Review Article

Human Papilloma Virus Related Head and Neck Squamous Cell Carcinoma-an updated review

Jaspreet Kaur[1]*

[1] Department of Neuroscience, University of Copenhagen, Copenhagen, Denmark
*jaspreet.kaur@sund.ku.dk

Abstract:

Human papilloma virus (HPV) related head and neck squamous cell cancer (HNSCC) has varying etiology, genetic as well as environmental factors involved and differential clinicicopathological features. HNSCC came in the limelight recently due to increased incidence rate and insufficient diagnostic methods. This review will comprehensively focus on the characteristics of HPV associated HNSCC. It will provide an updated review of our understanding of HPV role in Oral squamous cell carcinoma (OSCC) known to date. Curruntly, three vaccines are available (Gardasil, Gardasil 9 and Cervarix). These vaccines prevent infections with HPV types 16 and 18 HPV-16 is most common type associated with HNSCC. HPV related HNSCC has better prognosis, does not mutate but inactivatestumor suppressor genes and therefore has comparatively better treatment options. However, there is still a need to improve our methods of sampling, HPV molecular assay and type of specimen to be used.

Keywords:

HPV, oral carcinoma, prognosis, vaccines.

Introduction:

Head and neck cancer ranks 6th in most frequenct cancers all over the world [1]. The main risk factors include substance abuse e.g. smoking and alcohol. The association of Human papilloma virus (HPV) and cervical cancer is well-established [2], but it was in 1986, that HPV16 was detected for the first time in squamous cell carcinoma (SCC) of the HNSCC[3]. Recently, the association of HPV in the disease pathology of HNSCC has started to gain attention.

HPVs are tiny Deoxyribonucleic acid (DNA) viruses without any envelope having specific affinity for human squamous cell epithelium. Approximately, 180 different types of papilloma viruses; 150 of these are found in humans. HPVs may cause a variety of epithelial lesions, ranging from low malignant benign hyperplasia to pre-cancerous lesions having increased tendency to get malignant. HPV-1, 2 and 4 are linked with common warts and HPV-6 and 11 have role in the pathogenesis of papillomas in the respiratory tract. HPV-16, 18, 33 and 35 are considered as high risk HPVs [4]. In 1995, the International Agency for Research on Cancer identified HPV 16 and 18 associated with human cancers [5].

Epidemiology and Risk Factors:

HPV-16 was found in up to 86.7% cancers of oropharynx, 68.2% of oral cavity, and in 69.2% of the laryngeal cancers [6]. Squamous cell carcinomas comprise of more than 90% of malignant cancers of head and neck region. HPV prevalence in HNSCC ranges from 11-44% [7]. There is geographical variation in HPV type in different tumors occurring in different populations. HPV-18 is reported to be the highest common strain in Greece, whereas, , HPV-16 and 18 were found to be equally prevalent in India [8]. Developed countries with poor hygienic conditions and use of substandard tobacco, betel, chewing tobacco and alcohol seem to have higher incidence [9]. HPV is now well-known to cause only a subset of HNSCC. Although, smoking and drinking alcohol remain as an important risk factor for it, many studies suggest that more than

25% of HNSCC have HPV as a main etiology. Other risk factors can be early onset of sexual activity, polygamy, use of oral contraceptives and low socioeconomic status [2].

Molecular Mechanism of Action:

The replication of HPV depends crucially on the host-cell DNA. HPV DNA either integrated or episomal form lead to malignancy [4]. The genome of HPV encodes for a variety of proteins. L1 and L2 are responsible for the viral capsid proteins, whereas E6 and E7 proteins interferes with DNA replication. The malignancy of HPV is mainly due to its potential to incorporate E6 and E7 into host DNA. Consequently, the physiological function of tumor suppressor genes p53 and pRb is hindered. It is noteworthy to mention that the mechanism of action by which HPV disrupts p53 and pRb as compared to SCC caused by other factors e.g. smoking and alcohol use may vary (Figure 1)[10].

Route of Oral HPV Transmission:

HPV transmission mechanisms in HNSCC is not properly understood so far. Several theories of transmission have been proposed, which includes perinatal transmission, autoinfection from oral genital contact by hand. It has also been proposed that oral HPV infection is sexually transmitted [11]. Moreover, viral transmission by direct oral contact or by anyother means can not be overlooked. A research which involved married couples having healthy oral mucosa, oral route was observed as an important route for the transmission of HPV between them with a risk of 10 folds if one partner has an infection [12].

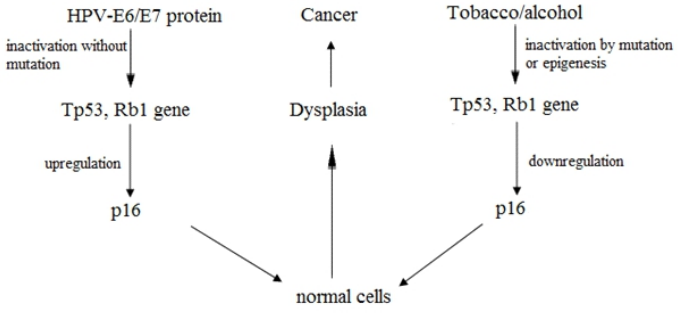

Figure 1: Mechanism of pathogenesis of HNSCC by two different risk factors

Site of Related Tumor:

HPV can cause cancers in several regions such as cervix, vulva, vagina, penis, anus and oropharynx [13]. HPV has been identified in 17-40% tumors at the base of tongue and in 21-100% of tonsillartumors with an estimated prevalence in tonsillar tumor of 50% [14, 8, 7]. Contrarily, only 2.3 - 25% of OSCCs were observed to be positive for HPV [15]. It is evident from studies that epithelial oropharynx including squamous columnar junction at the base of tonsillar crypts as well as transformation zone of uterine cervix have a greater tendency for the HPV related malignancies because they have more exposure of the basal cells. The origination of tonsillar carcinoma is either from crypt or surface epithelium is still not understood. However, it is known that HPV resides in the normal crypt epithelia. Another important fact that tonsillar tissue in Waldayer's ring is reservoir of HPV may not be ignored [15].

Lifetime risk of HNSCC with HPV infection:

It is not necessary that HPV infected patients will necessarily develop cancer. According to a research, 80% of women with cervical HPV infection get rid of it by normal immune system mechanisms. At the same time, association of HPV with carcinoma of head and neck region has not been established so far. A 14 fold increase in the risk of HNSCC was observed among patients positive for HPV-16 L1 protein [16].

Diagnosis of HPV infection:

HPV diagnosis in HNSCC is variable according to the method opted and the tissue under examination. The detection of DNA from HPV in oral samples during molecular assays is quite low. Hence, the detection methods for the low amounts of HPV-DNA should be highly sensitive, specific and accurate [17].

Tissue samples and exfoliated cells from the oral regions can be used for the detection of HPV [17]. Freshly frozen tissue (–70 o C) provides highest level and good quality of DNA contrary to the paraffin embedded formalin fixed tissue. The reason is degradation of DNA during the lengthy

storage periods as well as preservation methods [7]. Oral mucosal exfoliated cells can be obtained either by superficial brushing/scraping or oral rinse. Frozen tissue has increased efficiency as it gives more cell yield, DNA remains intact and is less invasive [18]. Commercial mouthwashes are also found to be better than glucose or saline in similar aspects [19].

Polymerase chain reaction (PCR) is the most sensitive and reliable test so far for HPV detection. Moreover, the quantitative PCR provides extra benefit of not only differentiating the low infection rate occurring from contamination but also determines the viral load in the specimen [20]. Southern blots and In-situ hybridization are not methods of choice as they lead to a lower HPV detection rate. High-risk E6/E7 mRNA or protein detected through test would be reliable for the classification of tumor on HPV basis. However, it is not recommended for formalin-fixed, paraffin-embedded tissue[17].

Expression of p16 is reported to be linked with HPV infection and therefore, determining its expression immunohistochemically could serve as a diagnostic and prognostic marker. But p16-positive/ HPV-negative HNSCC has also been detected, making it less reliable by decreasing the specificity and thus, remains controversial [21].

Classification of oropharyngeal squamous cell carcinomas:

Detection of HPV-DNA in an HNSCC is not clear indication to be the causative factor. Although HPV is a risk factor, but according to a study, among the HPV-DNA-positive cases, p16 expression is the determining factor for actual HPV association [14]. However, p16-positive/ HPV-negative HNSCC has also been detected and therefore its reliability as a surrogate marker has been controversial [21]. According to this study, only patients in class III (table 1) are HNSCC associated [17].

HPV Class	HPV expression	P16 expression	P53 and Rb gene expression
I	negative	Non expressing	high
II	positive	Non expressing	high
III	positive	expressing	lower

Table 1: Classification of HNSCC on the basis of HPV infection and p16 expression

Clinico-pathological features of HPV related OSCC:

HPV associated OSCC are different in terms of clinicopathologic features: more frequent in younger and male patients. The male to female ratio of patients is 4:1. They are presented mostly as a tiny primary tumor with late stage of disease. Histopathologically, they are non-keratinizing squamous cell carcinomas with basaloid features, excessive mitosis and comedo type necrosis. Immunohistochemically, they are featured by strong and diffuse p16 reactivity, low or negative p53 scores and strong expression of Ki67.

Clinical Implications of HPV:

Till to date, researchers could not differentiate HPV positive and negative patients on the basis of clinical profile. As a common observation, HPV infected oral SCC patients are non-smokers, non-drinkers and 5 years younger than the smoker group [22]. Initially, one hypothesis was that may be less consumption of tobacco and alcohol lead to HPV, but HPV has been observed to have additive effects with smoking and drinking alcohol and thus increases the risk of HNSCC. Patients who were involved in polygamy are more prone to have HPV positive tumors.

Any positive association between HPV positive carcinogenesis and clinicopathological characteristics of patients is not established so far, but the prognosis is better in HPV positive patients [7]. 5 years survival in HPV positive patients is 79%, while in HPV negative patients it is 20%, disease free survival was also 75% compared with 15% and the 5-year local recurrence was 14% compared with 45% (25). The good thing about HPV positive OSCC is the absence of p53 and PRB (is this pRb or Rb)mutations, no field cancerization (carcinogen

induced genetic changes)[23], high radiosensitivity and high expression of p16 protein [24].

Treatment options for HPV induced cancer:

As, the preventive HPV vaccines may not be of any help in treating the already infected patients, hence, it is important to explore new effective therapies. There can be two approaches, one is the development of vaccines and the second is gene therapy[25].

a) HPV Vaccination:Vaccination stimulates cytolytic T cell response against HPV-E6 and E7 proteins. But this strategy is not useful against carcinoma in situ and cervical carcinoma. Until 2007, two HPV vaccines were developed which provided protections against HPV types 6, 11, 16 and 18[26].

b) Currently, there are three vaccines (Gardasil, Gardasil 9, and Cervarix) approved by Food a Drug Administration (FDA) to prevent HPV infection [28, 29].It reduces genital warts along with cervical cancer. Cervarix is divalent and protects against HPV types 16 and 18and lessens the precancerous cervical lesions. Although, they provide 86-100% protection [26], yet a significant reduction in the incidence of cancer will not be significant for many years due to a prolonged course of cervical carcinogenesis. As HPV appears to be a causative agent in a certain percentage of head and neck cancer, the possibility of preventing this subset by thevaccine is worth considering.

c) **Gene therapy:** One of the methods in gene therapy is the use of E6 short interfering RNA, antisense RNA to E6 and E7 genes and mutated E2 protein that acts as cancer cell specific inducer of apoptosis[30] andp53 and pRbprotein expression is increased on the suppression of E6 and E7 protein expression, which results in apoptosis of HPV infected cells. So far there is less understanding regarding the mechanisms of HPV induced carcinogenesis and there is a need to further quest in the treatment via gene therapy.

Conclusions:

HPV-16 and other strains are a great risk factor for oral squamous cell carcinoma, more specifically from the tonsillar region. HPV-associated HNSCCs are most likely to have a good prognosis. HPV-16 type is considered as high risk and different studies are ongoing to develop effective vaccines against this virus. However, further studies are needed in this regard to explore the HPV infection in association with tobacco exposure and alcohol intake.

Recommendations:

1) Steps should be taken at government level of high risk countries to have a surveillance and statistical data after the screening of infected patients.

2) As there are different subtypes of HPV in different regions of the world, this knowledge needs to be explored leading to collaborative research to develop vaccines.

3) Policies should be developed and recommendations should be given to World Health Organization to start a global awareness, prevention and treatment plan.

4) There is further need to test for papilloma virus in healthy individuals as well as those at risk to investigate the high-risk type of HPV is in general population.

5) Improved methodsin terms of sampling, HPV molecular assay, diagnosis and type of specimen used should be designed.

6) As developing countries,especially the oriental region has a higher incidence of HPV infected HNSCC, steps should be taken for awareness, better hygienic conditions and education of the people.

7) As this disease is prevalent in developing countries, but the knowledge available regarding HPV linked HNSCC so far is from developed countries, there is a need to increase research in these high risk low income countries.

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