The clinical implications of circulating microRNAs as potential biomarkers in screening oral squamous cell carcinoma

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Background: Recent studies have highlighted the biomarker role of circulating miRNAs in oral squamous cell carcinoma (OSCC), indicating their potential application as early diagnostic markers for OSCC. However, the diagnostic results have proven inconclusive. This study was conducted to evaluate the diagnostic value of circulating miRNAs for OSCC diagnosis.

Methods: Eligible published studies were identified by a literature search carried out in several databases by using combinations of keywords associated with OSCC, circulating miRNAs, and diagnosis. The bivariate meta-analysis model was adopted to summarize the pooled parameters. Afterwards, we thoroughly explored the sources of heterogeneity after evaluating the risk of bias.

Results: A total of 60 studies focusing on 41 circulating miRNAs were included. The pooled sensitivity, specificity, and AUC were 0.75 (95%CI: 0.69–0.80), 0.76 (0.70–0.81), 0.82 (0.79–0.85), respectively. Subgroup analyses showed that miRNA combinations were more accurate than single miRNAs. Additionally, plasma may be a better matrix for miRNAs assays in OSCC diagnosis as the plasma-based miRNA assay had a higher level of diagnostic accuracy than serum-based miRNA assay. Subgroup analyses also suggested that using circulating miRNAs for OSCC diagnosis is more effective in Caucasians than in Asian ethnic groups. Finally, circulating miRNA assays based on large sample sizes have superior diagnostic accuracy than small sample sizes.
Conclusion: Circulating miRNAs might be applied as effective surrogate biomarkers for early diagnosis of OSCC. Nevertheless, future larger-scale prospective studies should be performed to enhance the diagnostic efficiency and investigate the miRNA combinations with more pronounced accuracy.

KEYWORDS
oral squamous cell carcinoma, microRNAs, biomarkers, diagnosis, circulating

Introduction

Oral squamous cell carcinoma (OSCC), accounting for more than 90% of total oral cancer, is often diagnosed at advanced stages and is characterized by a low survival rate (1). Approximately, 50-70% of OSCC patients died within 5 years after they were diagnosed with OSCC due to frequent metastases to regional lymph nodes (2). Although surgical and medical treatments for OSCC have been rapidly developed, the therapeutic efficacy and the five-year survival rate remain unsatisfactory. Early detection of OSCC is vitally significant to enhance the survival outcome, as rates as high as 80-90% can be achieved in the early stages of OSCC patients (3). Currently, diagnosis of OSCC still remains a challenge as the procedures mainly depend on imaging and histological biopsy, which are invasive and uncomfortable (4). Although noninvasive screening tools have been employed, no alternative predictors have been proved effective (5). To this regard, there is a need to explore novel and effective biomarkers for more advancement to promote early detection of OSCC and improve the treatment options.

Over the last decades, microRNAs (miRNAs) have emerged as a new set of biomarkers which play a vital part in oral carcinogenesis (6). MiRNAs are a class of endogenous, non-coding, 18-25 nucleotide length single-strand RNAs that regulate the gene expression at the post-transcriptional level and are highly vital for cell growth and proliferation, mediating critical pathways involved in cancer initiation and progression (7). A plethora of reports have shown that abnormal expressions of specific miRNAs are associated with numerous human diseases, and miRNAs may function as oncogenes or tumor suppressors in various cancer types (8). So far, a significant number of studies have been performed to explore the biomarker role of miRNAs in different cancer types and evidence gathered has revealed the potential use of miRNAs as biomarkers in cancer detection and prognosis (9). Moreover, miRNAs are highly stable in rough conditions and their isolation and quantification have been found to be easy, convenient, and reproducible (10). Therefore, these unique properties make miRNAs as promising diagnostic and prognostic biomarkers in malignant tumors including OSCC which can start from non-invasive specimen collection avoiding postoperative pain.

As we all know, the initiation of OSCC may originate from a multistep accumulation of heterogeneous genetic alterations (11, 12). In the past decades, the expression patterns of miRNAs in OSCC have been recognized as new directions in the exploration of oral carcinogenesis (13). Recent reports have also shown a good association between specific miRNA expression and clinical parameters including tumor metastasis, relapse, and survival in OSCC patients (14, 15). Moreover, a number of studies have demonstrated that miRNAs play an important part during the initiation and progression of OSCC (16, 17). Prominently, miR-21 stands out to be frequently associated with OSCC and may serve as a potential biomarker for diagnosis, prognosis or therapeutic target. Therefore, circulating miRNAs may serve as useful biomarkers for early detection of OSCC (18). However, the diagnostic accuracy of miRNA profiles for OSCC was still inconsistent or even contradictory across different studies, which may be caused by different study designs, different sample sources, different sample sizes and different races.

In this study, we performed a comprehensive meta-analysis of previously published miRNA expression profiling studies to evaluate and summarize the clinical results in the literatures, regarding the potential application of circulating miRNAs in plasma or serum as biomarkers for OSCC diagnosis.

Materials and method

Guidelines and searches

This study was performed according to the Preferred Reporting Items for Systematic Reviews and Meta-analysis (PRISMA) guidelines. We have developed a comprehensive search strategy to retrieve all available studies from PubMed, Embase, and Web of Science by using different combinations of keywords including oral, tumor, carcinoma, cancer, squamous cell carcinoma, OSCC, microRNAs, miRNAs, miR-, diagnosis, diagnostic, detect, detection, screen, screening, sensitivity, specificity, and ROC curve. The database was searched from its inception up to April 25, 2022. In addition, the reference lists of relevant reviews were independently scanned to avoid missing any potential studies.
Eligibility criteria

To be eligible for enrollment in the analysis, the studies had to comply with the following inclusion criteria: (1) original studies focused on the diagnostic value of circulating miRNAs in OSCC; (2) the OSCC diagnosis was achieved based on histopathology as the reference test; (3) studies detected the expression of miRNAs in whole blood, plasma, or serum; (4) the studies provided sufficient data for further calculation, including parameters including specificity, sensitivity, and area under the receiver operating characteristic (ROC) curve. Studies were excluded according to these exclusion criteria as follows: (1) studies were published as review articles, meta-analysis, letters, commentaries, or abstracts; (2) studies failed to provide sufficient information to allow construction of two-by-two table; (3) studies were performed on species other than humans; (4) the language was non-English.

Data extraction and quality assessment

Two investigators (H.G. and Y.S.) independently reviewed the full texts of included studies and gathered baseline characteristic and diagnostic data including (1) study characteristics: first author, year of study, and country; (2) patients’ demographic characteristics: ethnicity, number, mean age, and TNM stage; (3) miRNA features: miRNA signatures, detection methods, and sample types; (4) data used for further analyses: sensitivity and specificity or true positives (TP), true negatives (TN), false positives (FP), false negatives (FN), and area under the curve (AUC) values. In the present study, the qualities of studies were assessed independently by two reviewers using the revised Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2) criteria (19). If disagreements between the two reviewers occur, they will discuss together to achieve a consensus or consult with the third reviewer (Q.P.).

Statistical analysis

The bivariate meta-analysis model was adopted to summarize the sensitivity, specificity, positive likelihood ratio (PLR), negative likelihood ratio (NLR), and diagnostic odds ratio (DOR), respectively, together with their 95% confidence intervals (95% CIs) (20). Forest plots of summary statistics were constructed using the data from the enrolled studies. Afterwards, the Summary ROC (SROC) curves, which summarize the sensitivity and specificity of each enrolled study for the assessment of the overall diagnostic performance, were also established, while the AUC values were achieved from the constructed SROC curves (21). The Cochrane’s Q test and I² statistic were applied to judge the presence of statistical heterogeneity across studies (22). P-value less than 0.05 and I² value more than 50% indicated an obvious heterogeneity existing in the current study. When statistical heterogeneity existed between studies, subgroup analysis, meta-regression analysis and sensitivity analysis were adopted to seek the possible sources of heterogeneity (23). Moreover, the risk of publication bias of all the included diagnostic accuracy studies was measured by the Deeks’ funnel-plot asymmetry test (24). In our study, statistical analysis was undertaken using Stata 14.0 software. A p-value of <0.05 was considered statistically significant.

Results

Search results and characteristics of studies

Through searching the electronic databases with different combinations of the above used keywords, avoiding obviously unrelated titles and abstracts, and after careful consideration of all collected studies, 25 articles involving 60 different miRNAs tests met all inclusion criteria and were included in the final analysis (25–49). A flowchart detailing the selection process was illustrated in Figure 1.

All the included studies were performed between 2010 and 2021. Thirty of the selected studies were from China, sixteen from Japan, five from Canada, three from Germany, three from Italy and three from Iran. The number of samples evaluated by each study ranged from 20 to 380. Concerning the type of biological specimen, 32 studies collected from serum, 24 studies from plasma, while 4 studies were collected from whole blood. A total of 55 studies focused on single miRNAs while 5 studies focused on miRNA combinations. All included studies used quantitative real-time reverse transcription-PCR (qRT-PCR) technique to detect the expression of miRNAs. According to the QUADAS-2 assessment tool, the overall analytical quality was acceptable. Details of the enrolled studies in the current study were presented at Table 1.

Diagnostic accuracy of circulating miRNAs for OSCC

The forest plots describing the pooled sensitivity and specificity for circulating miRNA profiling in distinguishing OSCC patients from normal participants were illustrated at Figure 2. Overall, the circulating miRNAs indicated good performances for the detection of OSCC with the pooled sensitivity of 0.75 (95% CI: 0.69–0.80) and the pooled specificity of 0.76 (0.70–0.81). Moreover, the combined PLR, NLR, and DOR were estimated to be 3.2 (2.5–4.0), 0.33 (0.27–0.41), and 9 (6–14), respectively. In addition, the SROC curve of circulating miRNAs in detecting OSCC was presented at Figure 3A, and the AUC was calculated to be 0.82 (0.79–0.85).
FIGURE 1
Flow diagram of the identification and selection of the studies.

TABLE 1 The main features of the included studies for circulating miRNAs in the diagnosis of OSCC.

| Author      | Year | Country | Ethnicity | N-p | Age-p | Stage | N-C | Age-c | N Source | miRNA      | Methods | AUC   | SE | SP  |
|-------------|------|---------|-----------|-----|-------|-------|-----|-------|----------|------------|---------|-------|----|-----|
| Lin et al   | 2010 | China   | Asian     | 33  | NA    | I-IV  | 10  | NA    | 43 Plasma | miR-24 qRT-PCR | 0.82    | 0.70  | 0.92 |
| Liu et al   | 2010 | China   | Asian     | 43  | 54    | I-IV  | 21  | 51    | 64 Plasma | miR-31 qRT-PCR | 0.80    | 0.63  | 0.90 |
| Yang et al  | 2011 | China   | Asian     | 34  | NA    | I-IV  | 12  | NA    | 46 Plasma | miR-181a qRT-PCR | 0.84    | 0.94  | 0.67 |
| Yang et al  | 2011 | China   | Asian     | 34  | NA    | I-IV  | 12  | NA    | 46 Plasma | miR-181b qRT-PCR | 0.74    | 0.90  | 0.58 |
| Yang et al  | 2011 | China   | Asian     | 34  | NA    | I-IV  | 12  | NA    | 46 Plasma | miR-181a, miR-181b qRT-PCR | 0.89    | 0.78  | 0.86 |
| Lu et al    | 2012 | Canada  | Caucasian | 30  | 65    | NA    | 26  | 62    | 56 Serum  | miR-338 qRT-PCR | 0.82    | 0.80  | 0.80 |
| MacLellan et al | 2012 | Canada  | Caucasian | 30  | 65    | NA    | 26  | 62    | 56 Serum  | miR-29a qRT-PCR | 0.82    | 0.77  | 0.77 |
| MacLellan et al | 2012 | Canada  | Caucasian | 30  | 63    | NA    | 26  | 62    | 56 Serum  | miR-223 qRT-PCR | 0.81    | 0.96  | 0.60 |
| MacLellan et al | 2012 | Canada  | Caucasian | 30  | 63    | NA    | 26  | 62    | 56 Serum  | miR-16 qRT-PCR | 0.84    | 0.62  | 0.93 |
| MacLellan et al | 2012 | Canada  | Caucasian | 30  | 63    | NA    | 26  | 62    | 56 Serum  | let-7b qRT-PCR | 0.82    | 0.81  | 0.80 |
| Hung et al  | 2013 | China   | Asian     | 51  | NA    | I-IV  | 12  | NA    | 63 Plasma | miR-146a qRT-PCR | 0.86    | 0.79  | 0.92 |
| Liu et al   | 2013 | China   | Asian     | 65  | NA    | I-IV  | 24  | NA    | 89 Plasma | miR-196a qRT-PCR | 0.75    | 0.83  | 0.68 |
| Liu et al   | 2013 | China   | Asian     | 65  | NA    | I-IV  | 24  | NA    | 89 Plasma | miR-196b qRT-PCR | 0.59    | 0.37  | 0.98 |
| Ren et al   | 2014 | China   | Asian     | 58  | 61    | I-IV  | 32  | 61    | 90 Blood  | miR-21 qRT-PCR | 0.78    | 0.62  | 0.91 |
| Ries et al  | 2014 | Germany | Caucasian | 57  | 64    | I-IV  | 33  | 60    | 90 Blood  | miR-186 qRT-PCR | 0.69    | 0.60  | 0.79 |
| Ries et al  | 2014 | Germany | Caucasian | 57  | 64    | I-IV  | 33  | 60    | 90 Blood  | miR-3651 qRT-PCR | 0.82    | 0.84  | 0.67 |
| Ries et al  | 2014 | Germany | Caucasian | 57  | 64    | I-IV  | 33  | 60    | 90 Blood  | miR-494 qRT-PCR | 0.71    | 0.56  | 0.82 |
| Lu et al    | 2015 | China   | Asian     | 90  | 54    | I-IV  | 53  | 47    | 143 Plasma | miR-196a qRT-PCR | 0.864   | 0.67  | 0.96 |

(Continued)
| Author          | Year | Country | Ethnicity | N-p | Age-p | Stage | N-c | Age-c | N | Source       | miRNA               | Methods          | AUC  | SE  | SP  |
|-----------------|------|---------|-----------|-----|-------|-------|-----|-------|---|--------------|---------------------|-------------------|------|-----|-----|
| Lu et al        | 2015 | China   | Asian     | 90  | 54    | I-IV  | 53  | 47    | 143| Plasma       | miR-196b           | qRT-PCR          | 0.96 | 0.98| 0.81|
| Lu et al        | 2015 | China   | Asian     | 90  | 54    | I-IV  | 53  | 47    | 143| Plasma       | miR-196a, miR-196b | qRT-PCR          | 0.963 | 0.88| 0.92|
| Tachibana et al | 2016 | Japan   | Asian     | 31  | 75    | I-IV  | 31  | 75    | 62 | Plasma       | miR-223            | qRT-PCR          | 0.703 | 0.68| 0.61|
| Xu et al        | 2016 | China   | Asian     | 101 | 53    | I-IV  | 103 | 52    | 204| Serum        | miR-483            | qRT-PCR          | 0.85  | 0.85| 0.75|
| Liu et al       | 2017 | China   | Asian     | 63  | 54    | I-IV  | 26  | 54    | 89 | Plasma       | miR-187            | qRT-PCR          | 0.73  | 0.88| 0.50|
| Chang et al     | 2018 | China   | Asian     | 82  | 54    | I-IV  | 50  | 53    | 132| Plasma       | miR-150            | qRT-PCR          | 0.702 | 0.61| 0.77|
| Chang et al     | 2018 | China   | Asian     | 82  | 54    | I-IV  | 50  | 53    | 132| Plasma       | miR-423            | qRT-PCR          | 0.677 | 0.59| 0.73|
| Chang et al     | 2018 | China   | Asian     | 82  | 54    | I-IV  | 50  | 53    | 132| Plasma       | miR-222            | qRT-PCR          | 0.52  | 0.24| 0.87|
| Chang et al     | 2018 | China   | Asian     | 82  | 54    | I-IV  | 50  | 53    | 132| Plasma       | miR-150, miR-423   | qRT-PCR          | 0.749 | 0.71| 0.73|
| Chen et al      | 2018 | China   | Asian     | 121 | NA    | NA    | 176 | NA    | 160| Serum        | miR-99a            | qRT-PCR          | 0.911 | 0.80| 0.84|
| Sun et al       | 2018 | China   | Asian     | 80  | 55    | I-III | 80  | 54    | 160| Plasma       | miR-200b           | qRT-PCR          | 0.917 | 0.90| 0.89|
| Lu et al        | 2019 | China   | Asian     | 82  | 60    | I-IV  | 53  | 60    | 135| Serum        | miR-99a            | qRT-PCR          | 0.521 | 0.87| 0.25|
| Lu et al        | 2019 | China   | Asian     | 82  | 60    | I-IV  | 53  | 60    | 135| Serum        | miR-31             | qRT-PCR          | 0.661 | 0.70| 0.52|
| Lu et al        | 2019 | China   | Asian     | 82  | 60    | I-IV  | 53  | 60    | 135| Serum        | miR-138            | qRT-PCR          | 0.547 | 0.68| 0.51|
| Lu et al        | 2019 | China   | Asian     | 82  | 60    | I-IV  | 53  | 60    | 135| Serum        | miR-21             | qRT-PCR          | 0.579 | 0.64| 0.46|
| Lu et al        | 2019 | China   | Asian     | 82  | 60    | I-IV  | 53  | 60    | 135| Serum        | miR-375            | qRT-PCR          | 0.514 | 0.60| 0.50|
| Lu et al        | 2019 | China   | Asian     | 82  | 60    | I-IV  | 53  | 60    | 135| Serum        | miR-99a, miR-31, miR-138, miR-21, miR-375 | qRT-PCR          | 0.776 | 0.77| 0.74|
| Mahmood et al   | 2019 | China   | Asian     | 100 | NA    | NA    | 100 | NA    | 200| Serum        | miR-21             | qRT-PCR          | 0.829 | 0.91| 0.54|
| Crimi et al     | 2020 | Italy   | Caucasian | 10  | NA    | I-IV  | 10  | NA    | 20 | Plasma       | miR-133a           | qRT-PCR          | 0.86  | 0.90| 0.80|
| Crimi et al     | 2020 | Italy   | Caucasian | 10  | NA    | I-IV  | 10  | NA    | 20 | Plasma       | miR-375            | qRT-PCR          | 0.96  | 0.80| 0.90|
| Karimi et al    | 2020 | Iran    | Caucasian | 20  | 47    | NA    | 20  | 47    | 40 | Serum        | miR-24             | qRT-PCR          | NA    | 0.95| 0.95|
| Karimi et al    | 2020 | Iran    | Caucasian | 20  | 47    | NA    | 20  | 47    | 40 | Serum        | miR-29a            | qRT-PCR          | NA    | 0.80| 0.70|
| Bigagli et al   | 2021 | Italy   | Caucasian | 30  | 65    | I-IV  | 14  | 51    | 44 | Plasma       | miR-210            | qRT-PCR          | 0.951 | 0.93| 0.87|
| He et al        | 2021 | China   | Asian     | 184 | 56    | I-IV  | 196 | 56    | 380| Plasma       | miR-130a           | qRT-PCR          | 0.812 | 0.99| 0.46|
| Nakamura et al  | 2021 | Japan   | Asian     | 40  | 67    | I-IV  | 40  | 64    | 80 | Serum        | miR-23             | qRT-PCR          | 0.494 | 0.75| 0.17|
| Nakamura et al  | 2021 | Japan   | Asian     | 40  | 67    | I-IV  | 40  | 64    | 80 | Serum        | miR-24             | qRT-PCR          | 0.491 | 0.60| 0.35|
| Nakamura et al  | 2021 | Japan   | Asian     | 40  | 67    | I-IV  | 40  | 64    | 80 | Serum        | miR-423            | qRT-PCR          | 0.579 | 0.88| 0.38|
| Nakamura et al  | 2021 | Japan   | Asian     | 40  | 67    | I-IV  | 40  | 64    | 80 | Serum        | miR-19a            | qRT-PCR          | 0.659 | 0.63| 0.68|
| Nakamura et al  | 2021 | Japan   | Asian     | 40  | 67    | I-IV  | 40  | 64    | 80 | Serum        | miR-19b            | qRT-PCR          | 0.571 | 0.40| 0.88|
| Nakamura et al  | 2021 | Japan   | Asian     | 40  | 67    | I-IV  | 40  | 64    | 80 | Serum        | miR-20a            | qRT-PCR          | 0.637 | 0.75| 0.52|
| Nakamura et al  | 2021 | Japan   | Asian     | 40  | 67    | I-IV  | 40  | 64    | 80 | Serum        | miR-22             | qRT-PCR          | 0.58  | 0.63| 0.17|
| Nakamura et al  | 2021 | Japan   | Asian     | 40  | 67    | I-IV  | 40  | 64    | 80 | Serum        | miR-122            | qRT-PCR          | 0.609 | 0.20| 0.96|

(Continued)
Then, the Fagan’s nomogram was utilized to confirm the clinical utility of the circulating miRNAs in OSCC. As illustrated in Figure 4, when the pre-test probability of OSCC was 20%, a positive measurement could improve the post-test probability of suffering cancer to 44%, while the post-test probability will reduce to 8% if a negative measurement happened.

The above parameters revealed moderate discriminative ability of the application of circulating miRNAs as biomarkers for the early diagnosis of OSCC.

**TABLE 1** Continued

| Author          | Year  | Country | Ethnicity | N-p | Age-p | Stage | N-c | Age-c | N  | Source | miRNA | Methods | AUC   | SE   | SP   |
|-----------------|-------|---------|-----------|-----|-------|-------|-----|-------|-----|--------|-------|---------|-------|------|------|
| Nakamura et al  | 2021  | Japan   | Asian     | 40  | 67    | I-IV  | 40  | 64    | 80 | Serum  | miR-125 | qRT-PCR | 0.553 | 0.47  | 0.38 |
| Nakamura et al  | 2021  | Japan   | Asian     | 40  | 67    | I-IV  | 40  | 64    | 80 | Serum  | miR-144 | qRT-PCR | 0.588 | 0.45  | 0.80 |
| Nakamura et al  | 2021  | Japan   | Asian     | 40  | 67    | I-IV  | 40  | 64    | 80 | Serum  | miR-183 | qRT-PCR | 0.616 | 0.45  | 0.90 |
| Nakamura et al  | 2021  | Japan   | Asian     | 40  | 67    | I-IV  | 40  | 64    | 80 | Serum  | miR-150 | qRT-PCR | 0.546 | 0.22  | 0.90 |
| Nakamura et al  | 2021  | Japan   | Asian     | 40  | 67    | I-IV  | 40  | 64    | 80 | Serum  | miR-4419a | qRT-PCR | 0.546 | 0.45  | 0.77 |
| Nakamura et al  | 2021  | Japan   | Asian     | 40  | 67    | I-IV  | 40  | 64    | 80 | Serum  | miR-5100 | qRT-PCR | 0.706 | 0.50  | 0.90 |
| Nakamura et al  | 2021  | Japan   | Asian     | 40  | 67    | I-IV  | 40  | 64    | 80 | Serum  | miR-24, miR-20a, miR-122, miR-150, miR-4419a, miR-5100 | qRT-PCR | 0.844 | 0.55  | 0.93 |
| Wang et al 2021 | China | Asian   | 132       | 57  | I-IV  | 85    | NA | 217   | Serum | miR-206 | qRT-PCR | 0.846 | 0.81  | 0.73 |

N-p, number of patients; Age-p, age of patients; N-c, number of controls; Age-c, age of controls; N, number; AUC, area under curve; SE, sensitivity; SP, Specificity; OSCC, oral squamous cell carcinoma.
Heterogeneity and subgroup analyses

In this study, the heterogeneity analysis using $I^2$ suggested significantly higher heterogeneity in both pooled sensitivity ($I^2 = 91.49\%$; $P<0.001$) and pooled specificity ($I^2 = 89.17\%$; $P<0.001$). We first explored whether the heterogeneity may come from the threshold effect. The Spearman correlation coefficient between the sensitivity logarithm and (1-specificity) logarithm is calculated to be -0.24, and the P-value is 0.06, suggesting no obvious threshold effect.

Next, the subgroup analyses were carried out. As exemplified by Table 2, miRNA combination testing achieved a more overall promising accuracy than single miRNA assay, with a similar sensitivity of 0.75 (0.64-0.84) versus 0.75 (0.68-0.80), a higher specificity of 0.84 (0.75-0.91) versus 0.75 (0.69-0.81), a higher AUC of 0.87 (0.84-0.90) versus 0.82 (0.78-0.85), respectively. The SROC curves of single miRNAs and combination miRNAs were plotted at Figures 3C, D, respectively. Following subgroup analysis depending on sample sources, the plasma-based miRNA assay exhibited a higher level of diagnostic accuracy than serum-based miRNA assay accompanied by a pooled sensitivity of 0.82 (0.73-0.88) versus 0.69 (0.61-0.76), specificity of 0.81 (0.74-0.86) versus 0.72 (0.61-0.80) and AUC of 0.88 (0.85-0.91) versus 0.76 (0.72-0.79). Moreover, the diagnostic accuracy based on ethnicity was also assessed through subgroup analysis. The results indicated that the sensitivity, specificity, and AUC of circulating miRNAs for OSCC detection in the Caucasian group were higher than those in the Asian group due to higher sensitivity: 0.84 (0.74-0.90) versus 0.72 (0.65-0.78), higher specificity: 0.83 (0.77-0.88) versus 0.74 (0.67-0.80), and higher AUC: 0.88 (0.85-0.91) versus 0.79 (0.75-0.83). In addition, we also conducted a subgroup analysis to identify whether sample size had a potential influence on the whole diagnostic value of circulating miRNAs. The stratified analysis by sample size suggested that the diagnostic value of miRNAs based on large sample sizes ($N>80$) might be more significant than it is for small sample sizes ($N \leq 80$), with a pooled sensitivity of 0.78 (0.70-0.84) versus 0.71 (0.63-0.79),
specificity of 0.74 (0.66-0.81) versus 0.78 (0.70-0.85) and AUC of 0.83 (0.79-0.86) versus 0.81 (0.78-0.85). The SROC curves of these subgroups were also plotted at Figure 5.

Influence analysis and meta-regression

As heterogeneity observed in the present study, sensitivity analysis was performed (Figure 6). The influence analysis and outlier detection identified several outlier individual studies. After excluding seven outliers (34, 38, 41, 47, 48), the $I^2$ for heterogeneity decreased both in sensitivity (from 91.49% to 84.30%) and specificity (from 89.17% to 84.31%). However, the overall results showed only minimal changes and were similar to the original ones as the pooled specificity of the overall study increased from 0.76 to 0.78, PLR increased from 3.2 to 3.4, DOR decreased from 9 to 10, and AUC increased from 0.82 to 0.83, while sensitivity and NLR had no changes. The SROC curve of outliers excluded for circulating miRNAs was plotted at Figure 3B. The above results suggested that our study was robust.

Furthermore, a meta-regression analysis was applied to distinguish the potential sources of heterogeneity across studies by exploring research characteristics including specimen type, sample size, and ethnicity. The results suggested that ethnicity may be responsible for the heterogeneity while other factors revealed low likelihood of sources of inter-study heterogeneity.

Publication bias

Deeks’ funnel-plot asymmetry test (Figure 7) was performed to investigate the potential publication bias. The assessment of
To our knowledge, this is the most comprehensive study ever conducted on the role of circulating miRNAs in OSCC. A total of sixty studies were identified eligible and enrolled for the assessment of the diagnostic value of circulating miRNAs in OSCC. The overall analysis demonstrates moderate diagnostic accuracy as circulating miRNA profiling harbored a relatively high pooled diagnostic value in detecting OSCC, where the combined sensitivity and specificity were 0.75 and 0.76, respectively, corresponding with an AUC of 0.82. The stratified analysis by sample sources indicated that the plasma-based assay seemed to undergo a higher pooled sensitivity, specificity, and AUC than serum-based assay. Our results provided evidence that plasma may be a better matrix for diagnostic profiling of miRNAs in OSCC, which was also consistent with previous studies (50). Moreover, previous studies have suggested that genetic background may have some effect on the miRNA concentrations in body fluids or tumor lesions and the biomarker value of miRNAs varies among different ethnicities (51). In our pooled results by subgroup analysis, we found that the Caucasian population-based miRNA test harbored an overall higher accuracy compared than that of the Asians, suggesting that biomarker performance for circulating miRNAs in OSCC diagnosis may also be determined by different genetic background. Remarkably, the impact of genetic background on the diagnostic value of circulating miRNAs was also confirmed by a meta-regression analysis. Additionally, our previous study also reported that the diagnostic accuracies of miRNAs in different cancers were affected by sample sizes (52). More importantly, we observed a higher diagnostic accuracy in miRNA detection tests based on large sample size (n≥80) than investigations with small sample sizes (n ≤80), which may suggest that future translational and clinical investigations concentrating on large-scale prospective studies are worth performing to validate the diagnostic efficacy of circulating miRNAs in OSCC.

Accumulating evidence described that tumorigenesis is a complex cell processes involving multiple miRNAs (53). For OSCC, it is a highly heterogeneous cancer with a complicated
etiology and single miRNA is hard to diagnose OSCC with satisfactory performance (11). Combination biomarkers may be more comprehensive than single maker in dictating the complicated cancer evolutionary process and may be more powerful in cancer detection (54–56). There have been promising results yielded from studies that combination miRNAs had a higher level of diagnostic power than single miRNAs. However, since there were only five tests focusing on miRNA combination biomarkers in detecting OSCC, we could not answer the question that which and how

FIGURE 5
The SROC curve of the pooled analysis by some subgroups. (A) SROC curve for plasma miRNAs; (B) SROC curve for serum miRNAs; (C) SROC curve for circulating miRNAs based on Caucasian population; (D) SROC curve for circulating miRNAs based on Asian population; (E) SROC curve for circulating miRNAs based on large sample sizes; (F) SROC curve for circulating miRNAs based on small sample sizes.
many miRNAs should be combined together to enhance the diagnostic accuracy. This indicates that further research should be concentrated on the combined use of miRNA panels to investigate their effect on diagnostic accuracy.

There were 41 miRNAs utilized as diagnostic biomarkers involved in the present study, which may provide new insight into the early detection of OSCC. However, the exact role of miRNAs in the carcinogenesis of OSCC remains unclear. Recently, several researchers have demonstrated a functional role for miRNAs in the initiation and progression of OSCC, which may help us understand the potential biomarker role of miRNAs in OSCC. For example, Lin et al. reported that miR-31 played a pivotal part during the progression of OSCC through establishing a complicated network with its regulated genes and the signaling cascades such as EGF-AKT signaling axis, Hippo pathway, and Wnt signaling (57). Data from the study by Peng et al. revealed that downregulation of miR-130a could inhibit OSCC proliferation and metastasis by the Hippo-YAP pathway, which may provide potential target for OSCC therapy (58).

Previous studies have also demonstrated that miR-133a plays tumor suppressive role in OSCC by inhibiting the Notch signaling pathway via binding to CTBP2 (59). Moreover, mechanism research has suggested that miR-222 affects OSCC cell proliferation, migration, invasion, and apoptosis by targeting CDKN1B (60). In addition, previous evidence has demonstrated that miR-144 inhibits tumorigenesis of OSCC by targeting ERO1L/STAT3 signaling pathway (61). Besides, available data suggest that the expression of miR-196a increased in OSCC cells against normal oral squamous cells and downregulation of miR-196a could inhibit the malignant biological processes of OSCC cells through targeting FOXO1 (62). In a word, the altered expressions of miRNAs and the involved pathways may contribute to the biological behaviors in OSCC, ultimately leading to tumorigenesis. However, as for more detailed mechanisms of miRNAs in OSCC, further studies are needed to make further investigations.

Since circulating miRNAs possess the distinctive advantages of tumor specificity, stable, extracted easily and non-invasive, they may be applied as perfect non-invasive biomarkers for cancer detection. Our study also demonstrated that circulating miRNAs may represent potentially promising biomarkers for the detection of OSCC. However, there is still a long way to go before they can be used in clinical practice in OSCC diagnosis. For example, different studies from different laboratories or testing platforms used diverse cut-off values and different normalization in the detection of miRNAs, which may potentially affect the diagnostic efficacy and generate heterogeneity and uncertainty. Therefore, a consensus in the scientific community should be indispensable to establish the optimum cut-off values and detection methods. Moreover, current studies on miRNAs detection in OSCC were isolated and the future exploration of miRNA biomarker for OSCC should be rooted in systematical and dynamical manner to develop integrative diagnostic models based on specific miRNAs and combinations with more appropriate and better prediction capacity. In addition, studies included in our studies

**FIGURE 6**
Sensitivity analysis results. (A) Goodness of fit; (B) bivariate normality; (C) influence analysis; (D) outlier detection.
are still far from enough and the size of available samples in the individual studies are still relatively small. Thus, large-scale and well-designed prospective randomized controlled studies are required to confirm the actual diagnostic value of circulating miRNA assays for OSCC and promote the clinical application. Moreover, we believe that large adequately designed prospective studies may help determine a specific miRNA type or specific miRNA combinations that may be more appropriate and better used in clinical decision-making for OSCC patients.

The present study has some limitations that must be acknowledged. First, we did observe significant heterogeneity because of discrepancies among the different studies. Although several common check methods of evaluating the risk of bias assessment including subgroup, meta-regression, and sensitivity analysis were performed, we were still unable to clarify the precise factors contributing to the significant heterogeneity. Second, since some clinical factors such as gender difference, age distribution, and TNM stage were not detailed in some studies, we could not carry out any other subgroup analysis based on them. Third, though we had planned to investigate the impact of cut-off value on heterogeneity by using the meta-regression analyses, this could not be accomplished due to insufficient data and the different standards in different enrolled diagnostic tests. Moreover, as discussed in the subgroup analyses and meta-regression analysis, ethnicity might be considered as a source of heterogeneity among individual studies. However, all the diagnostic miRNA tests were conducted on the basis of Asian and Caucasian population, whereas African population should have been enrolled. Finally, the history of tobacco and alcohol consumption may influence the study results. However, these data have not been provided by some of the included studies and we cannot perform such analysis. Regardless of these limitations, ours is the most comprehensive study that aggregated available data on circulating miRNAs and analyzed their application in the field of OSCC detection.

Conclusions

In conclusion, our results indicated that circulating miRNAs has a relatively high diagnostic accuracy in the detection of OSCC and might be applied in noninvasive screening tests for OSCC. In addition, combination miRNA biomarkers have exhibited a prominent advantage over single miRNAs in improving diagnostic accuracy of OSCC. However, further
translational and clinical investigations based on large-scale prospective studies are necessary to validate the diagnostic efficacy of circulating miRNAs and promote their clinical application in OSCC.

**Data availability statement**

The original contributions presented in the study are included in the article. Further inquiries can be directed to the corresponding author.

**Author contributions**

HG, YS and ZF performed the computational analysis, and wrote the manuscript. YC, JY and YZ were responsible for the statistical analysis in meta-analysis part. QP conceived of the study, and took part in its design and coordination. All authors contributed to the article and approved the submitted version.

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**Conflict of interest**

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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**References**

1. Zini A, Czerninski R, Sgan-Cohen HD. Oral cancer over four decades: epidemiology, trends, histology, and survival by anatomical sites. *J Oral Pathol Med* (2010) 39(4):299–305. doi: 10.1111/j.1600-0714.2009.00845.x
2. Braakhuis BJ, Leemans CR, Visser O. Incidence and survival trends of head and neck squamous cell carcinoma in the Netherlands between 1989 and 2011. *Oral Oncol* (2014) 50(7):670–5. doi: 10.1016/j.oraloncology.2014.03.008
3. Chamoli A, Gosavi AS, Shirwadkar UP, Wangdale KV, Behera SK, Kurrey NK, et al. Overview of oral cavity squamous cell carcinoma: Risk factors, mechanisms, and diagnostics. *Oral Oncol* (2021) 121:105451. doi: 10.1016/j.oraloncology.2021.105451
4. Su YF, Chen YJ, Tsai FT, Li WC, Hsu ML, Wang DH, et al. Current insights into oral cancer diagnostics. *Diagnostics* (2021) 11(7):1287. doi: 10.3390/diagnostics11071287
5. Warnakulasuriya S, Kerr AR. Oral cancer screening: Past, present, and future. *J Dent Res* (2021) 100(12):1313–20. doi: 10.1177/00220345211014795
6. Dragomir MP, Knutsen E, Calin GA. Classical and noncanonical functions of miRNAs in cancers. *Trends Genet* (2022) 38(4):379–94. doi: 10.1016/j.tig.2021.10.002
7. Treiber T, Treiber N, Meister G. Regulation of microRNA biogenesis and its crosstalk with other cellular pathways. *Nat Rev Mol Cell Biol* (2019) 20(1):5–20. doi: 10.1038/s41580-018-0059-1
8. Rupaimoole R, Slack FJ. MicroRNA therapeutics: towards a new era for the management of cancer and other diseases. *Nat Rev Drug Discovery* (2017) 16(3):203–22. doi: 10.1038/nrd.2016.246
9. Preetha KA, Selvakumar SC, Ross K, Jayaraman S, Tuwibira D, Sekar D. Liquid biopsy: Exosomal microRNAs as novel diagnostic and prognostic biomarkers in cancer. *Mol Cancer* (2022) 21(1):54. doi: 10.1186/s12943-022-01525-9
10. Jet T, Gines G, Rondeler Y, Taly V. Advances in multiplexed techniques for the detection and quantification of microRNAs. *Chem Sci Rev* (2021) 50(6):4141–61. doi: 10.1039/D0CS00609B
11. Chai AWY, Lim KP, Cheong SC. Translational genomics and recent advances in oral squamous cell carcinoma. *Semin Cancer Biol* (2020) 61:71–83. doi: 10.1016/j.semcancer.2019.09.011
12. Prime SS, Cirillo N, Cheong SC, Prime MS, Parkinson EK. Targeting the genetic landscape of oral potentially malignant disorders has the potential as a preventative strategy in oral cancer. *Cancer Lett* (2021) 518:1058–12. doi: 10.1016/j.canlet.2021.05.025
13. Oso C, Chira S, Nutu AM, Braicu C, Baciu M, Korban SS, et al. The connection between MicroRNAs and oral cancer pathogenesis: Emerging biomarkers in oral cancer management. *Genes* (2021) 12(12):1989. doi: 10.3390/genes12121989
14. Ghosh RD, Patatheyil A, Roychoudhury S. Functional landscape of dysregulated MicroRNAs in oral squamous cell carcinoma: Clinical implications. *Front Oncol* (2020) 10:619. doi: 10.3389/fonc.2020.00619
15. Manzano-Moreno FJ, Costela-Ruiz VJ, Garcia-Reco E, Olmedo-Gaya MV, Ruiz C, Reyes-Botella C. Role of salivary MicroRNA and cytokines in the diagnosis and prognosis of oral squamous cell carcinoma. *Int J Mol Sci* (2021) 22(22):12215. doi: 10.3390/ijms22212215
16. Hsieh PL, Liao YW, Pichler M, Yu CC. MicroRNAs as theranostics targets in oral carcinoma stem cells. *Cancers* (2020) 12(12):340. doi: 10.3390/cancers1212340
17. Yeti S, Saranath D. MicroRNAs in oral cancer. Biomarkers with clinical potential. *Oral Oncol* (2020) 110:105002. doi: 10.1016/j.oraloncology.2020.105002
18. Wang J, Lx N, Lu X, Yuan R, Chen Z, Yu J. Diagnostic and therapeutic role of microRNAs in oral cancer (Review). *Oral Oncol Rep* (2021) 45(1):58–64. doi: 10.3892/oro.2020.7854
19. Whiting PF, Rutjes AW, Westwood ME, Mallett S, Deeks JJ, Reitsma JB, et al. QUADAS-2: a revised tool for the quality assessment of diagnostic accuracy studies. *Ann Intern Med* (2011) 155(8):529–36. doi: 10.7326/0003-4819-155-8-20111018-00009

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Biomarkers.

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Expression of miRNAs in the serum of patients with high-risk oral lesions.

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MicroRNA signature as a potential biomarker for the early detection of oral cancer.

MicroRNA-21 as a promising biomarker in the diagnosis and prognosis of oral squamous cell carcinoma.

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MicroRNA-31: a pivotal biomarker for lymph-node metastasis of oral squamous cell carcinoma.

Circulating serum 3 types of microRNA as biomarkers of oral squamous cell carcinoma; a pilot study.

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