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Review Article

Association between Human Papilloma Virus and Oral Squamous Cell Carcinoma: A Systematic Review

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***ABSTRACT*:**
Human Papilloma Virus (HPV) is an epitheliotropic virus with an affinity for keratinocytes and they are primarily found in the anogenital tract, urethra, skin, larynx and oral mucosa. Recent epidemiological evidence suggests that HPV may also be an independent risk factor for oropharyngeal cancer. The common mode of transmission of HPV is consequent to sexual activity, and or via mother to child, fomites and through skin contact. It can even act as primary oncogenic agent for inducing carcinogenesis in nonsmokers. HPV causes epithelial proliferation characterized by epithelial thickening, keratohyaline granules, acanthosis along with nuclear atypia,hyperchromasia, and double nucleation of superficial and intermediate cells being hallmark of productive HPV infection. The aim of this review is to highlight the genomic structure and possible mechanism of infection and carcinogenesis of HPV in oral mucosa. Emphasis has been laid to review the frequency of HPV prevalence in oral squamous cell carcinoma (OSCC).
Key words: Human papilloma virus, squamous cell carcinoma, carcinogenesis.

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Introduction:

Human papilloma virus (HPV) has gained much interest recently, because it is accepted as important correlates of cervical cancer. HPV has a wide disease spectrum affecting the cutaneous and mucosal areas of the body including anogenital tract, urethra, skin, larynx, tracheobronchial mucosa, nasal cavity, para- nasal sinus and oral cavity.[1]Studies have shown that HPV is associated with high risk of developing oro-pharngyeal and potentially malignant disorders. Oncogenic HPV are associated with oral malignancies, but its prevalence varies widely in differentstudies. Squamous cell carcinoma (SCC) is the most frequent oral cavity malignancy accounting for over 90%of oral cancers.[2] It is defined as "a malignant epithelial neoplasm exhibiting squamous cell differentiation as characterized by formation of keratin and/or presence of intercellular bridges".[3] 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.[4] In this paper biological aspects of HPV and their role in development of OSCC is briefly reviewed for the purpose of updating oral health professionals and raising the emerging relationships being established between HPV and some oral cancer. Alongwith, this article presents an update of HPV and its association with OSCC laying emphasis on its structure, pathogenesis, oncogenic potential, prevalence, and diagnostic methods.

Human Papilloma Virus:

It belongs to *Papillomavaviridiae* family of viruses which are capable of infecting mucosal and cutaneous epithelia in species specific manner and inducing cellular proliferations.HPVs are small non-enveloped DNA viruses with a diameter of 52-55nm. HPV genome contains a double stranded DNA molecule that is bound to cellular histones contained in a protein capsid. HPV DNA encodes approximately 8 open reading frames (ORFs).The ORF is divided into 3 functional parts: the early (E) region, the late (L) region and a long control region (LCR). The E region is necessary for replication, cellulartransformation and for control of viral transcription while

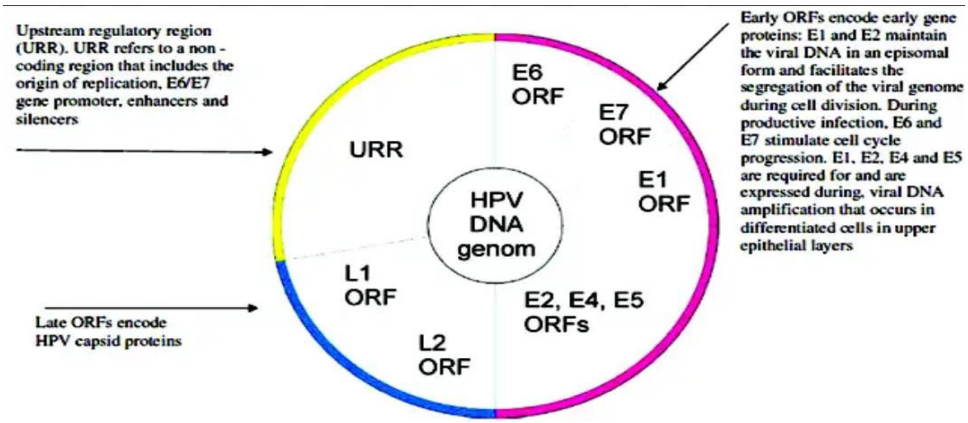

Figure 1: The circular organization of HPV DNA episome; depicting its morphogenic and functional abilities.[5]

the L region encodes the structural proteins (L1-L2) that take part in virion assembly. LCR helps in DNA transcription and replication (**Figure 1**).

The early region of ORF encodes 7 proteins: E1, E2, E3, E4, E5, E6 and E7.E1 is necessary for viral DNA replication. E2 has a role in viral gene transcription and replication. The function of E3 is still not understood. E4 protein interacts with keratin cytoskeleton and intermediate filaments and also facilitates the viral assembly in release. E5 protein interacts with the receptors of growth factors and stimulates cellular proliferation and inhibits apoptosis.E6 induces DNA synthesis, prevents cell differentiation, interacts with tumor suppressor proteins and repair factors; and E7 induces cell proliferation and interacts with negative regulator of cell cycle and tumor suppressor proteins. E6 and E7 proteins 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 and facilitates virion assembly (**Figure 1**).Based on their oncogenic potential HPV is divided into high risk (HR-HPV) like 16, 18, 31, 33, 35, 45, 51, 52, 56, 58, 59, 68, 73and 82 and low risk HPV like 6, 11, 42, 43, 44.[6]In cases of high risk HPV infection and under favorable conditions the viral genome is integrated into the host genome, which is a necessary event for keratinocyte immortality. During the process of integration, the circular form of viral genome breaks at the level of E1 and E2 regions. Loss of E2 during this process of integration produces the loss of E6 and E7 control. Therefore the sequences of E6 and E7 are directly involved in the cellular cycle by inhibiting the normal functions of p53 and pRb, respectively; which are proteins involved in regulation of cell cycle. In case of HPV infection, E6 suppresses the properties of p53 gene product, achieving the functional equivalent of the two hits required to knock out both alleles of a tumor suppressor gene.[7]Interactions of E7 with pRb causes the release of transcription factor E2F which is now free to act and can stimulate the cellular division. E7 is also able to bind and inactivate the protein kinase inhibitors p21 and p27, which further causes uncontrolled mitotic divisions in the cell cycle.

Prevalence:

Miller and Johnston[8] in a meta-analysis of OSCC observed that HPV may be a significant and independent risk factor. The prevalence of HPV in OSCC varies depending on several parameters such as geographic differences in population, type of specimen, selection of preparation method and use of HPV detection method. In gender wise distribution of HPV positive OSCC cases, Werness et al[9] found statistical correlation with male predominance. There was no significant correlation between site wise distribution of OSCC cases amongst HPV16 positive cases as per a study by Cruz et al.[10] D'Souza G et al[11] 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. Study by Sharma et al[12]categorised the association of HPV according to the grades of OSCC and resulted that moderately differentiated OSCC was most prevalent (81%) followed by well-differentiated OSCC (16.7%) and poorly differentiated carcinomas in association of HPV. The favorable outcome of HPV induced oropharyngeal cancers might be attributable to the absence of field cancerization or enhanced radiation sensitivity.[13]Luo et al[14]in 2007, in a sample of 51 cases of OSCC conducted a study via polymerase chain reaction(PCR) to confirm 21.5% cases of OSCC having HPV as a causativeagent. Likewise studies conducted by Ostwald et al[15], Balaram et al[16] , Elango et al[17] showed positive correlation between HPV and OSCC.

Transmission:

The common mode of transmission and acquisition of HPV is by horizontal transmission consequent to sexual activity, and vertical transmission that include- mother to child, fomites and skin contact. HPV transmission usually occurs via direct contact and through breaks in the epithelial layer and infects the basal epithelial cell layers where the virus is maintained in the nuclei of infected cells(**Figure 2**). HPV needs terminally differentiated epithelial cells, such as squamous cells for replication where the virus replicates and attains a high copy number.[18,19]

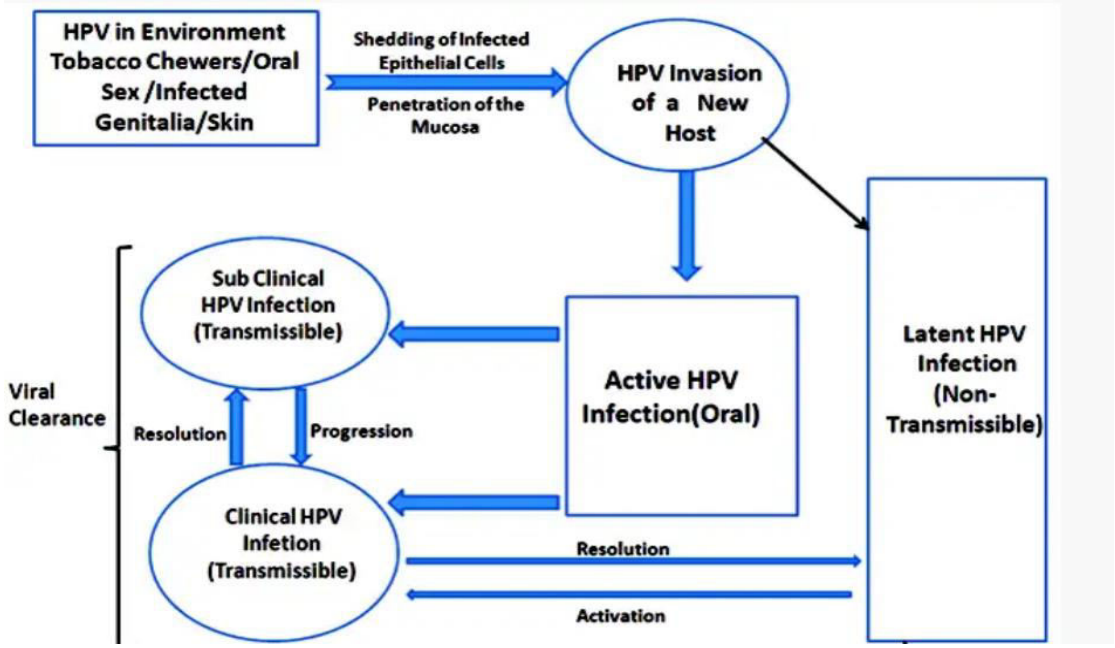

Figure-2: Flowchart depicting transmission of HPV leading to clinical and subclinical infection.

Normal Cell Cycle: The normal cell cycle consists of 5 phases: Go, G_1, S,G_2 and M. Cell grows and carries out normal metabolism; organelles duplicate in G_1phase. DNA replication and chromosome duplication occurs in S-phase.In G_2 phase cell grows and prepares for mitosis. M-phase is further divided into 5 phases: Prophase, Prometaphase, Metaphase, Anaphase, Telophase, during these phases mitosis occur. Go is a quiescent phase, where the cell has left the cycle and has stopped dividing.

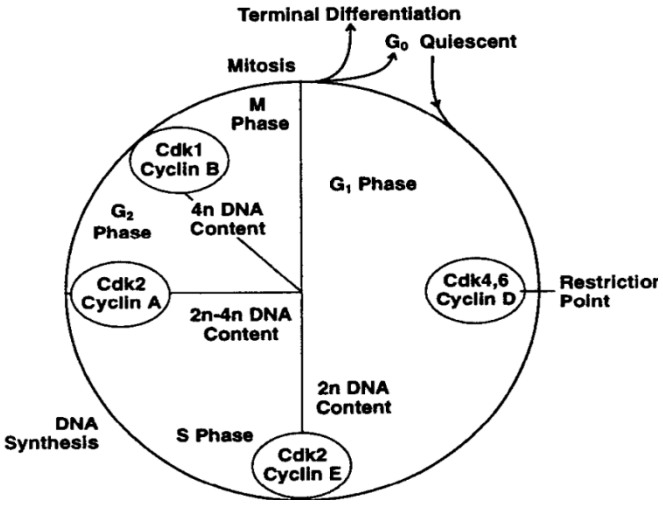

Figure 3: Schematic representation of normal cell cycle progression. After exiting from mitosis (M phase) cell can terminally differentiate, enter a quiescent state, or re enter the cell cycle. Progression through cell cycle is regulated by various cdk- cyclin complex.[21]

Cyclins and cdks play an important role in cell cycle. These are the engines that drive the cell cycle through various stages.Following an external signal (growth factors and integrins); MYC, RAS, and other genes get stimulated to activate CDK4 in presence of cyclin D to form an active complex. This activated complex then results in phosphorylation of Retinoblastoma (Rb) which binds with E2F and activates it. It ultimately results in - cyclin E / cdk2 active complex which helps in progression of the cell cycle **(Figure 3).**[20].

Oncogenic Mechanism:

p53: It is known as the "genome's guard"; its gene is located on seventeenth chromosome (17p13) and in case of DNA damage it can provoke the arrest of cellular division and assure the time necessary for DNA repair; making it a tumor suppressor gene.[22] It induces apoptosis and prevents the propagation of DNA damage in subsequent generations of cells in case where damage is irreparable. In normal cells, the p53 protein level is low. DNA damage and other stress signals may trigger the increase of p53 proteins, which have three major functions: growth arrest, DNA repair and apoptosis (cell death). The growth arrest stops the progression of cell cycle, preventing replication of damaged DNA. During the growth arrest, p53 may activate the transcription of proteins involved in DNA repair. Apoptosis is the "last resort" to avoid proliferation of cells containing abnormal DNA.[20]

Target Genes: p53 is a transcriptional activator, regulating the expression of Mdm2;the major regulator of p53, which can trigger its degradation by the ubiquitin system.

Genesinvolved in these interactions include: growth arrest (p21, Gadd45, 14-3-3s), DNA repair(p53R2) and apoptosis(Bax, Apaf-1, PUMA, NoxA).[20]**(Figure:4)**

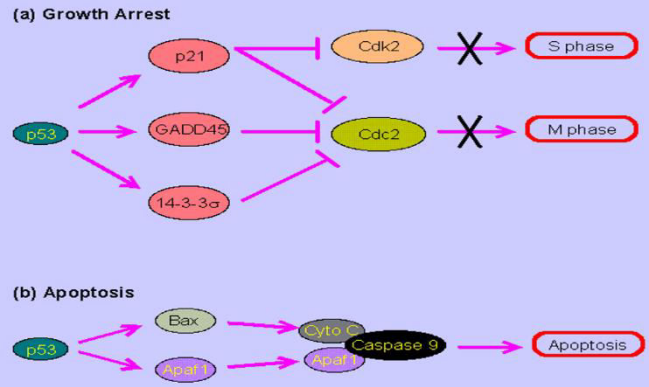

Figure 4: Role of P53 in growth arrest and apoptosis.

Rb: Retinoblastoma (Rb) is the first prototypic cancer suppressor gene to be discovered which is found to be located on chromosome 13q14. According to Knudson Two-Hit Hypothesis (1974); both the alleles of Rb locus must be inactivated (two hits) for the development of retinoblastoma.[23]

The Rb gene product is a DNA binding protein that exists in an active unphosphorylated state and an inactive phosphorylated state. It is proposed that in its active state Rb protein serves as brake on the advancement of cells from the Go/G_1 to S phase of cell cycle. When the cells are stimulated to divide; the Rb protein is inactivated by phosphorylation, the brake is released and the cell undergoes mitosis. During mitosis the dephosphorylated form of Rb is regenerated and the daughter cells enter G_1. If the Rb protein is absent or its normal functions are suppressed by mutation, the cell may continue to cycle and may progress to malignancy.[23] Various growth factors which promote the formation of cyclin D- cdk4 complex include EGF, PDGF, TGF, HGF. This complex phosphorylates Rb, changing it from an active (hypophosphorylated) to an inactive (hyperphosphorylated). Rb inactivation allows the cell to pass the G_2/S restriction point. Growth inhibitors such as TGF-B and p-53 cell cycle inhibitors prevent the Rb activation. Transforming proteins of oncogenic viruses bind hypophosphorylatedRb and cause functional inactivation.[20]The p16 protein is a cyclin dependent kinase inhibitor which regulates activity of CDK4 and CDK6. It inhibits hyper phosphorylation of pRb and prevents its dissociation from E2F transcription factor and the subsequent progression of S-phase of the cell cycle. In HPV infection p16 is up regulated by loss of the negative feedback control of pRb expression resulting in overexpression of p16 protein.[24]

HPV DETECTION METHODS:

The diagnosis of HPV infection can be made through cytological examination and biopsy. The cytological features of the HPV infection includes major and minor criteria.[4]

- **Major Criteria:** Koilocytes, perinuclear cytoplasmic halos and nuclear dysplasia.[4]
- **Minor Criteria:** Dyskeratocytes, atypical immature metaplasia, macrocytes and binucleation.[4]

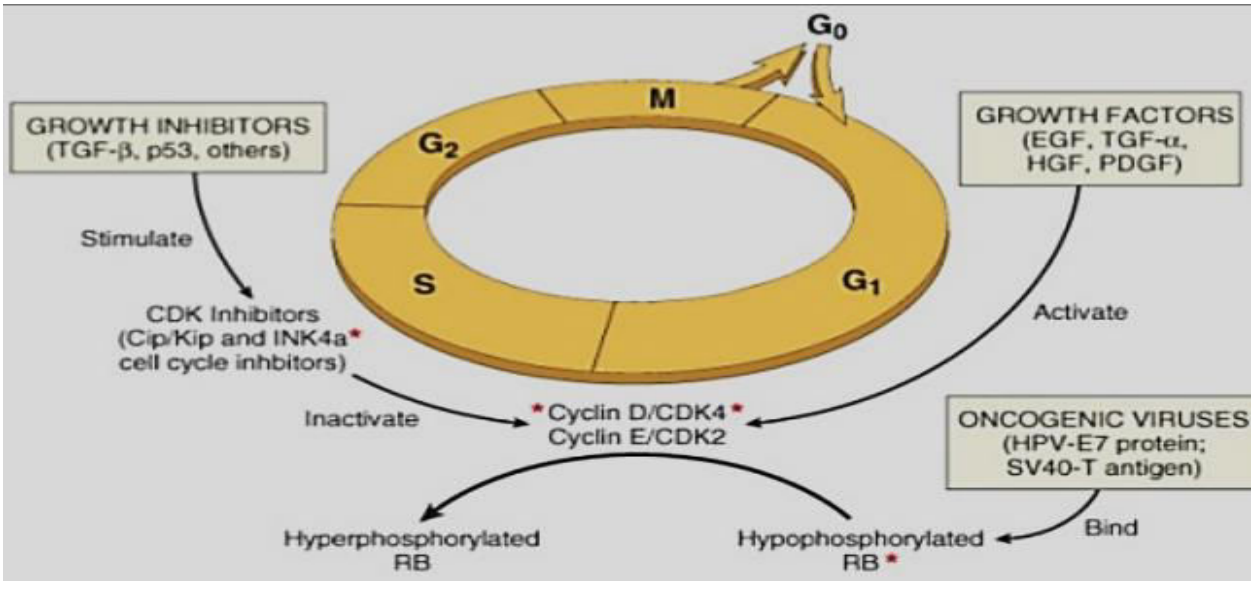

Figure 5: Role of Rb as a cell cycle regulator. [20]

Low Sensitivity Methods: Immunohistochemistry and In-situ hybridization (ISH) allows viral detection when present in more than 10 copies of viral DNA per cell.[4]
Moderate Sensitivity Methods: Southern Blot, dot blot, and reverse dot hybridization.
HighSensitivityMethods: PCR, because it detects virus in less than 1 copy of viral DNA per cell.[25]
Light microscopy: Papillomaviruses cause epithelial proliferation characterized by epithelial thickening, keratohyaline granules, acanthosis, and sometimes hyperkeratosis.[26] Koilocytes indicate the presence of productive HPV infection in exfoliated cells and biopsy specimen. The cells exhibits perinuclear clearing and increased density of surrounded cytoplasm. Nuclear atypia, hyperchromasia, and double nucleation of superficial and intermediate cells are hallmark of productive HPV infection.[27]

Electron microscopy: Viral particles can be demonstrated in HPV infections but HPV typing cannot be done. Virions were identified in the infected cells suggestive of HPV infection. But it's a laborious, time consuming and limited to productive infections only.[1]

Molecular methods: This technique is further divided into those that are not amplified (in situ hybridization,dot blot, and southern transfer hybridization) and those that utilize amplification (target amplification, signal amplification, probe amplification).[28]

- **Non- Amplified techniques:** In situ hybridization: It can be done directly on biopsies which allow localization of target sequences and correlation with clinical appearance and histopathology. The presence of HPV induced histologically equal focal lesions in biopsies can be confirmed by in situ hybridization.Viral transcription and integration can be studied with this technique in fixed tissue.[27,29]
- **Southern blotting and Dot blot hybridization:** This technique is used to classify newly identified viral types.[30] This process is entirely laboratory based and uses existing reagents and well established methodologies. Hence this process requires access to appropriate reagents and personnel skilled in advanced lab techniques. The HPV genome extracted is broken using enzymes. Then the product is subjected to Gel electrophoresis.
- **Dot Blot Hybridization:** It employs simpler laboratory methods as compared to southern blotting but is rarely used due to low sensitivity. does not include electrophoresis.[30]
- **Target Amplification:** PCR is the most commonly used tool in in-vitro method for primer corrected enzymatic amplification of specific DNA or RNA fragments with remarkable efficiency. It involves repeated cycles of heat denaturation of DNA, annealing of primers to their complementary sequences, and extension of annealed primers with DNA polymerase.[31] The strict laboratory procedures and controls are critical in producing contamination related false -positive findings.

Prevention:
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. Patients with positive oral cytology should be strongly advised to reduce or discontinue the use of tobacco and alcohol.[6] Patient education on risk factors for oral cancer including oral HPV transmission, should also be a part of oral cancer preventive strategy.HPV vaccine programs have been initiated all over the world targeting HPV types 16, 18, 6, 11. These vaccines are primarily designed for prevention of cervical cancer and genital warts but will also contribute to the reduction in the incidence of HPV related oral cancers.[6]

Conclusion:
It is evident that knowledge of relationship between HPV family of viruses and oral conditions is expanding amongst which the emerging evidence of causal relationship between HPV infection and OSCC is of particular significance. It is specially considered in younger patients with lesions on base of tongue and tonsillar region and where there is not history of exposure to the usual risk factors such as tobacco smoking and alcohol. .In future, the antiviral pharmaceutical approaches and therapeutic vaccination may allow effective, nontoxic therapy. More studies involving different population is required to know the exact mechanism of OSCC which could finally help in modifying the treatment plan.Still further research is needed in order to standardize a particular protocol for screening of patients with OSCC.

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