Review Article

Human papillomavirus associated head and neck squamous cell carcinoma: Controversies and new concepts

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ABSTRACT

High-risk human papillomavirus (HR-HPV) is a causative agent for an increasing subset of oropharyngeal squamous cell carcinoma. HPV 16 accounts for 90% of cases. The chance for malignant transformation due to infection with high-risk HPV is proportional to the expression of the viral oncogene products E6 and E7, which inactivate p53 and retinoblastoma (Rb) tumor suppressor functions. P16 is a surrogate marker of HPV associated HNSCC and 2+/3+ expression in more than 75% cells is diagnostic. Molecular demonstration of integrated virus by in situ hybridization is specific but has low sensitivity. HPV associated oropharyngeal carcinomas classically arise in the tonsillar crypts and commonly have basaloid morphology with a prominent lymphocytic response and minimal desmoplastic reaction. In situ vs invasive carcinomas may be difficult to distinguish in histology. The HPV positivity overrides traditional prognostic indicators such as tumour grade and histological subtype. Small cell morphology carries a poorer prognosis as does marked tumour anaplasia and multinucleation. Lymph node metastasis is extensive and frequently cystic however extranodal extension, laterality or nodal sizes do not carry prognostic implications as in conventional OSCC and OPSCC. Stage IV is reserved for distant metastasis. HPV-16-positive patients had significantly reduced overall and disease-specific mortality rates and an improved 3-year overall survival (OS) and disease-free survival (DFS) compared to patients with HPV negative tumors. Surgical treatment is the main option for primary and secondary HNSCC. Targeted therapies including drugs targeting EGFR and PIK3CA have shown some promising results. HPV pathway expressing tumors are less aggressive and may receive adequate curative intent therapy from a reduced radiation or chemotherapy dose revision. OSCC however fails to show a distinct difference between HPV associated and tobacco associated cancer and prognostic differences do not clearly exist.

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1. Introduction

Human Papilloma Virus associated Head and Neck carcinoma (HPV-HNSCC) has been established as a prognostically different entity in oropharynx in contrast to oral squamous cell carcinoma (OSCC) where HPV categories are associated with indistinct parameters. Current evidence supports oropharyngeal squamous cell carcinoma (OPSCC) as having identifiable risk factors and improved response to therapy. However controversies exist in the existence of a clearcut HPV associated OSCC with independent prognostic implications. Morphological alterations in HPV associated carcinomas are characteristic in a large fraction of cases. Further the diagnostic parameters for labelling a case as HPV associated, the tests to be used, molecular mechanisms and pathways involved and their implications for treatment and use of HPV prophylactic vaccines are grey zones in HPV-HNSCC. This review focuses on these controversial areas in HNSCC.

2. Human papilloma virus

Zur Hausen is credited with associating cervical cancer and condyloma accuminata with HPV.¹ HNSCC causation due to HPV was first investigated by Syrjanen in 1983.² HPV belongs to the family Papillomaviridae.¹ High-risk human papillomavirus (HPV) is a causative agent for an increasing subset of oropharyngeal squamous cell carcinomas (OPSCCs). There are numerous subtypes of this virus, but most have not been shown to be carcinogenic. Approximately 30 HPV types infect the anogenital and oral mucosa and can be further classified as “low-risk” and “high-risk” based on the clinical prognosis of their associated lesions. Low-risk HPVs cause benign epithelial hyperplasias (warts), while high-risk HPVs cause lesions that have a propensity for malignant progression. HPV subtypes 16 and 18 are the most-studied high-risk subtypes, but the majority of evidence in head and neck cancer has implicated HPV subtype 16 as an etiologic agent in more than 90% of HPV-related head and neck cancers.³

2.1. Incidence of HPV related HNSCC

Worldwide, an estimated 599,637 new cases of and 324,794 deaths from head and neck cancer (excluding nasopharyngeal cancer) occur every year.⁴ US data shows that the incidence of OPSCC associated with HPV is showing a steady rise since 1973. Among individuals aged 12 to 69 years HPV infection has an overall prevalence of approx 7%. Men have a higher prevalence of HPV at 10.1% as compared to women at 3.6%. Smokers and persons with multiple sexual partners from a significantly higher prevalence group at 20%. OPSCC is occurring increasingly in younger males it is predicted that OPSCC will surpass the most frequent commonest the HPV associated cancer viz cervical cancer by 2020 in USA. HPV prevalence in HNSCC varies with ethnicity and geographic origin. In Asiatic countries, Japan has the highest worldwide frequency. The lowest prevalence of HPV-positive HNSCC was in Africa.⁵ The proportion of HPV-positive OPSCC reported in another review was 56% in North America; 52% in Japan; 45% in Australia; 39% in Northern and Western Europe; 38% in Eastern Europe; 17% in Southern Europe and 13% in the rest of the world.⁶ (Table 1)

3. Molecular pathogenesis of HPV associated HNSCC

During carcinogenic progression the HPV genome frequently integrates into a host cell chromosome and, as a result, the viral oncoproteins, E6 and E7, are the only viral proteins that are consistently expressed in HPV positive carcinomas. These viral oncogenes have been shown to inactivate two crucial human tumor suppressor genes: *p53* (E6) and *pRb* (E7). This inactivation results in loss of cell-cycle control, impaired cell differentiation, increased mutations, and chromosomal instability.²⁷ HPV-OPSCCs are relatively low mutational density, wild-type cellular tumor antigen p53, and p16 protein overexpression.¹⁷ The interaction between E6 and p53 may also affect degradation of the Src family of proteins and thereby increase mitotic activity in the infected cells. Similarly, the E7 protein interacts with Rb through an E2F/Rb complex, which leads to the release of E2F-coded activators.²⁸ This in turn results in increased cell cycling even in the presence of DNA damage.^{29,30} A strong correlation between HPV infection and p16 protein expression has consistently been reported in OPSCC. Therefore, p16 can be presumed to be a surrogate biomarker for HPV infection in these high-risk tumors.³¹

In two cohorts compared in the Cancer Genome Atlas (TCGA) study cohort 1 with HPV positive OPSCC vs HPV negative cases with no p16 or HPV ISH negative reaction, miR-9 and miR-106b were the most significant miRNAs increased in positive cases while negative cases showed miR-9 levels were 16 fold lower.³² Detailed changes have been listed in Table 2. Developments of clinically useful markers that predict the neoplastic transformation of the host cell by the HPV virus and its subsequent progression and metastasis are the need of the day.

3.1. Demonstration of HPV in tumours

HPV status can be determined by using a combination of DNA multiplex tandem polymerase chain reaction (MT-PCR), RNA or in situ hybridization (ISH) and expression of p16.³⁷ Strengths of the p16-expression assay are that it is not specific for HPV type, unlike ISH assays, and therefore p16 expression status is a very good surrogate for tumor HPV status, it is widely available and easy to carry out and to interpret.³⁸ According to several studies, p16 may be the best single marker for prognosis in OPSCC.³⁹ Screening molecular HPV assays including PCR based HPV based assays carry the risk of false positive detection of endemic HPV infections not associated with cancer whereas the ISH test carries a low sensitivity. The cervista HPV HR and the Digene Hybrid Capture II (HC2) HR HPV DNA Test are FDA approved for IVD diagnosis and have been used in uterine cervical cancer screening.

4. Morphological spectrum of HPV-HNSCC

The HNSCC associated with HPV have a distinct morphological profile. Major histologic variants of HPV associated squamous cell carcinoma arising in the oropharynx include (a) Basaloid (b) Papillary (c) Lymphoepithelial and (d) Adenosquamous carcinoma.

HPV associated Oropharyngeal carcinomas classically arise in the tonsillar crypts. HPV-associated oral intraepithelial dysplasia is characterized by brightly eosinophilic compact ortho- or para-keratosis, epithelial hyperplasia with marked karyorrhexis and apoptosis present throughout the epithelium and surrounding

Table 1

Comparison of Demographic, Clinical, Histological, Genetic and Prognostic Parameters in HPV-OPSCC vs. TOBACCO ASSOCIATED OPSCC.

Features	HPV+ OPSCCs	HPV-OPSCCs	References
Age (mean)	Younger age (40–60)	Older age at presentation (>60)	Fakhry C et al. ⁷
Race	White >> Non white	White > Non white	Fakhry C et al. ⁷
Geographical distribution	Northern Europe and North America	Asia-Pacific	Forté T et al. ⁸
Gender	Men > Females (8:1)	Men > Females (3:1)	Gillison ML et al. ⁹
Socioeconomic status	Higher	Low–middle	Gillison ML et al. ⁹
Prevalence estimates and trend	Variable in regions	Stable	Hwang TZ et al. ¹⁰
Etiologic factors	13–56%, Increasing Nine subtypes of HPV	Known risk factors induce molecular changes and precancer.	Gillison ML et al. ¹¹
Risk factors	90–95% HPV-16 Sexual behavior (high number of sexual partners, history of oral-genital sex, and history of oral-anal sex)	No known etiologic factors Smoking, Alcohol, Areca nut, Smokeless tobacco, Betel quid chewing	Gillison ML et al. ¹² Heck JE et al. ¹³
Cofactors	Marijuana (Cannabinoids) smoking immunosuppression	Diet, oral hygiene, stress	Marks MA et al. ¹⁴
Site of origin	Reticulated epithelium	Squamous epithelium at all sites in oral and oropharyngeal cavity,	El-Mofty SK et al. ¹⁵
Preneoplastic Condition	lining of the tonsillar crypts lack precancerous HPV +ve mucosal changes	Keratinizing squamous dysplasia, field cancerization	Wenig BM. ¹⁶
Anatomical sites	Lingual and palatine tonsils, Base of tongue	All sites	Heck JE et al. ¹³
Mutational density	Low	High	Miller DL et al. ¹⁷
Oncoprotein expression:			Gondim DD et al. ¹⁸
Viral oncoproteins E6 & E7	Expressed	Not evident	El-Mofty SK et al. ¹⁵
p53 gene mutation	Wild type	Mutant	
Rb gene tumor repression function	Normal usually	Less frequency	
p16 gene product	Overexpressed	Usually not evident	
Pathological findings	Unassociated with dysplasia of the surface epithelium	Associated with dysplasia of surface epithelium, Keratinizing	Westra WH et al. ¹⁹
	Exhibit lobular growth		Wenig BM et al. ¹⁶
	Central necrosis, cystic degeneration infiltrating lymphocytes prominent “basaloid” morphology		
Regional lymph node involvement	Frequent, cystic	Frequent but less than HPV+ OPSCCs Necrotising or Solid	Yasui T et al. ²⁰
Prognosis	Favourable Small cell phenotype is associated with poor prognosis	Unfavourable	Ang KK et al. ²¹
ECOG performance status	Lower performance status (0 in 33% patients)	Higher performance status (0 in 66% patients)	Kraft S et al. ²²
Tumor surveillance	PCR, ISH techniques, IHC detection of HPV and surrogate markers	Evidence of keratinizing dysplasia, Molecular alterations in p53, Cyclin D1 More advanced T stage	Fakhry C et al. ⁷
T Stage	Early T stage (T3&T4 ~42%)	(T3&T4 ~67%) Early N stage (N2–N3 = 50%)	Smeets SJ et al. ²³ Fakhry C et al. ⁷
Nodal stage	Advance N stage (N2–N3 = 66%)		Fakhry C et al. ⁷
3 year Overall survival (months)	82.4% (95% CI, 77.2–87.6)	57.1% (95% CI, 48.1–66.1)	Ang KK et al. ²¹
3-year progression-free survival	73.7% (95% CI, 67.7–79.8)	43.4% (95% CI, 34.4–52.4)	Ang KK et al. ²¹
Overall response to treatment	94 (95% CI, 87–100)	58 (95% CI, 49–74)	Marur S et al. ²⁴
2-Year OS (%)	85 (95% CI, 74–99)	53 (95% CI, 36–67)	
2-Year PFS (%)			
Targeted therapy	MET overexpression (>80%), MET gene amplification (13%), and MET point mutation (14%) Her2, EGFR are underinvestigation	PIK3CA	Lechner M et al. ²⁵ Burtneess B et al. ²⁶

Table 2

Molecular profiles of HPV associated versus tobacco associated HNSCC.

Molecular features	HPV-associated HNSCC	Tobacco Associated HNSCC	References
MiRNA signature	Up-regulated: miR-320a miR-222-3p miR-93-5p Downregulated: miR-199a-3p//miR-199b-3p, miR-143, miR-145, and miR-126a	Age: hsa-miR-99a-5p hsa-miR-301b hsa-miR-182-5p hsa-miR-455-3p hsa-let-7c hsa-miR-32-5p hsa-miR-18a-5p hsa-miR-100-5p hsa-miR-130b-3p hsa-miR-331-3p Smoking: hsa-miR-1228-5p* hsa-miR-1207-5p hsa-miR-1224-5p hsa-miR-92b-5p hsa-miR-339-5p hsa-miR-1471 hsa-miR-324-5p hsa-miR-93-5p	Miller et al. ³²
mRNA signature	E6/E7 mRNA Present	Absent	Ndiaye C et al. ³³
Cell cycle and survival	%	%	Agrawal N et al. ³⁴
TP53 mutation	03	84	Cancer Genome Atlas Network. ³⁵
PI3K3CA	56	34	Lechner M et al. ²⁵
EGFR	06	15	
FGFR1	00	10	
PTEN	06	12	
HPV E6/E7	100	09	
MET	00	02	
CCND1	03	31	
CDKN2A	00	58	
Let-7c	17	40	
E2F1	19	02	
MYC	03	14	
Cell death			
TRAF3	22	01	
CASP8	03	01	
FADD	06	32	
Differentiation			
NOTCH1	17	26	
TP63	19	28	
FAT1	03	32	
AJUBA	00	07	
Oxidative stress			
NFE2L2	00	14	
Chromosomal Alterations	Chromosome 11q deletion Prominence of amplification of 3q Amplification at 20q11	Focal amplification on chromosome 7	Hayes DN et al. ³⁶
IHC signature molecule	P16 ^{INK4a}	P53overexpression, Cyclin D1 overexpression, Rb gene deletion	Ndiaye C et al. ³³
Mutation signature	TpC mutations (C >T) Increased APOBEC cytosine deaminase activity PIK3CA mutations in two hotspots (E542K, E545K)	Mutation at CpG sites more frequent PIK3CA mutations are seen throughout the gene and are seen much less commonly in the hot spots.	Hayes DN et al. ³⁶

cells with features of conventional dysplasia the dysplastic epithelium shows diffuse and strong p16 immunoreactivity while adjacent non-dysplastic epithelium is p16 negative. The transition between the tonsillar crypt epithelium and crypt epithelium is abrupt unlike the field cancerization with sequential dysplastic changes evident in tobacco associated carcinoma which is usually associated with a keratinizing type of dysplasia. HPV-HNSCC has a basaloid infiltrating morphology with a prominent lymphoid response and minimal desmoplastic reaction. The TILs are numerous and may sometimes impart a lymphoepithelial morphology to the tumour. The basaloid tumour cells lack intercellular bridging cytoplasmic keratinization and show a high nuclear cytoplasmic ratio. Lymphnode metastasis and central necrosis with cystic tumour degeneration is frequently encountered and the cysts may sometimes clinically mimic branchial cleft cysts. Classically demonstrated in HNSCC by disruption of the basement membrane may be difficult to distinguish in tonsillar crypts due to the porous nature of the epithelium and due to the deep location of the tonsillar crypt epithelium, lack of stromal desmoplasia and blurred interface between the epithelium and lymphoid stroma.

The dedifferentiated appearance as per the standard grading criteria would put HPV HNSCC in the moderate to poorly differentiated category. However it is significant to recognize HPV- HNSCC as a well differentiated neoplasm, and the role of keratinization in prognosticating these lesions is disputed. Predictors of poorer outcomes include anaplasia and tumour cell mutinucelation.^{19,40}

Morphological distinction of HPV SCC from classical basaloid SCC is difficult. The basaloid SCC has an aggressive clinical course and needs to be distinguished. Demonstration of HPV or surrogate markers may help distinguish these lesions and hence HPV testing may be a part of protocol in all oropharyngeal carcinoma but also in all basaloid morphology Oral SCC. The HPV positivity overrides traditional prognostic indicators such as tumour grade and histological subtype. However when small cell carcinoma morphology is associated with the HPV SCC phenotype it confers a poor prognosis to the patient. Both the small cell and SCC components of the tumour have been reported to show HPV positivity.^{19,41}

Lymphoepithelial (or undifferentiated) carcinoma occurs in the oropharynx (approximately 2–3%).⁴² Tumour cells are arranged

in syncytial sheets and sometimes singly dispersed tumor cells with a dense lymphoplasmacytic inflammatory infiltrate. The large undifferentiated tumour cells have vesicular nuclei with prominent nuclear membranes and macronucleoli. Cytoplasm has ill defined cell borders and is moderate amount and eosinophilic. The nuclei are classically vesicular with thick nuclear membranes and prominent single macro nucleoli. These can be very subtle, mixed in with the background lymphoid tissue of the tonsil and base of tongue. Between 95 and 100% of such tumors in the oropharynx are transcriptionally active high-risk HPV positive.^{43,44}

The morphology of the HPV SCC is retained at metastatic sites and may be accompanied by cystic change of nodes and HPV testing at these sites will also reveal a positive virus and surrogate marker profile.^{45,46} The high level of p16 expression is frequently used as an indirect evidence of HPV infection and may help in cases with a classical morphology. However in the absence of a classical morphology alternative testing methods including HPV hybridization may be used. This test is however limited by a low sensitivity.⁴⁷

4.1. Modification in staging of HPV positive OPSCC

The updated 8th edition of AJCC cancer defined a separate staging system for HPV mediated (p16 positive) OPSCC.⁴⁸ To categorize tumors in this group cut off for p16 is 2+/3+ intensity in >75% cells. Since lymphnode metastases with a similar morphology may occur in nasopharyngeal carcinoma molecular testing for both HR-HPV and EBER is recommended. The level of metastasis in OPSCC is usually level II and III cervical nodes Metastases may go upto the superior mediastinal (level VII) nodes which are also considered regional lymphnodes. A different N category is proposed in p16 positive cases where the extranodal extension, laterality or nodal size are not taken into consideration unlike conventional OSCC and OPSCC where these are key prognostication factors. The N3 category has been eliminated. N1 is metastasis in 4 or less nodes and N2 is involvement of more than 4 nodes. Stage IV is reserved for cases with distant metastasis. T categories are the same as for clinical and pathological staging.⁴⁸ Co-morbidity measures included in the Zubrod/Eastern Cooperative Oncology group (ECOG) score or the Karnofsky performance scale are recommended as specific measures of additional medical illnesses which have a level II evidence of being prognostic indicators along with standard staging. Tobacco use and smoking are also negative prognostic factors.⁴⁹

4.2. Prognosis

HPV induced cervical cancer has shown one of the most effective forms of cancer screening, early detection, and increased cure rates in cancer to date. HPV is now recognized as a likely cause of a subset of head and neck cancer, particularly in oropharyngeal and tonsillar lesions.⁵⁰ It is interesting that HPV-associated cancers tend to have increased survival and better outcomes. Schwartz and colleagues found that HPV-16-positive patients had significantly reduced overall and disease-specific mortality rates compared with other patients after adjustment for age, stage, treatment, smoking, alcohol, education, and comorbid disease.⁵¹ In a meta-analysis of HPV-HNSCC, Nichols et al. concluded that patients with HPV positive tumors had improved 3-year overall survival (OS) and disease-free survival (DFS) compared to patients with HPV negative tumors (90% vs 65%, $p=0.001$, and 85% vs 49%, $p=0.005$, respectively). There is evidence to support that patients with p16 expressing OPSCC have a better prognosis than those with p16 non-expressing OPSCC. The meta-analyses, which included overall survival, local recurrence, disease-free survival, disease-specific survival, and event-free survival, showed significantly

favorable rates for p16 expressing patients in all studies. Subgroup analyses with p16+/HPV DNA+ patients and p16+/HPV RNA+ patients were also favorable.⁵² (Table 1).

5. Treatment

The use of surgery, radiation, and/or chemotherapy depends on tumor resectability and location and feasibility of organ preservation with negative margins.⁵³ The Surgical treatment is the main option for primary and secondary HNSCC as well as recurrent disease.⁵⁴ Transoral laser surgery is the treatment of choice. In inoperable cases or where negative margins cannot be achieved primary radio-chemotherapy is an alternative. Radiation therapy combined with simultaneous 5-fluorouracil (5-FU), cisplatin, carboplatin, and mitomycin C as single drug or combinations of 5-FU with one of the other drugs results in a large survival advantage irrespective the employed radiation schedule. Cetuximab in combination with platinum/5-FU has emerged as a new alternative regimen for untreated patients based on results from the firstline treatment of recurrent or metastatic HNSCC trial.⁵⁵ The taxanes, docetaxel and paclitaxel are active in HNSCC. Radiation therapy intensity modulated (IMRT) has increasingly been shown to be advantageous compared with traditional techniques.⁵⁶ Novel therapies that target specific molecular components have been evaluated, for example, EGFR (Epidermal growth factor receptor), is overexpressed in more than 90% in the HNSCC, may be treated with tyrosine kinase inhibitors. In the event of failed conventional therapies uncommon mutations for example, PIK3CA mutations are seen in as approximately 5–10% of HNSCC and may be utilized as targets. Clinical trials have investigated drugs targeting PIK3CA and have shown some promising results. it is expected that targeted therapy will become a clinical option for treatment in HNSCC.⁵⁷

6. Controversies in the role of HPV in OSCC

Oral cancer has been defined as a neoplasm involving the oral cavity which begins at the lip and ends at the anterior pillar of the fauces. While the role of HPV in OPSCC is well defined and high frequency has been observed, the frequency of HPV in OSCC is low in Asia and worldwide and majority of OSCC are associated with a tobacco habit. We have observed in our studies on OSCC and risk factors that HPV positive cases show a confounding effect with tobacco. Role of HPV-related carcinogenesis in the etiology of oral squamous cell carcinoma (OSCC) is still vague in the Indian population. Use of p16 protein as a surrogate marker to assess the potential etiological role of HPV in OSCC in our population is not reliable.⁵⁸ In our attempt to elicit the differences in the expression pattern of p16 and p53 in HPV-positive and HPV-negative OSCC, if any we observed that risk factors including oral tobacco consumption and alcohol were present in all these ten p16-positive cases. Presence of mutant-type p53 and exposure to tobacco-related risk factors in both HPV-positive and negative cases suggest existence of p53-related carcinogenesis in HPV-positive cases in Indian population. We observed a basaloid morphology in only one case of 31 positive case while all other cases were keratinizing SCC (Fig. 1). Further survival of patients was not affected by HPV, p16 and p53 status. Hence unlike OPSCC, HPV positive OSCC are not always HPV associated cancers and hence do not form a separate prognostic and treatment group.⁵⁹

7. Prevention and vaccines

In the current context, primary prevention measures would include quitting cigarette smoking, limiting alcohol drinking, avoiding tobacco chewing, preventing exposure to second hand

Fig. 1. (A) HR-HPV positive OSCC showing a basaloid morphology (B) expressing >75% p16 in cell cytoplasm and nuclei with (C) overexpression of p53; (D) HR-HPV negative conventional keratinizing OSCC with (E) negative p16 expression and (F) overexpressed p53; (G) HR-HPV positive OPSCC with (H) strong p16 expression (I) and negative p53.

tobacco smoke, environmental carcinogens. Good oral health, nutritional habits and decreasing stress as well as proactive screening for HPV could be good primary measures for preventing or delaying HNSCC development.⁶⁰ The poor prognosis for HNSCC is primarily due to disease detection at advanced stages. Therefore, the understanding of the field cancerization and molecular genetics of HNSCC is essential to provide various biomarkers based tests with potential application for screening, diagnosis, staging, monitoring, and prognosticating.⁶¹ Prevention strategies for HPV positive HNSCC need to be framed as have been defined for uterine cervical carcinoma with cytological stratification and action flowcharts in the event of abnormality. The role of bivalent and quadrivalent vaccines in prevention of cervical cancer has also been advocated for young girls for cervical cancer prevention. In view of the increasing evidence of HPV-HNSCC in men the extension of the HPV vaccine schedule as a policy to boys as well may play a role in future decrease and prevention of HPV-OPSCC.

7.1. Screening for HPV in oral cavity

Promising molecular screening approaches have increased sensitivity for detection. Real-time PCR of HPV-associated DNA is now the standard for detection at low thresholds. However, use of these technologies as screening tests suffers from low sensitivity. Saliva screening for the HPV virus by Zhao and associates found a 45.6% incidence of tumor HPV positivity in HNSCC patients, of whom 57% had detectable salivary rinse HPV-16.⁶² Serological screening is limited by the number of HPV-seropositive patients who will not develop head and neck cancer or have seropositivity

from any number of other sources of infection. Competitive PCR combined with mass spectrometry gives positive results of one-copy number of DNA over real-time PCR, but the issue of false-positive nonpathologic detection of HPV exists.⁶³

8. Future scope for research

Important avenues include development of well defined biomarker profile tests to delineate the HPV associated and tobacco related HNSCC & development and testing of strategies based on randomised prospective data for differential treatment regimes in the two different types of HNSCC. Future clinical trials should be designed either to prospectively stratify patients by p16 status or other HPV related biomarkers or to conduct trials dedicated exclusively to HPV-HNSCC. Given the apparent less aggressive nature of the HPV related neoplasia and improved response to current treatment modalities, patients with HPV pathway expressing tumors may be able to receive adequate curative intent therapy from a reduced radiation or chemotherapy dose.

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