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The diagnostic potential of salivary microRNA for early detection of oral squamous cell carcinoma in healthy vs. affected patients: A systematic review.

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1. Abstract

Introduction: Oral squamous cell carcinoma (OSCC) is the most common oral malignancy, comprising over 90% of oral cancers. Despite advances in treatment, its late diagnosis and high recurrence contribute to poor prognosis. Conventional diagnostic methods often overlook early-stage disease. Salivary microRNAs (miRNAs), which are stable, non-coding RNAs involved in gene regulation, have emerged as promising non-invasive biomarkers for early OSCC detection.

Objectives: This systematic review identifies salivary miRNAs with diagnostic value in OSCC and compares their expression in affected patients versus healthy controls. It also explores intermediate phenotypes such as oral potentially malignant disorders (OPMD), oral lichen planus (OLP), and OSCC in remission (OSCC-R) to understand miRNA changes across the disease spectrum.

Materials and Methods: A systematic literature search of PubMed, Scopus, and Web of Science was conducted in line with PRISMA-P guidelines. Eligible studies included adult patients with OSCC, OPMD, OLP, or OSCC-R, and compared salivary miRNA expression with healthy individuals.

Results: Fourteen studies comprising 914 participants met inclusion criteria. Thirty-seven differentially expressed salivary miRNAs were identified. miR-21, miR-31, and miR-423-5p were consistently upregulated in OSCC, while miR-138, miR-424, and miR-30c-5p were downregulated. Several studies also examined OPMD, OLP, and OSCC-R, revealing intermediate expression patterns indicative of disease progression.

Conclusion: Salivary miRNAs exhibit distinct expression profiles between OSCC and healthy controls, underscoring their diagnostic potential. miR-21 and miR-31 show strong biomarker capabilities, while tumor-suppressive miRNAs like miR-138 and miR-145 further support risk stratification. Including intermediate phenotypes provides additional insights into early detection and monitoring. Standardized methodologies and large-scale validation are needed for clinical implementation.

Key words: *microRNA, salivary miRNA, OSCC, Oral squamous cell carcinoma, Early detection, Healthy controls*

2. Abbreviation

I. Oral squamous cell carcinoma (OSCC)
II. Head and neck cancer (HNC)
III. Reactive oxygen species (ROS)
IV. MicroRNA (miRNA)
V. Messenger RNA (mRNA)
VI. Primary miRNA (pri-miRNA)
VII. Precursor miRNA (pre-miRNA)
VIII. Exportin-5 (XPO5)
IX. Argonaute (AGO)
X. Real-time reverse transcription polymerase chain reaction (RT-PCR)
XI. Epithelial-mesenchymal transition (EMT)
XII. Sustainable development goal (SDG)
XIII. Human Papillomavirus (HPV)
XIV. Epstein-Barr Virus (EBV)
XV. Herpes Simplex Virus 1 (HSV-1)
XVI. Oral lichen planus (OLP)
XVII. Oral potentially malignant disorders (OPMD)
XVIII. Oral squamous cell carcinoma in remission (OSCC-R)
XIX. EV (Extracellular vesicle)
XX. AUC (Area under curve)
XXI. ROC (Receiver operating characteristics)
XXII. RT-qPCR (Reverse Transcription quantitative Polymerase Chain Reaction)
XXIII. TaqMan RT-qPCR (TaqMan Reverse transcription qPCR)
XXIV. RNASeq (RNA sequencing)
XXV. PTEN (phosphatase and tensin homolog)
XXVI. RECK (reversion-inducing cysteine-rich protein with Kazal motifs)

3. Keywords

I. Biomarker
II. Salivary biomarker
III. Salivary microRNA
IV. microRNA
V. miRNA
VI. Oral squamous cell carcinoma
VII. OSCC
VIII. Oral carcinoma
IX. Oral cancer
X. Healthy controls
XI. Early diagnosis
XII. Early detection
XIII. Diagnosis

4. Introduction

4.1 Oral Squamous cell carcinoma

Cancer is a chronic inflammatory disease characterized by uncontrolled and abnormal cell growth. In a healthy individual, cells follow a regulated cycle of growth, division, and apoptosis to maintain balance within the body. However, cancer cells bypass these controls, continuing to grow and divide indefinitely while disrupting the natural apoptotic process. As these malignant cells proliferate without control, they form solid masses known as tumours. These tumours can interfere with essential physiological systems, including the nervous, circulatory, and digestive systems. Additionally, cancer can dysregulate the endocrine system, leading to abnormal hormone production that further disrupts normal bodily functions (1).

Oral cancer in general encompasses a group of malignant tumours that can arise in various regions of the oral cavity, including the pharyngeal areas and salivary glands. However, the term is often used interchangeably with Oral squamous cell carcinoma (OSCC), which accounts for over 90% of all diagnosed oral neoplasms (2). OSCC originates from the mucosal epithelial tissue of the oral cavity and frequently presents as non-healing sores or ulcers. These lesions commonly occur on the mobile tongue, floor of the mouth, buccal mucosa, alveolar ridges, retromolar trigone, and hard palate (3,4).
The aggressive nature and high prevalence of OSCC have made it a central focus in oral cancer research, as scientists and clinicians work to improve understanding, diagnosis, and treatment. The widespread use of the term “oral cancer" to describe OSCC highlights the importance of distinguishing it from other, less common oral neoplasms, while also emphasizing the need for targeted approaches to combat this life-threatening disease. By addressing its unique characteristics and the specific tissues it affects, researchers and healthcare professionals aim to advance early detection and improve outcomes for individuals with OSCC.

4.1.1 Epidemiology of OSCC

Ranking as 16th most common cancer globally, OSCC is the most prevalent form of head and neck cancer (HNC). It is considered the most aggressive malignant tumour due to its metastatic potential and high relapse (5). According to the newest Global Cancer Observatory's 2022 data, cancers of the lip and oral cavity accounted for approximately 389,846 new cases and a total of 188,438 deaths globally. Interestingly, there is a higher prevalence in men, that may be due to lifestyle factors, such as higher consumption of tobacco and alcohol, both accounting for high risk factors in oral cancer (6).

4.1.2 Etiological risk factors of OSCC

The etiology of cancer is complex and multifactorial. Approximately one-third of all cases being associated with a combination of environmental and hereditary risk factors. Among the prominent environmental risk factors, tobacco use, alcohol consumption, and an elevated body mass index (BMI) play significant roles, alongside betel quid chewing, which is particularly prevalent in Southeast-Asian countries (5,7).

Tobacco use has been linked to various diseases, one of them being OSCC. As tobacco is consumed through the oral cavity, it directly affects the dental tissues. It alters the oral microbiome and can therefore increase the risk of periodontal disease, xerostomia and risk of infections. Whether through smoking or chewing, is a major risk factor especially in combination with betel quid, as it exposes individuals to carcinogenic nitrosamines and reactive oxygen species (ROS). These harmful compounds trigger genetic mutations and oxidative damage, promoting multistage oral carcinogenesis, with substances like benzo-α-pyrenes further enhancing the cancer risk (1).

Chewing betel quid, which contains areca nut and often tobacco, is a significant risk factor for oral cancer, increasing the likelihood by up to four times. This habit leads to the production of ROS, which damage oral mucosa by causing mutations or making it more susceptible to toxic compounds. ROS, generated in the alkaline environment of betel quid users' saliva, contributes to tumour formation by inducing genetic mutations and altering salivary proteins. Additionally, the nitrosation of areca alkaloids in saliva produces carcinogenic nitrosamines, further promoting oral cancer development (1,5).

Alcohol, primarily composed of ethanol, is a significant risk factor for oral cancer, with heavy consumption increasing the likelihood of developing the disease. The body metabolizes alcohol through enzymes that convert ethanol into acetaldehyde, a mutagenic compound linked to carcinogenic effects. Additionally, alcoholic beverages contain other harmful substances, such as polyphenols, acrylamide, and nitrosamines, which further contribute to cancer risk (1).

Body mass index (BMI) significantly influences OSCC prognosis, with underweight patients (<18.5 kg/m^2) showing lower survival rates due to malnutrition, while overweight patients (>25 kg/m^2) tend to fare better. Adequate nutrition may enhance treatment resilience, and this study found BMI to be a stronger predictor of survival than traditional adverse features like surgical margins and PNI. Maintaining a healthy BMI before treatment could improve OSCC outcomes (8).

The oral microbiome has been increasingly implicated in the development and progression of OSCC, with various bacterial species and viruses contributing to its carcinogenesis. Among these, *Porphyromonas gingivalis (P. gingivalis)* has been particularly associated with OSCC. Beyond inducing chronic inflammation, the oral microbiota plays a role in tumorigenesis through mechanisms such as oncometabolite production, epithelial-mesenchymal transition (EMT), apoptosis inhibition, and increased cell proliferation. Dysbiosis can shift the host-microbiota relationship from symbiotic to pathogenic, further accelerating OSCC progression. Additionally, the tumour-associated microbiota, present in both tumour and immune cells, interacts with the tumour microenvironment and influences key factors such as smoking and response to immunotherapy (7,9).

P. gingivalis promotes oral carcinogenesis by disrupting key cellular processes. It inhibits apoptosis via JAK1/STAT3 and PI3K/Akt signaling, suppressing pro-apoptotic factors and upregulating microRNA-203. Additionally, it enhances cell proliferation by activating β-catenin signaling and downregulating p53. Its role in invasion and EMT is evident through increased matrix metalloproteinase expression, facilitating metastasis. Furthermore, *P. gingivalis* induces chronic inflammation, promoting a tumour-supportive environment. Fusobacterium nucleatum (*F. nucleatum)* stimulates cell growth by upregulating kinases and cyclins, activating β-catenin signaling, and downregulating the tumour suppressor p53. Additionally, *F. nucleatum* enhances invasion and EMT by increasing the expression of matrix metalloproteinases (MMP-1, MMP-9, MMP-10, and MMP-13), which facilitate cancer cell migration and metastasis. The bacterium also induces chronic inflammation, promoting tumor progression through elevated production of pro-inflammatory cytokines (10).

Other fusobacteria, such as *Fusobacterium periodonticum,* are also found to increase in abundance as OSCC progresses, highlighting their critical role in the disease's development. Alongside fusobacteria, certain streptococcal species have been linked to OSCC. *Streptococcus anginosus* is found at higher levels in OSCC patients, suggesting its involvement in oral cancer carcinogenesis, while *Streptococcus mitis* shows a decrease in abundance as the disease advances. Other species, including Streptococcus constellatus and Streptococcus salivarius, also contribute to the growing evidence of the involvement of oral bacteria in OSCC development (7,9).

In addition to bacterial influences, viruses like Human Papillomavirus (HPV), Epstein-Barr Virus (EBV), and Herpes Simplex Virus 1 (HSV-1) have been linked to OSCC. HPV, especially HPV-16, has been increasingly recognized as a significant contributor to OSCC, with studies showing high prevalence rates in OSCC patients. EBV, associated with several cancers, including OSCC, has been shown to elevate the risk of OSCC by 2.5 times in infected individuals. Lastly, HSV-1, long recognized as associated with oral cancer, is linked to OSCC progression, with higher antibody levels found in patients with OSCC or precancerous lesions. Together, these microorganisms, both bacterial and viral, highlight the complex interplay of the oral microbiome and viral infections in OSCC pathogenesis, influencing disease progression and potentially offering new ways for diagnosis and therapeutic strategies (7,9).

4.1.3 Signs and symptoms of OSCC

Furthermore, patients often experience a range of symptoms that significantly disrupt their daily functions and overall quality of life. These symptoms can include difficulty eating, as well as challenges with speech, particularly dysarthria, making communication increasingly difficult. Additionally, many patients report experiencing pain during mastication, further complicating their ability to perform basic tasks, which can have a negative impact on their physical and mental well-being (4).

As shown in Table 1, which is based on the findings of Muthu et al. (3), there are several important warning signs that should be considered when attempting the diagnosis of OSCC in patients. These signs act as vital indicators for healthcare providers and may suggest the presence of the disease.

Table 1: Muthu et al. Warning signs and symptoms in patients with OSCC

Warning signs and symptoms in patients with OSCC
Non healing ulcer with or without induration / non-healing socket
White patch with firm consistency
Abnormal lump in the mouth with increase in size
Exophytic / ulcer-proliferative growth
Mass or lump in the neck and neighboring regions (Lymph node enlargement)
Mobility/ displacement/ non vital teeth
Periimplantitis
Tooth pain/referral pain
Bleeding from the mouth (hemorrhage)
Red or white
Painless

4.1.4 Treatment of OSCC

At present, the standard approach for treating OSCC primarily involves surgical resection, which is typically followed by adjuvant therapies such as chemotherapy or radiotherapy (5,11). These conventional methods, while effective to some extent, have limitations, prompting researchers and clinicians to explore alternative and innovative therapeutic strategies.

Among the advancements being investigated, monotherapy and combined pharmacological treatments have also gained significant attention. For instance, recent in-vivo studies have demonstrated the potential of Nimotuzumab, a therapeutic agent known for its ability to inhibit cell proliferation while promoting apoptosis in OSCC cells. This property of Nimotuzumab has been shown to substantially increase the cure rate, offering a promising avenue for more effective treatment. Similarly, the combination of Metformin and 4SC-202 has also been highlighted for its effectiveness in targeting cancer cell growth. This combination has been shown to inhibit the growth of cancerous cells while simultaneously inducing intrinsic apoptosis, further contributing to the advancement of treatment options for OSCC. The exploration of these therapeutic strategies holds great potential for improving patient outcomes and offering new hope for those battling this aggressive cancer (5).

Despite these innovations, surgical resection remains the primary option for OSCC treatment. Accurate staging is crucial and should involve a thorough clinical and physical examination, complemented by radiographic evaluations. The TNM is a standardized system that reflects the extent of tumour growth in the whole body and is based on assessment of the size of the primary tumour (T), the extent of regional lymph node involvement of (N), and metastases, meaning whether the cancer spread to other parts of the body (M) (11). Modern imaging techniques, such as CT and MRI, are currently the preferred methods for assessing loco-regional diseases (5).
Due to the aggressive nature of OSCC, early detection is essential for improving prognosis and treatment outcomes. Emerging research has identified salivary biomarkers, particularly microRNAs, as promising tools for the early detection of OSCC, which will be further explained in the following.

4.2 miRNA

miRNAs (microRNAs) are small, highly conserved, non-coding RNA molecules, about 22 nucleotides in length, that regulate gene expression by promoting messenger RNA (mRNA) breakdown or inhibiting its translation (12,13). Although less than 5% of expressed genes that produce mRNA are ultimately translated into proteins, miRNAs continue to maintain their full functionality within the cell cytoplasm. In this crucial location, miRNAs play an essential role in regulating key cellular and metabolic pathways, which include critical processes such as cell proliferation, differentiation, and survival (14). These regulatory functions have a far-reaching impact, influencing numerous vital cellular processes that are fundamental to maintaining cellular homeostasis and function (12).

MiRNAs play an essential role as molecular regulators, serving as a bridge between the genetic instructions encoded in DNA and the intricate process of protein synthesis. These small, non-coding RNA molecules are key players in gene expression regulation, orchestrating cellular processes with significant precision. As illustrated by *Muthu et al. (3)*, their biogenesis is a multi-step process that begins with their transcription from DNA sequences by RNA polymerases II and III, resulting in the formation of the so-called primary miRNAs (pri-miRNAs). These pri-miRNAs are long structures that undergo further processing to become functional. The initial processing of pri-miRNAs occurs within the nucleus, where the microprocessor complex, which is comprised of Drosha, an RNase III enzyme, and DGCR8, a double-stranded RNA-binding protein, playing a central role. Drosha cuts the pri-miRNA, transforming it into a shorter precursor miRNA (pre-miRNA) with a characteristic hairpin structure and a short 3' overhang. This step is crucial for preparing the miRNA for cytoplasmic transport. Once processed, the pre-miRNA is then exported from the nucleus to the cytoplasm by the Exportin-5 (XPO5)/RanGTP complex. In the cytoplasm, the enzyme Dicer, another RNase III protein, removes the terminal loop of the pre-miRNA, producing a mature miRNA duplex. This duplex consists of two strands, the guide strand and the passenger strand. The strand with lower thermodynamic stability at its 5' end, or one that contains a 5' uracil, is preferentially selected as the guide strand. This guide strand is loaded into an Argonaute (AGO) protein to form the RNA-induced silencing complex (RISC), while the passenger strand is degraded. The RISC complex, with the guide miRNA, is then directed to target mRNAs. Binding usually occurs at the 3' untranslated region (3' UTR) of the target mRNA, where the miRNA guides the RISC to regulate gene expression. This regulation is achieved through two primary mechanisms, by promoting the degradation of the mRNA or by inhibiting its translation into a protein. By controlling protein production, miRNAs ensure cellular balance and adaptability, acting as key regulators in the link between DNA and protein synthesis. Finally, their mature forms are highly stable in human biofluids, making them valuable candidates for diagnostic and prognostic biomarkers (12,13).

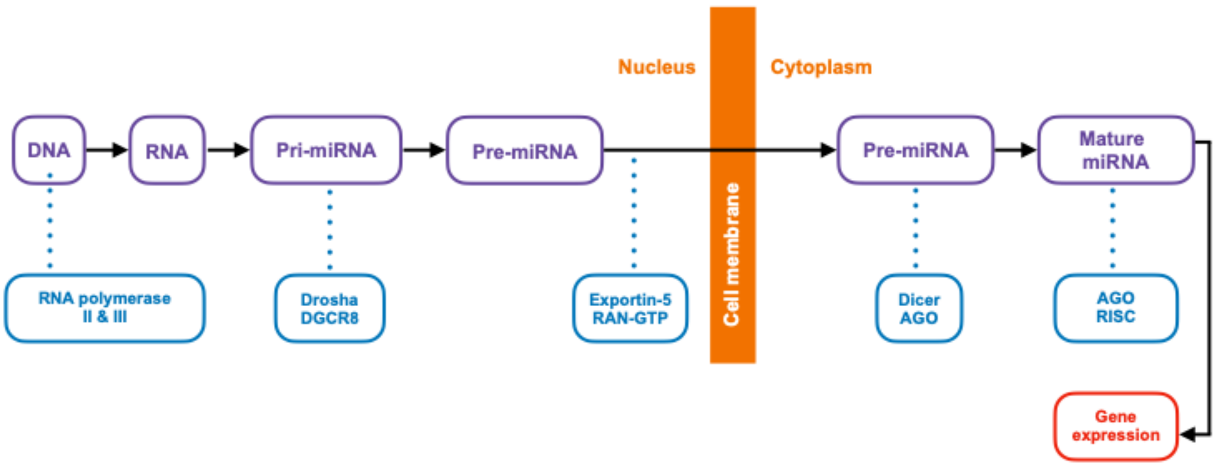

Figure 1: miRNA Biogenesis *(Figure created by author)*

4.3 Salivary microRNA in Oral Health

More than 2000 miRNAs are known, collectively regulating over 60% of the genome. Notably, among 12 human biofluids profiled, miRNAs are second in concentration in the whole saliva (15). Saliva is a highly complex biofluid composed of enzymes, antibodies, hormones, cytokines, antimicrobial agents and salivary miRNAs, which offers valuable insights for the early detection of diseases in oral health (16). Saliva, as a diagnostic fluid, has gained increasing attention, particularly during the COVID-19 pandemic, due to its non-invasive nature, ease of collection, and suitability for repeated sampling (17). As a diagnostic tool, saliva sampling provides significant advantages over blood testing, for its non-invasive, cost-effective alternative characteristics, delivering accurate information about physiological states. Its simplicity and affordability make it particularly suited for large-scale screenings and repeated sampling, supporting disease monitoring and early diagnosis (18). Techniques, such as sequencing, microarrays and RT-PCR, can be employed to analyze miRNA extracted from saliva, each offering unique insights. Sequencing is a method used to determine the exact sequence of nucleotides in a sample, providing detailed information about the genetic makeup. It allows for the identification of mutations, gene expression levels, and the presence of specific miRNAs in saliva (19).

Microarray is another technique used to measure the expression levels of multiple genes or miRNAs simultaneously. This approach uses a grid of tiny spots containing probes that bind to complementary genetic material in the sample, allowing for comprehensive profiling of gene or miRNA expression. Saliva is a suitable source for RNA extraction in microarray profiling, enabling the analysis of miRNA expression (20).

Real-time reverse transcription polymerase chain reaction (RT-PCR) is a laboratory method used to amplify and quantify specific RNA sequences. In the case of miRNAs, RT-PCR involves converting RNA into complementary DNA (cDNA) before amplification, allowing for the detection and precise measurement of miRNA levels in saliva (21). Together, these methods offer powerful tools for studying miRNAs in saliva, providing valuable insights into gene expression and potential biomarkers for disease diagnosis regarding oral health.

4.4. Salivary microRNA in Oral Squamous Cell Carcinoma

miRNAs play a crucial role in the development of OSCC due to their proximity to chromosomal abnormalities and their altered expression in a wide range of tumors. The overproduction of specific miRNAs can inhibit tumor suppressor genes, while the decreased expression of others can activate oncogenes, driving tumor progression (18).
Numerous studies have evaluated miRNAs as diagnostic biomarkers for OSCC, identifying differentially expressed miRNAs that could serve as potential indicators of the disease. Among the diagnostic tools available for OSCC, saliva stands out as particularly relevant since the tumors are directly in contact with the oral cavity and, therefore, with the saliva. This close interaction allows for the detection of both cellular and molecular changes that are linked to the presence of cancer. As a result, this makes saliva an ideal

medium for identifying miRNA biomarkers linked to OSCC, offering a promising tool for early detection and monitoring of the disease.

Extracellular miRNAs are released from cancer cells into body fluids through various mechanisms, including vesicle trafficking and the involvement of protein and lipid carriers. Once in the body fluids, these miRNAs have a significant role in regulating critical cellular processes, such as cell growth, movement, invasion, and the formation of new blood vessels, also known as angiogenesis. Depending on their specific functions, these miRNAs can either act as oncogenes, thereby promoting the progression of cancer, or as tumor suppressors, which work to inhibit the cancerous processes. What makes miRNAs particularly interesting is that their expression patterns are not only specific to the type of cancer but also vary depending on the tissue in which they are expressed. Furthermore, miRNAs exhibit a remarkable resistance to degradation by enzymes, which contributes to their stability in body fluids. This inherent stability ensures that the mature forms of miRNAs remain intact and detectable in bodily fluids, making them highly reliable biomarkers for cancer detection (17).

Considering the destructive behavior of OSCC and its poor prognosis, an early detection method is critical for improving effective treatment outcomes and patient survival. Early detection is essential, as it can significantly improve treatment outcomes as well as the long-term survival rates of patients. Taking this into account, several studies have been conducted to evaluate the potential of miRNAs as diagnostic biomarkers for OSCC. The aim of this systematic review is to combine the information gathered from multiple articles, that have identified various miRNAs with differential expression patterns, comparing values in patients with OSCC to healthy individuals.

5. Justification

This systematic review has focused on developing a specific approach to promote good health and well-being regarding the third sustainable development goal (SDG 3), which emphasizes the importance of improving health outcomes and reducing the burden of diseases, including cancer (22). This review is centered on determining the differential expression profile of salivary miRNAs in OSCC patients and healthy controls. Salivary miRNAs have emerged as promising biomarkers for the early detection and monitoring of OSCC, offering significant potential in improving both diagnosis and prognosis (17). These biomarkers provide molecular-level insights into disease progression and offer a non-invasive, painless, and easily accessible alternative to traditional diagnostic methods like tissue biopsies, which can be invasive, costly, and uncomfortable for patients (23).

By integrating available empirical research, this review seeks to determine whether specific salivary miRNAs hold promise to facilitate the earlier diagnosis of OSCC during those disease stages that are prone to effective intervention. The proposed methods of obtaining OSCC diagnostic molecular markers with the use of saliva are affordable and available (18). Thus, it contributes to the third SDG of ensuring healthy life and well-being for all. Early testing of clinical samples using salivary miRNAs is likely to yield more favorable clinical outcomes due to early treatment and increased survival chances.

6. Hypothesis

In patients with OSCC we hypothesize that salivary miRNA profiles will exhibit distinct alterations compared to those in healthy controls. Specifically, we anticipate that the differential expression of salivary miRNAs associated with OSCC pathogenesis will serve as potential biomarkers for enabling the early detection and diagnosis.

7. Objectives

General objective: Identify the most significant miRNA biomarkers in the early detection of oral squamous cell carcinoma by systematically reviewing and analizing existing literature comparing affected and healthy patients.

Specific objectives:

1. Identify and assess salivary miRNA with potential diagnostic value for early OSCC detection
2. Comparison of miRNA expression profiles between OSCC patients and healthy individuals.
3. Comparison of subjects with intermediate phenotypes OPMD, OLP, OSCC-R

8. Material and Methods

This article follows the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) (24) framework to ensure a transparent, structured, and reliable research process. The aim is to identify and evaluate microRNAs that show significant differences or changes, by focusing on the latest studies examining salivary miRNA profiles in patients with OSCC compared to healthy individuals. By carefully designing search strategies, assessing study eligibility, and extracting data systematically, this review highlights key microRNAs that could enhance the diagnosis of OSCC.

8.1 Identification of the PICO question

Articles with scientific relevance regarding the application of salivary miRNA in diagnosing oral squamous cell carcinoma were identified using the biomedical online databases PubMed, Scopus, and Web of Science. The search included publications indexed between January 2014 and January 2025, addressing the following research question: In patients with oral squamous cell carcinoma (P), how effective is the analysis of salivary microRNAs for early detection (I), compared to healthy individuals without carcinoma (C), in distinguishing between both groups (O)?

This research question was structured using the PICO framework (Population, Intervention, Comparison, Outcome) and was formulated as follows:

P: Patients with oral squamous cell carcinoma (OSCC)

I: Detection of salivary microRNA

C: Healthy patients without OSCC

O:

O1: Ability to differentiate between OSCC patient and healthy individuals for early diagnosis;

O2: Comparison of subjects with intermediate phenotypes OPMD, OLP, OSCC-R

8.2 Source of information and data base

A separate search was conducted on each of the selected platforms to gather articles that would address the PICO question and objectives. For all databases, a language filter was applied, restricting results to English-language articles. Additionally, the search was limited to articles published between January 2014 and December 2024. Boolean operators, such as ‘AND’ and ‘OR’ were utilized to combine the relevant keywords.

8.3 Inclusion and exclusion criteria

The following criteria were applied to choose the articles.

Inclusion Criteria:

1. Articles in English
2. Articles available in full text
3. Studies performed in Humans
4. Article from years 2014-2024

Exclusion criteria:

1. Bibliographic reviews,
2. Editorial material and Letters
3. Systematic reviews and Meta-Analysis

4. Animal studies
5. microRNA extracted from serum or tissue biopsies
6. Articles older from 2013 or older

8.4 Search Strategy

An automated search was carried out in the three databases Pub-Med, Scopus and Web of Science with the following keywords: (salivary microrna), (microRNA), (miRNA), (biomarker), (salivary biomarker), (oral squamous cell carcinoma), (oral carcinoma), (oral cancer), (oscc), (healthy control), (early diagnosis), (early detection). The keywords were combined with the Boolean operators AND, OR and NOT.

- On Pubmed the search was (saliva) AND ((microrna) OR (mirna)) AND ((oral squamous cell carcinoma) OR (oscc) OR (oral carcinoma) OR (oral cancer)) AND (healthy control) With this search, 44 articles were found.

- On Scopus the search included: (TITLE-ABS-KEY (salivary AND microrna) OR TITLE-ABS-KEY (salivary AND biomarker) AND TITLE-ABS-KEY (microrna) OR TITLE-ABS-KEY (mirna) AND TITLE-ABS-KEY (oral AND squamous AND cell AND carcinoma) OR TITLE-ABS-KEY (oral AND cancer) OR TITLE-ABS-KEY (oral AND carcinoma) AND TITLE-ABS-KEY (early AND diagnosis) OR TITLE-ABS-KEY (early AND detection)). With this search, 62 articles were found.

- On the Web of Science, the search was, ALL=((saliva*) and ((microrna*) or (mirna*)) and (("oral carcinoma") or ("oral squamous cell carcinoma") or ("oral cancer")) and (("early detection") or ("early diagnosis"))) With this search, 59 articles were found.

8.5 Study selection process

A three-stage selection process was implemented. During the first stage, the titles of articles were reviewed to remove those that were irrelevant for this systematic review. In the second stage, both titles and abstracts were assessed and filtered based on the study type and language. In the third stage, each article was read, and the data was taken according to the eligibility to be included in the systematic review.

8.6 Data base extraction

To obtain the results from the search the following criteria were included: authors, type of study, year of publication, language, patients with oral squamous cell carcinoma and healthy controls, salivary miRNA, and inclusion and exclusion criteria.

Main variables:

- Expression levels of salivary miRNA: The quantification of specific miRNA biomarkers in saliva, comparing their expression levels between OSCC patients and healthy controls. The measurement must be reported using standardised molecular techniques such as qRT-PCR, microarrays, or sequencing.
- Comparison between OSCC patients and healthy controls (differences in miRNA expression profiles).

Secondary variables:

- Comparison of salivary and tissue miRNA expression: Assessing whether miRNA expression in saliva reflects miRNA expression in OSCC tissue samples, based on paired analysis.
- Patient characteristics (age, gender, risk factors).
- Methods of miRNA detection (qRT-PCR, microarrays, sequencing).

8.7 Quality assessment

The assessment of the risk of bias was conducted by two reviewers (EN, ILR) to analyze the methodological quality of the included articles. Different tools were used depending on the study design. For the included observational cohort and cross-sectional studies, the NIH Quality Assessment Tool for Observational Cohort and Cross-Sectional (25). Studies was used. This checklist includes 14 questions that address various aspects of methodological rigor, such as the clarity of research objectives, population definition, exposure measurement, outcome assessment, confounding control, and statistical analysis. Each item was rated as “Yes,” “No,” or “Other” (which includes “Cannot Determine (CD),” “Not Applicable (NA),” or “Not Reported (NR)”). The overall quality of each study was judged based on the number and relevance of criteria fulfilled.

For the case-control studies, the JBI critical appraisal checklist was used (26). After addressing the checklist questions with responses like “Yes”,”No”, or “Can't tell”, the studies were classified based on their risk of bias as low, medium, or high. The Quality Assessment of Diagnostic Accuracy Studies version 2 (QUADAS-2) was applied to one observational diagnostic study. Answers were recorded as: “Yes” (low risk of bias), “No” (high risk of bias) or “Unclear” (insufficient information) (27).

9. Results

9.1 Study selection and flow-chart

A total of 97 articles were obtained from the initial search process: PubMed (n=44), Scopus (n=62) and Web of Science (n=59). Of these publications (129) were identified as potentially eligible articles by screening the titles and abstracts. Full text articles were subsequently obtained and thoroughly evaluated. As a result, (14) articles met the criteria for inclusion and were included in this systematic review (Fig.2). The information related to the excluded articles and the reason for their exclusion is presented in Table 2.

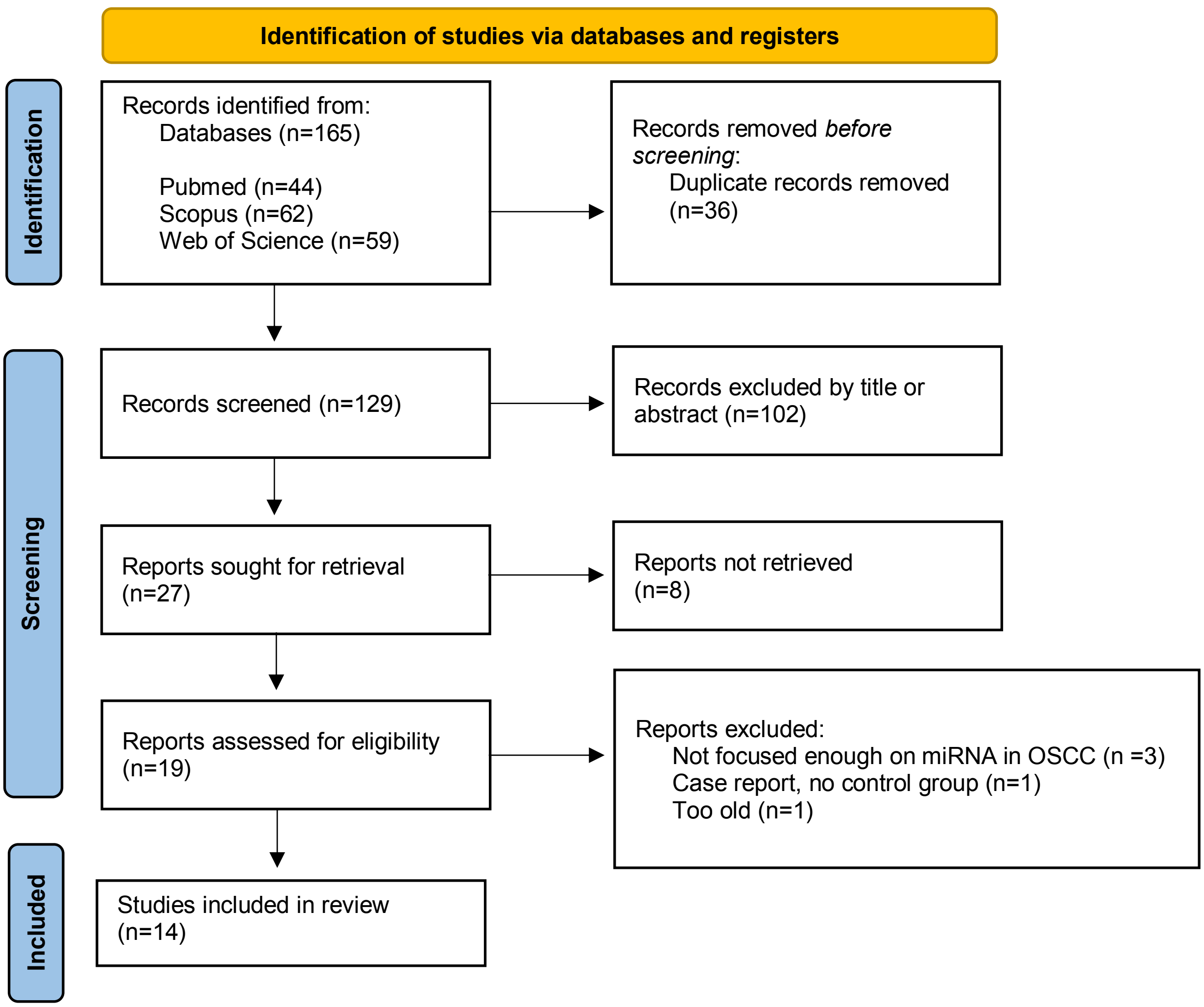

Figure 2: PRISMA Flow Chart of searching and selection process during the Systematic review

Table 2: Excluded studies and reasons for their exclusion from the present systematic review.

Author	Publication	Reason for exclusion	Reference
Kimura et al.	*Spandidos Publication*	Case study, no healthy controls	(28)
Mehdipour et al.	*BMC Oral Health*	Not focussed enough on OSCC	(29)
Park et al.	*Clinical Cancer Research*	Too old (10 > years)	(30)
Romani et al.	*Clinica Chimica Acta*	Not focussed enough on OSCC	(31)
Scheurer et al.	*Journal of Cranio-Maxillo-Facial Surgery*	Focussed on technical and biological reproducibility of different analytical procedures for salivary miRNA detection	(32)

(OSCC = Oral squamous cell carcinoma, miRNA = microRNA)

9.2 Analyisis of characteristics of the reviewed studies

This systematic review encompassed 14 articles that collectively investigated the diagnostic potential of salivary miRNAs as potential biomarkers for the early detection of OSCC. These studies together included a total of 914 participants, of which 483 were diagnosed with OSCC and 325 were healthy individuals without any oral mucosal pathology (Table 3). The remaining 106 participants were categorized into other relevant clinical groups, including those with OPMD, such as leukoplakia and erythroplakia, patients diagnosed with OLP, and individuals with a history of OSCC-R at the time of sampling.

While the primary comparison in most studies was between OSCC patients and healthy individuals, the inclusion of subjects with intermediate clinical phenotypes, such as OPMD and OLP, in several studies provided valuable insights into the dynamic regulation of miRNAs during oral carcinogenesis (Table 4). These comparative cohorts were essential in distinguishing miRNAs that are altered early in the malignant transformation process from those that become dysregulated only in later stages of tumor development. Additionally, the inclusion of OSCC-R participants in one study allowed for the investigation of miRNA signatures that are specific to active disease and not simply residual effects of treatment or prior malignancy.

This wide-ranging study population provided a valuable framework for comparative analysis, allowing for the exploration of miRNA expression profiles not only in overt malignancy but also across precancerous and post-treatment clinical states. Such an approach supports the translational goal of identifying miRNAs that can serve as early indicators of malignant transformation or recurrence.

Various diagnostic techniques were employed across the reviewed studies to measure and confirm miRNA expression levels. The most widely used approach was real-time quantitative polymerase chain reaction (qPCR), appreciated for its high sensitivity, specificity, and effectiveness in detecting low concentrations of salivary miRNAs. In addition, several studies incorporated microarray technology and next-generation sequencing (NGS) to facilitate high-throughput analysis and the identification of differentially expressed miRNAs. Notably, Momen-Heravi et al. (33) combined the NanoString nCounter platform with qPCR for validation, providing a comprehensive and scalable strategy for miRNA profiling. These advanced molecular methods played an essential role in ensuring the accuracy and reproducibility of miRNA-based diagnostics and reinforced their potential as non-invasive biomarkers for OSCC (Table 3).

Most of the articles were designed as case-control studies, typically comparing salivary miRNA expression levels between OSCC patients and healthy controls. Others adopted observational or prospective cohort designs, which enabled serial or stratified analysis based on disease progression or histological grading. Sample sizes across studies ranged from 25 to 116 participants, reflecting both focused pilot investigations and broader validation efforts. Despite this variability, all studies shared a common objective, which was to assess the diagnostic performance, clinical relevance, and biological plausibility of specific miRNAs or panels of miRNAs in identifying OSCC from non-malignant conditions using a non-invasive, saliva-based approach.

Table 3: Study characteristics of all 14 included studies

Author	Reference	Type of study	Participants	OSCC patients	Healthy controls	Method	microRNA
Romani et al., 2021	(39)	Case-control	116	58	58	miRNeasy Mini kit + RT-qPCR	miR-423-5p, miR-106b-5p, miR-193b-3p
Yap et al., 2018	(41)	Case-control	60	30	30	mirVana Kit + RT-qPCR	miR-31, miR-21, miR-99a, let-7c, miR-125b, miR-100
Rocchetti et al., 2024	(42)	Prospective cohort	25	14	5	RT-qPCR	miR-21, miR-31, miR-138, miR-145, miR-184, miR-424
Scholtz et al., 2022	(36)	Case-control	87	43	44	RT-qPCR	miR-31-5p, miR-345-3p, miR-424-3p
Tarrad et al., 2023	(40)	Observational diagnostic	36	12	12	RT-qPCR	miR-106a
Vageli et al., 2023	(43)	Case-control	44	23	21	RT-qPCR	miR-21, miR-136, miR-3928, miR-29B
Gai et al., 2023	(38)	Case-control	32	21	11	EV isolation + RT-qPCR	miR-302b-3p, miR-517b-3p, miR-512-3p, miR-412-3p
Di Stasio et al., 2022	(35)	Cohort	43	10	10	RT-qPCR	miR-21, miR-27b, miR-181b
Garg et al., 2023	(37)	Case-control	90	30	30	RT-qPCR	miR-21, miR-184
Mehterov et al., 2021	(44)	Case-control	45	33	12	TaqMan RT-qPCR	miR-30c-5p
Farshbaf et al., 2024	(45)	Cross-sectional	91	31	30	RT-qPCR	miR-3928
He et al., 2020	(46)	Case-control	59	45	14	Exosome isolation + RT-qPCR	miR-24-3p
Momen-Heravi et al., 2014	(33)	Cross-sectional	34	9	9	NanoString + RT-qPCR	miR-27b, miR-24
Patel et al., 2023	(34)	Exploratory + validation	70	50	20	RNASeq + RT-qPCR	miR-140-5p, miR-143-5p, miR-145-5p

(miR/miRNA = microRNA; OSCC = Oral squamous cell carcinoma; RT-qPCR = Reverse Transcription quantitative Polymerase Chain Reaction; TaqMan RT-qPCR = TaqMan Reverse transcription qPCR; RNASeq = RNA sequencing; EV isolation = Extracellular vesicle isolation)

Table 4: miRNAs in OSCC, OPMD, OLP and OSCC-R in comparison

Author	Reference	Healthy controls	OSCC	OPMD	OLP	OSCC-R	miRNA
Garg et al., 2023	(37)	30	30	30 (15 leukoplakia, 15 OSMF)	-	-	miR-21 miR-184
Di Stasio et al., 2022	(35)	10	10	23	included in OPMD	-	miR-21 miR-27b miR-181b
Farshbaf et al., 2024	(45)	30	31	-	30	-	miR-3928
Momen-Heravi et al., 2014	(33)	9	9	-	8	8	miR-27b miR-136
Rocchetti et al., 2024	(42)	5	14	6	included in OPMD	-	miR-138 miR-424

(miR/miRNA = microRNA; OSCC = Oral squamous cell carcinoma; OPMD = Oral potentially malignant disorders; OLP = Oral lichen planus; OSMF = Oral submucous fibrosis; OSCC-R = Oral squamous cell carcinoma patient in remission)

9.3 Sensitivity and specificity analysis

To assess diagnostic reliability, we extracted sensitivity and specificity data from the included studies. Most reported strong performance for salivary miRNAs in detecting OSCC. For example, Patel et al. (34) found a 3-miRNA panel (miR-143, miR-145, miR-140) with 98% sensitivity and 99% specificity. Di Stasio et al. (35) reported 94.1% sensitivity and 81.2% specificity for miR-181b, while Scholtz et al. (36) observed 86% sensitivity and 77% specificity for a miRNA panel. Momen-Heravi et al. (33) reported high accuracy for miR-27b with AUC values above 0.96. Garg et al. (37) found miR-21 and miR-184 yielded sensitivities and specificities around 70–80%. Gai et al. (38), while not reporting exact values, presented strong AUCs (0.847–0.871), further supporting the diagnostic value of miRNAs (Table 5). Several other studies included in this review did not report specific sensitivity or specificity values, and were therefore excluded from this analysis.

Table 5: Sensitivity and specificity analysis

Author	Reference	Sensitivity	Specificity
Patel et al. (2023)	(34)	98 %	99 %
Di Stasio et al. (2022)	(35)	94.1%	81.2%
Romani et al. (2021)	(39)	97.4%	94.2%
Momen-Heravi et al. (2014)	(33)	85.71–88.89%	83.33–100%
Scholtz et al. (2022)	(36)	86 %	77 %
Garg et al. (2023)	(37)	80 %	70 %
Tarrad et al. (2023)	(40)	100 %	70.8%–75%
Gai et al. (2023)	(38)	AUC-based: 0.847–0.871	AUC-based: High

(AUC = area under curve)

9.4 Assesment of methodological quality and risk of bias

For the case-control studies, the JBI checklist was applied. After addressing the 10 checklist questions with responses like “Yes”, “No”, “Unclear” or “Not Applicable”, the studies were classified based on their risk of bias as low, medium, or high. For the included observational cohort and cross-sectional studies, the NIH quality assessment tool was used. This checklist includes 14 questions that address various aspects of methodological rigor, such as the clarity of research objectives, population definition, exposure measurement, outcome assessment, confounding control, and statistical analysis. Each item was rated as “Yes,” “No,” or “Other” (“Cannot determine (CD),” “Not applicable (NA),” or “Not eported (NR)”). QUADAS-2 domain was applied to an observational diagnostic study. Answers were recorded as: Yes, No, or Unclear (insufficient information).

9.4.1 Case-control studies

The JBI critical appraisal checklist was used to assess the 9 case-control studies from this systematic review. In the following, the 10 questions from the checklist wil be answered in Table 5 with Yes, No, Unclear or Not applicable. The combined results indicate a low-moderate overall risk of bias due to a mix of mostly strong methodology quality, some unclear or missing reporting, a few critical "No" judgments (Table 6).

1) Were the groups comparable other than the presence of disease in cases or the absence of disease in controls?
2) Were cases and controls matched appropriately?
3) Were the same criteria used for identification of cases and controls?
4) Was exposure measured in a standard, valid and reliable way?
5) Was exposure measured in the same way for cases and controls?
6) Were confounding factors identified?
7) Were strategies to deal with confounding factors stated?
8) Were outcomes assessed in a standard, valid and reliable way for cases and controls?
9) Was the exposure period of interest long enough to be meaningful?
10) Was appropriate statistical analysis used?

Table 6: JBI-checklist for case-control studies

Criteria	Romani et al. 2021 (39)	Yap et al. 2018 (41)	Scholtz et al. 2022 (36)	Vageli et al. 2023 (43)	Gai et al. 2023 (38)	Garg et al. 2023 (37)	Mehterov et al. 2021 (44)	He et al. 2020 (46)	Patel et al. 2023 (34)
1.	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
2.	Yes	Yes	Yes	Yes	No	Yes	Yes	No	Yes
3.	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
4.	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
5.	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
6.	Yes	Unclear	Unclear	Yes	Yes	Yes	No	Unclear	Unclear
7.	Yes	No	Unclear	Yes	Unclear	Yes	No	Unclear	No
8.	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
9.	Not applicable	Not applicable	Not applicable	Not applicable	Not applicable	Not applicable	Not applicable	Not applicable	Not applicable
10.	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

9.4.2 Cohort and cross-sectional studies

The NIH Quality Assessment Tool for Observational Cohort and Cross-Sectional Studies checklist includes 14 questions that are listed below. The overall quality of each study was judged based on the number and relevance of criteria fulfilled. Di Stasio et al. (35) was rated as having a low risk of bias, as it clearly defined its study population, provided a sample size justification, reported a high participation rate, and demonstrated consistency in exposure and outcome measurements. Rocchetti et al. (42) and Farshbaf et al. (45) were judged to have a moderate risk of bias, primarily due to a lack of reporting on sample size calculations, confounding variable adjustment, and temporality between exposure and outcome. Momen-Heravi et al. (33) was assessed as having a high risk of bias, as several key criteria, including participation rate, control of confounding, and blinding, were not reported or could not be determined (Table 7).

1) Was the research question or objective in this paper clearly stated?
2) Was the study population clearly specified and defined?
3) Was the participation rate of eligible persons at least 50%?
4) Were all the subjects selected or recruited from the same or similar populations (including the same time period)? Were inclusion and exclusion criteria for being in the study prespecified and applied uniformly to all participants?
5) Was a sample size justification, power description, or variance and effect estimates provided?
6) For the analyses in this paper, were the exposure(s) of interest measured prior to the outcome(s) being measured?
7) Was the timeframe sufficient so that one could reasonably expect to see an association between exposure and outcome if it existed?
8) For exposures that can vary in amount or level, did the study examine different levels of the exposure as related to the outcome (e.g., categories of exposure, or exposure measured as continuous variable)?
9) Were the exposure measures (independent variables) clearly defined, valid, reliable, and implemented consistently across all study participants?
10) Was the exposure(s) assessed more than once over time?

11) Were the outcome measures (dependent variables) clearly defined, valid, reliable, and implemented consistently across all study participants?
12) Were the outcome assessors blinded to the exposure status of participants?
13) Was loss to follow-up after baseline 20% or less?
14) Were key potential confounding variables measured and adjusted statistically for their impact on the relationship between exposure(s) and outcome(s)?

Table 7: NIH Quality assesment for cohort and cross-sectional studies

Criteria	Rocchetti et al., 2024 (42)	Di Stasio et al., 2022 (35)	Farshbaf et al., 2024 (45)	Momen-Heravi et al., 2014 (33)
1.	Yes	Yes	Yes	Yes
2.	Yes	Yes	Yes	Yes
3.	Yes	Yes	Yes	CD
4.	Yes	Yes	Yes	Yes
5.	No	Yes	No	No
6.	CD	CD	CD	CD
7.	CD	CD	CD	CD
8.	CD	Yes	No	No
9.	Yes	Yes	Yes	Yes
10.	No	No	No	No
11.	NA	NA	NA	NA
12.	CD	CD	CD	CD
13.	NA	NA	NA	NA
14.	No	No	No	No

(CD = Cannot Determine NA = Not Applicable, NR = Not Reported)

9.4.3 Observational studies

The Quality Assessment of Diagnostic Accuracy Studies Version 2 (QUADAS-2). It is a standardized tool used in systematic reviews to assess the risk of bias and applicability of diagnostic accuracy studies. Each domain is assessed for risk of bias and concerns about applicability (how relevant the study is to your research question). Answers are recorded as: Yes (low risk of bias), No (high risk of bias) or Unclear (insufficient information). Based on the QUADAS-2 tool and the content of Tarrad et al. (40), the study appears to present a low-moderate risk of bias overall (Table 8).

Table 8: QUADAS-2 checklist for observational diagnostic study

Tarrad et al. (2023) (40)				
Domain	Question	Answer	Risk of Bias	Concerns about Applicability
Patient Selection	Was a consecutive or random sample of patients enrolled?	Yes	Low	Low
	Was a case-control design avoided?	No	High	High
	Did the study avoid inappropriate exclusions?	Yes	Low	Low
Index Test	Were the index test results interpreted without knowledge of the results of the reference standard?	Unclear	Unclear	Unclear
	If a threshold was used, was it pre-specified?	No	High	High
Reference Standard	Is the reference standard likely to correctly classify the target condition?	Yes	Low	Low
	Were the reference standard results interpreted without knowledge of the results of the index test?	Unclear	Unclear	Unclear
Flow and Timing	Was there an appropriate interval between index tests and reference standard?	Yes	Low	Low
	Did all patients receive a reference standard?	Yes	Low	Low
	Did all patients receive the same reference standard?	Yes	Low	Low
	Were all patients included in the analysis?	Yes	Low	Low

9.5 Synthesis of result

9.5.1 Salivary miRNA expression in OSCC compared to healthy controls

A total of 37 unique differentially expressed miRNAs were reported by the 14 studies, with miR-21 and miR-31 being the most frequently examined miRNAs found to be differentially expressed. Four of these differentially expressed miRNAs (11%) were reported in at least three studies, while the others have only been reported in one or two studies (Table 9). A big focus of all included studies was the comparison of salivary miRNA expression in OSCC patients versus healthy controls. The findings consistently revealed distinct patterns of miRNA dysregulation in OSCC, with several miRNAs being repeatedly identified as either upregulated (oncogenic) or downregulated (tumor-suppressive). In table 9, the results from all 14 studies are included.

Among the most frequently reported salivary miRNAs, miR-21 consistently emerged as the most upregulated in OSCC patients, confirmed across five studies (Yap et al. (41); Rocchetti et al. (42); Vageli et al. (43); Garg et al.(37); Di Stasio et al. (35)). Garg et al. (37) reported a 3.7-fold increase, and Vageli et al. noted even higher levels among smokers, suggesting lifestyle-linked modulation. Known for promoting tumor growth, invasion, and apoptosis resistance, miR-21 exemplifies a robust oncogenic biomarker. miR-31 was also frequently upregulated, as shown in studies by Yap et al. (2018), Rocchetti et al. (42) and Scholtz et al. (36) and was part of high-performance multi-miRNA panels. He et al. (46) identified a 5.73-fold increase in miR-24-3p expression in OSCC salivary exosomes, linking it to enhanced cell proliferation and reporting an AUC of 0.738. miR-423-5p, highlighted by Romani et al. (39), showed a log2 fold change of 1.34, with strong diagnostic performance (AUC = 0.98) and prognostic relevance due to its association with shorter disease-free survival.

Conversely, tumor-suppressive miRNAs were consistently downregulated. miR-138 and miR-424 were significantly reduced in OSCC saliva, particularly in studies by Rocchetti et al. (42) and Scholtz et al. (36). miR-30c-5p was notably decreased in OSCC patients, as shown by Mehterov et al. (44), with an AUC of 0.82. Garg et al. (37) reported that miR-184 expression dropped by 66% in OSCC patients, supporting findings by Rocchetti et al. (42). Although miR-184 appears consistently suppressed in OSCC, its role may vary depending on tumor stage or coexisting inflammation (Table 10). These quantifiable expression differences underscore the clinical relevance of salivary miRNAs as reliable, non-invasive biomarkers for early OSCC detection.

miR-21, miR-31, miR-423-5p, miR-138, and miR-106a showed statistically significant p-values in multiple studies, highlighting their potential as non-invasive biomarkers for the early detection of OSCC. For instance, Romani et al. (39) reported a highly significant p-value ($p < 0.001$) for miR-423-5p, while Tarrad et al. (40) observed p-values of 0.02 and 0.03 for miR-106a and LINC00657, respectively. Similarly, Scholtz et al. (36) reported significant upregulation of miR-31-5p and miR-424-3p with corresponding p-values below 0.05 (Table 12).

Interestingly, a few studies have reached different conclusions about the expression patterns of key miRNAs.

For example, Momen-Heravi et al. (33) reported that miR-27b was significantly overexpressed in the saliva of OSCC patients, pointing to its promise as a diagnostic biomarker. On the other hand, Di Stasio et al. (35) found miR-27b to be under-expressed in dysplastic lesions and not significantly different between OSCC patients and healthy controls, which challenges its potential diagnostic value.

Garg et al. (37) observed that miR-184 was reduced in both OSCC and OPMD compared to healthy controls, suggesting a tumor-suppressive role. The same article cites earlier studies that showed miR-184 was overexpressed in OSCC, highlighting its possible oncogenic function.

miR-424 offers another example of this inconsistency. Scholtz et al. (36) noted that while some earlier tissue studies showed increased expression of miR-424 in OSCC, their own salivary data revealed a decrease in expression in OSCC patients, suggesting that salivary levels may not always mirror tissue findings. Regarding miR-181b, Di Stasio et al. (35) found it to be upregulated in high-grade dysplasia but reduced again in OSCC, indicating its expression may vary depending on the stage of disease progression.

Table 9: Reported salivary miRNAs in OSCC: Study frequency and expression trends

miRNA	Authors	Frequently Reported	Expression in OSCC	Study Count
miR-21	Yap et al.; Rocchetti et al.; Vageli et al.; Garg et al.; Di Stasio et al.	Yes	Upregulated in OSCC	5
miR-31	Yap et al.; Rocchetti et al.; Scholtz et al.	Yes	Upregulated in OSCC	3
miR-423-5p	Romani et al.; Patel et al.	Yes	Upregulated in OSCC	2
miR-138	Rocchetti et al.; Scholtz et al.; Momen-Heravi et al.	Yes	Downregulated in OSCC	3
miR-106a	Tarrad et al.	No	Downregulated in OSCC	1
miR-24-3p	He et al.	No	Upregulated in OSCC	1
miR-31-5p	Scholtz et al.	No	Upregulated in OSCC	1
miR-345	Scholtz et al.	No	Upregulated in OSCC	1
miR-424-3p	Scholtz et al.	No	Upregulated in OSCC	1
miR-140	Patel et al.	No	Downregulated in OSCC	1
miR-143	Patel et al.	No	Downregulated in OSCC	1
miR-145	Patel et al.	No	Downregulated in OSCC	1
miR-30a	Patel et al.	No	Downregulated in OSCC	1
let-7i	Patel et al.	No	Downregulated in OSCC	1
miR-412-3p	Gai et al.	No	Upregulated in OSCC	1
miR-489-3p	Gai et al.	No	Upregulated in OSCC	1
miR-512-3p	Gai et al.	No	Upregulated in OSCC	1
miR-597-5p	Gai et al.	No	Upregulated in OSCC	1
miR-603	Gai et al.	No	Upregulated in OSCC	1
miR-27b	Momen-Heravi et al.	No	Upregulated in OSCC	1
miR-30c-5p	Mehterov et al.	No	Downregulated in OSCC	1
miR-106b-5p	Romani et al.	No	Downregulated in OSCC	1
miR-193b-3p	Romani et al.	No	Upregulated in OSCC	1
miR-184	Garg et al.; Scholtz et al.	No	Downregulated in OSCC	2
miR-191	Scholtz et al.	No	Upregulated in OSCC	1
miR-484	Gai et al.	No	Downregulated in OSCC	1
miR-720	Gai et al.	No	Downregulated in OSCC	1
miR-376c-3p	Gai et al.	No	Downregulated in OSCC	1
miR-27a-3p	Gai et al.	No	Upregulated in OSCC	1
miR-302b-3p	Gai et al.	No	Upregulated in OSCC	1
miR-337-5p	Gai et al.	No	Upregulated in OSCC	1
miR-373-3p	Gai et al.	No	Upregulated in OSCC	1
miR-494-3p	Gai et al.	No	Upregulated in OSCC	1
miR-517b	Gai et al.	No	Upregulated in OSCC	1
miR-520d-3p	Gai et al.	No	Upregulated in OSCC	1
miR-645	Gai et al.	No	Upregulated in OSCC	1
miR-125a	Mehterov et al.	No	Not significant	1

(miR/miRNA = microRNA; OSCC = Oral squamous cell carcinoma)

Table 10: Summary of salivary microRNA expression patterns and clinical relevance in OSCC vs. healthy controls

Author	Comparison	Upregulated miRNA	Downregulated miRNA	Clinical Relevance
Romani et al. (39)	Healthy vs OSCC	miR-423-5p, miR-106b-5p, miR-193b-3p		miR-423-5p overexpression associated with poor prognosis; AUC = 0.98
Yap et al. (41)	Healthy vs OSCC	miR-31, miR-21, miR-100	miR-99a, miR-125b, let-7c	miRNA panel showed high diagnostic accuracy (AUC = 0.95)
Rocchetti et al. (42)	Healthy vs OSCC	miR-21, miR-31	miR-138, miR-145, miR-424, miR-184	miR-138 and miR-424 as early suppressive biomarkers
Scholtz et al. (36)	Healthy vs OSCC	miR-31-5p, miR-345-3p	miR-424-3p	3-miRNA panel showed high discrimination (AUC = 0.87)
Tarrad et al. (40)	Healthy vs OSCC		miR-106a	miR-106a downregulation correlated with higher grade OSCC
Vageli et al. (43)	Healthy vs OSCC	miR-21, miR-136, miR-3928, miR-29B		miR-21 elevated in smokers; early OSCC marker
Gai et al. (38)	Healthy vs OSCC	miR-302b-3p, miR-517b-3p, miR-512-3p, miR-412-3p		miRNAs enriched in salivary EVs from OSCC patients
Di Stasio et al. (35)	Healthy vs OSCC		miR-27b, miR-181b	miR-181b up in high-grade dysplasia, down in OSCC
Garg et al. (37)	Healthy vs OSCC	miR-21	miR-184	Both miRNAs altered in OPMD and OSCC; early markers
Mehterov et al. (44)	Healthy vs OSCC		miR-30c-5p	Downregulated miR-30c-5p shows diagnostic value (AUC = 0.82)
Farshbaf et al. (45)	Healthy vs OSCC		miR-3928	Downregulation observed in OSCC and OLP; potential early biomarker
He et al. (46)	Healthy vs OSCC	miR-24-3p		Exosomal miR-24-3p promotes OSCC cell proliferation (AUC = 0.738)
Momen-Heravi et al. (33)	Healthy vs OSCC	miR-27b, miR-24		miR-27b specific to active OSCC, not in remission or OLP
Patel et al. (34)	Healthy vs OSCC		miR-140-5p, miR-143-5p, miR-145-5p	3-miRNA signature linked to EMT and prognosis

(miR/miRNA = microRNA; OSCC = Oral squamous cell carcinoma; OPMD = Oral potentially malignant disorders; OLP = Oral lichen planus; EV = Extracelluar vesicles; EMT = Epithelial-mesenchymal transition; AUC = Area under curve)

Table 11: Summary of frequently studied salivary miRNAs in OSCC: Expression trends, significance, and reported diagnostic accuracy across studies

miRNA	Authors	p-value	Expression in OSCC	Expression in healthy control	Diagnostic accuracy
miR-21	Scholtz et al., Di Stasio et al., Garg et al., Yap et al., Rocchetti et al., Romani et al.	0.945 (Di Stasio et al.)	↑	–	AUC = 0.95
miR-27b	Di Stasio et al., Gai et al., Momen-Heravi et al.	0.042 (Di Stasio et al.)	↑ in dysplasia	↓	ROC = Strong
miR-181b	Di Stasio et al.	0.006	↑ in dysplasia	↓	Not reported
miR-31	Scholtz et al., Rocchetti et al., Yap et al.	< 0.001 (Scholtz et al.)	↑	↓	AUC = 0.95
miR-345	Scholtz et al.	< 0.0001	↑	↓	AUC = 0.87
miR-424	Scholtz et al., Rocchetti et al.	< 0.01	↓	↑	Not reported
miR-184	Scholtz et al., Rocchetti et al., Garg et al.	> 0.05	↓	↑	ROC plotted
miR-191	Scholtz et al., Momen-Heravi et al.	Not significant	–	–	–
miR-106a	Tarrad et al., Romani et al.	< 0.05	↓	↑	AUC = 80.4%
miR-423-5p	Romani et al., Farshbaf et al.	< 0.001	↑	↓	AUC = 0.98
miR-138	Rocchetti et al.	< 0.05	↓	↑	Not reported
miR-145	Rocchetti et al., Patel et al.	Not specified	↓	↑	Functional validation (Patel et al.)
miR-3928	Vageli et al., Farshbaf et al.	< 0.05	↓	↑	Not reported
miR-24-3p	He et al., Yap et al., Momen-Heravi et al.	0.02	↑	↓	AUC = 0.738
miR-30c-5p	Mehterov et al., Yap et al.	0.04	↓	↑	AUC = 0.82
miR-125b	Yap et al.	< 0.01	↓	↑	Not reported
let-7c	Yap et al.	Not specified	↓	↑	AUC = 0.95

(miR/miRNA = microRNA; OSCC = Oral squamous cell carcinoma; AUC = Area under curve; ROC = Receiver operating characteristic; ↑ = upregulated; ↓ = downregulated; - = no information mentioned*)*

Table 12: Overview of included studies reporting differentially expressed salivary miRNAs in OSCC, with diagnostic performance and statistical significance

Study	miRNA(s) studied	Expression OSCC vs healthy control	p-value	Significant	Diagnostic accuracy
Romani et al. (39)	miR-423-5p, miR-106b	↑ OSCC	< 0.001	Yes	AUC = 0.98
Yap et al. (41)	miR-31, miR-21, let-7c	↑ miR-21/31 ↓ let-7c	< 0.01	Yes	AUC = 0.95
Scholtz et al. (36)	miR-31-5p, miR-345, miR-424	↑ OSCC	< 0.05	Yes	AUC = 0.87
Vageli et al. (43)	miR-21, miR-136, miR-3928	↑ OSCC, especially smokers	< 0.005	Yes	Not Reported
Gai et al. (38)	miR-512, miR-412, miR-302b	↑ OSCC EVs	< 0.01	Yes	ROC > 0.8 for miR-512
Farshbaf et al. (45)	miR-3928	↓ in OSCC/OLP	< 0.0001	Yes	Not Reported
He et al. (46)	miR-24-3p	↑ OSCC	0.02	Yes	AUC = 0.738
Di Stasio et al. (35)	miR-181b, miR-27b	↑ in dysplasia	0.006, 0.046	Yes	Not Reported
Garg et al. (37)	miR-21, miR-184	↑ miR-21 ↓ miR-184 OSCC	< 0.001	Yes	ROC plotted
Rocchetti et al. (42)	miR-138, miR-424	↓ in OSCC	< 0.05	Yes	Not Reported
Tarrad et al. (40)	miR-106a	↓ OSCC	< 0.05	Yes	AUC = 80.4%
Mehterov et al. (44)	miR-30c-5p	↓ OSCC	0.04	Yes	AUC = 0.82
Momen-Heravi et al. (33)	miR-27b, miR-24	↑ OSCC	< 0.01	Yes	ROC = Strong
Patel et al. (34)	miR-140, miR-143, miR-145	↓ OSCC	< 0.05	Yes	Functional validation too

(miR/miRNA = microRNA; OSCC = Oral squamous cell carcinoma; AUC = Area under curve; ROC = Receiver operating characteristic; ↑ = upregulated; ↓ = downregulated)

9.5.2 Comparison of miRNA expression in OSCC vs. other oral conditions

Beyond comparisons with healthy controls, five studies offered deeper insights by including participants with OPMD, OLP, or OSCC-R. These comparative data are crucial in understanding the progression of molecular changes across different stages of oral carcinogenesis. Garg et al. (37) evaluated salivary miRNA expression in OSCC, OPMD, and healthy individuals. Both miR-21 and miR-184 showed consistent trends across disease progression: miR-21 was upregulated and miR-184 downregulated in both OSCC and OPMD compared to controls, suggesting their early involvement in the malignant transformation cascade. Di Stasio et al. (35) provided a unique stratification of OPMD patients based on histopathological grading of dysplasia (low to high grade). Their results revealed that miR-181b levels increased with dysplasia severity but dropped significantly in OSCC, suggesting it may act as a transition-phase marker. In addition, miR-27b was reduced in dysplastic tissue, further indicating its early deregulation during pre-malignancy. Farshbaf et al. (45) studied miR-3928, finding it significantly downregulated in both OSCC and OLP relative to healthy controls. Since OLP is considered a potentially malignant condition, this suggests miR-3928 may serve as an early indicator of transformation risk. Momen-Heravi et al. (33) was the only study to include a remission group (OSCC-R). They demonstrated that miR-27b was markedly upregulated in active OSCC but remained unchanged in OLP and OSCC-R, suggesting its potential specificity as a marker of active disease rather than premalignancy or residual post-treatment changes. Rocchetti et al. (42) also confirmed that miR-138 and miR-424 were downregulated not only in OSCC but also in OPMD, reinforcing their possible role as early-stage suppressors and emphasizing their diagnostic relevance before clinically evident cancer, as demonstrated in Table 13.

Table 13: Summary of studies comparing salivary miRNA expression in OSCC, OPMD, OLP, and OSCC-R

Author	Compared Conditions	Key Findings
Garg et al. (37)	OSCC, OPMD, controls	↑ miR-21 ↓ miR-184 in OSCC & OPMD vs. controls
Di Stasio et al. (35)	OSCC, OPMD (graded dysplasia), controls	↑ miR-181b with dysplasia severity ↓ miR-181b in OSCC; ↓ miR-27b in dysplasia
Farshbaf et al. (45)	OSCC, OLP, controls	↓ miR-3928 in OSCC & OLP vs. controls
Momen-Heravi et al. (33)	OSCC, OSCC-R, OLP, controls	↑ miR-27b in OSCC only, unchanged in OLP & OSCC-R
Rocchetti et al. (42)	OSCC, OPMD, controls	↓ miR-138 & miR-424 in OSCC & OPMD vs. controls

(miR/miRNA = microRNA; OSCC = Oral squamous cell carcinoma; OPMD = Oral potentially malignant disorders; OLP = Oral lichen planus; OSCC-R = Oral squamous cell carcinoma patient in remission, ↑ = upregulated; ↓ = downregulated)

10. Discussion

This study has synthesized the available evidence on the differential expression of salivary miRNAs in patients with OSCC compared to healthy individuals. The findings consistently support the potential of these miRNAs as non-invasive biomarkers for the early detection of OSCC. In particular, recurrent patterns of upregulation were identified for oncogenic miRNAs such as miR-21, miR-31, miR-24-3p, and miR-423-5p, as well as downregulation of tumor-suppressive miRNAs including miR-138, miR-145, miR-424, miR-30c-5p, and miR-184.

Among the most consistently reported biomarkers was miR-21, noted in multiple studies (Yap et al. (41); Di Stasio et al. (35); Rocchetti et al. (42); Vageli et al. (43); Garg et al. (37); Scholtz et al. (36); Patel et al. (34)) for its significant upregulation in OSCC saliva. Its oncogenic role is well-established, promoting tumor proliferation and invasion through targets such as PTEN (phosphatase and tensin homolog) and RECK (reversion-inducing cysteine-rich protein with Kazal motifs). Notably, Vageli et al. (43) highlighted miR-21's stronger expression in OSCC smokers, suggesting its dual utility as both a diagnostic and risk stratification biomarker. This emphasizes the importance of accounting for patient lifestyle factors (e.g., smoking, alcohol consumption) when interpreting salivary miRNA profiles.

miR-31 was robustly validated in studies by Yap et al. (41), Scholtz et al. (36), and Rocchetti et al. (42) as consistently upregulated in OSCC saliva. Functionally, it plays a critical role in modulating EMT, cellular motility, and cytoskeletal reorganization, processes that underpin local invasion and metastasis in oral cancer. Scholtz et al. (36) further demonstrated its diagnostic strength as part of a three-miRNA panel including miR-345 and miR-424-3p, which collectively yielded an AUC of 0.87. This panel not only improved diagnostic sensitivity but also demonstrated potential for distinguishing OSCC from non-malignant oral conditions.

On the other hand, several miRNAs such as miR-138, miR-145, miR-140-5p, miR-30c-5p, and miR-143-5p, were consistently downregulated across studies (Mehterov et al. (44); Rocchetti et al. (42); Patel et al. (34)), aligning with their well-known tumor-suppressive roles. For instance, Mehterov et al. (44) found that downregulation of miR-

30c-5p corresponded with dysregulation in the p53 and Wnt signaling pathways, key axes in oral carcinogenesis. Similarly, Patel et al. (34) identified a 3-miRNA signature (miR-140-5p, miR-143-5p, miR-145-5p) whose suppression was associated with enhanced cell proliferation, EMT, and poor prognosis. These findings suggest that certain salivary miRNA signatures may not only serve diagnostic functions but also reflect real-time molecular changes driving tumor progression.

Discrepancies between studies also surfaced, particularly regarding miR-184. While both Garg et al. (37) and Rocchetti et al. (42) reported its downregulation in OSCC patients, other cancer types have shown contradictory trends. This variability may reflect context-dependent behaviors of miR-184, possibly influenced by tumor microenvironment, disease stage, or even technical factors such as sample processing and normalization strategies. Such inconsistencies underline the need for large, standardized, multicenter studies.

Less frequently reported miRNAs like miR-27b and miR-423-5p also demonstrated high diagnostic potential. Momen-Heravi et al. (33) identified miR-27b as significantly elevated in OSCC saliva compared to controls, OSCC-R, and patients with OLP, suggesting its potential to distinguish between malignant and potentially malignant lesions. Romani et al. (39) demonstrated that miR-423-5p had not only strong diagnostic power (AUC = 0.98) but also prognostic significance, being associated with reduced disease-free survival.

10.1 miRNA expression profiles: OSCC vs. healthy controls

In direct response to the second specific objective, comparing miRNA expression profiles between OSCC patients and healthy individuals, this review highlights robust differences in salivary miRNA expression across nearly all included studies. Regardless of methodological variation, the consensus supports the notion that OSCC is characterized by a specific miRNA expression signature, which differs substantially from that of non-malignant tissue. Upregulated miRNAs such as miR-21 and miR-31 were consistently reported in OSCC, whereas downregulated miRNAs such as miR-138, miR-145, miR-30c-5p, and miR-424 marked an absence of tumor-suppressive mechanisms.

Importantly, the dysregulation of these miRNAs was not only statistically significant but often functionally validated, reinforcing the biological credibility of the findings. Moreover, several studies demonstrated the improved diagnostic performance of multi-miRNA panels over individual biomarkers. Yap et al. (41) reported a 6-miRNA panel with an AUC of 0.95 in the training cohort and 0.86 in validation. Romani et al. (39) also presented a strong-performing panel with miR-423-5p, miR-106b-5p, and miR-193b-3p (AUC = 0.98). These findings suggest that combining miRNAs may capture a broader spectrum of tumor-related changes and provide a more stable and reproducible diagnostic tool.

10.2 Insights from OPMD, OLP, and OSCC-R comparisons

Several studies also included participants with OPMD, OLP, or OSCC-R, providing deeper insight into the dynamic regulation of miRNAs throughout the disease spectrum. Garg et al. (37) and Di Stasio et al. (35) showed that miR-21 and miR-184 were already altered in OPMD, supporting their use in screening high-risk populations. miR-181b, discussed by Di Stasio et al. (35), was upregulated in high-grade dysplasia but decreased in OSCC, suggesting a bell-shaped expression trajectory that mirrors the progression from dysplasia to carcinoma.

The study by Farshbaf et al. (45) revealed that miR-3928 was downregulated in both OSCC and OLP, suggesting shared molecular features and the possibility that this miRNA may serve as an early transformation marker. The inclusion of OSCC-R patients by Momen-Heravi et al. (33) added another dimension, as miR-27b was found to be elevated only in active OSCC, not in remission or OLP, suggesting specificity for active malignant disease and possible application in monitoring recurrence.

10. 3 Methodological reflections and analytical platforms

All studies utilized saliva as a diagnostic fluid, emphasizing the utility of non-invasive approaches in cancer screening. However, there were methodological differences in RNA extraction protocols, normalization strategies, and analysis platforms. While RT-qPCR was the most widely used technique, offering cost-effective and reliable quantification, several studies employed more advanced platforms: NanoString nCounter was used by Momen-Heravi et al. (33) for high-throughput multiplex detection without the need for amplification. Microarrays and RNA sequencing (RNA-seq), used by Romani et al. (39), Yap et al. (41), and Patel et al. (34), enabled genome-wide profiling and discovery of novel miRNA signatures. Several studies (He et al., Gai et al., Patel et al.) enriched salivary extracellular vesicles or exosomes prior to miRNA analysis, allowing for more tumor-specific signal capture. The heterogeneity in methodology may explain some of the variation in findings and underscores the need for standardized protocols in future studies to enhance reproducibility and facilitate clinical translation.

11. Limitations of the study

Despite the promising results, several limitations were evident across the studies reviewed. The most significant issue was methodological heterogeneity. Studies used different saliva collection protocols, RNA extraction kits, miRNA quantification methods (e.g. RT-qPCR, NanoString, RNA-seq), and normalization strategies. This lack of standardization limits direct comparability between studies and could contribute to variability in reported miRNA expression.

Sample sizes were relatively small in several studies, reducing statistical power and generalizability. Some studies lacked validation cohorts, and others did not include intermediate disease stages (e.g. OPMD, OSCC-R), which are essential for establishing the clinical utility of biomarkers for early detection or monitoring. Furthermore, demographic and lifestyle factors (e.g. tobacco, alcohol, betel nut use) were not consistently reported, which could influence miRNA expression and potentially confound results.

While several miRNAs were reported as statistically significant, functional analyses were limited. Few studies investigated the biological pathways regulated by these miRNAs or their causal role in oral cancer progression, making it difficult to determine whether they are direct contributors to disease or secondary markers of underlying processes.

12. Conclusions

Salivary miRNAs are differentially expressed in healthy individuals and OSCC patients, supporting their potential as non-invasive biomarkers for early detection and diagnosis. Several oncogenic miRNAs, including miR-21, miR-31, miR-24-3p, and miR-423-5p, are consistently upregulated in OSCC, while tumor-suppressive miRNAs such as miR-138, miR-145, miR-424, miR-30c-5p, and miR-184 are notably downregulated. Combined miRNA panels demonstrate high diagnostic accuracy, with many studies reporting AUC values above 0.85. Moreover, some miRNAs like miR-21 and miR-184 show altered expression in patients with oral potentially malignant disorders (OPMD), suggesting their involvement in early carcinogenesis, while miR-27b appears overexpressed exclusively in active OSCC and not in OLP or OSCC in remission, indicating potential as a marker of disease activity or recurrence. To strengthen these findings, future research should focus on large-scale, multicenter validation studies using standardized saliva collection and miRNA analysis protocols. It is also crucial to include intermediate diagnostic groups such as OPMD and OSCC-R to assess miRNA utility across the disease continuum. Longitudinal studies are needed to evaluate the predictive value of salivary miRNAs for disease progression, treatment response, and follow-up. Additionally, functional studies should investigate the mechanistic role of key miRNAs in OSCC development, which may reveal new therapeutic targets. Finally, integrating miRNA profiles with other molecular approaches, such as proteomics or DNA methylation analysis, could enhance diagnostic precision and support more personalized strategies for managing oral cancer.

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14. Annex

Section and Topic	Item #	Checklist item	Location where item is reported
TITLE			
Title	1	Identify the report as a systematic review.	1
ABSTRACT			
Abstract	2	See the PRISMA 2020 for Abstracts checklist.	5
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of existing knowledge.	8-17
Objectives	4	Provide an explicit statement of the objective(s) or question(s) the review addresses.	18-19
METHODS			
Eligibility criteria	5	Specify the inclusion and exclusion criteria for the review and how studies were grouped for the syntheses.	20-21
Information sources	6	Specify all databases, registers, websites, organisations, reference lists and other sources searched or consulted to identify studies. Specify the date when each source was last searched or consulted.	20
Search strategy	7	Present the full search strategies for all databases, registers and websites, including any filters and limits used.	21
Selection process	8	Specify the methods used to decide whether a study met the inclusion criteria of the review, including how many reviewers screened each record and each report retrieved, whether they worked independently, and if applicable, details of automation tools used in the process.	22
Data collection process	9	Specify the methods used to collect data from reports, including how many reviewers collected data from each report, whether they worked independently, any processes for obtaining or confirming data from study investigators, and if applicable, details of automation tools used in the process.	22
Data items	10a	List and define all outcomes for which data were sought. Specify whether all results that were compatible with each outcome domain in each study were sought (e.g. for all measures, time points, analyses), and if not, the methods used to decide which results to collect.	22
	10b	List and define all other variables for which data were sought (e.g. participant and intervention characteristics, funding sources). Describe any assumptions made about any missing or unclear information.	22
Study risk of bias assessment	11	Specify the methods used to assess risk of bias in the included studies, including details of the tool(s) used, how many reviewers assessed each study and whether they worked independently, and if applicable, details of automation tools used in the process.	23
Effect measures	12	Specify for each outcome the effect measure(s) (e.g. risk ratio, mean difference) used in the synthesis or presentation of results.	27
Synthesis	13a	Describe the processes used to decide which studies were eligible	24

methods		for each synthesis (e.g. tabulating the study intervention characteristics and comparing against the planned groups for each synthesis (item #5)).	
	13b	Describe any methods required to prepare the data for presentation or synthesis, such as handling of missing summary statistics, or data conversions.	24
	13c	Describe any methods used to tabulate or visually display results of individual studies and syntheses.	25
	13d	Describe any methods used to synthesize results and provide a rationale for the choice(s). If meta-analysis was performed, describe the model(s), method(s) to identify the presence and extent of statistical heterogeneity, and software package(s) used.	34
	13e	Describe any methods used to explore possible causes of heterogeneity among study results (e.g. subgroup analysis, meta-regression).	35
	13f	Describe any sensitivity analyses conducted to assess robustness of the synthesized results.	29
Reporting bias assessment	14	Describe any methods used to assess risk of bias due to missing results in a synthesis (arising from reporting biases).	23
Certainty assessment	15	Describe any methods used to assess certainty (or confidence) in the body of evidence for an outcome.	NR
RESULTS			
Study selection	16a	Describe the results of the search and selection process, from the number of records identified in the search to the number of studies included in the review, ideally using a flow diagram.	24
	16b	Cite studies that might appear to meet the inclusion criteria, but which were excluded, and explain why they were excluded.	25
Study characteristics	17	Cite each included study and present its characteristics.	25-26
Risk of bias in studies	18	Present assessments of risk of bias for each included study.	30-34
Results of individual studies	19	For all outcomes, present, for each study: (a) summary statistics for each group (where appropriate) and (b) an effect estimate and its precision (e.g. confidence/credible interval), ideally using structured tables or plots.	29-30
Results of syntheses	20a	For each synthesis, briefly summarise the characteristics and risk of bias among contributing studies.	35
	20b	Present results of all statistical syntheses conducted. If meta-analysis was done, present for each the summary estimate and its precision (e.g. confidence/credible interval) and measures of statistical heterogeneity. If comparing groups, describe the direction of the effect.	29-30
	20c	Present results of all investigations of possible causes of heterogeneity among study results.	45
	20d	Present results of all sensitivity analyses conducted to assess the robustness of the synthesized results.	29
Reporting biases	21	Present assessments of risk of bias due to missing results (arising from reporting biases) for each synthesis assessed.	23

Certainty of evidence	22	Present assessments of certainty (or confidence) in the body of evidence for each outcome assessed.	NR
DISCUSSION			
Discussion	23a	Provide a general interpretation of the results in the context of other evidence.	43-44
	23b	Discuss any limitations of the evidence included in the review.	45
	23c	Discuss any limitations of the review processes used.	46
	23d	Discuss implications of the results for practice, policy, and future research.	46-47
OTHER INFORMATION			
Registration and protocol	24a	Provide registration information for the review, including register name and registration number, or state that the review was not registered.	NR
	24b	Indicate where the review protocol can be accessed, or state that a protocol was not prepared.	NR
	24c	Describe and explain any amendments to information provided at registration or in the protocol.	NR
Support	25	Describe sources of financial or non-financial support for the review, and the role of the funders or sponsors in the review. *(Acknowledgements)*	4
Competing interests	26	Declare any competing interests of review authors.	NR
Availability of data, code and other materials	27	Report which of the following are publicly available and where they can be found: template data collection forms; data extracted from included studies; data used for all analyses; analytic code; any other materials used in the review.	NR

NR = Not reported

Declaration of the Use of articical intelligence (AI) in the preparation of the bachelors's thesis (TFG)

Tool: ChatGTP 4.0

Functions: AI tools were utilized for drafting, assitance in interpreting date and language editing.

Link: https://chatgpt.com

All AI-generated suggestions were reviewed carefully and adjusted.

All intellectual content and final decisions were made by the authors.

The diagnostic potential of salivary microRNA for early detection of oral squamous cell caricnoma in healthy vs. affected patients: A systematic review.

Running title: Salivary miRNAs for early detection of OSCC

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ABSTRACT

Introduction: Oral squamous cell carcinoma (OSCC) is the most common oral malignancy, comprising over 90% of oral cancers. Despite advances in treatment, its late diagnosis and high recurrence contribute to poor prognosis. Conventional diagnostic methods often overlook early-stage disease. Salivary microRNAs (miRNAs), which are stable, non-coding RNAs involved in gene regulation, have emerged as promising non-invasive biomarkers for early OSCC detection.

Objectives: This systematic review identifies salivary miRNAs with diagnostic value in OSCC and compares their expression in affected patients versus healthy controls. It also explores intermediate phenotypes such as oral potentially malignant disorders (OPMD), oral lichen planus (OLP), and OSCC in remission (OSCC-R) to understand miRNA changes across the disease spectrum.

Materials and Methods: A systematic literature search of PubMed, Scopus, and Web of Science was conducted in line with PRISMA-P guidelines. Eligible studies included adult patients with OSCC, OPMD, OLP, or OSCC-R, and compared salivary miRNA expression with healthy individuals.

Results: Fourteen studies comprising 914 participants met inclusion criteria. Thirty-seven differentially expressed salivary miRNAs were identified. miR-21, miR-31, and miR-423-5p were consistently upregulated in OSCC, while miR-138, miR-424, and miR-30c-5p were downregulated. Several studies also examined OPMD, OLP, and OSCC-R, revealing intermediate expression patterns indicative of disease progression.

Conclusion: Salivary miRNAs exhibit distinct expression profiles between OSCC and healthy controls, underscoring their diagnostic potential. miR-21 and miR-31 show strong biomarker capabilities, while tumor-suppressive miRNAs like miR-138 and miR-145 further support risk stratification. Including intermediate phenotypes provides additional insights into early detection and monitoring. Standardized methodologies and large-scale validation are needed for clinical implementation.

***Key words:** microRNA, salivary miRNA, OSCC, Oral squamous cell carcinoma, Early detection, Healthy controls*

INTRODUCTION

Oral squamous cell carcinoma (OSCC) accounts for over 90% of oral malignancies and is the most aggressive and prevalent subtype of head and neck cancers worldwide. Despite advances in treatment, OSCC remains associated with high morbidity and mortality, largely due to late diagnosis and frequent recurrence. In 2022, cancers of the lip and oral cavity ranked 16th globally, with approximately 390,000 new cases and 188,000 deaths, occurring more frequently in men due to higher exposure to risk factors such as tobacco and alcohol (1).

OSCC pathogenesis is multifactorial, involving environmental, microbial, and genetic contributors. Key risk factors include tobacco, alcohol, betel quid use, poor nutrition, and high body mass index (BMI). These exposures lead to the generation of reactive oxygen species and carcinogenic compounds, promoting epithelial dysplasia and mutagenesis (2,3). The oral microbiome also plays a role, with pathogens such as *Porphyromonas gingivalis* and *Fusobacterium nucleatum* contributing to carcinogenesis via inflammation, epethilial-mesenchymal transition (EMT), and immune evasion. Viral infections, particularly HPV, EBV, and HSV-1, have also been implicated (4,5).

Clinically, OSCC presents as persistent oral ulcers or lesions, often with pain, speech difficulties, or impaired chewing. Standard treatment involves surgery, often followed by radiotherapy or chemotherapy, though targeted agents like nimotuzumab and drug combinations (e.g., metformin with HDAC inhibitors) are under investigation (6).

Given OSCC's aggressive nature, early detection is critical. Salivary miRNAs have emerged as promising non-invasive biomarkers for early diagnosis. These small, conserved non-coding RNAs (~22 nucleotides) regulate gene expression post-transcriptionally and remain stable in biofluids like saliva, making them ideal candidates for liquid biopsy (7,8).

Saliva offers advantages over blood sampling, as it is non-invasive, cost-effective, and well-suited for large-scale screening. Advances in sequencing, microarrays, and qRT-PCR have enabled detailed profiling of salivary miRNAs in OSCC patients (9,10). Multiple miRNAs are consistently dysregulated in OSCC, functioning either as oncogenes or tumor suppressors. Their stability in saliva, due to encapsulation in vesicles or protein complexes, further enhances their diagnostic potential (11). This review aims to synthesize evidence from recent studies on salivary miRNAs in OSCC, evaluating their diagnostic accuracy, specificity, and clinical applicability, with the goal of advancing early detection and improving outcomes in this challenging disease.

MATERIAL AND METHODS

This systematic review was conducted in accordance with the PRISMA guidelines to ensure transparency and reproducibility. The objective was to identify salivary miRNAs with significant differential expression in patients with OSCC compared to healthy controls, emphasizing their diagnostic potential.

Identification of the PICO question

Relevant literature was identified through a structured PICO framework:

P: Patients with oral squamous cell carcinoma (OSCC)
I: Detection of salivary microRNAs
C: Healthy individuals without OSCC
O: O1: Ability to differentiate OSCC from healthy controls;
O2: Differentiation from intermediate phenotypes such as OPMD, OLP, and OSCC in remission (OSCC-R)

The guiding research question was: *In OSCC patients, how effective is salivary miRNA analysis in early detection compared to healthy individuals?*

Sources and Databases

Searches were independently conducted on PubMed, Scopus, and Web of Science, restricted to English-language articles published between January 2014 and December 2024. Boolean operators (AND, OR, NOT) were used to combine relevant terms.

Inclusion and Exclusion Criteria

The following criteria were applied to choose the articles.

Inclusion Criteria:

1. Articles in English

2. Articles available in full text
3. Studies performed in Humans
4. Article from years 2014-2024

Exclusion criteria:
1. Bibliographic reviews,
2. Editorial material and Letters
3. Systematic reviews and Meta-Analysis
4. Animal studies
5. microRNA extracted from serum or tissue biopsies
6. Articles older from 2013 or older

Search Strategy

An automated search was carried out in the three databases Pub-Med, Scopus and Web of Science with the following keywords: (salivary microrna), (microRNA), (miRNA), (biomarker), (salivary biomarker), (oral squamous cell carcinoma), (oral carcinoma), (oral cancer), (oscc), (healthy control), (early diagnosis), (early detection). The keywords were combined with the Boolean operators AND, OR and NOT.

Study Selection Process

A three-stage selection process was implemented. During the first stage, the titles of articles were reviewed to remove those that were irrelevant for this systematic review. In the second stage, both titles and abstracts were assessed and filtered based on the study type and language. In the third stage, each article was read, and the data was taken according to the eligibility to be included in the systematic review.

Data Extraction

To obtain the results from the search the following criteria were included: authors, type of study, year of publication, language, patients with oral squamous cell carcinoma and healthy controls, salivary miRNA, and inclusion and exclusion criteria.

Quality Assessment

To evaluate the methodological quality of the included studies, a structured risk of bias assessment was carried out independently by two reviewers. Different tools were applied based on study design. For observational cohort and cross-sectional studies, the NIH Quality Assessment Tool (12) was used, which includes 14 criteria covering aspects such as clarity of research objectives, population definition, exposure and outcome measurement, confounding control, and statistical analysis. Case-control studies were assessed using the JBI critical appraisal checklist (13), which evaluates key elements including case selection, matching, and exposure assessment. For diagnostic accuracy studies, the QUADAS-2 tool (14) was applied, focusing on risk of bias and applicability across four domains: patient selection, index test, reference standard, and flow and timing. Based on these assessments, each study was categorized as having a low, moderate, or high risk of bias, depending on the number and relevance of criteria met and the overall methodological rigor.

RESULTS

Study Selection and Flow Chart

A total of 165 articles were retrieved from PubMed (n=44), Scopus (n=62), and Web of Science (n=59). After screening titles and abstracts, 129 publications were deemed potentially eligible. Following full-text review, 14 studies met all inclusion criteria and were included in this systematic review (Figure 1).

Characteristics of the Included Studies

The 14 included studies assessed the diagnostic value of salivary miRNAs for OSCC, analyzing a total of 914 participants, 483 with OSCC, 325 healthy controls, and 106 with OPMD, OLP, or OSCC-R (Table 1).

Most studies compared OSCC patients with healthy individuals, while several also included intermediate clinical groups. This design enabled identification of miRNAs relevant to early malignant transformation or active disease. The use of real-time qPCR was common, with some studies incorporating microarrays or next-generation sequencing for broader profiling. For instance, Momen-Heravi et al. (15) employed the NanoString nCounter and qPCR for robust validation.

Most studies were case-control in design, while a few were prospective or observational. Sample sizes ranged from 25 to 116. Despite methodological diversity, all studies aimed to evaluate the diagnostic performance of salivary miRNAs in differentiating OSCC from non-malignant conditions using non-invasive methods.

Sensitivity and Specificity

High diagnostic accuracy was reported across several studies. Patel et al. (16) demonstrated a 3-miRNA panel (miR-143, miR-145, miR-140) with 98% sensitivity and 99% specificity. Di Stasio et al. (17) found miR-181b had 94.1% sensitivity and 81.2% specificity, while Scholtz et al. reported 86% sensitivity and 77% specificity. Gai et al. (18) and Romani et al. (19) also reported strong AUCs (up to 0.98), reinforcing the diagnostic utility of salivary miRNAs.

Risk of Bias

Risk of bias was assessed using the JBI checklist for case-control studies, the NIH Quality Assessment Tool for cohort and cross-sectional studies, and the QUADAS-2 tool for diagnostic accuracy studies. Most case-control studies showed good methodological quality, with a low to moderate risk of bias. Cohort and cross-sectional studies varied, ranging from low to high risk, primarily due to limited confounder control and incomplete reporting. The diagnostic accuracy study was rated as low to moderate risk. Overall, the studies included in this review demonstrated a low to moderate risk of bias.

Synthesis of Results

Salivary miRNA expression in OSCC vs. healthy controls

Across the 14 studies, 37 unique miRNAs were identified as differentially expressed (Table 2). The most frequently reported miRNAs were miR-21 and miR-31. miR-21 was consistently upregulated in OSCC patients in five studies, showing associations with tumor progression and smoking status. miR-31 was likewise elevated and included in several diagnostic panels. miR-423-5p and miR-24-3p were also reported with high diagnostic value, the latter showing a 5.7-fold increase and an AUC of 0.738.

On the other hand, tumor-suppressive miRNAs, such as miR-138, miR-424, miR-30c-5p, and miR-184, were consistently downregulated. For instance, Mehterov et al. (20)

reported an AUC of 0.82 for miR-30c-5p. These patterns confirm the clinical utility of salivary miRNAs as early diagnostic tools (Table 3).

Significant p-values were noted for miR-21, miR-31, miR-423-5p, miR-106a, and miR-138 across multiple studies, reinforcing their relevance (Table 3). However, discrepancies exist: miR-27b was found upregulated by Momen-Heravi et al. (15) but downregulated by Di Stasio et al. (17), and miR-184 showed both oncogenic and tumor-suppressive roles depending on study and context.

Comparison of OSCC with OPMD, OLP, and OSCC-R

Five studies extended comparisons beyond healthy controls. Garg et al. (21) showed consistent upregulation of miR-21 and downregulation of miR-184 in both OSCC and OPMD, indicating early deregulation. Di Stasio et al. (17) found miR-181b levels correlated with dysplasia severity, dropping in OSCC, suggesting it marks transitional disease stages.

Farshbaf et al. (22) demonstrated that miR-3928 was significantly reduced in both OSCC and OLP, supporting its role in early transformation. Momen-Heravi et al. (15) showed that miR-27b was overexpressed in active OSCC but unchanged in OSCC-R, highlighting its potential to distinguish active from past disease. Rocchetti et al. (23) observed that miR-138 and miR-424 were downregulated in both OSCC and OPMD, suggesting their value as early-stage biomarkers (Table 4).

DISCUSSION

This study has synthesized the available evidence on the differential expression of salivary miRNAs in patients with OSCC compared to healthy individuals. The findings consistently support their potential as non-invasive biomarkers for early detection. Oncogenic miRNAs such as miR-21, miR-31, miR-24-3p, and miR-423-5p were frequently upregulated, while tumor-suppressive miRNAs including miR-138, miR-145, miR-424, miR-30c-5p, and miR-184 were commonly downregulated.

Among these, miR-21 was the most consistently reported, with strong upregulation across multiple studies (Yap et al. (24); Di Stasio et al. (17); Rocchetti et al. (23); Vageli et al. (25); Garg et al. (21); Scholtz et al. (26); Patel et al. (16)). Elevated levels in

OSCC patients who smoke, according to Vageli et al. (25), suggest miR-21 may serve both diagnostic and risk stratification purposes.

miR-31 was also frequently validated (Yap et al. (24); Scholtz et al. (26); Rocchetti et al. (23)) and plays a key role in EMT and cellular invasion. Its inclusion in a three-miRNA panel with miR-345 and miR-424-3p improved diagnostic accuracy and helped distinguish OSCC from non-malignant conditions (26).

Several tumor-suppressive miRNAs, such as miR-138, miR-145, miR-140-5p, miR-30c-5p, and miR-143-5p, were consistently downregulated (16,20,23). Mehterov et al. (20) linked miR-30c-5p downregulation to disrupted p53 and Wnt signaling, while Patel et al. (16) identified a suppressed three-miRNA signature (miR-140-5p, miR-143-5p, miR-145-5p) associated with increased proliferation and poorer prognosis.

Some inconsistencies were noted, particularly for miR-184. While both Garg et al. (21) and Rocchetti et al. (23) reported its downregulation in OSCC, other cancer types have shown opposite trends. This variability may reflect biological context, disease stage, or technical differences, highlighting the need for standardized, multicenter validation.

Less commonly reported miRNAs also demonstrated high diagnostic potential. miR-27b, identified by Momen-Heravi et al. (15), was significantly elevated in OSCC but not in OSCC-R or OLP, suggesting specificity for active malignancy. Similarly, according to Patel et al. (16), miR-423-5p showed strong diagnostic performance (AUC = 0.98) and was associated with reduced disease-free survival emphasizing both its diagnostic and prognostic value.

Expression profiles: OSCC vs. healthy controls

Across all studies, distinct miRNA signatures consistently differentiated OSCC from healthy controls. Oncogenic miRNAs like miR-21 and miR-31 were repeatedly upregulated, while tumor suppressors such as miR-145, miR-138, and miR-30c-5p were downregulated. These changes were both statistically and functionally validated, supporting their diagnostic relevance. Several studies also showed that multi-miRNA panels outperformed single markers. For example, Yap et al. (24) reported a six-miRNA panel with an AUC of 0.95, while Romani et al. (19) identified another panel achieving an AUC of 0.98. Combining miRNAs may therefore offer a more accurate and reliable approach to OSCC detection.

Insights from OPMD, OLP, and OSCC-R

Several studies included participants with OPMD, OLP, or OSCC in remission, providing valuable insight into the dynamic regulation of salivary miRNAs across the disease spectrum. miR-21 and miR-184 were found to be altered even in OPMD, suggesting their potential as early indicators in high-risk populations. miR-181b displayed a stage-dependent pattern, upregulated in high-grade dysplasia but reduced in OSCC, which indicates it may reflect the transition from premalignant to malignant states. Similarly, miR-3928 was consistently downregulated in both OSCC and OLP, pointing to shared molecular features and highlighting its possible role as an early transformation marker. The inclusion of OSCC-R cases also revealed diagnostic distinctions: miR-27b was elevated only in active OSCC, but not in remission or OLP, suggesting its specificity for active disease. These findings support their potential use not only for early detection but also for recurrence monitoring and disease staging.

Methodological and Analytical Considerations

Although all studies employed saliva as a diagnostic medium, methodologies varied. While RT-qPCR remained the most commonly used platform due to its cost-effectiveness and sensitivity, high-throughput approaches such as NanoString, RNA-seq, and microarrays were also employed, allowing for broader miRNA discovery. Some studies (e.g., He et al. (27), Gai et al. (18), Patel et al. (16)) used extracellular vesicle (EV) enrichment to increase tumor-specific signal capture. Differences in saliva collection, RNA extraction, and normalization strategies likely contributed to variability in results and highlight the urgent need for standardization in future research.

Limitations

Despite promising results, several limitations were identified: Methodological heterogeneity across studies limits comparability. Sample sizes in several studies were modest, reducing generalizability. Some studies lacked validation cohorts or intermediate phenotypes (e.g., OPMD, OSCC-R), essential for biomarker stratification. Patient demographics, lifestyle factors (e.g., smoking, alcohol), and comorbidities were inconsistently reported, potentially confounding miRNA expression. Functional analyses of miRNA roles in OSCC were limited, restricting insight into their mechanistic relevance.

CONCLUSIONS

This review confirms that salivary miRNAs are differentially expressed in OSCC patients compared to healthy individuals, supporting their potential as non-invasive diagnostic biomarkers. Oncogenic miRNAs such as miR-21 and miR-31 were consistently upregulated, while tumor-suppressive miRNAs like miR-138 and miR-145 were downregulated. Multi-miRNA panels showed high diagnostic accuracy (AUC > 0.85), and some miRNAs, including miR-21 and miR-184, were also altered in premalignant conditions, suggesting value for early detection. miR-27b, notably elevated only in active OSCC, may serve as a marker of disease activity. These findings highlight the clinical promise of salivary miRNAs for OSCC detection and monitoring.

Future research should prioritize large, multicenter studies using standardized saliva collection and miRNA analysis protocols to improve consistency and comparability. Including intermediate groups such as OPMD and OSCC in remission will help clarify how miRNA expression changes throughout disease progression. Longitudinal studies are also needed to evaluate miRNAs as tools for predicting outcomes and monitoring treatment response. Additionally, functional studies should explore the biological roles of key miRNAs in OSCC development. Finally, integrating miRNA data with other molecular techniques, such as proteomics and epigenetics, may enhance diagnostic accuracy and support more personalized approaches to oral cancer care.

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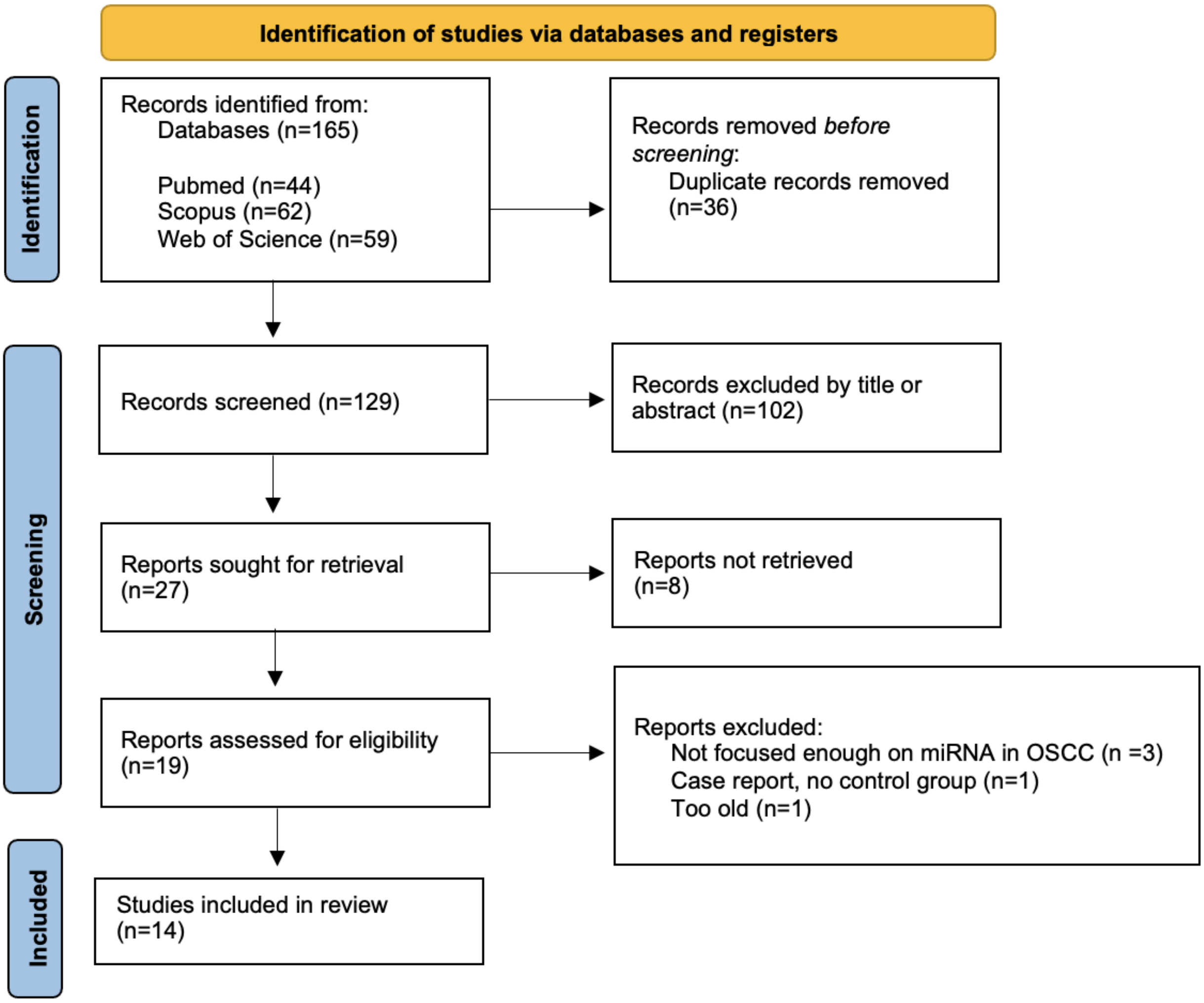

Figure 1: PRISMA Flow Chart of searching and selection process during the Systematic review

Table 1: Study characteristics of all 14 included studies

Author	Reference	Type of study	Participants	OSCC patients	Healthy controls	Method	microRNA
Romani et al., 2021	(19)	Case-control	116	58	58	miRNeasy Mini kit + RT-qPCR	miR-423-5p, miR-106b-5p, miR-193b-3p
Yap et al., 2018	(24)	Case-control	60	30	30	mirVana Kit + RT-qPCR	miR-31, miR-21, miR-99a, let-7c, miR-125b, miR-100
Rocchetti et al., 2024	(23)	Prospective cohort	25	14	5	RT-qPCR	miR-21, miR-31, miR-138, miR-145, miR-184, miR-424
Scholtz et al., 2022	(26)	Case-control	87	43	44	RT-qPCR	miR-31-5p, miR-345-3p, miR-424-3p
Tarrad et al., 2023	(28)	Observational diagnostic	36	12	12	RT-qPCR	miR-106a
Vageli et al., 2023	(25)	Case-control	44	23	21	RT-qPCR	miR-21, miR-136, miR-3928, miR-29B
Gai et al., 2023	(18)	Case-control	32	21	11	EV isolation + RT-qPCR	miR-302b-3p, miR-517b-3p, miR-512-3p, miR-412-3p
Di Stasio et al., 2022	(17)	Cohort	43	10	10	RT-qPCR	miR-21, miR-27b, miR-181b
Garg et al., 2023	(21)	Case-control	90	30	30	RT-qPCR	miR-21, miR-184
Mehterov et al., 2021	(20)	Case-control	45	33	12	TaqMan RT-qPCR	miR-30c-5p
Farshbaf et al., 2024	(22)	Cross-sectional	91	31	30	RT-qPCR	miR-3928
He et al., 2020	(27)	Case-control	59	45	14	Exosome isolation + RT-qPCR	miR-24-3p
Momen-Heravi et al., 2014	(15)	Cross-sectional	34	9	9	NanoString + RT-qPCR	miR-27b, miR-24
Patel et al., 2023	(16)	Exploratory + validation	70	50	20	RNASeq + RT-qPCR	miR-140-5p, miR-143-5p, miR-145-5p

(miR/miRNA = microRNA; OSCC = Oral squamous cell carcinoma; RT-qPCR = Reverse Transcription quantitative Polymerase Chain Reaction; TaqMan RT-qPCR = TaqMan Reverse transcription qPCR; RNASeq = RNA sequencing; EV isolation = Extracellular vesicle isolation)

Table 2: Reported salivary miRNAs in OSCC: Study frequency and expression trends

miRNA	Authors	Frequently Reported	Expression in OSCC	Study Count
miR-21	Yap et al.; Rocchetti et al.; Vageli et al.; Garg et al.; Di Stasio et al.	Yes	Upregulated in OSCC	5
miR-31	Yap et al.; Rocchetti et al.; Scholtz et al.	Yes	Upregulated in OSCC	3
miR-423-5p	Romani et al. 2021; Patel et al. 2023	Yes	Upregulated in OSCC	2
miR-138	Rocchetti et al.; Scholtz et al.; Momen-Heravi et al.	Yes	Downregulated in OSCC	3
miR-106a	Tarrad et al.	No	Downregulated in OSCC	1
miR-24-3p	He et al.	No	Upregulated in OSCC	1
miR-31-5p	Scholtz et al.	No	Upregulated in OSCC	1
miR-345	Scholtz et al.	No	Upregulated in OSCC	1
miR-424-3p	Scholtz et al.	No	Upregulated in OSCC	1
miR-140	Patel et al.	No	Downregulated in OSCC	1
miR-143	Patel et al.	No	Downregulated in OSCC	1
miR-145	Patel et al.	No	Downregulated in OSCC	1
miR-30a	Patel et al.	No	Downregulated in OSCC	1
let-7i	Patel et al.	No	Downregulated in OSCC	1
miR-412-3p	Gai et al.	No	Upregulated in OSCC	1
miR-489-3p	Gai et al.	No	Upregulated in OSCC	1
miR-512-3p	Gai et al.	No	Upregulated in OSCC	1
miR-597-5p	Gai et al.	No	Upregulated in OSCC	1
miR-603	Gai et al.	No	Upregulated in OSCC	1
miR-27b	Momen-Heravi et al.	No	Upregulated in OSCC	1
miR-30c-5p	Mehterov et al.	No	Downregulated in OSCC	1
miR-106b-5p	Romani et al.	No	Downregulated in OSCC	1
miR-193b-3p	Romani et al.	No	Upregulated in OSCC	1
miR-184	Garg et al.; Scholtz et al.	No	Downregulated in OSCC	2
miR-191	Scholtz et al.	No	Upregulated in OSCC	1
miR-484	Gai et al.	No	Downregulated in OSCC	1
miR-720	Gai et al.	No	Downregulated in OSCC	1
miR-376c-3p	Gai et al.	No	Downregulated in OSCC	1
miR-27a-3p	Gai et al.	No	Upregulated in OSCC	1
miR-302b-3p	Gai et al.	No	Upregulated in OSCC	1
miR-337-5p	Gai et al.	No	Upregulated in OSCC	1
miR-373-3p	Gai et al.	No	Upregulated in OSCC	1
miR-494-3p	Gai et al.	No	Upregulated in OSCC	1
miR-517b	Gai et al.	No	Upregulated in OSCC	1
miR-520d-3p	Gai et al.	No	Upregulated in OSCC	1
miR-645	Gai et al.	No	Upregulated in OSCC	1
miR-125a	Mehterov et al.	No	Not significant	1

(miR/miRNA = microRNA; OSCC = Oral squamous cell carcinoma

Table 3: Overview of included studies reporting differentially expressed salivary miRNAs in OSCC, with diagnostic performance and statistical significance

Study	miRNA(s) studied	Expression OSCC vs healthy control	p-value	Significant	Diagnostic accuracy
Romani et al. (19)	miR-423-5p, miR-106b	↑ OSCC	< 0.001	Yes	AUC = 0.98
Yap et al. (24)	miR-31, miR-21, let-7c	↑ miR-21/31 ↓ let-7c	< 0.01	Yes	AUC = 0.95
Scholtz et al. (26)	miR-31-5p, miR-345, miR-424	↑ OSCC	< 0.05	Yes	AUC = 0.87
Vageli et al. (25)	miR-21, miR-136, miR-3928	↑ OSCC, especially smokers	< 0.005	Yes	Not Reported
Gai et al. (18)	miR-512, miR-412, miR-302b	↑ OSCC EVs	< 0.01	Yes	ROC > 0.8 for miR-512
Farshbaf et al. (22)	miR-3928	↓ in OSCC/OLP	< 0.0001	Yes	Not Reported
He et al. (27)	miR-24-3p	↑ OSCC	0.02	Yes	AUC = 0.738
Di Stasio et al. (17)	miR-181b, miR-27b	↑ in dysplasia	0.006, 0.046	Yes	Not Reported
Garg et al. (21)	miR-21, miR-184	↑ miR-21 ↓ miR-184 OSCC	< 0.001	Yes	ROC plotted
Rocchetti et al. (23)	miR-138, miR-424	↓ in OSCC	< 0.05	Yes	Not Reported
Tarrad et al. (28)	miR-106a	↓ OSCC	< 0.05	Yes	AUC = 80.4%
Mehterov et al. (20)	miR-30c-5p	↓ OSCC	0.04	Yes	AUC = 0.82
Momen-Heravi et al. (15)	miR-27b, miR-24	↑ OSCC	< 0.01	Yes	ROC = Strong
Patel et al. (16)	miR-140, miR-143, miR-145	↓ OSCC	< 0.05	Yes	Functional validation too

(miR/miRNA = microRNA; OSCC = Oral squamous cell carcinoma; AUC = Area under curve; ROC = Receiver operating characteristic; ↑ = upregulated;
↓ = downregulated)

Table 4: Summary of studies comparing salivary miRNA expression in OSCC, OPMD, OLP, and OSCC-R

Author	Compared Conditions	Key Findings
Garg et al. (21)	OSCC, OPMD, controls	↑ miR-21 ↓ miR-184 in OSCC & OPMD vs. controls
Di Stasio et al. (17)	OSCC, OPMD (graded dysplasia), controls	↑ miR-181b with dysplasia severity ↓ miR-181b in OSCC; ↓ miR-27b in dysplasia
Farshbaf et al. (22)	OSCC, OLP, controls	↓ miR-3928 in OSCC & OLP vs. controls
Momen-Heravi et al. (15)	OSCC, OSCC-R, OLP, controls	↑ miR-27b in OSCC only, unchanged in OLP & OSCC-R
Rocchetti et al. (23)	OSCC, OPMD, controls	↓ miR-138 & miR-424 in OSCC & OPMD vs. controls

(miR/miRNA = microRNA; OSCC = Oral squamous cell carcinoma; OPMD = Oral potentially malignant disorders; OLP = Oral lichen planus; OSCC-R = Oral squamous cell carcinoma patient in remission, ↑ = upregulated; ↓ = downregulated)

El potencial diagnóstico de los microARN salivales para la detección temprana del carcinoma oral de células escamosas en pacientes sanos vs. afectados: Una revisión sistemática.

Titulo abbreviado: microARN salivales para la detección temprana del COCE

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RESUMEN

Introducción: El carcinoma oral de células escamosas (COCE) representa más del 90% de los cánceres orales y se asocia con un diagnóstico tardío y alta recurrencia, lo que limita el pronóstico. Los métodos diagnósticos actuales suelen ser insuficientes en etapas tempranas. Los microARNs salivales (miARNs), ARN no codificantes estables implicados en la regulación génica, han surgido como biomarcadores no invasivos prometedores para la detección precoz del COCE.

Objetivos: Esta revisión sistemática identifica miARNs salivales con valor diagnóstico en COCE y compara su expresión en pacientes frente a controles sanos. También se analizan fenotipos intermedios como desórdenes potencialmente malignos orales (DPMO), liquen plano oral (LPO) y COCE en remisión (COCE-R), para evaluar cambios progresivos en la expresión de miARNs.

Materiales y métodos: Se realizó una búsqueda sistemática en PubMed, Scopus y Web of Science, siguiendo las directrices PRISMA-P. Se incluyeron estudios con adultos diagnosticados con COCE, DPMO, LPO o COCE-R, que compararan la expresión salival de miARNs con la de individuos sanos.

Resultados: Catorce estudios con 914 participantes cumplieron los criterios de inclusión. Se identificaron 37 miARNs diferencialmente expresados. El miR-21, miR-31 y miR-423-5p estuvieron sobreexpresados en COCE, mientras que miR-138, miR-424 y miR-30c-5p se encontraron subexpresados. Estudios adicionales en DPMO, LPO y COCE-R revelaron patrones de expresión intermedios.

Conclusión: Los miARNs salivales muestran perfiles diferenciados entre COCE y controles sanos, destacando su potencial diagnóstico. El miR-21 y miR-31 destacan como biomarcadores clave, y los miARNs supresores tumorales como miR-138 y miR-145 aportan valor en la estratificación del riesgo. Se requiere validación clínica a gran escala.

Palabras clave: *microARN, miARN salival, COCE, Carcinoma oral de células escamosas, Detección temprana, Controles sanos.*

INTRODUCCIÓN

El carcinoma oral de células escamosas (COCE) representa más del 90% de las neoplasias malignas orales y es el subtipo más agresivo y prevalente de los cánceres de cabeza y cuello a nivel mundial. A pesar de los avances en el tratamiento, el COCE continúa asociado con una alta morbilidad y mortalidad, principalmente debido a su diagnóstico tardío y a la recurrencia frecuente. En 2022, los cánceres de labio y cavidad oral ocuparon el puesto 16 a nivel global, con aproximadamente 390,000 nuevos casos y 188,000 muertes, ocurriendo con mayor frecuencia en hombres debido a una mayor exposición a factores de riesgo como el tabaco y el alcohol (1).

La patogénesis del COCE es multifactorial, e involucra factores ambientales, microbianos y genéticos. Los principales factores de riesgo incluyen el tabaco, el alcohol, el consumo de nuez de betel, la malnutrición y un índice de masa corporal elevado. Estas exposiciones generan especies reactivas de oxígeno y compuestos carcinógenos que favorecen la displasia epitelial y la mutagénesis (2,3). El microbioma oral también desempeña un papel importante, con patógenos como *Porphyromonas gingivalis* y *Fusobacterium nucleatum* que contribuyen a la carcinogénesis mediante mecanismos inflamatorios, transición epitelio-mesénquima (EMT) y evasión inmune. También se han implicado infecciones virales, especialmente por VPH, VEB y VHS-1 (4,5).

Clínicamente, el COCE se presenta como úlceras o lesiones orales persistentes, frecuentemente acompañadas de dolor, dificultades para hablar o masticar. El tratamiento estándar incluye cirugía, usualmente seguida de radioterapia o quimioterapia, aunque agentes dirigidos como el nimotuzumab y combinaciones farmacológicas (por ejemplo, metformina con inhibidores de HDAC) están siendo investigados (6).

Dada la naturaleza agresiva del COCE, la detección temprana es fundamental. Los microARNs salivales han surgido como biomarcadores no invasivos prometedores para el diagnóstico precoz. Estos pequeños ARN no codificantes, conservados y de aproximadamente 22 nucleótidos, regulan la expresión génica a nivel post-transcripcional y permanecen estables en biofluidos como la saliva, lo que los convierte en candidatos ideales para biopsias líquidas (7,8).

La saliva ofrece ventajas sobre la toma de muestras sanguíneas, ya que es no invasiva, rentable y adecuada para tamizajes a gran escala. Los avances en secuenciación, microarrays y qRT-PCR han permitido un perfilado detallado de los miARNs salivales en pacientes con COCE (9,10).

Varios miARNs están consistentemente desregulados en el COCE, actuando como oncogenes o supresores tumorales. Su estabilidad en la saliva, debido a su encapsulamiento en vesículas o complejos proteicos, refuerza aún más su potencial diagnóstico (11). Esta revisión tiene como objetivo sintetizar la evidencia de estudios recientes sobre miARNs salivales en el COCE, evaluando su precisión diagnóstica, especificidad y aplicabilidad clínica, con la finalidad de avanzar en la detección temprana y mejorar los resultados en esta enfermedad desafiante.

MATERIALES Y MÉTODOS

Esta revisión sistemática se llevó a cabo conforme a las directrices PRISMA, con el fin de garantizar la transparencia y la reproducibilidad. El objetivo fue identificar microARNs salivales con expresión diferencial significativa en pacientes con COCE en comparación con controles sanos, haciendo énfasis en su potencial diagnóstico.

Identificación de la pregunta PICO

La literatura relevante se identificó mediante el uso de un marco estructurado PICO:

P: Pacientes con carcinoma oral de células escamosas (COCE)

I: Detección de microARNs salivales

C: Individuos sanos sin COCE

O:

- **O1**: Capacidad para diferenciar COCE de individuos sanos
- **O2:** Diferenciación de fenotipos intermedios como DPMO, LPO y COCE en remisión (COCE-R)

La pregunta de investigación orientadora fue: *En pacientes con COCE, ¿qué tan eficaz es el análisis de microARNs salivales para la detección temprana en comparación con individuos sanos?*

Fuentes y bases de datos

Se realizaron búsquedas independientes en PubMed, Scopus y Web of Science, limitadas a artículos en idioma inglés publicados entre enero de 2014 y diciembre de 2024. Se utilizaron operadores booleanos (AND, OR, NOT) para combinar los términos relevantes.

Criterios de inclusión y exclusión

Se aplicaron los siguientes criterios para seleccionar los artículos:

Criterios de inclusión:

1. Artículos en idioma inglés
2. Artículos disponibles en texto completo
3. Estudios realizados en humanos
4. Artículos publicados entre los años 2014 y 2024

Criterios de exclusión:

1. Revisiones bibliográficas
2. Material editorial y cartas al editor
3. Revisiones sistemáticas y metaanálisis
4. Estudios en animales
5. Estudios donde los microARNs fueron extraídos de suero o biopsias de tejido
6. Artículos publicados en 2013 o antes

Estrategia de búsqueda

Se realizó una búsqueda automatizada en las bases de datos PubMed, Scopus y Web of Science utilizando las siguientes palabras clave: (microrna salivales), (microARN), (miARN), (biomarcadores), (biomarcadores salivales), (carcinoma oral de células esquamosas), (carcinoma oral), (cancer oral), (coce), (controles sanos), (diagnosis temprana), (detección temprana). Las palabras clave se combinaron mediante operadores booleanos AND, OR y NOT.

Proceso de selección de estudios

Se implementó un proceso de selección en tres etapas. En la primera etapa, se revisaron los títulos de los artículos para eliminar aquellos irrelevantes para esta revisión sistemática. En la segunda etapa, se evaluaron los títulos y resúmenes, filtrando los estudios según el tipo y el idioma. En la tercera etapa, se realizó la lectura completa de cada artículo y se extrajeron los datos de acuerdo con los criterios de elegibilidad para su inclusión en la revisión sistemática.

Extracción de datos

Para obtener los resultados de la búsqueda, se consideraron los siguientes criterios: autores, tipo de estudio, año de publicación, idioma, pacientes con carcinoma oral de células escamosas y controles sanos, microARN salival, y criterios de inclusión y exclusión.

Evaluación de la calidad

Para evaluar la calidad metodológica de los estudios incluidos, se llevó a cabo una evaluación estructurada del riesgo de sesgo de forma independiente por dos revisores. Se aplicaron diferentes herramientas según el diseño del estudio. Para los estudios observacionales de cohorte y transversales, se utilizó la herramienta de Evaluación de Calidad del NIH (12), que incluye 14 criterios que abarcan la claridad de los objetivos, definición de la población, medición de la exposición y los resultados, control de factores de confusión y análisis estadístico. Los estudios de casos y controles se evaluaron utilizando la lista de verificación crítica del Instituto Joanna Briggs (JBI) (13), que valora aspectos clave como la selección de casos, emparejamiento y evaluación de la exposición. Para los estudios de precisión diagnóstica, se utilizó la herramienta QUADAS-2 (14), que se centra en el riesgo de sesgo y la aplicabilidad en cuatro dominios: selección de pacientes, prueba índice, estándar de referencia y flujo y cronología. Según estas evaluaciones, cada estudio fue clasificado como de bajo, moderado o alto riesgo de sesgo, en función del número y relevancia de los criterios cumplidos y del rigor metodológico general.

RESULTADOS

Selección de estudios y diagrama de flujo

Se recuperaron un total de 165 artículos de PubMed (n=44), Scopus (n=62) y Web of Science (n=59). Tras la revisión de títulos y resúmenes, 129 publicaciones fueron consideradas potencialmente elegibles. Luego del análisis de los textos completos, 14 estudios cumplieron con todos los criterios de inclusión y fueron incorporados en esta revisión sistemática (Figura 1).

Características de los Estudios Incluidos

Los 14 estudios evaluaron el valor diagnóstico de los microARNs (miARNs) salivales para el carcinoma oral de células escamosas (OSCC), analizando un total de 914 participantes: 483 con OSCC, 325 controles sanos y 106 con trastornos orales potencialmente malignos (OPMD), liquen plano oral (LPO) o en remisión de COCE (COCE-R) (Tabla 1).

La mayoría de los estudios compararon pacientes con COCE frente a individuos sanos, aunque varios también incluyeron grupos clínicos intermedios. Este diseño permitió identificar miARNs relevantes para la transformación maligna temprana o enfermedad activa. El uso de qPCR en tiempo real fue común, mientras que algunos estudios también emplearon microarreglos o secuenciación de nueva generación para un perfil más amplio. Por ejemplo, Momen-Heravi et al. (15) utilizó la plataforma NanoString nCounter junto con qPCR para una validación robusta. La mayoría de los estudios presentaron un diseño de casos y controles, mientras que unos pocos fueron prospectivos u observacionales. Los tamaños muestrales oscilaron entre 25 y 116 participantes. A pesar de la diversidad metodológica, todos los estudios buscaron evaluar el rendimiento diagnóstico de los miARNs salivales para diferenciar COCE de condiciones no malignas mediante métodos no invasivos.

Sensibilidad y Especificidad

Varios estudios informaron una alta precisión diagnóstica. Patel et al. (16) demostraron que un panel de 3 miARNs (miR-143, miR-145, miR-140) logró una sensibilidad del 98% y especificidad del 99%. Di Stasio et al. (17) encontraron que miR-181b presentó una sensibilidad del 94,1% y especificidad del 81,2%, mientras que Scholtz et al. reportaron valores de 86% y 77%, respectivamente. Gai et al. (18)

y Romani et al. (19) también reportaron valores elevados del área bajo la curva (AUC), hasta 0,98, lo que refuerza el valor diagnóstico de los miARNs salivales.

Riesgo de sesgo

El riesgo de sesgo se evaluó utilizando la lista de verificación JBI para estudios de casos y controles, la herramienta de evaluación de calidad del NIH para estudios de cohortes y transversales, y la herramienta QUADAS-2 para estudios de precisión diagnóstica. La mayoría de los estudios de casos y controles mostraron buena calidad metodológica, con un riesgo de sesgo bajo a moderado. Los estudios de cohortes y transversales variaron de bajo a alto riesgo, principalmente debido a un control limitado de factores de confusión y a informes incompletos. El estudio de precisión diagnóstica fue calificado con un riesgo de sesgo bajo a moderado. En general, los estudios incluidos presentaron un riesgo de sesgo bajo a moderado.

Síntesis de resultados

Expresión de miARNs salivales en OSCC vs. controles sanos

En los 14 estudios se identificaron 37 miARNs únicos con expresión diferencial. Los más frecuentemente reportados fueron miR-21 y miR-31. miR-21 se encontró consistentemente sobreexpresado en pacientes con COCE en cinco estudios, con asociaciones con progresión tumoral y tabaquismo. De forma similar, miR-31 también fue elevado e incluido en varios paneles diagnósticos. miR-423-5p y miR-24-3p también mostraron un alto valor diagnóstico; este último presentó un aumento de 5.7 veces y un AUC de 0,738 (Tabla 3).

Por otro lado, miARNs supresores tumorales como miR-138, miR-424, miR-30c-5p y miR-184 estuvieron consistentemente subexpresados. Por ejemplo, Mehterov et al. (20) reportaron un AUC de 0,82 para miR-30c-5p. Estos patrones confirman la utilidad clínica de los miARNs salivales como herramientas diagnósticas tempranas (Tabla 10).

Varios estudios reportaron valores p significativos para miR-21, miR-31, miR-423-5p, miR-106a y miR-138, lo que refuerza su relevancia diagnóstica (Tabla 3). Sin embargo, también se observaron discrepancias: miR-27b fue reportado como sobreexpresado por Momen-Heravi et al. (15) pero subexpresado por Di Stasio et al.

(17) mientras que miR-184 mostró funciones tanto oncogénicas como supresoras dependiendo del estudio y contexto.

Comparación de OSCC con OPMD, OLP y OSCC-R

Cinco estudios ampliaron las comparaciones más allá de los controles sanos. Garg et al. (21) mostraron una sobreexpresión constante de miR-21 y una disminución de miR-184 tanto en COCE como en DPMO, lo que indica una desregulación temprana. Di Stasio et al. (17) encontraron que los niveles de miR-181b se correlacionaban con la gravedad de la displasia, disminuyendo en COCE, lo que sugiere que podría marcar etapas transicionales de la enfermedad.

Farshbaf et al. (22) demostraron que miR-3928 estaba significativamente reducido tanto en COCE como en LPO, lo que respalda su papel en la transformación temprana. Momen-Heravi et al. (15) mostraron que miR-27b estaba sobreexpresado en COCE activo pero sin cambios en COCE-R, lo que resalta su potencial para distinguir enfermedad activa de pasada. Rocchetti et al. (23) observaron que miR-138 y miR-424 estaban disminuidos tanto en COCE como en DPMO, lo que sugiere su utilidad como biomarcadores en etapas tempranas (Tabla 4).

DISCUSIÓN

Este estudio ha sintetizado la evidencia disponible sobre la expresión diferencial de microARNs salivales en pacientes con COCE en comparación con individuos sanos. Los hallazgos respaldan de manera consistente su potencial como biomarcadores no invasivos para la detección temprana. Los microARNs oncogénicos como miR-21, miR-31, miR-24-3p y miR-423-5p se encontraron frecuentemente sobreexpresados, mientras que los microARNs supresores tumorales como miR-138, miR-145, miR-424, miR-30c-5p y miR-184 estuvieron comúnmente subexpresados.

Entre ellos, el miR-21 fue el más consistentemente reportado, con una fuerte sobreexpresión en múltiples estudios (Yap et al. (24); Di Stasio et al. (17); Rocchetti et al. (23); Vageli et al. (25); Garg et al. (21); Scholtz et al. (26); Patel et al. (16)). Los niveles elevados en pacientes con COCE fumadores, según Vageli et al. (25), sugieren que el miR-21 podría cumplir funciones tanto diagnósticas como de estratificación de riesgo.

El miR-31 también fue validado con frecuencia (Yap et al. (24); Scholtz et al. (26); Rocchetti et al. (23)) y desempeña un papel clave en la transición epitelio-mesénquima (EMT) y la invasión celular. Su inclusión en un panel de tres miARNs junto con miR-345 y miR-424-3p mejoró la precisión diagnóstica y ayudó a distinguir COCE de condiciones no malignas (26).

Varios microARNs supresores tumorales, como miR-138, miR-145, miR-140-5p, miR-30c-5p y miR-143-5p, estuvieron consistentemente subregulados (16, 20, 23). Mehterov et al. (20) vincularon la reducción de miR-30c-5p con la alteración de las vías de señalización de p53 y Wnt, mientras que Patel et al. (16) identificaron una firma de tres miARNs suprimidos (miR-140-5p, miR-143-5p, miR-145-5p) asociada con mayor proliferación y peor pronóstico.

Se observaron algunas inconsistencias, particularmente en el caso de miR-184. Aunque tanto Garg et al. (21) como Rocchetti et al. (23) informaron su disminución en COCE, otros tipos de cáncer han mostrado tendencias opuestas. Esta variabilidad podría reflejar diferencias en el contexto biológico, la etapa de la enfermedad o factores técnicos, lo que subraya la necesidad de validaciones estandarizadas y multicéntricas.

Algunos miARNs menos reportados también demostraron un alto potencial diagnóstico. El miR-27b, identificado por Momen-Heravi et al. (15), se encontró significativamente elevado en COCE pero no en COCE-R ni en LPO, lo que sugiere especificidad para la malignidad activa. De forma similar, según Patel et al. (16), el miR-423-5p mostró un sólido rendimiento diagnóstico (AUC = 0,98) y se asoció con una menor supervivencia libre de enfermedad, lo que enfatiza tanto su valor diagnóstico como pronóstico.

Perfiles de expresión: COCE vs. controles sanos

En todos los estudios, los perfiles de expresión de miARNs diferencian claramente a COCE de los controles sanos. miARNs oncogénicos como miR-21 y miR-31 estuvieron consistentemente sobreexpresados, mientras que miARNs supresores como miR-145, miR-138 y miR-30c-5p estuvieron disminuidos. Estos cambios fueron validados tanto estadísticamente como funcionalmente. Además, varios estudios mostraron que los paneles múltiples de miARNs superaron a los marcadores individuales en precisión diagnóstica. Por ejemplo, Yap et al. (24) reportaron un panel de seis miARNs con un AUC de 0,95, mientras que Romani et al. (19) identificaron otro panel con un AUC de 0,98. La combinación de miARNs puede ofrecer una herramienta diagnóstica más precisa y confiable.

Perspectivas desde DPMO, LPO y COCE-R

Varios estudios incluyeron participantes con DPMO, LPO o en remisión de COCE, proporcionando una visión más completa sobre la regulación dinámica de los miARNs salivales a lo largo del espectro de la enfermedad. miR-21 y miR-184 ya estaban alterados en DPMO, lo que sugiere su potencial como indicadores tempranos en poblaciones de alto riesgo. miR-181b mostró un patrón dependiente de la etapa, aumentando en displasia de alto grado y disminuyendo en COCE, lo que indica que podría reflejar la progresión de lesiones premalignas a cáncer. De forma similar, miR-3928 se redujo de forma consistente en COCE y LPO lo que apunta a características moleculares compartidas y sugiere un papel en la transformación temprana. Además, los casos en remisión revelaron diferencias diagnósticas: miR-27b solo se elevó en COCE activo, y no en remisión ni en LPO, lo que refuerza su especificidad como marcador de enfermedad activa. Estos hallazgos respaldan su posible utilidad no solo en la detección temprana, sino también en la vigilancia de recurrencias y estadificación de la enfermedad.

Consideraciones Metodológicas y Analíticas

Aunque todos los estudios utilizaron saliva como medio diagnóstico, las metodologías variaron. RT-qPCR fue la técnica más utilizada por su rentabilidad y sensibilidad, pero también se emplearon métodos de alto rendimiento como NanoString, RNA-seq y microarreglos, que permiten una detección más amplia. Algunos estudios utilizaron enriquecimiento de vesículas extracelulares (EV) para capturar señales más específicas del tumor. Las diferencias en la recolección de saliva, extracción de ARN y estrategias de normalización podrían explicar la variabilidad entre los resultados, lo que subraya la necesidad urgente de estandarización en futuras investigaciones.

LIMITACIONES

A pesar de los resultados prometedores, se identificaron varias limitaciones: la heterogeneidad metodológica entre los estudios dificulta su comparación directa. Los tamaños muestrales fueron reducidos en varios casos, lo que limita la generalización. Algunos estudios carecieron de cohortes de validación o de grupos intermedios como DPMO u COCE-R, necesarios para evaluar el valor clínico de los biomarcadores. Factores demográficos y de estilo de vida (como tabaquismo o consumo de alcohol) fueron reportados de forma inconsistente, lo que podría haber influido en la expresión de miARNs. Además, los estudios funcionales sobre los mecanismos biológicos de los miARNs fueron limitados.

CONCLUSIONES

Esta revisión confirma que los miARNs salivales presentan una expresión diferencial en pacientes con COCE frente a individuos sanos, lo que respalda su potencial como biomarcadores diagnósticos no invasivos. miARNs oncogénicos como miR-21 y miR-31 estuvieron consistentemente sobreexpresados, mientras que miARNs supresores como miR-138 y miR-145 estuvieron reducidos. Los paneles de miARNs mostraron alta precisión diagnóstica (AUC > 0,85), y algunos, como miR-21 y miR-184, también se alteraron en condiciones premalignas, lo que refuerza su valor para la detección temprana. miR-27b, elevado solo en COCE activo, podría servir como marcador de actividad tumoral. En conjunto, estos hallazgos destacan la promesa clínica de los miARNs salivales en la detección y el seguimiento del COCE.

Las futuras investigaciones deben centrarse en estudios multicéntricos a gran escala con protocolos estandarizados para la recolección de saliva y análisis de miARNs, mejorando así la consistencia y comparabilidad. Incluir grupos intermedios como DPMO y pacientes en remisión permitirá evaluar los cambios en la expresión de miARNs a lo largo del curso de la enfermedad. También se necesitan estudios longitudinales que analicen el valor predictivo de los miARNs en la progresión del cáncer y la respuesta al tratamiento. Además, se recomienda realizar estudios funcionales que exploren el papel biológico de los miARNs clave en el desarrollo del COCE. Finalmente, integrar los datos de miARNs con otras herramientas moleculares, como la proteómica y la epigenética, podría mejorar la precisión diagnóstica y apoyar un enfoque más personalizado en el manejo del cáncer oral.

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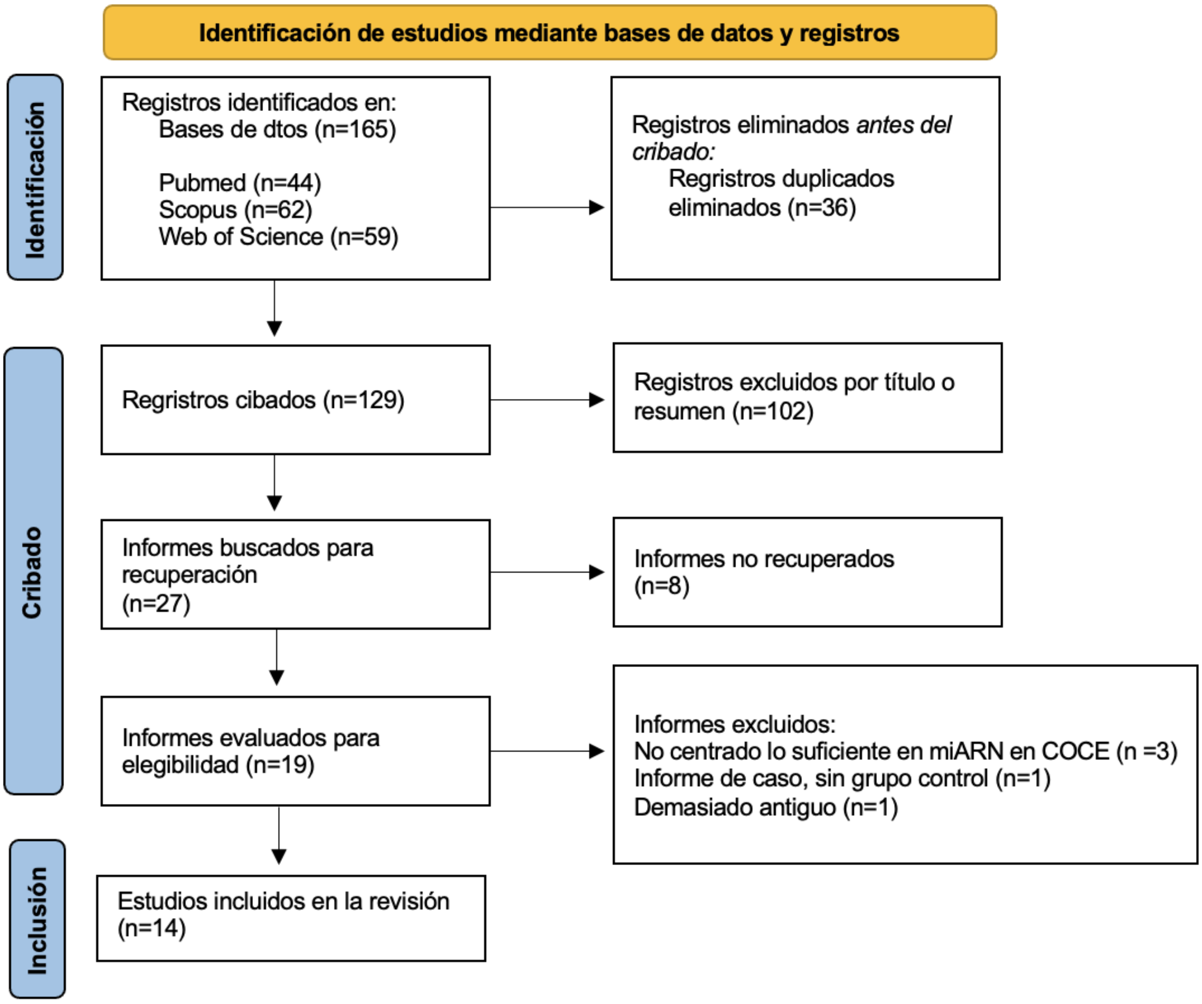

Figura 1: Diagrama de flujo PRISMA del proceso de búsqueda y selección durante la revisión sistemática.

Tabla 1: Study characteristics of all 14 included studies

Autor	Referencia	Tipo de estudio	Participantes	Pacientes COCE	Pacientes sanos	Método	microARN
Romani et al., 2021	(19)	Caso y controles	116	58	58	miRNeasy Mini kit + RT-qPCR	miR-423-5p, miR-106b-5p, miR-193b-3p
Yap et al., 2018	(24)	Caso y controles	60	30	30	mirVana Kit + RT-qPCR	miR-31, miR-21, miR-99a, let-7c, miR-125b, miR-100
Rocchetti et al., 2024	(23)	Cohorte prospectiva	25	14	5	RT-qPCR	miR-21, miR-31, miR-138, miR-145, miR-184, miR-424
Scholtz et al., 2022	(26)	Caso y controles	87	43	44	RT-qPCR	miR-31-5p, miR-345-3p, miR-424-3p
Tarrad et al., 2023	(27)	Diagnóstico observacional	36	12	12	RT-qPCR	miR-106a
Vageli et al., 2023	(25)	Caso y controles	44	23	21	RT-qPCR	miR-21, miR-136, miR-3928, miR-29B
Gai et al., 2023	(18)	Caso y controles	32	21	11	EV isolation + RT-qPCR	miR-302b-3p, miR-517b-3p, miR-512-3p, miR-412-3p
Di Stasio et al., 2022	(17)	Cohorte	43	10	10	RT-qPCR	miR-21, miR-27b, miR-181b
Garg et al., 2023	(21)	Caso y controles	90	30	30	RT-qPCR	miR-21, miR-184
Mehterov et al., 2021	(20)	Caso y controles	45	33	12	TaqMan RT-qPCR	miR-30c-5p
Farshbaf et al., 2024	(22)	Transversal	91	31	30	RT-qPCR	miR-3928
He et al., 2020	(28)	Caso y controles	59	45	14	Exosome isolation + RT-qPCR	miR-24-3p
Momen-Heravi et al., 2014	(15)	Transversal	34	9	9	NanoString + RT-qPCR	miR-27b, miR-24
Patel et al., 2023	(16)	Exploratorio + validación	70	50	20	RNASeq + RT-qPCR	miR-140-5p, miR-143-5p, miR-145-5p

(miR/miRNA = microRNA; OSCC = Oral squamous cell carcinoma; RT-qPCR = Reverse Transcription quantitative Polymerase Chain Reaction; TaqMan RT-qPCR = TaqMan Reverse transcription qPCR; RNASeq = RNA sequencing; EV isolation = Extracellular vesicle isolation)

Tabla 2: miARNs salivales reportados en COCE: frecuencia de estudios y tendencias de expresión

miARN	Autores	Frecuentemente reportado	Expresión dn COCE	Cantidad de estudios
miR-21	Yap et al.; Rocchetti et al.; Vageli et al.; Garg et al.; Di Stasio et al.	Si	Sobreexpresado en COCE	5
miR-31	Yap et al.; Rocchetti et al.; Scholtz et al.	Si	Sobreexpresado en COCE	3
miR-423-5p	Romani et al.; Patel et al.	Si	Sobreexpresado en COCE	2
miR-138	Rocchetti et al.; Scholtz et al.; Momen-Heravi et al.	Si	Subexpresado en COCE	3
miR-106a	Tarrad et al.	No	Subexpresado en COCE	1
miR-24-3p	He et al.	No	Sobreexpresado en COCE	1
miR-31-5p	Scholtz et al.	No	Sobreexpresado en COCE	1
miR-345	Scholtz et al.	No	Sobreexpresado en COCE	1
miR-424-3p	Scholtz et al.	No	Sobreexpresado en COCE	1
miR-140	Patel et al.	No	Subexpresado en COCE	1
miR-143	Patel et al.	No	Subexpresado en COCE	1
miR-145	Patel et al.	No	Subexpresado en COCE	1
miR-30a	Patel et al.	No	Subexpresado en COCE	1
let-7i	Patel et al.	No	Subexpresado en COCE	1
miR-412-3p	Gai et al.	No	Sobreexpresado en COCE	1
miR-489-3p	Gai et al.	No	Sobreexpresado en COCE	1
miR-512-3p	Gai et al.	No	Sobreexpresado en COCE	1
miR-597-5p	Gai et al.	No	Sobreexpresado en COCE	1
miR-603	Gai et al.	No	Sobreexpresado en COCE	1
miR-27b	Momen-Heravi et al.	No	Sobreexpresado en COCE	1
miR-30c-5p	Mehterov et al.	No	Subexpresado en COCE	1
miR-106b-5p	Romani et al.	No	Subexpresado en COCE	1
miR-193b-3p	Romani et al.	No	Sobreexpresado en COCE	1
miR-184	Garg et al.; Scholtz et al.	No	Subexpresado en COCE	2
miR-191	Scholtz et al.	No	Sobreexpresado en COCE	1
miR-484	Gai et al.	No	Subexpresado en COCE	1
miR-720	Gai et al.	No	Subexpresado en COCE	1
miR-376c-3p	Gai et al.	No	Subexpresado en COCE	1
miR-27a-3p	Gai et al.	No	Sobreexpresado en COCE	1
miR-302b-3p	Gai et al.	No	Sobreexpresado en COCE	1
miR-337-5p	Gai et al.	No	Sobreexpresado en COCE	1
miR-373-3p	Gai et al.	No	Sobreexpresado en COCE	1
miR-494-3p	Gai et al.	No	Sobreexpresado en COCE	1
miR-517b	Gai et al.	No	Sobreexpresado en COCE	1
miR-520d-3p	Gai et al.	No	Sobreexpresado en COCE	1
miR-645	Gai et al.	No	Sobreexpresado en COCE	1
miR-125a	Mehterov et al.	No	No significativo	1

(miR/miARN = microARN; COCE = Carcinoma oral de células escamosas)

Tabla 3: Resumen de los patrones de expresión de microARNs salivales y su relevancia clínica en OSCC vs. controles sanos

Autores	Comparación	miARN sobreexpresado	miARN subexpresado	Relevancia clínica
Romani et al., 2021 (19)	Sano vs. COCE	miR-423-5p, miR-106b-5p, miR-193b-3p		Sobreexpresión de miR-423-5p se asocia con mal pronóstico; AUC = 0,98
Yap et al., 2018 (24)	Sano vs. COCE	miR-31, miR-21, miR-100	miR-99a, miR-125b, let-7c	Panel de miARN mostró una alta precisión diagnóstica (AUC = 0,95)
Rocchetti et al., 2024 (23)	Sano vs. COCE	miR-21, miR-31	miR-138, miR-145, miR-424, miR-184	miR-138 y miR-424 como biomarcadores supresores tempranos
Scholtz et al., 2022 (26)	Sano vs. COCE	miR-31-5p, miR-345-3p	miR-424-3p	Panel de 3 miARNs mostró una alta capacidad de discriminación (AUC = 0,87)
Tarrad et al., 2023	Sano vs. COCE		miR-106a	Subexpresión de miR-106a se correlacionó con COCE de mayor grado.
Vageli et al., 2023 (25)	Sano vs. COCE	miR-21, miR-136, miR-3928, miR-29B		miR-21 elevado en fumadores; marcador temprano de COCE.
Gai et al., 2023 (18)	Sano vs. COCE	miR-302b-3p, miR-517b-3p, miR-512-3p, miR-412-3p		miARNs enriquecidos en vesículas extracelulares salivales de pacientes con COCE
Di Stasio et al., 2022 (17)	Sano vs. COCE		miR-27b, miR-181b	miR-181b elevado en displasia de alto grado, disminuido en COCE
Garg et al., 2023 (21)	Sano vs. COCE	miR-21	miR-184	Ambos miARNs alterados en OPMD y OSCC; marcadores tempranos
Mehterov et al., 2021 (20)	Sano vs. COCE		miR-30c-5p	Subexpresión de miR-30c-5p muestra valor diagnóstico (AUC = 0,82)
Farshbaf et al., 2024 (22)	Sano vs. COCE		miR-3928	Se observó subexpresión en COCE y LPO; posible biomarcador temprano.
He et al., 2020	Sano vs. COCE	miR-24-3p		El miR-24-3p exosomal promueve la proliferación de células de COCE (AUC = 0,738)
Momen-Heravi et al., 2014 (15)	Sano vs. COCE	miR-27b, miR-24		miR-27b específico de COCE activo, no presente en remisión ni en LPO
Patel et al., 2023 (16)	Sano vs. COCE		miR-140-5p, miR-143-5p, miR-145-5p	Firma de 3 miARNs asociada con EMT y pronóstico

(miR/miARN = microARN; COCE = Carcinoma oral de células escamosas; DPMO = Trastornos orales potencialmente malignos; LPO = Liquen plano oral; EV = Vesículas extracelulares; EMT = Transición epitelio-mesenquimal; AUC = Área bajo la curva)

Tabla 4: Resumen de los estudios incluidos que reportan microARNs salivales diferencialmente expresados en OSCC, con desempeño diagnóstico y significancia estadística.

Estudio	miARN(s) estudiados	Expresión en COCE vs. control sano	Valor-p	Significativo	Precisión diagnóstica
Romani et al. (19)	miR-423-5p, miR-106b	↑ COCE	< 0,001	Si	AUC = 0,98
Yap et al. (24)	miR-31, miR-21, let-7c	↑ miR-21/31 ↓ let-7c	< 0,01	Si	AUC = 0,95
Scholtz et al. (26)	miR-31-5p, miR-345, miR-424	↑ COCE	< 0,05	Si	AUC = 0,87
Vageli et al. (25)	miR-21, miR-136, miR-3928	↑ COCE, especialmente fumadores	< 0,005	Si	No reportado
Gai et al. (18)	miR-512, miR-412, miR-302b	↑ COCE EVs	< 0,01	Si	ROC > 0,8 para miR-512
Farshbaf et al. (22)	miR-3928	↓ in COCE/LPO	< 0,0001	Si	No reportado
He et al. (28)	miR-24-3p	↑ COCE	0,02	Si	AUC = 0,738
Di Stasio et al. (17)	miR-181b, miR-27b	↑ en displasia	0,006; 0,046	Si	No reportado
Garg et al. (21)	miR-21, miR-184	↑ miR-21 ↓ miR-184 COCE	< 0,001	Si	ROC trazada
Rocchetti et al. (23)	miR-138, miR-424	↓ in COCE	< 0,05	Si	No reportado
Tarrad et al. (27)	miR-106a	↓ COCE	< 0,05	Si	AUC = 80,4%
Mehterov et al. (20)	miR-30c-5p	↓ COCE	0,04	Si	AUC = 0,82
Momen-Heravi et al. (15)	miR-27b, miR-24	↑ COCE	< 0,01	Si	ROC = Fuerte
Patel et al. (16)	miR-140, miR-143, miR-145	↓ COCE	< 0,05	Si	Validación funcional

(miR/miARN = microARN; OSCC = Carcinoma oral de células escamosas; AUC = Área bajo la curva; ROC = Curva característica operativa del receptor; ↑ = sobreexpresado; ↓ = subexpresado)