A systematic review of proteomic biomarkers in oral squamous cell cancer

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Abstract

Background: Head and neck squamous cell cancer (HNSCC) is the most common cancer associated with chewing tobacco, in the world. As this is divided into sites and subsites, it does not make it to top 10 cancers. The most common subsite is the oral cancer. At the time of diagnosis, more than 50% of patients with oral squamous cell cancers (OSCC) had advanced disease, indicating the lack of availability of early detection and risk assessment biomarkers. The new protein biomarker development and discovery will aid in early diagnosis and treatment which lead to targeted treatment and ultimately a good prognosis.

Methods: This systematic review was performed as per PRISMA guidelines. All relevant studies assessing characteristics of oral cancer and proteomics were considered for analysis. Only human studies published in English were included, and abstracts, incomplete articles, and cell line or animal studies were excluded.

Results: A total of 308 articles were found, of which 112 were found to be relevant after exclusion. The present review focuses on techniques of cancer proteomics and discovery of biomarkers using these techniques. The signature of protein expression may be used to predict drug response and clinical course of disease and could be used to individualize therapy with such knowledge.

Conclusions: Prospective use of these markers in the clinical setting will enable early detection, prediction of response to treatment, improvement in treatment selection, and early detection of tumor recurrence for disease monitoring. However, most of these markers for OSCC are yet to be validated.

Keywords: Head and neck cancer, Proteomics, Biomarkers, Oral cancer

Background

Oral squamous cell cancer (OSCC) is the most common malignant neoplasm arising in the mucosa of oral cavity and includes subsites like the buccal mucosa, alveolus (upper and lower) tongue, palate, and lip [1]. Head and neck cancer accounts for more than 550,000 cases worldwide annually [2]. Oral cancers are more common in the Indian subcontinent, while cancer of the laryngopharynx is more common in other populations [3]. Overall, 57.5% of global oral cancers occur in Asia especially in India. It is 30% of all cancers in India, of which 60 to 80% of the patients present with advanced diseases as compared to 40% in developed countries, this also suggests lack of awareness and need for markers of early identification [4].

Almost all of these malignancies are squamous cell carcinomas (SSCs) which historically in the developed world was associated mostly with alcohol and tobacco consumption and the combination of the two, producing a synergistic increase in the risk. However, over the past 20 years, investigators have found a growing proportion of HNSCC patients with human papillomavirus (HPV) positive tumors that develop in younger people and those having a lower or no intake of tobacco and alcohol, the association in oropharynx is higher than oral cavity [5].

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Improvement in understanding the steps leading to carcinogenesis will enable the identification and prediction of malignant progression at an earlier stage of OSCC. Cancer signifies deviation from normal signaling network toward a dysregulated cellular proliferation. Proteins with linkages to various pathways when altered the functional state may shift the equilibrium of the signaling network to enhance the survival of the affected cells or reduce its apoptosis [6]. Searching for such proteins is the main purpose of cancer proteomics. Proteins being the common molecule that participate in the cellular function are often affected by disease, response to treatment, and being disease free. Development of novel protein biomarkers of OSCC in the light of proteomics can help in early cancer diagnosis, treatment, and prognosis.

Material and methods
This systematic review was performed as per PRISMA guidelines. A bibliographic search was performed for studies published till August 2021, using PubMed, Cochrane database, Google scholar, the National Library of Medicine, SpringerLink, and Science Open. The keywords used were “proteomic biomarkers,” AND “head and neck cancer,” AND “oral cancer.” The detailed search strategy for PubMed is detailed in Additional file 1. All relevant studies assessing proteomic characteristics of oral cancer and precancers were considered for analysis. Abstracts, incomplete articles, and non-comparative studies and article in language other than English were excluded. We performed a restriction of articles including only studies in humans; studies on cell line and animals were excluded.

The review also discusses proteomics-based techniques that are used in the identification of proteins that are altered in the disease process or in response to treatment or disease stage and course, and such information could be used to individualize therapy. Research findings in the review are highlights from articles focusing on proteomic approaches toward diagnosis and detection of oral cancer; identification of biomarkers through proteolytic analysis carried out using mass spectrometry, 2D electrophoresis, and other proteomic techniques.

Results
The search revealed 304 articles in English of these this systematic review includes a total of 112 articles (Fig. 1). The review articles were excluded, the two meta-analyses published on the subject has been discussed. These articles were categorized under subsections enumerated below followed by a list of all protein biomarkers identified and brief description of their importance.
of the enrichments include one-directional polyacrylamide gel electrophoresis (1D-PAGE), two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) among others [17].

Liquid chromatography, coupled to tandem MS (LC–MS/MS) is used to identify and quantify proteins from human tissues. This is based on interactions between protein, peptide, and column. First, the separation is done by liquid chromatography before identification by mass spectrometry (MS).

A mass spectrometer (MS) has mainly three components: an ionization source, a mass analyzer, and an ion detector [18, 19]. The most common ion sources used are electrospray ionization (ESI) and matrix-assisted laser desorption/ionization (MALDI). These sources produce ion from the sample which are then analyzed on mass spectrophotometer. The main ion analyzers used in proteomics are quadrupole (Q), time of flight (TOF), ion traps, and Fourier transform ion cyclotron (FT-ICR). The cellular localization and quantification are usually done by immunohistochemistry and ELISA; these are also used for validation of protein biomarkers.

OSCC biomarkers
As there is considerable variation in protein expression, there is a variety of potential biomarkers of OSCC. These can be broadly classified in to (i) tissue-based biomarkers, (ii) secretomes (plasma, saliva, blood, or other secretions), and (iii) autoantibodies.
Potential biomarkers

**Tissue-based biomarkers in head and neck cancer (Table 1)**

Majority of selected biomarkers investigated are tissue-based biomarkers by using different approaches and are summarized in Table 1. The approaches employed include LC-MS, RPLC-MS, SELDI-TOF MS, 2D DGE, iTRAQ, and 2DLC. Further, the results verified by using IHC, PCR, and western blot techniques as described above.

**Serum/plasma biomarkers/saliva/secretome (Table 2)**

Majority of selected serum/plasma-based biomarkers by using different approaches are summarized in Table 2. Only a few important ones are discussed.

**Epidermal growth factor receptor (EGFR)**

EGFR is an important member of the family of the membrane-bound tyrosine kinase receptors activated in tumor cells of epithelial origin. This receptor regulates cellular growth, proliferation, apoptosis, differentiation, migration, and secretion of certain proteins [94]. High EGFR expression has been observed in OSCC suggesting that an uncontrolled growth may be mediated by abnormal EGFR expression [82, 124].

**Vitamin D-binding protein**

Vitamin D-binding protein is a secreted transport protein which transports the vitamin D sterols in serum and prevents polymerization of actin. The level of vitamin D-binding protein level was significantly low in OSCC plasma. Plasma fibrinogen is a blood coagulation regulator associated with angiogenic and metastatic prediction in numerous tumors [50]. Vitamin D-binding protein has been used as a biomarker for breast cancer, thyroid cancer, and lung cancer [83]. In oral cancer, it has not been found to be increased in human plasma; however, higher concentrations are observed in mouse plasma [83]. Tung et al. (2013) [82] found vitamin D-binding protein to be reduced in OSCC plasma; these results suggested differential regulation in different species.

**Carcinoembryonic antigen (CEA)**

CEA is a glycoprotein produced by the cells of gastrointestinal tract during embryonic development and is involved in cell adhesion. The salivary and serum levels of CEA were found to be increased in malignant tumors than in healthy tissues [97]. It has been reported previously that the content of saliva CEA was significantly higher in oral-maxillofacial cancer patients and benign tumor than in normal persons (P < 0.01) [97]. Thus, saliva CEA is of guiding significance to a certain extent for identification of malignant and benign tumor, assisting clinical diagnosis and prognosis monitoring of treatment efficacy for cancer [97].

**Autoantibodies (Table 3)**

Majority of selected biomarkers investigated autoantibodies-based biomarkers by using different approaches are summarized in Table 3. Few important ones are discussed herein.

**P53 autoantibody**

p53 antibodies are found in serum and saliva of patients showing overexpression of p53 in their tumor tissues. This is an easy process as these can be detected from saliva [125].

**Hsp 70 autoantibody**

HSPs are frequently overexpressed in tumor cells. Autoantibodies directed against HSP70 can discriminate the risk condition between healthy and tumor cells. Its level increases from healthy controls to SCC, suggesting that autoantibodies might be used as both early marker and screening risk marker for SCC [126].

Discussion

Development of OSCC is a multistep process. Field carcinization is one of the hallmark of oral cancer, wherein the whole of the mucosa of the oral cavity and upper aerodigestive tract undergo molecular changes and is susceptible to develop cancer. Change in the protein expression profile can be a manifestation of the field carcinization and hence its identification is an important biomarker to predict risk of development of cancer, secondary primary or recurrence of OSCC.

Tobacco and alcohol consumption are the major independent risk factors for development of HNSCC that also show synergy when combined [128]. Oral cancer development risk is 3 to 9 times greater in those who smoke and drink than in those who consume neither of the two [6, 128]. The upper aerodigestive tract is first to make
| Sample | Technique | Biomarker [accession no.] | Confirmation by | Biomarker for | Discussion | Reference |
|--------|-----------|---------------------------|-----------------|---------------|------------|-----------|
| OSCC   | RPLC-MS/MS: MS | Desmoglein-3 [P32926] | IHC          | Carcinogenesis | Desmoglein 3 maintains structural integrity preferentially in oral epithelium. Changes in their relative levels might represent putative biomarkers of disease progression. | [18] |
| OSCC   | RPLC-MS/MS: MS | Cytokeratin 4 [P190013] | IHC          | Carcinogenesis | Expressed predominantly in suprabasal-nonkeratinizing layer of stratified epithelium of control normal tissues, whereas OSCC was restricted to only few well differentiated tumors. | [19–22] |
| OSCC   | RPLC-MS/MS: MS | Cytokeratin 16 [P08779] | IHC          | Carcinogenesis | Present in suprabasal layer of oral squamous epithelium in the normal tissue, whereas OSCC expresses positive in most moderate to well-differentiated cells | [18, 20, 23, 24] |
| OSCC   | RPLC-MS/MS: MS | Desmoplakin [P15924]; Vimentin [P08670] | IHC          | Carcinogenesis | Predominantly membranous with high immunoreactivity in suprabasal areas of squamous epithelium in normal tissues, whereas OSCC expresses dominantly along the well-differentiated areas of tumor cells | [25, 26] |
| OSCC   | RPLC-MS/MS: MS | Desmoglein-3 [P32926]; Cytokeratin 4 [P190013]; Cytokeratin 16 [P08779] | IHC          | Carcinogenesis | Involved in epithelial-mesenchymal transition. Malignant squamous cells in tumor cells show high immunoreactivity to vimentin in contrast to a few isolated normal cells. | [18, 26, 27] |
| Sample | Technique | Biomarker [accession no.] | Confirmation by | Biomarker for | Discussion | Reference |
|--------|-----------|--------------------------|-----------------|--------------|------------|-----------|
| OSCC   | IMAC30 protein arrays; SELDI-TOF MS | α-Defensins 1-3 [DEF1-3] | Tissue microarray; IHC | Tumor relapse | Major constituent of azurophilic granules of neutrophils. Normally do not express in epithelia. Plays vital role in mucosal innate immune defense to infectious diseases including epithelial cancers. In OSCC, increased expression of α defensin 1-3 has been described in neutrophils that infiltrate OSCC. In healthy mucosa defensin expression is limited to submucosal neutrophil granulocytes. Represents an important link between inflammation, angiogenesis, and cancer. | [25] |
| OSCC   | 2D DIGE | Keratin 4 [P19013] | IHC, ROC, KMS | OSCC premalignant tissue and second field cancer tissue | Prognostic | Low expression of keratin 4 in resection margins of surgically treated OSCC patients accurately predicts local relapse. Loss of keratin 4 expression is a valuable enrolment criterion for tertiary prevention trials in treated OSCC patients. | [19, 21, 28] |
| OSCC   | 2D DIGE | Keratin 13 [P13646] | IHC, ROC, KMS | OSCC premalignant tissue and second field cancer tissue | Keratin 13 is a protein involved in differentiation process, expression of which changes during the carcinogenic process. Keratin 13 and keratin 4 are such dimers that aggregate to form intermediate filaments of cytoskeleton in epithelial cells. | [19, 21] |
| OSCC   | 2D DIGE | Cornulin [Q9UBG3] | IHC, ROC, KMS | OSCC premalignant tissue and second field cancer tissue | Loss of expression in the surgical margin predicts the risk of local relapse. | [19] |
| Sample | Technique | Biomarker [accession no.] | Confirmation by | Biomarker for | Discussion | Reference |
|--------|-----------|---------------------------|-----------------|---------------|------------|-----------|
| OSCC   | 2D DIGE  | Small proline-rich protein 3 [Q9UBC9] | IHC; ROC; KMS | OSCC premalignant tissue and second field cancer tissue | Expresses high in normal mucosa and low in tumors. Belongs to protein group forming cornified envelope, which is an important protective barrier of mucosa and skin and is involved in the differentiation process. | [18] |
| OSCC   | 2D GE    | Stratifin [P31947]        | IHC             | OSCC          | Overexpressed in HNSCC. Stratifin protein recognizes phosphoserine/threonine-containing motifs to bind target proteins that play important roles in regulation of various cellular processes, including regulation of oncogenes and tumor suppressor genes in carcinogenesis | [18, 25, 29–32] |
| OSCC   | iTRAQ/MDLC | YWHAZ 14-3-3 zeta/delta [P63104] | IHC, WB; rt-PCR; Co-immunoprecipitation assays; ROC; KMS | OSCC          | Overexpressed in different stages of development of OSCC. Involved in cell signaling pathways in inflammation, cell proliferation and abrogation of apoptosis during oral carcinogenesis. Stratifin-YWHAZ heterodimer may serve as a plausible therapeutic strategy by using a small molecule modulator/peptide inhibitor that intervenes with 14-3-3 client protein interactions. | [29, 30] |
| OSCC   | iTRAQ/MDLC | S100-A7 [P31151]        | IHC, WB; rt-PCR; Co-immunoprecipitation assays; ROC; KMS | Prognostic    | A calcium-binding protein, originally identified in psoriatic keratinocytes and is upregulated in abnormally differentiated keratinocytes. Also identified in oral premalignant epithelium and is proposed to be a marker for invasion. | [29, 33–38] |
| Sample                     | Technique | Biomarker [accession no.] | Confirmation by | Biomarker for                        | Discussion                                                                                                                                                                                                                                                                                                                                                                                                  | Reference  |
|----------------------------|-----------|---------------------------|-----------------|--------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------|
| Oral premalignant (leucox- | iTRAQ     | hnRNPK [P61978]          | IHC, WB; rt-PCR; ROC | Epithelial dysplasia (leucoplakia)   | Heterogeneous nuclear ribonucleoprotein K is an RNA-binding protein that regulates gene expression at both transcriptional and translational level. It directly regulates the expression of COX2, implicated in the synthesis of prostaglandins, which are mediators of inflammatory response. hnRNPK is overexpressed aberrantly localized, whose transcriptional upregulation as reported in OSCC.  | [39–41]   |
| plakia)                    |           |                           |                 |                                      |                                                                                                                                                                                                                                                                                                                                                                                                             |           |
| Oral premalignant (leuco- | iTRAQ     | PTHA [P06454]            | IHC, WB; rt-PCR; ROC | Epithelial dysplasia (leucoplakia)   | Prothymosin alpha, overexpressed in oral premalignant lesions, proposed to be a proliferation marker of thyroid cancer.                                                                                                                                                                                                                                                                                                                                                   | [29, 42]  |
| plakia)                    |           |                           |                 |                                      |                                                                                                                                                                                                                                                                                                                                                                                                             |           |
| OSCC                       | 16O/18O-labeling; 2DLC | Thymidine phosphorylase [TYPH] [p19971] | WB, IHC        | Tissue markers for OSCC              | TYPH is overexpressed in wide variety of solid tumors and can be induced by several cytokines and contributes to angiogenesis.                                                                                                                                                                                                                                                                                                                               | [39, 40]  |
| OSCC                       | 16O/18O-labeling; 2DLC | Filamin-A [P21333]       | WB, IHC        | Tissue markers for OSCC              | Filamin A is involved in organization of extracellular matrix that assists the exchange of signals. Overexpressed in OSCC and is reported as a target for DNA-damage based cancer therapy.                                                                                                                                                                                                                                                                                          | [39, 43]  |
| OSCC; OSCC                 | 16O/18O-labeling; 2DLC | Fascin [Q16658]          | WB, IHC        | Tissue markers for OSCC              | Fascin is a globular actin-cross-linking protein that forms parallel actin bundles in cell protrusions. Fascin overexpression promotes cancer progression via AKT and MAPK pathways in OSCC.                                                                                                                                                                                                                                                               | [39, 44]  |
| Sample  | Technique                              | Biomarker [accession no.] | Confirmation by          | Biomarker for | Discussion                                                                                                                                                                                                 | Reference |
|---------|---------------------------------------|---------------------------|--------------------------|---------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------|
| OSCC    | 18O/18O-labeling; 2DLC                | Carbonic anhydrase 2 [P00918] | WB; IHC                  | Tissue markers for OSCC | Carboxylic anhydrases catalyze the equilibrium of carbon dioxide and carbonic acid. Found to be overexpressed in OSCC and can be used to predict local tumor growth in OSCC.                                    | [39, 45]  |
| OSCC    | 2D GE; Coomassie                      | Rack1 [P63244]            | WB; IHC; RT-PCR; Rack1 si-RNA | Severe dysplasia | Originally identified as an anchoring protein for protein kinase C, highly conserved among all eukaryotes and linked to translation initiation in all organisms. Overexpression of RACK1 in OSCC cancer cell lines suggests potential oncogenic property for RACK1 in oral carcinogenesis. | [45]      |
| OSCC    | LC-MS                                 | Keratin 13 [gi62897663]   | WB; IHC; rtPCR;           | OSCC          | Cancer demonstrated down regulation of keratin 13 in OSCC. Aberrant expression indicates dysregulation and cellular transformation of epithelial cells in OSCC.                                                 | [46]      |
| OSCC    | LC-MS                                 | Keratin 4 [109225249]     | WB; IHC; rtPCR;           | OSCC          | Keratin 4 expression was found to be significantly decreased in OSCC samples. Low expression of keratin 4 is associated with morphological changes in affected oral epithelium and can cause changes in cell shape and movement. | [46]      |
| OSCC    | LC-MS                                 | Transglutaminase 3 [gi80478896] | WB; IHC; rtPCR            | OSCC          | Significantly down regulated in cancer and correlated with loss of histological differentiation. Reduction in transglutaminase 3 expression is related with dedifferentiation, increase in invasive phenotype and poor prognosis. | [46]      |
Table 1 (continued)

| Sample                | Technique | Biomarker [accession no.] | Confirmation by | Biomarker for | Discussion                                                                 | Reference |
|-----------------------|-----------|---------------------------|-----------------|---------------|----------------------------------------------------------------------------|-----------|
| OSCC tongue cancer    | LC-MS     | Annexin I [gi442631]     | WB; IHC; rtPCR  | OSCC          | Carcinogenesis                                                             | [46–49]  |
|                       |           |                           |                 |               | Glucocorticoid-inducible protein, Annexin I has emerged as an important endogenous modulator of inflammation. Evident downregulation of ANXA1 in the cancerous lesions was observed. |          |
| OSCC                  | 2D GE     | Enolase1 [P06733]        | IHC; rt-PCR     | OSCC          | Glycolytic enzyme present in cytoplasm, acts as plasminogen receptor on the surface of cells. Detection of enolase 1 was observed significantly higher in OSCC patient saliva compared to healthy individuals. Useful as biomarker for OSCC | [50]     |
| Tongue cancer         | 2D-DIGE   | Cofilins                  | nLC-MS/MS       | Carcinogenesis | Actin binding protein participate in cellular motility severing actin filament, nucleating, depolymerizing, and bundling activities | [47, 51] |
| Tongue cancer         | 2D-DIGE   | Myosin light chain family members | nLC-MS/MS     | Carcinogenesis | Myosin light chains are members of the calmodulin (CaM) and CaM-related gene families involved in the mechanoenzymatic function of the myosin holoenzyme | [47, 52] |
| Tongue cancer         | 2D-DIGE   | Annexin 5                | nLC-MS/MS       | Carcinogenesis | Member of the calcium and phospholipid binding protein family act as immune check point inhibitor and tumor homing molecule | [47]     |
| Tongue cancer         | 2D-DIGE   | HSP A8                   | nLC-MS/MS       | Carcinogenesis | Heat shock cognate 71 act as repressor of transcriptional activator           | [47, 53] |
| Tongue cancer         | 2D-DIGE   | Carbonic anhydrase 1 (CA1) | nLC-MS/MS     | Carcinogenesis | Carbonic anhydrases 1 belong to family of zinc metalloenzymes that catalyze the reversible hydration of carbon dioxide | [47]     |
| Tongue cancer         | 2D-DIGE   | HSP5a (HSP70)            | nLC-MS/MS       | Carcinogenesis | Post-translational transport of small presecretory proteins across endoplasmic reticulum | [24, 47, 53] |
| Sample           | Technique   | Biomarker [accession no.] | Confirmation by | Biomarker for | Discussion                                                                 | Reference |
|------------------|-------------|---------------------------|-----------------|---------------|----------------------------------------------------------------------------|-----------|
| Tongue cancer    | 2D-DIGE     | Serpin B3                 | nLC-MS/MS       | Carcinogenesis | Papain-like cysteine protease inhibitor to modulate the host immune response against tumor cells | [47]      |
| Tongue cancer    | 2D-DIGE     | Tropomyosin alpha-4 chain TPM 4 | nLC-MS/MS   | Carcinogenesis | Binds to actin filament and participate in muscle contraction along with tropomycin complex and calcium dependent regulation | [47]      |
| Tumor            | IHC         | Cystatin B                | Targeted proteomics | Prognostic    | Cysteine protease inhibitors                                               | [54]      |
| OSCC             | iTRAQ       | Gelsolin                  | Immunoassay     | Prognostic    | Actin-modulating protein that participate in severing and capping cytoskeletal actin | [55]      |
| OSCC             | iTRAQ       | Fibronectin               | Immunoassay     | Prognostic    | Extracellular matrix glycoprotein binds to integrins                       | [55]      |
| OSCC             | iTRAQ       | Haptoglobin               | Immunoassay     | Prognostic    | Acute phase protein capable of binding hemoglobin                          | [55, 56]  |
| OSCC             | IHC         | Prothymosin α (PTMA)      | IHC             | Prognostic    | Major component of Thymosin Fraction 5 enhances cell-mediated immunity in humans | [33]      |
| OSCC             | IHC         | Heterogeneous nuclear ribonucleoproteinK (hnRNPK) | IHC         | Prognostic    | Binds to pre-messenger RNA as a component of heterogeneous ribonucleoprotein particles controls cell cycle progression | [33]      |
| OSCC             | Rho GDP-dissociation inhibitor alpha (RhoGDIα) | Prognostic | Regulates the GDP/GTP exchange reaction of the Rho proteins by inhibiting the dissociation of GDP and binding of GTP | [31, 57]  |
| OSCC             | 2D-PAGE/MS  | Annexin A2                | MS              | Carcinogenesis | Involved in cell motility, linkage of membrane-associated protein complexes to the actin cytoskeleton, endocytosis, fibrinolysis, ion channel formation, and cell matrix interactions | [58]      |
| OSCC             | MS          | Complement component C7   | MS              | HPV-induced carcinogenesis | Membrane attack complex (MAC) protein that plays a role in the innate and adaptive immune response | [59]      |
| Sample | Technique | Biomarker (accession no.) | Confirmation by | Biomarker for Discussion | Reference |
|--------|-----------|--------------------------|----------------|--------------------------|-----------|
| OSCC   | MS        | Apolipoprotein F (ApoF)  | MS             | HPV-induced carcinogenesis | [59]      |
| OSCC   | MS        | Galactin 3-binding protein | MS             | HPV-induced carcinogenesis | [59]      |
| OSCC   | AMIDA     | Cytokeratin 8            |                | Carcinogenesis            | [60]      |
| OSCC   | 2D-GE     | Heat shock protein 60    | MS             | Carcinogenesis            | [61]      |
| OSCC   | 2D-GE     | Heat shock protein 27    | MS             | Carcinogenesis            | [61]      |
| OSCC   | GeLC-MS/MS| Nidogen 1 (NID1)         | IHC            | Carcinogenesis            | [62]      |
| OSCC   | IHC       | Thrombospondin 2 (THBS2) |                | Prognosis                | [63]      |
| OSCC   | IHC       | End-binding protein (EB1) | IHC            | Carcinogenesis            | [64]      |
| OSCC   | IHC       | S100 A2                  | IHC            | Carcinogenesis            | [65, 66]  |
| OSCC   | IHC       | Scara5                   | IHC            | Carcinogenesis            | [67]      |
| OSCC   | IHC       | S100 A8                  | IHC            | HPV-induced carcinogenesis | [68, 69]  |
| OSCC   | IHC       | Galactin 7               | IHC            | Carcinogenesis            | [70]      |
| OSCC   | IHC       | Survivin                 | IHC            | Carcinogenesis            | [71]      |
| OSCC   | IHC       | SCC antigen              | IHC            | Carcinogenesis            | [71]      |
| OSCC   | IHC       | Keratin 1                |                | Carcinogenesis            | [48]      |
| Sample   | Technique | Biomarker [accession no.] | Confirmation by | Biomarker for   | Discussion                                                                 | Reference |
|----------|-----------|---------------------------|-----------------|-----------------|-----------------------------------------------------------------------------|-----------|
| OSCC     | IHC       | P53                       | IHC             | Carcinogenesis   | Protein product of tumor suppressor gene, participate in DNA repair        | [72]      |
| OSCC     | IHC       | Deleted in liver cancer (DLC1) | IHC             | Carcinogenesis   | Protein product of tumor suppressor gene regulates Rho GTPase-activating protein (GAP) domain | [73]      |
| OSCC     | IHC       | Carcinoembryonic antigen-related adhesion molecule 1 (CEACAM1) | IHC             | Carcinogenesis   | Mediates cell adhesion by homo and heterophilic bindings                   | [74]      |
| OSCC     | 2DE       | Proteosome activator PA28 a, b and g | MS              | Carcinogenesis   | Protein activator of the 20 S proteasome                                   | [75]      |
| OSCC     | MALDI TOF | NCOA7                     | IHC             | Carcinogenesis   | Nuclear receptor coactivator 7 enhances transcriptional activities and coactivate several other nuclear receptors | [76]      |
| OSCC     | IHC       | C6orf141 (chromosome 6 open reading frame 141) | IHC             | Carcinogenesis   | Cell membrane protein found in many cancers, associated with prognosis of breast and endometrial cancer | [77]      |
| OSCC     | IHC       | SOD2 superoxide dismutase 2 | IHC             | Lymph node metastasis | Member of the iron/manganese superoxide dismutase family               | [78]      |
| OSCC     | IHC       | BST2 bone marrow stromal cell antigen 2 | IHC             | Lymph node metastasis | Acts as a direct physical tether, holding virions to the cell membrane and linking virions to each other | [78]      |
| OSCC     | IHC       | ITGB6 integrin subunit beta 6 | IHC             | Lymph node metastasis | Receptor for fibronectin and cytotactin                                  | [78]      |
| OSCC     | IHC       | PRDX4 peroxiredoxin-4     | IHC             | Lymph node metastasis | Member of the peroxiredoxin family of antioxidant enzymes                | [78]      |
| OSCC     | MALDI     | LRP6 low-density lipoprotein receptor-related protein 6 | IHC             | Carcinogenesis   | Coreceptor of Wnt/beta-catenin signaling                                   | [79]      |
| OSCC     | Bioluminescence | Lactate dehydrogenase (LDH) | Bioluminescence | Carcinogenesis   | Catalyzes the conversion of lactate to pyruvate and back               | [80]      |

Note: IHC immunohistochemistry, WB western blot, ROC receiver operator characteristic analysis, KMS Kaplan-Meier survival analysis, iTRAQ isobaric tags for relative and absolute quantitation, RP reverse phase, OSCC oral squamous cell carcinoma

Modified from Schaaij-Visser BM. Biomarker discovery for head and neck cancer. A proteomics approach. Ipskamp Drukkers B.V., Enschede, The Netherlands ISBN: 978-90-393-5253-3 [81]
### Table 2: Potential protein biomarkers of head and neck cancers: Serum/plasma/saliva/secretome

| Sample                  | Technique             | Biomarker [Accession no.] | Confirmation by | Biomarker for | Discussion                                                                 | Reference |
|------------------------|-----------------------|---------------------------|-----------------|---------------|-----------------------------------------------------------------------------|-----------|
| OSCC                   | sDIGE&iTRAQ/2DLC      | EGFR [A8K2T7]             | ELISA; WB; IHC  | Carcinogenesis | High EGFR expression is observed in OSCC suggesting that uncontrolled growth may be mediated by abnormal EGFR expression. | [72, 82]  |
| OSCC                   | 2D-DIGE; MALDI-TOF MS | Vitamin D-binding protein [P02774] | ELISA; WB      | OSCC plasma marker          | Transport protein for Vitamin D sterols in serum that prevents polymerization of actin. The level of Vitamin D-binding protein level was significantly low in OSCC plasma. | [50, 72, 83] |
| OSCC plasma saliva     | 2D-DIGE; MALDI-TOF MS | Fibrinogen alpha chain [P02671] | ELISA; WB      | OSCC plasma marker          | Plasma fibrinogen is a blood coagulation regulator associated with angiogenic and metastatic prediction in numerous tumors. | [50, 84]  |
| OSCC                   | 2D-DIGE; MALDI-TOF MS | Fibrinogen beta chain [P02675] | ELISA; WB      | OSCC plasma marker          | Blood-borne glycoprotein, functions in inflammatory responses. Showed elevated expression in OSCC samples. | [50]      |
| OSCC                   | 2D-DIGE; MALDI-TOF MS | Fibrinogen gamma chain [Q9UC63] | ELISA; WB      | OSCC plasma marker          | Gamma component of fibrinogen has a major function in homeostasis. Can be considered tumor marker, as the protein shows significantly higher expression in OSCC samples compared to the healthy ones. | [50]      |
| OSCC                   | 2D-DIGE; MALDI-TOF MS | Haptoglobin [P00738]      | ELISA; WB      | OSCC plasma marker          | Plasma protein that binds to hemoglobin. Strong correlation was found between increasing levels of haptoglobin and clinical stages of OSCC. | [50, 55, 83] |
| OSCC                   | 2D-DIGE; MALDI-TOF MS | Leucine-rich alpha-2-glycoprotein/LRG1 [P02750] | ELISA; WB      | OSCC plasma marker          | This is involved in protein-protein interaction, signal transduction, cell adhesion and development. It is expressed during granulocyte differentiation. The expression was found up-regulated in the disease state. | [50]      |
| Sample   | Technique                                      | Biomarker [Accession no.]       | Confirmation by | Biomarker for | Discussion                                                                 | Reference |
|----------|-----------------------------------------------|---------------------------------|-----------------|---------------|-----------------------------------------------------------------------------|-----------|
| OSCC     | 2D-DIGE; MALDI-TOF MS                        | RSK2/Ribosomal protein S6 kinase alpha-3 [P51812] | EUSA; WB       | OSCC          | plasma marker                                                              | [50, 85–87]|
| OSCC saliva | Cation exchange/reversed phase LC, 2D GE       | S90K/Mac-2 binding protein (M28P) | EUSA; WB, ROC  | OSCC          | M2BP, a tumor antigen was significantly up-regulated in nasopharyngeal carcinoma. | [88, 89]  |
| OSCC saliva | Cation exchange/reversed phase LC, 2D GE       | S100-A9                         | EUSA; WB, ROC  | OSCC          | It is a calcium-binding protein which is significantly over-expressed in saliva of OSCC patients. | [88–90]  |
| OSCC saliva | Cation exchange/reversed phase LC, 2D GE       | CD59                           | EUSA; WB, ROC  | OSCC          | CD59 is one of the complement restriction factors that are overexpressed on the tumor cells and enable them to escape from complement-dependent and antibody-mediated killing. | [88, 91]  |
| OSCC saliva | Cation exchange/reversed phase LC, 2D GE       | Profilin                        | EUSA; WB, ROC  | OSCC          | Profilin is regulator of the microfilament system. Overexpressed in tumor cells. Involved in various signaling pathways via interaction with cytoplasmic and nuclear ligand. | [88]      |
| OSCC saliva | Cation exchange/reversed phase LC, 2D GE       | Catalase [P04040]               | EUSA; WB, ROC  | OSCC          | Catalase protects the cells against oxidative stress. Altered levels are evident in human tumors and involved in carcinogenesis and tumor progression. | [88]      |
| OSCC saliva | Peptide free flow electrophoresis, SCX         | Signal transducer and activator of transcription 3 [P40763] | WB             | OSCC          | STAT3 mediates cellular responses to different growth factors. Expression of STAT3 demonstrate its role of development of OSCC. | [92]      |
| Sample            | Technique                          | Biomarker [Accession no.]                                                              | Confirmation by | Biomarker for | Discussion                                                                 | Reference |
|-------------------|------------------------------------|----------------------------------------------------------------------------------------|-----------------|---------------|----------------------------------------------------------------------------|-----------|
| OSCC saliva       | Peptide Free Flow Electrophoresis; SCX | Thioredoxin-dependent peroxide reductase, mitochondrial [P30048]                        | WB              | OSCC          | Involved in redox regulation of cell. Expression of PRDX3 in whole saliva of patient confirmed the presence of the protein in saliva and can be considered a prospective biomarker of OSCC. | [92]      |
| OSCC saliva       | Peptide Free Flow Electrophoresis; SCX | Serpin B3 [P29508]                                                                     | WB              | OSCC          | Modulates the host immune response against tumor cells. Plays tumor inhibitor role. | [92]      |
| OSCC saliva       | 2D GE                              | Alpha-1 antitrypsin (AAT)                                                              | ELISA; IHC      | OSCC          | AAT is a serine protease inhibitor. The level of AAT increased in OSCC saliva. Useful for prediction of aggressive phenotypes in OSCC. | [84, 93] |
| OSCC saliva       | 2D GE                              | Complement C3                                                                          | ELISA; IHC      | OSCC          | Complement system helps antibodies fight of infections. Gauges the effectiveness of ongoing treatments for autoimmune disorder, cancers and infectious diseases. Level of C2 is proportional to aggression of tumor. | [93]      |
| OSCC saliva       | 2D GE                              | Hemopexin [HPX]                                                                        | ELISA; IHC      | OSCC          | HPX is plasma protein with highest binding affinity to heme. Distinctive high expression and tumor size parameter shows aggression of cancer. | [93]      |
| OSCC saliva       | 2D GE                              | Transthyretin [TTR]                                                                    | ELISA; IHC      | OSCC          | TTR is thyroid hormone-binding protein. Overexpressed in non-metastatic OSCC as compared to metastatic. | [51, 93] |
| OSCC Saliva       | MALDI-TOF MS                        | Zinc finger protein 510 (ZNF510)                                                       | IHC; ROC        | OSCC          | ZNF510 is involved in transcriptional regulation. Found to be expressed in high level in saliva of OSCC patients. | [34, 94] |
| OSCC Saliva       | 2D GE, MALDI-TOF                    | Transferrin                                                                             | WB; ELISA       | OSCC          | Transferrin is an iron transport protein whose levels were found elevated in saliva. The level of salivary transferrin shows a relation with size and stage of the disease. | [95, 96] |
| Sample          | Technique | Biomarker [Accession no.] | Confirmation by | Biomarker for | Discussion                                                                                                                                                                                                 | Reference |
|-----------------|-----------|---------------------------|-----------------|---------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------|
| OSCC saliva     | IHC       | Lactate dehydrogenase (LDH) | ELISA           | OSCC          | LDH concentration in saliva indicate cellular necrosis. In an oral OSCC lesion, the level of salivary LDH is expected to increase as the mitotic rate also increases with the aggressiveness of the lesion.                                | [96]      |
| OSCC saliva     | IHC       | Cyclin D1 (CycD1)         | ELISA           | OSCC          | CycD1 is a positive regulator of the transition from G1 to S phase in cell-cycle progression. Expression of CycD1 is amplified and over expressed in OSCC.                                                        | [96]      |
| OSCC saliva     | IHC       | Salivary carbonyls        | ELISA           | OSCC          | Protein carbonyls are markers of oxidative stress. Increase in salivary carbonyl points at increased free radical attack. In malignant tissues the degree of oxidative DNA damage is increased whereas total antioxidant capacity is decreased. | [96]      |
| OSCC saliva     | IHC       | Mammary serine protease inhibitor (Maspin) | ELISA           | OSCC          | Maspin suppress tumor growth and progression, angiogenesis, invasion and metastasis in various malignancies including OSCC.                                                                            | [96]      |
| OSCC saliva     | IHC       | 8-oxoguanine DNA glycosylase (OGG1) | ELISA           | OSCC          | Enzyme for repairing the oxidative DNA damage. Reduced activity of OGG1 is a risk factor for various cancers including OSCC.                                                                               | [96]      |
| OSCC saliva     | IHC       | Phosphorylated-SRC        | ELISA           | OSCC          | Src (a cytoplasmic kinase) drives adhesion changes that are associated with transition, proliferation and metastasis. It changes to phosphor-Src the inhibited form by oxidants by a reversible process. Src is expected to be increased while phosphor-Src is expected to be decreased to promote carcinogenesis in cancer patients. | [96]      |
| Sample        | Technique                  | Biomarker [Accession no.] | Confirmation by | Biomarker for | Discussion                                                                 | Reference  |
|--------------|---------------------------|---------------------------|-----------------|---------------|-----------------------------------------------------------------------------|------------|
| OSCC saliva  | IHC                        | Ki-67                     | ELISA           | OSCC          | A cell-cycle promoter that correlate with cellular proliferation, and tumor progression, metastasis and poor prognosis and are expected to increase in tumors. | [98]       |
| OSCC saliva  | Immunoradiometric analysis | Carcinoembryonic antigen  (CEA) | ELISA           | OSCC          | A type of glycoprotein produced by cells of gastrointestinal tract during embryonic development and involved in cell adhesion. Salivary and serum levels of CEA were found to be increased in malignant tumors than in healthy tissues. | [97]       |
| OSCC saliva  | Immunoradiometric analysis | Carcinoma associated antigen (CA 50) | ELISA           | OSCC          | A cancer-associated carbohydrate marker. CA50 is not organ-specific and its elevated levels in serum can be seen in variety of malignancies. CA 50 in saliva and serum showed significantly high levels in malignant tumors as compared to the healthy normal tissues. | [97]       |
| OSCC saliva  | Radial immunodiffusion     | Insulin growth factor-1 (IGF-1) | ELISA           | OSCC          | IGF plays significant role in carcinogenesis by modulating cancer-cell proliferation, survival, growth and apoptosis. Salivary IGF level was shown to be substantially raised in cancer patients. | [98]       |
| OSCC saliva  | Radial immunodiffusion     | Metalloproteinase (MMP2)   | ELISA           | OSCC          | Metalloproteinases participates in cancer pathogenesis by degrading type-IV collagen, elastin, and fibronectin. Highly expressed in stromal cells surrounding the invading front of metastasizing tumors and their levels are elevated in tumor endothelium. | [96, 98, 99] |
| OSCC saliva  | Radial immunodiffusion     | Metalloproteinase (MMP9)   | ELISA           | OSCC          | Level of MMP-9 showed an increase in OSCC patients. | [34, 96, 99, 100] |
| Sample                  | Technique                  | Biomarker [Accession no.]                  | Confirmation by | Biomarker for | Discussion                                                                 | Reference |
|------------------------|----------------------------|--------------------------------------------|-----------------|---------------|----------------------------------------------------------------------------|-----------|
| OSCC saliva            | WB; PCR                    | CD44 and soluble CD44                     | EUSA            | OSCC          | CD44 shows elevation in majority of HNSCC. Distinguishes cancer from benign disease with high specificity | [27, 37, 98, 101] |
| OSCC Serum and Saliva  | 2-D gel electrophoresis   | Tetranectin                                | Liquid chromatography/tandem mass spectrometry | Lymph node involvement | Plasminogen-binding protein with a C-type lectin domain may be involved in packaging molecules for exocytosis | [102] |
| OSCC Saliva            | ELISA                      | Interleukin 1b (IL1b)                      | ELISA           | Carcinogenesis | Cytokine mediator of inflammatory response                                  | [103–105] |
| OSCC saliva            | ELISA                      | Interleukin 8 (IL8)                       | ELISA           | Carcinogenesis | Chemoattractant cytokine mediates inflammatory response                     | [103–107] |
| OSCC Saliva            | ELISA                      | Mac-2 binding protein (M2BP)               | ELISA           | Carcinogenesis | Cell-adhesive protein of the extracellular matrix which self-assembles into ring-like structures and binds beta1 integrins, collagens, and fibronectin | [103] |
| OSCC saliva Plasma     | 2DE                        | Apolipoprotein A1 APOA1                   | MS              | Differentiate from leukoplakia | As component of HDL participate in lipid metabolism                          | [56, 108, 109] |
| OSCC saliva            | 2DE                        | Alpha amylase                              | MS              | Differentiate from leukoplakia | Hydrolyses alpha bonds of large, alpha-linked polysaccharides, such as starch and glycogen, yielding shorter chains thereof, dextrins, and maltose, normally present in saliva | [108] |
| OSCC saliva            | 2DE                        | Cystatins                                  | MS              | Differentiate from leukoplakia | Cysteine protease inhibitors                                                | [108] |
| OSCC saliva            | 2DE                        | Keratin 10                                 | MS              | Differentiate from leukoplakia | Intermediate filament protein of cytokeratin family. Fibrous protein forming the cellular framework | [108] |
| OSCC saliva            | LC-MRM/MS                  | Alpha-1-acid glycoprotein (A1AG1; AGP 1; OMD1) | MS              | Carcinogenesis | Inflammatory acute phase reactant                                           | [110] |
| OSCC saliva            | LC-MRM/MS                  | Alpha-1-antitrypsin A1AT                   | MS              | Carcinogenesis | Protease inhibitor                                                          | [110] |
| OSCC saliva            | LC-MRM/MS                  | Alpha-1-B Glycoprotein                     | MS              | Carcinogenesis | Glycoprotein of unknown function                                            | [110] |
| Sample                | Technique | Biomarker [Accession no.] | Confirmation by | Biomarker for | Discussion                                                                                                                                                                                                 | Reference |
|----------------------|-----------|---------------------------|-----------------|--------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------|
| OSCC saliva and plasma | MS        | Matrix metaalloprotein 1 (MMP1) | MS              | Cancerogenesis | Interstitial collagenase and fibroblast collagenase participate in breakdown of extracellular matrix                                                                                                    | [111, 112]|
| OSCC saliva and plasma | MS        | Matrix metaalloprotein 3 (MMP3) Stromelysin-1 | MS              | Cancerogenesis | Interstitial collagenase and fibroblast collagenase participate in breakdown of extracellular matrix. degrades collagen types II, III, IV, IX, and X, proteoglycans, fibronectin, laminin, and elastin | [111]     |
| OSCC saliva           | iTRAQ     | Complement factor H (CFH)  | MS              | Cancerogenesis | Regulators of complement activation family                                                                                                                                                            | [84]      |
| OSCC saliva           | Luminex-based multiplex | Tumor necrosis factor (TNF) | Cancerogenesis | Cytokine used by immune system for cell signaling                                                                                                                                                     | [104]     |
| OSCC saliva           | Luminex-based multiplex | Vascular endothelial growth factor (VEGF) | Cancerogenesis | Signaling protein that promotes angiogenesis                                                                                                                                                          | [104, 107]|
| OSCC saliva           | MS        | Actin                      | MS              | Cancerogenesis | Multi-functional protein form microfilaments in the cytoskeleton                                                                                                                                     | [113]     |
| OSCC saliva           | MS        | Myosin                     | Cancerogenesis | Motor proteins that interact with actin.                                                                                                                                                              | [31, 113]|
| OSCC saliva           | ELISA, IHC MALDI-TOF-MS | C-reactive protein (CRP) | Cancerogenesis | A protein whose concentration increase in response to inflammation—acute phase protein                                                                                                               | [52, 114]|
| OSCC saliva           | ELISA, IHC | Leucine-rich alpha-2-glycoprotein (LRG) | Cancerogenesis | Acute phase protein                                                                                                                                                                                   | [114]     |
| OSCC saliva           | 2D-GE     | Zinc-alpha-2-glycoprotein (ZAG) | Cancerogenesis | Participate in glycolipid metabolism                                                                                                                                                                 | [115]     |
| OSCC saliva           | 2D-GE     | Peroxiredoxin-2 (PRDX-2)   | Cancerogenesis | Catalyzes the reduction of peroxides                                                                                                                                                                 | [49, 115, 116]|
| OSCC saliva           | LC-MS/MS  | α-2-Macroglobulin-like protein 1 | Cancerogenesis | Inhibitor of proteases, role in autoimmune diseases                                                                                                                                                  | [117]     |
| OSCC saliva           | LC-MS/MS  | Kininogen -1               | Cancerogenesis | Cofactor for the activation of prekallikrein, factor XII and factor XI                                                                                                                                  | [117]     |
| OSCC saliva           | SDS-PAGE and MALDI-TOF/TOF | Annexin A8 | Cancerogenesis | Participate in membrane-cytoskeleton dynamics                                                                                                                                                        | [116]     |
| Sample       | Technique | Biomarker [Accession no.] | Confirmation by | Biomarker for | Discussion                                                                 | Reference |
|--------------|-----------|---------------------------|-----------------|---------------|----------------------------------------------------------------------------|-----------|
| OSCC Saliva  | ELISA     | IL6                       | ELISA           | Carcinogenesis | Acts as both a pro-inflammatory cytokine and an anti-inflammatory myokine  | [118]     |
| OSCC plasma  | MALDI-TOF | GIMAP7 GTPase, IMAP Family Member 7 | MS              | Carcinogenesis | Regulators of lymphocyte survival and homeostasis, GTP binding             | [119]     |
| OSCC plasma  | MALDI-TOF | Rabli3 RAB like protein 3  | MS              | Carcinogenesis | Unknown function, supposed to participate in cellular regulation          | [119]     |
| OSCC plasma  | MALDI-TOF | Heat shock protein 90 (HSP 90) | MS              | Carcinogenesis | Cell cycle control, cell survival, hormone and other signaling pathways   | [120]     |
| OSCC saliva  | SDS Page  | Resistin adipose tissue-specific secretory factor (ADSF) or C/EBP-epsilon-regulated myeloid-specific secreted cysteine-rich protein (XCP 1) | LS-MS           | Carcinogenesis | Increases production of LDL                                                | [121]     |
| OSCC brush biopsy | MS             | Secretory leukocyte protease inhibitor | MS              | Carcinogenesis | Anti-microbial and anti-inflammatory, found in saliva, breast milk, etc.  | [122]     |
| OSCC serum   | ELISA     | Guanylate-binding protein 1 (GBP1) | ELISA           | Carcinogenesis | Regulation of membrane, cytoskeleton, and cell cycle progression          | [123]     |

Note: IHC immunohistochemistry, WB western blot, ROC receiver operator characteristic analysis, KMS Kaplan-Meier survival analysis, iTRAQ isobaric tags for relative and absolute quantitation, RP reverse phase, OSCC oral squamous cell carcinoma, LC-MRM/MS multiplexed liquid chromatography multiple-reaction-monitoring mass spectrometry
contact with the harmful components of tobacco-like aromatic polycyclic hydrocarbon (PAH), nitrosamines, aromatic amines, and aldehydes that are responsible for malignant transformation [129]. The metabolism of chemicals occurs in two phases. In phase 1, reduction and oxidation reactions occur in cytochrome P-450 system, producing reactive and toxic substances. This oxidative stress induces glutathione S-transferase transcription to eliminate the toxic substances [130]. The toxic metabolites produced genetic instability, mutation, and may initiate the carcinogenesis. After the glucuronidation, sulfation, methylation, and conjunction reactions, the toxic agents are inactivated and become hydro soluble, and are excreted [131]. Mutation of $p53$ have been found to occur more frequently in tobacco and alcohol uses [132], suggesting that inactivation of $p53$ tumor suppressor gene may play an important role in tobacco-induced carcinogenesis.

Infection with human papilloma virus (HPV) is another risk factor specially for oropharyngeal cancer. This dsDNA virus has a 7 kB genome with number of early and late genes that synthesize proteins. Only a subset of more than 100 known HPV subtypes are oncogenic and high-risk types. HPV encodes E6 and E7 oncoproteins that inactivates p53 and Rb respectively, leading to failure of tumor suppressor mechanism [133]. Few HPV-associated biomarkers have also been identified.

Association of OSCC with genetic polymorphisms in genes encoding human enzymes related to toxic substance metabolism has also been reported [134] that affects the individual's susceptibility to noxious effects of cancer. Patients with Fanconi anemia (FA) are predisposed to develop OSCC [135]. Fanconi anemia is a recessive genetic disorder caused by biallelic mutation in a member of FA/BRC pathway [136]. These cancers usually develop at a young age [137]. Another predisposing factor for cancers of hypopharynx is Plumer-Vinson (also called Paterson-Kelly) syndrome, which results from iron-deficiency [138].

Arroyo et al. [139] in a recent meta-analysis found 11 biomarkers of which they did meta-analysis for 4. Of these, only carcinoembryonic antigens (CEA) and soluble fragment of cytokeratin 19 (CYFRA21) were found to be significantly associated with oral cancer. Kasradze [140] in their review found 44 relevant proteins. Of them, proteins (14-3-3γ, extracellular matrix metalloproteinase inducer, and PA28γ) were found to be most significant. Other studies reported only the number of proteins differentially expressed without any identification [141–144]. Li et al. [145] identified differential protein expression in oral cancer patients with or without lymph node metastasis. Levels of PF4V1 and F13A1 correlated with number of lymph nodes. Immunoglobulin (Ig) Kappa chain C region and Isoform 2 of fructose bisphosphate aldolase A are found to increase in tobacco users; however, these markers are not yet validated [146]. Other investigators found Serpin family of proteins to be overexpressed in tobacco users [147], while some just reported number of proteins with differential expression [148].

The OSCCs occur as a consequence of proto-oncogene activation or tumor suppressor gene inactivation. Promoter hypermethylation is an example of indirect mechanism [149]. The three main alterations in gene function

### Table 3: Potential protein biomarkers of head and neck cancers: autoantibodies

| Sample   | Technique | Biomarker [accession no.] | Confirmation by | Biomarker for | Discussion | Reference |
|----------|-----------|---------------------------|-----------------|---------------|------------|-----------|
| OSCC saliva | IHC  | P53 autoantibody | ELISA, IHC | OSCC | p53 antibodies found only in serum and saliva of patients showing overexpression of p53 in their tumor tissues. Checks for overexpression of p53 protein however do not differentiate between wild and mutant proteins. | [125] |
| OSCC | MALDI-TOF/TOF-MS | Heat shock protein 70 (HSP70) | MS | Carcinogenesis | Cellular network of molecular chaperones and folding catalysts assists in protein folding process. Identified as early marker and prognostic marker in OSCC | [126] |
| OSCC | MALDI-TOF-MS/2DE | Sideroflexin 3 (SFXN3) | MS | Response to therapy | Mitochondrial serine transporter participate in one-carbon metabolism pathway | [127] |

*Note: IHC immunohistochemistry, WB western blot, ROC receiver operator characteristic analysis, KMS Kaplan-Meier survival analysis, iTRAQ isobaric tags for relative and absolute quantitation, RP reverse phase, OSCC oral squamous cell carcinoma*
that occur in OSCC are (1) inactivation of p53 tumor suppressor gene, (2) inactivation of cyclin-dependent kinase (CDK) inhibitor p16, and (3) overexpression of epidermal growth factor receptor (EGFR); however, mutations in the EGFR genes occur with very low frequencies.

**Inactivation of p53 tumor suppressor gene**
p53 has a role in maintaining genomic stability, cell-cycle progression, cell differentiation, DNA repair, and apoptosis, and hence is aptly called the “guardian of the genome.” Mutations, deletions, and binding with viral proteins can produce p53 dysfunction [150]. It is found in approximately 50% of OSCC tumors and is one of the most common cancer development events [151] (Fig. 2).

**Inactivation of cyclin-dependant kinase (CDK) inhibitor p16**
CDK are important molecules responsible for regulation of the cell-cycle. A number of these proteins have been identified and some of these can be targeted. The function of CDK is regulated by number of genes like p16 and retinoblastoma gene. The effect is brought by regulating the phosphorylation of genes during G1 to S phase, through inhibition of CDK 4 and 6 [152]. The formation of CDK 4-6/cyclin D complex is inhibited by the p16 gene, and p21 gene (Fig. 1) thus leading to cell cycle arrest. Downregulation of these proteins is often associated with OSCC [153]. Regulation of phosphorylation of retinoblastoma gene by p16, p21, Cyclin D, and CDK leads to cell cycle arrest, DNA repair, and apoptosis if repair fails (Fig. 1).

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**Fig. 2** Line diagram showing p53-mediated downstream signaling pathway
Overexpression of EGFR

EGFR promotes epidermal cell growth and regulates cell proliferation, while regulation of metastasis and angiogenesis leads to development of OSCC. Therefore, EGFR proteins overexpression leads to increased tumor proliferation. EGFR ligand binding results in a molecular cascade that covers receptor-linked tyrosine kinase activation and other downstream pathways. EGFR family has four types of receptors and can have homo or heterodimers where in two similar members or different members bind to produce a dimer. EGFR controls many pathways however its overexpression is found to be associated with increased carcinogenesis [98, 127, 153, 154]. However, for targeting its mutations are normally looked at and mutant EGFR with chromosome 19-21 mutations are often targeted with tyrosine kinase inhibitors.

The biomarker data presented in the article show that this is still a new field and though a lot of the markers are identified, not much work has been done on validating these so far. Further, the data shows differences in the proteomic profile between continents and also between subsites. There is also a difference between tissue and secretome profile wherein more inflammatory markers are seen in saliva. The validation of diagnostic and prognostic biomarkers is a long-drawn process, and there is a need to have more proteomic research to identify better markers that will improve the diagnosis and prognostication of the patients.

Conclusion

Proteomic and genomic characterization of tumors is essential for identification of biomarkers of carcinogenesis, therapeutics, prognosis, progression, and metastasis. This is frequently been used in many tumors while their role in others is still under investigation. OSCC is an uncommon tumor in the west but is common in South East Asia; hence, very little work is done on it. In recent times, the newer evidence has come that shows p53 and ras mutations to be common, and these tumors have poor prognosis compared to that without it. Further work on proteomics will help identify more markers of carcinogenesis, prognosis, and therapeutic significance and will help identify newer targets.

Supplementary Information

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Additional file 1. Search strategy

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