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REVIEW

Updated overview of current biomarkers in head and neck carcinoma

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Abstract

Squamous cell cancer is the most common type of malignancy arising from the epithelial cells of the head and neck region. Head and neck squamous cell carcinoma (HNSCC) is one of the predominant causes of cancer related casualties worldwide. Overall prognosis in this disease has improved to some extent with the advancements in therapeutic modalities but detection of primary tumor at its initial stage and prevention of relapse are the major targets to be achieved for further improvement in terms of survival rate of patients. Latest achievements in basic research regarding molecular characterization of the disease has helped in better perception of the molecular mechanisms involved in HNSCC progression and also in recognizing and targeting various molecular biomarkers associated with HNSCC. In the present article, we review the information regarding latest and potential biomarkers for the early detection of HNSCC. A detailed molecular characterization, ultimately, is likely to improve the development of new therapeutic strategies, potentially relevant to diagnosis and prognosis of head and neck cancers. The need for more accurate and timely disease prediction has generated enormous research interests in this field.

Key words: Head and neck squamous cell carcinoma; Early detection; Prognosis; Biomarkers; Molecular level

Core tip: Early detection of head and neck squamous cell carcinoma is vital in improving the overall survival and prognosis. It can be achieved by use of latest biomarkers. With advancement in knowledge of molecular characteristics of this disease, various biomarkers acting at molecular level have been identified. This review compiles information regarding the potential players in this field.

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INTRODUCTION

The term head and neck carcinoma encompasses all malignancies arising in the nasal and oral cavities, pharynx, larynx and the paranasal sinuses. Majority of these (approximately 95%) epithelial cancers are squamous cell carcinomas[1]. Smoking and alcoholism are two well known predisposing factors[2]. Head and neck squamous cell carcinoma (HNSCC) is reported to be the sixth common cause of cancer mortality throughout the world[2].

There is no significant improvement in the mortality rates even with continuous research and trials in the field of diagnostics and therapeutics[3]. As compared to other cancers like breast, cervix and colorectal, the five-year survival rate of HNSCC after diagnosis is significantly lower[4,5]. The reason for this could be failure in early diagnosis and insufficient effectiveness of therapeutic modalities[6,7]. The predominant cause of mortality in HNSCC is regional and/or distant metastatic spreading of tumor cells from primary site[8]. Therefore, the vital area in the treatment of head and neck cancers is ability to diagnose it at an early stage.

EARLY DIAGNOSIS OF HNSCC

Till date only one third cases of HNSCC are being diagnosed at an early stage and rest land up with an advanced disease in the United States[9,10]. The major reason put forward for this trend include a lack of appropriate screening biomarkers[11]. The treatment of neoplasia is most effective in its early stage when the tumor size at primary site is lowest with least lymphatic and hematogenous spread. Therefore, early diagnosis and intervention is of utmost significance in the treatment of HNSCC. Here comes the role of biomarkers. Biomarkers may be analyzed in the tissue itself, plasma or other body fluids like saliva in case of HNSCC. The drawback of biomarkers may include lack of specificity and sensitivity but these may prove to be essential tools in timely diagnosis of the disease[12]. A variety of biomarkers have been reported in literature with a promising potential but these are still in the need of clinical validation. In this article, we present a review of different biomarkers which may be utilized in early diagnosis and timely decision-making for intervention in patients of HNSCC.

ALTERATION IN EXPRESSION OF CHEMOKINE RECEPTORS

Recently, the importance of chemokines and their cognate receptors in head and neck cancers is being reported by increasing number of studies.

CXC chemokine receptor 2

In the squamous cell carcinoma of the larynx, the expression of CXC chemokine receptor 2 (CXCR2) has been observed to be substantially higher in tumor tissue than that in the paraneoplastic tissue. The increased expression has been reported to be significantly related with lymph node metastasis, histological grade and 5-year survival of these patients. Thus, expression of CXCR2 can be considered as a potent prognostic marker for laryngeal squamous cell carcinoma[13].

CXCR4

The importance of CXCR4 in tumour progression and organ-specific metastasis in patients with HNSCC has been reported by a number of authors[14,15]. Wang et al studied the expression of CXCR4 in nasopharyngeal carcinoma tissues and found an increased CXCR4 expression in tumor tissues. Besides this, they also suggested that the increased expression of CXCR4 may be correlated with increased metastatic rates and poor overall survival of the patients[16]. This finding was consistent with another study which also reported significantly elevated CXCR4 mRNA in HNSCC tissues as compared to paraneoplastic tissues and that the increased expression was associated with increased risk of lymph node metastasis and distant metastasis[17]. Therefore, CXCR4 expression can also be used as a marker to predict prognosis and metastasis in patients with HNSCC.

CC chemokine receptor 7

CC chemokine receptor 7 (CCR7) is another CC chemokine receptor, which has been demonstrated to play a significant role in the migration of activated dendritic cells to regional lymph nodes. Its expression has also been reported to be elevated in HNSCC tumor tissues as compared to paraneoplastic tissues. Furthermore, the elevated expression of CCR7 has been found to correlate with lymph node metastasis and tumor tissue histological differentiation status[17]. Similar findings have been reported by another study which analyzed the expression of CCR7 in primary and metastatic tumor cell lines and also in biopsy material from both primary and metastatic lesions. They reported that CCR7 expression was increased in metastatic cells and tissues[18]. On the basis of these reports, an important role may be conferred to CCR7 in predicting the metastasis and prognosis in HNSCC patients.

HUMAN PAPILLOMA VIRUS

Human papilloma virus (HPV), especially HPV16, is considered one of the causing factors for HNSCC. HPV DNA has been found in 15% to 25% of HNSCC and the association differs depending on the site of the tumor[19]. HPV DNA is detected in 45%-67% of cases of cancers of the tonsil, in 13%-25% of hypopharyngeal cancer, in 12%-18% of the cancers of oral cavity and in 3%-7% of carcinoma larynx and it may be associated with prognosis of disease, especially in tonsillar cancers[20]. There are reports in literature suggesting that HNSCC with HPV has a favorable prognosis and that, it in fact, is a distinct clinicopathological entity[21]. HPV16 and HPV18

are considered to be the high risk HPVs, which produce E6 and E7 oncoproteins, implicated in transformation of cell and altering the control of cell cycle. Oncoprotein E7 binds to and induces the proteolysis of pRb while E6 inactivates p53 by accelerating its ubiquitin mediated degradation[22]. Thus, HPV DNA may act as a diagnostic and prognostic marker in patients of HNSCC.

It is of interest to know that adding $p16^{INK4A}$ immunostaining to HPV DNA detection may prove to be very useful in diagnosing HPV-related oral squamous cell carcinoma and it has been observed that HPV(+) and $p16^{INK4A}$(+) types of tumors have better prognosis[23]. As reported by Danish Head and Neck Cancer Group 5 trial, $p16^{INK4A}$(+) tumors appeared to be associated more strongly with poor histopathologic differentiation as compared to the $p16^{INK4A}$(-) ones, but the difference was not statistically significant, indicating that $p16^{INK4A}$ alone is not an adequate marker[24]. In the study of panitumamub efficacy in patients with recurrent and/or metastatic head and neck cancer (SPECTRUM), the authors reported that the $p16^{INK4A}$ status of the tumor might have significant bearings in designing future trials in cases of recurrent or metastatic HNSCC[25].

MICROSATELLITE INSTABILITY

Microsatellite instability (MSI) may be analyzed using different markers. Researchers have detected loss of heterozygosity (LOH) in tumor cell derived DNA (deoxyribonucleic acid) from mouth washing or lesion brushing samples in patients with T2N0M0 and T1N0M0 tumors[26]. MSI analysis in tumor cell DNA is of value in detection of pre-malignant conditions like erythroplakia and leukoplakia[1]. It has also been reported that LOH of 9p21 may be an initial event in HNSCC and may be associated with preneoplastic lesions as well as 30% of cases of squamous cell carcinoma[27]. Loss of chromosomal region 9p21 is seen in > 70% of cases, making it the most frequent genetic alteration seen in squamous cell dysplasia and HNSCC[27,28]. Some of the studies in which MSI was analyzed using different set of markers in patients with HNSCC have also reported MSI in 12.5%-35% of the cases while microsatellite alteration rate was detected to be 75%-95%[29-31]. Instability frequency has been reported to be related to the repeat unit length and overall size of the short tandem repeat (STR) affecting the probability of error during DNA replication. STR characteristics vary in different populations and those with longer average repeat size are more prone to instability than the ones having smaller repeat size[31].

MSI analyses have the disadvantage of lack of uniformity in selection of different methods or the type and number of markers evaluated[32]. A standard approach is yet to be developed for this marker to be useful as an early diagnostic marker in HNSCC patients.

METHYLATION

Gene activation due to hypermethylation of cytosine-phosphate-guanine (CpG)-rich promoter regions has been reported in early stages of HNSCC[30]. A specificity of 96% in salivary specimens for methylation specific polymerase chain reaction has been reported for detection of HNSCC[33]. Whereas it was observed to be 90% in salivary samples and 72% for serum samples in yet another study[34]. The lower rate of promoter hypermethylation may be due to dilution with normal, non-methylated DNA from normal mucosal areas[34]. It has also been reported that promoter hypermethylation may be associated with age and ethnicity of the patient or with history of chronic tobacco or alcohol consumption[35,36].

The disadvantages of methylation markers include lack of sensitivity, specificity, complexity and inconvenience in HNSCC detection in body fluids.

METALLOPROTEINASES

These include a large number of zinc and calcium dependent endopeptidases. These enzymes are implicated in extracellular matrix degradation leading to spread of the tumor cells out of the tissue of origin[37-39]. Besides migration of tumor cells, metalloproteinases (MMPs) play a significant role in providing a microenvironment conducive for the growth and angiogenesis of tumors. These also help in cellular differentiation, proliferation and apoptosis in tumor tissues[38]. Several types of MMPs, *e.g.,* MMP-1, the gelatinases (MMP-2 and MMP-9) and the stromelysins (MMP-3 and MMP-10) play a role in tissue invasion by cancer cells and metastasis[40,41]. Elevated levels of MMP-2 or MMP-9 have been observed in many types of cancers including HNSCC, lung, breast, colorectal and ovarian carcinoma indicating an association with tumor progression[42-45].

In HNSCC, patients have been found to have increased levels of MMP-3, MMP-8 and MMP-9[46] while MMP-1 and MMP-10 have been reported to be useful for detection of cancer of oral cavity and gingiva[47]. In another study, MMP-9 has been reported to be able to detect stage Ⅰ HNSCC disease with 80% positivity[48]. The disadvantage with MMP-9 lies in its poor specificity to discriminate cancer with benign disease[49].

INTERLEUKINS

Interleukin (IL)-6 and IL-8 have been linked with tumor progression and metastasis along with playing a role in the process of carcinogenesis[12]. IL-8 holds potential for acting as an early biomarker in salivary samples while IL-6 in serum samples for detection of oral cavity or oropharynx squamous cell carcinoma (OSCC)[50,51]. In some other studies, increased levels of IL-6 and IL-8 have been reported in a variety of specimens like cell line supernatants, tumor tissues and serum of patients with HNSCC[52,53].

Zimmermann *et al*[54] reported that four mRNAs (OAZ, SAT, IL-8 and IL-1β) in salivary samples have a collective sensitivity and specificity of 91% in detection of cancer of oral cavity. On the other hand, the levels

of salivary IL-8 were found to be raised in patients of OSCC as compared to controls but the difference was not statistically significant[55]. Thus, further studies are required to establish the sensitivity and specificity of IL-8 and IL-6 as biomarkers in patients of OSCC.

MICRO RNA

These are small non-coding RNA (ribonucleic acid) sequences playing a role in regulation of gene expression affecting a variety of physiological processes[56]. miRNAs, by virtue of their vast range of consequences may act both as oncogenes and tumor supressor genes[57]. In many types of cancers, dysregulation of genes for miRNAs has been reported and these can be used for detection and classification of different solid tumors[58]. The change in micro RNAs (miRNAs) in cancer cells as compared to normal cells has been reported to be many folds than the extent of change in mRNA[59].

It has been proposed that miR-106b-25 cluster and miR-375 may be involved in development and progression of HNSCC and that miR-451 could act as a prospective prognostic marker for recurrence in HNSCC patients. The same authors also observed one third of the miRNAs to be dysregulated in HNSCC[60]. Park *et al*[61] reported significantly lower levels of miR-125a and miR-200a in the saliva of OSCC patients as compared to controls. miR-205 has been found to have a variable expression in a number of tumor cells and, particularly, to be highly overexpressed in HNSCC cell lines and may prove helpful in detecting occult metastatic tumor deposits[62,63]. Deregulation of miR-138 has been commonly found in HNSCC and other types of cancer. A number of functional targets for miR-38 have been reported which include genes involved in initiation and progression of HNSCC[64]. It has also been demonstrated that restoration of transfected miR-34a mimics significantly inhibits the capability for epithelial-mesenchymal transition of cancer stem cell-phenotype and functionally decreases clonogenic and invasive capability in HNSCC cell lines[65].

Micro RNA biomarkers are superior to their mRNA counterparts. Because of their robust profiling and better stability in routine clinical samples, they may prove to be more suitable for analysis in some tissue samples[64]. Thus, miRNA may prove to be promising early biomarkers in detection of HNSCC but further research is needed to substantiate their role as screening tools.

MELANOMA-ASSOCIATED GENE

Melanoma-associated gene (MAGE) participates in the process of carcinogenesis by suppressing apoptosis[66]. Other similar tumor-specific shared antigen families like G antigen gene, B melanoma antigen gene and L antigen family 3 gene have been categorized at molecular levels[67-69]. These antigens, usually peptides in nature, may be significantly associated with tumor immunology as their expression has been found specific to tumor cells, *e.g.,* HNSCC, melanoma, carcinoma ovary, bladder cancer, carcinoma lung and colorectal cancer[70-73]. Expression of MAGE A3 and A4 has been found to be positive in early invasive carcinoma (by excisional biopsy) where brush and incisional biopsy was negative in a suspicious looking leukoplakic lesion[74]. Expression of MAGE has also been shown in the sputum samples of patients with HNSCC[75]. Therefore, it may be used as an early biomarker for HNSCC detection as it has not been observed to be expressed in normal healthy tissues with exception of testis[76]. Other studies have also reported 85.5%-90% expression rate of MAGE in HNSCC tissue[77,78]. It may help in initiating target specific immunotherapy in these patients[79]. According to a recent report by Lee *et al*[80] expression of MAGE-A1-6 in sputum predicts poor oncologic outcome in patients with squamous cell carcinoma of the larynx and hypopharynx. MAGE-A expression has been reported to be associated with poorer five year survival rate, thus, indicating its potential as a prognostic marker also[81].

CENTROSOME ABNORMALITIES

Centrosome abnormalities have also been observed in HNSCC. It has been reported that 17 out of 18 tumor samples analyzed from patients with HNSCC demonstrated centrosome hyperamplification. Based on these findings, it has been suggested by authors that centrosomal hyperamplification could be used as a marker for HNSCC[82]. Furthermore, the p53 suppressor gene, the most commonly mutated gene found in human cancers, has been reported to correlate with centrosome hyperamplification in HNSCC. Centrosome hyperamplification is either observed in tumors with mutated p53 or in tumours that retain wild type p53 but with an overexpressed Mdm2, an oncogene which is responsible for inhibiting the transactivation function of p53[83]. Increased frequency of centrosomal abnormalities has also been seen in OSCC in cells with spindle checkpoint protein CDC20 overexpression[84]. This may be because of the fact that in cancer cells, genes that encode for proteins involved in mitotic checkpoints/mitotic regulations are generally found mutated or overexpressed.

ACTIN AND MYOSIN

These are cytoskeletal proteins responsible for cell motility and invasion which are important components of epithelial tumorigenesis[85]. Increased expression of actin and myosin has been observed in exfoliated cells present in soluble saliva in patients with malignancy as compared to those with pre-cancerous lesions[86]. Increased actin isoforms have been observed in invasive basal cell carcinoma[87], squamous cell carcinoma of cervix[88] and esophagus[89] and invasive OSCC[90]. Increase in myosin abundance has been observed in proteomics of tissue from OSCC region[91]. However, Turhani

et al[92] have reported a lesser expression of myosin light chain in HNSCC contradicting the existing findings.

Thus, actin and myosin need rigorous research with larger sample groups including issues like sensitivity and specificity for their establishment as HNSCC biomarkers.

CYTOKERATINS

Cytokeratins (CKs) are one of the major components of intracellular filament network found in different tissues[93]. CKs are expressed in a number of combinations depending on the type of epithelial cell of origin[94]. They are further divided into two subtypes (Ⅰ and Ⅱ) that are generally coexpressed[95,96]. These are found to be overexpressed in OSCC tissue as compared to normal mucosa[93]. Overexpression of cytokeratins has been related with tumor progression and prognosis[97]. Constitutive expression of cytokeratin-17 (CK-17) in the lungs is only found in the normal basal cells[98]. It is now emerging as a tissue-specific immunohistochemical biomarker in squamous cell carcinoma of larynx[99]. CK-17 mRNA overexpression has been demonstrated in OSCC by few authors[96,99,100]. These studies were mainly performed in cancer tissue and not in saliva or serum samples. Increased expression of CK-17 has been demonstrated in respiratory syncitial virus infected epithelial cells also[95].

As CK-6 and CK-16 are found to be constitutively expressed in mucosal stratified squamous epithelia, they may be ragarded as markers of cellular hyperproliferation. CK-6 may also be considered as an additional squamous differentiation biomarker in poorly-differentiated cancers. Though CK-17 was also detected in most of the cases, its expression is not found to be uniform[101].

These markers need stringent workup with larger samples, including body fluids and also on issues regarding its specificity before their validation as biomarkers for HNSCC.

p53

It is the most frequently studied molecular marker in HNSCC[102]. The p53 pathway is activated when cells become old or damaged. The p53, a 53 kd protein, may then arrest cell cycle for DNA to be repaired or lead to apoptosis if damage is irreparable[103]. Alteration in function of p53 may be seen as a result of mutation or sequestration by other cellular proteins. Mutations of p53 gene are the most commonly encountered mutations in carcinomas including HNSCC[102,104]. p53 is associated with maintenance of cellular integrity and is regarded as guardian of genome[105]. Mutations in p53 in HNSCC patients have been reported by a number of researchers with an expression range of 50%-60% of the tumor cells[103,105-108]. Its expression can be conveniently studied with immunohistochemistry techniques for detection of cancers but complete role of p53 in pathogenesis of HNSCC is still not clear[109,110]. Survival rate has also been reported to be higher in p53 negative patients as compared to those who are positive for p53 mutations[102]. Thus, this marker has a fair potential for diagnostic and prognostic use in patients with HNSCC. Another interesting finding is that HPV infection rarely coexists with p53 mutation as both of them can independently lead to p53 inactivation implicated in HNSCC tumor[111].

EUKARYOTIC TRANSLATION FACTOR 4E

It is a protein involved in the initiation of protein synthesis[112]. Overexpression of eukaryotic translation factor 4E (eIF4E) has been found associated with different stages of carcinogenesis including metastasis. It is related with transformation of fibroblasts and primary epithelial cells[113,114]. Overexpression of this protein in mice has been found to be associated with a number of malignancies like lymphomas, angiosarcomas, hepatomas and carcinoma lung[115]. An expression of 100% in HNSCC has been reported in some studies[116,117]. Overexpression of eIF4E in cancers like breast, bladder, lung and HNSCC has been found to correlate with an increased risk of disease progression and poor prognosis[113,118-121]. Another study has reported overexpression of eIF4E in tumor free surgical margins to be related to loco-regional recurrence in patients of HNSCC[122].

Therefore, eIF4E may prove to be a significant independent prognostic predictor in terms of recurrence and survival in patients of HNSCC.

LOSS OF FUNCTION OF DNA REPAIR GENES

Effective DNA repair may be considered as a major determinant of cancer-free survival. Various mutations in DNA repair genes, especially, of the nucleotide excision repair (NER) group (XP genes in xeroderma pigmentosum patients), DNA crosslink repair (Fanconi anemia genes) mutations affecting the mismatch repair genes, and a number of others are the cause of several hereditary cancerous syndromes[123]. The dominant moderator of mismatch repair in HNSCC is promoter hypermethylation rather than direct mutation[124]. There is also limited data in HNSCC demonstrating a link between poly-(ADP-Ribose) polymerase overexpression and cisplatin resistance suggesting a possible role for chemoresistant tumours. Hyperphosphorylation of replication protein A, a single-strand DNA binding protein, that is, integral to HR, has also been implicated as a mechanism for cisplatin resistance in HNSCC cell lines[124]. Multiple (5-7) risk NER genotypes have been associated with a 2.4-fold increased relative risk of second primary HNSCC[124]. The inactivation of these DNA repair genes may be linked to carcinogenesis by decreasing genomic stability and producing certain genetic alterations[125] (Table 1).

Table 1 Comparison of different biomarkers of head neck squamous cell carcinoma

Marker	Mechanism	Type of specimen	Role	Limitations
Chemokine receptors	Increased expression in tumor tissue	Biopsy specimen	Prognosis and metastasis	Clinical validation by further research required
HPV	DNA associated HNSCC, oncoprotein production	Tumor tissue	Diagnosis and prognosis	Lack of sensitivity and specificity
MSI	LOH in tumor derived DNA	Mouth washings/lesion brushings	Detection of pre-malignant lesion	Lack of uniformity of method
Methylation markers	Gene inactivation following hypermethylation in promoter region	Saliva/serum	Early detection	Lack of sensitivity/specificity, complex methodology
MMPs	Provide conducive microenvironment for tumor growth, degrade ECM promoting tumor migration	Tumor tissue/saliva	Early detection	Poor specificity
Interleukins	Participate in process of tumor growth and metastasis	Tumor tissue/cell line supernatants/saliva/serum	Early and convenient biomarker	Lack of sensitivity and specificity
miRNA	Role in regulation of gene expression	Tumor tissue/saliva	Early detection	Clinical validation required
MAGE	Suppresses apoptosis	Biopsy specimen/saliva	Prognosis and in selecting targeted immunotherapy	Clinical validation required
Centrosome abnormalities	Mutation due to hyperamplification	Tumor tissue	Early detection	Further research required to understand molecular mechanism
Actin and myosin	Increased expression leading to greater invasiveness	Tumor tissue/saliva	Early detection	Lack of sensitivity and specificity
Cytokeratins	Over-expression associated with tumor progression	Tumor tissue/saliva/serum	Early detection and prognosis	Clinical validation required
p53	Mutation affects apoptosis/repair of malignant cells	Tumor tissue	Diagnosis, prognosis, convenient marker	Complete role in HNSCC yet to be deciphered
eIF4E	Overexpression associated with tranformation of fibroblasts and epithelial cells	Tumor tissue	Prognostic indicator	Lack of sensitivity and specificity

HNSCC: Head and neck squamous cell carcinoma; MSI: Microsatellite instability; LOH: Loss of heterozygosity; MMP: Metalloproteinase; ECM: Extracellular matrix; MAGE: Melanoma-associated gene; HPV: Human papilloma virus; miRNA: Micro RNA; eIF4E: Eukaryotic translation factor 4E.

ROLE OF IMAGING BIOMARKES

Besides biochemical and pathological biomarkers, a significant role is being played by imaging biomarkers in the early detection of head and neck oncology. To go into the details of these biomarkers is beyond the scope of the present article, but these are proving to be vital in initial staging, treatment planning, monitoring and follow-up of the patients with HNSCC non-invasively. ^{18}F-fluoro-2-deoxyglucose positron emission tomography/computerized tomography (PET/CT) proves to be more sensitive and specific as compared to magnetic resonance imaging (MRI) or CT alone[126,127]. Recently introduced regional PET/Gd (gadolinium-enhanced T1-weighted)-MRI combined with whole-body PET/MRI appears to be quite promising in detecting early lesions[128]. There is a need for further refinement and a concerted approach regarding imaging and molecular biomarkers for HNSCC which may help in early detection, targeted therapy and improved monitoring.

CONCLUSION

Understanding the molecular mechanisms of HNSCC is important to identify its biomarkers. Finding genetic alterations can lead to early detection of the disease. These can be detected in tumor tissue, saliva/body fluids washing the affected tissue or in the serum. A variety of molecular markers have been explained in literature. There may be a tremendous role of these markers in affecting the outcome of the disease by aiding in timely diagnosis and even in selecting specific therapy. Many of these have already shown their potential in this field like interleukins, MAGE, MSI, *etc.*, but still there are issues of specificity, sensitivity and clinical validation with some of these. With more standardised and uniform platform for sample selection, processing and data analysis along with stringent workup of the cases, these biomarkers may prove to be indispensable investigative tools in patients with HNSCC and may even help in better understanding of the pathogenesis of the disease. Thus, there is a strong hope that these molecular biomarkers or patterns of markers, alone or in co-ordination with imaging markers, could, in the future, be utilized for early detection of HNSCC, tumor metastasis and may aid in determining the best therapeutic modality for patient care.

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