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ADVANCEMENTS IN NON-INVASIVE SCREENING TECHNIQUES FOR ORAL POTENTIALLY MALIGNANT DISORDERS AND ORAL CANCER: A SYSTEMATIC REVIEW

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ABSTRACT:

Oral squamous cell carcinoma (OSCC) presents a significant health burden worldwide, ranking sixth among cancers and leading to substantial morbidity and mortality in the head and neck region. Early cancer treatment improves patient outcomes, emphasizing the importance of timely detection. However, achieving early diagnosis, particularly in remote or resource-limited areas, is challenging. While biopsy remains the gold standard, non-invasive screening techniques are gaining prominence due to their accessibility and effectiveness. These technologies offer the potential to extend screening capabilities to non-specialists and rural areas, enhancing analysis quality and objectivity while reducing the need for highly trained specialists. This shift towards non-invasive screening methods marks a promising step towards improving cancer detection and treatment outcomes globally. This systematic review focuses on a few advanced non invasive screening techniques such as confocal microscopy, DNA Image cytometry, Salivary Biomarkers and Artificial Intelligence.

KEY WORDS: Oral Squamous Cell Carcinoma, Oral Potentially Malignant Disorders, confocal microscopy, DNA Image cytometry, Salivary Biomarkers, Artificial Intelligence and Diagnostic Aids

INTRODUCTION

Clinical examination alone often proves insufficient for diagnosing oral premalignant disorders and oral cancer. Today, diagnostic aids serve as invaluable tools for early detection in such cases. However due to lack of knowledge about signs and symptoms, denial phase and reasons like diagnostic lag, ignorance and time between diagnosis and the start of therapy will lead to loss of time. Early detection, precise characterization, and timely intervention of oral precancerous and cancerous lesions are crucial factors for improving treatment outcomes and prognosis.[1] According to World Health Organization suspected lesions persisting beyond two weeks post-removal of local irritants necessitate biopsy, given histological examination's status as the gold standard in diagnosis. Yet, biopsies present numerous drawbacks, dissuading patients due to fears of pain, stress, and potential tissue damage, among others. Notably, precancerous lesions pose a heightened risk of progressing to oral cancer compared to healthy oral mucosa.[2] While the five-year survival rate has seen a modest increase from 53% to 60% over the last three decades, early diagnosis and treatment offer the potential for further enhancements.[3] Advanced diagnostic aids now offer promising avenues for detecting oral cancer promptly, hinting at brighter prospects for improving survival rates.

This review explores the current research on advanced diagnostics in oral premalignant and malignant diseases utilizing databases such as "PubMed," Scopus google scholar with strict inclusion criteria, focusing on original studies and reviews reported in English language literature while conference papers and posters were excluded(fig 1)

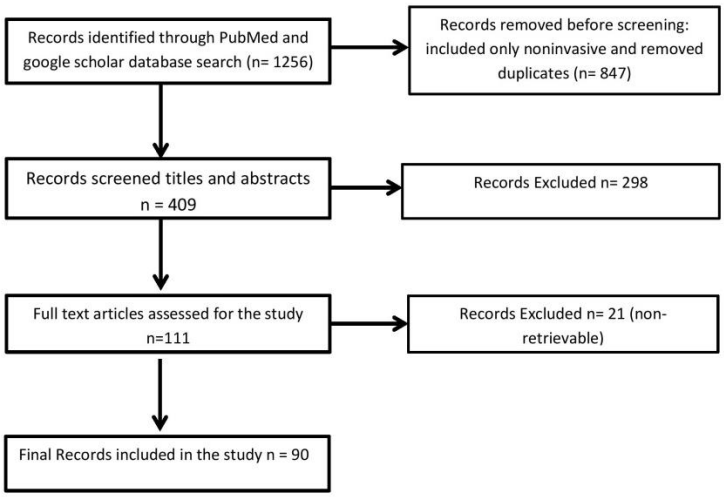

Fig:1 IDENTIFICATION OF STUDIES VIA DATABASE

REVIEW

Potentially malignant lesions like leukoplakia, erythroplakia, oral lichen planus, and actinic cheilitis are crucial due to their elevated risk of progressing into oral cancer. These lesions can undergo dysplastic changes, indicating the presence of abnormal cells and increasing the likelihood of oral cancer development if left unaddressed.[4] Therefore, early detection and proactive treatment significantly improves the chances of survival in oral cancer cases. Regular visual screenings are essential for detecting abnormalities in the oral cavity. Additionally, advancements in diagnostic adjuncts have enhanced early diagnosis. Chairside investigations such as vital tissue staining, Vizilite, chemiluminescence, VELscope, and brush cytology and few advanced diagnostic aids provide healthcare professionals with valuable tools for detecting potentially malignant lesions at an early stage.[5] These adjuncts allow for more accurate and precise diagnosis, enabling prompt intervention and treatment, which can ultimately improve patient outcomes and increase survival rates.

CONFOCAL MICROSCOPY

Confocal microscopy has emerged as a promising tool for high-resolution, real-time imaging of oral mucosal tissues. Unlike conventional imaging methods, confocal microscopy offers subcellular resolution and the ability to visualize tissue structures in vivo, making it invaluable for studying various pathological conditions affecting the oral cavity.[6] This non-invasive approach preserves tissue integrity and allows for non-destructive, real-time imaging of living tissues. confocal microscopy shows promise for the early detection and characterization of various lesions, including oral squamous cell carcinoma (OSCC) and oral epithelial dysplasia (OED).[7] By providing high-resolution images of cellular structures and tissue architecture, confocal microscopy enables clinicians to identify subtle changes indicative of malignancy or dysplasia, thereby facilitating timely diagnosis and treatment planning.[8,9] In Table 1 the in vivo studies, qualitative analyses predominated, with characteristic signs of OSCC identified. Higher diagnostic accuracy was observed among experienced assessors. One study employed quantitative analysis software, showing promising results in distinguishing between normal and malignant tissue. Ex vivo studies varied in their approaches, with some finding reasonable correlation between confocal imaging and histopathology, while others observed discrepancies. Assessment accuracy improved with increased confocal microscopy expertise. However, the lack of standardized assessment criteria poses challenges in protocol development for OSCC diagnosis.

DNA IMAGE CYTOMETRY

DNA-ICM, a sensitive method for DNA content analysis, detects near-diploid aneuploid cells. Brushings from oral mucosa offer non-invasive cell collection, though limited to superficial layers, hindering detection of mild dysplasia.[27] Cells typically possess two sets of all 23 chromosomes, termed diploid (2c), while those with a single set are haploid (1c). Euploid cells have complete multiples of the haploid set, whereas aneuploid cells lack a complete multiple of the basic chromosome set, potentially driving carcinogenesis. DNA-ICM detects DNA aneuploidy by staining cells with a DNA stoichiometric stain and measuring DNA content via integrated optical density (IOD).[28,29] Guidelines for DNA-ICM should adhere to the European Society for Analytical Cellular Pathology. Aneuploidy, a cytometric equivalent of chromosomal imbalance, may drive carcinogenesis, although the relationship between chromosomal instability and genetic mutation remains debated. Combining DNA-ICM with conventional cytology improves sensitivity and specificity, albeit with scalability and cost challenges. Patient acceptance of non-invasive brushings suggests DNA-ICM's potential as a cost-effective screening tool, especially in community settings.[30,31] Table 2 presents a diverse range of studies investigating the efficacy of DNA image cytometry (DNA-ICM) in diagnosing various oral lesions and potentially malignant disorders. These studies, spanning different methodologies and cases, collectively emphasize DNA-ICM's potential as a sensitive, specific, and non-invasive tool for

detecting early neoplastic changes in oral tissues. By comparing DNA-ICM with conventional methods and assessing parameters such as DNA content and nuclear morphology, the findings underscore its utility in improving diagnostic accuracy, aiding risk assessment, and guiding clinical decision-making.

SALIVA BIOMARKERS

Different efforts have been made to discover biomarkers to aid in the diagnosis of OPMDs and OSCC in order to predict the malignant transformation of precursor lesions, treatment response, and development of regional and distant metastases, among others [46]. Biomarkers have been obtained from tissues, urine, serum, blood, and saliva, among others. Saliva is probably the most attractive secretion for the finding of biomarkers for OSCC and OPMDs, as its collection is easy, non-invasive, fast, and cost-effective. Thus, different studies have explored the possibility of using salivary factors as biomarkers for the development and progression of OSCC and OPMDs.[47] Enzymes like salivary metalloproteinases (MMPs), particularly MMP-9, exhibit significant alterations, with MMP-9 distinguishing OSCC from OPMDs with high sensitivity and specificity. Other enzymes including lactate dehydrogenase (LDH), AKR1b10, and cathepsin V, alongside glycoproteins like CEA and CD44 variants, show potential for detecting OSCC. Cytokines such as IL-8 and TNF-α also demonstrate diagnostic significance. Moreover, microRNAs, metabolites, and other markers like ANG, ANG2, and KPNA2 exhibit alterations correlating with OSCC progression.[48,49] These biomarkers could aid in predicting OSCC outcome and malignant transformation of OPMDs, offering valuable insights for clinical management shown in Table 3.

ARTIFICIAL INTELLIGENCE

Artificial Intelligence (AI) is revolutionizing diagnostics by leveraging patient medical records and radiological images, significantly enhancing accuracy and minimizing errors in oncological assessments. Supervised machine learning stands at the forefront, empowering early detection of oral potentially malignant disorders (OPMD) and oral cancer.[94] The efficacy of AI-driven diagnostics lies in its ability to process and interpret complex medical information swiftly, augmenting the capabilities of healthcare professionals. Through supervised machine learning, AI algorithms learn from labeled data, enabling them to recognize patterns indicative of OPMD and oral cancer with high precision. This not only streamlines the diagnostic process but also enables early intervention. AI's role extends beyond mere pattern recognition. Deep learning, a sub-discipline of machine learning, enables AI systems to process large datasets and make complex decisions, mimicking human cognitive functions.[95] Artificial intelligence is revolutionizing cancer diagnosis, especially in oral cancer, with advanced image analysis techniques like deep convolutional neural networks (CNNs).[96,97] Studies demonstrate AI's superior sensitivity, accuracy,

and specificity compared to traditional screening methods, showcasing its potential to enhance precision and usability in cancer screening programs, ultimately improving diagnostic outcomesas shown in Table 4.

CONCLUSION

The emergence of non-invasive screening methods for oral potentially malignant disorders (OPMD) and oral cancer marks a significant advancement in healthcare. Technologies such as Confocal Microscopy,DNA Image cytometry Salivary Biomarkers and artificial intelligence (AI) analysis techniques offer enhanced sensitivity, accuracy, and specificity, surpassing traditional methods. Dentists' expertise in early lesion identification remains pivotal. With ongoing advancements and integration of technologies like AI, these non-invasive techniques hold promise for enhancing early detection, improving patient outcomes, and addressing global health challenges associated with oral cancers. However, further research, standardization, and larger-scale studies are needed for validation and refinement.

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TABLE: 1 Summary of the studies using confocal microscopy in diagnosis of oral premalignant and oral squamous cell carcinoma

STUDY	TYPE OF STUDY	CASES ASSESED	COMPARISION	PARAMETERS ASSESSED	CONCLUSION
Clark et al. (2002)[10]	Ex vivo	OSCC and dysplasia	Identification of confocal features in cervical biopsies and oral cavity	Cellular sizes, cell outlines, nuclei sizes, and nuclei shapes	Confocal microscopy's high-resolution imaging of sub-cellular morphology facilitates accurate diagnosis of normal and precancerous tissues, indicating its potential for in vivo dysplasia assessment
Clark et al. (2003) [11]	Ex vivo	OSCC	Histopathological correlation of normal and neoplastic oral mucosa with reflectance confocal microscopy	Cellular sizes, cell density, nuclear intensity, and signs of inflammation	Confocal microscopy holds significant potential in clinical evaluation of oral lesions, real-time tumor margin identification, and therapeutic treatment response monitoring
Carlson et al. (2007) [12]	Ex vivo	OSCC and dysplasia	Dual confocal imaging, Image analysis software and histopathologic correlation in ex vivo human tissues and in vivo animal models	Epithelium architecture, cellular and nuclear intensity (FLI), and nuclear cytoplasmic ratio (N/C ratio)	DCM stands as a crucial tool in our ongoing efforts to enhance non-invasive, in vivo imaging techniques, exploring diverse contrast agent conjugations and delivery methods to augment contrast differentiation between neoplastic and normal epithelial tissues.
Maitland et al. (2008) [13]	In vivo	OSCC	Histopathological correlation of normal, pre-neoplastic, and neoplastic oral tissue with confocal reflectance microscope	Dispersed nuclei, dense nuclei, and disordered tissue structure	In vivo fiber optic confocal reflectance microscopy presents a promising avenue for non-invasive detection of precancerous and cancerous lesions within the oral cavity, offering both sensitivity and specificity in clinical diagnostics.
Anuthama et al. (2010) [14]	Ex vivo	Betel Chewer's mucosa, Leukoplakia, OSMF, and OSCC	Image analysis Leica software and histopathologic correlation of suspected lesions of Oral precancer and Oral cancer with reflectance confocal microscopy	Keratin deposition, cell shape, nuclei, intensity of cell density, and nuclear density along with the mean intensity of different components of the epithelium	Reflectance confocal microscopy offers non-invasive imaging for precise evaluation of oral PMD and oral cancer and real-time detection of tumor margins

Ulrich et al. (2011) [15]	In vivo	AC	Defined scoring criteria & histopathologic correlation of actinic chelitis with reflectance confocal microscopy	Disruption of stratum corneum, hyperkeratotic scale, parakeratosis, cellular pattern, solar elastosis, blood vessel dilation, and inflammation	Reflectance confocal microscopy shows promise for non-invasive diagnosis and monitoring of actinic cheilitis, yet notable inflammation poses a potential challenge in accurate diagnosis.
Alessi et al. (2013) [16]	In vivo	OLP	RCM findings in desquamative gingivitis with conventional histopathology	Hyperkeratosis, Spongiosis, Honeycomb Keratinocyte Epithelial Structure, Mildly Bright, Round Structures and Mildly Bright, Stellate Structures	Reflectance confocal microscopy serves as a valuable tool in distinguishing between the three primary causes of desquamative gingivitis.
Nathan et al. (2014) [17]	In vivo	Neoplastic lesions from precancerous and cancerous lesions of head and neck.	Probe-based confocal laser endomicroscopy images were compared to histologic evaluation	Cellular shape, epithelial architecture, and vasculature	Probe-based confocal laser endomicroscopy holds potential as a promising technique for distinguishing between non-dysplastic, pre-cancerous, and cancerous lesions in the head and neck region.
Moore et al. (2016) [18]	In vivo	OED & OSCC	8 assessors (7 surgeons, 1 pathologist) agreement of pCLE offline images of noncancerous, precancerous, and cancerous lesions & histopathological correlation	Epithelial architecture and cellular shape	Probe-based confocal laser endomicroscopy has demonstrated high accuracy and reliability in distinguishing between normal mucosa, dysplasia, and invasive squamous cell carcinoma (SCCA), offering the potential to reduce sampling errors in head and neck lesions.
Oetter et al. (2016) [19]	In vivo	OSCC	6 assessors (3 expert, 3 non-expert) using developed scoring system (DOC-Score) based on specific patterns of tissue changes & histopathological correlation	Homogeneity of tissue architecture, intercellular gaps, cell morphology, fluorescence leakage, and vessels	CLE is a suitable and valid method for experts to diagnose oral cancer. Utilizing the DOC-Score system, an accurate chair-side diagnosis of oral cancer is achievable, showing results comparable to the gold standard of histopathology, even in daily clinical practice for non-experienced raters.

Contaldo et al. (2019) [20]	In vivo	OLP	Scan from the surface to the submucosa and compared with the literature.	Type of keratosis, size of keratinocytes, nuclear size and shape, acanthosis and spongiosis, necrotic keratinocytes, connective tissue papilla, and inflammatory cells	Incorporating RCM into routine clinical oral pathology tests is advised to prevent recurrence of oral lichen planus (OLP) and alterations in its response to therapy, thereby reducing the necessity for biopsies of lesions suspected of tumoral changes.
Shinohara et al. (2020) [21]	Ex vivo	OSCC	Measured autofluorescence intensity and histopathologic correlation	Nuclear size, shapes, and patterns of nuclei and cytoplasm between normal and SCC tissue	Real-time, in vivo imaging utilizing the newly developed CLE and conditions can potentially distinguish cancerous tissue from normal mucosa without the need for invasive biopsy procedures.
Contaldo et al. (2020) [22]	In vivo	OSCC and dysplasia	Cytoarchitectural findings in oral mucosae affected by OSCC and its precursors with Histopathological correlation	Thickness of epithelium, epithelial architecture, keratosis, size and shape of keratinocytes, and keratin pearls	RCM has the potential to detect dysplastic or neoplastic features present in oral lesions, thereby aiding in their diagnostic evaluation.
Peng et al. (2020) [23]	In vivo	OLP	The lesion sites and healthy adjacent sites were examined using *in vivo* RCM, with the lesion being histopathologically confirmed after RCM examination	Parakeratosis, acanthosis, liquefaction degeneration, inflammatory cell infiltration, and dilated blood vessels	The findings suggest that RCM holds promise as a non-invasive, in vivo technique for detecting oral lichen planus (OLP)
Shavlokhova et al. (2021b) [24]	Ex vivo	OSCC	Ex vivo fluorescence confocal microscopy (FCM) for detecting oral leukoplakia and to compare confocal images with gold-standard histopathology.	Cellular pleomorphism, nuclear hyperchromatism, prominent nucleoli, increase in nuclear cytoplasmic ratio, loss of cellular adhesion, and keratinisation	The results underscore the potential of ex vivo confocal microscopy in fresh tissue for rapid, real-time assessment of oral pathologies.

Sievert et al. (2021) [25]	In vivo	OSCC	4 assessors (3 surgeons, 1 pathologist) assessment of safe margins with confocal laser endomicroscopy (CLE) during oropharyngeal squamous cell carcinoma (OPSCC) surgery with histopathological correlation	Cellular size, shape, cytoplasmic membrane, and blood vessel architecture	CLE can be integrated into intraoperative settings, offering real-time, in-vivo microscopic imaging of the oropharynx for cancer evaluation and margin demarcation, potentially enabling less radical surgical approaches.
Zanoni DK et.al. (2024) [26]	In vivo	OSCC	Diagnostic accuracy of RCM against histopathology(2 assessors (1 a dermatologist experienced in reading RCM images, 1 pathologist)	pleomorphism, enlarged epithelial cell/multinucleated, hyperkeratinization, enlarged/elongated blood vessels, keratin pearls/tumor nests, and dyskeratotic cells	RCM imaging shows promise for non-invasive, in vivo diagnosis of OSCC and real-time intraoperative evaluation of mucosal surgical margins. However, its limitation lies in reduced capability to assess structures located deeper within the tissue.

TABLE 2 Summary of the studies using DNA image cytometry using brushings in diagnosis of oral premalignant and oral squamous cell carcinoma

Study	Type of study	Comparision	cases Assessed	parameters Assessed	Conclusion
Yang et al. (2017)[31]	Cross-sectional	Oral cytobrush biopsy with MotiSavant DNA image cytometer before scalpel biopsy of oral leukoplakia cases	oral leukoplakia (OL)	Nuclear morphometric analysis (DNA content amount, DNA index, nuclear area, nuclear radius, nuclear intensity, sphericity, entropy, and fractal dimension).	Image cytometry analyzes DNA content and nuclear morphology, aiding in early detection of oral leukoplakia progression by identifying abnormal cellular changes, thereby improving monitoring and treatment strategies for potential malignancy.

Xiao et al. (2018) [32]	Cross-sectional	correlation of DNA content using brush biopsy with image cytometry, and ewith the clinicopathologic features and OL-staging system.	oral leukoplakia (OL)	DNA index (DI) (e ratio of analyzed nuclear DNA content to the reference DNA content). DNA content abnormality if the test results met at least one of the following three conditions: (i) two separate G0–G1 peaks (diploid G0–G1 peak with DI of 0.98–1.02, aneuploid G0–G1 peak with DI of 1.05–1.9 or 2.1–3.8), (ii) more than 10% of the nuclei with DI of 1.25–2.3 and DI P 2.3, (iii) more than 3 nuclei (1% of a minimumof 300 nuclei) with DI ≥2.3.	Correlation between DNA content status and OL staging implies early DNA abnormalities in oral carcinogenesis, underlining image cytometry's potential for early detection and intervention in precancerous lesions.
Ma et al. (2020) [33]	Cross-sectional	diagnostic accuracy of brush biopsy with DNA-image cytometry (a noninvasive method) for potentially malignant oral disorders compared with tissue biopsy	potentially malignant oral disorders.	DNA diploid or DNA aneuploid	DNA-image cytometry serves as a valuable supplementary method for monitoring neoplastic epithelial cells in potentially malignant oral disorders, particularly facilitating repeated asynchronous examinations during the clinical therapeutic process for suspicious oral lesions.
Ng et al. (2017) [34]	Retrospective	Quantitative cytology (QC) in reference to clinicohistopathologic features in detecting abnormal DNA content and nuclear morphology in high-risk OPMLs	potentially malignant disorders/oral cancer	The DNA index DI is used to classify epithelial cell nuclei into groups wherediploid group has a DI of 0.85-1.15. The oral specimen was classified as DNA-ICM positive (DNA content abnormality) if there were> 4 nuclei (1% of a minimum of 400 nuclei) in the aneuploid group (DI> 2.3), 5% of total number of nuclei in the hyperdiploid group (1.15< DI < 1.7), or 10% of total number of nuclei in the tetraploid group (1.7 < DI < 2.3)	Quantitative cytology (QC) could function as an additional tool for identifying high-risk potentially malignant disorders (PMD) or squamous cell carcinoma (SCC), aiding in prompt clinical intervention when immediate care is necessary.

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Pektas et al. (2018)[35]	Cross-sectional	Cytomorphometric analysis via oral brush biopsy immediately before biopsy for oral malignancy detection	OSCC, potentially malignant disorders	DNA diploid or DNA aneuploid	Cytomorphometric analysis via oral brush biopsy is a valuable adjunct to biopsy for identifying premalignant and early-stage cancerous oral lesions. It offers a rapid and minimally invasive procedure with high specificity and sensitivity rates, requiring no topical or local anesthetic.
MacAulay et al. (2019)[36]	Retrospective	Quantitative cytometric analysis of oral brushing samples could facilitate the assessment of the risk compared to white light and FV	OSCC, carcinoma in situ or severe dysplasia; samples from sites with inflammation, infection, or trauma, and samples from normal sites	QTP Imaging System for Quantitative Cytology, (European Society of Analytical Cellular Pathology for ploidy analysis)	The data indicate that quantitative cytology has the potential to significantly decrease the number of visually suspicious lesions necessitating further biopsy assessment, potentially reducing the need by over 85%.
Kammerer et al. (2013)[37]	Cross-sectional	brush biopsies, analysed according to morphological criteria and by DNA-ICM (DNA image cytometry) vs. histological findings	potentially malignant disorders	AutoCyte QUIC-DNA software (DNA content of the cell, allowing ploidy analysis)	DNA-ICM enhances sensitivity in oral brush biopsy interpretation, yet methodological errors may limit its reliability, advocating caution, especially in clinically suspicious oral lesions.
Maraki et al. (2004)[38]	Prospective	diagnostic accuracy of exfoliative cytology (EC) and DNA-image cytometry applied to suspicious oral lesions compared with synchronous histology.	suspicious oral lesions	DNA-contents were measured using a TV image analysis system.	Cytology with DNA-cytometry is a highly sensitive and specific non-invasive method for early diagnosis of oral epithelial neoplasia, demonstrating excellent patient compliance.
Remmerbach et al (2001)[39]	Cross-sectional	Cytologic and DNA-cytometric compared with histological and/or clinical follow-ups	suspicious oral lesions	DNA-contents were measured after Feulgen restaining using a TV image analysis system.	DNA-image cytometry serves as a highly sensitive, specific, and objective adjunctive tool for the early

					detection of neoplastic epithelial cells in oral smears.
Kaur et al (2016)[40]	Prospective	DNA image cytometry with DNA image cytometry	suspicious oral lesions	Image Pro-Plus Version 6.1 software	The addition of DNA-ICM as an adjunct to brush cytology in diagnosing oral cancer proves invaluable. It mitigates false negative results in cytology and enhances objectivity, particularly in cases with cytological uncertainty.
Remmerbach et al (2009) [41]	Prospective	brush biopsies of suspicious oral lesions with histologic diagnoses	suspicious oral lesions	semiautomated multimodal cell analysis (MMCA)(morphology, DNA content, argyrophilic nucleolar organizer region counts)	MMCA (Multimodal Cytology Analysis) holds promise as a sensitive, highly specific, objective, and reproducible adjuvant diagnostic tool for identifying neoplastic changes in oral smears containing only a limited number of abnormal cells.
Velleuer E et.al. (2020) [42]	Prospective	oral brush biopsy-based cytology correlated to a long-term clinicopathological follow-up	Fanconi anemia	cytology, analysis of DNA ploidy	In Fanconi anemia patients, oral cavity inspection with brush biopsy-based cytology detects a significant portion of SCC and precursor lesions at noninvasive stages, while negative cytology or absence of DNA aneuploidy effectively excludes high-grade dysplasia or SCC, reducing the need for invasive biopsies.
Parfenova E et.al. (2021)[43]	Prospective	differentiating high grade from benign reactive oral lesions by oral brushing samples by ClearCyte®	oral cancer (n = 92), severe dysplasia (n = 20), reactive lesions (n = 52), and normal samples (n = 50)	Health Canada-approved DNA-ICM system, ClearCyte®	The iClearCyte test shows promise as a reliable non-invasive automated oral cancer screening tool, facilitating early detection and reducing unnecessary invasive biopsies.

Bechstedt N et.al (2022)[44]	Prospective	Brush biopsies first cytologically examined and categorized by a pathologist, second evaluated using DNA image cytometry, and finally compared to either histological biopsy result or clinical outcome.	Potentially Oral Malignant Diseases	DNA ploidy analysis	DNA ploidy analysis from conventional oral brush biopsies is a highly sensitive, non-invasive, and patient-friendly method, serving as an additional diagnostic tool for detecting malignant changes in the oral cavity.
Davidson SP et.al. (2023)[45]	Prospective	brush biopsy findings	tobacco-related lesions such as leukoplakia, tobacco pouch keratosis, and oral cancer	dysplastic changes	Brush biopsy demonstrates promising potential in detecting premalignant lesions and serves as a powerful diagnostic aid for the early detection of malignancy.

TABLE 3 Summary of the studies using salivary biomarkers in diagnosis of oral premalignant and oral squamous cell carcinoma

First author, year	Cases Assessed	Biomarkers	Method of analysis	conclusions
ArellanoGarcia,et.al. 2008[50]	OSCC, periodontitis, controls	IL8, IL1β	ELISA, Immune bead based assay	Both biomarkers were statistically higher in OC group than controls.
Babiuch, et.al. 2020[51]	OSCC, OED, OLP, controls	IL1α, IL6, IL8	ELISA	Higher salivary cytokine levels in OSCC patients compared to controls, with IL-8 showing particular importance in identifying malignant transformation from OPMDs

Bagan, et.al. 2016[52]	OSCC, PVL, controls	IL6	ELISA	Patients with oral cancer (OC) had the highest salivary biomarker levels, followed by potentially malignant lesions (PVL) and controls, proposing salivary IL6 as a potential biomarker for assessing disease progression in PVL.
Brailo, et.al. 2006[53]	Leukoplakia, controls	IL6	ELISA	the results of this study demonstrate that the patients with oral leukoplakia have an increase in salivary levels of IL6 which might indicate an altered immune response
Brailo, et.al. 2012[54]	OSCC, Leukoplakia, controls	IL1β, IL6	ELISA	Patients with oral cancer (OC) exhibit significantly elevated salivary concentrations of IL1β and IL6 compared to both patients with leukoplakia and healthy controls.
Brinkmanna,et.al. 2012[55]	OSCC, controls	IL1β, IL8,	ELISA, RT-qPCR	Combination of biomarkers from the proteome and transcriptome yielded the highest predictive power for OC
Csosz, et.al. 2017[56]	OSCC, controls	IL1α,IL1β, IL6, IL8	Millipex magnetic bead based assay	Salivary IL6 as a biomarker for oral squamous cell carcinoma (OSCC) in the Hungarian population, with the OC group showing notably higher biomarker levels.
Gleber-Netto, et.al. 2016[57]	OSCC, OPMD, controls	IL1β, IL8	ELISA	IL1β and IL8 levels both biomarkers are elevated in oral squamous cell carcinoma (OSCC) compared to potentially malignant disorders (OPMD) and controls. It suggests that combining salivary IL8 protein levels with other biomarkers and risk factors holds promise for the early detection of OSCC and OPMD.

JuretiŰ, et.al. 2013[58]	OSCC, OPMD, controls	IL6	ELISA	P values comparing study groups IL6 < 0.001, patients have higher salivary concentrations of biomarker compared to controls, this study suggest IL6 as a potential biomarker for OSCC
Katakura, et.al 2007[59]	OSCC, controls	IL1β,IL6,IL8	ELISA	P values comparing study groups IL 1β< 0.05, IL 6 < 0.05, IL 8 > 0.05, IL6 was significantly elevated in patients compared to controls, this study report IL6 as a potential biomarker in saliva for OSCC
Khyani, et.al. 2017[60]	OSCC, OPMD, controls	IL6, IL8	ELISA	P values comparing study groups IL8 < 0.05, IL6 no significant difference, IL6 and IL8 are probable biomarker for early detection of OSCC and OPMD in Pakistan population
Korostoff, et.al. 2011[61]	Tongue cancer, controls	IL1α,IL6, IL8	ELISA	P values comparing endophytic tongue cancer/controls IL1α < 0.0001, IL 6 < 0.0001,IL8 < 0.0001, all cytokines were markedly elevated in saliva of endophytic TC patients, this study propose the potential use of these biomarkers for screening, and prognosis of survival in tongue OSCC
Lee, et.al. 2018[62]	OSCC, controls	IL1α,IL1β,IL6, IL8, IL10	Millipex magnetic bead based assay	P values comparing study groups IL1α = 0.625,IL1β = 0.002, IL6 < 0.001, IL8 = 0.001,IL10 = 0.355.AUC for OSCC IL6 = 0.8, IL8 = 0.7, IL1β = 0.7, this study indicate that salivary biomarkers may serve useful as adjuncts for the early detection of OSCC
Lisa Cheng,et.al. 2014[63]	OSCC, OLP, Periodontitis, controls	IL6, IL8	ELISA	P values comparing study groups IL6 < 0.001, IL8 = 0.001, salivary biomarkers were higher in patients with OSCC, compared to OLP, CP and controls, this study propose that IL6 as a useful biomarker in the detection of OC, not influenced by OLP or CP

Panneer,et.al. 2015[64]	OSCC, Leukoplakia, controls	IL6	ELISA	P values for OSCC/controls, and OSCC/Leukoplakia <0.0001, biomarker levels were highest in OC, followed by leukoplakia and controls, this biomarker is proposed for further validation to assess its clinical utility
Punyani, et.al. 2013[65]	OSCC, OPMD, controls	IL8	ELISA	P values comparing OC/OPMD <0.0001, OC/controls <0.0001, OPMD/ controls = 0.7, IL8 is a potential biomarker for OSCC
Rajkumar, et.al. 2014[66]	OSCC, OPMD, controls	IL8	ELISA	P values comparing study groups <0.05, AUC 0.9, IL8 measurement in saliva is a better medium for OC prediction than serum
Rhodus, et.al. 2005[67]	OSCC, OLP, controls	IL1α,IL6, IL8	ELISA	IL1α, IL6, IL8, these biomarkers may be potential targets for disease monitoring in OLP
Sahebjamee, et.al. 2008[68]	OSCC, controls	IL1α, IL6, IL8	ELISA	IL1α, IL6, IL8, was elevated in OC compared to controls with statistical significance
Sharma, et.al.2011[69]	Leukoplakia, periodontitis, controls	IL6	ELISA	Leukoplakia patients with coexisting periodontitis had higher IL6 levels when compared with patients with periodontitis alone, this study report an increase in IL6 levels with severity of dysplasia grading, IL6 may be a useful biomarker for monitoring of leukoplakia
Singh,et.al. 2020[70]	OSCC, OPMD, controls	IL1β, IL8	ELISA	IL1β, IL8 for total OSCC were for both markers, for stage three and four OSCC, IL1β, IL8, these biomarker were significant in indian population, this study propose the potential use of studied biomarkers for screening and early detection in OSCC

de Jong et al., 2010[71]	Oral Squamous Cell Carcinoma (OSCC)	Increased abundance of myosin and actin	Mass spectrometry, Western blotting	Elevated myosin and actin levels in saliva correspond to increased abundance in cells shed from the mouth, indicating their potential as markers for detecting the progression from pre-malignant to malignant conditions in oral squamous cell carcinoma.
Hu et al., 2008[72]	Oral Squamous Cell Carcinoma (OSCC)	52 proteins present in diseased samples	Shotgun proteomics	Patient-based saliva proteomics holds promise in identifying biomarkers for oral squamous cell carcinoma (OSCC), potentially paving the way for a straightforward noninvasive diagnostic tool for oral cancer detection
Wang et al., 2014[73]	Oral Squamous Cell Carcinoma (OSCC)	Elevated levels of choline, betaine, pipecolinic acid, L-carnitine	Ultraperformance liquid chromatography-mass spectrometry (UPLC-MS)	This study confirms salivary metabolite biomarkers for early OSCC diagnosis, suggesting its use as a preclinical screening technique for the condition.
Camisasca et al., 2016[74]	Oral Leukoplakia	Apolipoprotein A1, alpha-amylase, cystatins, keratin 10, lysozyme precursor, CK10	Two-dimensional gel electrophoresis, Mass spectrometry, Immunohistochemistry	MS-based proteomics in saliva elucidates potential biomarkers for oral cancer precursors like leukoplakia, offering insights into disease development and surveillance strategies of oral cancer.
Flores et al., 2016[75]	Proliferative Verrucous Leukoplakia	Angiotensinogen (AGT), dipeptidyl peptidase 1 (DPP1)	Mass spectrometry	Both AGT and DPP1 may be involved in developmental mechanisms of PVL.
Duffy et al. 2008[76]	head and neck squamous cell carcinoma (HNSCC)	IL-6	ELISA	Proinflammatory and proangiogenic cytokines found as indicators of oral precancerous lesions progressing to oral cancer.
Zhong et al. 2005[77]	oral squamous cell carcinoma	telomerase activity	PCR- ELISA	Telomerase in saliva could be used as an assistant marker for oral squamous cell carcinoma.

Franzmann et al. 2007[78]	head and neck cancer	CD44, CD59, Profilin, MRP14	Immunoblot	CD44 and CD59 are highly sensitive markers for cancer and benign diseases differentiation..
Almadori et al. 2007[79]	head and neck cancer	Glutathione	HPLC	Epidemiological marker for chemoprevention, identifying the risk of OSCC development.
Jou et al. 2010[80]	oral cancer	transferrin, fibrin	gel electrophoresis (2DE) and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) analyses.	transferrin levels correlate with cancer size and stage, fibrin is involved in carcinogenic processes.
Kumar et al. 2015[81]	oral cancer	IgG, S100 calciumbinding protein, cofilin-1,	LC/MS	IgG inhibits apoptosis, S100A2 is a prognostic biomarker, cofilin is involved in cancer progression,
Righini et al. 2007[82]	head and neck cancer	α-1-antitrypsin (AAT)	2DE	Useful for prediction and determining the aggression of OSCC.
Reddy et al. 2012[83]	oral squamous cell carcinoma	SLPI, cystatin A, keratin 36, thioredoxin, HAP, salivary zinc finger, protein 510 peptide, α-amylase, albumin	MS-based proteomics	SLPI, cystatin A, keratin 36 are involved in preventive treatment of OSCC, thioredoxin mRNA levels are elevated in cancers, salivary zinc finger, protein 510 peptide, α-amylase, albumin aid in early OSCC detection.
Li et al. 2004[84]	oral cancer	Interleukin (IL)-1β, IL-8, Dual specificity phosphatase 1 (DUSP1), H3 histone family 3A (H3F3A)	ELISA, Quantitative PCR (qPCR) and microarrays followed by qPCR,	Implicated in angiogenesis, cell adhesion, chemotaxis, immune response, replication, signal transduction, proliferation, inflammation, and apoptosis.Linked to oxidative stress, protein modification, and signal transduction.Associated with DNA binding activity.
Elashoff et al.2012[85]	oral cancer	Interleukin (IL)-1β, IL-8	ELISA	Implicated in angiogenesis, cell adhesion, chemotaxis, immune response, replication, signal transduction, proliferation, inflammation, and apoptosis.

Tang et al.2013[86]	oral squamous cell carcinoma	Long noncoding HOTAIR	qPCR and microarrays followed by qPCR	Correlated with p53 gene expression and causes DNA damage.
Liu et al.2012[87]	oral squamous cell carcinoma	miR-31	qPCR and microarrays followed by qPCR	Involved in posttranscriptional regulation by RNA silencing complex, cellular growth, and elevated levels in proliferation in OSCC.
Park et al.2009[88]	oral cancer	miR-125a, miR-200a	qPCR and microarrays followed by qPCR	Saliva miRNAs can be used for oral cancer detection.
Wei et al.2011[89]	oral cancer and leukoplakia	γ-aminobutyric acid, phenylalanine, valine, *n*-eicosanoic acid and lactic acid	Capillary electrophoresis time-of-flight mass spectrometry (CE-TOFMS) and HPLC with quadrupole/TOF MS	Facilitates the clinical detection of OSCC, improves diagnosis, and serves as a stratification tool.
Ishikawa et al.2016[90]	oral cancer	Hypoxanthine, guanine, guanosine, trimethylamine N-oxide, spermidine, pipecolate, methionine	CE-TOF-MS	Discriminates controls from OSCC patients, with elevated levels in saliva, suitable for noninvasive oral cancer screening.
Abdul Aziz Shaikh S et.al. 2024[91]	oral submucosal fibrosis and squamous cell carcinoma	Tumor necrosis factor α (TNF-α)	ELISA	TNF-α shows a positive correlation with increasing stages of OSMF but lacks reliability as a biomarker for categorization in the same condition.
Gholizadeh N et.al. 2020[92]	oral squamous cell carcinoma, oral lichen planus and oral lichenoid reaction	lactate dehydrogenase	LDH kits (Pars Azmoun)	Patients with OSCC and OLRs had higher serum levels of LDH than OLP and control groups
Dikova V et.al. 2020[93]	oral squamous cell	IL-1α, IL-6, IL-8, IP-10, MCP-1, TNF-α, HCC-1, and PF-4 levels	sensitive bead-based multiplex immunoassay	Elevated levels of salivary IL-6, IL-8, TNF-α, HCC-1, and PF-4 were associated with the presence of neck metastases (NM), indicating their potential

	carcinoma (OSCC)			to discriminate between OSCC and metastatic disease.

TABLE 4 Summary of the studies using Artificial intelligence (AI) in diagnosis of oral premalignant and oral squamous cell carcinoma

STUDY	CASES ASSESSED	COMPARISION	Method	CONCLUSION
Haron et al., 2017[98]	oral potentially malignant disorders	decisions between dentists and oral medicine specialist (OMS)	phones with 3 DENTISTS 5 MP-13 MP resolutions	Teledentistry facilitates seamless communication between primary care providers and oral medicine specialists, enabling efficient collaboration and improving patient access to specialized care within clinical settings.
Song et al., 2018[99]	high-risk populations for oral cancer	Smartphone-based intraoral dual-modality immunofluorescence imaging platform and classification of images obtained.	deep learning neural networks	The efficacy of a smaller network with five convolutional layers surpasses that of a deeper network with 13 layers, likely due to the latter's increased complexity leading to overfitting with limited data. Strategies like transfer learning, data augmentation, and regularization techniques such as weight decay and dropout mitigate overfitting, enhancing learning performance while requiring fewer parameters and images for training.

Chan et al., 2019[100]	cancerous regions	To develop a texture map-based model for detecting cancerous regions and marking the Region of Interest (ROI).	SMOTE texture-map-based branch Network Wavelet transformation Gabor filtering Fully Convolutional Network (FCN) Feature Pyramidal Network (FPN)	By adjusting the probability criterion used in the detection branch, a balanced tradeoff between sensitivity and specificity can be achieved in the proposed model. The findings affirm the model's capability to automatically identify high-risk regions, thereby serving as a valuable tool for oral cancer screening.
Fu et al., 2020[101]	oral squamous cell carcinoma	oral squamous cell carcinoma (OSCC) using photograms.	cascaded convolutional neural networks	The automated detection of oral cavity squamous cell carcinoma (OCSCC) through deep-learning algorithms offers a rapid, non-invasive, cost-effective, and convenient approach.
Jeyaraj & Nadar, 2019[102]	oral cancer	To develop a deep learning algorithm for automated oral cancer detection using hyperspectral images compared the obtained results from another traditional medical image classification algorithm	Partitioned Deep CNN SVM Deep belief Network	The proposed regression-based partitioned CNN learning algorithm enhances the quality of diagnosis for complex medical images related to oral cancer.
Aubreville et al., 2017[103]	OSCC	deep learning on Confocal laser endomicroscopy (CLE) images compared against textural feature-based machine learning approaches	CLE Patch-extraction of images CNN RF-LBP; RF-GLCM	The patch probability fusion method in this paper outperforms conventional image texture-based classifiers and CNN-based transfer learning for image classification, signifying its significant advancement in accuracy and efficacy.

Heintzelman et al., 2000, [104]	OPMD/malignant	optimal excitation–emission wavelength combinations to discriminate normal and precancerous/cancerous tissue, and compared with e validation set	Xenon	These tools holds promise in equipping inexperienced practitioners with screening capabilities comparable to those of experienced practitioners, offering both sensitivity and specificity in diagnosis.
Wang C et al., 2003, [105]	healthy volunteers (NOM) and patients with oral lesions of submucous fibrosis (OSF), epithelial hyperkeratosis (EH), epithelial dysplasia (ED), and squamous cell carcinoma (SCC)	To evaluate whether algorithm could discriminate premalignant (ED) and malignant (SCC) tissues from ‘‘benign’’	Xenon (ƛ:330nm) PLS-ANN (partial least squares and ANN	The PLS-ANN classification algorithm, utilizing autofluorescence spectroscopy at 330-nm excitation, proves valuable for in vivo diagnosis, effectively identifying oral submucosal fibrosis (OSF) alongside premalignant and malignant oral lesions.
Majumder et al., 2005. [106]	OSCC	comparative evaluation of the diagnostic efficacy of the RVM algorithm with that based on support vector machine (SVM)	Bayesian framework of RVM formulation	The Bayesian framework of RVM allows for predicting the posterior probability of class membership, enabling discrimination between early SCC and normal squamous tissue sites in the oral cavity, contrasting with the dichotomous classification offered by non-Bayesian SVM.

Tanriver G et.al. 2021[107]	Benign, OPMD & Malignant	_	deep learning, a two-stage model ResNet-152	The proposed model offers a cost-effective, non-invasive, and user-friendly approach to screening for oral potentially malignant disorders (OPMD), enhancing early detection and treatment outcomes.