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Non-Smoker's Cancer- Human Papilloma Virus (HPV) Induced Head & Neck Squamous Cell Carcinoma (HNSCC)

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Abstract: Head and neck squamous cell carcinoma is recognized as 6th most common cancer worldwide. Etiological factors are generally cigarettes, alcohol consumption also tobacco products such as Ghutka, pan masala and betel quid also triggers the development of OSCC. Among several causes of cervical malignancies which the second most carcinoma is the world, infection with some types of human papilloma virus (HPV) is thought to be the greatest cervical cancer risk factor. Over 150 subtypes of HPV have been identified; from which more than 40 types of HPVs are typically transmitted through sexual contact and infect the anogenital region and oral cavity. HPV is suspected as the principle causative factor for oral malignancy in non-smoker and non-alcoholic patients. It was also reported to be associated with Head and neck squamous cell carcinoma in 1995. The recently introduced vaccine for HPV infection is effective against certain subtypes of HPV which are associated with cervical cancer, genital warts, and some less common cancers, including oropharyngeal cancer. The value of HPV vaccination for oral cancer prevention is still controversial and hypothetical; some evidence also supports the possibility that HPV vaccination may be preventive factor in reducing the incidence of oral cancer.

Keywords: Human Papilloma Virus, Oral Cancer, Oral Squamous Cell Carcinoma, Polymerase Chain Reaction, Virus-like particle, HPV vaccine

1. INTRODUCTION

Papilloma viruses (from the Latin "papilla" meaning pustule and from the Greek suffix "Oma") are classified in the Papillomaviridae family, officially recognized by the International Committee on Taxonomy of Viruses (ICTV). The human papilloma virus (HPV) is a small-sized DNA virus (diameter 50-55 nm) without envelope, which is resistant to heat, acids, and ether. Papilloma viruses have a high tropism for stratified squamous epithelial cells and replicate only in differentiating epithelial cells of the skin and mucous. [1-3] Oral cancer is classified as head and neck cancer, and head and neck squamous cell carcinoma (HNSCC). Oral squamous cell carcinoma exhibits more predilections towards males with the ratio of 1.7:1, which is the 10th most frequent cancer in males. Risk factors of "oral squamous cell carcinoma" are described as cigarettes, Ghutka, chewing of betel nut, alcoholism, viruses, age related or chronic irritation [4-5]. HPV seems to have a definite impact on prognosis of OSCC, so it is very crucial to probe the procedure of HPV related HN-SCC.

International Agency for Cancer Research found substantial proof for HPV-16, 18 playing a etiological role in pathophysiology of oral and pharyngeal carcinoma and a smaller group of oral neoplasms [6].

The evidence of HPV involvement was first given by Syrjanen in 1983 while they discovered koilocytotic atypia in neoplastic oral lesion. Evidences for involvement as:

1) HPV's well-documented wide epithelial tropism.

2) The oropharyngeal and vaginal epithelium exhibits morphological similarities.

3) In vitro immortalization of human oral keratinocytes.

4) The evidence of high-risk HPV in the cervical-SCC is well-established.

5) Recognizing HR-HPV genotypes in oral squamous cell cancer samples. [7]

Among 50 variations "HPV-16" and "HPV-18" are identified as a causative agent of oral squamous cell carcinoma. "E6 and E7" genes are responsible for the development of oral malignancy. High-risk HPV positive oral squamous cell carcinoma person would have low survival rate (approx. 5 years) than the low-risk HPV

positive person having oral squamous cell carcinoma. The cutaneous types are frequently associated with skin lesions; HPV 1, 2, and 4 are most prevalent in plantar warts, and HPV 5, 8, 9, 12, 14, 15, 17, 19-25, 36, 46, and 47 are usually found in epidermodysplasia verruciform. HPV 5 and 8 are also suspected to cause skin carcinomas. On the contrary, mucosal types infect the anogenital tract and upper aerodigestive tract and include HNSCC, OPSCC, and oral carcinoma. Based on oncogenic potential, mucosal types can be subdivided into low-risk and high-risk types. The most relevant low-risk types are HPV 6 and 11, and HPV 40, 42, 43, 44, 54, 61, 70, and 72 generally observed in benign genital mucosal lesions. HPV 31, 33, 35, 52, 58, and 67 are proposed to be moderate to high-risk, and among the high-risk types, HPV 16 and 18 are most common, and type 16 are predominant in various cancers such as cervical cancer, OPSCC, and penile carcinoma [8].

1.1 Structure of HPV

The HPV genome consists of a double-stranded DNA molecule which is bound to cellular histones and contained in a protein capsid. . The capsid contains 2 structural encoded proteins late L1 and L2. Protein combination of L1 and L2 or L1 alone is seen in appearance of virus like particles in human body. Occurrence of HPV-33, HPV-45 and HPV-52 is observed but HPV-16 and HPV-18 are frequently found among the maximum cases of OSCC. 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) [10, 11].

1.2 Transmission Route

Transmission of the infection occurs with three different methods of contact:

(i) Direct horizontal contact (saliva-saliva or genital mucous membranes during sexual intercourse): oral sexual contact is definitely one of the most frequent ways of transmission of the papilloma virus; a relationship has been demonstrated between the presence of HPV in the oral mucous membrane and the age of onset of sexual activity in the young; the presence of oral HPV count is also high in couples in which oral sex is practiced, compared to couples practicing genital sex. A 10 times higher risk of oral HPV infection is shown in patients who had more than 20 sexual partners in their lifetime compared to individuals with fewer than 3 partners [12].

(ii) Indirect contact (through contaminated medical instruments, utensils, or linen)

(iii) Vertical maternal-fetal transmission (during childbirth or postnatal) [13, 14].

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 along within them [15].

1.3 Entry of HPV in Host Cell

The virion is initially attached to the basement membrane before entrance to the basal keratinocyte cell surface. Glycosaminoglycans (GAGs), especially heparan sulfate found in the extracellular matrix (ECM) and on the surface of most cells, were proposed as initial binding receptors for HPV. Virions released from the stratum corneum and granulosum directly invade the basal layer through capsid synthesis and late and early promoter activation. A co-receptor like alpha 6-integrin is also associated in the internalization of HPV. Internalization of HPV is carried out via a clathrinid dependent endocytic mechanism. Once HPV infects the host tissue, its genome is integrated into the host genome and two products are formed – 'E6 protein' that forms a complex causing the degradation of p53 gene thereby inhibiting apoptosis via dysregulation of the cell cycle, and 'E7 protein' that interrupts the retinoblastoma tumor suppressor gene which finally leads to the increase of DNA synthesis. [16, 17] The elevated-risk HPV E6 and E7 oncoproteins, which are generated in HPV-infected squamous epithelial cells, are linked to enhanced proliferation and aberrant differentiation. The Genetic material from high-risk HPV-16 and HPV-18 is found in 70% HPV DNA positive oral tissue biopsy samples. Aside from the duration of HPV infection, with several HPV genotypes, viral DNA is detected epitomal and intracellular viral load may play a pivotal role in tumor development. In spite of being the key factor, HPV is insufficient to cause squamous cell carcinoma of the cervix of uterus by itself. HPV DNA is detected in the more than ninety percent of squamous cell carcinoma of the cervix biopsy specimens.

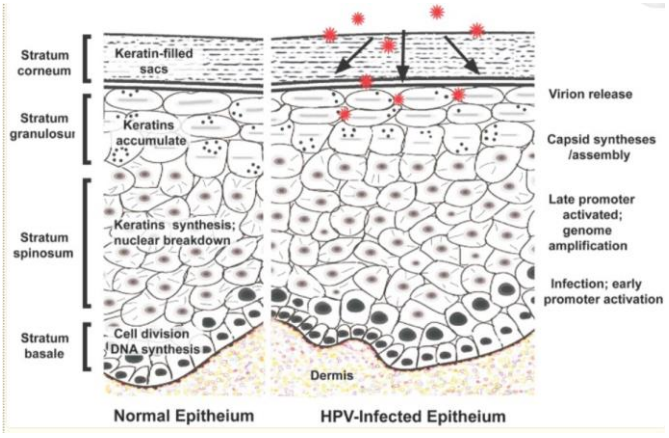

Figure 1. Comparison Normal Epithelium with HPV-Infected Epithelium

Comparison of normal and human papilloma virus (HPV) infected epithelia. Release of Virions from the stratum corneum and granulosum and directly infect the basal layer, involving capsid synthesis and late and early promoter activation. [18].

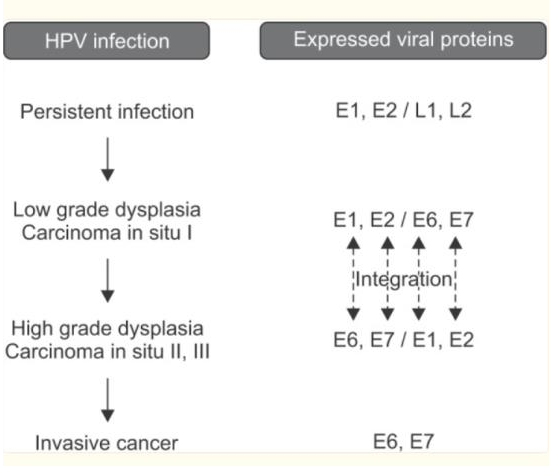

Figure 2. HPV Infection & Expressed Viral Proteins

Viral protein expression of human papilloma virus (HPV) infected tissue showing integration between E1 and E2 and between E6 and E7 [18].

2. METHODOLOGY

2.1 HPV Detection Methods

The diagnosis of HPV infection is detected through cytology and biopsy. The cytological features of the HPV infection are broadly divided as major and minor criteria. Sampling techniques for HPV usually include microscopy, ELISA, Southern blot, dot blot, hybrid capture, DNA microarray, and ligase chain reaction for probe amplification [19].

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

B. Minor criteria: Dyskeratoses, atypical immature metaplasia, macrocyte and binucleation. The methods are available 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; they are less sensitive as well as less expensive than PCR [20].

High Sensitivity methods: PCR, because it detects the virus in less than 1 copy of the viral DNA per cell. Over the years, Polymerase Chain Reaction (PCR) has emerged as the "gold standard" for identification of HPV types. Polymerase chain reaction (PCR), specifically the reverse transcription PCR (RT-PCR) is used to measure viral mRNA E6 and E7 in fresh tissue. It has a high sensitivity. It is even able to detect latent infections [21].

P16 is a protein preferred by some authors as a biomarker for HPV infection, which can be expressed after the integration of viral DNA into the host cell, which reflects the functional effects derived from the inactivation of pRb, induced by E7. It is perfectly detected by immunohistochemistry staining and it can be used as a predictor of HPV infection in OPSCC, even being proposed by some authors the detection of p16INK4A as an initial test, followed by the detection of HPV in which are positive for this [22, 23].

3. RESULTS AND DISCUSSION

3.1 HPV and Relevance to Head Neck Cancer (HPVHNC)

In the oral cavity, 24 types of HPV, 1, 2, 3, 4, 6, 7, 10, 11, 13, 16, 18, 30, 31, 32, 33, 35, 45, 52, 55, 57, 59, 69, 72, and 73 have been proved to be associated with benign lesions and 12 types, 2, 3, 6, 11, 13, 16, 18, 31, 33, 35, 52, and 57 with malignant lesions 31, 32 . A total of 99% of HPV infections in HNSCC are related to high-risk types 16, 18, 31, or 33, with HPV 16 as the most common subtype and HPV 33 accounting for less than 10% of cases. Clinically, HPV-associated tumors can present as a strawberry-like exophytic lesion, frequently at the ventral surface of the tongue or in the tonsillar fossa. Most of the lesions exhibit poorly differentiated pathologic findings and cystic changes in the metastatic neck lymph nodes. The transformation of normal oral mucosa in OSCC could be related to precancerous lesions, such as oral leukoplakia (OL), oral erythrolein (OE), oral lichen planus (OLP), nicotine palatini, tobacco pouch keratosis, and oral submucous fibrosis. The involvement of HPV in malignant transformation of precancerous lesions has not been confirmed yet, but OL has been known as the most frequent potentially malignant lesion, OE can be presented with severe epithelial dysplasia combined with carcinoma in situ or invasive carcinoma, the chronic mucocutaneous type of OLP is susceptible to HPV infection, and p16 with INK4a protein is a reliable precancerous marker in smokeless tobacco keratosis. [24, 25, 26].

A multicenter case-control study was conducted on oral cavity and oro-pharynx carcinomas in 9 countries. It was found that 70% of the tumor harbored HPV DNA. HPV16, usually detected in genital cancers was also the most common type observed in oral tumors. The study concluded that HPV appears to be the prime suspect in many cancers of the oropharynx and possibly a small subgroup of cancers of the oral cavity [27].

Jayaprakash et al. published in 2011 a meta-analysis about 458 oropharyngeal dysplasia's, estimating that the prevalence of HPV16/18 is 24.5%. Interestingly, they found

that HPV16/18 were 3 times more common in dysplastic lesions (OR, 3.29; 95% CI, 1.95-5.53%) and invasive cancers (OR, 3.43; 95% CI, 2.07-5.69%), when compared to normal biopsies. In addition, they also reported that, these two genotypes are at least 2.5 times more common in men. Within oral leukoplakia, proliferative verrucous leukoplakia is believed to have a definite relationship with HPV. (28) In gender wise predilection of HPV positive OSCC cases, Werness et al found statistical correlation with male predominance [29].

Different studies were performed by several investigators on HPV 16 significance in OSCC with clinical staging. Mellin et al found significant correlation between TNM staging and HPV positivity. (30) Agrawal et al in his study on the significance 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 in his study reported that stage and lymph nodal stage was significantly higher in the HPV positive group compared to the HPV negative group [32].

HPV-associated OSCC appears to be poorly differentiated, often basaloid in histology, and frequently present at an advanced stage. Agrarwal et al in his study on HPV 16 positivity and degree of differentiation reported that 71.44% lesions appear to be well differentiated, 14.28% belongs to moderate differentiation and 14.28% are poorly differentiated. [31] Study by Sharma et al revealed that moderately differentiated squamous cell carcinoma was most prevalent (81%) followed by well differentiated (16.7%) and poorly differentiated squamous cell carcinoma. [33] Dhanapal et al did not find any association between the presence of HPV and the grade of the tumor [34].

Many studies are conducted to find out the virus connection with benign lesions or even in normal mucosa. Prevalence is varied depending on the technique used, many times no PCR techniques are used, which may underestimate findings. They are summarized as mention [35-42] Appearance in normal mucosa: Varies between 0 and 81%. It may appear subclinical or latent, being detected by the higher sensitivity of the PCR and may be or not related to the occurrence of a future lesion. Squamous papilloma: Clinically indistinguishable most of the time from common warts. HPV genotypes 6 and 11 are most frequently involved, detected by ISH. Condyloma acuminatum: It is a sexually transmitted infection relevant to HPV 6 and 11 infections, varying its positivity between 75% and 85% in oral lesions. Sometimes it is also associated with the HPV 16. It is generally present in HIV positive patients.

Common wart (verruca vulgaris): Oral lesions usually occur due to autoinoculation from the fingers. It is usually seen in children. The HPV 2 is described as the most frequently related, followed by HPV 57, detected most of the time with no PCR techniques between 80% and 90%. Recent publications indicate more frequent detection of HPV2 and 4 109. Focal epithelial hyperplasia (Heck's disease): They are common in children and young adults. There is usually genetic predisposition. HPV13 (20%) and HPV32 (60%) are related to this lesion.

3.2 History of HPV Vaccine and Current Scenario

In 1991, Zhou et al. discovered essential virus-like particle (VLP) technology for HPV vaccination, in which 72 capsomers, each comprising five L1 proteins, are assembled into a VLP that resembles a virus-like structure. This VLP has higher safety and antigenicity and contains no foreign DNA. [43, 44] Zur Hausen then identified subtypes HPV 16 and 18 in cervical cancer, confirming that HPV infection is the key etiologic factor of 100% of cervical malignancies. [45] After these findings, different studies have found HPV infection in different sites of the human body, including the skin, urethra, nasal cavity, paranasal sinus, larynx, tracheobronchial mucosa, and oral cavity.

There are currently two U.S. Food and Drug Administration (FDA)-approved cervical cancer vaccines are available. Gardasil (Merck Co., Rahway, NJ, USA) is manufactured as a quadrivalent HPV (qHPV) vaccine, and Cervarix (GSK, Middlesex, UK) is produced by GlaxoSmithKline plc. as a bivalent HPV (biHPV) vaccine. The HPV L1 protein can efficiently self-assemble into VLPs, which are highly immunogenic. The qHPV vaccine, composed of VLPs from the viral L1 major capsid proteins of subtypes HPV 6, 11, 16, and 18 subtypes, is produced in yeast and uses aluminum hydroxy phosphate sulfate as an adjuvant. The biHPV vaccine consists of the VLP form of HPV 16 and 18 L1 proteins and uses an AS04 adjuvant, which is a combination of aluminum hydroxide and monophosphorylate lipid A (MPL) [46].

The development of the biHPV vaccine by GSK commenced in 1998, approved by FDA in 2009, and is now licensed in more than 100 countries and having approval in more than 60 countries. This biHPV vaccine includes the AS04 self-developed adjuvant system, which is composed of MPL, and triggers cellular and humoral immune responses with the general adjuvant aluminum hydroxide adsorbed form. MPL, the major constituent of AS04, is a modified Di phosphoryl lipid A extracted from Salmonella Minnesota R595 that removes phosphate and fatty acid groups.

Safety issue of the biHPV vaccine and the degree of immune response induction against monovalent /biHPV VLP types 16 and 18 were first assured through a phase I clinical study. A phase II clinical trial was carried out to evaluate the safety of vaccine composition and immunogenicity, followed by phase III studies to confirm the efficacy of the vaccine in HPV-infected and/or uninfected populations in the following age groups: 15-25 years, 10-25 years, 10-14 years, and 15-55 years [47, 48, 49]. The qHPV vaccine from Merck was initially developed as an HPV L1 VLP-based prophylactic vaccine. It was found to have the same structure as HPV 16, which was used as a main constituent in 1993, and was approved by the FDA in 2006 This vaccine has been

approved in 121 countries in 2011, and over 74,000,000 doses have been administered worldwide. [50, 51]

HPV 16 and 18 account for about 70% of cervical cancer cases worldwide. More than 90% of HPV-associated non-cervical cancers are also related to HPV 16 and 18, with HPV 16 accounting for the vast majority of cases. Smaller sample group trials of the qHPV vaccine in males have demonstrated protection against genital warts and premalignant anal neoplasia. There is rising incidence of OPSCC in both USA and Europe, including tonsillar and tongue base cancers, has been attributed to an epidemic of HPV infection. A decline in the incidence of cancer at HPV-unrelated sites has been reported in Japan, where about 35% of OPC and 25% of other oral cancers are HPV-positive. The vaccination against HPV-positive OSCC, including tonsillar fossa and ventral surface of tongue carcinomas, is expected to be effective for decreasing the prevalence of oral HPV infection in middle-aged adults even though vaccine efficacy against oral HPV infections presently lacks evidence [52, 53].

3.3 Prognosis of HPV-OSCC

Fakhry et al. in 2008 conducted a prospective phase 2 study of 96 patients with oral, oropharyngeal and laryngeal SCC. All patients were scheduled to receive two cycles of induction chemotherapy with paclitaxel and carboplatin followed by concurrent weekly paclitaxel and radiotherapy. HPV was detected (types 16, 33 and 35) with PCR and ISH in 40% of all patients. They compared their response to treatment with HPV-: OSCC HPV+ has better respond to chemotherapy (82% vs. 55%, difference = 27%, 95% CI, 9.3-44.7%; P = 0.01) and chemo-radiotherapy (84% vs. 57%, difference = 27%, 95% CI, 9.7- 44.3%; P = 0.007) [54].

In 2007, Ragin and Taioli performed a meta-analysis of 37 studies, where they concluded that patients with OSCC HPV+ had a lower risk of death (HR = 0.85 target; 95% CI, 0.7-1.0) and lower risk of recurrence (HR = 0.62% target; 95% CI, 0.5-0.8) than in HPV-. Regarding OPSCC they proposed that HPV+ had a reduced risk of death of 28% (Target HR 0.72; 95% CI, 0.5-1.0) compared with HPV- with a similar result for disease-free survival (Meta HR, 0.51; 95% CI, 0.4-0.7) [55].

In 2010, Ang et al. conducted a retrospective study of the association between tumor HPV status and survival among 743 patients with stage III or IV OPSCC who were enlisted in a randomized trial comparing treatment with accelerated-fractionation RT+ cisplatin vs. standard-fractionation RT+ cisplatin. Among 323 OPSCC, 63.8% were HPV+, which presented better 3-year rates of overall survival (82.4% vs. 57.1% among patients with HPV- negative tumours; P < 0.001 by the log-rank test) and a 58% reduction in the risk of death was observed (HR, 0.42; 95% CI, 0.27 to 0.66). They concluded that among patients with OPSCC, tumor HPV status is a strong and independent prognostic factor for survival [56].

Rosenthal et al. performed a retrospective assessment of the IMCL-9815 study, trying to detect differences in treatment patients with RT alone vs. RT+ cetuximab, in a series of 182 OSCC patients, in relation to the presence or absence of HPV and p16. They concluded that the addition of cetuximab to RT favored clinical outcomes regardless of p16 or HPV positivity. They also indicated that p16 does not predicted response to cetuximab [57].

4. CONCLUSION

There is a controversy about the carcinogenic potential of HPV. Hypothetically, the mechanism usually involves the pE7 and pE6 proteins, which can delete p53, p21 and pRb routes. HPV+ patients should be diagnosed at a younger age, mainly those with oropharyngeal tumors, presenting positivity first of all for HPV16 > HPV18, though it varies depending on the population. For diagnostic accuracy, the most advisable is to use the combination of several techniques. P16 positivity needs to be mentioned in special attention as a predictor of HPV infection in the OPSCC for their prognostic and therapeutic values. Despite of improvements over the last decade, five-year survival rates for head and neck squamous cell carcinoma still remains at 50%. It is crucial to distinguish HPV-associated HNSCC from tobacco/alcohol associated HNSCC. Molecular classification of tumors provides important new information that will allow a better understanding of prognosis and may have a definite influence on treatment decisions and also can be used for targeted therapy. OPSCC HPV+ tends to respond better to radio-chemotherapy treatments, considering the HPV positivity as a strong and independent survival prognostic factor. HPV vaccine is not implemented successfully yet now, but advancement in biomedical engineering in vaccinology may be a new hope to prevent OSCC early like Cervix Cancer.

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