

Diagnostic Potential of Salivary Biomarkers for the Early Detection of Oral Cancer

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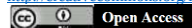
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ABSTRACT

This article analyzes the diagnostic potential of salivary biomarkers for the early detection of oral cancer, particularly oral squamous cell carcinoma (OSCC), based on contemporary scientific research. The diagnostic significance of saliva as a non-invasive and readily obtainable biological fluid was assessed, along with the presence of various molecular alterations in saliva that reflect the cancer process. The analysis examined the potential of salivary protein biomarkers, microRNAs, metabolites, and exosomal mRNAs for the detection of OSCC. Studies have demonstrated significant alterations in certain biomarkers associated with OSCC, with some showing high diagnostic performance. Furthermore, combining multiple biomarkers may improve diagnostic accuracy. In particular, salivary metabolomic studies have identified numerous metabolic alterations, while studies of exosomal mRNAs have demonstrated promising results for certain markers and their combinations. At the same time, methodological differences among studies, limited sample sizes, and insufficient independent clinical validation of biomarkers remain major limitations to their implementation in clinical practice. Overall, salivary biomarkers represent a promising approach for the early and non-invasive detection of oral cancer; however, large-scale, standardized, and multicenter studies are required to determine their true clinical utility in diagnosis.

Introduction

Oral cancer is one of the clinically significant malignant diseases of the head and neck region, with oral squamous cell carcinoma (OSCC) being its predominant histological type. The clinical course of the disease and the effectiveness of treatment largely depend on the stage at which the disease is diagnosed. Therefore, the early detection of the tumor process, particularly at stages when clinical manifestations are not yet pronounced, and the expansion of diagnostic capabilities represent important priorities in modern oncology. Although current clinical, instrumental, and histopathological methods constitute the basis of OSCC diagnosis, the search for complementary, patient-friendly, and non-invasive biomarkers continues.

In recent years, saliva has attracted considerable scientific interest as a promising biological material for this purpose. Its ease of non-invasive collection, the possibility of repeated sampling, and the presence of various biomolecules that may reflect molecular alterations associated with the tumor process make saliva a convenient source for diagnostic research. The studies reviewed have investigated the association of salivary protein biomarkers, microRNAs, metabolites, and exosomal mRNAs with OSCC. While some studies have demonstrated high diagnostic performance of individual biomarkers, others have shown the potential to improve diagnostic accuracy by combining multiple biomarkers. Systematic analyses of salivary metabolomics have also identified extensive metabolic alterations associated with OSCC.

Nevertheless, the available scientific evidence is not uniform in quality. Differences in sample size, composition of control groups, methods of saliva collection and processing, biomarker detection technologies, and statistical approaches make direct comparison of study findings difficult. Furthermore,

some promising biomarkers have been investigated in small study populations, and their diagnostic performance has not been adequately validated in independent and large clinical cohorts. Therefore, statistical significance of salivary biomarkers does not necessarily imply that they are ready for use as diagnostic tests in clinical practice.

The aim of this article is to analyze the diagnostic potential of salivary biomarkers for the early detection of oral cancer, particularly OSCC, based on scientific studies published between 2020 and 2026; to summarize promising biomarkers and their combinations; and to identify the limitations of the current evidence and priority areas for future research.

Main Body.

Al Ali et al. (2020) conducted a systematic review to evaluate the diagnostic accuracy of salivary CYFRA 21-1 and MMP-9 biomarkers for the detection of OSCC. The authors searched the Cochrane Library, MEDLINE, and Embase databases and analyzed data from six clinical studies that met the inclusion criteria, comprising a total of 775 participants. Of these, 355 participants were included in studies investigating CYFRA 21-1, while 420 were included in studies investigating MMP-9. All studies on CYFRA 21-1 were conducted in India, whereas studies on MMP-9 were conducted in Egypt, Germany, and Taiwan. For CYFRA 21-1, sensitivity ranged from 84% to 94% and specificity from 84% to 96%; for MMP-9, sensitivity ranged from 76% to 100% and specificity from 27% to 100%. However, the methodological quality of the studies was low, the risk of bias in patient selection was high, and the methods of saliva collection, processing, and determination of diagnostic cutoff values for the biomarkers were not standardized. Therefore, the authors concluded that the available evidence was insufficient to consider salivary CYFRA 21-1 or MMP-9 as clinically validated independent tests for OSCC screening. [10.1016/j.ijom.2020.01.020](https://doi.org/10.1016/j.ijom.2020.01.020)

Jain et al. (2021) conducted a study in India in which the salivary proteome of 50 patients with OSCC and 49 healthy controls was investigated. Proteomic analysis identified 135 differentially expressed proteins, and subsequent analyses demonstrated associations between OSCC and the proteins AHSG, KRT6C, AZGP1, KLK1, and BPIFB2. Among the individual biomarkers, AZGP1 demonstrated a diagnostic performance with an AUC of 72.8%. Combining biomarkers resulted in an AUC of 82.4% for the AHSG + KRT6C + AZGP1 panel across all OSCC cases. In the N0 group, the AHSG + KRT6C + AZGP1 + BPIFB2 panel achieved an AUC of 94.4%, with 100% sensitivity and 77.6% specificity. These findings suggest that salivary multi-biomarker panels may represent promising diagnostic tools for detecting OSCC, particularly in cases without lymph node metastasis. [10.1038/s41598-021-82635-0](https://doi.org/10.1038/s41598-021-82635-0)

Mehterov et al. (2021) investigated the diagnostic potential of salivary microRNAs for the detection of oral squamous cell carcinoma (OSCC) using clinical cohorts from Bulgaria and Italy. In the initial phase of the study, saliva samples from 33 histologically confirmed OSCC patients and 12 healthy controls at University Hospital St. George in Plovdiv, Bulgaria, were analyzed. Cell-free RNA was isolated from saliva, and the expression of eight cancer-associated miRNAs was assessed using a TaqMan assay. The results demonstrated significantly reduced expression of miR-30c-5p ($p=0.04$) and miR-34-3p ($p=0.03$) in the saliva of OSCC patients compared with healthy controls. For validation, salivary miRNA microarray data from an independent SCUB cohort in Brescia, Italy, comprising 50 OSCC patients and 42 healthy individuals, were analyzed. The reduction in miR-30c-5p expression was successfully validated ($p<0.001$), whereas no statistically significant difference was observed for miR-34-3p ($p=0.58$). Consequently, subsequent diagnostic analyses focused on miR-30c-5p. ROC analysis demonstrated that salivary miR-30c-5p discriminated OSCC patients from healthy individuals with an AUC of 0.82 (95% CI: 0.71–0.89), sensitivity of 86%, and specificity of 74%. In addition, analysis of 354 OSCC tumor tissues and 44 control tissues from The Cancer Genome Atlas (TCGA) database showed that, in contrast to the salivary findings, miR-30c-5p expression was significantly higher in tumor tissue ($p<0.001$). This finding suggests that tumor cells may not be the direct source of salivary miR-30c-5p. The authors concluded that reduced salivary miR-30c-5p may represent a potential biomarker for the non-invasive detection of OSCC. However, they emphasized the need for further validation in larger patient cohorts before clinical implementation. [10.3390/biomedicines9091079](https://doi.org/10.3390/biomedicines9091079)

Dikova et al. (2021) published a review article analyzing the diagnostic potential of non-invasive biomarkers, particularly salivary biomarkers, for the early detection of oral squamous cell carcinoma

(OSCC). Based on the available evidence, the authors examined alterations in salivary biomarkers associated with inflammation and immune responses in OSCC. The analyzed data demonstrated increased levels of IL-6, IL-8, TNF- α , MCP-1, HCC-1 (CCL14), and PF-4 (CXCL4) in patients with OSCC compared with healthy controls, with some of these alterations being observed even at early stages of the disease. In particular, increased expression of IL-6 and TNF- α was reported in association with OSCC development. However, the relationship between these biomarkers and clinical stages was not consistent across different studies. The authors suggested that salivary cytokines and chemokines may represent promising non-invasive biomarkers for the early detection of OSCC, while emphasizing the need for assessment of diagnostic accuracy, standardization, and validation in independent cohorts before their implementation in clinical screening. [10.3390/jcm10081658](https://doi.org/10.3390/jcm10081658)

Shaw et al. (2022) conducted a systematic review and meta-analysis evaluating the diagnostic accuracy of salivary biomarkers for the detection of early oral squamous cell carcinoma based on studies published between 2000 and 2020. Eighteen studies identified through PubMed, Google Scholar, and EBSCOhost were assessed for quality, and 13 were included in the meta-analysis, combining data from a total of 1,048 participants. The analysis investigated salivary biomarkers including mRNA, miRNA, DUSP1, S100P, IL-8, IL-1 β , TNF- α , and MMP-9. The highest diagnostic accuracy was observed for mRNA and miRNA biomarkers detected using PCR. For mRNA, sensitivity was 91%, specificity 90%, and AUC 0.96; for miRNA, these values were 91%, 91%, and 0.95, respectively. The diagnostic performance of IL-8 also varied considerably according to the detection method, with an AUC of 0.56 using ELISA compared with 0.94 using PCR. The authors concluded that salivary mRNA and miRNA biomarkers are among the most promising biomarkers for the non-invasive detection of early OSCC; however, further validation through standardized, high-quality diagnostic studies is required before clinical implementation. [10.31557/APJCP.2022.23.5.1483](https://doi.org/10.31557/APJCP.2022.23.5.1483)

Scholtz et al. (2022) conducted a multicenter study across four clinical centers in Hungary to evaluate the diagnostic potential of salivary microRNAs for the detection of oral squamous cell carcinoma (OSCC). Based on an initial literature review, six miRNAs potentially associated with OSCC - miR-345, miR-424-3p, miR-31-5p, miR-21-5p, miR-184, and miR-191-5p—were selected. Saliva samples from 43 OSCC patients and 44 control individuals were subsequently analyzed using real-time quantitative PCR. The results demonstrated significantly increased expression of miR-31-5p and miR-345 and decreased expression of miR-424-3p in the OSCC group. In contrast, no significant differences between the OSCC and control groups were observed for miR-21-5p, miR-191-5p, or miR-184. Because miR-184 was undetectable in many samples, it was considered unreliable as a biomarker. The diagnostic performance of individual miRNAs was limited, with the highest individual AUC being 0.77 for miR-345. However, a three-miRNA panel consisting of miR-345, miR-31-5p, and miR-424-3p discriminated OSCC from controls with an AUC of 0.87, sensitivity of 86%, and specificity of 77%. The authors considered this three-miRNA combination a promising biomarker panel for salivary detection of OSCC but emphasized the need for further evaluation in larger and independent cohorts because of the small sample size and variability in miRNA profiles across studies. [10.3390/pathogens11020229](https://doi.org/10.3390/pathogens11020229)

Da Costa et al. (2022) conducted an original study in Brazil evaluating combinations of salivary metabolites for OSCC detection using data mining and machine-learning approaches. The OSCC and control groups were matched for age, sex, and smoking status, and saliva samples were analyzed using gas chromatography–mass spectrometry (GC-MS). A total of 51 metabolites were evaluated, and their diagnostic relevance was assessed using the Random Forest algorithm with 10-fold cross-validation. Feature selection identified glucuronic acid, maleic acid, and batyl alcohol as the most important metabolites. A combination of these three metabolites discriminated OSCC from control samples with an AUC of 0.91 and an accuracy of 86.76%. In comparison, inclusion of all 51 metabolites in the model resulted in a decrease in AUC to 0.76. The authors considered the combination of glucuronic acid, maleic acid, and batyl alcohol to be a promising salivary biomarker panel for OSCC diagnosis. Nevertheless, further validation in independent populations is necessary before clinical application of the model. [10.1016/j.compbiomed.2022.105296](https://doi.org/10.1016/j.compbiomed.2022.105296)

Salivary molecular layers including RNA and metabolites may be used as diagnostic biomarker panels for discriminating OSCC.

Cai et al. (2022) conducted an original study in Guangzhou, China, investigating the expression of angiogenic factors and matrix metalloproteinases in tumor tissue and saliva from patients with OSCC. Tumor tissue and adjacent normal tissue samples were obtained from 10 patients with OSCC, and saliva samples from OSCC patients and healthy controls were also analyzed. Immunohistochemistry, RT-qPCR, and protein-chip array techniques were used. The results showed that salivary levels of HGF, PIGF, and VEGF were increased by 2.00-, 8.10-, and 1.38-fold, respectively, in patients with OSCC. In addition, the expression levels of MMP-1, MMP-3, MMP-8, MMP-9, MMP-10, MMP-13, and TIMP-2 were increased by 111.71-, 256.32-, 2.66-, 5.48-, 16.31-, 174.20-, and 1.34-fold, respectively. MMP-1, MMP-3, and MMP-13 were present at very low levels in healthy controls but were highly expressed in the OSCC group. The authors concluded that HGF, PIGF, VEGF, and certain members of the MMP family, particularly HGF and MMP-9, may represent promising biomarkers for the non-invasive detection and prognostic assessment of OSCC using saliva. However, they emphasized the need for further studies with larger sample sizes to establish clinically relevant cutoff values for HGF and MMP-9. [10.1186/s12885-022-09630-0](https://doi.org/10.1186/s12885-022-09630-0)

Bozyk et al. (2023) conducted a pilot study in Australia to evaluate the diagnostic potential of salivary exosomes for the early detection of oral squamous cell carcinoma (OSCC). The biomarker discovery phase included 20 healthy controls, 20 patients with oral potentially malignant disorders (OPMDs), and 10 patients with OSCC. Exosomes were isolated from saliva, and their protein composition was analyzed using mass spectrometry. A panel consisting of AMER3, LOXL2, and AL9A1 proteins discriminated healthy controls from the OPMD and OSCC groups with an AUC of 0.93, sensitivity of 80%, and specificity of 100%. For discrimination of OSCC from healthy controls, the combination of PSB7, AMER3, and LOXL2 demonstrated high diagnostic performance, with an AUC of 0.96, sensitivity of 100%, and specificity of 75%.

An important strength of the study was the investigation of **exosome-associated proteins**, rather than conventional salivary biomarkers alone, as well as the evaluation of combinations of multiple biomarkers. The inclusion of an OPMD group also provides additional clinical relevance in terms of identifying potentially malignant conditions that may precede the development of OSCC. The use of mass spectrometry for the identification of exosomal proteins enabled broader screening for potential biomarkers.

However, the principal limitation of the study was the **small sample size**, particularly the inclusion of only 10 patients in the OSCC group. Therefore, caution is required when extrapolating high diagnostic values, such as 100% sensitivity, to larger populations. As the study was exploratory and pilot in nature, external validation of the PSB7, AMER3, and LOXL2 biomarker panel in large, independent cohorts representing different clinical stages is necessary. Furthermore, the AUC of 0.96 was obtained in this small study population and cannot currently be interpreted as evidence of a clinically validated diagnostic test.

Overall, the findings indicate that **salivary exosomal protein panels represent a promising approach for the non-invasive detection of OSCC**. In particular, the high discriminatory performance of the PSB7, AMER3, and LOXL2 combination provides an important basis for future clinical investigations. Nevertheless, large-scale, independent, multicenter studies are required to establish the true diagnostic performance of this biomarker panel. [10.1016/j.oor.2023.100017](https://doi.org/10.1016/j.oor.2023.100017)

Khijmatgar et al. (2024) conducted a systematic review and network meta-analysis evaluating the diagnostic value of salivary biomarkers for the early detection of oral squamous cell carcinoma (OSCC) and head and neck squamous cell carcinoma (HNSCC). PubMed, Scopus, Web of Science, Embase, and the Cochrane Library were searched, and previously published systematic reviews and meta-analyses were reanalyzed. The final analysis included 37 original studies from four meta-analyses, comprising data from 5,893 patients, of whom 2,025 had OSCC and 3,868 had HNSCC. A total of 94 salivary biomarkers were analyzed.

For OSCC, Chemerin and MMP-9 demonstrated the highest sensitivity, with both showing a sensitivity of 0.94 (95% CI: 0.78–1.00) and a balanced accuracy of 0.93. Phytosphingosine also demonstrated high diagnostic performance, with a sensitivity of 0.91 (95% CI: 0.68–0.99) and a balanced accuracy of 0.87. Notably, for early-stage TNM I–II OSCC, Chemerin, MMP-9, Phytosphingosine, and pipercolinic acid were identified among the highest-ranked biomarkers. In the HNSCC group, Actin

demonstrated the highest sensitivity at 0.91 (95% CI: 0.68–0.99), with a specificity of 0.67 and overall accuracy of 0.79, followed by IL-1 β Singleplex and IL-8 ELISA. [10.1016/j.jdsr.2023.10.003](https://doi.org/10.1016/j.jdsr.2023.10.003)

Bastías et al. (2024) conducted an exploratory systematic review evaluating the diagnostic potential of salivary biomarkers for the early detection of oral squamous cell carcinoma (OSCC) and oral potentially malignant disorders (OPMDs). The study was conducted according to PRISMA and Joanna Briggs Institute scoping review guidelines. Clinical, cohort, and case-control studies involving human participants published between January 2015 and July 2022 were searched in EBSCO, PubMed/MEDLINE, Scopus, and Web of Science. Initially, 1,123 bibliographic records were identified. After removal of duplicates, 780 articles were assessed based on titles and abstracts, 97 proceeded to full-text assessment, and 35 articles were excluded for not meeting the inclusion criteria, resulting in 62 studies included in the final analysis.

These 62 studies included data from 5,278 participants: 2,546 patients with OSCC, 1,070 patients with OPMDs, and 1,662 healthy controls. More than 100 salivary molecules were investigated, with the majority focusing on early detection of OSCC. The most extensively and consistently studied biomarkers were TNF- α , IL-1 β , IL-6, IL-8, LDH, and MMP-9. The authors found that most studies investigating these biomarkers reported high sensitivity and specificity. In particular, eight studies evaluated MMP-9, and in some studies its AUC for discriminating OSCC from OPMDs reached 0.917. However, it is important to note that this AUC represents the result of an individual study. miRNAs were also considered promising biomarkers, although, apart from miR-21, many miRNAs had been investigated in only a single study.

However, because 55 biomarkers had been evaluated in only one study each, sufficient replicated evidence regarding their diagnostic value was lacking. Differences in patient characteristics, laboratory methods, biomarker detection techniques, and statistical approaches also made direct comparison between studies difficult. Furthermore, high sensitivity and specificity for some biomarkers were demonstrated only in individual studies with small sample sizes. Therefore, caution is required when generalizing such findings to larger populations.

The authors concluded that TNF- α , IL-1 β , IL-6, IL-8, LDH, and MMP-9 were among the most promising salivary biomarkers based on the available evidence. However, because a single molecule is unlikely to be sufficient for OSCC diagnosis, future research should focus on the development of panels combining multiple biomarkers. The authors also emphasized the need to validate these biomarkers in large cohorts and using more robust study designs before their implementation in clinical practice. [10.3390/ijms25052634](https://doi.org/10.3390/ijms25052634)

Senevirathna et al. (2024) conducted a preliminary clinical study in Sri Lanka evaluating the diagnostic potential of salivary IL-1 β , IL-8, SAT, S100P, and OAZ1 mRNAs for the early detection of oral squamous cell carcinoma (OSCC). The study included 40 participants: 9 with OSCC, 10 with oral submucous fibrosis (OSF), 11 with oral lichen planus (OLP), and 10 healthy controls. Extracellular RNA was isolated from saliva, and the expression of IL-1 β , IL-8, SAT, S100P, and OAZ1 mRNAs was determined using real-time quantitative reverse transcription polymerase chain reaction (RT-qPCR).

All five candidate biomarkers were elevated in the OSCC, OSF, and OLP groups compared with the control group. OAZ1 demonstrated the highest increase in the OSCC group, with a 198.956-fold increase in expression. S100P increased 24.996-fold, IL-8 and IL-1 β increased 14.083-fold, and SAT increased 7.148-fold. However, binary logistic regression analysis of the individual biomarkers showed that none independently predicted OSCC at a statistically significant level. Therefore, the researchers combined the five biomarkers into a single diagnostic model.

The combination of five biomarkers—IL-1 β , IL-8, SAT, S100P, and OAZ1—achieved an AUC of 1.000, 100% sensitivity, and 100% specificity for OSCC detection in ROC analysis ($p < 0.001$). The same panel achieved an AUC of 0.960, sensitivity of 80%, and specificity of 90% for OSF detection, whereas for OLP it demonstrated an AUC of 0.945, sensitivity of 90%, and specificity of 90.9%. These findings indicate that combining multiple salivary mRNA biomarkers may improve diagnostic discrimination compared with individual biomarkers. [10.1038/s41598-024-75735-0](https://doi.org/10.1038/s41598-024-75735-0)

Antonelli et al. (2024) conducted a systematic review evaluating the diagnostic potential of salivary metabolomics for the early detection of oral squamous cell carcinoma (OSCC). The authors searched the MEDLINE, Scopus, and Web of Science databases for scientific literature published up to

February 2024. Initially, 9,623 bibliographic records were identified, and after application of the predefined inclusion and exclusion criteria, 28 studies were included in the final systematic review. Methodological quality was assessed using the NIH checklist: two studies were classified as high quality, 22 as moderate quality, and four as low quality.

The included studies investigated the presence or concentration of more than 100 salivary metabolites associated with histologically confirmed OSCC. Some metabolites were evaluated individually as potential biomarkers, whereas others were assessed as components of combinations consisting of two or more metabolites. Sialic acid, neopterin, pyruvate, aspartate, glutamate, branched-chain amino acids (BCAAs), cortisol, 8-hydroxy-2-deoxyguanosine, and malondialdehyde were identified as promising candidates. All included studies reported a statistically significant association between at least one metabolite and OSCC.

Metabolomic analyses demonstrated alterations in several metabolic pathways in OSCC, including choline metabolism, amino acid metabolism, polyamine metabolism, the urea cycle, creatine metabolism, glycolysis, and glycerolipid metabolism. These findings suggest that metabolic reprogramming occurring during OSCC development may be reflected in the salivary metabolome and could potentially be exploited for the identification of diagnostic biomarkers. [10.1016/j.oor.2024.100657](https://doi.org/10.1016/j.oor.2024.100657)

Kumar et al. (2025) conducted a pilot study in India investigating the diagnostic and prognostic potential of mRNA biomarkers in salivary and serum exosomes for the detection of oral squamous cell carcinoma (OSCC) and assessment of its clinical and progressive characteristics. The study included 40 patients with histopathologically confirmed OSCC and 40 healthy controls, for a total of 80 participants. The researchers isolated exosomes from saliva and serum samples and characterized them using nanoparticle tracking analysis, transmission electron microscopy, zeta potential analysis, and Western blotting. The expression of exosomal mRNAs associated with inflammation (IL1, IL6, IL8, and TNF- α), proliferation (OAZ1, SAT, and S100P), and invasion and metastasis (MMP9 and Chemerin) was determined using qRT-PCR.

All nine mRNAs examined showed increased expression in salivary exosomes from OSCC patients compared with healthy controls. Among the individual diagnostic markers, TNF- α demonstrated the highest diagnostic performance, with 90% sensitivity, 80% specificity, and an AUC of 0.88. For OAZ1, sensitivity was 77.5%, specificity 87.5%, and AUC 0.87; for MMP9, the corresponding values were 72.5%, 85%, and 0.84. Combining multiple biomarkers improved diagnostic performance: the TNF- α + OAZ1 combination achieved an AUC of 0.89, sensitivity of 80%, specificity of 90%, and a Youden index of 0.70. The TNF- α + OAZ1 + MMP9 panel demonstrated an AUC of 0.89, sensitivity of 85%, and specificity of 80%.

In serum exosomes, increased expression of OSCC-associated mRNAs was also observed; however, overall diagnostic performance was slightly lower than that observed in saliva. The best individual serum marker was IL1, with a sensitivity of 87.5%, specificity of 70%, and AUC of 0.86. The IL1 + OAZ1 + MMP9 combination achieved an AUC of 0.89, sensitivity of 80%, and specificity of 85%. At the same time, the salivary TNF- α + OAZ1 panel demonstrated a sensitivity of 80% and a Youden index of 0.70, suggesting that salivary exosomes may represent a somewhat more advantageous biological matrix for OSCC diagnosis.

Prognostic analysis demonstrated associations between certain salivary exosomal mRNAs and tumor grade. In particular, MMP9 expression increased from grade I to grade III, with statistically significant differences between grades I and III and between grades II and III ($p=0.001$ for both comparisons). IL8 expression also progressively increased with increasing tumor grade. In addition, IL6 expression was positively correlated with lymph node metastasis. These findings suggest that salivary exosomal mRNAs may have potential prognostic significance not only for OSCC detection but also for assessing tumor aggressiveness and clinical progression.

Overall, the findings indicate that the combination of TNF- α and OAZ1 mRNAs in salivary exosomes represents a promising biomarker panel for the non-invasive detection of OSCC. The associations of MMP9 and IL6 with tumor grade and lymph node metastasis suggest that these markers may also have prognostic value. Nevertheless, the authors emphasized the need for validation in large-scale, multicenter, and independent cohorts before these findings can be translated into clinical practice. [10.1038/s41598-025-22246-1](https://doi.org/10.1038/s41598-025-22246-1)

Conclusion.

The analyzed scientific studies demonstrate that various biomolecules present in saliva can reflect molecular alterations associated with OSCC, and that some of these molecules show promise as diagnostic biomarkers. Among the biomarkers investigated, proteins, microRNAs, metabolites, and exosomal mRNAs are of particular importance. While some studies have reported high diagnostic performance for individual biomarkers, combining multiple biomarkers has, in certain cases, improved sensitivity and specificity. These findings suggest that, rather than relying on a single universal biomarker, an approach based on biomarker panels reflecting different biological processes may be a promising strategy for OSCC detection.

At the same time, caution is required when considering the direct translation of the available evidence into clinical practice. A substantial proportion of the reviewed studies have limitations, including small sample sizes, comparisons restricted primarily to healthy controls, and insufficient inclusion of patients with benign oral diseases and potentially malignant conditions. In addition, the lack of standardized protocols for saliva collection, storage, and laboratory processing limits comparability between studies. Although some biomarkers have demonstrated high AUC values, sensitivity, or specificity, these findings have not yet been adequately externally validated in independent populations. Therefore, at the current stage, it is scientifically more appropriate to regard the identified biomarkers as promising candidate biomarkers rather than as validated clinical diagnostic tests for OSCC.

Future research should primarily focus on large-scale, multicenter, prospective studies. Such studies should include not only healthy individuals but also patients with inflammatory oral diseases, potentially malignant disorders, and other oral neoplasms in the control groups. This approach would help determine the true clinical specificity of the biomarkers. Furthermore, standardization of saliva collection and storage procedures, as well as biomarker isolation and detection methods, would improve the comparability of findings across different studies.

External validation of promising biomarker panels in independent cohorts and assessment of their diagnostic performance across different clinical stages and during early tumor development are also important research priorities. For molecular biomarkers such as exosomal mRNAs and microRNAs, longitudinal studies are needed to determine their associations with disease progression, tumor grade, and metastatic processes. In addition, integrating metabolomic, proteomic, and nucleic acid biomarkers into a single multimodal biomarker panel may represent a promising direction for future research.

Overall, salivary biomarkers have considerable scientific potential for the non-invasive and early detection of OSCC. However, the current evidence is insufficient to establish their definitive clinical diagnostic utility. Future high-quality, standardized studies with independent external validation may enable promising biomarkers to be developed into reliable diagnostic panels and facilitate their eventual integration into clinical practice.

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