid antigens stain positive in papillomas that have been shown to contain DNA or RNA. HPV 2, 4, 6, and 11 are associated with and cause the benign warts of oral mucosa and vermilion epithelia; the role of HPV 16, 18 and other oncogenic genotypes in the pathogenesis of oral carcinoma is equivocal.
To establish a wart or papilloma, the virus must infect a basal epithelial cell. Knowledge of the initial steps in the replication cycle such as attachment (1), uptake (2), endocytosis (3) and transport to the nucleus and uncoating of the viral DNA (4) is limited. E-region transcription (5), translation of the E proteins (6) and steadystate viral DNA replication (7) all occur in the basal cell and in the infected suprabasal epithelial cell. Events in the viral life cycle that lead to the production of virion particles occur in the differentiated keratinocyte: vegetative viral DNA replication (8), transcription of the L region (9), production of the capsid proteins L1 and L2 (10), assembly of the virion particles (11), nuclear breakdown (12) and release of virus (13).
Human Papilloma virus and Oral Papillary or Verrucous Lesions

<table>
<thead>
<tr>
<th>Lesion</th>
<th>Associated HPV Genotypes</th>
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<tbody>
<tr>
<td>1) Verruca vulgaris</td>
<td>2, 4, 40</td>
</tr>
<tr>
<td>2) Squamous papilloma</td>
<td>6, 11</td>
</tr>
<tr>
<td>3) Condyloma acuminatum</td>
<td>6, 11</td>
</tr>
<tr>
<td>4) Focal epithelial hyperplasia</td>
<td>13, 32</td>
</tr>
<tr>
<td>5) Squamous cell carcinoma</td>
<td>16, 18, 31, 33, 35</td>
</tr>
<tr>
<td>6) Proliferative verrucous leukoplakia</td>
<td>6, 11, 16</td>
</tr>
<tr>
<td>7) Verrucous carcinoma</td>
<td>2, 6, 11, 16,18</td>
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The specific types of solitary papillary lesions that occur in the oral cavity include the common squamous papilloma, verruca vulgaris, Condyloma acuminatum, and keratoacanthoma. Condylomas may also occur in crops of multiple papillomas, either clustered or widely separated. Proliferative verrucous leukoplakia (PVL), verrucous carcinoma, and papillary exophytic squamous cell carcinoma are diffuse sessile lesions.

**Squamous papilloma**

The squamous papilloma is the most common benign epithelial neoplasm of oral epithelium. The HPV type 6 and 11 are associated with squamous papilloma. It may occur anywhere in the mouth with a predilection for the ventral tongue and fraenum area, palate, and mucosal surface of the lips. Nonkeratinized lesions appear coral pink; if keratinized, they are white. Some have a cauliflower surface whereas others have discrete finger-like projections. They may be pedunculated or sessile in configuration. Papillomas are typically single, yet occasionally, more than one may occur. They occur at any age and are frequently seen in children and adolescents. There is no clearly defined mode of transmission, most occurring spontaneously. The microscopic appearance of the papilloma is characteristic of many long, thin, finger like projections extending above the surface of the mucosa. Each finger like projection is made up of stratified squamous epithelium and contains thin connective tissue core. The important feature of the lesion is proliferation of the spinous cells in a papillary pattern. Koilocytes may be found in the superficial layer of the epithelium.9

**Verruca vulgaris**

The common skin wart may be seen on the vermilion border, or less often, in the oral cavity. This type of oral wart is the least common and may occur at any age, being more frequent in children and adolescents. In the mouth, verruca vulgaris has a tendency to arise most frequently on the keratinized surfaces of the gingiva and palate. Verrucae are sessile, oval, and white, owing to the thickened keratin layer on the surface. In children with warts on their fingers, autoinoculation may occur to the lips in those with a thumb-sucking habit. Rarely, there may be more than one lesion present.
Condyloma acuminatum
Sexually transmitted warts tend to occur in multiples yet may be single lesions. They are usually broad-based and sessile. They occur in both sexes but are more common in homosexual males. Although oral Condylomas can occur on any mucosal surface, they are more commonly found on the lips, commissural region, and gingival. Condyloma acuminatum is predominantly seen on the skin and mucosal surfaces of the anogenital tract. Oral Condyloma acuminatum lesions occur as a result of oral sex or from autoinoculation of the virus in adults. When multiple, they tend to arrange themselves into regional clusters, although some cases are widely distributed throughout the oral mucosa, particularly in human immunodeficiency virus (HIV)-infected patients. Most are coral pink in colour, have a warty appearance, but are slightly keratinized. These lesions of the oral mucosa are often transmitted by oral, genital, and anal sex. Multiple papillary lesions in a child should arouse suspicion of sexual abuse, warranting investigation of persons in contact with that child.

Focal Epithelial Hyperplasia
It is one of the most contagious of the oral papillary lesion. It is induced by HPV -13 and 32. It occurs more commonly in children and young adults. It involves labial, buccal, and lingual mucosa. It appears clinically as papule and plaque, which is similar to the colour of the oral mucosa. It gives rise to cobblestone or fissured appearance clinically. Microscopically there is presence of thickened mucosa extending upwards not down into the underlying connective tissue, hence the lesional rete ridges are widened sometimes club shaped. The superficial keratinocytes show a Koilocytes change, while others demonstrate collapsed nucleus which resembles a mitotic figure (mitosoid cell).

Oral Leukoplakia
The term leukoplakia essentially means a white patch. Not all oral white patches are potentially malignant. A mucosal white patch (leukoplakia) that histologically exhibits a varying degree of epithelial dysplasia can be considered a potentially malignant lesion; 3–6% of ‘dysplastic’ white patches show malignant transformation. Oral leukoplakia carrying malignant potential can present a varied clinical appearance. It is seen as a well demarcated white/grey keratotic patch which may appear flat, smooth, fissured, granular or nodular in appearance. Sometimes a red and white mixed plaque (called erythro leukoplakia or speckled leukoplakia) may be seen. Known aetiological factors of oral leukoplakia include long-term use of tobacco and/or alcohol, chronic friction, electro-galvanic reaction caused by two dissimilar metallic restorative materials and ultraviolet radiation from chronic sun exposure. HPV type 16 and 18 have been identified in Leukoplakia lesions by several investigators.

Oral Lichen Planus
Lichen planus (LP) is a chronic mucocutaneous disorder which is immunologically mediated. Often it involves only the oral mucosa with white hyperkeratotic or red erosive lesion patterns. Oral
lichen planus (OLP), particularly of the erosive variant has been considered as a potentially malignant disorder with less than 2% of OLP lesions showing malignant transformation over a period of 10 years. Although considered to be an immunologically derived disorder, many aspects of its aetiopathogenesis are not yet clear. Among other factors, viral aetiology of LP has been proposed in recent years. Ostwald et al. reported the presence of HPV DNA in 15.4% of OLP lesions.

**Squamous cell Carcinoma**

Head and neck cancers include cancers of the oral cavity, oropharynx, hypo pharynx, larynx, Sino nasal tract and nasopharynx. Collectively, they are the sixth most common types of cancer worldwide. More than 90% of these cancers are squamous cell carcinomas of the mucous membranes of the mouth and oropharynx. Other than tobacco and alcohol as a risk factor HPV is also found as one of the risk factor for causing squamous cell carcinoma. Oral infection with high risk HPV has been found to be associated with a significant increased risk of developing oropharyngeal cancer when adjusted for tobacco use and alcohol consumption. In a review of head and neck carcinoma samples, HPV was detected by PCR method in 34.5%; by in situ hybridization method in 15.8%; and by Southern blot method in 24.5% of cases. In most HPV-related oral cancers, HPV oncogenes act synergistically with chemical carcinogens in alcohol, tobacco and betel quid resulting in malignant transformation of oral keratinocytes.

On the other hand, a small number of HPV-related oral cancers may result from HPV E6 and E7 activity in the absence of chemical carcinogens. In vitro studies on squamous cell carcinoma cell lines showed HPV 16 DNA sequences suggesting that HPV may have a ‘hit and run’ function in oral carcinogenesis. HPV has been identified aetiologically with cancers of the tonsils and base of the tongue. It has also been observed that HPV-associated base of tongue-tonsillar squamous cell carcinomas are poorly differentiated, HPV 16 positive are radiosensitive and have a better prognosis.

**Proliferative verrucous leukoplakia (PVL)**

Proliferative verrucous leukoplakia (PVL) is a distinct form of oral leukoplakia. Gingiva and alveolar ridges are the favoured sites of PVL. It is a slow growing hyperkeratotic lesion that tends to spread and become multifocal, and develops as a wart-like lesion over time. PVL has a higher rate of malignant transformation. PVL is of unknown aetiology. An association with HPV has been suggested by some investigators. Available literature reveals a 0-89% range for the association of HPV with PVL.

**Verrucous Carcinoma**

Oral verrucous carcinoma (OVC), also known as Ackerman’s tumour, is a variant of oral squamous cell carcinoma. OVC presents as an exophytic, soft, fungating, painless, slow growing and locally aggressive tumour. A strong aetiologica association with tobacco and alcohol has been reported for the development of OVC. HPV DNA
types 6, 11, 16 and 18 have also been shown to be associated with OVC by some investigators.  

**Keratoacanthoma**
Lesions which resemble squamous cell carcinoma clinically and pathologically is a low grade malignancy that originates in pilosebaceous gland. HPV types 9,11,13,16,18,24,25,33,37,57 are one of the causative factors. Clinically it appears as solitary, firm, round papule which rapidly progresses to dome shaped nodule with central crateriform ulceration with keratin plug which may project like a horn. Histologically the lesion appears as hyperplastic squamous epithelium growing into the underlying connective tissue. The surface is covered by thickened layer of Parakeratin or orthokeratin with central plugging.  

**Detection of HPV in Oral lesions and treatment**
The early detection of any oral lesion that shows clinical characteristics of malignancy or carries malignant potential is important. Detection of such lesions can be carried out by combining HPV typing with exfoliative cytology. As chemical carcinogens in tobacco and alcohol appear to enhance HPV transforming activity, patients with positive oral cytology should be strongly advised to reduce or discontinue their use. Patient education on risk factors for oral cancer and OPMDs, which among other things include oral HPV transmission, should also be a part of an oral cancer preventive strategy. HPV vaccination programmes all over the world have been targeted primarily at females, but studies reveal that the vaccines also elicit a strong humoral immune response in males. Commercial vaccines currently available are quadrivalent Gardasil® (Merk & Co, USA) and bivalent Cervarix® (GlaxoSmithKine Group of Companies, Australia). These vaccines prevent infection with HPV types 16, 18, 6 and 11, and are primarily designed for the prevention of cervical cancer and genital warts. They do not cure HPV infection or cervical cancer. Vaccines are recommended for females aged 9–25 who have not been exposed to HPV. It is possible that currently available HPV vaccines designed to prevent cervical cancers and genital warts will also contribute to the reduction in the incidence of HPV related oral cancers. Therapeutic HPV vaccines which are being developed for cervical cancer may also be of benefit in the management of HPV related oral cancer.  

**Conclusion**
This paper briefly reviews the molecular, clinical, histopathological aspects of oral lesions caused by HPV.  

**References**


