Title: CYFRA 21-1 and MMP-9 as Salivary Biomarkers for The Detection of Oral Squamous Cell Carcinoma (OSCC): A Systematic Review of Diagnostic Test Accuracy

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Key words:
Oral cancer, oral squamous cell carcinoma, OSCC, salivary biomarkers, CYFRA 21-1, MMP-9
Abstract

Tissue biopsy with histopathological examination is still considered as the gold standard to diagnose oral-squamous-cell-carcinoma (OSCC). This systematic review explores the diagnostic test accuracy of two salivary biomarkers in adults suspected of OSCC.

Cochrane Library, MEDLINE and EMBASE were searched for clinical studies evaluating the diagnostic accuracy of salivary biomarkers in detecting OSCC. Studies were eligible for inclusion if only singular salivary biomarkers were evaluated in three or more studies. Studies investigating combined salivary biomarkers or assessing patients with oral-potentially-malignant-disorders only were excluded. The reporting of the review follows the PRISMA checklist.

Six studies, recruiting 775 participants, were included in this review for only two salivary biomarkers, CYFRA 21-1 and MMP-9. The sensitivity and specificity values (with 95% CI) for CYFRA 21-1 studies ranged from 0.84 (0.75-0.91) to 0.94 (0.83-0.99) and 0.84 (0.71-0.93) to 0.96 (0.80-1.00) respectively. While in MMP-9 studies, the sensitivity values (with 95% CI) ranged from 0.76 (0.67-0.83) to 1.00 (0.78-1.00) and specificity values from 0.27 (0.12-0.46) to 1.00 (0.78-1.00). The overall quality of the included studies was poor.

Due to a lack of strong and high-quality of evidence, considerable uncertainty remains surrounding the use of singular salivary biomarkers for the detection of OSCC.
1. **INTRODUCTION**

Many different techniques and tests delivered in a variety of settings are available for the detection of oral squamous cell carcinoma (OSCC). Conventional oral examination (COE) carried out by a general dental practitioner has traditionally been recommended by various health and medical/dental organisations as a part of oral cancer (OC) screening programs e.g. BDA. A Cochrane systematic review has evaluated the diagnostic accuracy of a variety of tests as adjunct tests to the COE in a dental setting including vital staining, oral cytology, light-based detection or oral spectroscopy in patients presenting with clinically evident lesions. Their findings showed that cytology tests appeared to have the greatest potential, and warranted further investigation. However, the overall quality of the evidence was variable, with insufficient evidence to justify the replacement of the current ‘gold standard’ diagnostic test of scalpel biopsy with histopathological examination in addition to the COE.

Comparatively, saliva, also known as the “mirror of the body”, is an easy medium to be collected for bioanalysis. Sample/specimen collection is non-invasive, inexpensive and simple, which makes it ideal method for diagnostic purposes. Saliva contains an extensive number of compounds, is safe to handle and store, does not clot, and as such, it is deemed a promising diagnostic biofluid. Two systematic reviews have evaluated the performance of a wide range of salivary biomarker expression in OSCC. However, these systematic reviews mainly included ‘case-control’ type accuracy studies, which can be prone to bias. Studies which compare altered expression of a specific salivary biomarker in ‘cases’ with OSCC with those in healthy ‘control’ participants can deliver misleading results when the methods of participant recruitment are not taken into consideration. If participants representing only...
subgroups of the spectrum of disease and spectrum of non-diseased are included, the resulting estimates of diagnostic test accuracy may not be applicable to the clinical question. (8)

OSCC is an increasing disease globally. Several factors have been implicated in the development of the disease such as tobacco, betel quid, alcohol, and others like human papillomavirus (HPV) and Epstein-Barr virus (EBV). The survival rate of individuals with OSCC is approximately 90% when detected early, compared to only 50% after delayed diagnosis. (9) Hence, both prevention and early detection of disease are crucial in reducing OSCC mortality.

This systematic review aims to identify and summarise the diagnostic accuracy of salivary biomarkers currently being used in the detection of OSCC in adults. The reporting of the review follows the PRISMA checklist. (10)
2. MATERIALS AND METHODS

2.1 Study Criteria

Studies were eligible for inclusion in the review if they were evaluating salivary biomarkers that reported measures of diagnostic test accuracy such as sensitivity and specificity in detecting OSCC; in adults (aged 16 years or over) suspected of having OSCC with no prior treatment such as chemotherapy or radiotherapy.

2.1.1 Inclusion and Exclusion Criteria

Only studies evaluating the performance of singular salivary biomarkers (index test) against any type of tissue biopsy with histopathological examination (reference standard) for the diagnosis of OSCC were eligible for inclusion. Studies were eligible for inclusion in the review if only singular salivary biomarkers were evaluated and where the performance of a salivary biomarker was assessed in three or more studies. Studies investigating combined salivary biomarkers or assessing patients with oral potentially malignant disorders (PMDs) only were excluded.

2.2 Search Protocol and Study Selection

An electronic search was performed on 14 June 2018 for studies published within the last five years (from 2014 to 2018) using the following databases: Cochrane Library, MEDLINE via OVID, and EMBASE via OVID. The search strategy used: (oral cancer OR mouth cancer OR oral squamous cell carcinoma OR OSCC).mp AND (diagnos* OR detect*).mp AND (saliva* AND (marker* OR biomarker* OR test)).mp. Following the removal of duplicates, the titles or/and abstracts of the retrieved records were screened for relevancy, and full-text articles were
obtained if necessary. Next, full-text articles were assessed for eligibility. Studies were included following consensus among all three authors.

2.3 Data Extraction

The following data were extracted from each included study: (1) Study information and setting (country, design, sample size, test used), (2) Type of salivary biomarker, (3) Study results: true positive (sensitivity) and true negative (specificity), and positive and negative predictive values where reported; a 2x2 table was constructed for each study (reference standard against the index test) using Review Manager 5.3. (11) The corresponding author of a study was contacted via e-mail where further information or explanation was needed.

2.4 Assessment of Methodological Quality

By using a tailored QUADAS-2 tool, the quality of the included studies (risk of bias and applicability concerns) was assessed through its 4 domains: patient selection, index test, reference standard and flow and timing of participants through the study. (12) The indicators of the assessment of quality (QUADAS-2) are summarized in Table 1 as adapted from Macey et al. (2) The results then were graphically presented using RevMan. (11)

2.5 Statistical Analysis and Data Synthesis

The target condition (disease positive) was OSCC. The true positive, true negative, false positive and false negative values were used to construct 2x2 tables for each study. (12) The diagnostic test accuracy results of each biomarker were expressed as sensitivity and specificity with 95% confidence intervals. Results were presented graphically as coupled forest plot for each salivary biomarker.
3. RESULTS
Following the removal of duplicates, the electronic search yielded a total of 250 records, of which 224 were excluded, and 26 were further assessed for eligibility (Appendix 1). Only 6 studies met the inclusion criteria in this review as illustrated in the PRISMA flow-diagram in Figure 1. (10) The main reason for exclusion was the low number of studies that investigated each salivary biomarker (Appendix 2). Only if a biomarker had 3 or more studies was it eligible to be included in the review. Thus, only six studies reported the diagnostic accuracy of salivary biomarkers CYFRA 21-1 (13-15) and MMP-9 (16-18) in detecting OSCC were included.

3.1 Description of Studies
The included studies evaluated data from a total of 775 patients (355 patients for CYFRA 21-1 and 420 patients for MMP-9). From the total number, 356 (46%) patients were diagnosed with OSCC according to the reference standard of biopsy and histopathological examination (180 (54%) patients for the CYFRA 21-1 studies and 176 (42%) patients for MMP-9), while the remaining 419 patients were classed as disease free or non-OSCC (175 patients for CYFRA 21-1 and 244 patients for MMP-9). The three CYFRA 21-1 studies were of Indian origin (13-15), while the three MMP-9 studies were from Egypt (16), Germany (17), and Taiwan (18). Salivary sample collection was carried out in a hospital setting (academic or dental/medical centres) between 2008 and 2014.

Classification and reporting of OSCC varied across studies, making meta-analysis inappropriate; two studies reported OSCC stages only (I to IV) (16, 18); one study reported OSCC grades only (1 and 2) (14); two studies reported both OSCC stages (0 through IVA) with...
3.2 Methodological Quality of Included Studies

Figures 2 and 3 summarise the results of the tailored quality assessment of the included studies for CYFRA 21-1 and MMP-9 respectively utilizing the QUADAS-2 tool. (12) The figures show a summary of the risk of bias and applicability concerns by the authors’ judgement in each of the four domains for each included study (Appendix 3 includes comprehensive quality assessment performed for each study).

None of the included studies could be classified as being at low risk of bias for all four domains. Patient selection was considered as “high” risk of bias in all of the studies, which was mainly due to the method of patients’ enrolment (13), the nature of the study design (13-18), and implementing inappropriate exclusions. (13, 14, 16)

The index test was considered to be at “low” risk of bias in only one study (18), “unclear” in one study (14), but “high” in 4 studies. (13, 15-17) The variation across the level of bias differs due to insufficient details reported whether the results of the index test were interpreted without prior knowledge of the reference standard results; lack of pre-specification of a test-positive threshold; and statement of conflict of interest. Similarly, the reference standard domain was considered to be at “low” risk of bias in only one study (18), and “unclear” in the other 5 studies. All of the 5 studies were judged as “unclear” due to the inadequate reporting of whether the biopsy and histopathological diagnoses were made without any prior
knowledge of the index test results. Additionally, 3 studies (13, 16, 17) failed to comprehensively report the details of the reference standard.

The flow and timing domain was considered as “unclear” risk of bias across all the studies due to insufficient details reported regarding the interval periods between the index and reference standard (inadequately reported in all six studies), whether all patients received the same reference standard (13), and if all patients have been included in the analysis. (14) Only 3 studies (15, 16, 18) were assessed as “low” concern for applicability according to patient selection, the index test and the reference standard. The remaining three studies were assessed as “unclear” concern for applicability due to lack of details regarding the patient selection (14) and conduct of the reference standard. (13, 17)

3.3 Findings

The sensitivity and specificity of CYFRA 21-1 (with 95% CI) ranged from 0.84 (0.75 to 0.91) to 0.94 (0.83 to 0.99) and 0.84 (0.71 to 0.93) to 0.96 (0.80 to 1.00) respectively. Whereas the sensitivity and specificity of MMP-9 (with 95% CI) ranged from 0.76 (0.67 to 0.83) to 1.00 (0.78 to 1.00) and specificity values from 0.27 (0.12 to 0.46) to 1.00 (0.78 to 1.00). Table 2 shows a summary of the data extracted from the included studies. The overall quality of the included studies for both CYFRA 21-1 and MMP-9 was poor and mainly limited by selective patient selection, a lack of assessors to the results of previous assessments, and small sample sizes. Table 3 shows a summary of the findings and quality assessment for both CYFRA 21-1 and MMP-9 studies.
Due to the small number of studies, differences in index test techniques, and variability of positivity thresholds across the studies, meta-analysis was not conducted. Results were presented graphically as coupled forest plots for each salivary biomarker as shown in figures 4 and 5.

4. DISCUSSION

4.1 Study Design, Methodology, and Reporting

Most of the studies included were at high risk of selection bias arising from the use of a two-gate or ‘case-control’ study design. All but one of the included studies recruited OSCC participants alongside healthy (disease-free), age-and-gender-matched cases, or both (healthy age-and-gender-matched cases). One study recruited a sample of participants with OSCC and healthy or low-risk PMDs cases, which is a more representative spectrum of diseased and non-diseased individuals in the population. (18)

In addition, patients’ sampling and/or recruitment into the studies were insufficiently reported. Only Yu study has sufficiently reported the patient selection process, which was randomly selected. (18) Moreover, inappropriate exclusion criteria were applied in some of the included studies, such as excluding patients with chronic inflammatory diseases, autoimmune disorders, or individuals with history of consumption of drugs with anticholinergic effects, which all could be within a spectrum of common diseases and drugs used with patients presenting in clinical setting; thus questioning the validity was inevitable.
All studies used a biopsy and histopathological examination as a reference standard and a salivary analysis, with different techniques, as an index test. However, insufficient detail provided and lack of clarity in reporting the studies made it difficult to assess the risk of bias. Therefore, the use of STARD (19) checklist in reporting the primary studies could have facilitated quality appraisal.

Across all the studies included, different thresholds values for the classification of disease-positive were used; only one study used a pre-specified cut-off (18), and one study (14) incompletely reported the process of cut-off value determination. Selecting the cut-off values after performing the test to maximise the test performance can impact the accuracy estimates especially in small studies.(19)

In terms of the conditions and methods of saliva sample collection, process, and storage, there was no particular standard method agreed and followed across the included studies. Without process standardization, comparing, and validating the studies for OSCC salivary biomarkers would be difficult.(20)

### 4.2 Geographical Impact

The CYFRA-21-1 studies (13-15) were solely conducted in India, while the MMP-9 studies (16-18) were conducted in different geographical locations, namely Egypt, Germany, and Taiwan. The sensitivity estimates for the CYFRA 21-1 studies were similar, as were the specificity estimates, whereas the sensitivity and specificity from the MMP-9 studies were more heterogeneous. It is important to bear in mind that India alone accounts for a fifth of all OC cases worldwide and all OC cases were developed from potentially malignant disorders seen
in patients including betel quid users. (21, 22) Studies showed that chemicals in betel quid have cytotoxic and genotoxic effects on mucosal epithelial cells due to the generation of reactive oxygen species (ROS), genetic damage, and micronuclei formation. (23)

Csősz et al. (24) argued that protein biomarkers that were identified in one population may not necessarily be suitable in another population; this argument was supported by some studies that identified IL-8, S100A9, and catalase as biomarkers in certain countries but not in others. It has been also suggested that applying world-wide general protein biomarkers for OSCC detection would be very difficult, thus, the need for conducting population-tailored proteomics studies should be emphasized. (24)

4.3 Biomarkers Expression

Saliva as a diagnostic fluid has been shown to manifest altered expression of biomarkers not only in OSCC but in various oral and systemic diseases. (25-29) Historically, serum CYFRA 21-1 was originally being investigated as a biomarker for non-small-cell lung carcinoma (NSCLC) then evaluated in other malignant and non-malignant diseases. (30-32) Several studies have reported the usefulness of serum CYFRA 21-1 as a biomarker in different conditions including breast cancer, gastric cancer, intrahepatic cholangiocarcinoma (ICC), colorectal cancer, cervical cancer, urinary bladder cancer, and malignant mesothelioma. (33-41) In the head and neck region, serum CYFRA 21-1 could also be a useful biomarker in nasopharyngeal carcinoma, hypopharyngeal carcinoma, oropharyngeal cancer, and OSCC. (14, 15, 41, 42) Malhotra (14) and Rajkumar (15) studies, which are both included in this review, have evaluated the correlation between the serum and salivary CYFRA 21-1 in OSCC. Rajkumar (15) found that
salivary CYFRA 21-1 levels were threefold higher when compared to serum levels (P < 0.001) and increased in stage IV in both serum and saliva. Whereas Malhotra (14) found a significant correlation between serum and salivary CYFRA 21-1 (P= 0.002) and showed that both salivary and serum CYFRA 21-1 levels were significantly elevated in grade II OSCC compared to grade I. At the same time, previous studies showed various results regarding the differences in CYFRA 21-1 levels according to the clinical stages (43-45) while other studies have claimed no correlation. (46, 47)

Similarly, MMP-9 altered expression in tissues and biofluids has also been evaluated in extensive number of studies that explored its role in different diseases and malignancies. Studies showed that tissue MMP-9 level was elevated in many types of malignancies such as SCC of the uterine cervix, ovarian cancer, endometrial cancer, breast cancer, adenocarcinoma of the lung, NSCLC, papillary thyroid cancer, gastric cancer, colorectal cancer, and salivary gland cancer. (48-57) While serum MMP-9 was also elevated in breast cancer, pancreatic ductal adenocarcinoma, and NSCLC. (58-61) Moreover, MMP-9 can be found in urine, plasma and cerebrospinal fluid in various types of malignancies, and was found to be elevated in rheumatoid arthritis in gingival crevicular fluid (GCF) as well. (62, 63) In the head and neck region, MMP-9 is shown to be overexpressed not only in saliva but in plasma, tissue and GCF in oral PMDs such as oral lichen planus, leukoplakia, oral submucous fibrosis, and chronic periodontitis. (63-67)

Since CYFRA 21-1 and MMP-9 can be expressed in various cellular stages and diseases such as inflammatory diseases and malignancies, it is important to explore their effectiveness as diagnostic biomarkers in each condition and to correlate their levels in different biofluids. Due
to the nature of the existence of these biomarkers in various conditions, it is extremely challenging to consider them specific biomarkers for oral cancer. Therefore, the ability of these biomarkers to distinguish oral cancer from other co-morbidities is the keystone for the sake of clinical usefulness and future applications and development. One of the ideas that could help in reaching this goal is combining multiple biomarkers as demonstrated in several studies, however, further studies are needed to investigate the effectiveness of multiple marker combinations. (24, 68-70)

In conclusion, salivary biomarkers in general may have a great potential toward the early detection of OSCC and other diseases as well. However, due to the presence of biases and other limitations in the studies reviewed, there is no conclusive evidence of value of singular salivary CYFRA 21-1 or MMP-9 analyses as screening tools for OSCC at the present time. Therefore, further standardised diagnostic test accuracy studies which minimise potential sources of bias through rigorous design, conduct and reporting are needed. Reporting guidelines for primary diagnostic studies (71) should be strictly followed, and studies should address all potential sources of bias and applicability concerns as indicated in the QUADAS-2 tool. (12) Future research should focus on the accuracy of the current potential salivary biomarkers in the detection of OSCC with clear and robust methodology. Futures studies in oral cancer could be informed by studies of the diagnostic accuracy of combined salivary biomarkers panels in other diseases, which could have similar singular biomarkers’ altered expressions.
Declarations

Funding: No funding

Competing Interests: There are no conflicts of interest

Ethical Approval: Ethical approval was not needed

Patient Consent: Patient consent was not needed
References:


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CYFRA 21-1 and MMP-9 as salivary biomarkers for the detection of oral squamous cell carcinoma: a systematic review of diagnostic test accuracy

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Abstract

Tissue biopsy with histopathological examination is still considered the gold standard to diagnose oral squamous cell carcinoma (OSCC). This systematic review explored the diagnostic test accuracy of two salivary biomarkers in adults suspected of OSCC. The Cochrane Library, MEDLINE, and Embase databases were searched for clinical studies evaluating the diagnostic accuracy of salivary biomarkers in detecting OSCC. Studies were eligible for inclusion if only singular salivary biomarkers were evaluated in three or more studies. Studies investigating combined salivary biomarkers or assessing patients with oral potentially malignant disorders only were excluded. The reporting of the review follows the PRISMA checklist. Six studies, recruiting 775 participants, were included in this review for only two salivary biomarkers, cytokeratin 19 fragment (CYFRA 21-1) and matrix metalloproteinase 9 (MMP-9). The sensitivity and specificity (with 95% confidence intervals) for CYFRA 21-1 studies ranged from 0.84 (0.75–0.91) to 0.94 (0.83–0.99) and from 0.84 (0.71–0.93) to 0.96 (0.80–1.00), respectively. In MMP-9 studies, sensitivity (with 95% confidence intervals) ranged from 0.76 (0.67–0.83) to 1.00 (0.78–1.00) and specificity from 0.27 (0.12–0.46) to 1.00 (0.78–1.00). The overall quality of the included studies was poor. Due to a lack of strong and high-quality evidence, considerable uncertainty remains surrounding the use of singular salivary biomarkers for the detection of OSCC.
**Key words:** oral cancer, oral squamous cell carcinoma, OSCC, salivary biomarkers, CYFRA 21-1, MMP-9

**Introduction**

Many different techniques and tests delivered in a variety of settings are available for the detection of oral squamous cell carcinoma (OSCC). Conventional oral examination performed by a general dental practitioner has traditionally been recommended by various health and medical/dental organizations as a part of oral cancer screening programmes, e.g., the British Dental Association\(^1\). A Cochrane systematic review has evaluated the diagnostic accuracy of a variety of tests as adjunct tests to the conventional oral examination in a dental setting, including vital staining, oral cytology, light-based detection, and oral spectroscopy in patients presenting with clinically evident lesions\(^2\). The results showed that cytology tests appeared to have the greatest potential and warranted further investigation. However, the overall quality of the evidence was variable, with insufficient evidence to justify the replacement of the current ‘gold standard’ diagnostic test of scalpel biopsy with histopathological examination in addition to the conventional oral examination.

Comparatively, saliva, also known as the ‘mirror of the body’, is an easy fluid to collect for bioanalysis. Sample/specimen collection is non-invasive, inexpensive, and simple, which makes it an ideal method for diagnostic purposes\(^3\). Saliva contains an extensive number of compounds, is safe to handle and store, does not clot, and as such, it is deemed a promising diagnostic biofluid\(^4\). Two systematic reviews have evaluated the performance of a wide range of salivary biomarkers (biomarker
expression) in OSCC\textsuperscript{5,6}. However, these systematic reviews mainly included ‘case–control’ type accuracy studies, which can be prone to bias. Studies that compare altered expression of a specific salivary biomarker between ‘cases’ with OSCC and healthy ‘control’ participants can deliver misleading results when the methods of participant recruitment are not taken into consideration\textsuperscript{7}. If participants representing only subgroups of the spectrum of disease and spectrum of non-diseased are included, the resulting estimates of diagnostic test accuracy may not be applicable to the clinical question\textsuperscript{8}.

OSCC is an increasing disease globally. Several factors have been implicated in the development of the disease, such as tobacco, betel quid, and alcohol use, as well as others like human papillomavirus (HPV) and Epstein–Barr virus (EBV) infections. The survival rate of individuals with OSCC is approximately 90\% when detected early, compared to only 50\% after delayed diagnosis\textsuperscript{9}. Hence, both prevention and early detection of disease are crucial in reducing OSCC mortality.

The aim of this systematic review was to identify and summarize the diagnostic accuracy of salivary biomarkers currently being used in the detection of OSCC in adults. The reporting of the review follows the PRISMA checklist\textsuperscript{10}.

**Materials and methods**

**Study criteria**

Studies were eligible for inclusion in the review if they evaluated salivary biomarkers and reported measures of diagnostic test accuracy such as sensitivity and specificity in
detecting OSCC in adults (age ≥16 years) suspected of having OSCC, with no prior treatment such as chemotherapy or radiotherapy.

**Inclusion and exclusion criteria**

Only studies evaluating the performance of singular salivary biomarkers (index test) against any type of tissue biopsy with histopathological examination (reference standard) for the diagnosis of OSCC were eligible for inclusion. Studies were eligible for inclusion in the review if only singular salivary biomarkers were evaluated and where the performance of a salivary biomarker was assessed in three or more studies. Studies investigating combined salivary biomarkers or assessing patients with oral potentially malignant disorders (PMDs) only were excluded.

**Search protocol and study selection**

An electronic search was performed on June 14, 2018 for studies published within the last 5 years (from 2014 to 2018) using the following databases: Cochrane Library, MEDLINE via OVID, and Embase via OVID. The search strategy used was as follows: (oral cancer OR mouth cancer OR oral squamous cell carcinoma OR OSCC).mp AND (diagnos* OR detect*).mp AND (saliva* AND (marker* OR biomarker* OR test)).mp. Following the removal of duplicates, the titles and/or abstracts of the retrieved records were screened for relevancy, and full-text articles were obtained if necessary. Next, full-text articles were assessed for eligibility. Studies were included following consensus among all three authors.
Data extraction

The following data were extracted from each included study: (1) study information and setting (country, design, sample size, test used), (2) type of salivary biomarker, (3) study results: true-positive (sensitivity) and true-negative (specificity), and positive and negative predictive values (PPV and NPV) where reported. A 2 × 2 table was constructed for each study (reference standard against the index test) using Review Manager 5.3. The corresponding author of a study was contacted via email where further information or explanation was needed.

Assessment of methodological quality

Using a tailored QUADAS-2 tool, the quality of the included studies (risk of bias and applicability concerns) was assessed through its four domains: patient selection, index test, reference standard, and flow and timing of participants through the study. The indicators of the assessment of quality (QUADAS-2) are summarized in Table 1, as adapted from Macey et al. The results were then graphically presented using RevMan.

[Table 1 here]

Statistical analysis and data synthesis

The target condition (disease positive) was OSCC. The true-positive, true-negative, false-positive, and false-negative values were used to construct 2 × 2 tables for each
study. The diagnostic test accuracy results for each biomarker were expressed as sensitivity and specificity with 95% confidence intervals (CI). Results were presented graphically as a coupled forest plot for each salivary biomarker.

**Results**

Following the removal of duplicates, the electronic search yielded a total of 250 records, of which 224 were excluded; 26 were further assessed for eligibility (Supplementary Material Appendix 1). Only six studies met the inclusion criteria in this review, as illustrated in the PRISMA flow diagram in Fig. 1. The main reason for exclusion was the low number of studies that investigated each salivary biomarker (Supplementary Material Appendix 2). Only if a biomarker had three or more studies was it eligible to be included in the review. Thus, only six studies reporting the diagnostic accuracy of salivary biomarkers cytokeratin 19 fragment (CYFRA 21-1) and matrix metalloproteinase 9 (MMP-9) in detecting OSCC were included.

[Figure 1 here]

**Description of studies**

The included studies evaluated data from a total of 775 patients (355 patients for CYFRA 21-1 and 420 patients for MMP-9). Of the total 775 patients, 356 (46%) were diagnosed with OSCC according to the reference standard of biopsy and histopathological examination: 180 (54%) patients in the CYFRA 21-1 studies and 176 (42%) patients in the MMP-9 studies. The remaining 419 patients were
classed as disease-free or non-OSCC (175 patients for CYFRA 21-1 and 244 patients for MMP-9). The three CYFRA 21-1 studies were from India\textsuperscript{13–15}, while the three MMP-9 studies were from Egypt\textsuperscript{16}, Germany\textsuperscript{17}, and Taiwan\textsuperscript{18}. Salivary sample collection was conducted in a hospital setting (academic or dental/medical centre) between 2008 and 2014.

The classification and reporting of OSCC varied across studies, making meta-analysis inappropriate: two studies reported OSCC stages only (I–IV)\textsuperscript{16,18}, one study reported OSCC grades only (1 and 2)\textsuperscript{14}, two studies reported OSCC stage (0–IVA) with grade (1–3)\textsuperscript{13,15}, and one study reported OSCC grade (1–4), but reported stages in the TNM system separately\textsuperscript{17}.

**Methodological quality of included studies**

Figs. 2 and 3 summarize the results of the tailored quality assessment of the included studies for CYFRA 21-1 and MMP-9, respectively, utilizing the QUADAS-2 tool\textsuperscript{12}. The figures show a summary of the risk of bias and applicability concerns according to the authors’ judgement in each of the four domains for each included study

**Supplementary Material** Appendix 3 includes the comprehensive quality assessment performed for each study).

[Figures 2 and 3 here]

None of the included studies could be classified as being at low risk of bias for all four domains. Patient selection was considered as high risk of bias in all of the
studies, which was mainly due to the method of patient enrolment\textsuperscript{13}, the nature of the study design\textsuperscript{13–18}, and implementing inappropriate exclusions\textsuperscript{13,14,16}.

The index test was considered to be at low risk of bias in only one study\textsuperscript{18}, unclear in one study\textsuperscript{14}, but high in four studies\textsuperscript{13,15–17}. The variation across the level of bias differed due to insufficient details reported as to whether the results of the index test were interpreted without prior knowledge of the reference standard results, lack of pre-specification of a test-positive threshold, and statement of conflict of interest. Similarly, the reference standard domain was considered to be at low risk of bias in only one study\textsuperscript{18}, and unclear in the other five studies\textsuperscript{14–17}. All of the five studies were judged as unclear due to the inadequate reporting of whether the biopsy and histopathological diagnoses were made without any prior knowledge of the index test results. Additionally, three studies failed to comprehensively report the details of the reference standard\textsuperscript{13,16,17}.

The flow and timing domain was considered as having an unclear risk of bias across all of the studies due to insufficient details reported regarding the interval period between the index and reference standard (inadequately reported in all six studies), whether all patients received the same reference standard\textsuperscript{13}, and whether all patients had been included in the analysis\textsuperscript{14}. Only three studies were assessed as being of low concern for applicability according to patient selection, the index test, and the reference standard\textsuperscript{15,16,18}. The remaining three studies were assessed as being of unclear concern for applicability due to the lack of detail regarding patient selection\textsuperscript{14} and conduct of the reference standard\textsuperscript{13,17}.

**Findings**
The sensitivity and specificity of CYFRA 21-1 (with 95% CI) ranged from 0.84 (0.75–0.91) to 0.94 (0.83–0.99) and from 0.84 (0.71–0.93) to 0.96 (0.80–1.00), respectively. The sensitivity and specificity of MMP-9 (with 95% CI) ranged from 0.76 (0.67–0.83) to 1.00 (0.78–1.00) and from 0.27 (0.12–0.46) to 1.00 (0.78–1.00), respectively. Table 2 shows a summary of the data extracted from the included studies. The overall quality of the included studies for both CYFRA 21-1 and MMP-9 was poor and mainly limited by selective patient selection, a lack of assessors to the results of previous assessments [Au?3], and small sample sizes. Table 3 shows a summary of the findings and quality assessment for both CYFRA 21-1 and MMP-9 studies.

[Tables 2 and 3 here]

Due to the small number of studies, differences in index test techniques, and variability of positivity thresholds across the studies, a meta-analysis was not conducted. The results are presented graphically as coupled forest plots for each salivary biomarker in Figs. 4 and 5.

[Figures 4 and 5 here]

Discussion

Study design, methodology, and reporting
Most of the studies included were at high risk of selection bias arising from the use of a two-gate or ‘case–control’ study design. All but one of the included studies recruited OSCC participants alongside healthy (disease-free), age and sex-matched cases, or both (healthy age and sex-matched cases) [Au?4]. One study recruited a sample of participants with OSCC and healthy or low-risk PMD cases, which is a more representative spectrum of diseased and non-diseased individuals in the population18.

In addition, patient sampling and/or recruitment into the studies were insufficiently reported. Only the study by Yu et al. had sufficiently reported the patient selection process, which was randomly selected18. Moreover, inappropriate exclusion criteria were applied in some of the included studies, such as excluding patients with chronic inflammatory diseases, autoimmune disorders, or individuals with a history of consumption of drugs with anticholinergic effects, which all could be within a spectrum of common diseases and drugs used in patients presenting in the clinical setting; thus questioning the validity was inevitable.

All studies used a biopsy and histopathological examination as the reference standard and a salivary analysis (with different techniques) as the index test. However, insufficient detail provided and lack of clarity in reporting the studies made it difficult to assess the risk of bias. Therefore, use of the STARD19 checklist in reporting the primary studies could have facilitated the quality appraisal.

Across all of the studies included, different threshold values for the classification of disease-positive were used; only one study used a pre-specified cut-off18, and one study incompletely reported the process of cut-off value determination14. Selecting the cut-off value after performing the test to maximize the test performance can impact the accuracy estimates, especially in small studies19.
In terms of the conditions and methods of saliva sample collection, processing, and storage, there was no particular standard method agreed and followed across the included studies. Without process standardization, comparing and validating the studies for OSCC salivary biomarkers would be difficult\textsuperscript{20}.

**Geographical impact**

The CYFRA 21-1 studies were solely conducted in India\textsuperscript{13–15}, while the MMP-9 studies were conducted in different geographical locations, namely Egypt, Germany, and Taiwan\textsuperscript{16–18}. The sensitivity estimates for the CYFRA 21-1 studies were similar, as were the specificity estimates, whereas the sensitivity and specificity from the MMP-9 studies were more heterogeneous. It is important to bear in mind that India alone accounts for a fifth of all oral cancer cases worldwide, and all oral cancer cases developed from potentially malignant disorders seen in patients including betel quid users\textsuperscript{21,22}. Studies have shown that chemicals in betel quid have cytotoxic and genotoxic effects on mucosal epithelial cells due to the generation of reactive oxygen species (ROS), genetic damage, and micronuclei formation\textsuperscript{23}.

Csősz et al. argued that protein biomarkers that were identified in one population may not necessarily be suitable in another population\textsuperscript{24}; this argument was supported by some studies that identified interleukin (IL)-8, S100A9, and catalase as biomarkers in certain countries but not in others. It was also suggested that applying worldwide general protein biomarkers for OSCC detection would be very difficult; thus, the need to conduct population-tailored proteomics studies should be emphasized\textsuperscript{24}.
Biomarker expression

Saliva as a diagnostic fluid has been shown to manifest altered expression of biomarkers not only in OSCC but in various oral and systemic diseases\textsuperscript{25–29}. Historically, serum CYFRA 21-1 was originally investigated as a biomarker for non-small-cell lung carcinoma (NSCLC) and was then evaluated in other malignant and non-malignant diseases\textsuperscript{30–32}. Several studies have reported the usefulness of serum CYFRA 21-1 as a biomarker in different conditions including breast cancer, gastric cancer, intrahepatic cholangiocarcinoma (ICC), colorectal cancer, cervical cancer, urinary bladder cancer, and malignant mesothelioma\textsuperscript{33–41}. In the head and neck region, serum CYFRA 21-1 could also be a useful biomarker in nasopharyngeal carcinoma, hypopharyngeal carcinoma, oropharyngeal cancer, and OSCC\textsuperscript{14,15,41,42}. The studies by Malhotra et al.\textsuperscript{14} and Rajkumar et al.\textsuperscript{15}, which were both included in this review, evaluated the correlation between the serum and salivary CYFRA 21-1 in OSCC. Rajkumar et al.\textsuperscript{15} found that salivary CYFRA 21-1 levels were three-fold higher when compared to serum levels ($P < 0.001$) and increased in stage IV in both serum and saliva. Malhotra et al.\textsuperscript{14}, on the other hand, found a significant correlation between serum and salivary CYFRA 21-1 ($P = 0.002$) and showed that both salivary and serum CYFRA 21-1 levels were significantly elevated in grade II OSCC compared to grade I. At the same time, other previous studies have shown various results regarding the differences in CYFRA 21-1 levels according to the clinical stage\textsuperscript{43–45}, while other studies have claimed no correlation\textsuperscript{46,47}.

Similarly, altered MMP-9 expression in tissues and biofluids has also been evaluated in an extensive number of studies that have explored its role in different diseases and malignancies. Studies have shown elevated tissue MMP-9 levels in many
types of malignancy such as squamous cell carcinoma of the uterine cervix, ovarian cancer, endometrial cancer, breast cancer, adenocarcinoma of the lung, NSCLC, papillary thyroid cancer, gastric cancer, colorectal cancer, and salivary gland cancer\textsuperscript{48–57}. Furthermore, serum MMP-9 has also been shown to be elevated in breast cancer, pancreatic ductal adenocarcinoma, and NSCLC\textsuperscript{58–61}. Moreover, MMP-9 can be found in urine, plasma, and cerebrospinal fluid in various types of malignancy, and has been found to be elevated in the gingival crevicular fluid (GCF) of patients with rheumatoid arthritis as well\textsuperscript{62,63}. In the head and neck region, MMP-9 overexpression has been shown not only in saliva but also in plasma, tissue, and GCF in oral PMDs such as oral lichen planus, leukoplakia, oral submucous fibrosis, and chronic periodontitis\textsuperscript{63–67}.

Since CYFRA 21-1 and MMP-9 can be expressed in various cellular stages and diseases such as inflammatory diseases and malignancies, it is important to explore their effectiveness as diagnostic biomarkers in each condition and to correlate their levels in different biofluids. Due to the nature of the existence of these biomarkers in various conditions, it is extremely challenging to consider them specific biomarkers for oral cancer. Therefore, the ability of these biomarkers to distinguish oral cancer from other co-morbidities is the keystone for the sake of clinical usefulness and future applications and development. One of the ideas that could help in reaching this goal is combining multiple biomarkers, as demonstrated in several studies; however, further studies are needed to investigate the effectiveness of multiple marker combinations\textsuperscript{24,68–70}.

In conclusion, salivary biomarkers in general may have great potential in the early detection of OSCC and other diseases as well. However, due to the presence of biases and other limitations in the studies reviewed, there is no conclusive evidence of
the value of singular salivary CYFRA 21-1 or MMP-9 analyses as screening tools for OSCC at the present time. Therefore, further standardized diagnostic test accuracy studies that minimize potential sources of bias through rigorous design, conduct, and reporting are needed. Reporting guidelines for primary diagnostic studies should be followed strictly\textsuperscript{71}, and studies should address all potential sources of bias and applicability concerns as indicated in the QUADAS-2 tool\textsuperscript{12}. Future research should focus on the accuracy of the current potential salivary biomarkers in the detection of OSCC with clear and robust methodology. Futures studies in oral cancer could be informed by studies of the diagnostic accuracy of combined salivary biomarker panels in other diseases, which could have similar altered expression of singular biomarkers.

**Funding**

No funding.

**Competing interests**

There are no conflicts of interest.

**Ethical approval**

Ethical approval was not needed.

**Patient consent**

Patient consent was not needed.
References


55. Sier CF, Kubben FJ, Ganesh S, Heerding MM, Griffioen G, Hanemaaijer R, et al. [Au?] Tissue levels of matrix metalloproteinases MMP-2 and MMP-9 are related


**Figure captions**

Fig. 1. PRISMA flow diagram of the search strategy. [Au?11]

Fig. 2. Summary of the risk of bias and applicability concerns for each included study on CYFRA 21-1. [Au?12]

Fig. 3. Summary of the risk of bias and applicability concerns for each included study on MMP-9.

Fig. 4. Forest plot showing the sensitivity and specificity of CYFRA 21-1 studies. [Au?12]

Fig. 5. Forest plot showing the sensitivity and specificity of MMP-9 studies.
63 records from Cochrane
94 records from MEDLINE
200 records from EMBASE

357 records identified through electronic database searching
107 duplicates removed

224 of records excluded:
Irrelevant (n:95)
No Control group (n:2)
Not assessing diagnostic accuracy of salivary biomarkers (n: 84)
No data reported (n:9)
Not available (n:3)
ineligible study design (n:31)

250 of records screened by title and/or abstract

26 of full-text assessed for eligibility
No biomarkers in the 20 eligible studies were evaluated in three or more studies

6 of studies included

Figure 1
<table>
<thead>
<tr>
<th></th>
<th>Risk of Bias</th>
<th>Applicability Concerns</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Patient Selection</td>
<td>Index Test</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Awashti 2017</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Malhotre 2016</td>
<td>-</td>
<td>?</td>
</tr>
<tr>
<td>Rajkumar 2015</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

- High
- Unclear
- Low

Figure 2
<table>
<thead>
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<th></th>
<th>Applicability Concerns</th>
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</thead>
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<tr>
<td></td>
<td>Patient Selection</td>
<td>Index Test</td>
<td>Reference Standard</td>
<td>Flow and Timing</td>
</tr>
<tr>
<td>Ghallab 2017</td>
<td>● ● ? ?</td>
<td>● ● ●</td>
<td>+ + +</td>
<td>+ + +</td>
</tr>
<tr>
<td>Yu 2016</td>
<td>● + + ?</td>
<td>● + +</td>
<td>● + +</td>
<td>● + +</td>
</tr>
</tbody>
</table>

- ●: High
- ?: Unclear
- +: Low

*Figure 3*
<table>
<thead>
<tr>
<th>Study</th>
<th>TP</th>
<th>FP</th>
<th>FN</th>
<th>TN</th>
<th>Sensitivity (95% CI)</th>
<th>Specificity (95% CI)</th>
<th>Sensitivity (95% CI)</th>
<th>Specificity (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Awashil 2017</td>
<td>27</td>
<td>1</td>
<td>3</td>
<td>24</td>
<td>0.90 [0.73, 0.98]</td>
<td>0.96 [0.80, 1.00]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Malhotre 2016</td>
<td>47</td>
<td>8</td>
<td>3</td>
<td>42</td>
<td>0.94 [0.83, 0.99]</td>
<td>0.84 [0.71, 0.93]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rajkumar 2015</td>
<td>84</td>
<td>5</td>
<td>16</td>
<td>95</td>
<td>0.84 [0.75, 0.91]</td>
<td>0.95 [0.89, 0.98]</td>
<td></td>
<td></td>
</tr>
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</table>

*Figure 4*
<table>
<thead>
<tr>
<th>Study</th>
<th>TP</th>
<th>FP</th>
<th>FN</th>
<th>TN</th>
<th>Sensitivity (95% CI)</th>
<th>Specificity (95% CI)</th>
<th>Sensitivity (95% CI)</th>
<th>Specificity (95% CI)</th>
</tr>
</thead>
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<tr>
<td>Challab 2017</td>
<td>15</td>
<td>0</td>
<td>0</td>
<td>15</td>
<td>1.00 [0.78, 1.00]</td>
<td>1.00 [0.78, 1.00]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peisker 2017</td>
<td>30</td>
<td>22</td>
<td>0</td>
<td>8</td>
<td>1.00 [0.88, 1.00]</td>
<td>0.27 [0.12, 0.46]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yu 2016</td>
<td>99</td>
<td>79</td>
<td>32</td>
<td>120</td>
<td>0.76 [0.67, 0.83]</td>
<td>0.60 [0.53, 0.67]</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Figure 5*
Table 1. QUADAS-2 indicators adapted for the current systematic review.

<table>
<thead>
<tr>
<th>Domain</th>
<th>Patient selection</th>
<th>Index test</th>
<th>Reference standard</th>
<th>Flow and timing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Signalling questions</td>
<td>Was a consecutive or random sample of patients enrolled?</td>
<td>Were the index test results interpreted without knowledge of the results of the reference standard?</td>
<td>Is the reference standard likely to correctly classify the target condition?</td>
<td>Was there an appropriate interval between the index test and reference standard?</td>
</tr>
<tr>
<td>(Yes, No, or Unclear)</td>
<td>Yes: if consecutive patients or a random sample of individuals were recruited</td>
<td>Yes: if the biopsy was independently confirmed by at least two qualified pathologists</td>
<td>Yes: if the biopsy was not independently confirmed by at least two qualified pathologists, or there was a lack of agreement between pathologists</td>
<td>No: if the delay between the index test and reference standard was considered unacceptable for the majority of participants</td>
</tr>
<tr>
<td></td>
<td>No: if non-consecutive patients or a non-random sample of individuals were recruited</td>
<td>No: if the biopsy was independently confirmed by at least two qualified pathologists, or there was a lack of agreement between pathologists</td>
<td>No: if the biopsy was not independently confirmed by at least two qualified pathologists, or there was a lack of agreement between pathologists</td>
<td>Yes: if the delay between the index test and reference standard was considered acceptable for the majority of participants</td>
</tr>
<tr>
<td></td>
<td>Unclear: if patient selection was not clearly described</td>
<td>Unclear: if the study did not provide any information on whether interpreters of the index tests were blinded to biopsy/histopathology</td>
<td>Unclear: if the study did not state who confirmed the biopsy</td>
<td>Unclear: if the delay between the index test and reference standard was not explicitly stated</td>
</tr>
<tr>
<td>Question</td>
<td>Yes</td>
<td>No</td>
<td>Unclear</td>
<td></td>
</tr>
<tr>
<td>------------------------------------------------------------------------</td>
<td>----------------------------------------------------------------------</td>
<td>----------------------------------------------------------------------</td>
<td>------------------------------------------------------------------------</td>
<td></td>
</tr>
<tr>
<td>Was a case–control design avoided?</td>
<td>If a threshold was used, was it pre-specified?</td>
<td>Were the reference standard results interpreted without knowledge of the results of the index tests?</td>
<td>Did all patients receive the same reference standard?</td>
<td></td>
</tr>
<tr>
<td>Yes: if a case–control design was avoided</td>
<td>Yes: if the threshold was pre-specified</td>
<td>Yes: if pathologists clearly did not know the index test results when interpreting biopsied tissues</td>
<td>Yes: if the same reference standard was used for all participants</td>
<td></td>
</tr>
<tr>
<td>No: if a case–control design was not avoided</td>
<td>No: if the threshold was not pre-specified</td>
<td>No: if pathologists knew the results of the index test when interpreting biopsied tissues</td>
<td>No: if the same reference standard was not used for all participants</td>
<td></td>
</tr>
<tr>
<td>Unclear: if no clear type of study design was reported</td>
<td>Unclear: if it was unclear whether the threshold was pre-specified</td>
<td>Unclear: if the study did not provide any information on whether the pathologists were blinded to the index test results</td>
<td>Unclear: if it was unclear whether different reference standards were used</td>
<td></td>
</tr>
<tr>
<td>Did the study avoid inappropriate exclusions?</td>
<td>Was conflict of interest avoided?</td>
<td>Were all patients included in the analysis?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes: if patients with any stage of OSCC regardless of their other</td>
<td>Yes: if the study declared no conflict of interest</td>
<td>Yes: if all patients were included in the analysis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall risk of bias</td>
<td>Could the selection of patients have introduced bias?</td>
<td>Could the conduct or interpretation of the index test have introduced bias?</td>
<td>Could the reference standard, its conduct, or its interpretation have introduced bias?</td>
<td>Could the patient flow have introduced bias?</td>
</tr>
<tr>
<td>---------------------</td>
<td>-----------------------------------------------------</td>
<td>-----------------------------------------------------------------</td>
<td>-----------------------------------------------------------------</td>
<td>---------------------------------</td>
</tr>
<tr>
<td>High</td>
<td>If answered ‘No’ to any question</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>If answered ‘Yes’ to all questions</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unclear</td>
<td>If answered ‘Unclear’ to all questions or accompanied by any ‘Yes’</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Concerns regarding applicability: High, Low, or Unclear
- Are there concerns that the included patients do not match the review question?
- Are there concerns that the index test, its conduct, or interpretation differ from the review question?
- Are there concerns that the target condition, as defined by the system, differs from the review question?
OSCC, oral squamous cell carcinoma.
<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Study design</th>
<th>City, country</th>
<th>Sample size (OSCC/control)</th>
<th>Technique/test</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
<th>Cut-off value</th>
<th>AUC</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYFRA 21-1</td>
<td>Two-gate&lt;br&gt;(Case–control)</td>
<td>Lucknow, India</td>
<td>55 (30/25)</td>
<td>ELISA</td>
<td>90%</td>
<td>97%</td>
<td>96.4%</td>
<td>91.7%</td>
<td>8.7 ng/ml</td>
<td>0.994</td>
</tr>
<tr>
<td></td>
<td>(Case–control)</td>
<td>New Delhi, India</td>
<td>100 (50/50)</td>
<td>ECLIA</td>
<td>93.8%</td>
<td>84.3%</td>
<td>85.5%&lt;sup&gt;c&lt;/sup&gt;</td>
<td>93.3%&lt;sup&gt;c&lt;/sup&gt;</td>
<td>8.5 ng/ml</td>
<td>NR</td>
</tr>
<tr>
<td></td>
<td>(Case–control)</td>
<td>Chennai, India</td>
<td>200 (100/100)</td>
<td>ELISA</td>
<td>83.6%</td>
<td>95%</td>
<td>94.4%&lt;sup&gt;c&lt;/sup&gt;</td>
<td>85.6%&lt;sup&gt;c&lt;/sup&gt;</td>
<td>&gt;7.91 ng/ml</td>
<td>0.895</td>
</tr>
</tbody>
</table>

Cytokeratin 21-1 (CYFRA 21-1) was detected using ELISA and ECLIA in salivary fluid from patients with oral squamous cell carcinoma (OSCC). Sensitivity and specificity were reported for each study. The cut-off values and area under the curve (AUC) for each study are also provided.

<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Year conducted: 2014&lt;sup&gt;16&lt;/sup&gt;</td>
<td><strong>Two-gate&lt;sup&gt;b&lt;/sup&gt;</strong> Cairo, Egypt 30 (15/15) ELISA 100% 100% 100% 100% 260.32 1.000 ng/ml</td>
</tr>
<tr>
<td>Year conducted: Unknown&lt;sup&gt;17&lt;/sup&gt;</td>
<td><strong>Two-gate&lt;sup&gt;b&lt;/sup&gt;</strong> Jena, Germany 60 (30/30) ELISA 100% 26.7% 57.7%&lt;sup&gt;c&lt;/sup&gt; 100%&lt;sup&gt;c&lt;/sup&gt; &gt;0.104 NR</td>
</tr>
<tr>
<td>Years conducted: 2008 to 2013&lt;sup&gt;18&lt;/sup&gt;</td>
<td><strong>Two-gate&lt;sup&gt;b&lt;/sup&gt;</strong> Taiwan 330 (131/199) Multiplex LC- MRM-MS 75.6% 60.3% 55.6%&lt;sup&gt;c&lt;/sup&gt; 79%&lt;sup&gt;c&lt;/sup&gt; NR 0.726</td>
</tr>
<tr>
<td>(Case–control)</td>
<td>AUC, area under the curve; CYFRA 21-1, cytokeratin 19 fragment; ECLIA, electrochemiluminescence immunoassay; ELISA, enzyme-linked immunosorbent assay; MMP-9, matrix metalloproteinase 9; NPV, negative predictive value; NR, not reported; OSCC, oral squamous cell carcinoma; PPV, positive predictive value;</td>
</tr>
</tbody>
</table>
According to histopathological examination.

According to Rutjes et al.\textsuperscript{7} [Au?14].

Values obtained using the RevMan calculator when not reported, or when our calculation differed from the reported value.
What is the diagnostic accuracy of the current salivary biomarkers used in the detection of OSCC?

**Table 3. Summary of the findings and quality assessment.**

<table>
<thead>
<tr>
<th>Patient population</th>
<th>Adults (aged ≥16 years) suspected of having OSCC with no prior treatment such as chemotherapy or radiotherapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Index test</td>
<td>Salivary analysis for CYFRA 21-1 and MMP-9 singular biomarkers only (not combined)</td>
</tr>
<tr>
<td>Reference standard</td>
<td>Biopsy with histopathological examination</td>
</tr>
<tr>
<td>Target condition</td>
<td>Oral squamous cell carcinoma (OSCC)</td>
</tr>
<tr>
<td>Included studies</td>
<td>$N = 6$ two-gate (case–control) studies</td>
</tr>
<tr>
<td>Quality assessment</td>
<td>None of the included studies could be classified as being at low risk of bias in all of the four domains, which are patient selection, index test, reference standards, and flow and timing. The overall quality of the included studies for both CYFRA 21-1 and MMP-9 was poor and mainly limited by a poor patient selection process, unclear blinding implementation, and small sample sizes.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Sensitivity (95% CI) Range</th>
<th>Specificity (95% CI) Range</th>
<th>Number of total participants/OSCC cases/non-OSCC cases</th>
<th>Quality</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYFRA 21-1</td>
<td>0.84 (0.75–0.91) to 0.94 (0.83–0.99)</td>
<td>0.84 (0.71–0.93) to 0.96 (0.80–1.00)</td>
<td>355/180/175</td>
<td>High risk of bias</td>
</tr>
</tbody>
</table>
MMP-9 0.76 (0.67–0.83) to 1.00 0.27 (0.12–0.46) to 1.00 420/176/244 High risk of bias
(0.78–1.00) (0.78–1.00)

CI, confidence interval; CYFRA 21-1, cytokeratin 19 fragment; MMP-9, matrix metalloproteinase 9; OSCC, oral squamous cell carcinoma.