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Review

Salivary extracellular vesicle-derived RNA biomarkers for early detection of esophageal squamous cell carcinoma: A systematic review

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Received: 10 October 2026 | Approved: 27 October 2026 | Online: 27 October 2026

Abstract

Aim: To systematically evaluate the diagnostic potential of salivary extracellular vesicle (EV)-associated RNA biomarkers for esophageal squamous cell carcinoma (ESCC), with particular emphasis on early-stage detection.

Methods: PubMed, Web of Science, Scopus, Cochrane Library, ProQuest, and Google Scholar were systematically searched through July 29, 2026. Eligible studies evaluating salivary EV-associated RNA biomarkers for ESCC detection were selected using

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predefined criteria. Methodological quality was assessed using the Joanna Briggs Institute (JBI) diagnostic test accuracy checklist. Findings were synthesized narratively because of clinical and methodological heterogeneity.

Results: Four studies met the inclusion criteria, investigating a salivary exosomal chimeric RNA, a two-small-RNA signature, a six-microRNA panel, and a tri-RNA signature. The chimeric RNA assay showed sensitivities of 85.3%-89.1% and specificities of 89.1%-91.4% across training and validation cohorts. The two-small-RNA signature achieved 90.5% sensitivity and 94.2% specificity. The six-microRNA panel yielded an area under the receiver operating characteristic curve (AUROC) of 0.969 for stage I/II ESCC in the training cohort. The tri-RNA signature achieved AUROCs of 0.987 and 0.964 for overall ESCC detection in training and validation cohorts, respectively, and 0.975 and 0.950 for distinguishing early-stage, lymph node-negative ESCC from healthy controls.

Conclusion: Salivary EV-associated RNA biomarkers, particularly multi-RNA signatures, show promising diagnostic performance for ESCC. However, methodological heterogeneity, potential cohort overlap, and limited geographically diverse validation warrant further independent prospective studies before clinical implementation.

Keywords: Extracellular vesicles, salivary biomarkers, esophageal squamous cell carcinoma, early detection, non-coding RNA, liquid biopsy, exosomes, diagnostic biomarkers

INTRODUCTION

Esophageal squamous cell carcinoma (ESCC) continues to pose a significant public health issue worldwide, especially in regions like Eastern Asia and parts of Eastern and Southern Africa, where it represents a large share of the global cases of esophageal cancer[1,2]. As reported by GLOBOCAN 2022, there were around 511,000 new diagnoses and 445,000 fatalities linked to esophageal cancer globally[3]. The incidence is expected to rise further in the upcoming years, highlighting the urgent need for effective prevention and early detection strategies[2,3]. The primary hurdle in clinical practice is not just identifying ESCC, but doing so at an early stage to enhance patient outcomes[4]. In its early phases, ESCC may not exhibit noticeable symptoms, while more advanced stages significantly limit treatment alternatives and are tied to a poor prognosis[2,4]. Conversely, lesions identified at an early stage could potentially be treated successfully through

endoscopic or surgical means[4]. This creates a pressing demand for reliable biomarkers that can detect molecular changes associated with the disease before it becomes symptomatic or reaches an advanced stage[2,5]. Presently, endoscopic evaluations combined with histopathological analysis are the cornerstone of ESCC diagnosis[5]. However, while endoscopy allows for direct observation and tissue sampling, its invasive nature, reliance on specialized skills and facilities, burdensome procedures, and limited ability to scale for wider population screening hinder its effectiveness as a straightforward tool for screening large groups[5]. These challenges have sparked increased interest in developing minimally invasive and non-invasive biomarkers that could work alongside endoscopy to support earlier diagnosis[2,5].

Saliva stands out as a promising medium for discovering non-invasive biomarkers due to its ease of collection, pain-free handling, low cost, and ability to be obtained multiple times[6]. This biological fluid comprises proteins and extracellular vesicles (EVs)[7]. Recent studies indicate that salivary EVs might hold diagnostic value that extends beyond oral health issues, potentially aiding in the detection of gastrointestinal cancers like esophageal cancer[8]. Nevertheless, standardizing methodologies and validating findings on a large scale are crucial steps before these biomarkers can be integrated into everyday clinical use[6,8].

Extracellular vesicles transport a wide range of molecular materials, including messenger RNAs, microRNAs, transfer RNA fragments, long non-coding RNAs, and other regulatory molecules[9,10]. The molecular contents of these vesicles may mirror the features of the cells they originate from[9]. As a result, RNA associated with EVs has surfaced as a promising avenue for finding minimally invasive cancer biomarkers and could play a role in liquid biopsy techniques[11,12].

However, the diversity within EV populations and variations in their isolation and characterization methods pose significant methodological hurdles[13]. Various studies have identified salivary EV-related RNA patterns that may hold diagnostic significance for esophageal squamous cell carcinoma (ESCC), such as chimeric RNAs, small non-coding RNAs, and multi-miRNA combinations[8,14,15]. Specific candidates for biomarkers include the salivary exosomal GOLM1-NAA35 chimeric RNA, tRNA-GlyGCC-5, and sRESE[8,14-16]. Additionally, a newly discovered six-miRNA signature from salivary

extracellular vesicles and particles showed remarkable capability in distinguishing between ESCC patients and healthy controls, even at the early stages of the disease (stage I/II)[14]. These results indicate that RNA linked to salivary EVs might provide critical molecular indicators useful for the early detection of ESCC[14,16].

Although the findings are encouraging, the research surrounding RNA biomarkers derived from salivary extracellular vesicles (EV) for esophageal squamous cell carcinoma (ESCC) is still scattered and not comprehensively integrated, particularly in relation to early detection. Thus, it is essential to conduct a targeted systematic review to ascertain if the existing data supports the use of salivary EV-derived RNA as a reliable method for the early identification of ESCC. This review should scrutinize the properties of potential RNA biomarkers, the techniques for isolating and characterizing EVs, the demographics of study participants and the stages of disease, as well as approaches for discovery and validation, diagnostic efficacy, and the quality of methodologies employed, while also pinpointing obstacles to clinical application[8,15]. Consequently, the purpose of this systematic review is to thoroughly identify and consolidate the existing evidence on salivary EV-derived RNA biomarkers aimed at the early detection of ESCC, paying particular attention to potential RNA candidates, EV isolation and characterization methods, the processes of biomarker discovery and validation, diagnostic effectiveness in early-stage disease, and the quality of research methodologies alongside the limitations for translation into clinical practice[14,16].

METHODS

Study design and reporting guidelines

This systematic review was conducted and reported in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 statement[17]. The review aimed to systematically identify, critically appraise, and synthesize the available evidence on salivary extracellular vesicle (EV)-derived RNA biomarkers for the early detection of esophageal squamous cell carcinoma (ESCC). The protocol for this systematic review was prospectively registered on PROSPERO (CRD420261485929).

Research question and review objective

This systematic review aimed to synthesize the available evidence regarding the diagnostic potential of salivary extracellular vesicle-associated RNA biomarkers for esophageal squamous cell carcinoma (ESCC), with particular emphasis on early-stage disease. The primary research question was formulated according to the PICO framework: What is the diagnostic performance of salivary extracellular vesicle-associated RNA biomarkers for detecting esophageal squamous cell carcinoma, particularly early-stage disease?

- Population: Patients with ESCC and appropriate non-ESCC controls.
- Index test: Salivary EV-associated RNA biomarkers.
- Comparator: Non-ESCC controls.
- Outcome: Diagnostic performance, particularly sensitivity, specificity, and AUROC, with emphasis on early-stage ESCC.

The primary objective was to identify and critically synthesize studies investigating salivary extracellular vesicle-associated RNAs as potential biomarkers for ESCC detection. Specifically, this review aimed to: (1) identify the types and molecular profiles of salivary extracellular vesicle-associated RNAs reported in association with ESCC; (2) evaluate their diagnostic performance, including sensitivity, specificity, and area under the receiver operating characteristic curve (AUROC), where reported; (3) assess their associations with relevant clinical and pathological characteristics and, where reported, prognostic outcomes of ESCC; and (4) characterize methodological heterogeneity across studies, including approaches to extracellular vesicle isolation, RNA extraction and profiling, biomarker identification, and molecular validation.

Eligibility criteria

Eligibility criteria were predefined according to the review question and applied consistently during study selection, as summarized in Table 1. Studies investigating salivary EV-associated RNA biomarkers were considered eligible; studies involving extracellular vesicles and particles (EVPs) were evaluated according to the extent to which their findings were relevant to EV-associated RNA biomarkers.

Table 1. Inclusion and exclusion criteria for study selection.

Criterion	Inclusion criteria	Exclusion criteria

Population	Human participants with histologically or clinically confirmed esophageal squamous cell carcinoma (ESCC)	Animal studies, in vitro studies, or studies without an ESCC population
Biological specimen	Saliva or salivary samples containing extracellular vesicles (EVs) or exosome-enriched fractions	Non-salivary specimens without a salivary EV component, such as blood, plasma, serum, urine, or tissue alone
Index biomarker	RNA biomarkers derived from salivary EVs/exosomes, including miRNAs, small non-coding RNAs, tRNA-derived fragments, chimeric RNAs, and other EV-associated RNA species	Protein, metabolite, DNA, or non-EV-associated biomarkers
Target condition	Esophageal squamous cell carcinoma, with particular interest in early-stage disease	Other malignancies or esophageal diseases without separately reported ESCC data
Purpose	Detection, discrimination, or diagnosis of ESCC using salivary EV-derived RNA biomarkers	Studies without a diagnostic or discriminatory objective
Outcomes	Diagnostic performance measures such as sensitivity, specificity, AUROC, predictive values, or other measures of diagnostic discrimination	Studies without sufficient information to assess the diagnostic relevance of the biomarker
Study design	Original human observational, diagnostic, or biomarker studies	Reviews, systematic reviews, meta-analyses, editorials,

	reporting relevant primary data	letters, case reports, conference abstracts without sufficient data, and other non-original publications
Publication status	Full-text articles with sufficient methodological and outcome information	Articles for which the full text or essential diagnostic information was unavailable

Information sources and search strategy

Information Sources and Search Strategy A comprehensive literature search was conducted on 29 July 2026 in six electronic databases: PubMed, Web of Science, Scopus, Cochrane Library, ProQuest, and Google Scholar, to identify studies investigating salivary extracellular vesicle (EV)-associated RNA biomarkers for the detection of esophageal squamous cell carcinoma (ESCC). The search strategy was developed using five core concepts: RNA/transcriptomic biomarkers, saliva or oral fluids, extracellular vesicles or exosomes, diagnosis/early detection, and ESCC. Within each concept, relevant synonyms, abbreviations, related terms, and word variations were combined using the Boolean operator OR, while the five concepts were combined using AND. Truncation (*) was applied where appropriate to capture variations of search terms.

The core search syntax was structured as follows:

(“Transcriptomic Biomarker*” OR RNA OR Transcriptom* OR miRNA OR microRNA OR lncRNA OR circRNA OR “Circular RNA” OR mRNA OR piRNA OR snoRNA OR snRNA OR tsRNA OR tRF OR ncRNA OR “non-coding RNA”) AND (Saliva* OR “Oral Fluid*” OR “Mouth Fluid*” OR “Oral Biofluid*”) AND (Exosome* OR “Extracellular Vesicle*” OR “Extracellular Particle*” OR Exovesicle* OR “Apoptotic Bod*”) AND (Diagnos* OR “Early Detection” OR Screening OR Detection) AND (“Esophageal Squamous Cell Carcinoma” OR ESCC OR “Esophageal Cancer*” OR “Cancer of Esophagus” OR “Esophageal Neoplasm*” OR “Oesophageal Squamous Cell Carcinoma” OR “Esophageal Tumor*” OR “Esophageal Carcinoma” OR “Esophagus Neoplasm*”)

The detailed search strategies tailored for each database, encompassing the precise search terms, restrictions on fields, and the records obtained, can be found in Appendix A. For PubMed, the search terms were utilized specifically within the Title and Abstract sections.

In contrast, Scopus, Web of Science, Cochrane Library, and ProQuest employed their unique field-specific parameters in accordance with their respective search platforms. Google Scholar was examined using a set combination of five fundamental concepts. Beyond electronic searches, we also manually reviewed the reference lists of all studies included and pertinent review articles to uncover any potentially qualifying studies that might have been missed in the electronic searches. All retrieved records were compiled, and duplicate records were removed before title and abstract screening. Other records removed before screening, if any, were documented separately in the PRISMA flow diagram.

Study selection

All records found via electronic database searches were transferred into a reference management system, and any duplicates were eliminated before the screening process began. The selection of studies occurred in two distinct phases. Initially, two reviewers independently evaluated titles and abstracts based on specific eligibility criteria. Studies deemed possibly relevant were then fully retrieved and assessed for eligibility by the same reviewers. Any differences in opinion between the reviewers were settled through discussion, and a third reviewer was consulted when needed. The reasons for excluding studies at the full-text review stage were carefully recorded. The overall study selection process was illustrated using a PRISMA flow diagram.

Data extraction

Two reviewers independently gathered data using a standardized and tested data extraction form created specifically for this systematic review. The collected information encompassed various study attributes, such as the name of the first author, year of publication, country, and research design. Additionally, details about the participants, including characteristics and sample size, along with clinical features of the studied population, were included. The type of salivary extracellular vesicle-associated RNA biomarker analyzed, the methods for isolating and characterizing extracellular vesicles, and the techniques for RNA extraction and profiling were also documented. Furthermore, the methods for detecting and validating biomarkers, the comparator groups, and the diagnostic or prognostic outcomes reported were extracted as well. When available, diagnostic performance metrics such as sensitivity, specificity, and the area under the receiver operating characteristic curve (AUC) were noted, along with any associations

with clinicopathological features. Any differences in the data collection process were addressed through discussion and mutual agreement.

Quality appraisal and risk of bias assessment

The quality of methods and potential biases in the studies analyzed were evaluated independently by two reviewers following the relevant Joanna Briggs Institute (JBI) critical appraisal checklist tailored to the design of each study included[18]. This evaluation focused on essential methodological areas that are crucial for the credibility and relevance of biomarker research. These areas included how participants were chosen, how the main biomarker was measured and understood, the suitability of comparison groups, the thoroughness of outcome assessments, and any possible biases. When the reviewers had differing opinions, they reached a resolution through discussion to achieve a consensus. The outcomes of this critical assessment were integrated into the interpretation of the results rather than being used as the sole reason for excluding any studies.

Data synthesis

Due to the considerable differences in clinical and methodological aspects among the studies analyzed, a quantitative meta-analysis was deemed unsuitable. These discrepancies stemmed from variations in study groups, types of biomarkers, methods for isolating and characterizing extracellular vesicles, techniques for RNA profiling and detection, strategies for validation, and the outcomes reported. As a result, the findings were integrated through a structured narrative approach. The salivary RNA biomarkers associated with extracellular vesicles were categorized based on their molecular class and clinical usage, and their diagnostic or prognostic effectiveness, along with their connections to clinicopathological features, were compared across different studies. Special focus was placed on the reliability of biomarker results, differences in methodologies, and the degree to which each biomarker had been validated independently. The overall evidence was evaluated considering the methodological quality and potential biases present in the studies examined.

RESULTS

Study selection

The selection of studies was carried out in line with the PRISMA 2020 standards. On July 29, 2026, an extensive search of the literature was conducted across specified electronic

databases using a well-structured search method that integrated keywords relating to transcriptomic or RNA biomarkers, saliva, extracellular vesicles or exosomes, and the early identification or diagnosis of esophageal squamous cell carcinoma (ESCC). Detailed strategies for searching within each database are listed in Appendix A. After eliminating duplicate entries, titles and abstracts were evaluated based on established eligibility criteria. Articles deemed potentially relevant were then examined in their entirety. Studies were omitted from consideration if they did not explore salivary extracellular vesicle- or exosome-related RNA biomarkers, did not focus on esophageal cancer or ESCC, or failed to supply pertinent diagnostic or early-detection information. In the end, four studies fulfilled all the eligibility requirements and were included in the systematic review. These studies examined various categories of salivary extracellular vesicle-associated RNA biomarkers, such as chimeric RNA, small non-coding RNAs, microRNAs, and a tri-RNA signature. The four studies that were included are: (1) a study that analyzed salivary exosomal chimeric GOLM1-NAA35 RNA as a potential biomarker for esophageal carcinoma (Lin *et al.*, 2019); (2) a multicenter prospective study that evaluated a saliva-derived exosomal small RNA signature for esophageal carcinoma (Li *et al.*, 2022); (3) a clinical investigation into salivary extracellular microRNAs for the early detection and prognosis of esophageal cancer (Li *et al.*, 2023); and (4) a multicenter study that developed and validated a salivary exosomal tri-RNA liquid biopsy for esophageal carcinoma (Lin *et al.*, 2026). The overall process of study selection, which includes the numbers of records identified, screened, assessed for eligibility, and ultimately included, is illustrated in the PRISMA 2020 flow diagram [Figure 1].

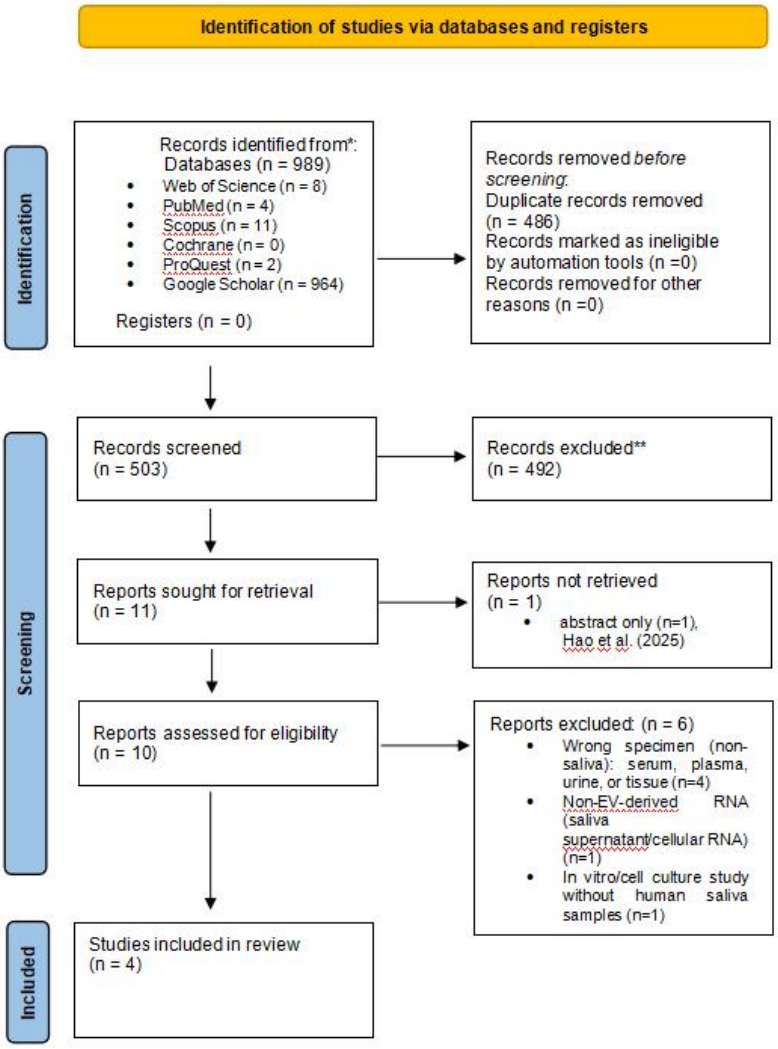


Figure 1. PRISMA 2020 flow diagram of the study selection process.

Characteristics and main findings of included studies

Between the years 2019 and 2026, four studies that met the necessary criteria were selected for this systematic review. Each of these studies focused on analyzing RNA biomarkers found in salivary extracellular vesicles or exosomes to aid in the detection or clinical evaluation of esophageal squamous cell carcinoma (ESCC). The research was conducted in China and examined various types of extracellular vesicle-associated RNA, including chimeric RNA, small exosomal non-coding RNAs, and microRNAs derived from extracellular vesicles or particles.

The initial study, authored by Lin and colleagues in 2019, explored the potential of salivary exosomal GOLM1-NAA35 chimeric RNA (seG-NchiRNA) as a biomarker for

ESCC. This study comprised a discovery cohort, a training cohort, and a validation cohort. In the prospective cohorts, seG-NchiRNA was quantified using quantitative reverse-transcription polymerase chain reaction (qRT-PCR). Results indicated that this biomarker was significantly higher in patients diagnosed with ESCC compared to healthy controls and had a notable correlation with the tumoral G-NchiRNA levels. In both the training and validation cohorts, seG-NchiRNA showed sensitivities ranging from 85.3% to 89.1% and specificities from 89.1% to 91.4% in detecting ESCC. Notably, this biomarker also proved effective in distinguishing early-stage ESCC patients from healthy individuals, highlighting its promising potential for early detection.

The second research, conducted by Li and colleagues in 2022, employed small-RNA sequencing of saliva-derived exosomes to pinpoint small RNAs associated with cancer in esophageal squamous cell carcinoma (ESCC). They identified two candidate small RNAs, tRNA-GlyGCC-5 and sRESE, which were then combined to create a bi-sesncRNA signature. The investigation consisted of both pilot and discovery groups, followed by prospective multicenter groups from two different institutions. This bi-sesncRNA signature effectively differentiated ESCC patients from control subjects, achieving a sensitivity of 90.50% and a specificity of 94.20%. Beyond its diagnostic capabilities, this signature was linked to overall survival and progression-free survival, indicating its potential to help identify patients who might gain from adjuvant therapy.

In a separate study published in 2023, Li and his team explored microRNAs sourced from salivary extracellular vesicles and particles (EVPs). Initially, they analyzed a pilot group through microarray profiling, which was followed by candidate validation using qRT-PCR and the creation of diagnostic models across training, internal validation, and external validation cohorts. Ultimately, six specific miRNAs were merged to form a diagnostic signature. This 6-EVP miRNA signature exhibited remarkable ability to distinguish early-stage ESCC, recording an AUROC of 0.969 in the training cohort while retaining robust diagnostic performance in both internal and external validation cohorts. Additionally, the same miRNA panel was employed to establish a prognostic signature for risk stratification based on progression-free and overall survival.

The most recent study, conducted by Lin and colleagues (2026), combined three previously reported RNA biomarkers—seG-NchiRNA, tRNA-GlyGCC-5, and sRESE—

into a tri-RNA diagnostic signature. As these biomarkers had been investigated in earlier studies, the extent of independence between the contributing research cohorts warrants consideration. This multicenter study included a training cohort of 222 patients with ESCC and 137 healthy controls (n = 359), and a validation cohort of 151 patients with ESCC and 74 healthy controls (n = 225). The combined RNA signature demonstrated excellent diagnostic performance, achieving AUROCs of 0.987 and 0.964 in the training and validation cohorts, respectively, outperforming individual RNA biomarkers. It also distinguished patients with early stage ESCC without lymph node metastasis (N0) from healthy controls, with AUROCs of 0.975 and 0.950 in the training and validation cohorts, respectively. Furthermore, the signature showed potential for improved prognostic risk stratification.

Collectively, despite heterogeneity in RNA species, biomarker discovery strategies, and analytical platforms, all four included studies consistently demonstrated the promising diagnostic potential of salivary extracellular vesicle-associated RNA biomarkers for ESCC. Notably, Lin *et al.* (2026) extended previous biomarker investigations by evaluating a combined tri-RNA signature in multicenter training and validation cohorts and examining its prognostic and treatment-related associations. The main characteristics and key findings of these studies are summarized in Table 2.

Table 2. Characteristics, Biomarker Profiles, and Main Findings of the Included Studies.

Characteristic	Lin *et al.* (2019)	Li *et al.* (2022)	Li *et al.* (2023)	Lin *et al.* (2026)
Country	China (multi-center: Shantou & Anyang)	China (multi-center: Shantou & Anyang)	China (multi-center: Shantou & Anyang)	China (multi-center: Shantou & Anyang)
Study design	Prospective observational	Prospective multicenter observational	Prospective multi-cohort	Prospective multicenter observational
Sample type	Saliva (exosomes)	Saliva (exosomes)	Saliva (extracellular	Saliva (exosomes)

			vesicles/particles, EVPs)	
EV / RNA type	Exosomal chimeric RNA	Exosomal small non-coding RNAs (tsRNA + novel RNA)	EVP-derived microRNAs	Exosomal Tri- RNA signature
Biomarker(s)	seG-NchiRNA (GOLM1-NAA35)	Bi-sesncRNA signature: tRNA-GlyGCC-5 + sRESE	6-miRNA panel: miR-1972, miR-4274, miR-4701-3p, miR-6126, miR-1268a, miR-4505	Tri- RNA signature: seG- NchiRNA + tRNA- GlyGCC-5 + sRESE
Cohorts / Sample size	Training: 220 ESCC + 55 controls Validation: 102 ESCC + 35 controls Total: 322 ESCC + 90 controls	Training (CHSUM): 200 ESCC + 120 controls Validation (ATH): 140 ESCC + 60 controls Total: 340 ESCC + 180 controls	Training: 222 ESCC + 120 controls Internal validation: 140 ESCC + 67 controls External validation: 159 ESCC + 67 controls Total: 521 ESCC + 254 controls	Training (CHSUMC): 222 ESCC patients + 137 healthy controls (n = 359) Validation (ATH): 151 ESCC patients + 74 healthy controls (n = 225) Total: 373 ESCC patients + 211 healthy controls (N = 584)
Analytical method	qRT-PCR, logistic regression, ROC analysis, Kaplan-Meier, Cox regression	RNA-seq, qRT-PCR, logistic regression, Kaplan-Meier,	Microarray, qRT-PCR, logistic regression, LASSO,	qRT- PCR, logistic regression, ROC analysis, Kaplan- Meier, Cox regression

		Cox regression, nomogram	Kaplan-Meier, Cox regression	
Main diagnostic finding	Sensitivity: 85.3%–89.1% Specificity: 89.1%–91.4% AUROC: 0.912 Early-stage (I/IIa) sensitivity: 73.8%–81.6%	Sensitivity: 90.50% Specificity: 94.20% AUROC: 0.933 Stage I detection: positive	AUROC (all-stage): 0.968 AUROC (early-stage I/II): 0.969 Early-stage sensitivity: 92.0% (training), 90.3% (internal), 91.1% (external) Early-stage specificity: ~89%–91%	Overall ESCC detection: AUROC = 0.987 (training cohort) and 0.964 (validation cohort), outperforming individual RNA biomarkers. Early-stage, lymph node-negative (N0) ESCC detection: AUROC = 0.975 (training cohort) and 0.950 (validation cohort).
Other key findings	• Decreased after chemoradiation • Predicted PFS ($p < 0.001$) • Detected recurrence earlier than imaging	• RSP predicted OS (HR 4.95) & PFS (HR 3.69) • Adjuvant therapy benefited only high-RSP patients	• RS independent prognostic factor (HR 2.74) • PPV up to 90% in high-risk populations • Risk stratification for	• improved risk stratification

	• No circadian variability	• 3-year OS nomogram (C-index 0.718)	personalised management • Proposed clinical algorithm	
Clinical trial registration	Not registered (ethics approval only)	ChiCTR2000031507	ChiCTR2000031507	ChiCTR2000031507

Quality appraisal and risk of bias

The methodological quality of the studies was evaluated using the Joanna Briggs Institute (JBI) checklist designed for assessing the accuracy of diagnostic tests. Each of the four studies included received a score of 7 out of 10, indicating that their methodological quality was generally satisfactory, although the diagnostic thresholds were derived from training cohorts rather than being pre-specified. Notable strengths of these studies included the sequential recruitment of patients, careful avoidance of case-control designs, the employment of a reliable reference standard (histopathology), and the inclusion of independent validation cohorts. These factors contributed positively to the trustworthiness of the diagnostic findings reported. However, certain limitations were noted across all studies, mainly concerning the unclear reporting of blinding during the interpretation of both the index test and the reference standard (criteria 4 and 7), as well as the lack of predetermined threshold values for the index test (criterion 5). These issues are frequently encountered in exploratory studies utilizing EV-based biomarkers. Although the use of training and validation cohorts allowed further assessment of diagnostic model performance, the possibility of overfitting and optimistic performance estimates cannot be completely excluded, particularly in the absence of fully independent external validation. A comprehensive breakdown of the JBI critical appraisal results for each study is provided in Table 3.

Table 3. Methodological Quality and Risk of Bias of the Included Studies Based on the JBI Critical Appraisal Checklist for Diagnostic Test Accuracy Studies.

JBI criterion	Lin *et al.*, 2019	Li *et al.*, 2022	Li *et al.*, 2023	Lin *et al.* (2026)
1. Was a consecutive or random sample of patients enrolled?	Yes	Yes	Yes	Yes
2. Was a case control design avoided?	Yes	Yes	Yes	Yes
3. Did the study avoid inappropriate exclusions?	Yes	Yes	Yes	Yes
4. Were the index test results interpreted without knowledge of the results of the reference standard?	Unclear	Unclear	Unclear	Unclear
5. If a threshold was used, was it pre-specified?	No	No	No	No
6. Is the reference standard likely to correctly classify the target condition?	Yes	Yes	Yes	Yes
7. Were the reference standard results interpreted without knowledge of the results of the index test?	Unclear	Unclear	Unclear	Unclear
8. Was there an appropriate interval between index test and reference standard?	Yes	Yes	Yes	Yes
9. Did all patients receive the same reference standard?	Yes	Yes	Yes	Yes
10. Were all patients included in the analysis?	Yes	Yes	Yes	Yes
Yes responses	**7/10**	**7/10**	**7/10**	**7/10**

DISCUSSION

This systematic review identified four clinical studies investigating RNA biomarkers associated with salivary extracellular vesicles (EVs) or exosomes for the detection of esophageal squamous cell carcinoma (ESCC). Although the number of included studies was limited, the findings suggest that salivary EV associated RNA biomarkers may have diagnostic potential for ESCC. The studies evaluated four diagnostic approaches involving different RNA biomarkers and their combinations, including an exosomal chimeric RNA, a signature comprising two small noncoding RNAs, a panel of microRNAs derived from salivary extracellular vesicles and particles, and a combined signature incorporating three previously identified RNA biomarkers. All four studies reported promising discrimination between patients with ESCC and control participants. Combined RNA signatures also demonstrated encouraging diagnostic performance, although their superiority over individual biomarkers and their clinical applicability require further independent validation.

Lin *et al.* (2019) reported that salivary exosomal GOLM1-NAA35 chimeric RNA could distinguish patients with ESCC from healthy controls, with sensitivities ranging from 85.3% to 89.1% and specificities ranging from 89.1% to 91.4% across the training and validation cohorts[15]. The assay also showed sensitivities of 73.8% to 81.6% for detecting early stage ESCC. Li *et al.* (2022) developed a signature comprising two small noncoding RNAs, tRNA-GlyGCC-5 and sRESE, which achieved a sensitivity of 90.5% and a specificity of 94.2% in the training cohort. The study also reported associations with overall survival (HR = 4.95) and progression free survival (HR = 3.69)[8]. Subsequently, Li *et al.* (2023) developed a six microRNA signature that achieved an AUROC of 0.969 for stage I/II ESCC in the training cohort, with favorable diagnostic performance also reported in the internal and external validation cohorts[14]. More recently, Lin *et al.* (2026) combined three previously identified RNA biomarkers into a single diagnostic signature, achieving AUROCs of 0.987 and 0.964 for overall ESCC detection in the training and validation cohorts, respectively. For distinguishing early stage, lymph node negative (N0) ESCC from healthy controls, the corresponding AUROCs were 0.975 and 0.950, respectively[16].

Taken together, these findings suggest that combining multiple RNA biomarkers may improve diagnostic discrimination compared with individual biomarkers, although further independent validation is needed to establish the added diagnostic value of

combined signatures[14,16]. The methodological appraisal using the JBI Checklist for Diagnostic Test Accuracy Studies identified both strengths and potential sources of bias across the four included studies. Although the included studies incorporated prospective recruitment or validation components and used histopathology to confirm ESCC, their diagnostic findings primarily reflect discrimination between patients with established ESCC and controls. Accordingly, the reported performance in early-stage disease should not be interpreted as evidence of preclinical detection or effectiveness in asymptomatic screening populations[8,14,15]. Furthermore, the development of diagnostic thresholds within study cohorts may contribute to optimistic estimates of diagnostic performance. Blinding procedures were not consistently or clearly reported, which introduces uncertainty regarding potential interpretation bias [Table 3]. Future studies should follow the STARD reporting guidelines to improve transparency and facilitate a more reliable assessment of diagnostic accuracy[19].

Compared with approaches such as Cytosponge™, which requires swallowing a capsule[20], transnasal endoscopy, which requires specialized equipment and trained personnel[21], and plasma derived circulating tumor DNA analysis, which may have limited sensitivity in early stage malignancies[22], saliva collection may provide a convenient approach for obtaining biological samples[23]. However, the diagnostic and clinical performance of salivary extracellular vesicle associated RNA biomarkers has not been directly established as superior to these alternative approaches. The feasibility of repeated saliva collection may facilitate future longitudinal studies evaluating biomarker changes and risk assessment over time[23]. Although salivary RNA biomarkers could potentially contribute to screening strategies in populations at increased risk of ESCC, their clinical utility, implementation requirements, and cost effectiveness remain to be established through prospective comparative studies and formal health economic evaluations[24].

Despite these promising findings, several limitations should be considered. The small number of eligible studies limits the breadth of the evidence; therefore, the findings should be interpreted as preliminary and hypothesis-generating rather than definitive evidence sufficient to support changes in clinical practice. Although recent studies have reported diagnostic potential for salivary extracellular-vesicle (EV) RNA signatures, the available results do not by themselves establish clinical utility in routine practice[8,23].

Evidence from studies conducted in selected Chinese populations or high-incidence regions may not be generalizable to populations with different demographic, ethnic, and epidemiological characteristics[8]. Methodological differences in EV isolation, characterization, RNA quantification, and biomarker-model development are recognized barriers to reproducibility and comparability across EV studies[25]. Although some studies have used internal or external validation cohorts, diagnostic performance in case-control or clinically recruited samples does not establish performance in asymptomatic, population-based screening cohorts[14,26]. Accordingly, the ability of salivary EV RNA biomarkers to detect ESCC before clinical diagnosis, and their clinical utility in screening, remain to be established[26]. Further progress will require standardized and transparent laboratory workflows, prospective evaluation in diverse populations, and independent validation in screening-relevant cohorts before routine clinical application can be recommended[26].

The available evidence suggests that salivary extracellular vesicle associated RNA biomarkers remain investigational. Although several studies report promising discrimination between patients with ESCC and control participants, including early-stage disease, their results do not establish effectiveness in asymptomatic population screening or routine clinical decision making[8,14]. A potential future role may be risk stratified triage to help identify individuals who should undergo further diagnostic evaluation, particularly in high incidence settings. However, clinical utility requires evidence that biomarker guided care improves meaningful outcomes at acceptable cost and harm[26].

Future research should use standardized and transparently reported procedures for EV separation and characterization, alongside validated RNA measurement and normalization methods. MISEV2023 provides updated recommendations on EV production, separation, characterization, and reporting, while also noting the strengths and limitations of available approaches[27]. Because MISEV does not prescribe methods for molecular quantification of EV cargo, RNA assays should also follow appropriate analytical-quality guidance[28]. Priorities include prospective, multicenter evaluation in geographically and ethnically diverse populations; validation in asymptomatic participants; and assessment of diagnostic performance in the intended screening context, rather than relying solely on case-control results[26]. Studies should also compare

biomarkers with existing diagnostic approaches and assess clinical utility and cost, since high diagnostic accuracy alone does not demonstrate benefit from implementation[26]. Longitudinal studies using samples collected before diagnosis would help determine whether these biomarkers can identify ESCC before clinical presentation; the sources reviewed here do not establish that capability[26].

In conclusion, salivary EV-associated RNA biomarkers show promise for ESCC detection, including discrimination of early-stage cases from controls, but they have not yet been established as routine screening tests[14,26]. Standardized methods, independent validation, and prospective evaluation in the populations for whom screening is intended are needed before routine clinical use can be recommended[26,27]. Until then, these biomarkers should be regarded as investigational rather than clinically validated screening tests[26].

CONCLUSION

This systematic review identified four clinical studies reporting promising diagnostic performance of salivary extracellular vesicle associated RNA biomarkers for the detection of esophageal squamous cell carcinoma (ESCC), including early stage disease. Both individual RNA biomarkers and combined signatures demonstrated potential for distinguishing patients with ESCC from healthy controls. However, the limited number of studies, methodological heterogeneity, recruitment of participants exclusively in China, and lack of independent validation in asymptomatic screening populations restrict the generalizability and clinical applicability of these findings. Further multicenter prospective studies using standardized laboratory procedures, predefined diagnostic thresholds, and diverse populations are needed to establish the clinical utility of these biomarkers. At present, salivary extracellular vesicle associated RNA biomarkers should be considered promising investigational tools rather than validated alternatives to established diagnostic methods.

DECLARATIONS

Authors' contributions

Conceptualization, data extraction, methodological assessment, and writing of the original draft: Shabani Panbeh Choleh S;

Supervision, methodological review, and critical revision of the manuscript: Kazeminejad E;

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All authors reviewed and approved the final version of the manuscript.

Availability of data and materials

Data is available on request.

AI and AI-assisted tools statement

During the preparation of this manuscript, the authors used ChatGPT (OpenAI, Plus subscription) to assist with English language editing and improve the clarity and readability of the text. All AI-assisted revisions were carefully reviewed and verified by the authors, who take full responsibility for the accuracy, integrity, and final content of the manuscript.

Financial support and sponsorship

This research received no specific funding from any public, commercial, or nonprofit funding agency.

Conflict of interest

The authors declare that they have no conflicts of interest related to this work.

Ethical approval and consent to participate

This systematic review was based exclusively on previously published studies and did not involve the direct recruitment of human participants or the use of animals by the authors. Therefore, ethical approval was not required.

Consent for publication

Not applicable.

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