

Salivary biomarkers for the early detection of oral squamous cell carcinoma: Progress and challenges

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Abstract: Biomarker-oriented research represents an emerging research area within oncology. As a complex body fluid mainly secreted by salivary glands, saliva is intrinsically linked to the oral cavity and carries abundant biological information. Saliva not only serves as a vast repository of biomarkers, but salivary diagnostics have also emerged as a new diagnostic technique, offering advantages such as non-invasiveness, ease of collection, and low costs. Nevertheless, the oral microenvironment is highly dynamic and heterogeneous, which can readily alter biomarker concentrations. For this reason, validating genuinely reliable salivary biomarkers still poses considerable technical obstacles. This study systematically collates currently available clinical evidence to elaborate the biological profiles of salivary biomarkers applicable for oral squamous cell carcinoma, including the inflammatory mediators IL-8 and TNF- α , as well as CD44 and mRNA-related molecular indicators. This comprehensive review summarizes current research progress, providing practical insights for the optimization of early diagnostic schemes and therapeutic strategies for oral cancer.

Keywords: Saliva; biomarker; interleukin-8; tumor necrosis factor- α , oral squamous cell carcinoma

1. Introduction

Head and neck squamous cell carcinoma (HNSCC) accounts for approximately 890,000 patients new cases annually, with an overall five-year survival rate hovering around 50%^[1,2]. Among all head and neck malignant tumors, oral cancer represents the most prevalent subtype. Squamous cell carcinoma constitutes about 90% of all oral malignancies. For patients suffering from oral squamous cell carcinoma, early identification, timely diagnosis and appropriate therapeutic intervention remain essential to prolonging survival and preserving postoperative quality of life. In clinical practice, definitive diagnosis of OSCC relies not only on physicians' clinical experience, but also on a combination of auxiliary imaging tests and pathological biopsy, including ultrasound and cone-beam computed tomography (CBCT). Nevertheless, such conventional examination methods can only identify tumor lesions after they have developed to a measurable size. This leads to delayed early screening for occult lesions and recurrent tumors in patients who have undergone tissue flap reconstruction, making early detection of recurrence extremely difficult. The continuous advancement of molecular biology and immunological techniques has greatly promoted in-depth exploration of tumor biomarkers. Specifically, emerging studies on OSCC-associated biomarkers have verified their promising clinical prospects, which can be applied to auxiliary clinical diagnosis and long-term prognostic monitoring of OSCC^[3].

Histopathologically, entire saliva includes exfoliated oral mucosal epithelial cells from across the whole oral cavity. This makes it a non-invasive sample that draws from the same epithelial lineage in which HNSCC originates, but covers a much broader anatomical area than a single biopsy site^[4]. Particularly, tumor markers may have certain clinical value in the diagnosis and prognosis of OSCC. As a detection medium, saliva has the characteristics of safe, painless, and non-traumatic sampling, simple operation, and repeated sampling, which is easily accepted by patients^[5]. It has been confirmed that the changes of tumor markers related to OSCC can be detected in the saliva of OSCC patients, such as IL-8, TNF- α ^[6], mRNA^[7], CD44 as well as Cyfra21-18^[8]. These studies suggest that it is of great scientific significance to find important indicators of systemic diseases from saliva and assist the early prediction, diagnosis, and treatment of OSCC.

2. Diagnostic Advantages of Salivary Tumor Markers in OSCC

From a clinical standpoint, biopsies are the main diagnostic technique used by numerous clinician for detecting initial-stage OSCC. Tissue biopsy is esteemed as the premier method for identifying OSCC due to its invasive nature, which may lead to issues like pain, bleeding, and infection; additionally, sampling bias may cause incorrect negative outcomes. The challenge in applying tissue biopsy for extensive early screening of high-risk individuals and ongoing surveillance of precancerous lesions from low patient adherence and the hassle of multiple tests, leading researchers to persistently investigate innovative non-invasive liquid biopsy approaches. The advantages of using salivary tumor marker screening instead of serum screening include: 1) It is non-invasive^[9]; the sampling procedure is safe, completely painless, and trauma-free, thereby avoiding anxiety caused by fear of trauma; 2) Simple sampling—operators can master the collection technique with only appropriate training; 3) Low cost; 4) Convenient storage and transportation; 5) Easy acquisition of large sample volumes, allowing for long-term follow-up testing^[3]. Based on these advantages, saliva-based screening has become a relatively ideal method for detecting tumor markers. As an ideal screening strategy, it facilitates the work of both physicians and patients, reduces the need for many invasive procedures, and holds potential value for the periodic monitoring and screening of tumors.

3. Salivary molecular indicators derived from patients diagnosed with OSCC

Low-molecular-weight glycoproteins can be synthesized by both immune cells and malignant epithelial cells in human tissues, among which cytokines serve as core mediators regulating immune responses. Cytokines have attracted significant attention in the scientific community because they mediate signal transduction and regulate intercellular interactions, and are regarded as “molecular messengers” of cellular communication^[6]. Cytokines have a wide range of functions and take part in biological processes such as inflammatory responses, cell apoptosis, immune regulation, angiogenesis, and matrix remodeling. In the microenvironment of oral squamous cell carcinoma lesions, persistent chronic inflammatory stimulation promotes the release of large amounts of pro-inflammatory cytokines into the secretions of oral glands, which can ultimately be detected in saliva. These inflammation-related molecules not only contribute to the formation of a tumor-promoting microenvironment, driving the progression of abnormal epithelial hyperplasia to malignant lesions, but also serve as important candidate salivary biomarkers for non-invasive OSCC screening.

3.1 IL-8

IL-8, a classic pro-inflammatory factor in the chemokine family, can be released from neutrophils and macrophages responding to different external stressors. Various stimulatory signals promote the transcription and expression of IL-8 by activating the NF- κ B pathway. IL-8 primarily acts on two receptors, CXCR1 and CXCR2, both of which are expressed on the surfaces of tumor-associated macrophages, neutrophils, and tumor cells. The expression of IL-8 receptors on tumor cells confirms that IL-8 acts as a vital regulatory factor inside the tumor microenvironment.^[10]

Mounika et al^[11]. collected saliva samples from 120 patients, of whom 60 with OSCC served as the experimental group and 60 as the healthy control group. After testing, IL-8 was isolated from the saliva, and the results showed a mean concentration of 22.76 ± 5.11 in the experimental group, compared to 7.45 ± 2.56 in the control group ($P < 0.05$). In addition, Kalpani et al.^[10], also isolated IL-8 in their study of oral mucosal fibrosis and oral lichen planus (14.083-fold for OSCC; 15.475-fold for OSF; 2.260-fold for OLP), suggesting that submucosal fibrosis of the oral mucosa shares molecular expression characteristics similar to those of OSCC, which may explain the higher malignant potential of OSF. According to multiple published investigations, the concentration of salivary IL-8 remains extremely low among people without oral malignancies, but they increase significantly in patients with OSCC. Therefore, inflammatory mediators play a crucial role in oral cancer.

3.2 TNF- α

Tumor necrosis factor- α acts as a vital pro-inflammatory cytokine involved in regulating various biological processes, including the inflammatory response, apoptosis, and angiogenesis. Elevated concentrations of salivary TNF- α can be observed in patients suffering from oral squamous cell carcinoma and oral premalignant lesions. However, TNF- α is not a tumor-specific marker; periodontal disease and chronic inflammation of the oral mucosa can also cause its upregulation. Detecting TNF- α

alone makes it difficult to distinguish between benign inflammatory lesions, precancerous lesions, and early-stage oral squamous cell carcinoma; therefore, it is often used as one component of a combination of biomarkers for diagnostic purposes^[12].

Kumar et al.^[13] found that TNF- α is a highly promising tool for diagnosing OSCC; TNF- α was detected in saliva specimens obtained from 40 OSCC patients. Patients with TNM stage II and III malignancies exhibited markedly elevated cytokine levels up to 6.0-fold ($P = 0.0001$). The sensitivity of TNF- α was 90%, and its specificity was 80% (AUC: 0.88). Additionally, in a study by Dikova et al.^[14] on oral leukoplakia—a precancerous lesion—researchers analyzed saliva samples and found TNF- α levels of 17.01 pg/mL (sensitivity 86.36%, specificity 100%). Furthermore, 33 samples of early oral squamous cell carcinoma and 33 samples of advanced oral squamous cell carcinoma were tested by U test, and the corresponding test results were 59.82 ± 11.56 and 122.52 ± 20.68 , respectively. Several studies have also shown that Tnf- α concentration in patients with oral lichen planus (OLP) is significantly higher than that in control populations. Given that Tnf- α is involved in the regulation of inflammatory response and signaling pathways, the expression level of Tnf- α is expected to become a clinical diagnostic marker with application value.

3.3 CD44

As a key adhesion molecule mediating cell and extracellular matrix interactions, CD44 presents elevated expression across numerous malignant tumors and is well-accepted as a typical biomarker for tumor stem cells^[15]. In normal physiological states, CD44 is capable of binding with hyaluronic acid, thereby maintaining stable adhesion between cells and extracellular matrix. Moreover, tumor cells can interact with hyaluronic acid in the extracellular matrix through CD44^[16]. Proteolytic cleavage of the CD44 extracellular domain takes place in numerous malignancies, lowering its surface-resident abundance. Disruption of CD44 weakens adhesive contacts between tumor cells and the extracellular matrix. Tumor cells consequently detach from the primary lesion, gain migratory potential, and go on to facilitate local invasion as well as distant metastatic spread^[17].

Petrovic et al.^[8], in their study of the expression of candidate tumor stem cell markers in oral squamous cell carcinoma, found that sustained high expression of CD44 across all patient specimens (positive expression rate range from 83.8% to 99.0%, with a median value of 96.4%). Expression was more pronounced in advanced-stage tumors. At the same time, as a tumor stem cell marker, high CD44 expression not only contributes to the onset and invasive progression of OSCC but is also associated with a poor prognosis^[18]. Patients with overexpressed CD44 tend to have shorter overall survival, increased locoregional recurrence rates, and reduced tumor sensitivity to radiation therapy^[19]. Therefore, in addition to its use in early screening, salivary CD44 holds great potential for assisting the evaluation of patient prognosis and therapeutic response.

3.4 miRNA

Exosomal microRNAs (miRNAs) show great potential for clinical translation in the early risk prediction, disease progression assessment and dynamic therapeutic monitoring of various malignant tumors^[20]. With high tissue specificity, miRNAs are capable of negatively regulating approximately 60% of human protein-coding genes. Therefore, screening for miRNA-derived biomarkers and elucidating the miRNA-mRNA regulatory networks that underlie abnormalities in various biological processes and signaling pathways holds significant research value^[21-23].

Through miRNA expression profiling analysis, Patel et al.^[24] found that significant enrichment of differentially expressed miRNAs in both primary tumor tissues and salivary exosomes isolated from OSCC patients. Compared with normal control samples, a total of 188 differentially expressed miRNAs were identified in tumor tissue and 181 in oral leukoplakia samples. In salivary exosomes, 316 altered miRNAs were screened from OSCC patients and 69 from leukoplakia cases, and 69 in salivary exosomes from patients with leukoplakia. Among these dysregulated molecules, 47 molecules exhibited consistent differential expression across all sample types and disease stages, including premalignant leukoplakia lesions. The study results confirm that the miRNA signature derived from saliva brushings possesses good diagnostic value; Dysregulated miRNA expression facilitates the transformation from normal epithelial phenotypes to malignant phenotypes in the oral cavity, rendering these signatures promising biomarkers for the early diagnosis of OSCC.

3.5 Cyfra21-1

CYFRA21-1 represents a soluble fragment of cytokeratin 19 (CK19), which is an acidic polypeptide that constitutes the cytoskeleton of squamous epithelial cells and is the cytokeratin with the lowest molecular weight in squamous cell carcinoma. Under normal conditions, CK19 fragments exist in the form of oligomers at extremely low levels^[25-27]. During the processes of necrosis and apoptosis in tumor cells, CK19 is cleaved by proteases to form the soluble CYFRA21-1 fragments, which are then released into the extracellular space. CYFRA 21-1 remains stable in bodily fluids such as saliva and serum and is a classic protein-based salivary biomarker for oral squamous cell carcinoma research^[28-30].

Ping et al^[31] collected saliva samples from 30 patients with OSCC to detect CYFRA21-1. The results revealed that the average level of CYFRA21-1 in OSCC group was 85.95 ± 78.00 $\mu\text{g/L}$. The mean value of salivary CYFRA21-1 in the healthy control group was 42.27 ± 48.84 $\mu\text{g/L}$, suggesting that the salivary CYFRA21-1 level in the patients with OSCC was significantly elevated than that in the healthy people. The team further analyzed the association between salivary CYFRA21-1 expression and patient prognosis. 10 of the 30 patients in the study had tumor recurrence, and these patients had significantly higher salivary CYFRA21-1 levels before treatment than those without recurrence; However, there was no significant difference in saliva test results between dead patients and survival patients. A number of existing studies have confirmed that the expression of salivary CYFRA21-1 in patients with OSCC is notably increased compared with healthy individuals, and its concentration is also related to tumor stage and tissue differentiation degree, which has the application potential in auxiliary clinical diagnosis and disease dynamic monitoring. However, the clinical specificity of this marker is not ideal, and chronic oral inflammation, benign epithelial hyperplasia and other conditions can also cause a slight increase in CYFRA21-1. It is difficult to complete the accurate identification of early cases only relying on this single marker.

Collectively, a comprehensive analysis of published data on the detection of salivary cytokines in oral cancer studies reveals that chemokines play a critical signaling role throughout the entire process of tumor initiation, malignant proliferation, and disease progression. Compared with other types of pro-inflammatory mediators, chemokines are more strongly associated with localized oral neoplastic lesions and can help shape a local microenvironment conducive to tumor development. As a liquid biopsy sample with practical application potential, saliva is easy to collect, non-invasive, readily accepted by study participants, and the overall cost of testing remains within an acceptable range. Leveraging these unique advantages, biomarker testing using saliva shows considerable potential for auxiliary applications in the preliminary screening of high-risk populations for oral squamous cell carcinoma, the adjuvant diagnosis of clinical lesions, and the dynamic monitoring of treatment efficacy during the therapeutic phase. However, a review of the literature reveals a practical challenge: due to various confounding factors—including subject baseline conditions, sample processing procedures, and testing platforms—there remain significant discrepancies between the results of tests for the same class of molecular biomarkers reported by different research teams.

4. Conclusions

As a type of non-invasive liquid biopsy sample, saliva shows great potential for the early adjuvant diagnosis of oral squamous cell carcinoma. Compared to traditional tissue biopsy methods, saliva biomarker testing does not cause injuries such as wounds or bleeding and can effectively avoid sampling bias; samples can be collected multiple times, which is more conducive to screening high-risk populations and long-term follow-up monitoring of patients, and is more readily accepted. Various salivary molecules—including the inflammatory mediators IL-8 and TNF- α , the cell adhesion molecule CD44, exosome-derived miRNAs, and CYFRA21-1—exhibit abnormal expression levels in both patients with oral squamous cell carcinoma and those with oral precancerous lesions. These molecules reflect the inflammatory state of the tumor microenvironment, the biological characteristics of tumor stem cells, and signaling pathways associated with cellular malignant transformation, providing valuable insights for disease diagnosis and prognosis assessment.

Nevertheless, individual biomarkers still suffer from a lack of specificity. Benign oral conditions such as periodontal inflammation, oral lichen planus, and submucosal fibrosis can also cause upregulation of these molecules, making it difficult to distinguish between ordinary inflammation, precancerous lesions, and early-stage oral squamous cell carcinoma based solely on a single indicator. The comparability of results across different studies is poor, primarily due to inconsistencies in subject inclusion criteria, saliva collection protocols, testing equipment, and cutoff values. Currently, the industry has yet to establish

standardized operating procedures or unified criteria for the detection of salivary biomarkers.

It is evident that relying on a single indicator alone does not meet the practical requirements for clinical diagnosis. In the future, we should advance large-scale, multicenter clinical studies to identify saliva-based candidate biomarkers with greater stability, establish a multi-parameter combined diagnostic system, improve the sensitivity and specificity of detection methods, and promote the clinical translation of saliva-based liquid biopsy technology, thereby providing a simple and feasible method for early-stage risk screening and prognostic assessment of oral squamous cell carcinoma.

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References

- [1] Bray F, Laversanne M, Sung H, et al. *Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries*[J]. *CA: A Cancer Journal for Clinicians*, 2024, 74(3), 229–263.
- [2] Johnson D E, Burtneß B, Leemans C R, et al. *Head and neck squamous cell carcinoma*[J]. *Nature reviews. Disease primers*, 2020, 6(1): 92.
- [3] GONG Tao. *Research progress of salivary tumor markers* [J]. *Journal of Sichuan University (Medical Edition)*, 2023, 54(3): 462.
- [4] Rapado-González Ó, Rodríguez-Ces A M, López-López R, et al. *Liquid biopsies based on cell-free DNA as a potential biomarker in head and neck cancer*[J]. *The Japanese Dental Science Review*, 2023, 59: 289-302.
- [5] Huang L. *Research progress of salivary tumor markers*[J]. *International Journal of Stomatology*, 2020, 47(1): 68.
- [6] Sahibzada H A, Khurshid Z, Khan R S, et al. *Salivary IL-8, IL-6 and TNF- α as Potential Diagnostic Biomarkers for Oral Cancer*[J]. *Diagnostics*, 2017, 7(2): 21.
- [7] Shaw A, Garcha V, Shetty V, et al. *Diagnostic Accuracy of Salivary Biomarkers in Detecting Early Oral Squamous Cell Carcinoma: A Systematic Review and Meta-Analysis*[J]. *Asian Pacific Journal of Cancer Prevention*, 2022, 23(5): 1483-1495.
- [8] Petrović A, Mojsilović S, Bugarski D, et al. *Expression of Putative Cancer Stem Cell Markers in Oral Squamous Cell Carcinoma: Correlation with Clinicopathological Features*[J]. *International Journal of Molecular Sciences*, 2025, 26(22): 10939.
- [9] Zhang A, Sun H, Wang X. *Saliva Metabolomics Opens Door to Biomarker Discovery, Disease Diagnosis, and Treatment*[J]. *Applied Biochemistry and Biotechnology*, 2012, 168(6): 1718-1727.
- [10] Senevirathna K, Mahakapuge T A N, Jayawardana N U, et al. *Diagnostic potential of salivary IL-1 β , IL-8, SAT, S100P, and OAZ1 in oral squamous cell carcinoma, oral submucous fibrosis, and oral lichen planus based on findings from a Sri Lankan cohort*[J]. *Scientific Reports*, 2024, 14(1): 27226.
- [11] Yeladandi M, Suliman N M A, Rastogi J G, et al. *Oral Squamous Cell Cancer Early Detection Using Salivary Biomarkers*[J]. *Annals of African Medicine*, 2026.
- [12] Bastías D, Maturana A, Marín C, et al. *Salivary Biomarkers for Oral Cancer Detection: An Exploratory Systematic Review*[J]. *International Journal of Molecular Sciences*, 2024, 25(5): 2634.
- [13] Kumar S K, Kanaparthi N, Annes S B, et al. *Evaluation of salivary and serum Exosomal mRNAs as biomarkers for the diagnosis and prognosis of oral squamous cell carcinoma*[J]. *Scientific Reports*, 2025, 15(1): 38310.
- [14] Dikova V, Jantus-Lewintre E, Bagan J. *Potential Non-Invasive Biomarkers for Early Diagnosis of Oral Squamous Cell Carcinoma*[J]. *Journal of Clinical Medicine*, 2021, 10(8): 1658.
- [15] Sadeghi H, Saffar H, Taheri P, et al. *Prognostic Significance of Cancer Stem Cell Markers in Patients With Salivary Gland Carcinomas*[J]. *Applied Immunohistochemistry & Molecular Morphology*, 2022, 30(4): 284-290.
- [16] Gualtero D F, Suarez Castillo A. *Biomarkers in saliva for the detection of oral squamous cell carcinoma and their potential use for early diagnosis: a systematic review*[J]. *Acta Odontologica Scandinavica*, 2016, 74(3): 170-177.
- [17] Seyedmajidi S, Seyedmajidi M, Zahedpasha A, et al. *Comparison of Salivary and Serum Soluble CD44 Levels between Patients with Oral SCC and Healthy Controls*[J]. *Asian Pacific Journal of Cancer Prevention*, 2018, 19(11): 3059-3063.
- [18] Juodzbals G, Kasradze D, Cicciù M, et al. *Modern molecular biomarkers of head and neck cancer*.

Part I. Epigenetic diagnostics and prognostics: Systematic review[J]. Cancer Biomarkers, 2017, 17(4): 487-502.

[19] Cohen E R, Reis I M, Gomez-Fernandez C, et al. CD44 and associated markers in oral rinses and tissues from oral and oropharyngeal cancer patients[J]. *Oral Oncology*, 2020, 106: 104720.

[20] Ventura T M O, Ribeiro N R, Taira E A, et al. Radiotherapy changes the salivary proteome in head and neck cancer patients: evaluation before, during, and after treatment[J]. *Clinical Oral Investigations*, 2022, 26(1): 225-258.

[21] Pepe G, Parca L, Viviani L, et al. Variation in the co-expression profile highlights a loss of miRNA-mRNA regulation in multiple cancer types[J]. *Non-coding RNA Research*, 2022, 7(2): 98-105.

[22] Fu J, Tang W, Du P, et al. Identifying MicroRNA-mRNA regulatory network in colorectal cancer by a combination of expression profile and bioinformatics analysis[J]. *BMC Systems Biology*, 2012, 6(1): 68.

[23] Zhang W, Edwards A, Fan W, et al. miRNA-mRNA Correlation-Network Modules in Human Prostate Cancer and the Differences between Primary and Metastatic Tumor Subtypes[J]. *PLoS ONE*, 2012, 7(6): e40130.

[24] Patel A, Patel P, Mandlik D, et al. A novel 3-miRNA network regulates tumour progression in oral squamous cell carcinoma[J]. *Biomarker Research*, 2023, 11(1): 64.

[25] AlAli A M, Walsh T, Maranzano M. CYFRA 21-1 and MMP-9 as salivary biomarkers for the detection of oral squamous cell carcinoma: a systematic review of diagnostic test accuracy[J]. *International Journal of Oral and Maxillofacial Surgery*, 2020, 49(8): 973-983.

[26] Kumar P, Singh K, Garg A, et al. Salivary IL-8, CYFRA 21-1, and CD44 for early detection of oral squamous cell carcinoma: a systematic review and meta-analysis[J]. *Clinica Chimica Acta*, 2025, 577: 120471.

[27] Stamatovski A, Monevska D P, Pancevski G, et al. Baseline and treatment-induced change of salivary CYFRA 21 – 1 as independent predictors of recurrence in oral squamous cell carcinoma[J]. *Clinical Oral Investigations*, 2026, 30(6): 282.

[28] Zhang X, Guo H, Wong L S, et al. A highly sensitive Love-wave surface acoustic wave immunosensor for point-of-care detection of Cyfra21-1 in saliva[J]. *The Analyst*, 2026, 151(8): 2269-2279.

[29] Malhotra R, Urs A B, Chakravarti A, et al. Correlation of Cyfra 21-1 levels in saliva and serum with CK19 mRNA expression in oral squamous cell carcinoma[J]. *Tumor Biology*, 2016, 37(7): 9263-9271.

[30] Beladiya J, Mehta D, Patel Y, et al. Salivary protein biomarkers for the diagnosis of oral squamous cell carcinoma: a systematic review and meta-analysis[J]. *Diagnosis*, 2025.

[31] Zhong Laiping. *A Study on the Expression of Tumor Markers in the Saliva of Patients with Oral Squamous Cell Carcinoma and Its Mechanisms [D]. Zhejiang University, 2006.*