

A Systematic Review on Histopathological and Molecular Biomarkers in Oral Pathology: Advancing Early Diagnosis of Precancerous and Cancerous Lesions

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ABSTRACT

Background: Precancerous and cancerous oral lesions (leukoplakia and erythroplakia) have shown a significant diagnostic challenge and were often faced with challenges due to clinically diverse presentations and overlapping with benign features. Despite the gold standard of histopathology, there were limitations in the subjective interpretation and the detection of early molecular changes. This systematic review evaluated the histopathological and molecular biomarkers to enhance the early diagnosis of oral lesions.

Methods: A comprehensive search of studies published between 2015 and 2024 was conducted, following PRISMA guidelines. The electronic databases employed were Google Scholar, PubMed, and Science Direct. Studies that used histopathological or molecular biomarkers to assess diagnostic accuracy, sensitivity, specificity, and area under the curve (AUC) on humans were present in the criteria. The studies were excluded if they had inadequate data, examined non-oral lesions, or were based on animal models. Microsoft Excel 2022 was used to perform data extraction, and SPSS 2024 software was used to assess study quality. The risk of bias was assessed using the Evidence Project Risk of Bias Tool.

Results: 15 studies were selected out of 98 studies screened, with predictive accuracies of 90.1% for Ki-67 and 96.2% for CK19, which were histopathological biomarkers. The noninvasive diagnostic potential of molecular biomarkers such as IL-6, LDH-A, and ctDNA was also promising (AUC > 0.8). Limitations included variability in methods, small sample sizes, and lack of standardization.

Discussion: This review highlighted the role of histopathological and molecular biomarkers in the early diagnosis of oral lesions. Clinical implementation was hindered by inconsistencies in methodologies used and limited validation despite encouraging results.

Keywords: Oral Cancer, Precancerous Lesions, Histopathology, Molecular Biomarkers, Early Detection.

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INTRODUCTION

Oral precancerous and cancerous lesions such as leucoplakia, erythroplakia, and oral lichen planus have become a crucial health challenge globally¹. It was due to their potential to progress to malignancy and their effect on the patients' outcomes. A most common type of oral cancers worldwide was these lesions, which also contributed to a substantial burden in healthcare². Despite the advances in diagnostic and therapeutic approaches towards these lesions, early detection remained a crucial problem due to their numerous clinical phenotypes and overlaps with benign conditions. Focusing on early recognition and intervention was critical to reduce morbidity and improve survival³.

Histopathology has been the mainstay for diagnosing precursor and malignant lesions while offering valuable insights into cellular architecture and tissue morphology⁴. However, in cases where lesions exhibited borderline features, histopathological assessments became limited due to subjective interpretation and variability among pathologists⁵. In addition, histology might not fully capture molecular changes that preceded visible morphological alteration alone, and thus, complementary means of diagnosis were also needed⁶. Such limitations have led to the emergence of molecular biomarkers as promising tools. Molecular biomarkers such as Ki-67, TP53, cytokeratin, and interleukins have shown potential in diagnosing early molecular changes that lead toward malignancy⁷. Advanced techniques, including immunohistochemistry, quantitative PCR, next-generation sequencing, and liquid biopsies, had also broadened the scope of biomarker research, allowing for the discovery of genetic, proteomic, and metabolic signatures that were linked with disease progression⁸. These approaches have shown the potential for more objective, precise, and non-invasive diagnosis in comparison to traditional ones.

Despite these developments, clinical use of biomarkers in oral pathology was still constrained by many challenges, which included small sample size, variability in study designs, and lack of standardization of methodologies⁹. In addition, there were many biomarkers that were still in their

experimental phase, and validation had been restricted mainly due to being tested on narrow populations and not on diverse populations and clinical settings¹⁰. These gaps underscore the need for a systematic review based on existing evidence regarding the diagnostic accuracy, clinical utility, and limitations of both histopathological and molecular biomarkers in the early detection of precancerous and cancerous lesions.

The scope of this systematic review was to identify, describe, and critically assess histopathological and molecular biomarkers used in oral pathology to advance early diagnosis of lesions. This review aimed to analyze the findings from various studies to identify the biomarkers that held promise for clinical implementation, highlight areas where current research was lacking, and suggest directions for future research. Ultimately, this work sought to fill that gap between basic research and clinical practice, providing insights into precancerous conditions that could turn into potential malignancy and improved outcomes for those who were at risk of malignant transformation.

METHODS

This systematic review followed PRISMA guidelines to systematically search, evaluate, and synthesize literature pertinent to histopathological and molecular biomarkers for early detection of oral precancerous and cancerous lesions. Comprehensive searches on three electronic databases, Google Scholar, PubMed, and ScienceDirect, were conducted for studies published between 2015 and 2024, with the last search conducted in Dec 2024. The search terms included combinations of words, 'oral precancerous lesions,' 'oral squamous cell carcinoma,' 'histopathology,' 'molecular biomarkers,' and 'diagnosis.' Boolean operators AND, OR were applied. Filters and truncations were also applied, including year and publication type (abstract and conference papers were excluded).

The inclusion criteria were based on original studies on the diagnostic, prognostic, or predictive value of histopathological or molecular biomarkers in oral precancerous and cancerous lesions of human samples and reporting metrics such as sensitivity, specificity, and area under the curve AUC. Studies that met the eligibility criteria included

experimental, case-control, cohort, or observational study designs and were published in English or translated. No study older than the last 8 years was included. Studies on non-oral lesions, biomarkers unrelated to histopathology or molecular biology, reports, and abstracts only were ruled out by exclusion criteria. Studies that lacked sufficient data were conducted on animal or in vitro models only, or redundant studies were also excluded. The criteria used in the selection of these studies are based on the collection of relevant and high-quality research. The process of study selection involved the removal of 15 duplicates, the screening of 98 records gathered, and the elimination of 39 records based on relevance. Of 59 studies retrieved, 19 were not available, so 40 full-text articles were screened for eligibility assessment. Fifteen studies, which met methodological quality and relevance criteria, were finally included. These studies involved seven experimental designs, three retrospective case-control studies, three reviews, 1 cohort study, and one observational study.

Two reviewers applied the inclusion criteria, screened 98 records, and carried out data collection using a piloted Microsoft Excel 2022 sheet independently. This sheet was designed to extract study characteristics such as sample size, study design, biomarkers investigated, methodology, clinical relevance, and key findings, and table was made out of the data collected. Discrepancies in data extraction were resolved by discussion or in conjunction with a third reviewer. Extracted data were cross-verified by both reviewers to ensure consistency. No attempt to contact authors for clarification or missing data was made.

To assess quality, SPSS 2024 software was used by focusing on biases, statistical rigor, and replicability.

The risk of bias for both randomized and non-randomized studies was assessed using the Evidence Project Risk of Bias Tool. Statistical analysis methods used in the included studies varied based on each study. Predictive accuracy measures such as sensitivity, specificity, and area under the curve were reported frequently. For example, biomarkers such as Ki-67 and TP53 showed predictive accuracies that range from 90.1% to 96.2%. Some studies employed machine learning models, such as the logistic regression model, to evaluate associations between the expression of biomarkers and lesion progression, while others used machine learning models for pattern recognition and stratification of risks. The variance in data and methodologies reflects the diversity of approaches to analyze biomarkers. This highlighted the need for standardization in future research. GRADE framework was used to assess the certainty of evidence.

RESULTS

Findings from 15 studies investigating histopathological and molecular biomarkers in oral lesion diagnosis were synthesized. The studies studying salivary IL-6 in oral cancer with animal models were excluded because of the non-human samples, such as the study by Chia et al. (2020). Similarly, studies on TP53 in leukoplakia, which were outside the publication date range (2015–2024), were also removed. For instance, a study by Ogmundsdóttir et al. (2009) examined TP53 mutations in oral lesions but was excluded from being published before 2015. The studies ^{11,12,13,14,15,16,17,18,19,20,21,22,23,24,25} was

selected to be included in the systematic review table, as shown in Table 1, and the process through which 15 studies were filtered is shown in **Figure 1**.

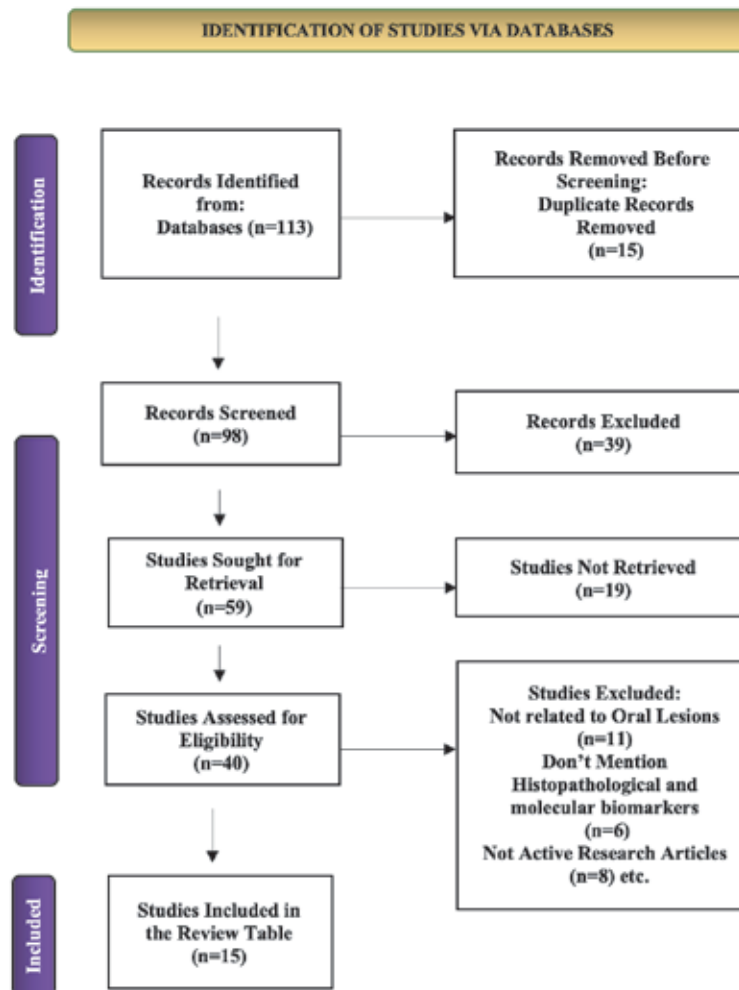


Figure 1: Flow of the process of Study Selection Based on Inclusion and Exclusion Criteria Determined by PRISMA

In terms of high diagnostic accuracy biomarkers, histopathological biomarkers were highlighted, especially Ki-67, which had a predictive accuracy of 90.1% for epithelial hyperplasia and 96.2% for carcinoma in situ. Likewise, lesion severity correlated strongly with CK19 expression, making CK19 a useful serum marker of risk stratification. Limitations that were observed across the studies included the lack of standardized thresholds and interobserver variability in histopathological interpretation.

Molecular biomarkers were shown to be emerging as promising complementary tools. Differentiation between precancerous and cancerous lesions was suggested using inflammatory markers such as IL-6 and TNF- α and metabolic markers such as LDH-A. In several studies, salivary biomarkers such as mRNA markers MAOB and NAB2 and proteins KRT6C and AZGP1 were used as a non-invasive approach and exhibited high diagnostic performance (e.g., AUC>0.8). Early lesion detection and disease progression monitored by the liquid biopsy-based

technologies, including circulating tumor DNA (ctDNA) and microRNAs (e.g., miRNA-133a-3p and miRNA-375-3p), were explored. In these studies, diagnostic performance was measured by statistical analyses for sensitivity, specificity, Area under the curve (AUC), and correlation coefficients.

Some of these studies also featured the latest technologies and methodologies, such as multiplex assays, next-generation sequencing, and machine learning. These methods helped in identifying complex biomarker signatures and pinpointed the location of diagnosis. For instance, the successful inclusion of histopathological and molecular data in machine learning models to improve diagnostic accuracy and risk assessment. However, small sample sizes, heterogeneity in biomarker expression, and variability in statistical methods across studies limited the generalizability of the findings.

The most common design among the 15 studies was experimental (n=7), followed by retrospective

case-control studies (n=2), reviews (n=4), one prospective multicenter study, and one observational study. Robust statistical analyses were given in many studies, but others lacked sufficient sample sizes to prove the results. Furthermore, variations in analyses of biomarkers, such as differences in threshold definitions, techniques (e.g., immunohistochemistry, qPCR), and control group selection, were also observed. The existence of these inconsistencies highlighted the need for standardized protocols and larger multi-centered studies to validate these promising biomarkers.

The overall certainty of evidence for histopathological biomarkers, in particular for markers such as Ki-67, with consistent diagnostic performance across studies, was high. However, the

certainty of evidence for molecular biomarkers such as ctDNA and microRNAs was moderate to low due to small sample sizes, methodological heterogeneity, and no independent validation. Finally, there is much future work to be done to build the evidence that will be based on these limitations. Within a subset of studies, subgroup analyses found significant differences in biomarker performance dependent on lesion type, with Ki-67 having significantly improved accuracy in the setting of carcinoma in situ ($p < 0.01$) compared to epithelial hyperplasia. Very few biomarker studies reported sensitivity analyses to address methodological variability, such as differences in threshold values and control groups, further highlighting the importance of standardized approaches in biomarker research.

Table 1: Systematic Review Table Showing 15 Selected Studies That Met the Inclusion Criteria

Study Details (Author, Year, Country)	Study Design	Sample Size	Biomarkers & Methodology	Clinical Relevance	Findings
Ikeda et al., 2020, Japan ¹¹	Retrospective analysis	200 patients	Ki-67, TP53, CK13, CK17 (Immunohistochemistry)	Early detection of oral premalignant lesions and carcinoma in situ (CIS) for OSCC diagnosis and treatment	High predictive accuracy (EHP: 90.1%, CIS: 96.2%); Ki-67 and TP53 were identified as key markers.
Hirsch et al., 2023, Sweden ¹²	Prospective multicentre study	39 biomarkers analysed	Microsatellite DNA analysis, PCR, and immunohistochemical staining	Identifying molecular biomarkers for assessing malignant transformation risk in oral leukoplakia (OL) and keratinocytes	Significant biomarkers: heterozygosity on 3p, 9p, 11q, and 17p, and DNA ploidy.
Scholtz et al., 2020, Hungary ¹³	Experimental	23 OLP patients, 12 OLK patients	IL6, MMP1, MMP10, CXCL11, CXCL10, CCL11 (Proximity extension assay, quantitative PCR, ELISA)	Differentiation between OSCC and precancerous lesions (OLP, OLK) using salivary biomarkers	Elevated IL6 and MMP levels in precancerous and OSCC groups; chemokines linked to chronic inflammation and carcinogenesis.
Wang et al., 2022, China ¹⁴	Review	7 biomarkers	GLUT1, GLUT3 (Histology), Lactate levels (Mass Spectrometry), Glutamine & Arginine (Mass Spectrometry)	Identified important molecular biomarkers for early oral lesion detection	OSCC exhibits metabolic reprogramming, with upregulated GLUT1, GLUT3, and lactate accumulation supporting tumor progression.
Bai et al., 2020, Japan ¹⁵	Review	10 biomarkers	E-cadherin (Histology), Vimentin (Immunohistochemistry)	Understanding molecular changes in precancerous lesions	Loss of epithelial adhesion (E-cadherin) and gain of mesenchymal markers (Vimentin) signals the transition to OSCC.
Sharma et al., 2023, India ¹⁶	Experimental	16 patients	IL6 (Proteomics), LDH-A (Mass spectrometry)	Differentiating precancerous lesions from malignancies	Identified IL6 and LDH-A as markers of metabolic and inflammatory changes signaling malignancy.
Ali et al., 2021, Canada ¹⁷	Retrospective case-control	145 samples from 95 patients	Invadopodia markers (Cortactin, Tks5, MMP14) via FIHC	Early detection of OSCC progression in oral potentially malignant disorders (OPMD)	Sensitivity: 75–100%; specificity: 72–76%. The INV score identifies lesions at high risk of transformation.

Rajeswari et al., 2020, India ¹⁸	Observational	50 samples (10 per group: normal, hyperplasia, dysplasia, OSCC)	Cytokeratin 19 via immunohistochemistry (IHC)	Predicting malignant transformation in epithelial dysplasias	Increased CK19 expression correlates with lesion severity.
Oh et al., 2020, South Korea ¹⁹	Case-control	67 saliva samples (34 controls, 33 OSCC patients)	Salivary mRNA biomarkers (MAOB, NAB2, CYP27A1) via qPCR	Non-invasive early detection of OSCC	Identified MAOB and NAB2 as key markers. AUC: 0.84 for CYP27A1 + SIAE combination.
Dikova et al., 2021, Spain ²⁰	Case-control	157 samples: Healthy (n=25), HL (n=33), PVL (n=33), OSCC early (n=33), OSCC advanced (n=33)	Salivary cytokines (IL-6, IL-8, TNF- α , HCC-1, PF-4) via multiplex immunoassay	Non-invasive biomarkers for OSCC detection and differentiation between OSCC stages, OL, and controls	Significant IL-6, TNF- α , IL-8, and PF-4 elevations in OSCC vs. controls; AUC > 0.8 for certain markers.
Li et al., 2022, China ²¹	Experimental	129 samples (23 normal, 15 OLK, 91 OSCC)	Genes: DCT, KRT3, KRT76, FAM3B (GEO datasets GSE85195, GSE25099)	Identifying molecular changes in OLK to OSCC transformation, potential therapeutic targets	DCT, KRT3, KRT76, and FAM3B genes have diagnostic models with AUC = 0.963 for early detection.
Vaidya et al., 2022, India ²²	Review	15+ studies (ref ^{29-40, 55-74}), 10+ biomarkers	Keratins (K1, K4, K8, K10, K13, K18, K19; proteomic, immunohistochemical)	Prognostic indicators for malignant transformation of oral precancerous lesions	Altered keratin expression is associated with oral SCC progression.
Gattuso et al., 2022, Italy ²³	Review	50+ studies (ref ^{59,73,82,87,88, 145,150, 235,240}), 100+ biomarkers	Liquid biopsy-based markers: ctDNA, miRNAs, exosomes, proteins (qPCR, ddPCR, NGS, microarray, ELISA)	Non-invasive techniques for early detection and monitoring of OSCC	Promising biomarkers like miRNA-133a-3p and miRNA-375-3p for OSCC.
Jain et al., 2021, India ²⁴	Experimental	50 OSCC cases, 49 controls, 12 biomarkers	Salivary proteins (KRT6C, AHSG, AZGP1, BPIFB2, KLK1; validated using PRM)	Non-invasive early detection and the distinction between stages of OSCC	AHSG, KRT6C upregulated in advanced OSCC; AZGP1, KLK1, BPIFB2 downregulated. High sensitivity and specificity.
de Souza et al., 2022, Brazil ²⁵	Case-Control	42 patients	Histopathology (H&E Staining), Immunohistochemistry (PD-L1), ELISA (IL-6, IL-10, IL-12, IFN- γ)	Smoking, lesion size, inflammatory infiltrate, and PD-L1 as biomarkers for malignancy	Significant association of smoking, lesion size, inflammatory infiltrate, and PD-L1 with malignancy.

Table 2: Risk of Bias Table Using Evidence Project Risk of Bias Tool

Study	Cohort	Control or Comparison Group	Pre/Post Intervention Data	Random Assignment of Participants to the Intervention	Random Selection of Participants for Assessment	Follow-up Rate of 80% or More	Comparison Groups Equivalent on Sociodemographic	Comparison Groups Equivalent at Baseline on Disclosure
Ikeda et al., 2020, Japan ¹¹	No	Yes	No	No	No	No	No	No

Hirsch et al., 2023, Sweden ¹²	Yes	Yes	Yes	No	No	Yes	No	No
Scholtz et al., 2020, Hungary ¹³	Yes	Yes	No	No	No	No	No	No
Wang et al., 2022, China ¹⁴	No	No	No	No	No	NA	NA	NA
Bai et al., 2020, Japan ¹⁵	No	No	No	No	No	NA	NA	NA
Sharma et al., 2023, India ¹⁶	Yes	Yes	Yes	No	No	No	No	No
Ali et al., 2021, Canada ¹⁷	No	Yes	No	No	No	No	No	No
Rajeswari et al., 2020, India ¹⁸	Yes	Yes	No	No	No	No	No	No
Oh et al., 2020, South Korea ¹⁹	No	Yes	Yes	No	No	No	No	No
Dikova et al., 2021, Spain ²⁰	Yes	Yes	Yes	No	No	No	No	No
Li et al., 2022, China ²¹	Yes	Yes	Yes	No	No	No	No	No
Vaidya et al., 2022, India ²²	No	No	No	No	No	NA	NA	NA
Gattuso et al., 2022, Italy ²³	No	No	No	No	No	NA	NA	NA
Jain et al., 2021, India ²⁴	Yes	Yes	Yes	No	No	No	No	No
de Souza et al., 2022, Brazil ²⁵	No	Yes	No	No	No	No	No	No

Yes: Indicates the presence of the specified characteristic, no: Indicates the absence of the specified characteristic, NA: Not applicable, NR: Not reported.

DISCUSSION

To evaluate the role of histopathological and molecular biomarkers in the early diagnosis of oral precancer and cancerous lesions, this systematic review was conducted. Across a series of studies in diverse populations, this study was able to identify a range of biomarkers and methodologies that showed promise to improve diagnostic accuracy. These findings determined the potential of biomarker-driven approaches to overcome the limitations of more traditional diagnostic methods, which may lead to more timely intervention and better patient outcomes.

Histopathological biomarkers continue to be foundational in diagnosing precancerous lesions and cancer transformations²⁶. Research studied Ki67 and TP53 and found that these were reliable

markers showing high predictive accuracy in epithelial hyperplasia (90.1%) and carcinoma in situ (96.2%). The correlation between CK19 expression and lesion severity found in these findings is in agreement with that of a study that showed correlation between CK19 expression and lesion severity, and this study emphasized the relevance of histopathological markers in risk stratification²⁷. However, across these studies, there were consistent limitations of variability in histological interpretation and a lack of standardized diagnostic thresholds.

Complementary information about the pathogenesis of oral lesions comes from molecular biomarkers like interleukins, matrix metalloproteinases, and chemokines²⁸. In one study, it was found that elevated levels of IL6 and MMPs in patients with precursor lesions and OSCC

compared with normal mucosa indicated their action in inflammation-driven carcinogenesis. Another study mentioned the metabolic and inflammatory changes reported by IL-6 and LDH-A in cancers²⁹. These findings were consistent with those of another study that characterized cytokines IL-6, IL-8, and TNF-alpha as useful for differentiating stages of OSCC. However, these markers fell short of promise because changes in the system's conditions affected their measurements as demonstrated in various studies, and it was therefore necessary to identify more specific, context-sensitive biomarkers³⁰.

There has been an increasing traction of salivary biomarkers as noninvasive diagnostic tools. In a study, it was demonstrated that salivary mRNA markers MAOB and NAB2 were efficient for early OSCC detection, with an AUC of 0.84 for CYP27A1 and SIAE in combination. One study also validated the potential of salivary proteins KRT6C and AZGP1 to discriminate OSCC stages. These findings are in agreement with another study's work, which stressed the potential of liquid biopsy-based markers such as ctDNA and miRNAs for the monitoring of disease progression³¹. Nevertheless, the use of salivary biomarkers in clinical practice was hampered by challenges conferred on their use, such as salivary biomarker variability, which was influenced by dietary and environmental factors³².

While biomarker-driven diagnostics offered great promise there remained several challenges. As noted by one review study, the variability in biomarker expression across studies reflected differences in oral lesion heterogeneity and patient populations³³. The small sample sizes of the samples in two of the studies limited the statistical capability and generalization of findings. Furthermore, studies that regularly employed retrospective and case control designs had limitations to causal inferences and could be unduly affected by biases³⁴.

There were significant barriers that were applied by methodological inconsistencies. Due to the lack of standardization in techniques for biomarker analysis, such as immunohistochemistry, qPCR, mass spectrometry, etc, the comparison of results between studies was hindered³⁵. For example, differences in thresholds for the biomarker's positivity and the use of varying control groups prevented the establishment of universal diagnostic criteria³⁶. Two studies in the review showed high variability due to variations in the method of analysis and population characteristics.

Future research should focus on the standardization of biomarker evaluation to overcome these challenges. To improve the reproducibility and comparability of findings, uniform protocols for

sample collection, analysis, and interpretation needed to be established. Similarly, large-scale, multi-center prospective studies were needed to validate the clinical utility of promising biomarkers, as exemplified by one study, which relied on limited datasets for model validation³⁷. Integration of biomarkers into the routine diagnostic workflow required a multidisciplinary approach. Histopathological and molecular markers might be combined in diagnostic algorithms to enhance the sensitivity and specificity of lesion risk, resulting in a more comprehensive analysis of risk assessment³⁸. Artificial intelligence and machine learning have been increasingly integrated in biomarker analysis, such as in pattern recognition in histopathological slides and omics data. It presented an exciting avenue for improving diagnostics precision³⁹.

Particular promise resided in non-invasive diagnostic modalities, such as saliva-based biomarker assays, for population-based screenings. Future studies should refine these techniques to adjust for confounding variables and improve their specificity for oral lesions. In addition, longitudinal studies to track biomarker changes with respect to time would help assess the predictive value of these biomarkers as well as their utility in monitoring disease progression⁴⁰.

This systematic review underscored the game changing potential of histopathological and molecular biomarkers in the timely recognition of oral precancerous and cancerous lesions. These biomarkers built a bridge between conventional diagnostic methods and emerging molecular technologies to offer more precise, objective, and personalized diagnostic pathways. However, achieving this potential to break current limitations would require more rigorous standardization, larger validation studies, and interdisciplinary collaboration.

Ultimately, the promise of biomarker-driven diagnostics to support earlier detection, prompt intervention, and better patient outcomes lay within the integration of these biomarkers into clinical practice, where patients were at risk of malignant transformation. The future of oral pathology would belong to the realm of a synergistic application of histopathological and molecular biomarkers, which would play a significant role in shaping it.

CONCLUSION

This systematic review demonstrated the critical importance of the use of histopathological and molecular biomarkers for early detection and risk stratification of oral precancerous and cancerous lesions. Histopathological biomarkers remained essential, but molecular biomarkers brought additional insights into lesion pathogenesis, thus

facilitating more specific and non-invasive diagnostic approaches. These promising findings had yet to be integrated into clinical practice due to the variability of methodology, lack of standardization, and insufficient large-scale validation studies. Future biomarker research should focus on the standardization of evaluation protocols, multicenter studies with heterogeneous populations, and the development of non-invasive techniques such as salivary diagnostics. With the combination of these histopathological and molecular biomarkers, along with emerging technologies such as artificial intelligence, oral pathology would provide a transformative path in treatment, allowing for early diagnosis and improved patient outcomes in oral pathology.

LIST OF ABBREVIATIONS

AUC: Area Under the Curve

CK: Cytokeratin

IL-6: Interleukin-6

LDH-A: Lactate Dehydrogenase-A

MAOB: Monoamine Oxidase B

NAB2: NGFI-A Binding Protein 2

O SCC: Oral Squamous Cell Carcinoma

PCR: Polymerase Chain Reaction

PRISMA: Preferred Reporting Items for Systematic Reviews and Meta-Analyses

CONFLICT OF INTERESTS

None

AUTHORS' CONTRIBUTIONS

All participants participated equally as per the ICMJE.

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