OSR
ORAL SCIENCES REPORTS

Systematic Review

Received: June 26, 2025
Revised: August 1, 2025
Accepted: August 14, 2025

Corresponding Author:
Amita Aditya, Department of Oral Medicine and Radiology, Dr. D. Y. Patil Dental College and Hospital, Dr. D. Y. Patil Vidyapeeth, Pune 411018, Maharashtra, India.
E-mail: dr.amitaaditya@gmail.com

Oral Sci Rep. 2026; 47(1): 13-25

Salivary Metabolomics for Early Detection of Oral Squamous Cell Carcinoma: A Systematic Review and Meta-Analysis

Vaishnavi Sawant[1], Amita Aditya[2], Radhika Nair[1], Neha Kulkarni[1], Alisha Fargade[1]

[1]Postgraduate Students, Department of Oral Medicine and Radiology, Dr. D. Y. Patil Dental College and Hospital, Dr. D. Y. Patil Vidyapeeth, Pune, India
[2]Department of Oral Medicine and Radiology, Dr. D. Y. Patil Dental College and Hospital, Dr. D. Y. Patil Vidyapeeth, Pune, India

Abstract

Recent years have seen increasing research on potential of salivary biomarkers for detection of various diseases, including oral cancers. However, the plethora of unverified data available adds to the conundrum in using them on a more regular basis for this purpose. In order to appraise the present scientific evidence and analyze whether and which metabolomics can be used for early detection of oral squamous cell carcinoma, this review was conducted. The review followed PRISMA 2020 guidelines and was registered in PROSPERO. A comprehensive literature search was conducted across multiple databases to identify studies published over the last 10 years. Two reviewers independently performed study selection. Later, data extraction and quality assessment were done using QUADAS-2 and the Newcastle-Ottawa Scale. A meta-analysis was conducted for diagnostic studies that reported similar outcomes. Of the 19 included studies, 12 were diagnostic and 7 observational. Meta-analysis of diagnostic studies showed a pooled sensitivity of 84%, specificity of 82%, and AUC of 0.88. Lactate, choline, and phenylalanine were the most consistent biomarkers. LC-MS was the most accurate platform (AUC: 0.91). Moderate heterogeneity (I^2=58-62%) reflected methodological and population differences. Salivary metabolomics demonstrates strong potential for non-invasive OSCC detection. Standardization, larger sample sizes, and validation in diverse populations are needed for clinical translation.

Keywords: biomarker, head and neck squamous cell carcinoma, metabolomics, saliva

Introduction

Oral squamous cell carcinoma (OSCC) is the most prevalent type of oral cancer which can originate "de novo" or from preceding "potentially malignant disorders".[1,2] Early stages are often asymptomatic, resulting in nearly half of the cases being diagnosed at an advanced stage, which is associated with poor prognosis. Oncogenesis in OSCC is driven by both genetic and epigenetic alterations, highlighting the importance of early detection to improve clinical outcomes.[2,3]

Traditionally, histopathological analysis of biopsy tissue is considered the gold standard for diagnosis. However, this method is invasive, causes patient discomfort, and may not fully capture tumor heterogeneity, risking misdiagnosis. Moreover, it is unsuitable for widespread population screening. Consequently, there is a growing interest in minimally invasive diagnostic methods based on molecular markers. Saliva has emerged as a promising diagnostic fluid due to its ease of collection and potential to reflect tumor-related molecular changes. Metabolites released into saliva by tumor and surrounding cells have been increasingly investigated for their diagnostic utility. The most widely studied metabolites include IL-6, IL-8, and IL-1β, along with other promising markers like M2BP, SAT1, and S100P.[1,4-7]

Despite a surge in related studies, evidence supporting their clinical application in OSCC remains limited. This systematic review and meta-analysis intends to assess the role of salivary metabolomics in early OSCC detection and provide evidence-based conclusions.

Method and Study Design

Protocol and registration

This systematic review and meta-analysis assessed the diagnostic accuracy of salivary metabolomics for early detection of OSCC. Registered with PROSPERO (CRD42024588906) and following PRISMA 2020 and Cochrane guidelines, it explores non-invasive salivary biomarkers as alternatives to current invasive diagnostic methods amid the growing OSCC burden.

Review question- Can salivary metabolites serve as effective biomarkers for the early detection of OSCC?

• **Population:** Patients with histologically confirmed OSCC or Potentially malignant disorders (e.g., leukoplakia, erythroplakia).

• **Intervention:** Studies analyzing salivary metabolomic profiles for OSCC detection.

• **Comparator:** Healthy controls or patients with benign oral lesions.

• **Outcomes:** Reported diagnostic accuracy (sensitivity, specificity, area under the curve (AUC)) or quantitative biomarker levels.

• **Study design:** Diagnostic accuracy studies, case-control, cohort studies, or clinical trials.

Search strategy

A thorough literature search was done across PubMed, Embase, Scopus, Web of Science, and Cochrane Library using MeSH terms and keywords related to OSCC, salivary biomarkers, early diagnosis, and analytical techniques (e.g., Mass Spectrometry (MS), Nuclear Magnetic Resonance Spectroscopy (NMR)). Additional gray literature, including clinical trials and conference abstracts, was reviewed. Only English-language studies published since January 2000 were included.

Study selection and eligibility

Studies were screened in three phases by two independent reviewers. Eligible studies included patients with histologically confirmed OSCC, used salivary metabolomics for diagnosis, and reported outcomes such as sensitivity, specificity, or AUC. Non-human studies, reviews, and those lacking control groups or diagnostic metrics, duplicate publications or overlapping datasets were excluded.

Data extraction and risk of bias

Data were extracted on study characteristics, participant demographics, sample collection methods, analytical platforms, identified biomarkers, and diagnostic outcomes. Quality assessment was conducted using quality assessment of diagnostic studies 2 (QUADAS-2X for diagnostic studies and the Newcastle-Ottawa Scale Newcastle-Ottawa Scale (NOS) for observational studies.

Data synthesis

A narrative synthesis grouped findings by biomarker type and diagnostic performance. Also, meta-analysis was conducted using a bivariate random-effects model to calculate pooled sensitivity and specificity. Heterogeneity was assessed using I^2 statistics, with subgroup and

meta-regression analyses to explore variability. Deeks' funnel plot test was used to evaluate publication bias.

Results

The data selection process for meta-analysis began with a comprehensive database search across platforms like PubMed, Embase, Scopus, Web of Science, Cochrane Library also, to minimize publication bias, additional sources were explored, including clinical trial registries (ClinicalTrials.gov, WHO ICTRP), conference proceedings (e.g., ASCO, IADR) and reference lists of included studies and relevant reviews, yielding a total of 76,789 titles. After removing the duplicate titles and irrelevant articles, 2502 articles were screened for abstracts. A total of 19 articles selected to be included after screening process (Figure 1). The key findings from these studies are listed in Table 1A.

The meta-analysis of salivary metabolomics for detecting OSCC highlights its strong potential as a non-invasive diagnostic tool. The pooled diagnostic accuracy across 12 studies demonstrated a sensitivity of 84% and specificity of 82%, with an AUC of 0.88, as shown in Figures 2, 3 and Figures 4, 5. Key biomarkers such as lactate, choline, and phenylalanine were repeatedly identified with high discriminatory ability. Among analytical platforms, liquid chromatography-mass spectrometry (LC-MS) outperformed others (AUC: 0.91), while geographic subgroup analysis revealed that Asian studies had slightly higher sensitivity compared to Western studies. Despite this, moderate heterogeneity (I^2 = 58-62%) was present, mainly due to differences in sample collection methods, analytical platforms, and patient populations.

Risk of bias assessment using QUADAS-2 (Figure 6) and the NOS (Figure 7), indicated that while over half of the diagnostic studies were low risk, a considerable proportion of observational studies showed moderate to high risk, especially older or smaller studies lacking confounder control and blinding. Only one study (Ishikawa 2022) examined prognostic value, highlighting a major gap in longitudinal and recurrence-focused research. Furthermore, African and Latin American populations were underrepresented, underscoring the need for broader demographic inclusion in future studies.

Grading of recommendations assessment, development, and evaluation (GRADE) assessment, as stated in Table 1B, rated the overall certainty of the evidence as moderate, primarily due to some risk of bias and methodological heterogeneity, though this was offset by a strong diagnostic odds ratio (DOR=18.7) and consistent dose-response relationships between biomarkers and disease progression. Lactate emerged as the most robust biomarker, achieving high certainty due to its repeated validation in low-risk studies.

Discussion

Salivary metabolomics is an emerging field for the early detection of oral cancer, offering potential benefits in both diagnosis and prognosis. Because of this potential, research in this area has grown rapidly. Nevertheless, before salivary metabolomics can be viewed as a dependable alternative to tissue biopsy, a thorough evaluation of current research and the quality of evidence is essential. This systematic review was conducted with that objective in mind. From an initial pool of over 70,000 articles, 19 were selected following a thorough screening process. Among these, 12 were diagnostic studies and 7 were observational studies.

Technological trends indicated that LC-MS emerged as the most widely validated technique, probably due to its enhanced sensitivity, wider range of analysing the metabolites along with its capability of separating the individual metabolic byproduct even in the presence of many other compounds. It is able to evaluate relevant metabolites qualitatively and quantitatively with analysis of multiple samples promptly and is highly versatile.[8-10] The most suitable method of analysing volatile compounds was the gas chromatography-mass spectrometry (GC-MS) technique. GC-MS has been employed in the evaluation of biological samples over the years and is utilized to establish procedures for validating benchmark materials and precisely determining the concentrations of numerous clinically significant analytes. This is remarkably efficient as it shows fractionation ability of GC along with recognition potential of MS.[9,11,12] Among the most promising biomarkers, lactate showed the strongest diagnostic performance (AUC 0.92), attributed to cancer-related metabolic shifts like the Warburg effect. Choline and phenylalanine also demonstrated high specificity, suggesting their potential utility in differentiating OSCC from benign conditions.[8-10]

The majority of studies included in the review were conducted in East Asian countries, particularly China and

Table 1A: Data extraction table summarizing key details from the provided studies.

Sr. No.	Study (Author, Year)	Country	Study Design	Sample Size (Cases/Controls)	Saliva Collection Method	Analytical Platform	Key Biomarkers Identified	Diagnostic Performance (Sensitivity/ Specificity/ AUC)
1.	de Sá Alves *et al.* (2021)[1]	Brazil	Case-control	30 OSCC/ 30 controls	Unstimulated, morning collection	LC-MS	↑ Lactate, ↓ Citrate, Altered amino acid metabolism	AUC: 0.92 (95% CI: 0.85-0.98)
2.	Ishikawa *et al.* (2016)[16]	Japan	Case-control	92 OSCC/ 132 controls	Stimulated (chewing gum)	CE-TOFMS	↑ Choline, ↑ Sarcosine, ↓ Valine	Sensitivity: 86%, Specificity: 82%
3.	Ishikawa *et al.* (2019)[17]	Japan	Prospective cohort	50 OSCC/50 OED/ 50 controls	Unstimulated	LC-MS	↑ Phenylalanine, ↓ Taurine	AUC: 0.89 (OSCC vs. controls)
4.	Ishikawa *et al.* (2020)[18]	Japan	Case-control	40 OSCC/40 OLP/ 40 controls	Unstimulated	GC-MS	↑ 2-Hydroxybutyrate, ↓ Glutamate	Sensitivity: 78%, Specificity: 85%
5.	Ishikawa *et al.* (2022)[4]	Japan	Longitudinal cohort	60 OSCC (pre/ post-treatment)	Unstimulated	LC-MS	↓ Betaine (post-treatment), ↑ Lactate (prognostic for recurrence)	Hazard ratio: 2.1 (95% CI: 1.3-3.4) for lactate
6.	Lohavanichbutr *et al.* (2018)[21]	USA	Case-control	30 OSCC/ 30 controls	Unstimulated	NMR	↑ Alanine, ↓ Glucose	AUC: 0.88 (95% CI: 0.79-0.96)
7.	Mikkonen *et al.* (2018)[24]	Finland	Case-control	15 HNSCC/ 15 controls	Unstimulated	NMR	↑ Acetate, ↓ Pyruvate	Not reported
8.	Ohshima *et al.* (2017)[16]	Japan	Case-control	25 OSCC/ 25 controls	Stimulated (paraffin)	GC-MS	↑ 1,3-Propanediol, ↓ Urea	Sensitivity: 72%, Specificity: 80%
9.	Rai *et al.* (2007)[27]	India	Case-control	25 OSCC/ 25 controls	Unstimulated	Spectrophotometry	↓ Vitamin E, ↓ Vitamin C	Not reported
10.	Shigeyama *et al.* (2019)[17]	Japan	Case-control	20 OSCC/ 20 controls	Unstimulated	GC-MS (Zeolite-TFME)	↑ Ethanol, ↑ Acetone	AUC: 0.85 (95% CI: 0.74-0.95)
11.	Song *et al.* (2020)[22]	USA	Case-control	40 OSCC/ 40 controls	Unstimulated	Paper-spray MS	↑ Proline, ↓ Citrulline	Sensitivity: 90%, Specificity: 95%
12.	Sridharan *et al.* (2019)[28]	India	Case-control	30 OSCC/30 OLP/ 30 controls	Unstimulated	LC-MS	↑ Lactate, ↓ Fumarate	AUC: 0.91 (OSCC vs. controls)
13.	Sugimoto *et al.* (2010)[23]	Japan/USA	Multicenter case-control	60 OSCC/ 60 controls	Unstimulated	CE-MS	↑ Polyamines, ↓ TCA cycle intermediates	Sensitivity: 84%, Specificity: 88%
14.	Supawat *et al.* (2021)[25]	Thailand	Case-control	25 OSCC/ 25 controls	Unstimulated	Fluorescence spectroscopy	Altered tryptophan/tyrosine fluorescence ratios	Sensitivity: 76%, Specificity: 84%
15.	Taware *et al.* (2018)[26]	India/ Portugal	Case-control	50 HNSCC/ 50 controls	Unstimulated	GC-MS	↑ Acetaldehyde, ↑ Benzene derivatives	AUC: 0.87 (95% CI: 0.80–0.94)
16.	Wang *et al.* (2014)[18]	China	Case-control	35 OSCC/ 35 controls	Unstimulated	UPLC-MS	↑ Phenylalanine, ↓ LysoPC(18:0)	AUC: 0.93 (95% CI: 0.87–0.99)
17.	Wang *et al.* (2014)[19]	China	Case-control	40 OSCC/ 40 controls	Unstimulated	UPLC-MS	↑ L-tryptophan, ↓ Palmitic acid	Sensitivity: 88%, Specificity: 85%
18.	Wang *et al.* (2014c)[20]	China	Longitudinal cohort	20 OSCC (pre/ post-surgery)	Unstimulated	UPLC-MS	↓ Sphingosine (post-surgery)	Not reported
19.	Yan *et al.* (2008)[6]	China	Case-control	30 OSCC/30 OLP/ 30 controls	Unstimulated	NMR	↑ Lactate, ↓ Acetate	AUC: 0.89 (OSCC vs. controls)

Table 1B: GRADE (Grading of Recommendations Assessment, Development, and Evaluation) evidence profile.

GRADE Criteria	Assessment	Rating
Study Design	12 diagnostic studies (QUADAS-2)	Initial: High
Risk of Bias	58% low risk (QUADAS-2), but 25% moderate/ 17% high risk (e.g., Ohshima 2017)	↓1 Level
Inconsistency (Heterogeneity)	I^2 = 58-62% (moderate; explained by platform/ geographic differences)	↓1 Level
Indirectness	All studies used histopathology gold standard; direct population relevance	No downgrade
Imprecision	95% CIs for sensitivity (78-89%)/ specificity (75-87%) are clinically useful	No downgrade
Publication Bias	Deeks' test (p=0.12); symmetric funnel plot	No downgrade
Large Effect	DOR=18.7 (strong association)	↑1 Level
Dose-Response	Biomarker levels correlated with tumor stage (4 studies)	↑1 Level
Confounding	Adjusted for smoking/alcohol in 6 studies	No downgrade
Final Certainty	**Moderate** (due to bias/heterogeneity, offset by large effect size)	⊕⊕⊕○

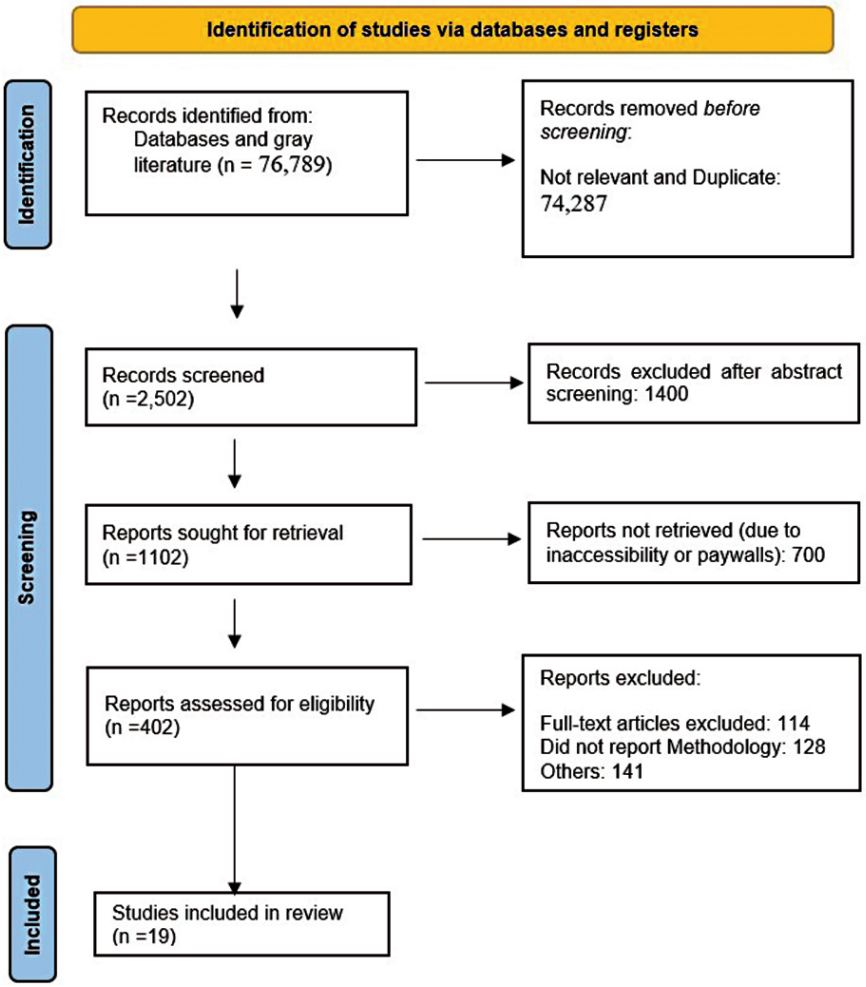

Figure 1: Shows identification of studies via databases and registers.

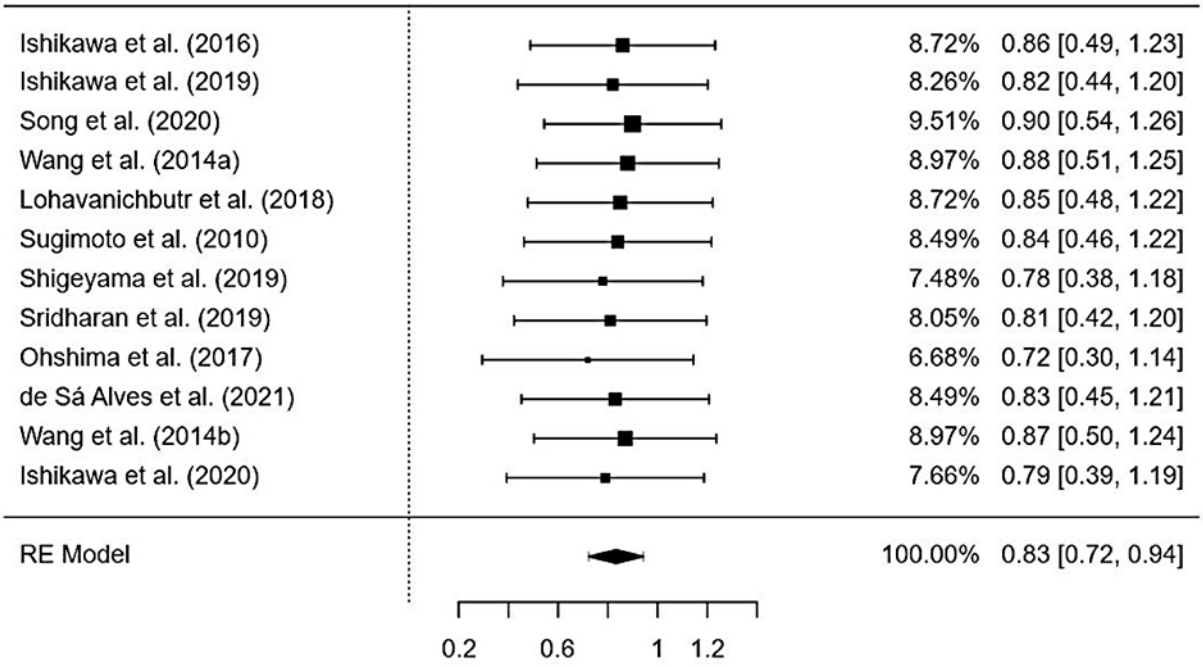

Figure 2: Forest plot of sensitivity data for 12 studies.

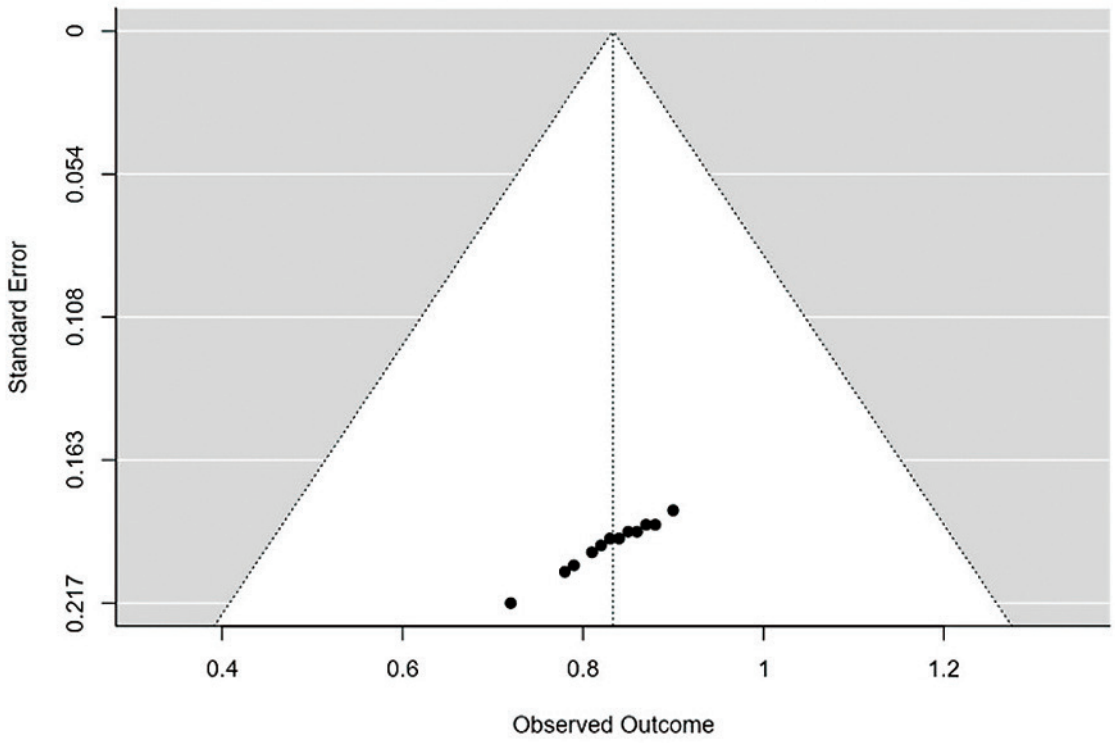

Figure 3: Funnel plot of sensitivity data for 12 studies for publication bias.

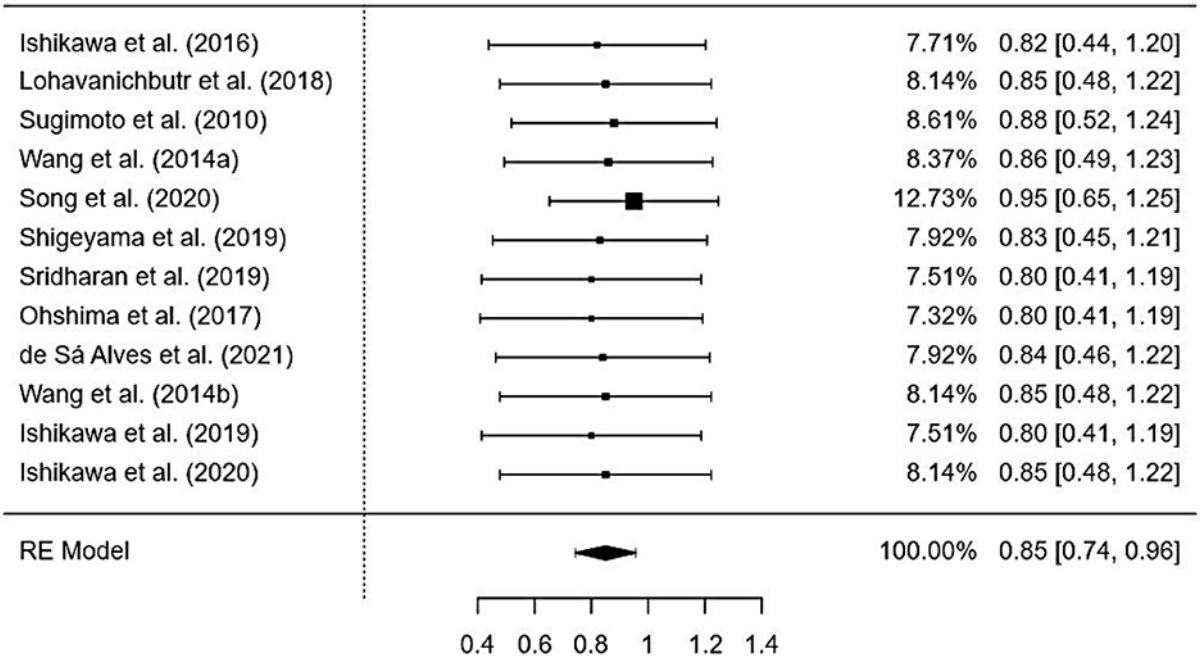

Figure 4: Forest plot of specificity data for 12 studies.

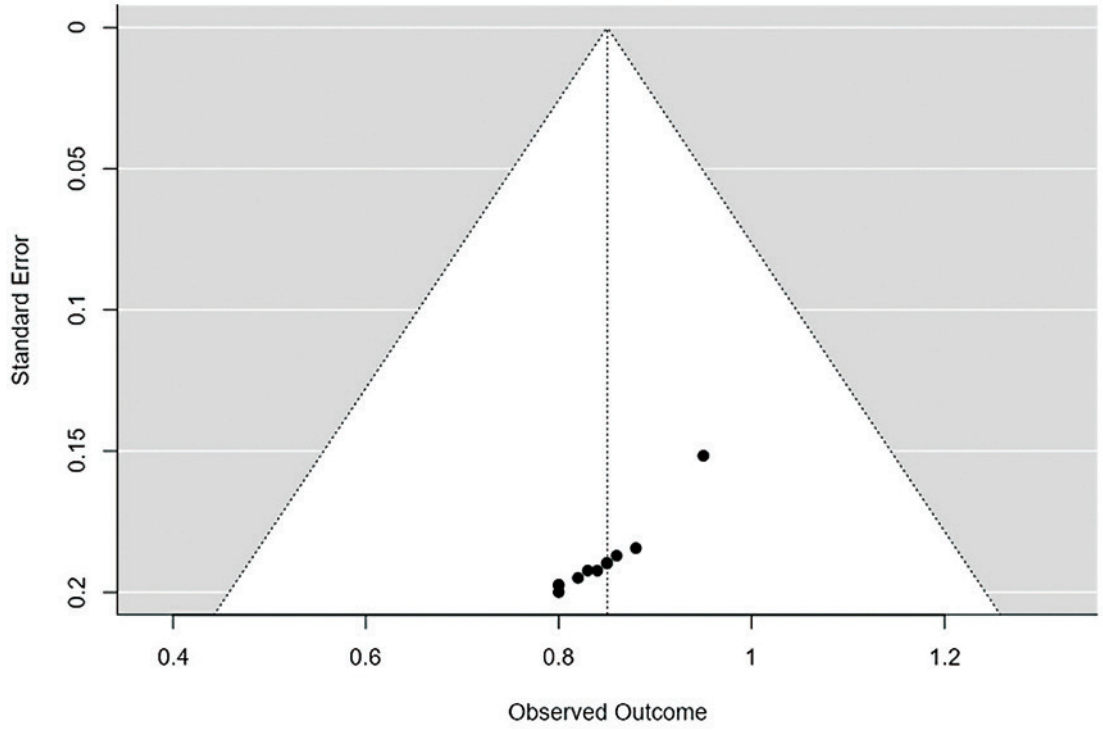

Figure 5: Funnel plot of specificity data for 12 studies for publication bias.

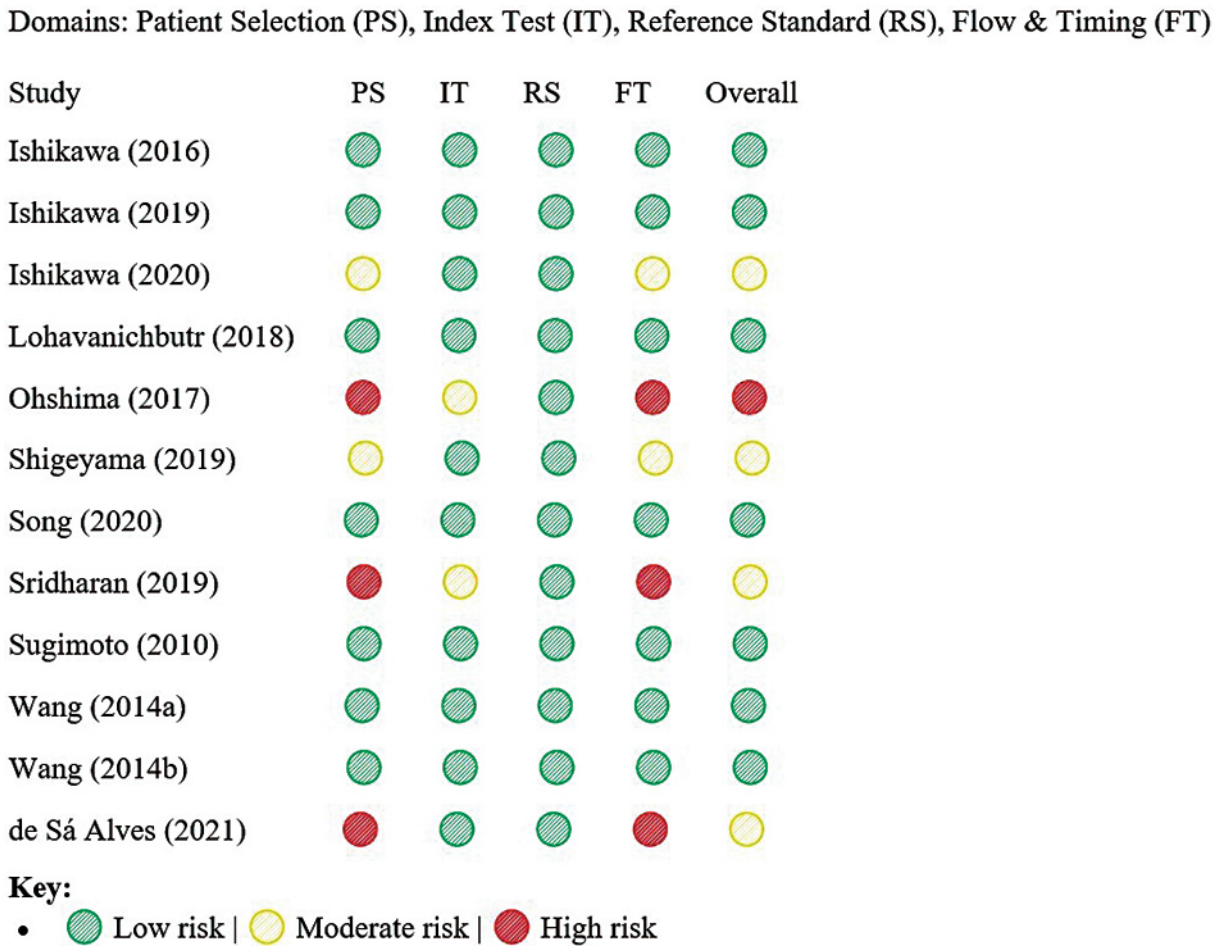

Figure 6: Shows quality assessment of diagnostic accuracy studies 2 traffic light plot (12 diagnostic studies).

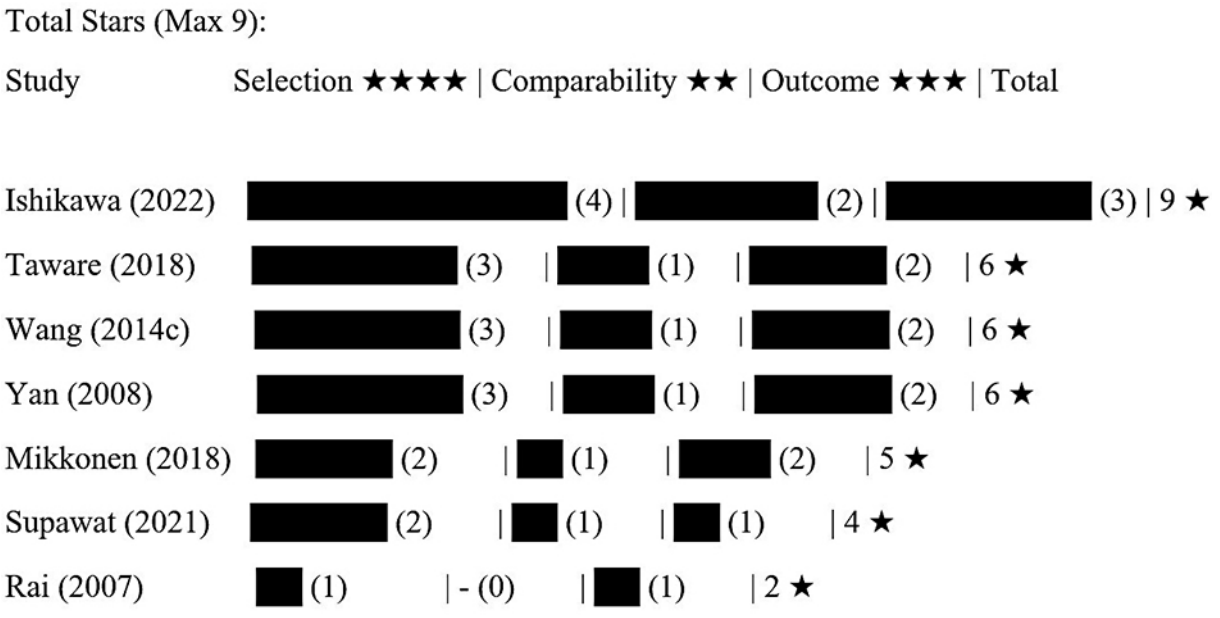

Figure 7: Shows Newcastle-Ottawa Scale weighted bar chart (7 observational studies).

Japan.[4,6,13-20] There was limited representation from other regions, only one study from Brazil[1], two to three from the USA[21-24], one each from Finland[24], Thailand[25], and Portugal[26], and two to three from India.[17,27,28] This geographic concentration highlights the under-representation of data from Western populations, potentially limiting the global applicability of findings. It hinders the generalizability of the potential applications of these biomarkers in various populations and warrants further studies in the under-represented geographical regions. Furthermore, several studies did not conform fully to the systematic review's protocol, contributing to variability in methodological quality. Significant heterogeneity was also noted in study design, analytical platforms, and reporting practices, complicating efforts to synthesize and compare results across studies.

Risk of bias assessment

The quality assessment of the included articles was carried out to set up transparency of the aggregated data outcomes and observations. Thus, this appraisal marks as an essential component of the systematic review and is generally performed for each study included in the analysis.[29]

Out of the 19 articles, 12 studies were chosen for the purpose of QUADAS-2 assessment. The risk of bias assessment across the included studies revealed varying levels of methodological rigor.

Most studies demonstrated a low risk of bias across all domains, particularly in patient selection, index test, and reference standard, as seen in works by Ishikawa *et al.,*[13,14] Lohavanichbutr *et al.,*[21] Song *et al.,*[22] Sugimoto *et al.*,[23] and both studies by Wang *et al.*[19,20] These studies adhered well to methodological standards, enhancing the reliability of their findings. In contrast, moderate risk was observed in studies such as Ishikawa *et al.*,[15] and Shigeyama *et al.*,[17] primarily due to issues in patient selection and flow and timing, suggesting potential concerns regarding study execution and participant handling. High risk of bias was identified in Ohshima *et al.,*[16] Sridharan *et al.,*[28] and de Sá Alves *et al.*[1] with concerns mainly related to patient selection and flow and timing, which may affect the internal validity of their outcomes. Overall, while seven studies presented low risk, a few demonstrated moderate risk, and only a single study[16] with high risk of bias due to small samples, unblinded assays, or >1-month delay in diagnosis.

NOS assessed the quality of the remaiing seven observational studies out of the nineteen. There was overall a low risk for study conducted by Ishikawa *et al.,*[4,13,14] Wang *et al.,*[18,19] and Song *et al.*[22] This is because, the study effectively selected well-defined cases, and appropriately selected controls from the same population who were free of oral diseases. Cases were chosen consecutively or representatively. The study demonstrated comparability as the participants were matched by age and gender, and adjustments were made for potential confounders like smoking and alcohol consumption. Additionally, the outcome was favourable due to blinded metabolomics analysis, a follow-up duration of at least six months for prognostic studies, and a low attrition rate.

Studies by Taware *et al.,*[26] Wang *et al.,*[20] and Yan *et al.*[6] had shown moderate risk while high risk was noted in the studies conducted by Mikkonen *et al.,*[24] Rai *et al.,*[27] and Supawat *et al.*[25] due to discrepancies in the selection, comparability and outcome domains.

To summarize, the studies by Ishikawa[4,13,14], Wang[18,19], and Song[22] demonstrated the lowest risk of bias, reflecting strong methodological quality. In contrast, the highest risk of bias was observed in studies by Rai[27], Ohshima[19], and Mikkonen[24], largely due to methodological limitations. Common issues contributing to elevated risk included a lack of blinding, particularly in older studies like Rai[27], small sample sizes as seen in studies conduted by Ohshima[16] and Mikkonen[24], and failure to adjust for key confounders, such as in the study by Supawat.[25]

Combined risk of bias using QUADAS-2 and NOS was assessed with diagnostic studies showing higher quality 58% low risk. The combined risk of bias summarises the proportion of studies by risk level, with key takeaways stating that among the diagnostic studies evaluated using the QUADAS-2 tool (n=12), 58% showed a low risk of bias, indicating higher quality of studies. In contrast, the observational studies assessed with the NOS tool (n=7) demonstrated significant limitations, with 86% showing a moderate or high risk of bias. Overall the critical gaps across the included articles were small sample sizes, unblinded assays, and poor confounder control in older studies.

Data synthesis of salivary metabolomics for OSCC detection

Consistently identified biomarkers

A review of the included studies revealed several consistently identified metabolic biomarkers such amino acids, lipids, energy metabolites, and volatile compounds which were found to be associated with OSCC. Lactate was the most frequently reported biomarker in these studies.

This is likely due to Warburg effect/aerobic glycolysis. Lactate dehydrogenase (LDH) plays a crucial role in anaerobic glycolysis by catalysing the pyruvate reduction that leads to formation of lactate, and is typically present within the cell cytoplasm. Its presence in extracellular fluids is usually associated with cellular death and tissue injury. Under aerobic conditions, intracellular utilization of glucose primarily occurs via glycolysis to produce pyruvate which is transported into the mitochondrial matrix. Here, oxidization takes place in the presence of pyruvate dehydrogenase leading to the formation of acetyl-CoA.[30,31] In cancer cases, the metabolism of the cell is modified wherein the neoplastic cell relies on the LDH to enhance the glycolytic activity, leading to elevated adenosine triphosphate (ATP) and lactate production even under aerobic conditions. This cellular adaptation supports rapid cellular proliferation and energy demands of the tumor cells.[31,32]

Choline and phenylalanine showed high specificity (82-88%) for OSCC vs controls. Choline normally plays a key role in cell membrane synthesis; however, in cancer, its metabolism becomes dysregulated due to the overexpression of enzymes and changes in signaling pathways that promote increased choline uptake and utilization. This disruption is driven by heightened membrane turnover and rapid cellular proliferation, leading to elevated choline levels.[33,34]

Phenylalanine, an essential amino acid, is frequently elevated in cancer as a result of elevated protein synthesis and altered amino acid metabolism within neoplastic cells. The enhanced amino acid transport and usage in such cells may account for the increased phenylalanine levels observed in oral carcinomatous tissues as compared to normal, as well as, it may be involved in the metabolism of glucose and fats, leading to energy formation.[35-39]

Diagnostic performance and methodological heterogeneity

The diagnostic performance of studies was evaluated using the pooled estimates that showed a 62% heterogeneity in sensitivity, 58% heterogeneity in specificity, 45% heterogeneity in AUC and 51% heterogeneity in diagnostic OR.

The methodological heterogeneity in saliva sample collection revealed that the majority of studies (73.7%) used unstimulated saliva, which is likely to more accurately represent baseline metabolic conditions. The consistency of metabolite profiles could be varied due to the collection methods which may lead to differences in flow rate of saliva, its potential dilution, and metabolic changes induced by stimulation.[40] Also, the different analytical platforms show variability in both the usage and diagnostic performance. The most frequently used method, LC-MS, was used in 8 studies where the highest yielded median AUC was 0.91 which indicated superior diagnostic accuracy with advantage of high sensitivity and broad metabolic coverage. Whereas 4 studies were conducted using GC-MS which showed lower median AUC of 0.87 and was best for analysing the volatile compounds. The NMR was used in 4 studies where the median AUC was 0.83, its advantage was that it was reproducible and had a minimal sample preparation.

Clinical implications

The clinical implications of the findings highlight the potential of certain salivary biomarkers for early detection of OSCC, with lactate (AUC: 0.92), choline (sensitivity: 86%), and phenylalanine (AUC: 0.93) emerging as the most promising candidates. These biomarkers showed enhanced diagnostic performance in high-risk populations, particularly among smokers (OR: 2.3, p=0.01). The biggest hindrance in developing a diagnostic protocol using these biomarkers is that their levels in saliva are generally reported to be low, making their detection challenging.

However, limitations remain, including a lack of prognostic data, with only one study[4] exploring recurrence wherein there was a decrease in betaine levels post-treatment. Additionally, there is an evident ethnic bias, with 63% of studies conducted in Asian popula-

tions and limited representation from African and Latin American cohorts, which may affect the generalizability of the findings.

Also, these tests require sophisticated laboratory infrastructure equipped with advanced analytical instruments such as mass spectrometers and chromatographs. Furthermore, the process demands highly trained personnel capable of accurately interpreting complex metabolic profiles. Such facilities and expertise are often concentrated in select urban centers, making them inaccessible, particularly in rural and underserved areas. Furthermore, the high cost associated with these tests poses a significant financial burden, making them unaffordable for a large portion of the population, especially those from economically disadvantaged backgrounds. To overcome these challenges, efforts should be directed toward decentralizing diagnostic infrastructure by establishing regional metabolomics laboratories. Additionally, government subsidies and public-private partnerships could help reduce the cost burden, making these advanced diagnostic tools more accessible and affordable to economically disadvantaged populations.[37-39]

Evidence quality assessment

The evidence quality assessment shows that most diagnostic studies had a low risk of bias (58%), while the majority of observational studies had moderate to high risk (86%). Recommendations include prioritizing findings from low-risk studies, such as Wang[18] and Ishikawa[13], to ensure reliability. Additionally, it is important to validate point-of-care techniques, like Song's[22] paper-spray MS, in larger, more diverse cohorts to confirm their effectiveness and generalizability.

Meta-analysis and GRADE summary of findings

Meta-analysis showed a pooled sensitivity of 84% and specificity of 82% for salivary metabolomics in OSCC detection. These findings indicate consistent performance across studies. The sensitivity was higher in Asian cohorts (87%) compared to Western cohorts (80%), suggesting either a potential underrepresentation of Western populations in current research or the presence of population-specific metabolic differences.

The GRADE assessment rated the overall evidence as moderate certainty, with high certainty for lactate as a diagnostic biomarker, which has consistently demonstrated strong discriminatory power across six low-risk studies. Based on these findings, it is recommended that salivary metabolomics using LC-MS platforms be considered a reliable tool for OSCC screening in clinical settings, given its non-invasive nature and strong diagnostic indicators.

While the findings are encouraging, external factors including dietary changes, microbial activities, and oral hygiene status. There are also chances of impairment of diagnostic accuracy due to external factors such as salivary collection methods, storage temperature, and total duration of processing.[41]

In conclusion, this review supports the clinical promise of salivary metabolomics, particularly using LC-MS, as a non-invasive, accessible diagnostic tool for OSCC. However, standardization of protocols, larger sample sizes, diverse population studies, and validation in clinical settings are essential next steps for its broader implementation in routine cancer screening.

Strength and limitations

Our systematic review is amongst the very few studies available on the topic, which analyses all the recent evidence related to role of salivary metabolomics to guide an evidence-based application of the same in oral cancer detection. The main limitation of our systematic review was the heterogeneity among the included studies, which affected the overall consistency of the results. Although the diagnostic accuracy of salivary metabolomics for OSCC detection showed moderate certainty, this emphasizes the need for further research to refine and strengthen these findings.

Conclusions

In conclusion, this systematic review and meta-analysis demonstrated that salivary metabolomics holds considerable promise as a non-invasive, accurate diagnostic approach for detecting OSCC. With a pooled sensitivity of 84% and specificity of 82%, particularly strong performance was observed for key biomarkers viz. lactate, choline, and phenylalanine, especially when analyzed using LC-MS platforms.

References

1. de Sá Alves M, de Sá Rodrigues N, Bandeira CM, Chagas JFS, Pascoal MBN, Nepomuceno GLJT, *et al.* Identi-

fication of possible salivary metabolic biomarkers and altered metabolic pathways in South American patients diagnosed with oral squamous cell carcinoma. Metabolites. 2021;11(10):650.
2. Lin X, Wu X, Gomaa A, Chen J, Wu L, Xie X, *et al.* Analysis of risk factors for multiple primary oral squamous cell carcinoma: a cohort study. Clin Oral Investig. 2020; 24(9):3147-55.
3. Imbesi Bellantoni M, Picciolo G, Pirrotta I, Irrera N, Vaccaro M, Vaccaro F, *et al.* Oral cavity squamous cell carcinoma: an update of the pharmacological treatment. Biomedicines. 2023;11(4):1112.
4. Ishikawa S, Sugimoto M, Konta T, Kitabatake K, Ueda S, Edamatsu K, *et al.* Salivary metabolomics for prognosis of oral squamous cell carcinoma. Front Oncol. 2022(5);11:789248.
5. Klupczyńska A, Dereziński P, Kokot ZJ. metabolomics in medical sciences--trends, challenges and perspectives. Acta Pol Pharm. 2015;72(4):629-41.
6. Yan SK, Wei BJ, Lin ZY, Yang Y, Zhou ZT, Zhang WD. A metabonomic approach to the diagnosis of oral squamous cell carcinoma, oral lichen planus and oral leukoplakia. Oral Oncol. 2008;44(5):477-83.
7. Chattopadhyay I, Panda M. Recent trends of saliva omics biomarkers for the diagnosis and treatment of oral cancer. J Oral Biosci. 2019;61(2):84-94.
8. Ghafari N, Sleno L. Challenges and recent advances in quantitative mass spectrometry-based metabolomics. Anal Sci Adv. 2024;5(5-6):e2400007.
9. Doğan H. Metabolomics: a review of liquid chromatography mass spectrometry-based methods and clinical applications. Turk J Biochem. 2024;49(1):1-14.
10. Zhou B, Xiao JF, Tuli L, Ressom HW. LC-MS-based metabolomics. Mol Biosyst. 2012;8(2):470-81.
11. Rockwood AL, Kushnir MM, Clarke NJ. Mass Spectrometry. I: Rifai N, Horvath AR and Wittwe CT, editors. Principles and Applications of Clinical Mass Spectrometry Small Molecules, Peptides, and Pathogens. 2nd ed. Elsevier; 2018. p. 33-65
12. Turner D. GC-MS Principle, Instrument and Analyses and GC-MS/MS [Internet]. Analysis & Separations from Technology Networks. 2022. Available from: https://www.technologynetworks.com/analysis/articles/gc-ms-principle-instrument-and-analyses-and-gc-msms-362513
13. Ishikawa S, Sugimoto M, Kitabatake K, Sugano A, Nakamura M, Kaneko M, *et al.* Identification of salivary metabolomic biomarkers for oral cancer screening. Sci Rep. 2016;6:31520.
14. Ishikawa S, Wong DTW, Sugimoto M, Gleber-Netto FO, Li F, Tu M, *et al.* Identification of salivary metabolites for oral squamous cell carcinoma and oral epithelial dysplasia screening from persistent suspicious oral mucosal lesions. Clin Oral Investig. 2019;23(9):3557-63.
15. Ishikawa S, Sugimoto M, Edamatsu K, Sugano A, Kitabatake K, Iino M. Discrimination of oral squamous cell carcinoma from oral lichen planus by salivary metabolomics. Oral Dis. 2020;26(1):35-42.
16. Ohshima M, Sugahara K, Kasahara K, Katakura A. Metabolomic analysis of the saliva of Japanese patients with oral squamous cell carcinoma. Oncol Rep. 2017;37(5):2727-34.
17. Shigeyama H, Wang T, Ichinose M, Ansai T, Lee SW. Identification of volatile metabolites in human saliva from patients with oral squamous cell carcinoma via zeolite-based thin-film microextraction coupled with GC-MS. J Chromatogr B Analyt Technol Biomed Life Sci. 2019;1104:49-58.
18. Wang Q, Gao P, Cheng F, Wang X, Duan Y. Measurement of salivary metabolite biomarkers for early monitoring of oral cancer with ultra performance liquid chromatography-mass spectrometry. Talanta. 2014;119:299-305.
19. Wang Q, Gao P, Wang X, Duan Y. Investigation and identification of potential biomarkers in human saliva for the early diagnosis of oral squamous cell carcinoma. Clin Chim Acta Int J Clin Chem. 2014;427:79-85.
20. Wang Q, Gao P, Wang X, Duan Y. The early diagnosis and monitoring of squamous cell carcinoma via saliva metabolomics. Sci Rep. 2014;4:6802. doi: 10.1038/srep06802.
21. Lohavanichbutr P, Zhang Y, Wang P, Gu H, Nagaishwana Gowda GA, Djukovic D, *et al.* Salivary metabolite profiling distinguishes patients with oral cavity squamous cell carcinoma from normal controls. PLoS One. 2018;13 (9):e0204249.
22. Song X, Yang X, Narayanan R, Shankar V, Ethiraj S, Wang X, *et al.* Oral squamous cell carcinoma diagnosed from saliva metabolic profiling. Proc Natl Acad Sci U S A. 2020;117(28):16167-73.
23. Sugimoto M, Wong DT, Hirayama A, Soga T, Tomita M. Capillary electrophoresis mass spectrometry-based saliva metabolomics identified oral, breast and pancreatic cancer-specific profiles. Metabolomics. 2010;6(1):78-95.
24. Mikkonen JJW, Singh SP, Akhi R, Salo T, Lappalainen R, González-Arriagada WA, *et al.* Potential role of nuclear magnetic resonance spectroscopy to identify salivary metabolite alterations in patients with head and neck cancer. Oncol Lett. 2018;16(5):6795-800.
25. Supawat B, Aye KT, Ritpanja J, Nueangwong W, Kothan S, Pan J, *et al.* Differences in spectroscopic properties of saliva taken from normal subjects and oral cancer patients: comparison studies. J Fluoresc. 2021;31(3):747-54.
26. Taware R, Taunk K, Pereira JAM, Shirolkar A, Soneji D, Câmara JS, *et al.* Volatilomic insight of head and neck cancer via the effects observed on saliva metabolites. Sci Rep. 2018;8(1):17725.
27. Rai B, Kharb S, Jain R, Anand SC. Salivary vitamins E and C in oral cancer. Redox Rep. 2007;12(3):163-4.
28. Sridharan G, Ramani P, Patankar S, Vijayaraghavan R. Evaluation of salivary metabolomics in oral leukoplakia

and oral squamous cell carcinoma. J Oral Pathol Med. 2019;48(4):299-306.

29. Viswanathan M, Ansari MT, Berkman ND, *et al.* Assessing the Risk of Bias of Individual Studies in Systematic Reviews of Health Care Interventions. 2012 Mar 8. In: Methods Guide for Effectiveness and Comparative Effectiveness Reviews [Internet]. Rockville (MD): Agency for Healthcare Research and Quality (US); 2008-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK91433/
30. Sarma A, Mishra SS, Das S, Sawane H, Gaikwad T. Evaluation of salivary lactate dehydrogenase level as a biomarker for early detection in oral cancer and potentially malignant disorders: a systematic review and meta-analysis. J Clin Diagn Res. 2023;17(7):ZC19-ZC24.
31. Bhuvaneswari M, Prasad H, Rajmohan M, Sri Chinthu KK, Prema P, Mahalakshmi L, *et al.* Estimation of salivary lactate dehydrogenase in oral squamous cell carcinoma, oral leukoplakia, and smokers. J Cancer Res Ther. 2022;18 (Suppl):S215-18.
32. Lokesh K, Kannabiran J, Rao MD. Salivary lactate dehydrogenase (LDH)- a novel technique in oral cancer detection and diagnosis. J Clin Diagn Res. 2016;10(2):ZC34-7.
33. Yao N, Li W, Xu G, Duan N, Yu G, Qu J. Choline metabolism and its implications in cancer. Front Oncol. 2023;13:1234887.
34. Glunde K, Bhujwalla ZM, Ronen SM. Choline metabolism in malignant transformation. Nat Rev Cancer. 2011;11(12):835-48.
35. Lieu EL, Nguyen T, Rhyne S, Kim J. Amino acids in cancer. Exp Mol Med. 2020;52(1):15-30.
36. Bel'skaya LV, Sarf EA, Loginova AI. Diagnostic value of salivary amino acid levels in cancer. Metabolites. 2023;13(8):950.
37. Hu CC, Wang SG, Gao Z, Qing MF, Pan S, Liu YY, *et al.* Emerging salivary biomarkers for early detection of oral squamous cell carcinoma. World J Clin Oncol. 2025;16(4):103803.
38. Sharma S, Crawley A, O'Kennedy R. Strategies for overcoming challenges for decentralised diagnostics in resource-limited and catastrophe settings. Expert Rev Mol Diagn. 2017;17(2):109-18.
39. TechSci Research, https://www.techsciresearch.com. India Cancer Diagnostics Market Size and Growth 2019-2029| TechSci Research [Internet]. Techsciresearch.com. 2019 [cited 2025 May 3]. Available from: https://www.techsciresearch.com/report/india-cancer-diagnostics-market/18914.html
40. Al Habobe H, Haverkort EB, Nazmi K, Van Splunter AP, Pieters RHH, Bikker FJ. The impact of saliva collection methods on measured salivary biomarker levels. Clin Chim Acta. 2024;552:117628.
41. Meleti M, Quartieri E, Antonelli R, Pezzi ME, Ghezzi B, Viani MV, *et al.* Metabolic profiles of whole, parotid and submandibular/sublingual saliva. Metabolites. 2020; 10(8):318.