Human Papilloma Virus: Important Etiological Factor in Head and Neck Squamous Cell Carcinogenesis

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Abstract

Human papilloma viruses (HPVs) are involved not only in anogenital but also in head and neck carcinogenesis. So far almost 100 sub-types of HPV have been identified but HPV16, 18 and 31 are most common high risk types in oro-pharyngeal cancers. Since HPVs are involved in cancers, therefore, preventive and therapeutic options will reduce the prevalence, mortality and morbidity associated with HPV induced head and neck squamous cell carcinomas.

INTRODUCTION AND EPIDEMIOLOGY

Squamous cell carcinomas (SCC) of oral cavity, oropharynx, nasopharynx, hypopharynx and larynx have been categorized as head and neck squamous cell carcinoma (HNSCC). SCC constitutes around 90% of all the malignancies of head and neck region [1]. Annual incidence of SCC across the globe is around ~633,000 and about 355,000 people lose their lives to this disease annually. These statistics rank HNSCC as the sixth most common malignancy [2]. Global incidence of HNSCC varies with the region and it is the most common malignancy in India i.e. accounting up to 40% of the total malignancy load [3]. In head and neck region, SCC of oral cavity and oropharynx is more common. In 2008, SCC of oral cavity and oropharynx collectively accounted for 263,900 fresh cases and 128,000 deaths across the globe [4]. In the early stages HNSCCs are symptom free and by the time they are diagnosed they have already metastasized but if detected at an early stage HNSCCs are curable. The common modalities for treatment of HNSCCs include surgical removal, radiation therapy, chemotherapy or combinations of these. At the time of diagnosis 43% regional lymph node involvement and 10% distant metastasis leads to 5 year survival of the subjects is less than 60%, even after the above mentioned therapies [5].

Risk factors of HNSCC development

Development of HNSCC is multifactorial and tobacco is a major risk factor because it induces mutagenesis that leads to HNSCC. Approximately 80% of HNSCC have been related to tobacco [6] and risk of HNSCC is 10 times higher in smokers as compared to non-smokers [7]. Alcohol consumption is another risk factor [8] and its consumption and use of tobacco act synergistically to increase the risk of HNSCC [9]. Tobacco use has been reduced in the second half of 20th century by the introduction and implementation of public health awareness programs which resulted decrease in the incidence of HNSCC [6]. However, tobacco use remains an important risk factor for HNSCC in many countries. Decrease in overall incidence of HNSCC is largely related to the reduction in the use of tobacco. HPV has emerged as a serious risk factor for oro-pharyngeal Squamous cell carcinoma (OPSCC) [10,11] which is being linked to the changes in the sexual behaviours.

Incidence of head and neck cancers associated with HPV (wqw33) is significantly higher among males as compared to females. Although factors, contributing to this male predilection, are not completely understood but it is suggested that HPV is more prevalent in cervical region as compared to penial region, thus male performing oral sex are far more at risk [10]. Indeed, data suggested higher incidence of HPV in the oral cavity of men as compared to women [12]. The recently published HPV in men (HIM) cohort study reported a similar incidence of HPV in men as had been previously described in anogenital region of women [13]; however, this study investigated the incidence of HPV in the anogenital region rather than the oral cavity. It is possible that the natural history of oral HPV infection may differ from the infection of genitalia [10]. Approximately 100 HPV subtypes are recognized, but the high-risk oncogenic subtype, HPV-16 accounts for >90% of HPV-related HNSCC [14,15].
Papilloma Virus (PV)

Epithelial linings of various body cavities in vertebrates are infected by papilloma viruses and these infections could remain asymptomatic or may lead to neoplastic proliferations. DNA genomes of PVs have a circular double stranded structure and typically consist of eight genes approximate size of 8kb. Principal capsid protein of the virus is encoded by a single L1 gene and this gene can produce virus-like particles currently being used for vaccine production. On the basis of L1 nucleotide sequence, PVs have been categorised into “types”. A single type has a full length PV genome but its L1 nucleotide sequence is atleast 10% dissimilar to any other PV type. L1 gene is well conserved and it can be aligned for all types making it very useful for the classification and construction of phylogenetic trees [16]. In the 7th report of the International Committee on Taxonomy of Viruses (ICTV), PVs have been named as a separate family called “Papilloma viridae” [17]. The study group of papilloma viruses of ICTV and PV research community, classified family papilloma viridae into 92 human papilloma viruses (HPV) and 24 animal papilloma virus types [16,18].

Human Papilloma Virus

Genomes of more than 150 HPV types have been sequenced till date. Based on the DNA sequencing, HPV types have been further classified into 5 different genera. These types have characteristic life cycles and disease associations [19,20]. Many HPV types especially those of Beta and Gamma genera which are detected in skin swabs and some Gamma types in mucosal rinses are harmless and cause asymptomatic infections in healthy population [21,22]. Such viruses in most instances do not cause any disease and complete their life cycles without causing disease [23].

High and low risk HPV types and their associations with cancers

The low risk mucosal HPVs are generally not involved in tumorigenesis, but can cause genital cutaneous lesions. In immuno-compromised patients especially those suffering from EV (epidermodysplasia verruciformis) and SCID (Severe Combined Immunodeficiency Disease) and in those on immunosuppressive therapy cutaneous lesions by low-risk HPV types become difficult to treat [24,25]. The high risk HPV strains are associated with carcinomas. World Health Organization (WHO) has listed 12 different types of HPV including 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58 and 59 under the heading of high risk cancer causing HPV types and two additional types namely 68 and 73 under “possible” cancer causing HPV types. On the basis of evolutionary similarity, several other types of HPV are also included in the high risk group but up till now their association with cancer has not been established [26,27]. Two viral oncogenes, E6 and E7 are considered as the mainstream initiators of cell transformation and are encoded by the high risk HPV types [28].

HPV types associated with HNSCC

Over the last 30 years a decrease in the incidence of cervical cancer has been observed partly due to Pap smear screening but on the other hand an increased incidence of anal, oro-pharyngeal and vulvar cancers has been observed [29]. The IARC Working Group has named the same 12 HPV types (namely 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58 and 59) categorized in a high risk group by WHO as carcinogenic in humans and HPV type 68 as possible carcinogenic [30]. All the carcinomas of the cervix and several carcinomas of anal, vaginal, penile, vulvar and oro-pharyngeal region are associated with these above mentioned HPV types [31].

Pathogenesis of HPV associated HNSCC

The transfer of HPV infection to cancer is a multistep process and is influenced by multiple viruses related, host dependent and environmental factors. Virus persistence, its load and its production by the host cells after their reprogramming by HPV are some of the virus related factors. In some cases, events following HPV infection may lead to the integration of HPV genome in host DNA leads to unregulated expression of E6 and E7 which are viral oncogenes [28] e.g. HPV associated cervical cancers [32]. In addition to these virus-related factors, genetic susceptibility to viral infection, increasing age of the host, and other epigenetic and lifestyle factors, such as smoking, chronic inflammation and co-infection with other sexually transmitted organisms particularly chlamydia trachoma is increases the risk of cervical cancer in HPV-infected women [5,6,8,33]. Most of our knowledge related to HPV infection and its occurrence has been derived from studies on cervical infections.

It is known that type of HPV and related host factors such as immune status of the individual predicts the possible outcome of an infection but most of the HPV infections are cleared spontaneously in approximately 12-24 months. Virus can go into latent phase and remain undetectable and can reactivate later in life or it may be cleared completely from the host. Benign lesions (verrucae and papillomas) are caused by low risk HPV infections out of these low grade lesions and some high grade ones regress spontaneously while some high grade lesions may progress to invasive carcinoma but it is a rare event and can be prevented by appropriate management and surgical removal of high grade lesions [34].

The HPV genome consists of three different sections: “long control region” (LCR) which regulates the expression and replication of genes and is none coding, the other two regions are protein-coding namely early (E) region and late (L) region. The former is involved in the coding of proteins which are required for the expression, replication and survival of genes while proteins coded by the later are capsid proteins [21,22].

E5, E6 and E7 are viral onco-proteins which are encoded by three early genes. E6 and E7 play an important role in tumourogenesis. E6 of different HR types and especially of HPV 16 can degrade p53 protein via ubiquitin pathway which is a tumour suppressor protein I [35]. During cellular stress or DNA damage p53 which is a transcription factor induces apoptosis or cell cycle arrest [36]. E6AP is a cellular protein to which viral oncoprotein E6 binds and the resulting E6/E6AP complex is involved in ubiquitination and proteasomal disintegration of p53 which plays a major role in maintaining integrity of genome. Malfunctioning of p53 is a common feature of many human malignancies. In most instances mutation of p53 is associated with HNSCCs but in carcinomas associated with HPV infection,
degradation of wild type functional p53 occurs. In addition to p53 degradation, cells which express E6 onco-protein of HPV 16 demonstrate chromosomal instability which is advancement in the malignant transformation [35].

E7 viral onco-protein of some HR types and especially of HPV 16 is involved in the inactivation of pRb, which controls the transition from G1-S phase of cell cycle by binding to E2F. E2F is a transcription factor and under physiological conditions its release occurs in response to phosphorylation of pRb which is mediated by cyclin dependent kinases (CDK). This inactivation of pRb by E7 viral onco-protein results in the release of E2F and increased transition from G1-S phase of cell cycle [37]. In addition to this, transcription of multiple genes occurs e.g. cyclin E and A which are involved in the progression of cell cycle. This pRb inactivation also induces over expression of p16INK4A which can be detected on immunohistochemistry and is generally used as a diagnostic marker for HPV infection [35]. Many researchers have reported controversy about the role of p16 as a predictive marker for HPV infection. Harris et al. (2015) reported over expression of p16 in oral tongue squamous cell carcinoma and it was a marker of favorable prognosis but they observed that p16 could not be used as a reliable predictor of HPV positivity [38]. Similarly, Reyes et al. (2013) also could not detect association of p16 with HPV infection [39]. Binding of pRb with E7 depends on the type of HPV producing E7, E7 from low-risk HPV binds weakly in comparison to E7 of high risk HPV types e.g. HPV 18 [35].

Persistent HR HPV infection is a prerequisite for cervical precancerous lesions and invasive cancer. In fact, in order to induce stable malignant transformation of the host cells, E6 and E7 of HR HPVs need to cooperate with activated cellular proto-oncogenes. Proliferation-associated mutagenesis induced by persistent expression of viral oncogenes E6 and E7 may finally lead to activation of proto-oncogenes resulting in the fully malignant phenotype [35].

Carcinogenic mechanisms in HPV-associated oropharyngeal cancers (OPSCC) may be similar to cervical cancers, but since the oral cavity/oropharynx are exposed to higher levels of chemical carcinogens in comparison to genital tract, it is likely that different mechanisms are implicated in cervical and oropharyngeal carcinogenesis. Probably due to differences in viral load and/or viral oncogene expression, HPV DNA positive OPSCCs are heterogeneous in biological and clinical behaviours [40]. Low levels of HPV DNA and absence of viral transcriptional activity are likely to have no or limited biologic significance, and could indicate that HPV does not play a pathogenetic role in these malignancies [41].

Clinical features of HPV associated HNSCC

OPC associated with HPV occur at a younger age as compared to OPC negative for HPV [42]. Nasman et al. documented that at the time of tumour diagnosis, patients with HPV associated tonsillar carcinoma had a mean age of 59 years as compared to 68 years of HPV negative patients [43]. Furthermore HPV can be transmitted via body fluids during kissing and sexual intercourse. There is a higher probability of HPV associated OPC in individuals of six or more sexual partners [44]. There is relationship among HPV associated OPC with STDs (sexually transmitted diseases), sexual exposure at a young age and lack of using protection during oral sex [44]. Caucasians have a higher tendency of HPV related HNSCC as compared to Blacks [45]. Other factors like poor brushing habits and increased use of certain mouthwashes which disturbs normal flora of mouth causes increased vulnerability for oral HPV infections [46]. The following table 1 shows the most common clinical symptoms of HPV associated HNSCC.

<table>
<thead>
<tr>
<th>Table 1: Common symptoms of HPV positive head and neck cancer [47].</th>
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<tbody>
<tr>
<td>Symptoms</td>
</tr>
<tr>
<td>Neck mass</td>
</tr>
<tr>
<td>Sore throat</td>
</tr>
<tr>
<td>Visible oral lesion</td>
</tr>
<tr>
<td>Difficulty in swallowing</td>
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<tr>
<td>Lump in the throat</td>
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</table>

Diagnosis and prognosis of HPV associated HNSCC

Diagnostic tests are available for the screening of HPV positive cervical cancers but for the determination of mouth or throat HPV infection there is no FDA approved test [48]. HPV cannot be cultured in the laboratories but multiple tests can be used for the diagnosis of HPV infection e.g. colposcopy and acetic acid test, biopsy, cytological smears (Pap smears), DNA tests (PCR, southern blot hybridization, in situ hybridization) [49]. However all these test have limited use in diagnosing oral HPV infection. There are no visible signs and symptoms of oral HPV infection. There are many kits to diagnose oral HPV infections but being tested positive once do not mean persistence of HPV infection because that is important in cancerous transformation of normal tissues [50].

Regarding prognosis, HPV associated HNSCC responds well as compared to HPV negative HNSCC [51]. HPV associated HNSCC responds well to all the current treatments such as surgery, radiotherapy, chemotherapy and combination therapy [52-55]. Christian et al. also reported a significantly improved survival rate in HPV positive oropharyngeal cancers as compared to negative cases (Log rank p=0.03) [56].There is available ample data supporting the hypothesis that patients suffering from HPV related HNSCC show better survival rates as compared to HPV negative HNSCC due to a higher sensitivity to chemo-radiation therapy [57]. There can be multiple reasons for better prognosis of HPV positive tumours but actual pathway is yet to be elucidated. HPV related tumours show fewer genetic alterations than negative ones which may confer sensitivity to cytotoxic agents [58]. Some other possible reasons for better prognosis can be that TP53 is just non-functional in HPV positive tumours while it is mutated in negative ones that give chemoradio-sensitivity to HPV associated tumours [59]. Favorable prognostic role of p16 in HPV related HNSCC has already been discussed above. Newer treatment strategies for HPV related HNSCC include dose reduction of radiotherapy, use of cetuximab instead of cisplatin and less invasive surgical therapy [60]. However there is still little consensus on optimal treatment for HPV associated HNSCC. Focus of current clinical trials is on reduction of treatment related toxicities and development of HPV targeted therapies. The
following (tables 2,3) show summary of the features associated with HPV positive and negative HNSCCs, and year wise prognosis.

**CONCLUSION**

The research and clinical data mentioned above emphasizes the need for ongoing research into the underlying mechanisms involved in HPV associated carcinogenesis. All over the world HNSCC carcinoma is a growing malignancy. Although smoking, tobacco chewing, wet snuff, use of alcohol and exposure to ultra violet radiation are the contributing factors for HNSCC but HPV becomes the most important causative factor especially for oropharyngeal SCC. It is due to changing trends in sexual behaviours of young generation in Western countries. People should also be educated by launching a media campaign at mass level about maintaining good hygiene measures and safe sexual habits to reduce the risk of HPV related benign and malignant diseases. More research is needed to actually explore how HPV is passed on and also if screening for oro-pharyngeal HPV will have health benefits. Patient should be educated and encouraged to discuss with his dentist or physician about any symptoms that could suggest early signs of head and neck cancer. Until now, no research have been performed on how HPV associated oropharyngeal SCC can be prevented. However by adopting preventive measures during oral sex (condom use) and dental treatment (rubber dam use), person to person transmission of HPV infection can be stopped and it will ultimately lessen the burden on economy due to morbidity and mortality caused by HPV associated cancers. Researchers have successfully formulated the vaccines to prevent cervical cancer and other less common genital cancers associated with HPV. There is still a wider room for researchers to study HPV transmission, disease causing mechanism, preventive and therapeutic vaccines.

**REVIEW CRITERIA**

We adopted a liberal method for inclusion of research articles in this review. Actually this review does not aim to be wholly covering all the available literature and research data on OSCC, OPSSC or HNSCC; rather, it highlights and discusses the important findings from some relevant studies. The data for this review article have been gathered without applying any exclusion criteria when deciding which scientific article would be included in this review. We selected the articles published with no time limit from “Pub Med” and “Science Direct” databases by searching the key terms “HPV”, “OSCC”, “Oro-pharyngeal Squamous cell carcinoma” and “HNSCC”. Only the articles published in English language were considered and reviewed systematically and thoroughly. The selected full articles were prioritized according to relevant content and the references were reconfirmed.

**REFERENCES**

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**Table 2:** HPV positive and HPV negative head and neck cancer [61].

<table>
<thead>
<tr>
<th>Important Parameters</th>
<th>HPV Negative</th>
<th>HPV Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Incidence</td>
<td>Decreases</td>
<td>Increases</td>
</tr>
<tr>
<td>Common risk factors</td>
<td>Tobacco, Alcohol</td>
<td>HPV</td>
</tr>
<tr>
<td>Most common site</td>
<td>Tonsil, Soft palate</td>
<td>Base of tongue, Tonsil</td>
</tr>
<tr>
<td>Age</td>
<td>Older</td>
<td>Younger</td>
</tr>
<tr>
<td>Education level</td>
<td>Lower</td>
<td>Higher</td>
</tr>
<tr>
<td>African-American</td>
<td>Common</td>
<td>Rare</td>
</tr>
<tr>
<td>Tobacco use</td>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td>Survival</td>
<td>Poor</td>
<td>Good</td>
</tr>
</tbody>
</table>

Murphy B, Day TA. MUSC ENT Update: Sept 2014

**Table 3:** Differences in overall survival between HPV positive and HPV negative head and neck cancer [55].

<table>
<thead>
<tr>
<th>Times in years</th>
<th>HPV Positive</th>
<th>HPV Negative</th>
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<tbody>
<tr>
<td>1</td>
<td>97%</td>
<td>90%</td>
</tr>
<tr>
<td>2</td>
<td>95%</td>
<td>62%</td>
</tr>
<tr>
<td>3</td>
<td>86-93%</td>
<td>58%</td>
</tr>
<tr>
<td>4</td>
<td>84%</td>
<td>50%</td>
</tr>
<tr>
<td>5</td>
<td>79-82%</td>
<td>46%</td>
</tr>
</tbody>
</table>


CDC. Genital HPV Infection Fact Sheet. 2015.


61. Murphy B, Day TA. Oropharyngeal cancers.