Indian J Otolaryngol Head Neck Surg
(October 2019) 71(Suppl 1):S1002–S1011; https://doi.org/10.1007/s12070-019-01683-1

ORIGINAL ARTICLE

Biomarkers in Head and Neck Cancer an Update

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Received: 19 February 2019 / Accepted: 4 June 2019 / Published online: 19 June 2019

Abstract The study is aimed at establishing the purpose of tumour markers, their application, classification, diagnostic and therapeutic roles in the management of head and neck cancer. A literature review using Medline, Scopus, Google Scholar, the Cochrane Database of Systematic Reviews and the Cochrane central register of controlled trials for articles published between 1993 and 2016 on tumour markers and their role in head and neck cancer was performed. A broader search of prognostic markers in head and neck cancer was also carried out to avoid missing other pertinent markers. Natural history, tumour biology, stage and prognostic factors influence the outcome of management in patients with Head and Neck Squamous cell carcinoma (HNSCC). Evaluation of the cellular lineage and histogenic origin of diverse neoplasms can be done using tumour biomarkers. Identifying predictive tumour markers can lead to improvement in preventive management of HNSCC. There has been remarkable advancement in molecular technology with gene expression and proteomic profiling. Integration of specific tumour markers into routine clinical practice requires substantiation through well designed clinical trials. The investigation of tumour markers is imperative as they influence the prognosis of HNSCC and provide the potential to improve outcomes of treatment through targeted therapy. We have outlined recent tumour biomarkers in this review which have significant role in diagnosis, screening and prognostication in HNSCC. Recent advancement in clinical applications, therapeutic strategies of tumour markers has been highlighted.

Keywords Tumour marker · Biomarker · Head and neck cancer · Oral cancer · Squamous cell carcinoma · Human papilloma virus · EGFR

Introduction

Head and neck squamous cell carcinoma (HNSCC) represents 6% of all newly diagnosed cancer cases. As the sixth most common type of cancer it affects squamous epithelium of the upper aerodigestive tract. HNSCC causes 350,000 cancer related mortalities worldwide and 650,000 new cases are diagnosed every year [1].

Although the overall 5-year survival is approximately 50%, those patients who present with stage 1 and 2 HNSCC do well. In contrast, the 65% of patients who present with advanced stage have significantly compromised survival [2].

The relative higher incidence of advanced stage tumours could be related to limited symptomatology in patients with early stage or swift progression from early to advanced stage in HNSCC tumours. Up to 40% of cN0 necks harbor occult metastatic disease. Hence, developing tumour biomarkers to detect metastasis at early stage is essential. Tumour markers play a significant role in secondary prevention. Tumour differentiation can be quantified using biochemical and immunological representation as tumour markers. Currently, the FDA has approved 28 biomarkers after robust in vitro tests for clinical use [3]. However,

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there is no protein or mutation marker approved for diagnosis or prognosis in HNSCC by the FDA.

Tumour Markers

Tumour marker can be defined as a measurable molecular factor that signifies the presence of tumour and allows its quantification in the host aiding its differentiation from normal tissues of the host as determined by quantitative and qualitative measurements through molecular, biochemical and immunological investigation for neoplasia in body fluids, cells and tissues [4, 5].

Tumour markers help in detecting pre-malignant lesions, predicting tumour growth, its invasive biology and in ascertaining its metastatic potential. Analysis of tumour antigens can be done using immunohistochemistry, cytogenetics, fluorescent in situ hybridization (FISH), reverse transcriptase and polymerase chain reaction [6].

Clinical Application of Tumour Markers

1. Screening in both asymptomatic and symptomatic population and identifying possible diagnostic differentials in the latter population
2. *Early Detection of Cancer* p53 mutation, Loss of heterozygosity (LOH) on chromosomes 3p, 9p, 17p, 18q and promoter hypermethylation detected in saliva, could be markers useful for early detection of HNSCC. The ribonucleoprotein enzyme, Telomerase, activity is found in more than 90% of invasive head and neck cancer, 100% of premalignant lesions, and 100% head and neck cancer cell lines, whereas normal tissues exhibit no activity. Detection of telomerase activity in saliva serves as a molecular marker for early diagnosis in high risk HNSCC [7]. Salivary Interleukin-8(IL-8) and melanoma associated antigens (MAGE) showed good sensitivity, specificity, in the early detection of HNSCC [8].
3. *Diagnosis of lymph node metastases* Immunohistochemistry can identify 5–50% of positive lymph node metastasis deemed negative through histological analysis. Reverse Transcriptase (RT-PCR), when combined with sentinel node biopsy in quantitative analysis of Cytokeratin and E48 antigen expressions, has the potential to improve sensitivity and specificity of nodal staging thereby minimizing the number of neck dissection required for cN0 patients. Gene expression profiling helps as a prognosticator of lymph node metastasis.
4. *Detecting tumour cell dissemination* Immunocytochemical analysis enables quantification of Cytokeratin and E48 expression, as biomarkers for detection of distant metastasis.
5. *Surgical margin analysis* Negative surgical margins in advanced tumours does not lower the local recurrence rate, which is astoundingly still 10–30% [9, 10]. p53 mutation, tumor suppressor gene mutations or LOH on chromosomes 3p, 9p, 17p, 18q in histologically clear margins can be used to predict increased risk of local recurrence. eIF4E overexpression, hypermethylation of p16 and O6-methylguanine- DNA-methyl transferase (MGMT) genes in negative surgical margins are reliable prognosticators [7].
6. *Cancer staging* Although TNM staging helps in prognostication and selecting treatment strategies, but tumour behaviour is highly variable based on intrinsic tumour characteristics that is variable between different stages. Predictive tumour markers that help to identify development, metastasis, invasion and tumour burden facilitates accurate staging, prognostication and select appropriate interventional strategies for that particular tumour incorporating both clinical and pathological staging. Somatic alterations in cancer cells are being used to devise diagnostics, facilitate staging and formulate cancer specific treatment strategies.
7. *Estimation of tumor volume* Innovations in biomarker technology with mass spectrometry, 18F PET-CT radiotracers incorporating hypoxia and proliferation tracers, advanced MRI characteristics helps us to study the tumour volume, treatment response, metastasis and recurrence more accurately.
8. Prognostic indicators for tumour progression (Table 1)
9. *Detecting recurrence of cancer* A clinically sensitive tumor marker will reflect the amount of viable tumor burden, assuming homogeneity of production in tumor cell population [5].
10. *Prognosis and monitoring effects of therapy* Assessing the effectiveness of surgery by measuring the half-life of tumor marker and if prolonged, signifies the presence of residual disease.
11. *Radioimmunolocalisation of tumour masses* Radioactive labeled antibody specific tumour marker injected intravenously or into the lymphatic system gets accumulated in metastatic tumor cells detected by isotope scanning.
12. *Targets and direction for immunotherapy* Biochemical alterations causing genetic instability leading to HNSCC can be detected at genetic, epigenetic, nuclear and mitochondrial DNA level. These transcriptional abnormalities affecting p53, pRb, EGFR, VEGF, PI3 K, MAPK, JAK/STAT pathways, Beta Catenin expression could be used for

Table 1 Prognostic Indicators for tumour progression

Markers to predict response to therapy	A. Steroid-regulated proteins- Cathepsin D and pS2
Markers to monitor drug resistance	P-glycoprotein (a transmembrane protein)
Growth factors and receptors	Epidermal growth factor receptors, erb-2 oncoprotein, insulin and insulin-like growth factor receptors, transforming growth factor - receptors, fibroblast growth factor receptors and the somatostatin receptors
Tumour angiogenesis	Microvascular density-independent prognosticator
Tumour growth fraction	Ki 67 Antibody, Proliferating Cell Nuclear Antigen (PCNA) & P27 KiP1 Gene
Tumour suppressor genes	pRb, p53
Anti-apoptosis genes	bcl-2
Nm23 Anti-metastasis gene	Higher in melanoma cell line with low metastasis
DNA repair genes- Microsatellite instability(MSI)	
Miscellaneous	K-ras and c-myc oncogenes transforming factors-TGF-a, TGG-b Adhesion proteins-E-cadherin and CD 44 Matrix metaloproteases and inhibitors

targeted inhibitors with monoclonal antibodies in immunotherapy.

13. *Developing vaccines*, such as Gardasil, targeted against HPV 6,11,16,18, for use in girls and young women against cervical cancer and young men (age, 9-26) against genital warts and HPV-associated cancers including HNSCC.
14. Tumour Markers in HNSCC.

Advancements in the analysis of genetic alteration and malignant transformation of cells help to understand the fundamental biochemical basis of head and neck carcinogenesis with a wide array of tumour markers available for various tumour subsites.

Oral cavity(CK19,CK8,Beta 2-microglobulin,CD 44,CD 80,1-ACT,CA125,Cyfra21-1, Cyclin D1, Ki6758, CKD2,MIB, C-erb2), Oropharyngeal(p16,pRb,HPV DNA,E6/7 viral genes,elF4E, p53), Laryngeal/Hypopharyngeal(TGF-alpha,Cathepsin-D,EGFR,VEGF,PD-ECGF,FGF).

Classification of Tumour Markers

Tumour markers can be categorized by their origin into tumour derived (tumour specific) and tumour associated (host response) [11]. They are classified based on molecular structure and functions into diagnostic or prognostic markers. Classification of tumour markers proposed earlier were inconsistent due to everchanging new array of molecular markers identified which makes contemporaneous classification systems obsolete within short period [12, 13]. It is difficult to devise a standardized and globally acceptable classification system for tumour markers. A comprehensive organization of the tumour markers by their type of tissue interaction was given by Scully and Burkhardt (1993) [14]. An updated classification was given in 2003 by Schliephake H and Chimenos-Küstner E classified markers based on tumour growth, tumour suppression and anti-tumour response, tumour invasion and metastasis, angiogenesis, cell surface and intracellular markers, markers of aberrant keratinization, arachidonic acid products and enzymes [15, 16].

HNSCC

Signaling pathways involving p53, pRb, EGFR, P13-kinase, mTOR, DNA repair, angiogenesis, genetic instability, dissociation, invasion, cellular adhesion, migration and metastasis are commonly affected in HNSCC [17]. Approximately 75% of HNSCC cases are directly related to tobacco and alcohol abuse [18, 19].

Tobacco and alcohol cause genetic changes in tumour suppressor genes(TSG), affects chromosomal segregation, telomere stability, alteration in genome copy number, p53 mutation affecting gene expression and alters notch signaling pathways. High expression of Notch1 is found in metastatic oral cancer. Tobacco causes loss of heterozygosity(LOH) in TSG detected by polymorphism studies. DNA disruption causes mutation by destabilizing the DNA damage response that triggers the downstream pathway to control genomic integrity. These genetic changes could be used to develop diagnostic and targeted therapeutic modalities in oral cancer.

Hashibe et al. from a pooled analysis reported that population attributable risk for alcohol alone was 4%, tobacco alone was33% and 35% for both. The synergistic risk association of tobacco and alcohol with HNSCC was reported with odds ratio of developing HNSCC to be 1.06(0.88–1.28) for tobacco alone users, 2.37(1.66–3.39)

Table 2 HNSCC relevant tumour markers and their applications

Tumour marker	Purpose	Use
Alpha-1-antichymotrypsin (1-ACT) & factor XIIIa	Oral cavity—giant cell lesion	Diagnosis
antibodies		
BCL-2 GST-pi, p53, bax expression	HNSCC	Detecting pathological response and prognostic
Beta 2-Microglobulin	Oral submucous fibrosis, oral cancer	Diagnosis
Cytokeratin-CK19, CK8	Oral SCC	Premalignant lesion
ENDOGLINS-	Oral SCC	Low CD44 = Decreased Survival
CD44	Oral SCC	Low CD80 = High Tumorogenecity
CD80	Adenoid cystic Salivary Neoplasm	CD105 in Vessel = Metastatic risk
CD105		
Cathepsin-d	Cervical lymph node metastasis in HNSCC	Prognosis
CEA, CA19-9, CA125, SCC-Ag	CEA in Adenoid cystic carcinoma	Prognosis
	CA125 in Oral SCC	Diagnosis
	Cyfra 21-1 in Oral SCC	Diagnosis
Carbohydrate associated antigens	Markers of salivary glandular differentiation	Diagnosis
C-erb2	Oral SCC	Progression free survival and overall survival decreased in recurrent HNSCC
		Predict high risk of recurrence of tongue SCC
Cyclin D1, Ki6758, CKD2,MIB	Oral SCC, Precancerous Lesions	Surrogate endpoint marker in chemoprevention trial, Prognosis
TGF-alpha	HNSCC	Increased relapse and adverse survival

for alcohol users and 5.73(3.62–9.09) for users of both [20].

Prediction of clinical outcomes in HNSCC by p53, EGFR, TGF-a, and cyclin D1 is supported by substantial evidence [21] (Table-2). In early preneoplastic lesions, LOH of 9p21 is seen in 30% of squamous hyperplasia [6]. Loss of chromosomal region 9p21 (70–80%) and loss of 3p chromosomal region is seen in squamous dysplasia and HNSCC. Aggressive tumours exhibit amplification of 11q13 and over-expression of cyclin D1 (30%) [6].

P16 is a transcript encoded by CDKN2A gene locus of chromosome 9p21, and it regulates cell cycle whereas p14 inactivates p53. P16 is inactivated in HNSCC by either homozygous deletion or promoter methylation. P16 +/ p53wild type is a positive prognosticator in HNSCC associated with HPV and further research is required to analyse the benefit of surgical ablative treatment in patients with this phenotype.

Tumor suppressor gene TP53 with point mutations and LOH of 17p are detected in approximately 50% of HNSCC. These mutations appear to occur at a later stage of transformation from acute dysplasia to invasive carcinoma [6].

Specific Applications of Tumour Markers in HNSCC

Epithelial Tumour Marker

Cytokeratin- Currently 54 out of 60 cytokeratin genes identified in human genome are functional genes. They differ in their tissue reactive pattern and immunoreactivity. They help in the diagnosis of spindle cell tumours, malignant melanoma, HNSCC, leiomyosarcoma, ameloblastoma (solid multicystic, desmoplastic with squamous differentiation, adenomatoid-ductal, tubular, whorled patterns), mixed salivary gland tumour. Merkel Cell Carcinoma is differentiated from round cell sarcoma and lymphoma by the presence of paranuclear keratin. It is infrequently seen in haemagiopericytoma, malignant fibrohistiocytoma, hemangiopericytoma, angiosarcoma, rhabdomyosarcoma, hemangioendothelioma and liposarcoma [22].

Growth Factors

EGFR- Epidermal growth factor receptor (EGFR) is overexpressed in more than 90% of HNSCC cases. [23]

This overexpression corresponds to tumour growth and progression, resistance to therapy and poor outcome for patients with HNSCC [24]. Although many molecular agents are available in the treatment of HNSCC, Cetuximab was the first to be used in standard practice. It is a monoclonal antibody targeting extracellular part of EGFR and its expression. The improvement in survival rates in patients treated with combined cetuximab and conventional radiotherapy highlights the importance of biomarker application [25].

EGFRvIII- Not yet validated in clinical use but may have an effect on sensitivity to cetuximab [26].

EGFR kinase domain mutations- Low prevalence, unclear relevance [26].

Tyrosine kinase inhibitors targeting EGFR (erlotinib, gefitinib) and VEGF (sunitinib, sorafenib, vandetanib, semaxanib, and foretinib) are undergoing phase II and III clinical trials currently. EphB4 and EphrinB2 belong to tyrosine kinase-Eph receptor family. Reduced overall survival in HNSCC can be identified with overexpression of these markers. Hence, they can be utilized for targeted therapy [27].

Growth factors correlate well as prognosticators of advanced tongue cancer [28] (Table 3). Although vascular endothelial growth factor(VEGF) is a vital angiogenic factor in HNSCC [29] it does not correlate with field cancerization or transition to dysplasia [30]. Bevacizumab (a monoclonal antibody to VEGF) and Sorafenib or Sunitinib (multitarget receptor tyrosine kinase inhibitors) have promising results in HNSCC [31, 32].

VEGF-C or LVD (lymphatic vessel density) can diagnose lymphatic metastasis of oral SCC effectively. [21] Fibroblast growth factor (FGF) proves to be a significant prognostic factor in advanced HNSCC [33].

p53

Mutation in p53 tumour suppressor gene and its expression provides an invaluable marker for detection of recurrence and second primary in HNSCC. It can also predict the tumour resistance to radiotherapy. Preoperative serum p53 antibody is an important prognostic marker for nodal metastasis of HNSCC [34].

Cyclin D1/EMS1 (11q13), EGFR and Neu

Lymph node metastasis in HNSCC [35].

E-cadherin

Nodal metastasis can be detected by the failure of expression of E-Cadherin in primary tumour.

CD 44

The tendency of tumors to metastasize has been strongly correlated with down regulation of CD44v6 expression in HNSCC [36] and CD44 h expression in laryngeal [37] and tongue cancer [38].

ERCC1 (Excision Repair Cross-complementation Group 1) Polymorphisms

ERCC1 expression prior to therapy has an inverse relationship with survival and response to platinum agents as it repairs the damaged DNA following cisplatin treatment. Although not validated yet, this potential of ERCC1 can be utilized to prognosticate the efficacy of radiotherapy treatment. ERCC1 and its mRNA expression associated with nucleotide excision repair pathway (NER) were connected with better reaction to correlates better response to chemotherapy. The genes related to NER could be used as potential markers in the treatment of HNSCC.

β-Tubulin

Taxanes such as docetaxel is used with cisplatin and 5-fluorouracil in the induction therapy for HNSCC. Few isotypes of β-Tubulin has an inverse relation with the response to taxanes, but needs further validation [26, 39].

Table 3 Biomarkers in HNSCC diagnosis, screening, prognosis and treatment

Marker	Detection method	Species detected	Usage
HPV DNA	PCR	DNA	Diagnosis/Prognosis
HPV E6/E7	ISH	RNA	Diagnosis/Prognosis
Expression profile	Array/RT-PCR	RNA	Diagnosis/Prognosis
EGFR	IHC	Protein	Treatment
VEGF	IHC	Protein	Treatment

k-RAS Mutations

Has a low prevalence rate in HNSCC and doesn't have relevant predictive value [26].

Salivary IL 8 and Serum IL 6

It is 99% sensitive and 90% specific in detecting oropharyngeal cancer. It is 91% sensitive and specific in early detection of oral cancer [8, 40].

MAGE A1-A6(Melanoma-Associated Gene)

Studied from oral tumour specimen is found to be 85.5% sensitive and 100% specific in early detection of oral cancer and 99% sensitive and 90% specific in detecting oropharyngeal cancer [8, 41]. There was no correlation found between elevated levels of CYFRA 21-1, SCC antigen, CEA and lymph node metastasis in advanced HNSCC [42].

Chemokine Receptors

Altered expression of chemokine receptors (CXCR2-Laryngeal SCC, CXCR4, CCR7) help predict prognosis and metastasis in HNSCC patients [43].

Miscellaneous Markers

1. Proto-oncogene eIF4E (4E)—Useful in Laryngeal premalignant lesions.
2. IL1RN, MAL and MMP1 (matrix metalloproteins)—Tumour diagnosis in HNSCC
3. Podoplanin-helps predict poor clinical outcome in HNSCC and lymph node metastasis.
4. Heat stable alkaline phosphatase, Galectin, Serum Ferritin—Prognostic predictors in Hnscc.

Genetic and Epigenetic Expressions in HNSCC

Currently, the concept of primary tumour gene expression and their signatures in prognosticating metastasis is controversial. Expression profiles of certain genes differ significantly between HPV-positive and HPV-negative tumors. Validation of these gene expression signatures is vital to correlate with clinical outcome. Genetic polymorphisms as well as molecular tumour markers help validate and enhance accuracy in predicting outcomes. A recent meta-analysis identified polymorphisms in CCND1, FGFR and XRCC1 influencing survival in HNSCC. Significant relationship is also seen between polymorphism of GSTM1-null genotype, epoxide hydrolase EPHX1 (codon 113 Tyr/His and His/His) genotypes, ALDH2*1/*2, p53 (codon 72 Pro/Pro) and increased risk of HNSCC [44, 45].

Hypermethylation of CDH1 in HNSCC was found to enhance overall survival in low smokers and it's believed that a co-factor plays a significant role in epigenetic alteration related to tumourigenesis [46].

A recent discovery in Florida identified CCT 1a (Choline phosphate cytidylyltransferase-a), the second antigen identified by an anti-ERCC1 monoclonal antibody, 8F1, to be a promising prognosticator of survival in HNSCC [47].

The oncogene FOXM1-induced epigenetic markers that involve DNA methylation of genes showed GLT8D1 to be hypomethylated and C6orf136 hypermethylated in tissues of HNSCC.

They are promising biomarkers, predictive of pre-cancer diagnosis. The advantage of epigenetic DNA marker includes detection from non-invasive specimens, e.g., saliva, serum, mucosal brushings [48].

Mutations in PI3 K pathway mutations can be used as prognostic markers aiding treatment planning in HNSCC [49].

Loss of heterozygosity (LOH) can dysregulate Kruppel-like transcription factor (KLF6) gene. This tumor suppressor gene (TSG) can be used as a biomarker to identify tumour recurrence in HNSCC and predict patient survival [50].

Biomarkers that predict local recurrence are vital as they can be used clinically to identify patients who can benefit from either concomitant chemoradiation approaches or altered fractionation schedules. Despite avoiding performance of unrewarding neck dissection post-radiation in non-beneficial patients it can also facilitate the development of new molecular targeted therapy [50].

Field cancerization could cause second primary tumours in approximately 4% of HNSCC patients annually [51]. Developing a biomarker of field cancerization and related targeted therapy may be the way forward to prevent recurrence and second primary tumour.

Hpv

HPV, especially HPV16 is known to be an independent, causative risk factor for oral and oropharyngeal HNSCC. The HPV genomic DNA is found in 15–67% of HNSCC [26]. This association differs depending on tumour site. The oropharyngeal cancer (64%), and more specifically, cancer of the tonsil (45–67%) and base of tongue (38.4%) are found to be significantly associated with HPV DNA. It is less often found in Hypopharyngeal cancer (13–25%), and very rarely in oral cavity (12–18%) and larynx (3–7%) [52–55].

The oncogenic variant HPV-16 is found in 90% of HPV DNA related HNSCC [56]. The oncoproteins, E6 and E7, encoded by high risk HPV16 and HPV18, promote cellular transformation and alteration of the cell cycle control. pRb is bound by E7 thereby undergoes proteolysis. E6 enhances ubiquitin mediated proteolysis and degrades p53 [57].

Transcription, translation and eventually viral replication takes place following viral genome integration into host causing disruption of transcription repressor gene E2Fs. This inhibition is triggered by viral-host genome integration in 84% of HPV-Cervical Carcinoma and 21–43% in OPSCC thereby allowing epigenetic regulated disruption of E2Fs in the remaining proportion of cases. Hence detection methods targeting presence of HPV DNA and transcriptional products E6/E7 help in high yield of accurate diagnostic rates.

Tests to detect presence of HPV DNA in tumour tissue include PCR and chromogenic in situ hybridization (CISH) or DNA in situ hybridization (DISH). There is poor correlation between PCR and CISH. Tests that detect biological activity in tumour cells include Reverse transcriptase PCR to detect E6/E7 transcription products and using immunohistochemistry(IHC) to detect p16, a tumour suppressor protein, overexpressed when unregulated E7 oncogene expression causes impairment of pRb causing phosphorylation and losing inhibition on tumour suppressor gene E2Fs [58].

P16

Detecting p16 overexpression could be overestimated as the downstream stimulation of pRb can also happen through non-viral mechanism [59]. IHC is cheap and widely available. p16 expression correlates well with outcomes but has relatively low specificity for HPV infection. The diagnostic algorithm initiated from John Hopkins Institute by Westra in 2009 has been widely utilized and has undergone new modifications with technological advancement. Due to variations in sensitivity and specificity of individual tests, variability in detection rates influenced by different diagnostic modalities used and detection of biological activity being critical, effective diagnostic algorithm are designed to detect presence of HPV and its biological/transcriptional activity in the most reliable, accurate and cost-effective pragmatic way [60]. First, IHC for p16 is performed and if positive HPV 16 specific CISH/DISH is done and if both positive then a diagnosis of HPV 16 + is made. However, discordant cases (p16 +/HPV16-) undergo CISH/DISH to detect other high-risk genotypes. The discordant rates have been reported to be about 20% [60, 61]. Further, the role of E6/E7 mRNA insitu hybridization (RISH) in detecting HPV status in OPSCC was found to be very helpful, particularly in discordant cases [62]. RISH identified E6/E7 in 88% of discordant cases and concurred 100% of concordant cases with good interobserver reproducibility rates. RISH detects the presence of HPV DNA and its transcriptional and biological activity in a single assay. HPV as a strong prognostic factor, warrants dedicated trial designs. Results of de-escalation trials will change the paradigm in managing HPV positive patients.

Advances in Biomarker Technology

Novel biomarkers are being discovered using advanced molecular technologies such as proteomic profiling and gene expression. Identifying changes in gene expression prior to oncological transformation serves a key step to discover biomarkers that helps in diagnosing early stage malignancy. These biomarkers, by predicting behavior of various tumours, help in specific targeted therapy [6]. Transforming laboratory systems into automated miniature card/chip form helps simplifying and advancing the diagnostic methodologies in HNSCC. These techniques have been implemented in performing cell lysis, PCR, product sizing, and microarray analysis. Analysis of a sample of saliva placed on the chip can detect specific cancerous cells based on a series of biomarkers [6].

It is almost unlikely that one marker can conclusively ascertain metastasis due to the complexity it involves. Compiling several markers may help in better prognostication than a lone isolated marker. Microarray technology allows gene profiling at RNA and DNA level for nearly all expressed genes, thus enabling investigation of the prognostic or therapeutic significance of multiple biomarkers simultaneously in various tumours [6]. Thus far, many technologies including microarray have yet to be employed outside laboratory in HNSCC [6].

Advances in the proteomic analysis have led to the discovery of proteins and their expression related to carcinogenesis that can be quantified from the tissue as well as saliva or serum samples. Proteins can be detected by ELISA in body fluids or further by immunohistochemistry (IHC) in tissue hence making them very attractive biomarkers. ELISA and IHC are easy techniques, cost-effective and commonly applied in clinical pathology [2].

A mass spectrometry (MS) based method called SRM (selected reaction monitoring), introduced recently, and has been useful in selecting, quantifying and validating potential biomarkers. It may also replace antibody-based methods in future [2]. In a recent discovery, proteomic profiling, based on MS, was performed on HNSCC cell lines (FaDu, UTSCC8 and UTSCC42a) in conditioned media (secretome). This proteomic profiling along with gene expression and microarray technology identified high

tumor expression of MMP14, PLAU, and THBS1and IGFBP7. The findings correlated with decreased disease-free survival and increased relapse [63].

There is ongoing development in utilizing PET/CT as imaging biomarker through discovery of novel 18F-based radiotracers. This is done using complimentary mechanism-specific disease characterization. The radiotracers of HNSCC under study include proliferation tracer 30-deoxy-30-18F fluorothymidine and the hypoxia tracer 18F-fluoromisonodazole (F-MISO). Dynamic contrast enhanced-MRI (DCE-MRI) and diffusion-weighted MRI can be used to observe the response of tumor via localization, detect recurrence and measure tumor perfusion changes and vascular integrity quantitatively [64].

Limitation in the Use of Tumour Markers

The heterogeneity of tumors, differences in results interpreted by different techniques employed to study the same biomarker, variability in scoring patterns of biomarkers are some of the limiting factors of biomarkers [6]. Biopsy material may not be useful to study biomarkers dependent on tumour host interaction. Variability in biomarker expression reflects disparity in tumour biology related to different head and neck subsites. Variations of tumour marker levels are present between cancer patients and controls. The sensitivity and specificity of a tumour marker must be at least above 95% for it to be a valid screening tool, as the number of false positives created otherwise could suffer significant anxiety and psychological stress [65]. Regardless of the specificity of some tumor markers, tumour recurrence cannot be ruled out by a negative marker value.

Conclusion

Identifying specific molecular changes in different premalignant lesions and malignant tumours that could potentially guide management has been made possible by advances in understanding the genetics and molecular basis of human malignancies. Absence of overlap in molecules with good predictive prognostication amid similar studies, variation of tumor specific characteristics and smaller sample size are the major factors influencing criticisms on studies pertaining to development of biomarkers. Studies with larger sample size, validation of any patterns or differences in gene expression profiling and uniform tumour specimen characteristics are required to overcome these drawbacks. Biomarkers combined with imaging modalities help in predicting regional nodal metastasis and helps to select high-risk patients for elective neck management. Pending further clinical studies, ERCC1 and HPV will revolutionize the way we manage HNSCC.

Management of HNSCC has become more specific and treatment pathway is inherently dependant on patient selection which could be guided by validated predictive and prognostic biomarkers. With new discoveries and validations each year, the future of biomarker use in routine clinical practice is already in the horizon.

Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

Human and Animal Rights This article does not contain any studies with human participants or animals performed by any of the authors.

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