Emerging histological and serological biomarkers in oral squamous cell carcinoma: Applications in diagnosis, prognosis evaluation and personalized therapeutics (Review)

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Abstract. Oral squamous cell carcinoma (OSCC) is the most common malignancy of the oral cavity and accounts for >90% of all oral cancers. Despite advances in diagnostic procedures and therapeutic interventions, overall survival has not improved significantly in recent decades, primarily due to late diagnosis, locoregional recurrence and treatment resistance. Identifying reliable biomarkers for early detection, prognosis evaluation and treatment response prediction is critical for improving clinical outcomes in patients with OSCC. In the present review, the prognostic and predictive utility of circulating biomarkers, such as circulating tumour cells, serological biomarkers and histological and genetic biomarkers, were explored in the context of OSCC. In addition, the potential role of immune checkpoints in the treatment of OSCC was highlighted and the rapidly evolving field of liquid biopsy and its potential to revolutionize diagnosis, prognosis evaluation and treatment were examined. The existing evidence for the clinical utility of these biomarkers was critically evaluated and the challenges and limitations associated with their introduction into routine clinical practice were addressed. In conclusion, the present review highlights the promising role of biomarkers in improving the current understanding of the pathogenesis of OSCC and offers potential avenues for improving patient care through personalized medicine approaches.

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1. Introduction: Epidemiology, clinical presentation, diagnosis and treatment

Oral squamous cell carcinoma (OSCC) affects ~600,000 patients per year, accounting for ~4% of all cancer cases, and this disease has high cancer-related morbidity and mortality rates. The overall 5-year survival rate for OSCC remains as low as ~50-60%, with early-stage cancers having a considerably better prognosis than advanced-stage tumours. The presence of regional lymph node metastasis is a key

predictor of survival, with 5-year survival rates dropping to ~30-40% in such cases (1,2). OSCC is the most common type of oral cancer, accounting for >90% of all oral malignancies. It should be noted that the global incidence and mortality rates of OSCC vary significantly across geographic regions, with higher rates observed in South Asia, Southeast Asia and Europe than in other regions. OSCC affects men more frequently than women and its incidence increases with age (3). These variations in OSCC burden across populations may be attributed to differences in exposure to risk factors, genetic predisposition and health care access.

It should be highlighted that several risk factors have been identified for OSCC, including smoking or smokeless (chewing) tobacco use, which is the leading risk factor for OSCC, contributing to ~75% of all cases (4). Other risk factors that may be highlighted are excessive alcohol intake, which is an independent risk factor for OSCC. The combined effect of tobacco and alcohol use exponentially increases the risk of dysplasia and cancer, and it should be noted that in recent years, the importance of human papillomavirus (HPV) infection in the oral cavity, which has been recognized as an aetiological factor for squamous neoplasia, primarily affecting the oropharynx, has been observed. While most HPV infections are asymptomatic and transient, there are high-risk HPV types, such as HPV-16 and HPV-18, that have been linked with the development of various malignancies, including cervical, anogenital and oropharyngeal cancers, and have an important role in OSCC (5,6). It should be noted that the risk factors for HPV-positive OSCC are different from those of HPV-negative OSCC; the former is more frequently associated with younger age, fewer lifetime sexual partners, and a lower prevalence of tobacco and alcohol use (7) Other risk factors, such as poor oral hygiene and diets low in fruits and vegetables, which have been linked to an increased risk of chronic inflammation and cancer, are not as important as previous factors, but have an important role in OSCC, increasing the risk of squamous dysplasia in the oral cavity (8).

The clinical presentation of OSCC may vary among patients. Early-stage OSCC may present with nonspecific signs and symptoms, such as an indurated, painless, nonhealing ulcer, which is one of the most common early signs of OSCC (9). The ulcer may be accompanied by raised, rolled or everted edges and may have irregular margins. Other early signs include the presence of leukoplakia and erythroplakia (the latter with a higher risk of malignant transformation), which may be precursors of OSCC or represent early-stage lesions (10). Patients may also report oral pain, discomfort or a burning sensation that may be persistent or intermittent and associated with unexplained tooth mobility or tooth changes. In cases involving the base of the tongue or oropharynx, this may present as persistent sore throat or hoarseness (11).

On the other hand, patients with advanced OSCC may have an enlarging mass or growth in the oral cavity, which is often firm with irregular margins. This is a characteristic finding in advanced OSCC associated with dysphagia, odynophagia or dysarthria due to tumour infiltration or obstruction of the oral cavity or oropharynx. It may also present as facial swelling or asymmetry with involvement of cervical lymphadenopathy (12).

It is important to note that the diagnosis of OSCC is based on clinical examination, biopsy and imaging techniques to determine the extent of the disease and histologic confirmation of malignancy. The first step is clinical examination with visual inspection of the oral cavity and oropharynx, which may identify lesions suggestive of OSCC, such as leukoplakia and erythroplakia, palpation of the oral cavity, and evaluation of cranial nerves to identify any neurologic deficits that may indicate tumour infiltration or compression of adjacent nerves. It is important to emphasize that a biopsy is required to confirm the diagnosis of OSCC by histopathologic analysis (13).

Of note, symptom-based panendoscopy (laryngoscopy, bronchoscopy and oesophagoscopy) may be performed in the first assessment and the incidence rate of second primary upper aerodigestive tract tumours with this method is 2.4 to 4.5%; this method may also provide information about synchronous lesions. On the other hand, fine-needle aspiration biopsy (FNAB) combined with ultrasound has an important role in the evaluation of cervical lymph nodes and subsequent cytological evaluation is performed to assess suspicious lymph nodes (14). FNAB has proven to be an invaluable diagnostic tool in the management of OSCC, particularly in the evaluation of suspicious cervical lymph nodes, with a diagnostic accuracy of 89-98%, and may be used to evaluate potential recurrence or residual disease in patients with OSCC after treatment, particularly when imaging results are inconclusive (15). It may also be performed with excisional biopsy of the lesion with a surrounding margin of healthy tissue in easily accessible lesions and may be used both diagnostically and therapeutically in selected patients.

Imaging studies have a critical role in the diagnosis and staging of OSCC by providing detailed information about the size of the tumour, its location and possible invasion of adjacent structures. This usually includes computed tomography (CT), which is useful for assessing bone invasion, regional lymphadenopathy and distant metastases. Contrast-enhanced CT scans may improve the visualization of soft tissue involvement and vascular structures and positron emission tomography (PET) is frequently combined with CT (PET-CT) to perform OSCC staging, detect regional lymph node involvement and identify distant metastases or synchronous primary tumours (16). In this context, it should be emphasized that magnetic resonance imaging (MRI) provides a better soft-tissue resolution than CT, making it the tool of choice for assessing tumour extension, perineural spread and involvement of vital structures, such as nerves and blood vessels. MRI is also useful for distinguishing tumour recurrence from posttreatment changes such as fibrosis (17). With regard to metastatic disease, it is important to assess distant invasion in OSCC. The incidence of metastatic disease ranges from 2 to 26% and varies according to locoregional extent, lymphatic involvement and histologic grade (18). Typically, distant metastases are asymptomatic and the most common sites are the lungs, liver and bones, with the CT scan being the most sensitive method for screening metastases in patients with OSCC, revealing malignant findings in 4-19% of newly diagnosed cases (19).

In terms of treatment, advances in diagnostic and therapeutic approaches over the years have improved patient outcomes, but the prognosis remains poor, particularly in advanced-stage cases. Current treatment strategies for OSCC

Table I. Summary of different biomarkers in oral squamous cell carcinoma.

Biomarker	Type of analysis	Utility	(Refs.)
p16	Immunohistochemical	Prognosis	(31-35)
PD-L1	Immunohistochemical	Treatment, prognosis	(36-42)
EGFR	Immunohistochemical	Treatment, prognosis	(43-47)
Cyclin D1	Immunohistochemical	Treatment, prognosis	(50-56)
Ki-67	Immunohistochemical	Prognosis	(58-65)
CEA	Serological	Diagnosis, prognosis	(66-68)
VEGF	Serological, immunohistochemical	Diagnosis, prognosis	(70-73)
CA-125	Serological	Diagnosis, prognosis	(75-78)
CYFRA 21-1	Serological	Diagnosis, prognosis	(81-83)
Circulating tumor cells	Serological	Diagnosis, prognosis, treatment	(85-91)
Salivary biomarkers	Multimodal use of cytokines and proteins.	Diagnosis, prognosis	(84-99)

PD-L1, programmed death-ligand 1; EGFR, epidermal growth factor receptor; CEA, carcinoembryonic antigen; VEGF, vascular endothelial growth factor; CA-125, cancer antigen 125; CYFRA 21-1, cytokeratin 19 fragment antigen.

are based on locoregional and distant disease classification according to the TNM staging classification system, which was updated in 2018 by the American Joint Committee on Cancer and the Union for International Cancer Control (20). The primary treatment for early-stage OSCC remains surgery, which includes wide local excision of the tumour and lymphadenectomy with reconstructive surgery when appropriate. It is effective, but potential complications include difficulty swallowing, speech problems and aesthetic issues that may affect patients' quality of life (21). In certain cases, radiation therapy may be used as a complementary treatment after surgery or as the only treatment for patients who are not candidates for surgery, and it includes intensity-modulated radiotherapy and stereotactic body radiotherapy, which aim to deliver high doses of radiation to the tumour while sparing surrounding normal tissue but are usually associated with acute toxicities, such as mucositis, xerostomia and dysphagia (22,23). Chemotherapies such as cisplatin, carboplatin and 5-fluorouracil are often used in conjunction with radiotherapy and immunotherapy for locally advanced cases and are preferred for symptomatic patients (24). In recent years, the importance of various biomarkers has been highlighted, which has enabled the development of targeted agents and immunotherapies. For instance, agents targeting the epidermal growth factor receptor (EGFR), such as cetuximab, have shown promise in improving survival when combined with radiotherapy and recently, immune checkpoint inhibitors (ICIs) have also shown promise. ICIs such as pembrolizumab and nivolumab have been approved for relapsed/metastatic OSCC and represent the first-line treatment for locoregional and metastatic disease in patients with OSCC (25-27).

In conclusion, OSCC is a complex disease that requires a multifaceted approach to its prevention, diagnosis and treatment. The introduction of new diagnostic tools and continuing advances in treatment modalities require ongoing research and clinical trials, with the aim of improving early detection, optimizing treatment and increasing survival rates and quality of life for patients with OSCC.

2. Role of immunohistochemical biomarkers

Currently, one of the most promising approaches to improve OSCC treatment is the identification and application of histological biomarkers. These biomarkers may provide information regarding prognosis, predict the response to treatment and monitor disease progression or recurrence. In the context of OSCC, histological biomarkers may provide a deeper understanding of the biological behaviour of the tumour, which is important for understanding the underlying pathophysiology of the tumour and facilitates clinical decision making and personalized treatment. All of these biomarkers are summarised in Table I and Fig. 1.

Among the various histological biomarkers available in OSCC are a number of molecular alterations in proteins or genes related to cell proliferation, apoptosis, angiogenesis, immune response and metastatic invasion. In recent years, several potential histological biomarkers of $p16^{INK4A}$ have been identified and their functions in OSCC have been studied in detail. These include markers of cell cycle regulation ($p16^{INK4A}$, cyclin D1), EGFR, cell proliferation (Ki-67), immune checkpoint proteins [programmed cell death 1 (PD-1) ligand 1 (PD-L1)] and others involved in tissue invasion and metastasis, such as metalloproteinases (28).

p16. The protein $p16^{INK4A}$ or p16, encoded by the cyclin-dependent kinase (CDK) inhibitor 2A gene, has a critical role in the regulation of the cell cycle by inhibiting CDK4 and CDK6, which are essential for the transition from the G1 phase to the S phase of the cell cycle. Dysregulation of $p16^{INK4A}$ is frequently observed in numerous types of cancer, including OSCC (29).

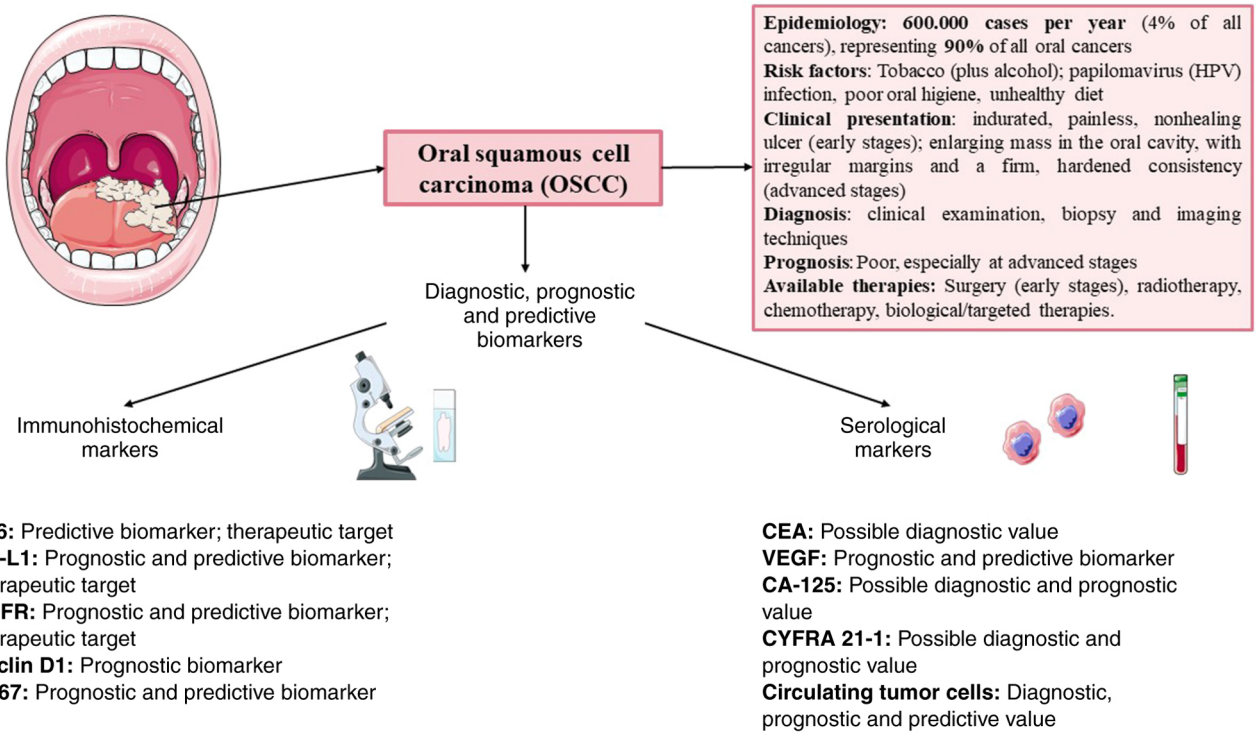

Figure 1. Overview of the main biomarkers currently explored in oral squamous cell carcinoma and their potential uses. PD-L1, programmed death-ligand 1; EGFR, epidermal growth factor receptor; CEA, carcinoembryonic antigen; VEGF, vascular endothelial growth factor; CA-125, cancer antigen 125; CYFRA 21-1, cytokeratin 19 fragment antigen.

HPV expresses the oncoproteins E6 and E7, which deactivate tumour suppressor proteins. The E6 protein induces the degradation of P53, whose function is to halt cell division at the G1 level and induce apoptosis through ubiquitin-mediated proteolysis, inactivating its activity (30-33). Cells expressing E6 are unable to respond to DNA damage signaling mediated by P53 and are thus susceptible to genomic instability. The E7 protein binds and inactivates the retinoblastoma tumour suppressor gene product (pRB), causing the cell to enter S phase, leading to cell cycle disruption, proliferation and malignant transformation. Inactivation of pRB leads to upregulation of $p16^{INK4a}$ as a cellular response to uncontrolled cell cycle progression (30).

Its importance as a prognostic marker is related to HPV infection. The prevalence of HPV in patients with OSCC has been examined in various research studies. According to Vergori *et al* (30), HIV-positive individuals are more likely to harbour HPV in the oral cavity than HIV-negative individuals.

A relevant study on an HIV-infected population have indicated an overall prevalence of HPV DNA in the oral cavity ranging from 20 to 45%, with the oncogenic type HPV16 found in 12 to 26% of cases (31). A study by Castro and Filho (32) found that HPV is an independent risk factor for OSCC. The prevalence of HPV in oral cancer, particularly SCC, was observed to be higher for HPV16 (32). One of the most significant studies regarding the relevance of p16 in OSCC is a meta-analysis of 31 studies including ~5,000 patients conducted by Katirachi *et al* (33). The overall prevalence of HPV+ OSCC in this meta-analysis was ~6% (95% CI; 3-10%), and only one study identified a significant correlation between HPV and OSCC (33).

From a morphological perspective, it has been observed that HPV+ OSCC originates more frequently in the tonsillar crypts and presents more often in advanced stages (III or IV) than HPV-OSCC. On the other hand, the prognostic utility of p16 in OSCC differs among studies given its low prevalence, although according to studies such as that by Doll *et al* (34), P16 expression is connected to a better survival rate in individuals undergoing primary surgery with adjuvant radio(chemo) therapy (34). However, meta-analyses such as that of Christianto *et al* (35), which evaluated 22 articles comprising 3,065 patients with OSCC, observed that HPV-positive OSCC is associated with significantly decreased survival.

Therefore, dysregulation of $p16^{INK4A}$ is associated with various types of malignancies, including OSCC, where its importance is directly associated with HPV infection. As mentioned above, several studies have examined the prevalence of HPV in OSCC and found considerably high rates of p16 positivity. While certain reports suggest better outcomes for patients with P16-expressing OSCC undergoing specific therapies, meta-analyses offer a more nuanced perspective and indicate decreased outcomes in HPV-positive cases. This intricate interplay underscores the need for specialized research and approaches. Understanding these processes has the potential to offer more effective defences against OSCC, reflecting the evolving nature of cancer treatment.

PD-L1. PD-L1, also known as CD274 or B7-H1, is a protein that has an important role in oncology, particularly in the context of immunotherapy. In OSCC, the role of PD-L1 is increasingly recognized as crucial, not only in terms of understanding tumour biology but also for determining

prognosis and establishing therapeutic strategies. PD-L1 is typically expressed on the surface of certain cells, including cancer cells, where it interacts with its receptor, PD-1, found on T cells (36). This interaction inhibits the immune response and allows cancer cells to evade the immune system. This mechanism of immune evasion is particularly important in OSCC, as there is growing evidence that PD-L1 expression is associated with more aggressive disease features, such as advanced tumour stage, nodal metastases and unfavourable overall survival. In recent years, immune checkpoint inhibitors that block this interaction have shown promising results in various cancer types, including OSCC (37,38). This is because PD-L1 expression appears to be regulated by multiple signalling pathways, including MAPK, PI3K and Akt/PKB, which are commonly altered in head and neck carcinoma. As a consequence of these molecular interactions, it is a dynamic biomarker that is subject to variations (39).

In the last decade, several immune checkpoint inhibitors targeting PD-1 or PD-L1 have been approved for the treatment of recurrent or metastatic OSCC. Among these drugs are pembrolizumab and nivolumab (targeting PD-1) and atezolizumab and durvalumab (targeting PD-L1). These inhibitors have shown promising results in terms of response rates and survival, particularly in patients with PD-L1-positive tumours, changing the treatment paradigm for this patient population. For instance, pembrolizumab was approved as a first-line treatment for relapsed or metastatic OSCC with a PD-L1 combined positive score of 1 or higher according to the KEYNOTE-048 trial (40,41). This study demonstrated that pembrolizumab improved overall survival compared to that with the standard therapy EXTREME (cetuximab with platinum-based chemotherapy) (42).

Therefore, PD-L1+ tumours tend to exhibit better response rates to anti-PD-1/PD-L1 therapies compared to PD-L1-tumours (40). It is important to note that, albeit to a lesser extent, PD-L1-tumours also benefit from these treatments (41), indicating the need to consider additional factors, such as HPV status or tumour mutational burden, when initiating these treatments.

However, studies such as CHECKMATE-141 failed to show a significant association between PD-L1 expression and tumour response or survival when evaluating nivolumab in platinum-resistant patients (42). The lack of concordance in these results obtained from different studies may be explained by the heterogeneity in biomarker expression, as well as the lack of uniformity in assays and the variability in thresholds used to define PD-L1 positivity, leading to the establishment of harmonization projects for PD-L1 assays by the scientific community and regulatory bodies (41,42).

Anti-PD-L1 therapies, such as atezolizumab and durvalumab, are also being studied at various stages of clinical trials for OSCC and are showing promising results. Despite these advances, overall response rates to monotherapies against PD-1/PD-L1 remain relatively modest, underscoring the need for strategies to improve their efficacy (43). The combination of immune checkpoint inhibitors with other therapies, such as cytotoxic chemotherapy, radiotherapy or other immunotherapeutic agents, is a promising approach under active investigation (44).

EGFR. EGFR is a transmembrane receptor that belongs to the ErbB family of receptor tyrosine kinases. This receptor has a critical role in the pathogenesis and progression of several cancers, including OSCC. EGFR is frequently overexpressed in OSCC and its upregulation is associated with aggressive tumour behaviour, increased invasiveness and poor prognosis (45). In this sense, it correlates with advanced tumour stage, lymph node metastasis and lower overall survival rates. Therefore, the determination of EGFR expression levels in OSCC may serve as a valuable prognostic indicator to aid in treatment planning and patient management (46).

EGFR is not only a prognostic marker but also an emerging therapeutic target in OSCC. Several strategies, including monoclonal antibodies (such as cetuximab) and small molecule tyrosine kinase inhibitors (such as erlotinib), have been developed to inhibit EGFR signalling. Cetuximab has been shown to be clinically effective in combination with radiotherapy or chemotherapy for locally advanced OSCC, leading to improved survival rates. It is important to highlight that in recent years, EGFR therapies have been shown to have the potential to sensitize tumours to other treatments, improve the efficacy of chemotherapy and radiotherapy, and overcome treatment resistance (47). Related to treatment resistance, several molecular alterations, such as EGFR gene amplification, mutations and activation of downstream signalling pathways, remain a major challenge in these patients and represent one of the main limitations of these therapies (48).

EGFR has a critical role in the pathogenesis and progression of OSCC. Its overexpression is associated with aggressive tumour behaviour and poor prognosis. Targeting EGFR represents a promising therapeutic approach for OSCC that may improve treatment outcomes. However, further research is needed to refine patient selection criteria, overcome resistance mechanisms and optimize treatment strategies to fully realize the therapeutic potential of EGFR inhibition in OSCC.

Cyclin D1. Cyclin D1 is an important regulatory protein involved in cell-cycle progression and has been extensively studied in various cancers, including OSCC. Cyclin D1 has a critical role in regulating the transition from G1 to S phase of the cell cycle by forming complexes with CDKs. Overexpression of cyclin D1 has been observed in OSCC, which is associated with impaired cell cycle control, increased proliferation and tumour progression (49). Multiple molecular alterations, including gene amplifications, mutations and dysregulated signalling pathways, contribute to the upregulation of cyclin D1 in OSCC (50).

Numerous studies have investigated the prognostic significance of cyclin D1 expression in OSCC. Increased expression of cyclin D1 has been associated with unfavourable clinicopathological features, including advanced tumour stage, lymph node metastasis and poor overall survival. Its overexpression serves as an independent prognostic marker for aggressive tumour behaviour and may aid in risk stratification and treatment planning (51-53).

Cyclin D1 is closely involved in the interaction with various oncogenic signalling pathways in OSCC, such as p53, RB and components of the Wnt/β-catenin pathway, thus influencing cell-cycle progression, apoptosis and tumour growth (54). Despite the growing evidence for the link between cyclin D1

and OSCC, several challenges remain to be overcome. These include standardization of cyclin D1 determination methods, identification of predictive biomarkers for treatment response, and understanding the complex network of interactions involving cyclin D1 in OSCC (55,56).

In conclusion, cyclin D1 has a critical role in the pathogenesis of OSCC and serves as a prognostic marker. Its dysregulation contributes to tumour progression and poor clinical outcomes. Further exploration of the molecular mechanisms underlying cyclin D1 involvement in OSCC and clinical validation of therapeutic approaches targeting cyclin D1 will pave the way for more effective treatment strategies and improved outcomes for OSCC.

Ki-67. Ki-67 is a nuclear protein associated with cell proliferation and has been shown to be a valuable biomarker in cancer research, including OSCC. Ki-67 is commonly used as a marker of cell proliferation, as it is present in the active phases of the cell cycle (G1, S, G2 and mitosis) (57). OSCC is characterized by dysregulated cell proliferation and Ki-67 expression levels reflect proliferation activity in the tumour. High Ki-67 expression in OSCC is associated with aggressive clinicopathological features in various cancers, such as breast cancer (58,59).

Numerous studies have investigated the prognostic value of Ki-67 expression in head and neck cancer. Increased Ki-67 expression has been associated with decreased overall survival and disease-free survival, and increased risk of recurrence (60,61). Its evaluation may be helpful in risk stratification, treatment planning and posttreatment surveillance. Ki-67 expression has been shown to be particularly valuable in identifying high-risk subgroups among patients with early-stage OSCC, for whom accurate prognosis prediction is difficult. Ki-67 expression has also been studied as a predictive marker of treatment response in OSCC (62). Low Ki-67 expression has been associated with a better response to therapy, including surgery, radiotherapy and chemotherapy (63). Conversely, high Ki-67 expression may indicate treatment resistance and the need for more aggressive therapeutic approaches. Incorporating Ki-67 assessment into treatment decision-making may help personalize treatment strategies and optimize outcomes (64).

Combining Ki-67 assessment with other molecular biomarkers has the potential to improve risk stratification and treatment planning in OSCC. Integration of Ki-67 with factors such as those previously discussed (PD-L1, cyclin D1, EGFR and p16) may lead to a more comprehensive understanding of tumour behaviour and enable personalized therapeutic approaches (65).

In summary, the evaluation of histologic biomarkers in OSCC, including p16, EGFR, PD-L1, cyclin D1 and Ki-67, provides valuable insight into tumour behaviour, prognosis and response to treatment. These biomarkers have the potential to aid in risk stratification, treatment planning and the development of targeted therapies for OSCC.

3. Serological markers

Serological markers are biomolecules present in the blood that can indicate cancer development and progression and response to treatment. The potential of combining multiple markers to improve diagnostic accuracy and specificity is also a key point, and the integration of diverse markers into routine clinical practice may aid in early detection. In this sense, a diverse group of molecules has been described in OSCC. All of these biomarkers are summarised in Table I.

Carcinoma-specific carcinoembryonic antigen (CEA). CEA is a high-molecular-weight glycoprotein, isolated from a tumour cell extract, and is found in the cytoplasmic membrane of numerous glandular cells. The term carcinoembryonic is due to its presence in foetal colonic mucosa (66). Its physiological function is unknown. Due to its structural similarity with members of the immunoglobulin family, it may be involved in cellular recognition mechanisms or cell adhesion mechanisms.

It is usually present at very low concentrations in human serum, generally below 5 ng/ml. However, of note, higher levels (5-10 ng/ml) may be found in healthy smokers. Initially, CEA was thought to be a specific marker for digestive tract tumours; later studies revealed that it is elevated in various types of tumour, including OSCC (67). It may also be elevated in nontumor diseases, such as renal insufficiency, liver cirrhosis, pancreatitis and inflammatory bowel disease.

Several studies have evaluated the diagnostic value of CEA in OSCC. For instance, a study investigated the diagnostic value of serum tumour markers, including CEA, in patients with OSCC, and the results showed that CEA levels were significantly higher in these patients than in patients with benign oral tumours and healthy controls (68). This suggests that CEA may be a useful biomarker for its detection, as its overexpression in these types of cancer may be related to the malignant transformation of epithelial cells (67). However, due to the variability in CEA levels among patients and the possibility of elevations in other types of diseases, CEA is not used as a sole marker for the diagnosis of head and neck cancer but rather as part of a more comprehensive diagnostic approach (68).

In addition to its diagnostic value, CEA levels may also be useful for monitoring treatment response in patients with head and neck cancer. A decrease in CEA levels during or after treatment may indicate a favourable treatment response (67,68).

Apart from CEA, other tumour markers have also been studied in OSCC. For instance, a study identified serum autoantibodies against sideroflexin 3 as a potential tumour marker for OSCC (67). Furthermore, another study revealed that CEA levels in saliva increase in the presence of oral cavity malignancies, including OSCC (69). These findings suggest that CEA, along with other tumour markers, may be of diagnostic value.

It is important to note that the diagnostic accuracy of CEA and other tumour markers in OSCC may vary depending on the specific marker and the studied population. For instance, the diagnostic accuracy of progastrin-releasing peptide (ProGRP), CEA, cytokeratin 19 fragment antigen (CYFRA 21-1) and neuron-specific enolase in the differential diagnosis of small cell lung cancer demonstrated that ProGRP exhibited higher detection accuracy than CEA in lung cancer (68). Therefore, further research is needed to determine the specific diagnostic value of CEA in OSCC.

Vascular endothelial growth factor (VEGF). VEGF has a crucial role in OSCC by promoting angiogenesis, invasiveness, aggressiveness and proliferation of cancer cells (70). The overexpression of VEGF has been associated with tumour progression, lymph node metastasis and poor prognosis in OSCC (71,72). Several studies have investigated the expression and role of VEGF in OSCC.

The expression of VEGF-A and its receptor VEGFR-2 has been evaluated in patients with OSCC. The results showed that VEGF-A gene expression and serum levels were significantly higher in patients with OSCC than in controls. In addition, VEGFR-2 expression was observed in tumour tissues. These findings suggest that VEGF-A and VEGFR-2 may have a role in the pathophysiology of OSCC (71).

Furthermore, the correlation between clinicopathological factors and VEGF expression in OSCC was examined. Immunohistochemical staining and reverse transcription-quantitative (RT-q)PCR were performed to assess VEGF expression. The results showed that high-level staining of VEGF was observed in poorly differentiated and invasive OSCC. There were significant correlations between VEGF expression and histologic differentiation and tumour size. These findings suggest that VEGF expression may be associated with tumour aggressiveness in OSCC (72).

In addition to its role in angiogenesis, VEGF has been implicated in the epithelial-to-mesenchymal transition (EMT) process in OSCC. Neuropilin-1 (NRP1), a coreceptor of VEGF, has been shown to promote EMT by activating the NF-κB pathway (72). In this sense, NRP1 overexpression promotes EMT in OSCC. Inhibition of the NF-κB pathway reverses the NRP1-mediated EMT process. These findings suggest that VEGF-NRP1 signalling may contribute to the invasive and metastatic potential of OSCC (73).

Furthermore, Alsafadi and Manadili (74) investigated the expression of VEGF in OSCC after inducing it in hamsters and subjecting them to radiotherapy. The results showed that VEGF expression was not significantly different between the group that received radiotherapy and the group that did not. In addition, tumour cells that were resistant to radiotherapy showed positive expression of VEGF. These findings suggest that VEGF expression may be associated with radioresistance in OSCC (74). In conclusion, VEGF has a significant role in the pathogenesis and progression of OSCC. Its overexpression has been associated with tumour aggressiveness, lymph node metastasis and poor prognosis in OSCC.

Cancer antigen 125 (CA-125). CA-125 is a glycoprotein initially isolated from ovarian tumours and produced under normal conditions by structures derived from Müller's ducts (fallopian tube, endocervix and vaginal fundus), as well as in the pleural, pericardial and peritoneal mesothelia. Concentrations <35-40 U/ml are considered normal. CA-125 is the marker of choice for epithelial ovarian tumours (except mucinous ovarian tumours), but several studies have investigated the expression of CA-125 in patients with OSCC and its potential as a biomarker with diagnostic, predictive and prognostic utility (75,76).

The serum concentration of CA-125 accurately reflects the malignant degree of the tumour mass, and thus, pretreatment serum values are directly related to the tumour stage in patients (75). However, higher levels of CA-125 may be detected in benign conditions, such as endometriosis, benign ovarian tumours, cysts or tuberculosis.

Saliva has also been investigated as a diagnostic medium to detect biomarkers in patients with OSCC. Zhang *et al* (75) reported that an increase in CA-125 levels in saliva may be considered a salivary biomarker for cancers of the oral cavity. Similarly, Roi *et al* (76) found that CA-125 levels were significantly elevated in the saliva of patients with oral cancer. These findings suggest that CA-125 may be a potential salivary biomarker for OSCC (75,76).

Furthermore, Goldoni *et al* (77) found that CA-125 levels were significantly elevated in saliva samples from patients with OSCC analysed using the immunoblot technique. They also reported that elevated levels of soluble CD44 in saliva were present in the majority of OSCC cases and could distinguish cancer from benign tumours with high specificity (77).

Furthermore, a review mentioned that CA-125 has been studied as a diagnostic marker in head and neck SCC, including OSCC. They emphasized the importance of protein markers, such as CA-125 in the diagnosis and progression of various cancer types (78).

In this context, the available literature suggests that CA-125 may be a potential biomarker for OSCC, but further research is needed to validate its diagnostic and prognostic value in patients with OSCC. Saliva-based diagnostic methods, including the analysis of salivary biomarkers such as CA-125, show promise for the early detection of OSCC.

CYFRA 21-1. CYFRA 21-1 is a soluble form of cytokeratin 19, a structural protein found in epithelial cells. Research has been conducted to determine the clinical value of CYFRA 21-1 in the diagnosis, prognosis and follow-up of head and neck cancer.

Rajkumar *et al* (79) investigated the levels of salivary and serum CYFRA 21-1 in patients with oral precancer and OSCC. The study found that CYFRA 21-1 levels were increased in both salivary and serum samples of patients with OSCC compared to healthy controls (79). This suggests that CYFRA 21-1 may serve as a potential biomarker for the detection of OSCC. In reference to its prognostic utility, Liu *et al* (80) highlighted that high levels of CYFRA 21-1 were significantly associated with shorter overall survival.

CYFRA 21-1 is not specific to OSCC and has been studied in other types of cancer as well. However, the studies mentioned above provide evidence for the potential utility of CYFRA 21-1 as a biomarker in OSCC.

SCC antigen (SCCA). SCCA is a member of the serine protease inhibitor family located in the serine protease inhibitor (serpin) group on chromosome 18q21.3. It is a glycoprotein with a molecular weight of ~48 kDa, a molecule initially described from cervical tissue. Molecular studies demonstrate that SCCA is transcribed by two nearly identical genes (SCCA1 and SCCA2), and it has been observed that both SCCA1 and SCCA2 are expressed in moderately and well-differentiated tumours. It is thought that SCCA has a role in protecting tissue against enzymatic degradation. However, its exact function remains to be fully elucidated. It has been shown that SCCA2 inhibits chymotrypsin-like proteinases, such as cathepsin

G and mast cell chymase, while SCCA1 inhibits cysteine proteinases such as cathepsins K, L and S (81,82).

In terms of its clinical utility, its use as a marker for the diagnosis, prognosis, monitoring and histological differentiation of certain squamous lineage cancers, such as lung SCC, cervical cancer, OSCC and anal cancer, has been explored (81). This is particularly useful, since ~90% of head and neck cancer cases are of a squamous lineage.

Regarding its diagnostic utility, in certain cases, elevated levels of SCCA in the blood may suggest the presence of SCC. Furthermore, the presence of SCCA has been associated with unfavourable prognosis in certain cases, serving as a guide for clinical decision making (82).

This antigen may be physiologically found in saliva, hair and skin particles; thus, there is a certain risk of contamination that may lead to inaccurate SCCA values in current assays. Furthermore, it may be present in other benign conditions and its levels may vary based on the method of blood sample collection for subsequent analysis (83).

In OSCC, SCCA is useful for aiding in the diagnosis in certain cases, as elevated levels of SCCA in the blood may suggest the presence of SCC. In addition, the presence of SCCA has been associated with a poorer prognosis in certain cases. Due to its short half-life, which is <24 h, it may assist in postoperative monitoring; in this way, SCCA levels in the blood generally decrease after tumour resection. It also aids in monitoring the response to a specific therapy, as it may decrease in responsive tumours and increase with recurrence (82,83). The increased SCCA2/SCCA1 mRNA ratio in a primary tumour is also associated with cancer recurrence; hence, it is interesting to detect both SCCA1 and SCCA2 to determine total SCCA expression (82). However, currently available assay systems cannot differentiate between the two isoforms in this manner, as the assays were not developed for this specific purpose (81).

It is worth noting that currently, there is no firm evidence or reports on the interaction between HPV status and SCCA in relation to OSCC prognosis (83).

Research on SCCA remains active, with the aim of better understanding its function, clinical utility and limitations. In addition, more sensitive and specific assay methods for its detection and quantification are being developed and evaluated (82).

4. Circulating tumour cells (CTCs)

CTCs are those cells that break away from a primary tumour and enter the blood or lymphatic circulation, giving them the potential to spread to other parts of the body. Techniques used to detect and analyse CTCs include immunocapture, which uses specific antibodies to identify and isolate CTCs, and techniques based on nucleic acid amplification, such as PCR and RT-qPCR, to amplify and detect specific markers of CTCs. Currently, the screening test approved by the Food and Drug Administration is based on the detection of epithelial cell adhesion molecule)/cytokeratin-expressing cells in the blood using antibodies through the CellSearch platform (84,85).

Molecular analysis of CTCs provides information on the genetic and molecular characteristics of the primary tumour; in this way, specific genetic mutations may be examined, such as mutations in the TP53 gene, which is common in head and neck cancer. In addition, gene expression and chromosomal alterations in CTCs may be analysed to gain a more detailed understanding of the specific characteristics of the tumour (85,86).

In OSCC, the detection and characterization of CTCs have been studied to gain insight into tumour biology and to develop novel therapeutic strategies. Studies have indicated that the presence of CTCs is associated with poor prognosis and an increased risk of metastasis in patients with OSCC. The quantification and analysis of CTCs may provide valuable information about tumour progression, treatment response and the development of resistance (86,87).

Certain specific markers have been identified in CTCs, such as increased expression of Ki-67 (a cell proliferation marker) or genes associated with invasion and metastasis, such as Twist1 or Snail, which have been associated with unfavourable prognosis and a higher probability of relapse in this tumour type (88,89).

Another aspect to consider is that CTCs may acquire characteristics that confer resistance to conventional cancer treatments. This may be due to genetic and molecular changes that occur in tumour cells during the metastasis process. For instance, CTCs may develop mutations in genes related to drug response, such as genes involved in DNA repair or in the EGFR signalling pathway. One of the characteristics that confers these resistances to CTCs is their heterogeneity; certain CTCs may be inherently resistant to drugs, while others may develop resistance during treatment or may alter the expression of genes that are involved in the response to these therapies. Therefore, the study of CTCs and their resistance to treatments may provide key information to develop therapeutic strategies aimed at overcoming these resistances. Drug combinations may also be evaluated to address the heterogeneity of CTCs and target multiple survival pathways (90,91).

Another useful application of CTC analysis is the evaluation of the efficacy of treatments and the monitoring of tumour response over time. Changes in the number and characteristics of CTCs may indicate a positive or negative response to treatment. For instance, a decrease in the number of CTCs or changes in their molecular profile may suggest a favourable response. Conversely, persistent or increased CTCs during or after treatment may indicate a suboptimal response and poor prognosis. This may be particularly useful in the early detection of tumour progression and in therapeutic decision-making; for instance, the persistence of CTCs after treatment has been associated with an increased risk of relapse (92,93).

5. Salivary biomarkers

Although biopsies or serological markers have been considered the 'gold standard' for oral cancer diagnosis, other noninvasive techniques are being implemented to predict its development. Body fluids, such as saliva, have a specific structural composition for each condition or disease. Studies have been conducted on predictive, diagnostic or prognostic biomarkers found in saliva, with positive results for carcinomatous, inflammatory and genetic oral diseases. Furthermore, it has been observed that saliva may be useful for assessing certain systemic diseases with varying sensitivity and specificity (94).

One of the possible sample types is gingival crevicular fluid (GCF), a biological fluid found in the space between the tooth and the gum, known as the gingival sulcus. GCF is a clear, watery liquid that originates from periodontal tissues and contains a variety of components, such as proteins, enzymes, hormones, immune cells and tissue breakdown products. This fluid has an important role in maintaining periodontal health and may vary in composition based on oral conditions, such as gingival health, inflammation and periodontal disease. The analysis of GCF may provide valuable information about the health status of the gums and periodontal tissues. Another fluid of interest is dentinal tubular fluid (DTF), which has a fundamental role in protecting the pulp from microbial invasion when the pulp-dentin complex is injured. With the onset of the inflammatory cascade, the exudate of dentinal fluid mainly contains polymorphonuclear leukocytes, migrating macrophages, B cells and T cells. Therefore, the characterization of DTF may provide estimates of the extent of dentopulpal injury, the degree of pulpal inflammation or the efficacy of dental restoration (94,95).

Salivary biomarkers are proteomic or genomic macromolecules; >100 salivary biomarkers have been identified; these include DNA, RNA, mRNA, defensin-1, P53, Cyfra 21-1, tissue-specific polypeptide antigen, dual-specificity phosphatase, spermidine/spermine N1-acetyltransferase, profilin, cofilin-1 and transferrin. However, to date, no single marker has been agreed upon due to a lack of research and consensus among researchers. However, there are promising results regarding cytokines (96).

Cytokines are a group of low-molecular-weight glycoproteins produced by immune and nonimmune cells that have a crucial role in the regulation, signalling, maintenance and induction of most cellular interactions. They have roles in phenotypes and processes such as pleiotropy, inflammation and cellular apoptosis. Oral conditions, both benign (such as aphthous ulcers or periodontitis) and malignant (such as dysplastic lesions and oral and pharyngeal carcinomas), contribute to elevated cytokine levels. Among the most studied cytokines are interleukin 8 (IL8) and IL6, as well as tumour necrosis factor α (TNF-α) (97).

Among the cytokine analysis techniques for saliva samples, enzyme-linked immunosorbent assays are used for quantitative evaluation, and PCR is used for qualitative evaluation. Additional techniques include western blotting, migration assays, immunohistochemical staining, liquid chromatography and commercial colorimetric methods. Luminex bead-based multiplex assays are used to discoverdiagnostic biomarkers in human saliva and plasma responsible for tumour progression in patients with OSCC, reporting that IL-1β, macrophage inflammatory protein 1β, IFN-γ, TNF-α, IL-6, IL-8 and eotaxin are plausible salivary biomarkers (97,98).

Certain studies determined the presence of IL-6 and IL-8 cytokines in patients with OSCC, potentially malignant lesions (PML), and a control group. A significant increase in IL-6 and IL-8 values was observed in saliva samples from patients with OSCC compared to the control group. However, in the PML group, only IL-8 was found to be elevated. Furthermore, in the OSCC patient group, the ratio of these cytokines in serum and blood was compared, revealing that the levels of IL-8 were similar in serum and saliva samples, while the levels of IL-6 were higher in serum than in saliva samples from patients diagnosed with OSCC (98). However, in other studies, although these biomarkers were elevated in the OSCC group compared to the control group, no statistically significant differences were found (99).

While IL-8 levels may be altered due to lifestyle, geographical distribution, ethnic differences, genetic differences, gingivitis or periodontitis, none of these causes have led to levels as high as those observed in cases of OSCC. This is because IL-8 possesses angiogenic properties and contributes to tumour progression. Other cytokines, such as IL-6 and TNF-α, are also elevated in patients with oral leukoplakia, oral lichen planus and oral submucosal fibrosis, making them possible diagnostic markers for precancerous oral lesions (100).

Patients with OSCC are commonly diagnosed at advanced stages of malignancy and therefore have an unfavourable prognosis. Thus, identified salivary biomarkers may have a valuable role as a complement in the early detection and management of OSCC.

6. Conclusions

The study of histological and serological biomarkers in OSCC has shown great promise in improving the diagnosis, prognosis and management of this deadly disease. Histological examination remains the gold standard for diagnosing OSCC, providing valuable insight into tumour characteristics, such as tumour size, grade, invasion and lymph node involvement. However, the limitations of histology, including subjectivity and intraobserver variability, have prompted researchers to explore alternative approaches, such as serological biomarkers. Serological biomarkers, which may be easily measured in blood samples, offer a noninvasive and cost-effective means of detecting and monitoring OSCC. Various biomarkers, including CTCs, circulating tumour DNA and tumour-specific antigens, have been investigated. These biomarkers hold tremendous potential in aiding in early detection, prediction of the treatment response, monitoring of disease progression or identification of patients at high risk of recurrence; furthermore, the integration of histological and serological biomarkers has the potential to revolutionize OSCC management. Combining the morphological information obtained from histological analysis with the molecular insight offered by serological biomarkers may enhance diagnostic accuracy, enable personalized treatment and guide clinicians in making informed decisions about patient care. This multimodal approach may also facilitate the identification of novel therapeutic targets and the development of targeted therapies, leading to improved patient outcomes. The use of saliva for early cancer detection in the quest for new clinical markers is a promising approach since sample collection is simple and noninvasive.

On the other hand, it is essential to acknowledge the existing challenges and limitations in the field of histological and serological biomarkers in OSCC. Standardization of sample collection and processing and analysis methods is crucial to ensure reliable and reproducible results. In addition, large-scale multicentre studies are necessary to validate the clinical utility and establish the optimal cut-off values for different biomarkers.

To summarize, these biomarkers have the potential to transform the landscape of OSCC diagnosis, prognosis and treatment by providing valuable information on tumour characteristics and patient outcomes, but further research and collaboration are needed to refine these biomarkers and establish their clinical utility in the management of OSCC.

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Authors' contributions

LP, MJGG, ASC, JC, TP, JA, RDP and MAO were involved in the conceptualization of the study. MAO, MAM and JA were involved in funding acquisition. MAO and JA were involved in project administration. LP, MJGG, ASC, JC, TP, OFM, CGM, LLG, ARP, MAM, JA, RDP and MAO were involved in the investigative aspects of the study. All authors have read and agreed to the published version of the manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Cadoni G, Giraldi L, Petrelli L, Pandolfini M, Giuliani M, Paludetti G, Pastorino R, Leoncini E, Arzani D, Almadori G and Boccia S: Prognostic factors in head and neck cancer: A 10-year retrospective analysis in a single-institution in Italy. Acta Otorhinolaryngol Ital 37: 458-466, 2017.
2. Reyes-Gibby CC, Anderson KO, Merriman KW, Todd KH, Shete SS and Hanna EY: Survival patterns in squamous cell carcinoma of the head and neck: Pain as an independent prognostic factor for survival. J Pain 15: 1015-1022, 2014.
3. Barsouk A, Aluru JS, Rawla P, Saginala K and Barsouk A: Epidemiology, risk factors, and prevention of head and neck squamous cell carcinoma. Med Sci (Basel) 11: 42, 2023.
4. Gormley M, Creaney G, Schache A, Ingarfield K and Conway DI: Reviewing the epidemiology of head and neck cancer: Definitions, trends and risk factors. Br Dent J 233: 780-786, 2022.
5. Kim SM: Human papilloma virus in oral cancer. J Korean Assoc Oral Maxillofac Surg 42: 327-336, 2016.
6. Sathish N, Wang X and Yuan Y: Human papillomavirus (HPV)-associated oral cancers and treatment strategies. J Dent Res 93 (Suppl 7): 29S-36S, 2014.
7. Oliveira AC, Cavalcanti de Lima IC, Frez Marques VM, Alves de Araújo WH and De Campos Ferreira C: Human papillomavirus prevalence in oral and oropharyngeal squamous cell carcinoma in South America: A systematic review and meta-analysis. Oncol Rev 16: 552, 2022.
8. Petti S: Lifestyle risk factors for oral cancer. Oral Oncol 45: 340-350, 2009.
9. Candotto V, Lauritano D, Nardone M, Baggi L, Arcuri C, Gatto R, Gaudio RM, Spadari F and Carinci F: HPV infection in the oral cavity: Epidemiology, clinical manifestations and relationship with oral cancer. Oral Implantol (Rome) 10: 209-220, 2017.
10. Bagan J, Sarrion G and Jimenez Y: Oral cancer: Clinical features. Oral Oncol 46: 414-417, 2010.
11. Gileva OS, Libik TV and Danilov KV: Oral precancerous lesions: Problems of early detection and oral cancer prevention. AIP Conf Proc 1760: 020019, 2016.
12. Güneri P and Epstein JB: Late stage diagnosis of oral cancer: Components and possible solutions. Oral Oncol 50: 1131-1136, 2014.
13. Baykul T, Yilmaz HH, Aydin U, Aydin MA, Aksoy M and Yildirim D: Early diagnosis of oral cancer. J Int Med Res 38: 737-749, 2010.
14. McGuirt WF: Panendoscopy as a screening examination for simultaneous primary tumors in head and neck cancer: A prospective sequential study and review of the literature. Laryngoscope 92: 569-576, 1982.
15. Fulciniti F, Califano L, Zupi A and Vetrani A: Accuracy of fine needle aspiration biopsy in head and neck tumors. J Oral Maxillofac Surg 55: 1094-1097, 1997.
16. Burkill GJC, Evans RM, Raman VV and Connor SEJ: Modern radiology in the management of head and neck cancer. Clin Oncol (R Coll Radiol) 28: 440-450, 2016.
17. Dai YL and King AD: State of the art MRI in head and neck cancer. Clin Radiol 73: 45-59, 2018.
18. Irani S: Distant metastasis from oral cancer: A review and molecular biologic aspects. J Int Soc Prev Community Dent 6: 265-271, 2016.
19. El-Naaj IA, Leiser Y, Shveis M, Sabo E and Peled M: Incidence of oral cancer occult metastasis and survival of T1-T2N0 oral cancer patients. J Oral Maxillofac Surg 69: 2674-2679, 2011.
20. Zanoni DK, Patel SG and Shah JP: Changes in the 8th edition of the American joint committee on cancer (AJCC) staging of head and neck cancer: Rationale and implications. Curr Oncol Rep 21: 52, 2019.
21. Shah JP and Gil Z: Current concepts in management of oral cancer-surgery. Oral Oncol 45: 394-401, 2009.
22. Huang SH and O'Sullivan B: Oral cancer: Current role of radiotherapy and chemotherapy. Med Oral Patol Oral Cir Bucal 18: e233-e240, 2013.
23. Lang K, Baur M, Held T, Shafie RE, Moratin J, Freudlsperger C, Zaoui K, Bougatf N, Hoffmann J, Plinkert PK, *et al*: Definitive radiotherapy for squamous cell carcinoma of the oral cavity: A single-institution experience. Radiol Oncol 55: 467-473, 2021.
24. Hartner L: Chemotherapy for oral cancer. Dent Clin North Am 62: 87-97, 2018.
25. Kimura I, Kitahara H, Ooi K, Kato K, Noguchi N, Yoshizawa K, Nakamura H and Kawashiri S: Loss of epidermal growth factor receptor expression in oral squamous cell carcinoma is associated with invasiveness and epithelial-mesenchymal transition. Oncol Lett 11: 201-207, 2016.
26. Wang T, Sun S, Zeng X and Li J: ICI-based therapies: A new strategy for oral potentially malignant disorders. Oral Oncol 140: 106388, 2023.
27. Doumas S, Foukas PG, Economopoulou P, Kotsantis I and Psyrri A: Atypical patterns of responses in the era of immune checkpoint inhibitors in head and neck cancer. Oral Oncol 100: 104477, 2020.
28. Mohanty V, Subbannayya Y, Patil S, Puttamallesh VN, Najar MA, Datta KK, Pinto SM, Begum S, Mohanty N, Routray S, *et al*: Molecular alterations in oral cancer using high-throughput proteomic analysis of formalin-fixed paraffin-embedded tissue. J Cell Commun Signal 15: 447-459, 2021.
29. Tokuzen N, Nakashiro KI, Tojo S, Goda H, Kuribayashi N and Uchida D: Human papillomavirus-16 infection and p16 expression in oral squamous cell carcinoma. Oncol Lett 22: 528, 2021.

30. Vergori A, Garbuglia AR, Piselli P, Del Nonno F, Sias C, Lupi F, Lapa D, Baiocchini A, Cimaglia C, Gentile M, *et al*: Oral human papillomavirus DNA detection in HIV-positive men: Prevalence, predictors, and co-occurrence at anal site. BMC Infect Dis 18: 25, 2018.
31. Sritippho T, Chotjumlong P and Iamaroon A: Roles of human papillomaviruses and p16 in oral cancer. Asian Pac J Cancer Prev 16: 6193-6200, 2015.
32. Castro TPPG and Bussoloti Filho I: Prevalence of human papillomavirus (HPV) in oral cavity and oropharynx. Braz J Otorhinolaryngol 72: 272-281, 2006.
33. Katirachi SK, Grønlund MP, Jakobsen KK, Grønhøj C and von Buchwald C: The prevalence of HPV in oral cavity squamous cell carcinoma. Viruses 15: 451, 2023.
34. Doll C, Steffen C, Beck-Broichsitter B, Richter M, Neumann K, Pohrt A, Lohneis P, Lehmann A, Heiland M, Stromberger C, *et al*: The prognostic significance of p16 and its role as a surrogate marker for human papilloma virus in oral squamous cell carcinoma: An analysis of 281 cases. Anticancer Res 42: 2405-2413, 2022.
35. Christianto S, Li KY, Huang TH and Su Y: The prognostic value of human papilloma virus infection in oral cavity squamous cell carcinoma: A meta-analysis. Laryngoscope 132: 1760-1770, 2022.
36. Miranda-Galvis M, Rumayor Piña A, Sales de Sá R, Almeida Leite A, Agustin Vargas P, Calsavara VF, Lópes Pinto CA, Teng Y and Kowalski LP: PD-L1 expression patterns in oral cancer as an integrated approach for further prognostic classification. Oral Dis 27: 1699-1710, 2021.
37. Lenouvel D, González-Moles MÁ, Ruiz-Ávila I, Chamorro-Santos C, González-Ruiz L, González-Ruiz I and Ramos-García P: Clinicopathological and prognostic significance of PD-L1 in oral cancer: A preliminary retrospective immunohistochemistry study. Oral Dis 27: 173-182, 2021.
38. Dave K, Ali A and Magalhaes M: Increased expression of PD-1 and PD-L1 in oral lesions progressing to oral squamous cell carcinoma: A pilot study. Sci Rep 10: 9705, 2020.
39. Wu T, Tang C, Tao R, Yong X, Jiang Q and Feng C: PD-L1-mediated immunosuppression in oral squamous cell carcinoma: Relationship with macrophage infiltration and epithelial to mesenchymal transition markers. Front Immunol 12: 693881, 2021.
40. Burtness B, Harrington KJ, Greil R, Soulières D, Tahara M, de Castro G Jr, Psyrri A, Basté N, Neupane P, Bratland Å, *et al*: Pembrolizumab alone or with chemotherapy versus cetuximab with chemotherapy for recurrent or metastatic squamous cell carcinoma of the head and neck (KEYNOTE-048): A randomised, open-label, phase 3 study. Lancet 394: 1915-1928, 2019.
41. Ries J, Agaimy A, Wehrhan F, Baran C, Bolze S, Danzer E, Frey S, Jantsch J, Möst T, Büttner-Herold M, *et al*: Importance of the PD-1/PD-L1 axis for malignant transformation and risk assessment of oral leukoplakia. Biomedicines 9: 194, 2021.
42. Vermorken JB, Mesia R, Rivera F, Remenar E, Kawecki A, Rottey S, Erfan J, Zabolotnyy D, Kienzer HR, Cupissol D, *et al*: Platinum-based chemotherapy plus cetuximab in head and neck cancer. N Engl J Med 359: 1116-1127, 2008.
43. Botticelli A, Cirillo A, Strigari L, Valentini F, Cerbelli B, Scagnoli S, Cerbelli E, Zizzari IG, Rocca CD, D'Amati G, *et al*: Anti-PD-1 and anti-PD-L1 in head and neck cancer: A network meta-analysis. Front Immunol 12: 705096, 2021.
44. Fasano M, Corte CMD, Liello RD, Viscardi G, Sparano F, Iacovino ML, Paragliola F, Piccolo A, Napolitano S, Martini G, *et al*: Immunotherapy for head and neck cancer: Present and future. Crit Rev Oncol Hematol 174: 103679, 2022.
45. Wongpattaraworakul W, Gibson-Corley KN, Choi A, Buchakjian MR, Lanzel EA, Rajan Kd A and Simons AL: Prognostic role of combined EGFR and tumor-infiltrating lymphocytes in oral squamous cell carcinoma. Front Oncol 12: 885236, 2022.
46. Chen IH, Chang JT, Liao CT, Wang HM, Hsieh LL and Cheng AJ: Prognostic significance of EGFR and Her-2 in oral cavity cancer in betel quid prevalent area cancer prognosis. Br J Cancer 89: 681-686, 2003.
47. Kang JJ, Ko A, Kil SH, Mallen-St Clair J, Shin DS, Wang MB and Srivatsan ES: EGFR pathway targeting drugs in head and neck cancer in the era of immunotherapy. Biochim Biophys Acta Rev Cancer 1878: 188827, 2023.
48. Bossi P, Resteghini C, Paielli N, Licitra L, Pilotti S and Perrone F: Prognostic and predictive value of EGFR in head and neck squamous cell carcinoma. Oncotarget 7: 74362-74379, 2016.
49. Moharil R, Khandekar S, Dive A and Bodhade A: Cyclin D1 in oral premalignant lesions and oral squamous cell carcinoma: An immunohistochemical study. J Oral Maxillofac Pathol 24: 397, 2020.
50. Lam KY, Ng IO, Yuen AP, Kwong DL and Wei W: Cyclin D1 expression in oral squamous cell carcinomas: Clinicopathological relevance and correlation with p53 expression. J Oral Pathol Med 29: 167-172, 2000.
51. Ahmed MAS, Omer N, Suliman AM and Ellaithi M: Expression of cyclin D1 in oral squamous cell carcinoma. Sudan J Med Sci 16: 558-566, 2021.
52. Saawarn S, Astekar M, Saawarn N, Dhakar N and Gomateshwar Sagari S: Cyclin D1 expression and its correlation with histopathological differentiation in oral squamous cell carcinoma. ScientificWorldJournal 2012: 978327, 2012.
53. Acero-Mondragón E, Rodríguez-Farías R, Salazar-Hernández M and Figueroa-Avendaño J: Histological correlation of Ki-67 and D1 cycline markers expression in squamous cell carcinoma in the oral cavity. Rev Odontol Mex 20: e225-e229, 2016.
54. Ramos-García P, González-Moles MÁ, González-Ruiz L, Ayén Á, Ruiz-Ávila I, Bravo M and Gil-Montoya JA: Clinicopathological significance of tumor cyclin D1 expression in oral cancer. Arch Oral Biol 99: 177-182, 2019.
55. Huang SF, Cheng SD, Chuang WY, Chen IH, Liao CT, Wang HM and Hsieh LL: Cyclin D1 overexpression and poor clinical outcomes in Taiwanese oral cavity squamous cell carcinoma. World J Surg Oncol 10: 40, 2012.
56. Awawdeh MA, Sasikumar R, Aboalela AA, Siddeeqh S, Gopinathan PA, Sawair F and Khanagar SB: Evaluation of prognostic significance of the expression of p53, cyclin D1, EGFR in advanced oral squamous cell carcinoma after chemoradiation-A systematic review. Appl Sci 13: 5292, 2023.
57. Takkem A, Barakat C, Zakarea S, Zaid K, Najmeh J, Ayoub M and Seirawan MY: Ki-67 prognostic value in different histological grades of oral epithelial dysplasia and oral squamous cell carcinoma. Asian Pac J Cancer Prev 19: 3279-3286, 2018.
58. Davey MG, Hynes SO, Kerin MJ, Miller N and Lowery AJ: Ki-67 as a prognostic biomarker in invasive breast cancer. Cancers (Basel) 13: 4455, 2021.
59. Li LT, Jiang G, Chen Q and Zheng JN: Ki67 is a promising molecular target in the diagnosis of cancer (review). Mol Med Rep 11: 1566-1572, 2015.
60. Xie S, Liu Y, Qiao X, Hua RX, Wang K, Shan XF and Cai ZG: What is the prognostic significance of Ki-67 positivity in oral squamous cell carcinoma? J Cancer 7: 758-767, 2016.
61. Meyer HJ, Gundermann P and Surov A: Associations between FDG-PET and Ki 67-index in head and neck cancer: A meta-analysis. Medicine (Baltimore) 98: e17472, 2019.
62. Lopes VKM, de Jesus AS, de Souza LL, Miyahara LAN, Guimarães DM, Pontes HAR, Pontes FSC and Carvalho PL: Ki-67 protein predicts survival in oral squamous carcinoma cells: An immunohistochemical study. Braz Oral Res 31: e66, 2017.
63. Mohamed AA, EL-Shall OS and EL-Kilani NS: Role of expression Ki-67 in diagnosis of oral cancer. Al-Azhar Dent J Girls 7: 441-445, 2020.
64. Govindaraj PK, Kallarakkal TG, Mohd Zain R, Tilakaratne WM and Lew HL: Expression of Ki-67, Cornulin and ISG15 in non-involved mucosal surgical margins as predictive markers for relapse in oral squamous cell carcinoma (OSCC). PLoS One 16: e0261575, 2021.
65. Pan W, Zhang C, Chen M, Min S, Xu L and Chi Z: Expression of Ki-67 and P16 are related with HPV in squamous cell carcinoma of the external auditory canal. J Otolaryngol Head Neck Surg 51: 40, 2022.
66. Jun W, Shaobo O, Xianhua Z, Siyu Z, Mingyang C, Xin F, Ying C and Lan L: Deregulation of hsa_circ_0001971/miR-186 and hsa_circ_0001874/miR-296 signaling pathways promotes the proliferation of oral squamous carcinoma cells by synergistically activating SHP2/PLK1 signals. Sci Rep 11: 20561, 2021.
67. Murase R, Abe Y, Takeuchi T, Nabeta M, Imai Y, Kamei Y, Kagawa-Miki L, Ueda N, Sumida T, Hamakawa H and Kito K: Serum autoantibody to sideroflexin 3 as a novel tumor marker for oral squamous cell carcinoma. Proteomics Clin Appl 2: 517-527, 2008.
68. Mauro C, Passerini R, Spaggiari L, Galetta D, Radice D, Lentati P and Sandri MT: New and old biomarkers in the differential diagnosis of lung cancer: Pro-gastrin-releasing peptide in comparison with neuron-specific enolase, carcinoembryonic antigen, and CYFRA 21-1. Int J Biol Markers 34: 163-167, 2019.

69. Deepa T and Thirrunavukkarasu N: Saliva as a potential diagnostic tool. Indian J Med Sci 64: 293-306, 2010.
70. Johnstone S and Logan RM: The role of vascular endothelial growth factor (VEGF) in oral dysplasia and oral squamous cell carcinoma. Oral Oncol 42: 337-342, 2006.
71. Edirisinghe ST, Weerasekera M, De Silva DK, Devmini MT, Pathmaperuma S, Wijesinghe GK, Nisansala T, Maddumage A, Huzaini H, Rich AM, *et al*: Vascular endothelial growth factor A (VEGF-A) and vascular endothelial growth factor receptor 2 (VEGFR-2) as potential biomarkers for oral squamous cell carcinoma: A Sri Lankan study. Asian Pac J Cancer Prev 24: 267-274, 2023.
72. Kim SK, Park SG and Kim KW: Expression of vascular endothelial growth factor in oral squamous cell carcinoma. J Korean Assoc Oral Maxillofac Surg 41: 11-18, 2015.
73. Loh CY, Chai JY, Tang TF, Wong WF, Sethi G, Shanmugam MK, Chong PP and Looi CY: The E-cadherin and N-cadherin switch in epithelial-to-mesenchymal transition: Signaling, therapeutic implications, and challenges. Cells 8: 1118, 2019.
74. Alsafadi R and Manadili A: The correlation between CD44 and angiogenesis in oral squamous cell carcinoma induced in buccal pouch in syrian hamster that underwent radiotherapy. Asian Pac J Cancer Prev 23: 3571-3576, 2022.
75. Zhang CZ, Cheng XQ, Li JY, Zhang P, Yi P, Xu X and Zhou XD: Saliva in the diagnosis of diseases. Int J Oral Sci 8: 133-137, 2016.
76. Roi A, Rusu LC, Roi CI, Luca RE, Boia S and Munteanu RI: A new approach for the diagnosis of systemic and oral diseases based on salivary biomolecules. Dis Markers 2019: 8761860, 2019.
77. Goldoni R, Scolaro A, Boccalari E, Dolci C, Scarano A, Inchingolo F, Ravazzani P, Muti P and Tartaglia G: Malignancies and biosensors: A focus on oral cancer detection through salivary biomarkers. Biosensors (Basel) 11: 396, 2021.
78. Konings H, Stappers S, Geens M, De Winter BY, Lamote K, van Meerbeeck JP, Specenier P, Vanderveken OM and Ledeganck KJ: A literature review of the potential diagnostic biomarkers of head and neck neoplasms. Front Oncol 10: 1020, 2020.
79. Rajkumar K, Ramya R, Nandhini G, Rajashree P, Ramesh Kumar A and Nirmala Anandan S: Salivary and serum level of CYFRA 21-1 in oral precancer and oral squamous cell carcinoma. Oral Dis 21: 90-96, 2015.
80. Liu L, Xie W, Xue P, Wei Z, Liang X and Chen N: Diagnostic accuracy and prognostic applications of CYFRA 21-1 in head and neck cancer: A systematic review and meta-analysis. PLoS One 14: e0216561, 2019.
81. Liu Y, Cao Y, He S and Cai W: Technical and clinical performance of two methods to detect squamous cell carcinoma antigen levels for comparing pathological diagnosis coincidence rates in lung, cervical, and head and neck cancers. Clin Lab 66, 2020.
82. Yasumatsu R, Nakano T, Hashimoto K, Kogo R, Wakasaki T and Nakagawa T: The clinical value of serum squamous cell carcinoma antigens 1 and 2 in head and neck squamous cell carcinoma. Auris Nasus Larynx 46: 135-140, 2019.
83. Obata K, Yutori H, Yoshida K, Sakamoto Y, Ono K and Ibaragi S: Relationships between squamous cell carcinoma antigen and cytokeratin 19 fragment values and renal function in oral cancer patients. Int J Oral Maxillofac Surg 52: 417-422, 2023.
84. Lin D, Shen L, Luo M, Zhang K, Li J, Yang Q, Zhu F, Zhou D, Zheng S, Chen Y and Zhou J: Circulating tumor cells: Biology and clinical significance. Signal Transduct Target Ther 6: 404, 2021.
85. Deng Z, Wu S, Wang Y and Shi D: Circulating tumor cell isolation for cancer diagnosis and prognosis. EBioMedicine 83: 104237, 2022.
86. Kulasinghe A, Hughes BGM, Kenny L and Punyadeera C: An update: Circulating tumor cells in head and neck cancer. Expert Rev Mol Diagn 19: 1109-1115, 2019.
87. Perumal V, Corica T, Dharmarajan A, Sun Z, Dhaliwal SS, Dass CR and Dass J: Circulating tumour cells (CTC), head and neck cancer and radiotherapy; future perspectives. Cancers (Basel) 11: 367, 2019.
88. Liskova A, Samec M, Koklesova L, Giordano FA, Kubatka P and Golubnitschaja O: Liquid biopsy is instrumental for 3PM dimensional solutions in cancer management. J Clin Med 9: 2749, 2020.
89. Anjum A, Shree R.V B, Jayaram Thambiah L and Rodrigues C: A comparitive and prognostic study of circulating tumor cells and vegfr-2 in different histological grades of oral squamous cell carcinoma. Int J Adv Res 10: 745-754, 2022.
90. Qayyumi B, Bharde A, Aland G, D'Souza A, Jayant S, Singh N, Tripathi S, Badave R, Kale N, Singh B, *et al*: Circulating tumor cells as a predictor for poor prognostic factors and overall survival in treatment naïve oral squamous cell carcinoma patients. Oral Surg Oral Med Oral Pathol Oral Radiol 134: 73-83, 2022.
91. Gauthier A, Philouze P, Lauret A, Alphonse G, Malesys C, Ardail D, Payen L, Céruse P, Wozny AS and Rodriguez-Lafrasse C: Circulating tumor cell detection during neoadjuvant chemotherapy to predict early response in locally advanced oropharyngeal cancers: A prospective pilot study. J Pers Med 12: 445, 2022.
92. Curtin J, Thomson P, Wong G, Lam A and Choi SW: The impact of surgery on circulating malignant tumour cells in oral squamous cell carcinoma. Cancers (Basel) 15: 584, 2023.
93. Zhang X, Ekanayake Weeramange C, Hughes BGM, Vasani S, Liu ZY, Warkiani ME, Hartel G, Ladwa R, Thiery JP, Kenny L and Punyadeera C: Application of circulating tumour cells to predict response to treatment in head and neck cancer. Cell Oncol (Dordr) 45: 543-555, 2022.
94. Sahibzada HA, Khurshid Z, Khan RS, Naseem M, Siddique KM, Mali M and Zafar MS: Salivary IL-8, IL-6 and TNF-α as potential diagnostic biomarkers for oral cancer. Diagnostics (Basel) 7: 21, 2017.
95. Khurshid Z, Zafar MS, Khan RS, Najeeb S, Slowey PD and Rehman IU: Role of salivary biomarkers in oral cancer detection. In: Advances in Clinical Chemistry. Vol. 86. Elsevier, pp23-70, 2018. http://dx.doi.org/10.1016/bs.acc.2018.05.002.
96. Khurshid Z, Warsi I, Moin SF, Slowe PD, Latif M, Zohaib S and Zafar MS: Biochemical analysis of oral fluids for disease detection. In: Advances in Clinical Chemistry. Vol. 100. Elsevier, pp205-253, 2021. http://dx.doi.org/10.1016/bs.acc.2020.04.005.
97. Chu HW, Chang KP, Hsu CW, Chang IY, Liu HP, Chen YT and Wu CC: Identification of salivary biomarkers for oral cancer detection with untargeted and targeted quantitative proteomics approaches. Mol Cell Proteomics 18: 1796-1806, 2019.
98. Chiamulera MMA, Zancan CB, Remor AP, Cordeiro MF, Gleber-Netto FO and Baptistella AR: Salivary cytokines as biomarkers of oral cancer: A systematic review and meta-analysis. BMC Cancer 21: 205, 2021.
99. St John MA, Li Y, Zhou X, Denny P, Ho CM, Montemagno C, Shi W, Qi F, Wu B, Sinha U, *et al*: Interleukin 6 and interleukin 8 as potential biomarkers for oral cavity and oropharyngeal squamous cell carcinoma. Arch Otolaryngol Head Neck Surg 130: 929-935, 2004.
100. Cheng YSL, Rees T and Wright J: A review of research on salivary biomarkers for oral cancer detection. Clin Transl Med 3: 3, 2014.