Natural Resources for Human Health
eISSN: 2583-1194

Original Research

Received 18 Jan 2024
Revised 09 Feb 2024
Accepted 27 Feb 2024

Natural Resources for Human Health 2024, Vol. 4 (4): 535-543

KEYWORDS:
oral cancer, oral squamous cell carcinoma, salivary diagnostics, saliva; biomarkers, liquid biopsy, circulating tumour DNA, microRNA.

Role of Salivary Diagnostics in Oral Cancer Detection: Narrative Review

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ABSTRACT: Oral squamous cell carcinoma remains a significant global health burden, with late-stage diagnosis driving poor five-year survival rates that have improved only marginally over recent decades. Conventional detection relies on clinical examination followed by tissue biopsy, a pathway that is invasive, resource-intensive, and poorly suited to population-level screening—particularly in low-resource settings where the disease burden is highest. Saliva has emerged as an attractive alternative biospecimen in this context, owing to its non-invasive and repeatable collection, minimal patient discomfort, low handling cost, and the fact that it bathes the oral cavity directly and continuously, making it a plausible reservoir of tumour-derived material. Consequently, salivary diagnostics has attracted substantial research interest as a potential tool for early detection, risk stratification, and post-treatment surveillance in oral cancer. This narrative review synthesises the current evidence on salivary biomarkers for oral cancer detection, encompassing circulating tumour DNA and mitochondrial DNA alterations, tumour-related transcripts and non-coding RNAs—notably the widely investigated microRNA panels—as well as protein and metabolite signatures detectable by mass spectrometry and immunoassay platforms. Technological advances in point-of-care devices and lab-on-a-chip systems are also examined, alongside the analytical challenges that currently limit clinical translation: substantial inter-individual variability in salivary composition, lack of standardised collection and processing protocols, absence of validated diagnostic thresholds, and the confounding influence of periodontal disease and tobacco exposure.

INTRODUCTION

Oral cancer remains a substantial global health burden, with oral squamous cell carcinoma (OSCC) accounting for approximately 90% of all malignant neoplasms of the oral cavity. Despite advances in surgical oncology, radiotherapy, and systemic therapy, the five-year survival rate for OSCC has remained disappointingly static, largely because a significant

proportion of cases are diagnosed at an advanced stage when curative intervention is no longer feasible [1]. The conventional diagnostic pathway—visual inspection, followed by scalpel biopsy and histopathological examination—is invasive, dependent on specialised clinical infrastructure, and poorly suited to population-level screening, particularly in low-resource settings where the burden of OSCC is greatest [2]. These constraints have generated sustained interest in minimally invasive, molecularly informative diagnostic alternatives that can intercept oral carcinogenesis before macroscopic and microinvasive disease becomes established [2].

Saliva has emerged as the most conceptually attractive biofluid for oral cancer diagnostics. It is produced by the major and minor salivary glands, bathes the entire oral mucosa, and continuously accumulates cellular and molecular material shed from both healthy and malignant oral epithelium. Unlike blood, saliva is collected without needles, requires no trained phlebotomist, and can be obtained repeatedly with minimal patient discomfort, making it uniquely suited to serial monitoring and community-based screening [3]. The diagnostic value of saliva is further reinforced by the observation that salivary circulatory tumour DNA (ctDNA) concentrations in OSCC may exceed corresponding plasma levels, reflecting the direct anatomical proximity of the biofluid to the tumour microenvironment [4].

The term "salivaomics" encompasses the proteomic, transcriptomic, genomic, epigenomic, metabolomic, and microbiomic constituents of saliva, and it is precisely this multi-layered molecular architecture that makes saliva a plausible substrate for early cancer detection [1]. Over the past decade, candidate biomarkers spanning cytokines, cytokeratin fragments, microRNAs, cell-free DNA methylation marks, exosomal cargo, and metabolite signatures have been reported with varying degrees of diagnostic accuracy [2]. Concurrently, point-of-care biosensing platforms and multi-omics analytical strategies have begun to move salivary diagnostics from the research laboratory toward clinically actionable tools [5]. Nevertheless, the field remains characterised by a proliferation of promising pilot data rather than validated, regulatory-approved assays, and the gap between analytical performance and clinical implementation remains the central unresolved problem [6].

Saliva as a Diagnostic Biofluid: Biological Rationale and Composition

The diagnostic appeal of saliva rests on three intersecting properties: anatomical proximity, molecular richness, and procedural accessibility. Anatomically, the oral cavity is the site of origin for most OSCCs, and malignant or dysplastic epithelial cells release DNA, RNA, proteins, and extracellular vesicles directly into the salivary pool. This contrasts with blood-based liquid biopsy, where tumour-derived analytes must survive dilution in the systemic circulation and hepatic clearance before reaching a sampling site [4]. The result is that saliva can be enriched in tumour-associated molecules that are present at only trace concentrations in plasma, particularly for lesions of the tongue, floor of mouth, buccal mucosa, and gingiva [4].

Saliva is also compositionally complex. It contains electrolytes, mucins, immunoglobulins, antimicrobial peptides, enzymes, hormones, cytokines, shed epithelial cells, leucocytes, bacterial communities, and a diverse population of extracellular vesicles [1]. This complexity is both an asset and a liability. On one hand, it provides multiple orthogonal molecular layers that can be interrogated for diagnostic signal. On the other hand, the dynamic range of salivary analytes is enormous, and low-abundance tumour-derived signals can be masked by abundant host and microbial proteins, a problem analogous to the "matrix effect" encountered in plasma proteomics [7]. Salivary flow rate, circadian rhythm, hydration status, diet, smoking, oral hygiene, periodontal inflammation, and collection technique all influence analyte concentrations, and these pre-analytical variables have emerged as among the most serious obstacles to reproducible biomarker measurement [8].

Notwithstanding these challenges, saliva offers practical advantages that are difficult to overstate in the context of global oral cancer control. Collection is non-invasive and can be performed by the patient or a minimally trained health worker; no cold-chain is required for many downstream assays if appropriate stabilisation buffers are used; and samples can be transported in simple collection devices [3]. These features make saliva-based testing particularly attractive for screening programmes in South and Southeast Asia, Eastern Europe, and parts of Latin America, where OSCC incidence is high and where access to specialist biopsy services is limited [9]. The biological rationale is therefore strong, but the operational validity of salivary diagnostics depends on whether robust, standardised, and clinically validated assays can be constructed from this complex and variable biofluid [8].

Pre-Analytical Variability and the Standardisation Problem

The single most consistent message across recent reviews of salivary diagnostics is that pre-analytical heterogeneity remains a rate-limiting barrier to clinical translation [6,10]. Saliva can be collected as unstimulated whole saliva, stimulated whole saliva,

gland-specific saliva, or oral rinse, and each modality yields a different molecular profile. Stimulation with citric acid or chewing paraffin increases flow rate but dilutes certain analytes and alters the composition of extracellular vesicles [7]. Collection time relative to food intake, smoking, and oral hygiene practices introduces additional variance. Processing variables—centrifugation speed and duration, filtration, storage temperature, freeze–thaw cycles, and the presence or absence of protease and nuclease inhibitors—further compound the problem [8].

The consequences of this variability are not merely technical. When candidate biomarkers are measured using different collection protocols and analytical platforms, the resulting diagnostic thresholds are often cohort-specific and not transferable across studies. A systematic review of salivary biomarkers for OSCC and oral potentially malignant disorders (OPMDs) concluded that the lack of harmonised thresholds for candidate markers, ranging from cytokines to genetic and epigenetic markers, undermines biosensor calibration and restricts the scalability of computational diagnostic tools [10]. Even biomarkers with strong biological plausibility may exhibit inconsistent diagnostic odds ratios across studies because the pre-analytical pipeline, rather than the biology, drives a substantial proportion of the measured variance [6].

Several remedies have been proposed. The first is the adoption of a consensus reference protocol for saliva collection, processing, and storage, analogous to the standardised operating procedures that underpin plasma-based liquid biopsy [8]. The second is the use of internal controls—for example, total salivary protein concentration, a housekeeping miRNA, or a panel of stable abundant proteins—to normalise for collection-related dilution and degradation [7]. The third is the development of device-integrated collection systems that stabilise analytes at the point of collection and reduce operator-dependent variability [5]. None of these solutions is yet universally adopted, and the absence of a regulatory-grade standard remains a structural weakness of the field.

Proteomic and Cytokine Biomarkers

Proteins and cytokines constitute the most extensively studied class of salivary biomarkers for OSCC. Interleukin-6 (IL-6) and interleukin-8 (IL-8) are inflammatory cytokines that are elevated in the saliva of patients with OSCC and OPMDs, and they have been repeatedly evaluated as diagnostic candidates. A 2026 review reported that salivary IL-6 and IL-8 achieve sensitivities of approximately 70–90% and specificities of 75–95% for OSCC, although these estimates vary considerably across studies and assay platforms [11]. A validation study in an Egyptian population confirmed that salivary IL-6, IL-8, and soluble CD44 (sCD44) levels increase progressively from healthy mucosa through dysplasia to OSCC, and that the three markers correlate with histological grade and established risk factors [12]. The same study emphasised that sCD44 had particular discriminating power between normal and dysplastic tissue, suggesting a role not only in cancer detection but also in risk stratification of OPMDs [12].

Cytokeratin 19 fragment (CYFRA 21-1) and CD44 have emerged as complementary markers. A systematic review and meta-analysis of 20 studies evaluated salivary IL-8, CYFRA 21-1, and CD44 individually and in combination. Pooled areas under the summary receiver operating characteristic curve were 0.88 for IL-8, 0.90 for CYFRA 21-1, and 0.91 for CD44. When at least two of the three markers were combined, the pooled area under the curve rose to 0.92, with a sensitivity of 88% and a specificity of 90% [13]. These figures are encouraging, but the meta-analysis also exposed moderate heterogeneity and highlighted the need for prospective validation in clinically relevant populations rather than convenience samples of advanced disease [13].

Other proteomic candidates include matrix metalloproteinases, vascular endothelial growth factor (VEGF), tumour necrosis factor-alpha (TNF-α), and a growing list of proteins identified through mass spectrometry-based discovery workflows. A proteomic study of an Indian population identified NUCB2, ITIH4, ITIH2, and RBP4 as potential markers for early OSCC detection and lymph node invasion, and reported enrichment of protein domain families including lipocalins, SERPINs, and annexin repeats [14]. These discovery-phase findings are biologically interesting but require targeted validation in independent cohorts before they can be considered diagnostic candidates.

Table 1. Selected salivary protein and cytokine biomarkers for OSCC detection

Biomarker	Molecular class	Reported diagnostic performance	Key caveats	Representative references

IL-8	Chemokine	Pooled AUC 0.88; sensitivity and specificity in the 70–95% range across studies	Inflammatory confounding; variable across assay platforms	[11,13]
IL-6	Cytokine	Sensitivity 70–90%, specificity 75–95% in review-level synthesis; progressive elevation across dysplasia–carcinoma sequence	Overlap with periodontal inflammation	[11,12]
CYFRA 21-1	Cytokeratin fragment	Pooled AUC 0.90	Limited standalone specificity; best in panels	[13]
CD44 / sCD44	Adhesion molecule	Pooled AUC 0.91; discriminating power between normal and dysplastic tissue	Assay standardisation unresolved	[12,13]
Combined IL-8 + CYFRA 21-1 + CD44	Multi-marker panel	Pooled AUC 0.92; sensitivity 88%, specificity 90%	Heterogeneity across included studies	[13]
NUCB2, ITIH4, ITIH2, RBP4	Discovery-phase proteins	Identified as potential markers of early OSCC and nodal invasion	Require independent validation	[14]

The proteomic literature therefore supports a cautiously optimistic conclusion. Individual cytokines and cytokeratin fragments demonstrate moderate-to-good diagnostic accuracy, and multi-marker panels consistently outperform single analytes. However, the field has not yet produced a prospectively validated, standardised protein panel that is ready for regulatory submission, and inflammatory confounding remains a particular concern because periodontal disease and oral lichen planus can elevate the same cytokines that are proposed as cancer markers [11].

Nucleic Acid Biomarkers: MicroRNAs, Cell-Free DNA, and Epigenetic Marks

Nucleic acid-based markers offer several theoretical advantages over proteins. MicroRNAs (miRNAs) are short, stable non-coding RNAs that regulate post-transcriptional gene expression and are frequently dysregulated in OSCC. They are protected from RNase degradation within extracellular vesicles and protein complexes, and they can be amplified with high analytical sensitivity using quantitative PCR or isothermal amplification methods [3]. Salivary miRNAs such as miR-21, miR-31, miR-184, miR-423-5p, and others have been associated with OSCC diagnosis and prognosis, and review-level evidence indicates that salivary miRNAs can achieve areas under the curve of 0.85 or higher [11]. A 2025 review of salivary miRNAs and cytokines concluded that these two classes of molecules are closely associated with OSCC progression and that their combined measurement may improve both diagnosis and prognostic stratification [3].

Cell-free DNA (cfDNA) and circulating tumour DNA (ctDNA) in saliva represent a second nucleic acid avenue. Saliva contains DNA shed from oral epithelial cells, leucocytes, and microorganisms, and tumour-derived DNA can be detected through somatic mutations, copy-number alterations, and cancer-specific methylation patterns [4]. The epigenetic route is particularly attractive because DNA methylation changes are early, stable, and tumour-specific. A prospective study of 30 OSCC patients used high-resolution melting analysis to detect methylation marks in five genes—ASCL1, MEOX2, CBLN1, OLIG2, and SOX14—in pre-operative saliva. At least one methylation mark was detectable in 70% of pre-operative OSCC cases, and a combination of ASCL1, MEOX2, and OLIG2 was sufficient to detect the same proportion. After surgical resection, methylation levels decreased significantly for ASCL1, MEOX2, and OLIG2, and persistence of at least one mark was associated with close or positive surgical margins [15]. This study is important because it demonstrates not only diagnostic potential but also a plausible role for salivary cfDNA methylation in post-treatment surveillance and margin assessment [15].

The biological rationale for salivary cfDNA is strengthened by the observation that saliva may be enriched in tumour DNA relative to plasma in OSCC, particularly for locally advanced lesions [4]. However, cfDNA-based assays face several technical hurdles: cfDNA is present at low concentration, fragmentation patterns vary, and the proportion of tumour-derived DNA can be very small relative to host and microbial DNA. Sensitive enrichment methods, such as methylation-specific PCR, targeted

bisulphite sequencing, and hybrid-capture approaches, are therefore necessary. The absence of detectable methylation marks in approximately 30% of OSCC cases in the study cited above also indicates that no single epigenetic panel will achieve universal sensitivity, reinforcing the case for multi-analyte strategies [15].

Salivary Exosomes and Extracellular Vesicles

Extracellular vesicles (EVs), including exosomes, are nanoscale membrane-bound structures released by cells into the extracellular space and biological fluids. Salivary exosomes carry a cargo of proteins, lipids, messenger RNAs, miRNAs, and DNA fragments that reflects the molecular state of their cell of origin, and they are more stable than free nucleic acids and proteins because their lipid bilayer protects cargo from enzymatic degradation [7]. These properties have made salivary exosomes one of the most actively investigated frontiers in oral cancer diagnostics.

Several exosomal biomarkers have shown diagnostic promise. Increased levels of the exosomal marker protein Alix have been reported in the saliva of OSCC patients, and exosomal miRNAs isolated from OSCC saliva show distinct expression profiles compared with healthy controls [7]. A study evaluating salivary and serum exosomal mRNAs found that a panel of exosomal TNF-α and OAZ1 was promising for OSCC diagnosis, while MMP9 and IL6 were informative for tumour grading [16]. Exosomal IL-6 and IL-8 have also been linked to smoking-related inflammatory pathways and oral carcinogenesis, suggesting that exosomal cytokine profiling may capture both tumour and microenvironmental signals [17].

The translational pathway for exosome-based diagnostics is, however, constrained by four interconnected bottlenecks. First, pre-analytical variability in saliva collection, processing, and storage directly affects exosome yield and cargo integrity. Second, vesicle enrichment methods involve trade-offs between yield and purity, and different isolation techniques—ultracentrifugation, size-exclusion chromatography, affinity capture, and precipitation—produce preparations with different contaminant profiles. Third, low-abundance tumour-derived vesicles are masked by abundant background vesicles of salivary gland, leucocyte, and microbial origin. Fourth, biomarker heterogeneity across cohorts limits the robustness of exosomal signatures [7]. Nanotechnology-enabled solutions, including microfluidic and affinity-based enrichment, nucleic acid amplification circuits, and optical or electrochemical transduction, are being developed to address these bottlenecks, but they remain largely at the proof-of-concept stage [7].

Metabolomic, Microbial, and Viral Biomarkers

Metabolomics interrogates the small-molecule complement of saliva and captures downstream biochemical consequences of malignant transformation, including altered polyamine metabolism, amino acid catabolism, lipid metabolism, and choline metabolism. A targeted metabolomics study of salivary monoacetylated polyamines found that N^1-acetylspermidine and N^1-acetylspermine increased progressively from oral leukoplakia to early and advanced OSCC, with areas under the curve of 0.87 and 0.81, respectively. A two-marker logistic model achieved a cross-validated area under the curve of 0.85 [18]. Other studies have identified cadaverine, N-acetylcadaverine, choline, glycine, tryptophan, acetone, and 5-aminopentanoic acid as dysregulated metabolites, and a four-metabolite panel has been proposed as a candidate diagnostic signature [19]. These findings are biologically coherent because polyamines and amino acid metabolites are intimately linked to cell proliferation, oxidative stress, and tumour metabolism, but the metabolomic literature is characterised by small sample sizes and limited external validation [19].

The oral microbiome and viral constituents add a further dimension. Dysbiosis of the oral bacterial community has been associated with malignant transformation and progression, and high-risk human papillomavirus (HPV) genotypes, particularly HPV-16 and HPV-18, are established risk factors for a subset of oropharyngeal and oral cancers [4]. Salivary detection of HPV DNA and microbial community signatures therefore represents a complementary strategy that may be particularly relevant for HPV-associated disease and for risk stratification in populations with high betel quid and tobacco exposure [4]. The diagnostic performance of microbial and viral markers as standalone tests is generally modest, but their integration into multi-domain panels may improve specificity by capturing aetiological heterogeneity [4].

Table 2. Emerging salivary biomarker domains beyond proteins and cytokines

Biomarker domain	Representative analytes	Reported diagnostic signal	Principal challenges	References

MicroRNAs	miR-21, miR-31, miR-184, miR-423-5p	AUC ≥0.85 in review-level synthesis; stable within vesicles	Normalisation strategy; pre-analytical variability	[3,11]
Cell-free DNA methylation	ASCL1, MEOX2, OLIG2, CBLN1, SOX14	Detectable in ~70% of pre-operative OSCC; decreases after resection	Low tumour fraction; 30% false-negative rate in one cohort	[15]
Exosomal cargo	Exosomal TNF-α, OAZ1, MMP9, IL6, Alix, miRNAs	Promising diagnostic and grading signals	Yield–purity trade-offs; background vesicle masking	[7,16]
Metabolites	N^1-acetylspermidine, N^1-acetylspermine, cadaverine, choline, tryptophan	Two-marker model cross-validated AUC 0.85; four-metabolite panels under study	Small cohorts; limited external validation	[18,19]
Microbiome and HPV	HPV-16/18 DNA; dysbiotic bacterial signatures	Complementary aetiological and risk-stratification information	Modest standalone accuracy; confounded by oral hygiene	[4]

Multi-Marker Panels and Integrated Diagnostic Models

The consistent lesson from single-analyte studies is that no individual salivary biomarker combines sufficiently high sensitivity and specificity to function as a stand-alone diagnostic test for OSCC. This has driven a shift toward multi-marker panels that combine analytes from different molecular domains. The meta-analytic evidence for a three-marker protein panel of IL-8, CYFRA 21-1, and CD44 is the clearest example: the pooled area under the curve of 0.92 for the combined panel exceeded the performance of any individual marker [13]. Similarly, combining miRNAs with cytokines, or cfDNA methylation with protein markers, has been proposed as a strategy to capture both tumour-specific and microenvironment-derived signals [3,15].

Integrated models also allow clinical risk factors to be incorporated alongside molecular data. A multivariate model combining salivary markers with established risk factors—tobacco use, alcohol consumption, age, and lesion site—has been reported to improve discrimination between OSCC and controls compared with molecular markers alone [7]. This is an important methodological development because it acknowledges that diagnostic probability in a real clinical population depends on pre-test probability, not only on assay performance. The use of risk-factor-weighted algorithms may be particularly valuable in screening programmes targeting high-incidence populations, where the prevalence of OPMDs and early OSCC is sufficient to justify population-level testing [7].

However, multi-marker panels introduce new challenges. The number of candidate combinations grows rapidly with the number of analytes, and many published panels have been derived from small, retrospective cohorts without independent validation. Overfitting is a serious risk when the ratio of markers to events is high, and the reported diagnostic accuracy of a panel in its discovery cohort is often substantially higher than in subsequent external validation [6]. The field therefore needs prospective, pre-registered studies that lock down the panel composition and thresholds before evaluation in independent populations [6].

Point-of-Care Biosensing and Microfluidic Platforms

The clinical and public-health value of salivary diagnostics ultimately depends on the availability of platforms that can measure biomarkers rapidly, reliably, and at low cost outside specialist laboratory settings. Point-of-care (POC) biosensors have therefore become a central area of translational research. Electrochemical, optical, and transistor-based biosensors targeting salivary IL-8, CYFRA 21-1, cancerous inhibitor of PP2A (CIP2A/P90), and high-risk HPV genotypes have been reported, with limits of detection frequently in the femtomolar range [5]. These analytical sensitivities are impressive, but a scoping review of the biosensor literature found that reporting of assay time, sample volume, and clinical diagnostic accuracy is inconsistent, and that translation to routine use will require standardised analytical validation and well-designed multicentre clinical studies [5].

Several recent platform innovations illustrate the direction of travel. A portable biosensor integrating an autocycling primer extension reaction with a bioinspired photonic-crystal microchip has been developed for quantitative detection of salivary miRNA-31, with the explicit aim of enabling early, non-invasive OSCC screening [20]. A quartz crystal microbalance

immunosensor array has been reported for sensitive detection of CYFRA 21-1 in saliva, with a limit of detection of 0.2 ng/mL across a 0.1–100 ng/mL dynamic range [21]. Disposable microelectrodes functionalised with hybrid poly(methacrylic acid-co-acrylamide)–polythiophene nanofibres have been used to detect cholic acid, a candidate OSCC biomarker, in human saliva [22]. These platforms demonstrate that the analytical chemistry required for salivary OSCC detection is feasible; the remaining question is whether they can be manufactured, validated, and deployed at a cost and scale compatible with screening in high-burden settings [22].

Microfluidic integration is particularly important because it addresses the sample-preparation bottleneck that has limited many biosensor prototypes. Whole saliva contains cells, debris, mucins, and microbial aggregates that can foul sensor surfaces and generate matrix effects. Microfluidic devices that incorporate filtration, enrichment, and detection on a single chip can reduce operator handling and improve reproducibility [7]. Lab-on-a-chip systems that combine exosome capture with nucleic acid amplification and optical readout are being developed for salivary exosome analysis, although most remain at technology-readiness levels that are several years from clinical deployment [7].

Artificial Intelligence and Multi-Omics Integration

The multi-dimensional nature of salivary data—proteomic, transcriptomic, epigenetic, metabolomic, and microbiomic—creates both an opportunity and a computational challenge. Artificial intelligence (AI) and machine learning (ML) methods are increasingly used to integrate these data layers, identify diagnostic signatures, and build predictive models. Machine-learning classifiers applied to salivary multi-omics datasets have shown improved discrimination compared with single-domain analyses, and AI-based approaches are being explored for biomarker discovery, panel optimisation, and image-based cytology [23].

However, the use of AI in salivary diagnostics is constrained by the same data-quality problems that affect the underlying biomarkers. Machine-learning models depend on consistent, high-quality input data, and when biomarker concentrations and diagnostic thresholds vary substantially between studies, algorithms may overfit to cohort-specific idiosyncrasies and fail to generalise [10]. The absence of harmonised reference ranges and standardised reporting frameworks makes it difficult to integrate salivary biomarkers into digital diagnostic ecosystems, and the risk of algorithmic bias is substantial when training cohorts under-represent the populations in which the test will ultimately be used [10]. For AI to deliver on its promise in this field, it must be coupled to pre-analytical standardisation, prospective validation, and transparent reporting of model performance across diverse populations [10].

Clinical Translation: Barriers, Guidelines, and Regulatory Pathways

Despite two decades of biomarker discovery, no salivary assay has received FDA approval for oral cancer detection [4]. This is the starkest indicator that the field's translational pathway remains incomplete. A 2026 living evidence-informed guideline on early detection of OSCC and OPMDs concluded that the near-total absence of diagnostic accuracy data in the target clinical population, combined with the lack of randomised controlled trials evaluating the effect of salivary tests on patient-important outcomes, justified the recommendation that salivary adjunct tests should not be used in regular clinical practice outside of a research setting [24]. This recommendation is not a rejection of the technology but a statement about the current evidence base: the available studies are predominantly small, retrospective, and enriched for advanced disease, and they do not establish that salivary testing improves outcomes when applied to the population in which screening would actually occur [24].

The barriers to translation can be grouped into four categories. The first is **pre-analytical variability**, as discussed above: differences in collection, processing, and storage generate inconsistent measurements and impede the development of universal thresholds [8]. The second is **lack of prospective multicentre validation**: most studies are single-centre and retrospective, and independent external validation is rare [6]. The third is **regulatory and evidentiary uncertainty**: regulatory agencies require analytical validation, clinical validation, and evidence of clinical utility, and the field has not yet produced the large, prospectively designed studies needed to satisfy these standards [4]. The fourth is **health-economic uncertainty**: the cost-effectiveness of salivary screening has not been established, and without evidence that testing improves survival or reduces treatment costs, payers and health systems are unlikely to adopt it [24].

The fragmented-threshold problem is particularly pernicious because it creates a self-reinforcing cycle. Without standardised thresholds, multicentre studies cannot pool data; without pooled data, thresholds cannot be harmonised; and without harmonised thresholds, biosensor calibration and AI model development remain cohort-specific [10]. Breaking this cycle will require coordinated action by professional societies, regulatory bodies, industry, and academic consortia. Initiatives such as the

COST Action CA21140—INTERCEPTOR, which aims to intercept oral carcinogenesis through collaborative innovation in molecular diagnostics and surveillance technologies, represent early attempts to build the cross-sector infrastructure needed for standardisation and multicentre validation [10].

Future Directions

Several priorities emerge for the next phase of salivary diagnostics research. First, the field needs **prospective, population-relevant cohort studies** that enrol participants with OPMDs and early-stage OSCC, rather than convenience samples dominated by advanced disease. Such studies should be pre-registered, use a consensus pre-analytical protocol, and report diagnostic accuracy against histopathology as the reference standard [6]. Second, **standardisation** must move from aspiration to implementation: a minimal reporting dataset for salivary biomarker studies, analogous to REMARK for tumour markers, would improve comparability and facilitate meta-analysis [10]. Third, **multi-omics integration** should be pursued not as an end in itself but as a means of identifying biologically coherent signatures that are robust across platforms and populations [2]. Fourth, **point-of-care platforms** must be validated in clinical settings, not only in buffered solutions, and must demonstrate acceptable performance with whole saliva rather than processed samples [5]. Fifth, **clinical utility studies** are needed to determine whether salivary testing, used as a triage tool before biopsy or as a surveillance test after treatment, improves outcomes or reduces costs [24].

A particularly promising future direction is the concept of **liquid TNM staging**, in which salivary and blood-based biomarkers are combined with imaging and histopathology to produce a more comprehensive assessment of tumour burden and biological aggressiveness [4]. Salivary cfDNA methylation has already shown potential for monitoring surgical margin status and detecting recurrence, and exosomal cargo analysis may provide real-time information about tumour evolution during therapy [7,15]. If these applications can be validated, salivary diagnostics may find its most durable clinical role not as a replacement for biopsy but as a complement to it—a tool for risk stratification, treatment monitoring, and post-treatment surveillance.

CONCLUSION

Salivary diagnostics for oral cancer detection has matured from a speculative concept into a biologically grounded and technologically sophisticated field. The evidence reviewed here establishes that saliva contains a rich repertoire of tumour-associated molecules—cytokines, cytokeratin fragments, miRNAs, methylated cfDNA, exosomal cargo, and metabolites—and that multi-marker panels can achieve areas under the curve exceeding 0.90 in meta-analytic synthesis. Point-of-care biosensors with femtomolar limits of detection and microfluidic platforms for exosome analysis demonstrate that the analytical chemistry is no longer the principal bottleneck. Yet the field has not crossed the threshold into clinical practice, and the reasons are structural rather than scientific: pre-analytical variability, absent standardisation, retrospective and underpowered study designs, and the lack of prospective evidence that salivary testing improves patient outcomes.

The path forward requires a deliberate shift from biomarker discovery to biomarker qualification. That means consensus protocols, multicentre prospective cohorts, independent validation, regulatory engagement, and health-economic evaluation. Saliva is too accessible, too biologically informative, and too well suited to low-resource settings to remain confined to research laboratories. The question is no longer whether salivary diagnostics can detect oral cancer, but whether the field can build the evidence infrastructure needed to make that detection clinically meaningful.

REFERENCES

[1] Starska-Kowarska K. Salivaomic biomarkers—an innovative approach to the diagnosis, treatment, and prognosis of oral cancer. Biology. 2025 Jul 13;14(7):852.

[2] Tan WS, Kuo H, Jiang CG, Lu MH, Lu YH, Wang YL, Wang CS, Vo TT, Lee IT. Salivary biomarkers and their link to oncogenic signaling pathways in oral squamous cell carcinoma: diagnostic and translational perspectives in a narrative review. Oncology Research. 2025 Dec 30;34(1):6.

[3] Qin Y, Dong X, Li B. Salivary miRNAs and cytokines associated with diagnosis and prognosis of oral squamous cell carcinoma. Frontiers in Cell and Developmental Biology. 2025 Jan 22;13:1531016.

[4] Ebrahim NA, Soliman SM, Farghaly TA. Saliva-based molecular diagnostics in oral squamous cell carcinoma (OSCC): a non-invasive frontier in oncology. European Archives of Oto-Rhino-Laryngology. 2025 Dec 29:1-6.

[5] Syawqie A, Sovira GD, Ichwan SJ. Biosensors for oral cancer detection: a scoping review. Diagnostic Pathology. 2026 Jun 22.

[6] Patadiya HH, Upadhyayula S, Adeshara VT. Saliva-based genomic signatures for early detection of oral squamous cell carcinoma: the state and perspectives of translation. Frontiers in Genetics. 2026 Oct 9;17:1831378.
[7] Zhao LX, Wang ET, Jia WH, Wang MH, Pan Z, Dong L, Wen HQ, Xu WH, Xu R. Emerging salivary exosome nanodiagnostics for early oral squamous cell carcinoma: platforms, biomarkers, and future perspectives. International Journal of Nanomedicine. 2026 Dec 31:577306.
[8] Moulahoum H, Ghorbanizamani F. Fragmented Thresholds, Unified Vision: Advancing Salivary Diagnostics for OSCC and OPMD. Journal of Oral Pathology & Medicine. 2026 Apr 15.
[9] Pateel DG, Fan CY, Rai NP, Fakhra I, Asif MK. Advances in early detection technologies of oral cancer: a narrative review. Oncologie. 2026 Jul 6(0).
[10] Moulahoum H, Ghorbanizamani F. Fragmented Thresholds, Unified Vision: Advancing Salivary Diagnostics for OSCC and OPMD. Journal of Oral Pathology & Medicine. 2026 Apr 15.
[11] Cardoso SD, Pedroso CM, de Goes MF, da Silva VT, Ribeiro AC, Santos-Silva AR. Treatment planning software to protect dental structure, salivary glands, and nontarget tissue in head and neck cancer patients: a scoping review. Oral Surgery, Oral Medicine, Oral Pathology and Oral Radiology. 2026 Feb 5.
[12] Ghalwash D, Ammar A, Abou-Bakr A, Diab AH, El-Gawish A. Validation of salivary proteomic biomarkers for early detection of oral cancer in the Egyptian population. Future Science OA. 2025 Dec 31;11(1):2432222.
[13] Kumar P, Singh K, Garg A, Urs AB, Augustine J. Salivary IL-8, CYFRA 21-1, and CD44 for early detection of oral squamous cell carcinoma: a systematic review and meta-analysis. Clinica Chimica Acta. 2025 Sep 1;577:120471.
[14] Singh A, Sharma P, Balasubramani A, Shetty SP, Roy S, Vasudeva SB, Patil S, Vaish R, Nair S, Srivastava S. Identification of Potential Salivary Biomarkers and Enriched Protein Domain/Motif Families for Early Detection and Lymph Node Invasion in OSCC Patients of the Indian Population. Journal of Proteome Research. 2025 Sep 18;24(10):5204-15.
[15] Singh A, Sharma P, Balasubramani A, Shetty SP, Roy S, Vasudeva SB, Patil S, Vaish R, Nair S, Srivastava S. Identification of Potential Salivary Biomarkers and Enriched Protein Domain/Motif Families for Early Detection and Lymph Node Invasion in OSCC Patients of the Indian Population. Journal of Proteome Research. 2025 Sep 18;24(10):5204-15.
[16] Kumar SK, Kanaparthi N, Annes SB, Bendre RV, Balakrishna N, Kumar S, Hakeem AH, Sasidhar MV. Evaluation of salivary and serum Exosomal mRNAs as biomarkers for the diagnosis and prognosis of oral squamous cell carcinoma. Scientific Reports. 2025 Nov 3;15(1):38310.
[17] Bano A, Vats R, Yadav P, Kamboj M, Bhardwaj R. Smoking-induced shifts in salivary exosomal cytokines and amino acid profiles as potential early biomarkers for oral cancer. Cytokine. 2025 Mar 1;187:156857.
[18] Huang M, Magalhaes M. Reply to the Letter to the Editor: the importance of integrating histopathologic, clinical, radiographic and ancillary findings. Oral surgery, oral medicine, oral pathology and oral radiology. 2026 May;141(5):725.
[19] Hsieh YJ, Chang Y, Yang C, Teng NH, Yu JS, Chang KP, Chen WC, Chu LJ, Chen YT. Systematic verification of saliva-based biosignatures for oral cancer via targeted metabolomics. Journal of Food and Drug Analysis. 2026 Jun 15;34(1):88.
[20] Li J, Zhang W, Liu Y, Chang R, Lan Y, Qiu D, Chang C, Huang J, Xu Q. Sensitive Point-of-Care Detection of Oral Squamous Cell Carcinoma-Associated Salivary miRNA-31 Enabled by Autocycling Primer Extension Reaction and a Bioinspired Photonic-Crystal Microchip. ACS sensors. 2026 Mar 4;11(3):2252-60.
[21] Zhang X, Guo H, Wong LS, Xiong H, Kong L, Ren G, Hu Y, Zhou Y, Zheng S, Zhang L, Hsia KJ. DMSN@ AuNPs-enhanced QCM immunosensor array for non-invasive and highly sensitive detection of Cyfra21-1 in saliva. Analytica Chimica Acta. 2026 Feb 12:345226.
[22] Akhtar M, Mujahid A, Afzal A. Hybrid Poly (Methacrylic Acid-Co-Acrylamide)-Polythiophene Nanofibers for Salivary Diagnosis of Oral Squamous Cell Carcinoma. Macromolecular Bioscience. 2025 Jul;25(7):2400420.
[23] Thangasamy B, Sivagnanam A. Salivary biomarkers and their diagnostic importance in oral diseases. Archives of Oral Biology. 2026 Apr 12:106595.
[24] Wei W, Zhang R, Zhang Z. Circulating Biomarkers with Artificial Intelligence for Clinical Risk Assessment and Therapeutic Monitoring: Evidence, Limitations, and Translation. Therapeutics and Clinical Risk Management. 2026 Dec 31:612081.