ISSN: 0975-3583,0976-2833 VOL 16, ISSUE 11, 2025

Diagnostic accuracy of salivary biomarkers in identifying early oral squamous cell carcinoma– A Systematic Review and Meta-analysis

1. **Dr. Aditi Dilip Vaidya**
Post graduate student
Department of Oral & Maxillofacial Pathology and Microbiology.
Dr. DY Patil Deemed to be University, School of Dentistry, Navi Mumbai 400706, Maharashtra, India
Mobile no.- +7506071333
Email ID: aditivaidya567@gmail.com
Contribution towards the manuscript: Concepts, design, definition of intellectual content, manuscript preparation, manuscript review, manuscript editing.

2. **Dr. Treville Pereira**
Professor
Department of Oral & Maxillofacial Pathology and Microbiology.
Dr. DY Patil Deemed to be University, School of Dentistry, Navi Mumbai 400706, Maharashtra, India
Mobile no.- +919821281458
Email ID: treville.pereira@dypatil.edu
Contribution towards the manuscript: Concepts, design, definition of intellectual content, manuscript preparation, manuscript editing.

3. **Dr. Mayura Mahajan**
Associate Professor
Department of Oral & Maxillofacial Pathology and Microbiology.
Dr. DY Patil Deemed to be University, School of Dentistry, Navi Mumbai 400706, Maharashtra, India
Mobile no.- +919819562211
Email ID: mayurachande1234@gmail.com
Contribution towards the manuscript: Design, definition of intellectual content, manuscript preparation, manuscript editing.

4. **Dr. Pooja Kamath**
Associate Professor
Department of Oral & Maxillofacial Pathology and Microbiology.
Dr. DY Patil Deemed to be University, School of Dentistry, Navi Mumbai 400706, Maharashtra, India
Mobile no.- +919833967354
Email ID: drpoojak1@gmail.com
Contribution towards the manuscript: Definition of intellectual content, manuscript preparation, manuscript review, manuscript editing.

5. **Dr.Swati Gotmare**
Professor
Department of Oral & Maxillofacial Pathology and Microbiology.
Dr. DY Patil Deemed to be University, School of Dentistry, Navi Mumbai 400706, Maharashtra, India
Mobile no.- +919322794067
Email ID: swati.gotmare@dypatil.edu
Contribution towards the manuscript: Definition of intellectual content, manuscript preparation, manuscript editing.

6. **Dr. Nasreen Akhai**
Post graduate student
Department of Oral & Maxillofacial Pathology and Microbiology.
Dr. DY Patil Deemed to be University, School of Dentistry, Navi Mumbai 400706, Maharashtra, India
Mobile no.- +918080668871

ISSN: 0975-3583,0976-2833 VOL 16, ISSUE 11, 2025

Email ID: itsnasreen26@gmail.com
Contribution towards the manuscript: Definition of intellectual content, manuscript preparation, manuscript editing.
Name and Address of Corresponding Author:
Dr. Aditi Dilip Vaidya
Department of Oral & Maxillofacial Pathology and Microbiology.
Dr. DY Patil Deemed to be University, School of Dentistry, Navi Mumbai 400706, Maharashtra, India
Mobile no.- +917506071333
Email ID: aditivaidya567@gmail.com

Abstract

Objective: To summarize and compare the existing evidence on diagnostic accuracy of salivary biomarkers with their estimation method in detecting early oral squamous cell carcinoma (OSCC) in adults through a novel meta-analysis.

Method: The review was performed in accordance with preferred reporting items for systematic review and meta-analysis and protocol is registered under PROSPERO(CRD42023457292). Electronic databases were searched from January 2000 to November 2023 to identify the screening potential of major salivary biomarkers: S100P, DUSP-1, IL-8, IL-1B, MMP, miRNA, mRNA and TNF-a. Raw data like true-positive, false-positive, true-negative, false-negative, sensitivity, specificity values were extracted or calculated if not present for each study. Quality of selected studies was evaluated based on Quality assessment of diagnostic accuracy studies (QUADAS)- 2 tool through review manager (RevMan version 5.3). Meta-analysis was performed in Meta-Disc 1.4 software through a bivariate model parameter for the pooled sensitivity and specificity, summary receiver operating curve (SROC), overall area under curve (AUC) and likelihood ratio (positive and negative likelihood ratio (LR).

Results: Twenty-one studies were included for qualitative synthesis and eighteen for meta-analysis. All the included studies had a presence of low to moderate risk of bias. The sensitivities and specificities for studies ranged from 62% to 100% and from 36% to 98%. Highest sensitivity was observed for MMP with 100% by ELSIA while lowest specificity was observed for S100P with 36% estimated by ELISA. Positive likelihood ratio (PLR) was of range 1.03 – 7.79 and the highest was seen for mRNA by PCR (7.79) while negative likelihood ratio (NLR) was of range 0.09 – 0.80 and the highest -LR was seen for TNF-a by ELISA. The highest AUC was seen for IL-8 by ELISA (0.85) and miRNA by PCR (0.79) which was

ISSN: 0975-3583,0976-2833 VOL 16, ISSUE 11, 2025

considered overall good and the lowest AUC was seen for IL-1B and TNF-a by ELISA (0.56) and DUSP and mRNA (0.50) estimated by PCR which was considered poor.

Conclusion: Early detection of OSCC was best achieved by screening for salivary IL-8 by ELISA and miRNA estimated by PCR. These novel biomarkers and salivary biomarkers may be potentially used for non-invasive detection of early OSCC under early diagnosis and prompt treatment.

Keywords: accuracy, diagnosis, meta-analysis, oral cancer, saliva, salivary biomarker

Introduction

Oral cancer has become a disease of concern worldwide with up-to 400,000 new cases per year with almost 13,00,00 deaths annually (Hovarth et al., 2017). Oral squamous cell carcinomas (OSCC) accounts for 90% of all oral cancers, of which 80% occur in Southeast Asia. Oral cancer accounts for over thirty per cent of all cancers reported in the country (Yang Li et al., 2004). Hence, control and management of oral cancer has become a top priority in the health sector. Early detection, mass-screening, and easy follow-ups would improve survival, and decrease mortality and morbidity associated with OSCC (Hu et al., 2008).

Though biopsy is the gold standard for diagnosis of OSCC, it is not convenient for screening and follow-up due to its invasive nature, high cost, and need for specially trained medical personnel and equipment (Arellano et al., 2008). Moreover, the current tools of diagnosis are not enough for detecting high risk PMODs (potentially malignant oral disorders) or in post-treatment phases during follow-up, as DNA mutations have been observed even in epithelial cells with no evidence of histopathological changes (Lee et al., 2017). Thus it is of utmost importance to develop newer, non-invasive and easy to use diagnostic medium and tools for the detection of OSCC. The detection of discriminatory biomarkers in saliva samples is considered to be the most promising answer at this stage (Singh et al., 2020).

The use of body fluids, i.e., saliva or serum/plasma, has shown considerable promise for the early diagnosis of cancers, including breast cancer, prostate cancer, and lung cancer, among others (Peisker et al., 2017). Human saliva is a multi-component oral fluid that may play a potential role in the diagnosis of cancer. Saliva has gained notable attention as a diagnostic

ISSN: 0975-3583,0976-2833 VOL 16, ISSUE 11, 2025

fluid because of its simple collection and processing, minimal invasiveness and low costs (Ghallab et al., 2014). Many researchers have studied salivary proteins as potential diagnostic markers for various diseases such as breast cancer, ovarian cancer, Sjögrens syndrome, hepatocellular carcinoma, leukoplakia and oral cancer (Shiptzer et al., 2009).

Salivary biomarkers have recently become an emerging field for monitoring oral diseases and systemic diseases (Saleem et al., 2021). In carcinogenesis, overexpression of certain biomarkers could result in downregulation of tumour suppressor genes, while under-expression of certain biomarkers could cause oncogene upregulation (Smriti et al., 2019). This fact indicates that biomarkers may play a role as either tumour suppressors or oncogenes. Moreover, a high fraction of discovered biomarkers are found to be located at fragile sites or in cancer-associated genomic regions, including minimal regions of loss of heterozygosity or minimal amplicons. Therefore, salivary biomarkers screening emerges as a novel diagnostic method for detection of human cancers, especially at early stages (Smriti et al., 2019).

Salivary biomarker offers a promising diagnostic adjunct due to its simple non- invasive collection method and can be employed to screen large populations (Heravi et al., 2014). Analysis of literature reveals that current investigative approaches for improving oral cancer detection consists of salivary proteins, salivary proteases, salivary RNA, transcriptomic and proteomic classes of biomarkers which includes mRNA, miRNA, DUSP100, s100P, IL-8, IL-1B, TNF-a, MMP-9 (Shaw et al., 2022).

Understanding the diagnostic accuracy would help clinicians to reach the correct diagnosis and choose the most effective treatment. Diagnostic accuracy includes sensitivity, specificity and summary receiver operating characteristics (SROC) analysis (Shaw et al., 2022).

Sensitivity and specificity explain the diagnostic ability of a test to correctly identify diseased and non- diseased respectively. They are independent of disease prevalence which refers to the probability of disease in a specific population at a given time and summary receiver operating characteristics (SROC) analysis is used to evaluate the predictive power for diagnosis (Shaw et al., 2022).

Till date, no study has provided a comprehensive, quantitative analysis of salivary biomarkers on which diagnostic reasoning of early oral squamous cell carcinoma can be established. Therefore, we updated our research and conducted this systematic review with the aim to

ISSN: 0975-3583,0976-2833 VOL 16, ISSUE 11, 2025

compare the diagnostic accuracy of various salivary biomarkers estimated by polymerase chain reaction (PCR) and enzyme linked immunosorbent assay (ELISA) for early diagnosis of OSCC in adults through a meta- analysis.

Methodology

Protocol and Registration

The systematic review and meta-analysis protocol was registered at the international prospective register of systematic reviews (PROSPERO-CRD42023457292) and performed in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analysis – Diagnostic Test Accuracy (PRISMA- DTA) checklist (Salameh et al., 2020).

Study Design

The following focused research question in the Participants (P), Index test (I), reference standard (R) and target condition (T) format was proposed “Is there a difference in the diagnostic accuracy of salivary biomarkers (Index Test) compared to biopsy (gold standard) for the early detection of oral squamous cell carcinoma (OSCC) in adults?

Eligibility Criteria: studies were selected based on the following criteria’s

Inclusion Criteria. The inclusion criteria were as follows:

(1) Study Design: In-vivo studies- Observational studies or Clinical trials comparing the diagnostic accuracy of salivary biomarkers with biopsy.

(2) Participant characteristics: patients diagnosed with oral squamous cell carcinoma aged 18 years and older

(3) Outcome measurements: Diagnostic accuracy including sensitivity, specificity, accuracy, determined using different methods irrespective of the methods of quantifying the outcomes.

(4) Articles written in English language

ISSN: 0975-3583,0976-2833 VOL 16, ISSUE 11, 2025

(5) Articles from January 2000 – November 2023 and available as free full text

Exclusion Criteria. The exclusion criteria were as follows:

(1) Non-clinical studies, in-vitro studies, and animal studies. Studies reporting about a single intervention were also excluded.
(2) Studies done on individuals less than 18 years of age.
(3) Studies not fully available in the database.
(4) Article reporting only abstracts were also excluded.
(5) Studies not reporting primary outcomes of accuracy, sensitivity, and specificity as well as where primary outcomes are not possible to calculate from the given raw data.

Search protocol and study selection

A comprehensive electronic search was performed till November 2023 for the studies published within the last 23 years (from 2000 to 2023) using the following databases: PubMed and EBSCOhost to retrieve articles in the English language. The searches in the clinical trials database, cross-referencing and grey literature were conducted using Google Scholar, Greylist, and OpenGrey. In addition to the electronic search, a hand search was also made, and reference lists of the selected articles were screened.

Search Strategy

Appropriate key words and Medical Subject Heading (MeSH) terms were selected and combined with Boolean operators like AND. The search strategy used was as follows: (salivary biomarkers AND sensitivity AND specificity AND oral cancer), (saliva AND biomarkers AND diagnosis).

The search and screening, according to the previously established protocol, were conducted by two review authors. A two-phase selection of articles was conducted. In phase one, two reviewers reviewed titles and abstracts of all articles. Articles that did meet inclusion criteria were excluded. In phase-two, selected full articles were independently reviewed and screened by the same reviewers. Any disagreement was resolved by discussion. When mutual agreement between two reviewers was not reached, a third reviewer was involved to make the final decision. The final selection was based on consensus among all three authors.

Data extraction

ISSN: 0975-3583,0976-2833 VOL 16, ISSUE 11, 2025

For all included studies, following descriptive study details were extracted by two independent reviewing authors (and) using pilot-tested customized data extraction forms: authors, study year, mean age of participants (cases and controls), sample size of cases and controls, type of salivary biomarker (index test used), method of salivary biomarker detection (ELISA and PCR), main study results like sensitivity, specificity, true positive, true negative, false positive, false negative and conclusion. Quantitative data of sensitivity and specificity were compiled from each study and using these quantitative data, values like true positive, true negative, false positive and false negatives were calculated manually for the studies using the below formula's where the data was not provided by authors. The corresponding authors were contacted via email where further information was needed.

a) False positive = (1-specificity) x (1- diseased cases/ total sample)

b) True negative = specificity x (1- diseased cases/total sample)

c) True positive = sensitivity x diseased cases/ total sample

d) False negative = (1- sensitivity) x diseased cases/total sample

Assessment of methodological quality

The methodological quality or the risk of bias was evaluated using Quality Assessment for Diagnostic Accuracy Studies -2 (QUADAS-2) tool (Whiting et al., 2011). The QUADAS-2 is a revised tool developed to assess quality of diagnostic studies through its four domains: patient selection, index test, reference standard, flow and timing of participants. Each domain had signalling questions with options of "Yes", "No" or "Unclear". The overall risk of bias was assessed as high: if answered 'No' to any question, Low: if answered 'Yes' to all questions and Unclear: if answered 'Unclear' to all questions or accompanied by any 'Yes'. Risk of bias summary and applicability concern was graphically plotted using Review Manager (RevMan) software version 5.3.

Statistical analysis and data synthesis

Raw data was used to calculate sensitivity and specificity for each biomarker with their estimation method. For overall accuracy, we calculated pooled sensitivity, pooled specificity with 95% confidence interval, area under summary receiver operating characteristic. (Interpretation of AUC values were as follows: values above 80% were considered as excellent, between 70% and 80% as good, between 60% and 69% as fair and below 60% as poor outcomes

ISSN: 0975-3583,0976-2833 VOL 16, ISSUE 11, 2025

for a diagnostic test (Jones et al., 2005). To assess the impact of heterogeneity, Higgins I^2 test was used. This test represents the proportion of variability due to heterogeneity rather than due to sampling error (Lijmer et al., 2002). According to I^2 test statistic the heterogeneity could be low (I^2 <50%) or high (I^2 >50%). Subgroup analysis was also carried out. Results were presented graphically as a coupled forest plot for each salivary biomarker with their estimation method using Meta-Disc 1.4 software.

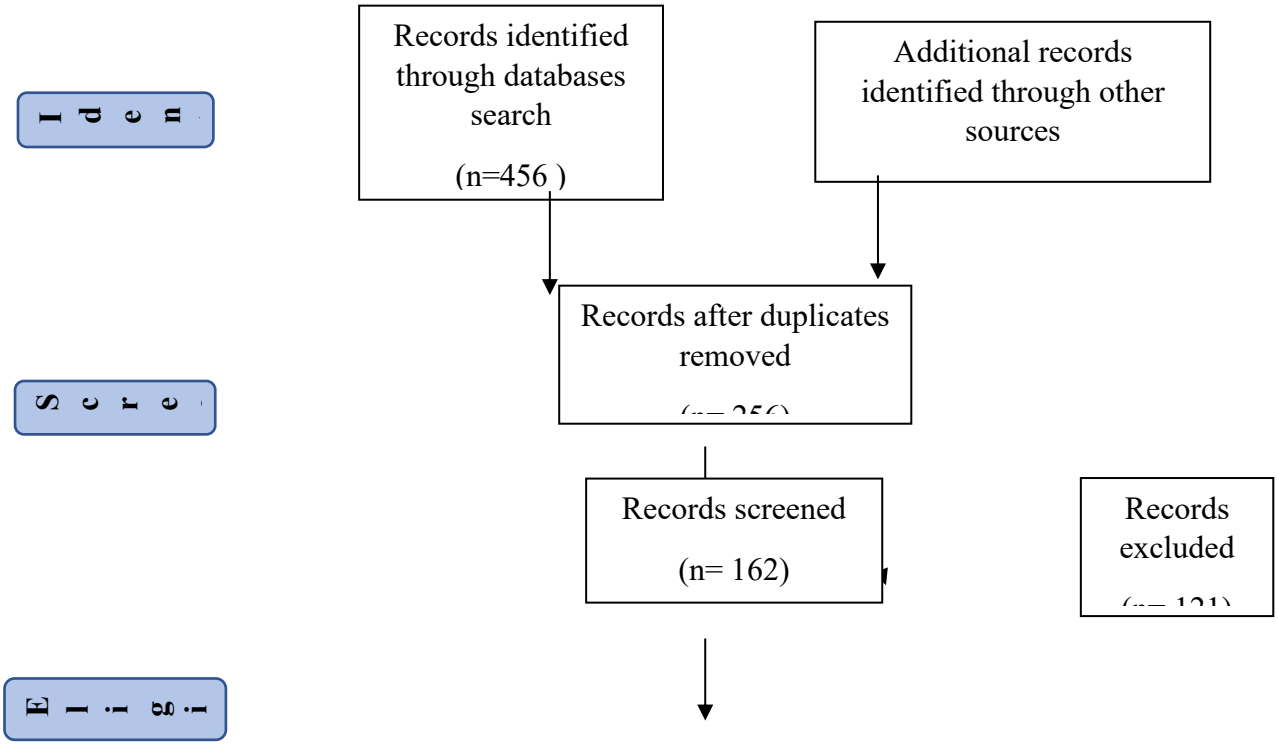
Additional analysis

Additional analysis was performed with positive likelihood ratio (PLR) and negative likelihood ratio (NLR) using DerSimonian-Laird's estimator considering a random effect model. Positive likelihood ratio (PLR) in range of 2-5, 5-10 and >10 represents small, moderate and large increase in probability of disease when test is positive while Negative likelihood ratio (NLR) in range of 0.2-0.5, 0.2-0.1 and <0.1 represents small, moderate and large decrease in probability of disease when test is negative (Grimes et al., 2005).

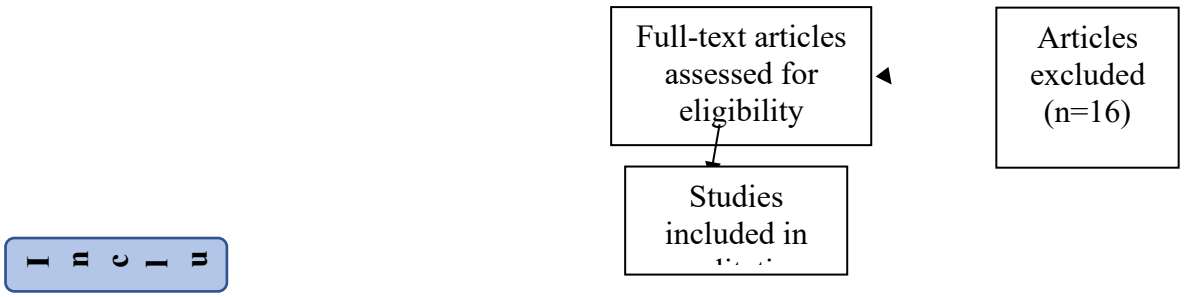
Results

Study Selection

A flowchart of identification, inclusion and exclusion of studies is shown in **Figure 1**. After duplicates removal, a reference list of all included studies was screened. Of which 121 studies were excluded. After this full text articles were assessed for eligibility and articles that did not meet inclusion criteria were excluded. Only twenty-one studies fulfilled eligibility criteria and were included in qualitative synthesis and eighteen studies for meta-analysis.



ISSN: 0975-3583,0976-2833 VOL 16, ISSUE 11, 2025



Study Characteristics

A summary of descriptive characteristics of all included 21 studies is provided in **(Supplemental Table 1)**. Data was evaluated from aggregate of 1531 patients with mean age of 63.15 years. The articles were published between 2000 to 2023 and conducted in eleven countries: seven studies (Yang li et al., 2004; Hu et al., 2008; Arellano et al., 2008; John et al., 2004; Heravi et al., 2014; Spielman et al., 2010; Zimmerman et al., 2007) in USA, four studies (Rajkumar et al., 2014; Singh et al., 2020; Smriti et al., 2019; Deepthi et al., 2019) in India, two studies (Lee et al., 2017; Yu et al., 2016) in Taiwan, one study (Hovarth et al., 2017) in Hungary, one study (Peisker et al., 2017) in Germany, one study (Ghallab et al., 2014)in Egypt, one study (Shiptzer et al., 2009), one study (Saleem et al., 2021) in Pakistan, one study (Mehterov et al., 2021) in Bulgaria and one study (Zahran et al., 2015) in Saudi Arabia. Three studies (Yang li et al., 2004; Hovarth et al., 2017; Hu et al., 2008) evaluated S100P biomarker, two studies (Yang li et al., 2004; Hovarth et al., 2017) evaluated DUSP-1 biomarker, four studies (Arellano et al., 2008; Rajkumar et al., 2014; John et al., 2004; Lee et al., 2017) evaluated IL-8 biomarker, two studies (Lee et al., 2017; Singh et al., 2020) evaluated IL-1B biomarker, six studies (Peisker et al., 2017; Ghallab et al., 2014; Yu et al., 2016; Shiptzer et al., 2009; Saleem et al., 2021; Smriti et al., 2019) evaluated MMP biomarker, three studies (Heravi et al., 2014; Zahran et al., 2015; Spielman et al., 2010) evaluated miRNA biomarker, two studies (Young et al., 2020; Zimmerman et al., 2007) evaluated mRNA biomarker and two studies (Deepthi et al., 2019; Lee et al., 2017) evaluated TNF-a biomarker. Salivary biomarkers

ISSN: 0975-3583,0976-2833 VOL 16, ISSUE 11, 2025

were estimated by ELISA in 11 studies (Hu et al., 2008; Arellano et al., 2008; Rajkumar et al., 2014; Lee et al., 2017; Singh et al., 2020; Peisker et al., 2017; Ghallab et al., 2014; Shiptzer et al., 2009; Saleem et al., 2021; Smriti et al., 2019; Deepthi et al., 2019) and by PCR in 10 studies (Yang li et al., 2004; Hovarth et al., 2017; John et al., 2004; Heravi et al., 2014; Zahran et al., 2015; Spielman et al., 2010; Young et al., 2020, Zimmerman et al., 2007). All studies utilized the same reference standard: tissue biopsy and histopathological investigations.

Assessment of methodological quality of included studies

Although none of the included studies were classified as low risk of bias for all four domains. Patient selection was considered as high risk of bias majorly in seven studies (Ghallab et al., 2014; Hu et al., 2008; John et al., 2004; Peiskar et al., 2017; Saleem et al., 2021; Spielman et al., 2011; Zimmerman et al., 2007), which was mainly due to method of patient enrollment, nature of study design and implementing inappropriate exclusion.

The index test was considered to be at high risk of bias in (li et al., 2004). High risk of bias was reported with respect to index test domain due to insufficient details reported as to whether results of index test were interpreted without prior knowledge of reference standard results, lack of pre-specification of a test-positive threshold and statement of conflict of interest.

The reference standard was at high risk for (Li et al., 2004; Heravi et al., 2014; Rajkumar et al., 2014; Zahran et al., 2015) and flow and timing domain was considered at low risk in all studies.

The risk of bias and applicability concern summary and graph is depicted in **Figure 2.**

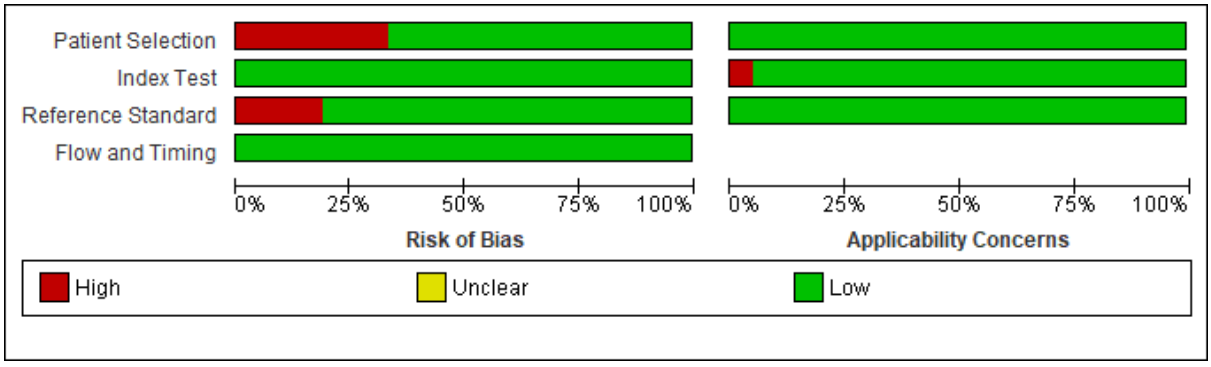


Figure 2. Risk of bias and applicability concerns graph: review authors' judgements about each domain presented as percentages across included studies

ISSN: 0975-3583,0976-2833 VOL 16, ISSUE 11, 2025

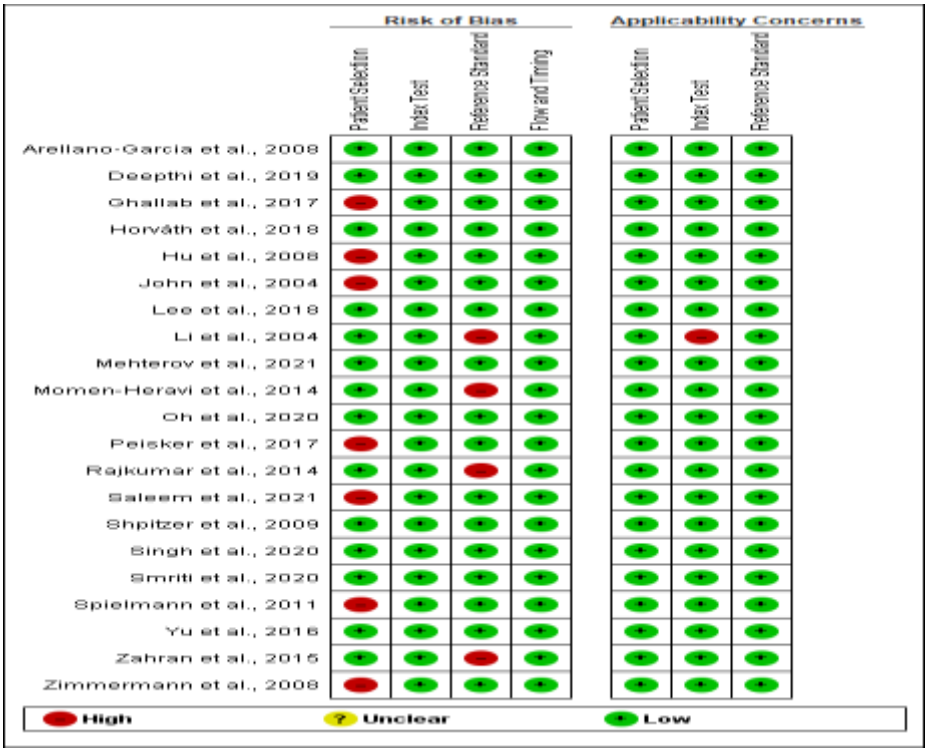


Figure 3. Risk of bias and applicability concerns summary: review authors' judgements about each domain for each included study

Synthesis of Results/ meta-analysis

The meta-analysis was conducted to assess the diagnostic ability of salivary biomarkers in terms of pooled sensitivity, pooled specificity, likelihood ratio and overall accuracy through area under the curve (AUC) **as shown in figures below.**

A) S100P by PCR

S100P salivary biomarker estimation by PCR: a total of 282 patients from three studies (Yang li et al., 2004; Howarth et al., 2017; Hu et al., 2008) investigated accuracy of IL-1B estimated by ELISA. The pooled sensitivity was 0.69 (CI 0.01- 1.00) and pooled specificity was 0.36 (CI 0.00- 0.98) as shown in **Figure 4**.

ISSN: 0975-3583,0976-2833 VOL 16, ISSUE 11, 2025

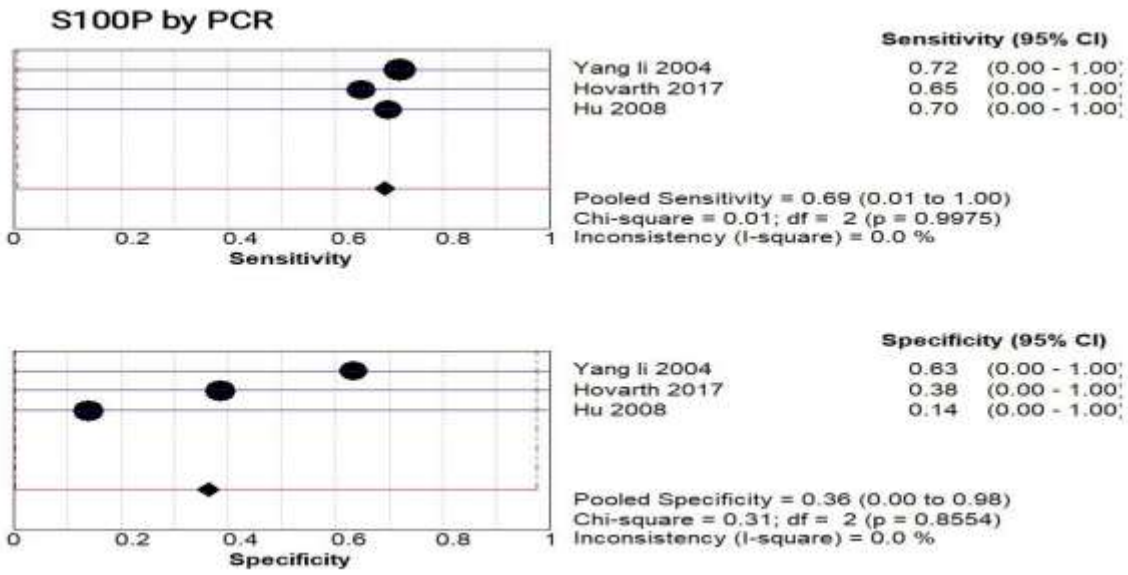
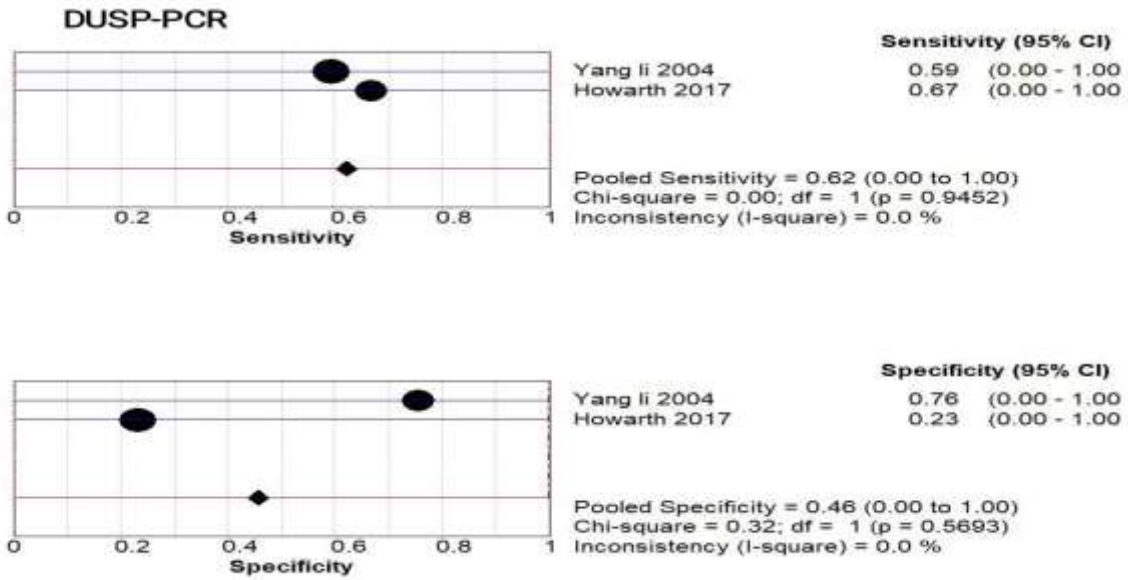


Figure 4. Pooled sensitivity and specificity for S100P salivary biomarker

B) DUSP PCR

DUSP salivary biomarker estimation by PCR: a total of 154 patients from three studies (Yang Li et al., 2004; Howarth et al., 2017)) investigated accuracy of DUSP estimated by PCR. The pooled sensitivity was 0.62 (CI 0.00- 1.00) and pooled specificity was 0.46 (CI 0.00- 1.00) as shown in **Figure 5**.



ISSN: 0975-3583,0976-2833 VOL 16, ISSUE 11, 2025

Figure 5. Pooled sensitivity and specificity for DUSP salivary biomarker by PCR

C) IL-8 ELISA

IL-8 salivary biomarker estimation by ELISA: a total of 305 patients from three studies (Arellano et al., 2008; Rajkumar et al., 2014; Lee et al., 2017) investigated accuracy of IL-8 estimated by ELISA. The pooled sensitivity was 0.79 (CI 0.05- 1.00) and pooled specificity was 0.78 (CI 0.01- 1.00) as shown in **Figure 6**.

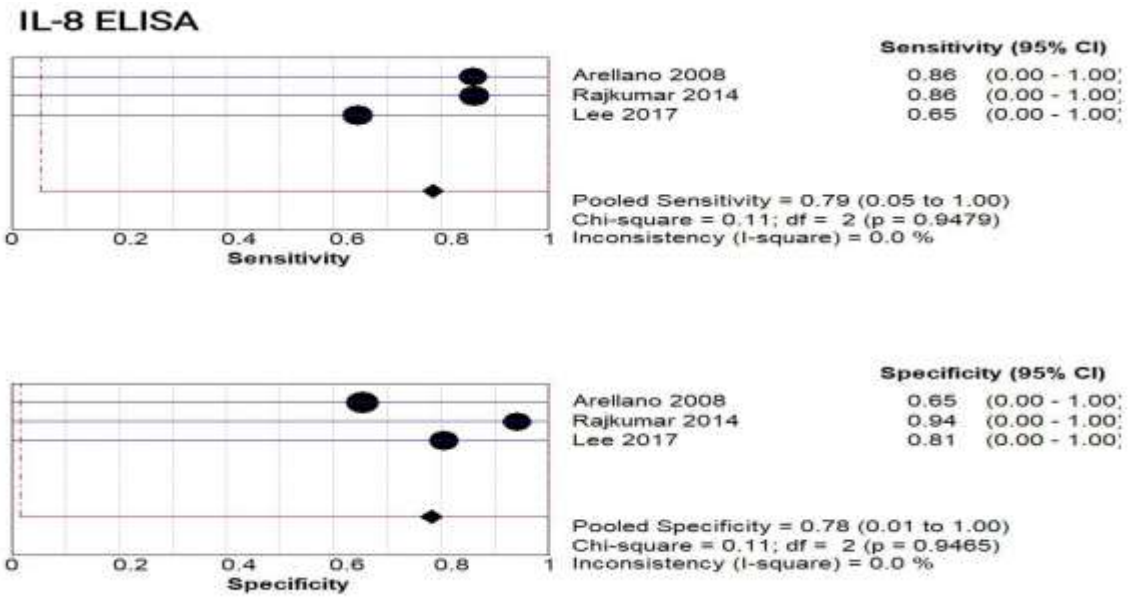


Figure 6. Pooled sensitivity and specificity for IL-8 salivary biomarker by ELISA

D) IL-1B ELISA

IL-1B salivary biomarker estimation by ELISA: a total of 165 patients from two studies (Lee et al., 2017; Singh et al., 2020) investigated accuracy of IL-1B estimated by ELISA. The pooled sensitivity was 0.66 (CI 0.01- 1.00) and pooled specificity was 0.69 (CI 0.00- 1.00) as shown in **Figure 7**.

ISSN: 0975-3583,0976-2833 VOL 16, ISSUE 11, 2025

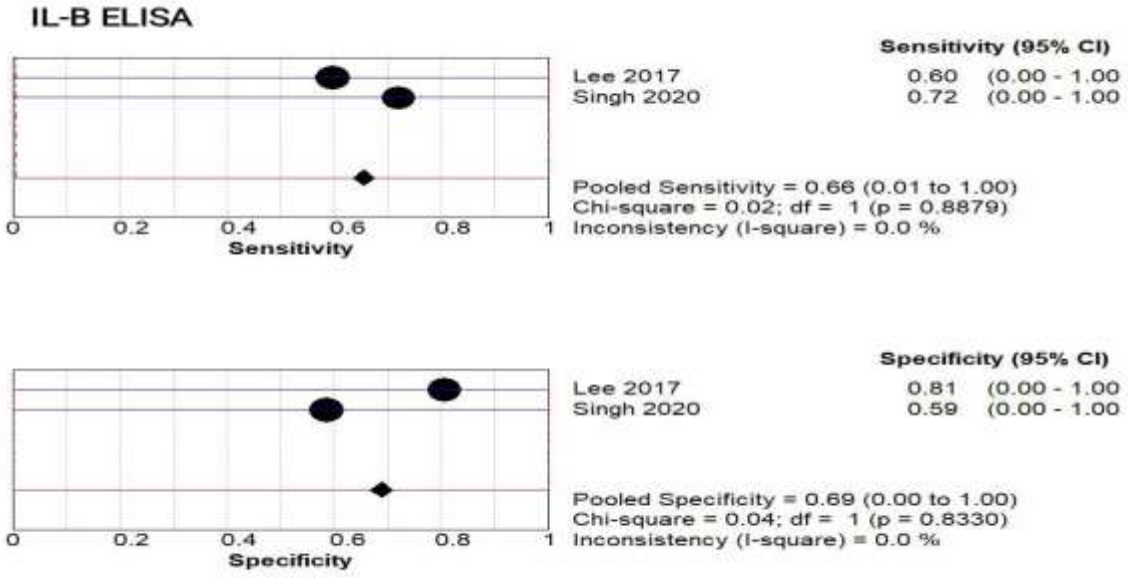
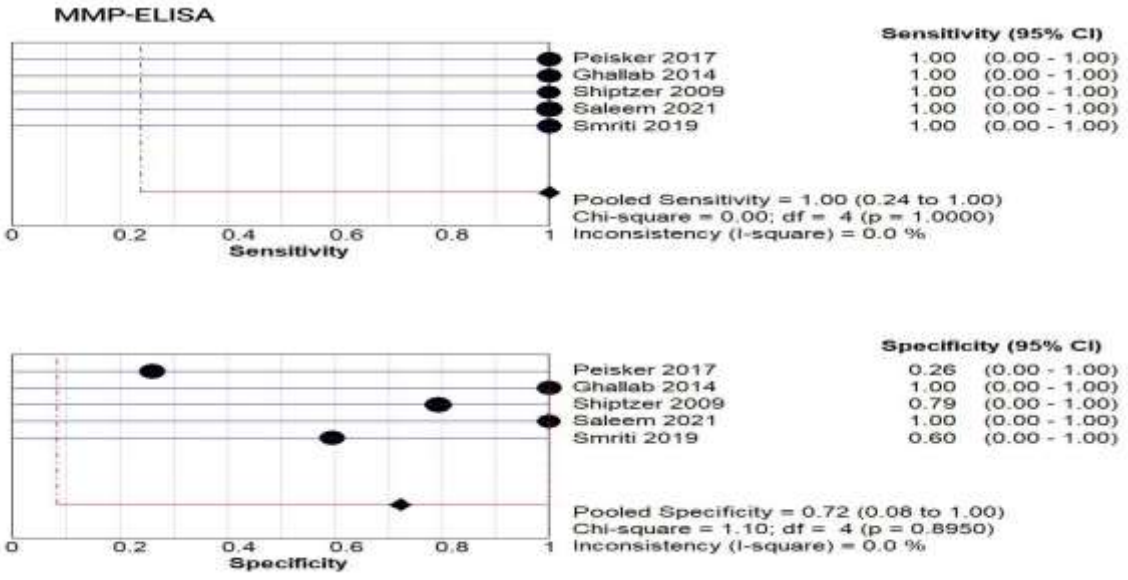


Figure 7. Pooled sensitivity and specificity for IL-1B salivary biomarker by ELISA

E) MMP- ELISA

MMP salivary biomarker estimation by ELISA: a total of 196 patients from five studies (Peisker et al., 2017; Ghallab et al., 2014; Shiptzer et al., 2009; Saleem et al., 2021, Smriti et al., 2019) investigated accuracy of MMP estimated by ELISA. The pooled sensitivity was 1.00 (CI 0.24- 1.00) and pooled specificity was 0.72 (CI 0.08- 1.00) as shown in **Figure 8**.



ISSN: 0975-3583,0976-2833 VOL 16, ISSUE 11, 2025

Figure 8. Pooled sensitivity and specificity for MMP salivary biomarker by ELISA

F) mRNA -PCR

mRNA salivary biomarker estimation by PCR: a total of 131 patients from two studies (Young et al., 2020; Zimmerman et al., 2007) investigated accuracy of mRNA estimated by PCR. The pooled sensitivity was 0.92 (CI 0.02- 1.00) and pooled specificity was 0.88 (CI 0.01- 1.00) as shown in **Figure 9**.

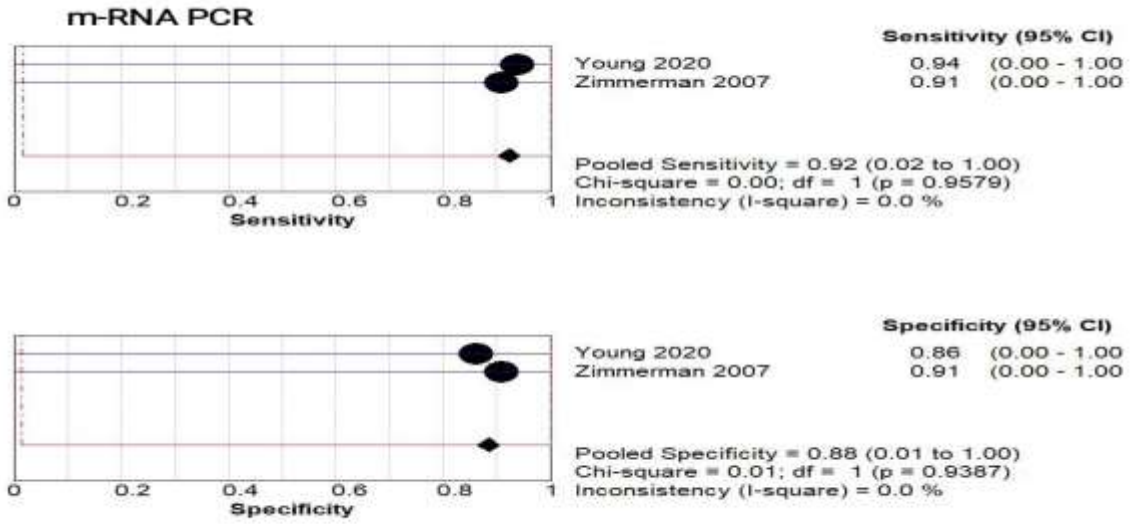


Figure 9. Pooled sensitivity and specificity for mRNA salivary biomarker by PCR

G) TNF-a – ELISA

TNF-a salivary biomarker estimation by ELISA: a total of 125 patients from two studies (Deepthi et al., 2019; Lee et al., 2017) investigated accuracy of TNF-a estimated by ELISA. The pooled sensitivity was 0.66 (CI 0.00- 1.00) and pooled specificity was 0.98 (CI 0.01- 1.00) as shown in **Figure 10**.

ISSN: 0975-3583,0976-2833 VOL 16, ISSUE 11, 2025

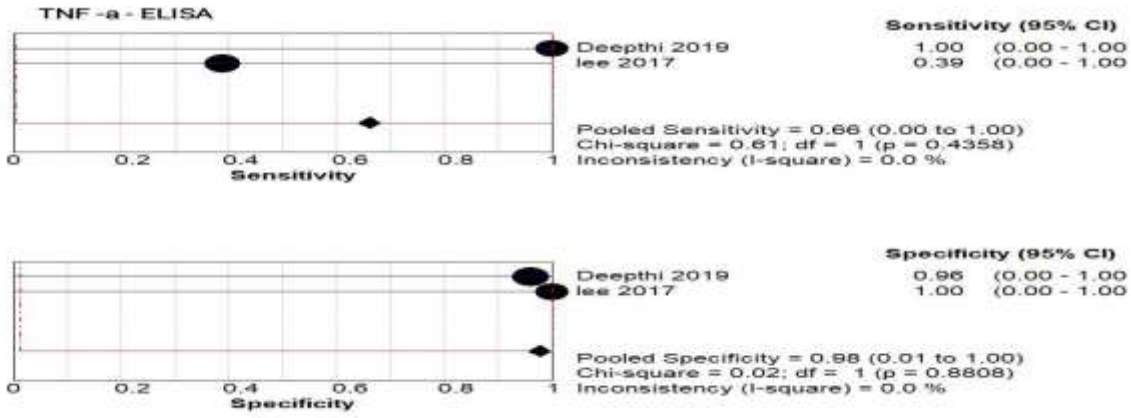


Figure 10. Pooled sensitivity and specificity for TNF-a salivary biomarker by ELISA

H) <u>miRNA – PCR</u>

miRNA salivary biomarker estimation by PCR: a total of 122 patients from three studies (Heravi et al., 2014; Zahran et al., 2015; Spielman et al., 2010) investigated accuracy of miRNA estimated by PCR. The pooled sensitivity was 0.87 (CI 0.05- 1.00) and pooled specificity was 0.89 (CI 0.05- 1.00) as shown in **Figure 11**.

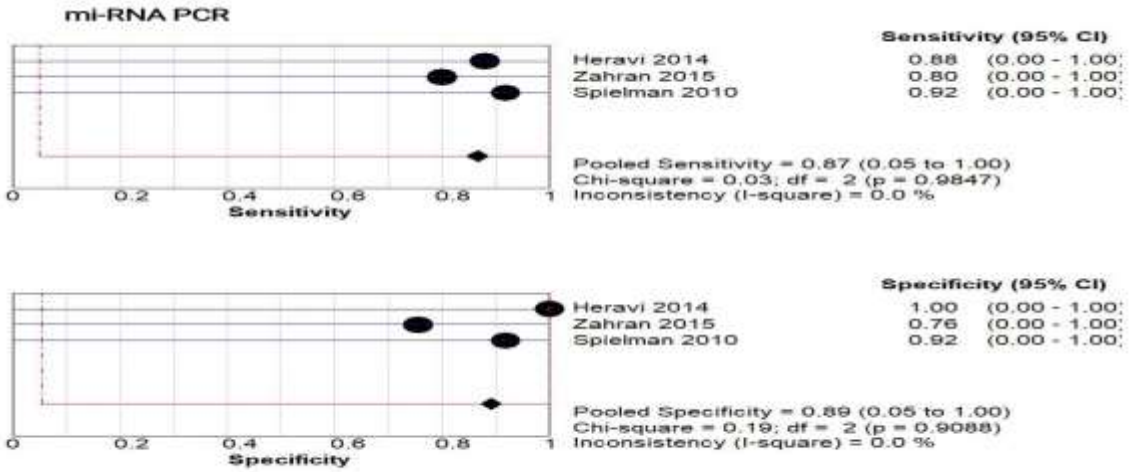
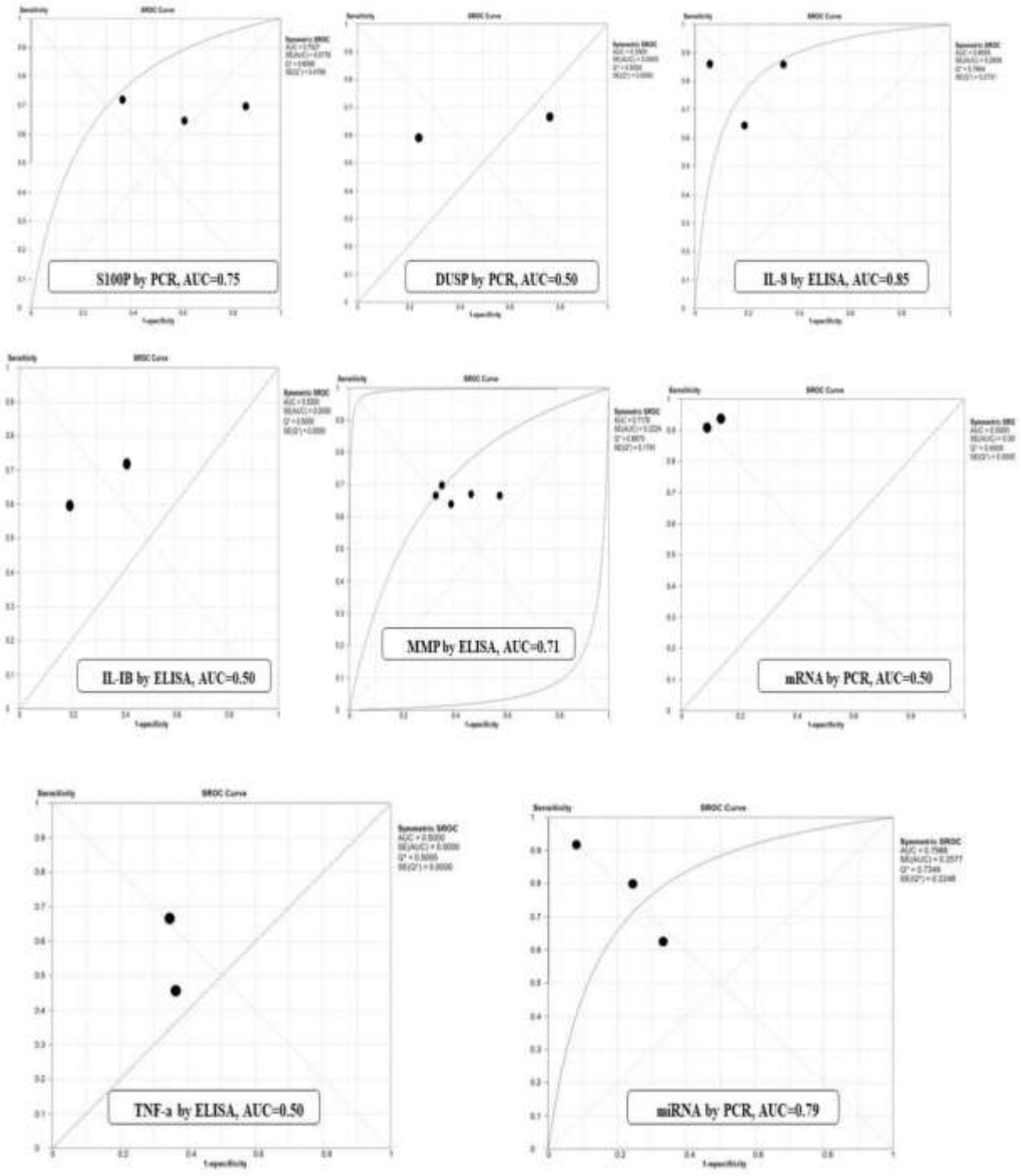


Figure 11. Pooled sensitivity and specificity for mi-RNA salivary biomarker by PCR

The sensitivities and specificities for studies ranged from 62% to 100% and from 36% to 98%. The area under the curve (AUC) with summary receiver operating characteristics (SROC) curve was plotted for all the biomarkers with their estimation method as shown in **Figure 12.**

ISSN: 0975-3583,0976-2833 VOL 16, ISSUE 11, 2025



The highest AUC was seen for IL-8 by ELISA (0.85) and miRNA by PCR (0.79) which was considered overall good and the lowest AUC was seen for IL-1B and TNF-a by ELISA (0.56) and DUSP and mRNA (0.50) estimated by PCR which was considered poor.

Additional analysis

ISSN: 0975-3583,0976-2833 VOL 16, ISSUE 11, 2025

Likelihood ratio was estimated which signifies the ability of the index test to predict the test results (positive / negative) when the disease condition in actual is present or absent. As shown in **figure 13 - 20,** pooled positive likelihood ratio (PLR) was of range 1.03 – 7.79 and the highest +LR was seen for mRNA by PCR (7.79) while negative likelihood ratio (NLR) was of range 0.09 – 0.80 and the highest -LR was seen for TNF-a by ELISA. Pooled PLR suggested that test result is associated with absence of disease when the disease is present while pooled NLR suggested that the test result is associated with presence of disease when the disease is absent.

A) <u>S100P PCR</u>

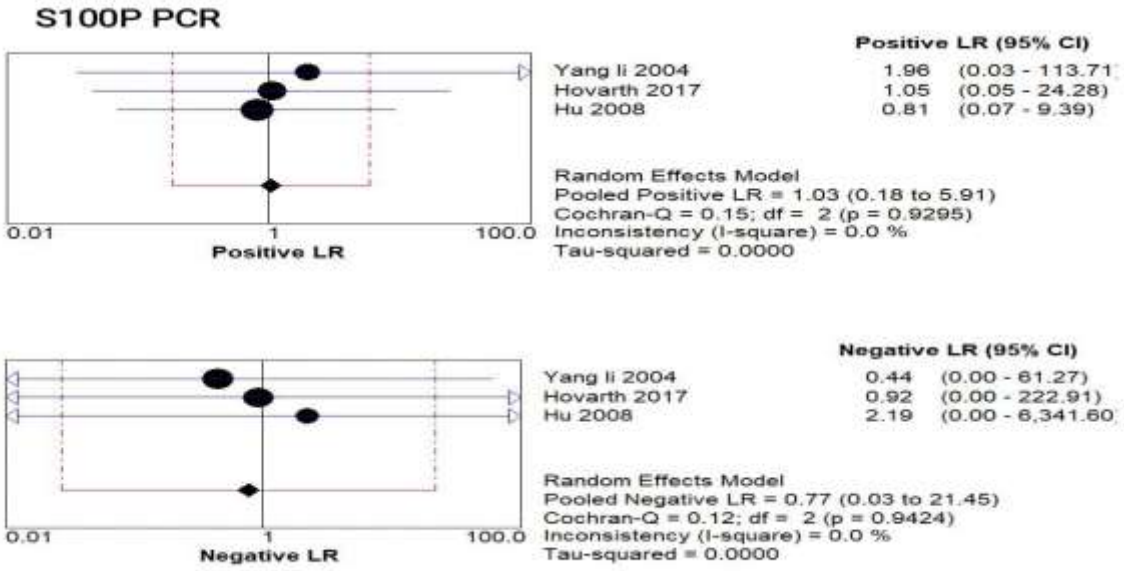


Figure 13: Pooled PLR and NLR of S100P estimated by PCR

B) <u>DUSP PCR</u>

ISSN: 0975-3583,0976-2833 VOL 16, ISSUE 11, 2025

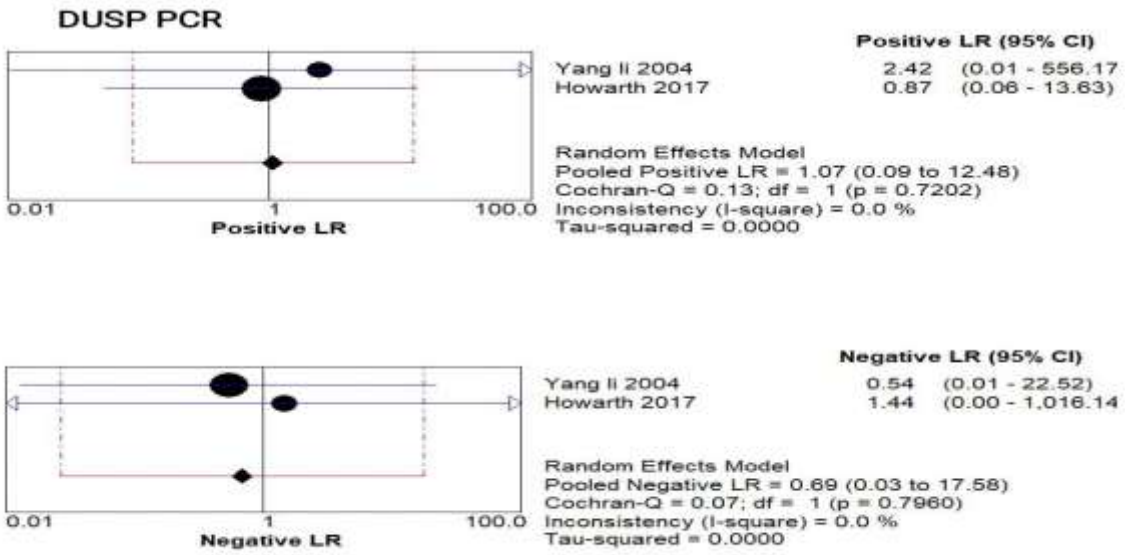


Figure 14: Pooled PLR and NLR of DUSP estimated by PCR

C) IL-8 ELISA

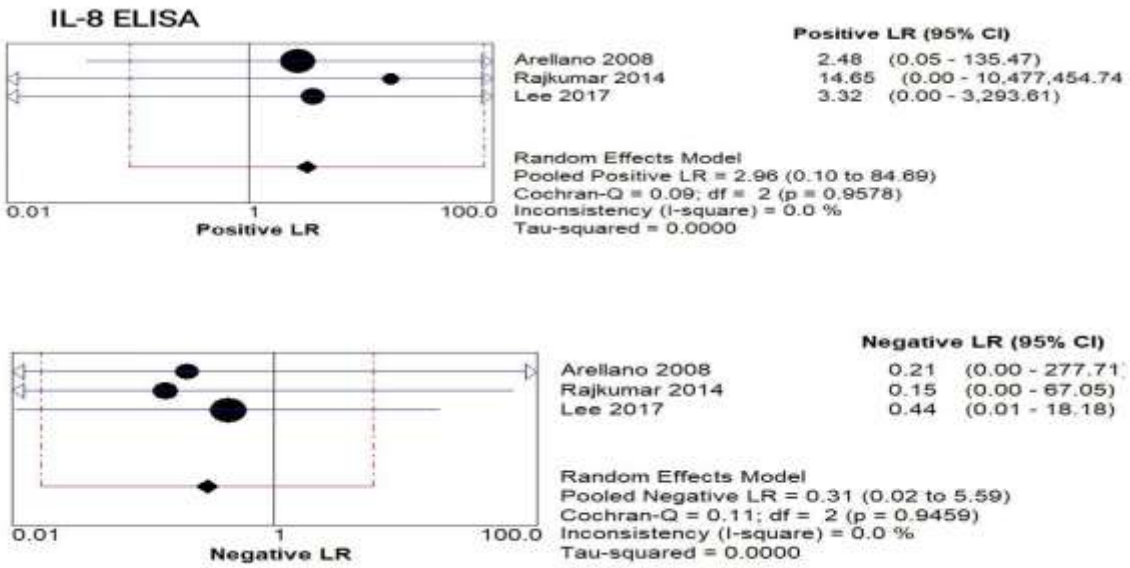


Figure 15: Pooled PLR and NLR of IL-8 estimated by ELISA

D) IL-1B ELISA

ISSN: 0975-3583,0976-2833 VOL 16, ISSUE 11, 2025

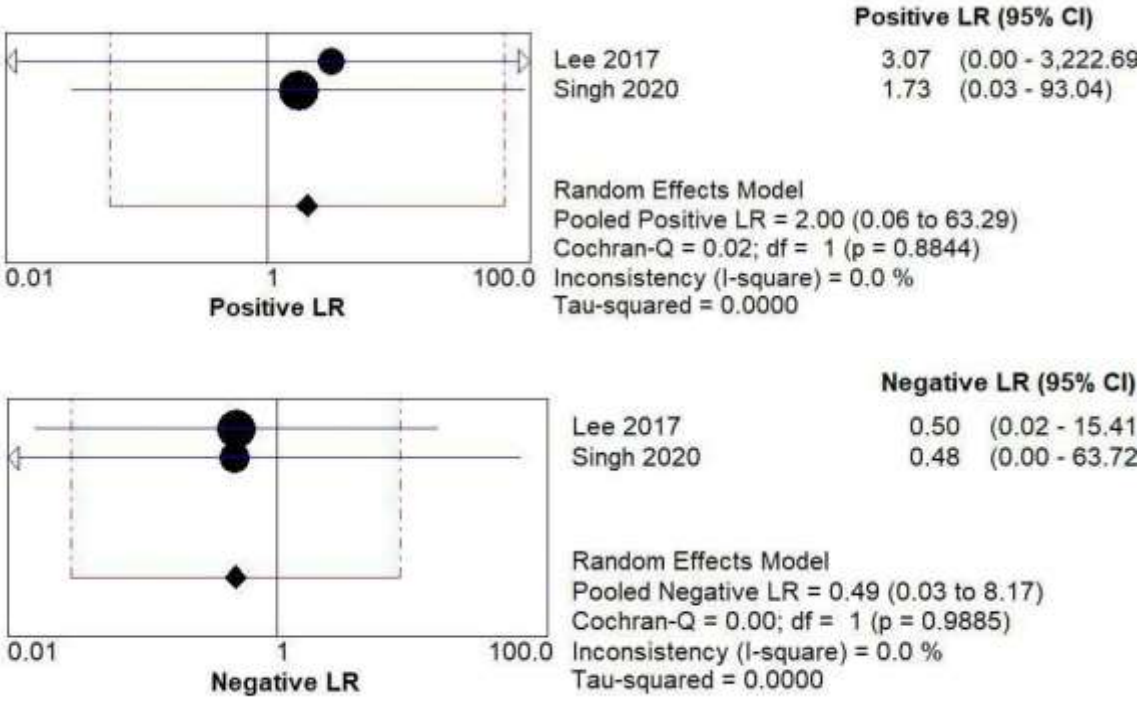


Figure 16: Pooled PLR and NLR of IL-1B estimated by ELISA

E) MMP- ELISA

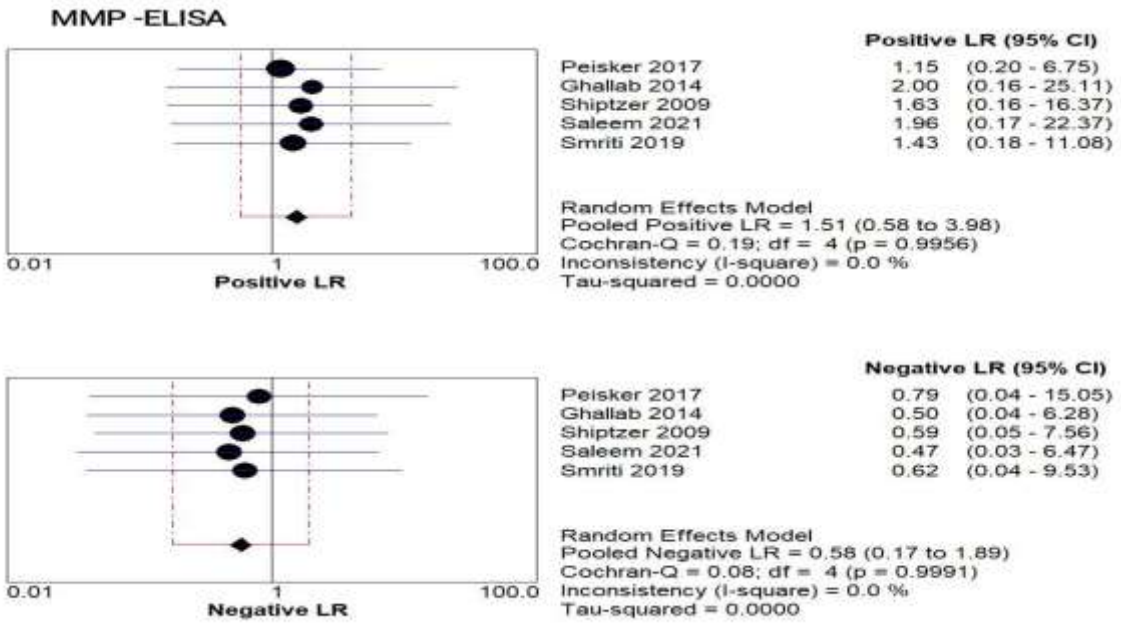


Figure 17: Pooled PLR and NLR of MMP estimated by ELISA

ISSN: 0975-3583,0976-2833 VOL 16, ISSUE 11, 2025

F) mRNA -PCR

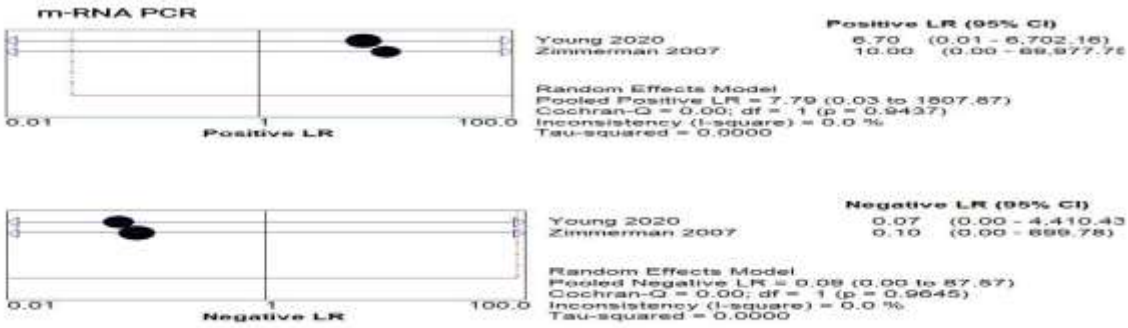


Figure 18: Pooled PLR and NLR of mRNA estimated by PCR

G) TNF-a – ELISA

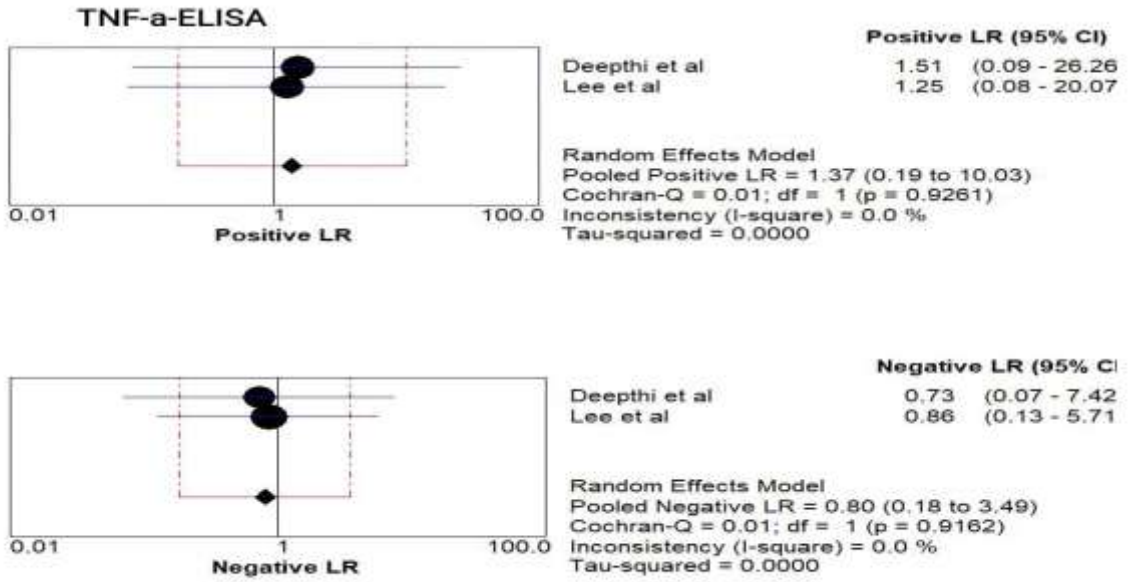


Figure 19: Pooled PLR and NLR of TNF-a estimated by ELISA

ISSN: 0975-3583,0976-2833 VOL 16, ISSUE 11, 2025

H) miRNA – PCR

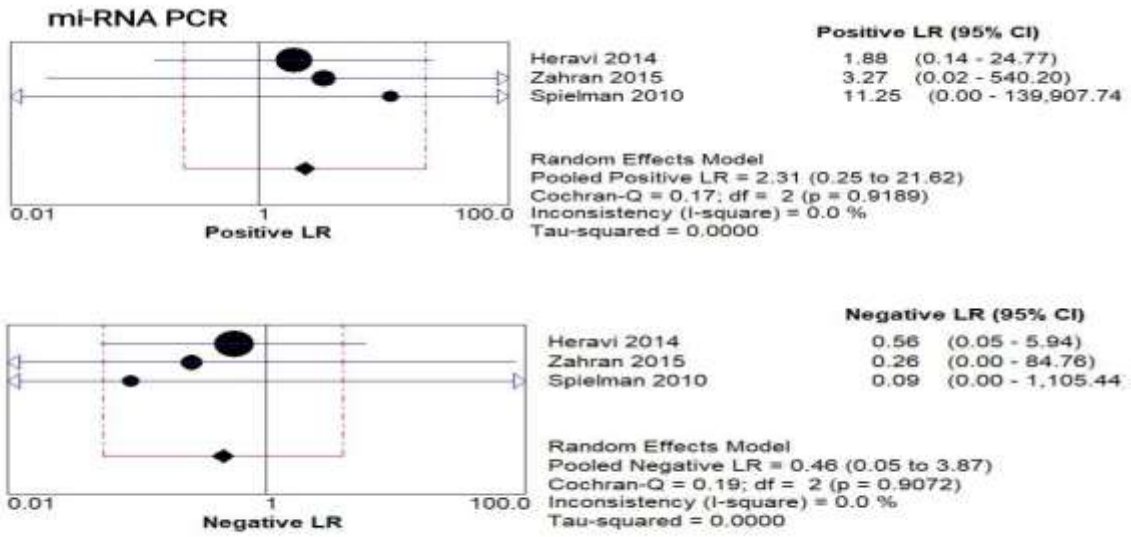


Figure 20: Pooled PLR and NLR of miRNA estimated by PCR

Discussion

Majority of oral cancers are OSCC, which when found early can have 80–90% survival rate. WHO has reported oral cancer as having the highest mortality ratios amongst other malignancies with a death rate at five years from diagnosis at 45% (Lee et al., 2017). The high morbidity and mortality rate can therefore be due to the delayed diagnosis of the disease (Arellano et al., 2008). The commonly used clinical techniques such as biopsy, tissue processing and staining and cytology can be used only on small groups of patients who come to seek advice and treatment. Such methodology has certain limitations (Ghallab et al., 2014). The aim of using salivary biomarkers for OSCC detection is that they can be useful for large scale screening purposes so that lesions can be detected easily without being expensive or invasive and can be used by non-trained individuals (Zimmerman et al., 2007). For this saliva screening methods must have sufficient sensitivity and specificity. In the recent past a large array of accessible salivary biomarkers have been reported for OSCC detection. These markers now need to be validated to make them clinically applicable. This would further facilitate the development of point of care devices in order to provide easy to use diagnostic technology using salivary biomarkers (Deepthi et al., 2019).

ISSN: 0975-3583,0976-2833 VOL 16, ISSUE 11, 2025

(Shaw et al., 2022) conducted a systematic review to summarize and compare the existing evidence on diagnostic accuracy of salivary biomarkers with their estimation method in detecting early oral squamous cell carcinoma. Electronic databases were searched from 2000 to 2020 to identifying the screening potential of eight salivary biomarkers: mRNA, miRNA, DUSP100, s100P, IL-8, IL-1B, TNF-a and MMP-9. Eighteen studies were included for review and thirteen studies for meta-analysis with sensitivity and specificity for mRNA being 91% and 90% with 0.96 AUC, miRNA had 91% and 91% with 0.95 AUC for PCR. IL-1B had 46% and 60% with 0.61 AUC, S100p had 45% and 90% with 0.57 AUC for ELISA. IL-8 had 54% and 74% for ELISA and 89% and 90% for PCR with 0.79 AUC and DUSP1 had 32% and 87% for ELISA and 76% and 83% for PCR with 0.83 AUC respectively.

The aim of this systematic review and meta-analysis is to provide an update on existing evidence on various salivary biomarkers and to compare and evaluate their diagnostic ability in early oral squamous cell carcinoma in adults. To the best of our knowledge, this is the first systematic review and meta-analysis which provides a comprehensive quantitative analysis of salivary biomarkers in early oral squamous cell carcinoma diagnosis. Most of the salivary biomarkers overall had good diagnostic accuracy. Databases were searched till November 2023 which yielded 21 studies. Quality assessment through QUADAS-2 tool showed presence of low to moderate level of bias. Meta-analysis showed sensitivity and specificity in range of 62% to 100% and 36% to 98% with overall AUC value ranging from 0.50-0.85.

Seven studies (Ghallab et al., 2014; Hu et al., 2008; John et al., 2004; Peiskar et al., 2017; Saleem et al., 2021; Spielman et al., 2011; Zimmerman et al., 2007) were at high risk of selection bias arising from use of a ‘case-control’ study design. In addition, patient sampling and/or recruitment into studies were insufficiently reported. All studies used biopsy as reference standard and salivary biomarkers as index test. However, insufficient detail and lack of clarity in reporting studies made it difficult to assess risk of bias. Therefore, use of STARD (Cohen et al., 2016) checklist in reporting primary studies could have facilitated the quality appraisal. Reporting guidelines for primary diagnostic studies should be followed strictly and studies should address all potential source of bias and applicability concern as indicated in QUADAS-2 tool (Whiting et al., 2011).

Saliva as a diagnostic fluid has shown to express altered levels of biomarkers not only in OSCC but also in various oral and other systemic disease (Singh et al., 2020; Saleem et al., 2021).

ISSN: 0975-3583,0976-2833 VOL 16, ISSUE 11, 2025

Salivary biomarkers offer to be a promising tool for oral cancer screening and diagnosis with high sensitivity and specificity. A number of molecular screening tests have been established to detect cancer in early stages (Yang Li et al., 2014).

This study provides information on the accuracy and applicability of salivary biomarkers in improving cancer detection through dynamic and non-invasive method. Overall sensitivity and specificity were in range of 62% to 100% and from 36% to 98%. Highest sensitivity was observed for MMP with 100% by ELSIA while lowest specificity was observed for S100P with 36% estimated by ELISA. Also, the pooled positive likelihood ratio (PLR) of 7.79 was highest for mRNA estimated by PCR, indicating that patients with oral cancer have a 7.79 folds higher chance of having a positive test result compared to cancer free patients. By contrary the pooled negative likelihood ratio (NLR) was 0.80, indicating the probability of a patient having cancer is 10% if the test shows negative result. The individual sensitivity and specificity with an overall holistic AUC value of 0.85 for IL-8 by ELISA and 0.79 for miRNA biomarkers makes them as more accurate overall. Analysis of other biomarkers revealed comparatively moderate to low sensitivity and specificity value compared to MMP and TNF-a biomarkers. The higher AUC value for these biomarkers suggests a more easily interpretable and meaningful measure of performance in correctly diagnosing the target condition.

This study is limited by overall quality of included studies. Further standardised diagnostic test accuracy studies that minimises potential sources of bias through rigorous design, conduct and reporting are needed. Future research must focus on the accuracy of current potential principal salivary biomarkers in detection of OSCC with clear and robust methodology.

The adherence to the PRISMA guidelines, the thorough unrestricted literature search, utilization of reliable methodology with regard to the qualitative synthesis of data, the quality assessment of evidence with the QUADAS-2 tool included studies strengthens this systematic review. The quality assessment of all the included studies had high overall quality, specifying lack of potential and inevitable sources of bias with limited variability and reporting deficiencies.

A systematic review is a transparent and repeatable procedure for identifying, selecting and critically assessing published or unpublished data to address a well-defined research question. Meta-analyses, a statistical analysis that incorporates numerical data from related studies, are frequently paired with systematic reviews. The best evidence is generally regarded as

ISSN: 0975-3583,0976-2833 VOL 16, ISSUE 11, 2025

systematic reviews and meta-analyses. However, the calibre of the included studies has an impact on how strong the evidence is. In the present review, sufficient studies with a brief observation period and a known risk of bias were included. As a result, the presently available evidence is sufficient to make therapeutic recommendations in response to the current systematic review's focus question.

Sr.No	AUTHOR	INTERVENTION	NO. OF PARTICIPANTS	TYPE OF STUDY	RESULT
1)	2004, Yang Li[9] - California, The United States of America.	Salivary biomarker - S100P	64 OSCC: 32 Healthy controls: 32		S100P exhibits at least a 3.5 fold elevation in saliva of OSCC patients (*P=<0.01)*.
2)	2008, Shen Hu[11] - California, The United States of America.	Salivary biomarker - S100P	128 OSCC: 64 Healthy controls: 64		Upregulated levels of S100P in saliva samples of OSCC patients was observed.
3)	2017, Jozsef Horvath[12] - Debrecen, Hungary.	Salivary biomarker - S100P	90		Expression pattern of S100P was similar between OSCC and age matched control patients which was attributed to high intra group variability characteristics. But, Comparative analysis of samples derived from young control patients vs OSCC showed S100P was present in significantly higher quantities in saliva samples of OSCC patients.
4)	2004, Yang Li[9] - California, The United States of America.	Salivary biomarker - DUSP1	64 OSCC: 32 Healthy controls: 32		DUSP1 exhibits at least a 3.5 fold elevation in saliva of OSCC patients (*P<0.01)*.
5)	2017, Jozsef Horvath[12] - Debrecen, Hungary.	Salivary biomarker - DUSP1	90		Expression pattern of DUSP1 was similar between OSCC and age matched control patients which was attributed to high intra-group variability characteristics. But, Comparative analysis of samples derived from young control patients vs OSCC showed DUSP1 was present in significantly higher quantities in saliva samples of OSCC patients.
6)	2004, Maie A. R. St. John[13] - The United States of America.	Salivary biomarker - IL-8	64 OSCC: 32 Healthy controls: 32		IL-8 was detected at higher concentration in saliva in patients with OSCC (*P<0.01)*. These findings indicate that IL-8 in saliva is a promising biomarker for

ISSN: 0975-3583,0976-2833 VOL 16, ISSUE 11, 2025

					OSCC. A saliva based test could be a cost effective adjunctive tool in diagnosis and follow-up of patients with OSCC.
7)	2008, ME Arellano Garcia[14] - California, The United States of America.	Salivary biomarker - IL-8	40 OSCC: 20 Healthy controls: 20		Results obtained for IL-8 using singleplex , multiplex assays and ELISA showed IL-8 was expressed at significantly higher levels in OSCC subjects than in matched healthy control subjects.
8)	2014, K. Rajkumar[15] - Chennai, India.	Salivary biomarker - IL-8	200 OSCC: 100 Healthy controls: 100		Significant increase in levels of IL-8 was found in OSCC subjects *(P<0.0001)* compared to healthy controls. IL-8 has superior sensitivity in detecting OSCC. Also, increasing levels of IL-8 based on histopathological grading of OSCC was also observed.
9)	2017, L. T. Lee[16] - Taipei, Taiwan.	Salivary biomarker - IL-8	65 OSCC: 41 Healthy controls: 24		Levels of salivary IL-8 differed significantly between OSCC patients and healthy controls. *(P<0.0002)*
10)	2017, L. T. Lee[16] - Taipei, Taiwan.	Salivary biomarker - IL-1B	65 OSCC: 41 Healthy controls: 24		Levels of salivary IL-1B differed significantly between OSCC patients and healthy controls. *(P<0.0004)*
11)	2020, Prerana Singh[17] - Kanpur, India.	Salivary biomarker - IL-1B	100 OSCC: 58 Healthy controls: 42		Levels of salivary IL-1B were significantly elevated in OSCC subjects compared to healthy controls. *(P <0.05)*
12)	2009, T Shpitzer[18] - Haifa, Israel.	Salivary biomarker - MMP	19		Levels of MMP-9 were found to be increased significantly in saliva of OSCC patients by 39% as compared with controls. *(P<0.014)*
13)	2016, Andre Peisker[19] - Jena, Germany.	Salivary biomarker - MMP	60 OSCC: 30 Healthy controls: 30		MMP-9 value was significantly increased in OSCC patients than in the healthy controls by + 19.2% *(P=0.008).*
14)	2016, Jau-Song Yu [20] - Taiwan.	Salivary biomarker - MMP	227 OSCC: 131 Healthy controls: 96		The risk score significantly increased from healthy controls (0.16 ± 0.19) to OSCC group (0.75 ± 0.24) *(P<0.0001).*
15)	2016, Noha A. Ghallab[21] - Guiza, Egypt.	Salivary biomarker - MMP	30 OSCC: 15 Healthy controls: 15		The results of this investigation indicated that salivary levels of MMP-9 in OSCC patients were significantly elevated as compared to their levels in the healthy control group. *(P < 0.05)*

					Cut off/thresholds of 260.3 ng/ml was chosen for detecting early stage OSCC.
16)	2019, Smriti[22] - India.	Salivary biomarker - MMP	46 OSCC: 24 Healthy controls: 22		Subjects with OPMD and OSCC had significantly higher mean MMP-9 levels than control groups. *(P<0.001)*.
17)	2021, Saleem[23] - Pakistan.	Salivary biomarker - MMP	60 OSCC: 30 Healthy controls: 30		The study results demonstrate expression of salivary MMP-12 was higher in OSF and OSCC patients as compared to healthy controls. *(P<0.001)*. Therefore estimation of salivary MMP-12 serves as a valuable non-invasive early diagnostic tool in diagnosing OSF and OSCC.
18)	2017, L. T. Lee[16] Taipei, Taiwan.	Salivary biomarker - TNF-a	65 OSCC: 41 Healthy controls: 24		Levels of salivary TNF-a differed significantly between OSCC patients and healthy controls. *(P<0.0004)*
19)	2019, Deepthi[24] - Hyderabad, India.	Salivary biomarker - TNF-a	60 OSCC: 30 Healthy controls: 30		The result demonstrates higher levels of salivary TNF-a in individuals with OSCC compared to leukoplakia and healthy control subjects. The test showed a highly significant *(P= 0.00)* difference between the two groups *(P <0.01)*. ROC curve analysis along with diagnostic parameter calculation reveals salivary TNF-a to be a better medium for detecting OSCC.
20)	2008, Zimmermann[25] - California, The United States of America.	Salivary biomarker - mRNA	64 OSCC: 32 Healthy control: 32		7 transcripts were confirmed to be elevated in OSCC with statistical significance *(P<0.05)* Combinations of these biomarkers displayed a sensitivity and specificity of up to 91% in distinguishing patients from controls, which places them amongst the most discriminatory panels of cancer biomarkers from body fluids.
21)	2020, Su Young Oh[26] - Kyungpook, South Korea.	Salivary biomarker - mRNA	67 OSCC: 33 Healthy control: 34		Normalized mRNA levels of 6 genes were significantly lower in saliva of OSCC patients. Saliva samples were divided into two groups using a 60-year cut-off, with OSCC patients and controls evaluated together, the AUC of MAOB–NAB2 was more predictive of OSCC in the under-60 group (AUC = 0.91) than any

ISSN: 0975-3583,0976-2833 VOL 16, ISSUE 11, 2025

					other gene combination. These results are expected to aid the early diagnosis of OSCC, especially in patients under 60 years of age.
22)	2010, Nadine Spielmann[27] - California, The United States of America.	Salivary biomarker - miRNA	64 OSCC: 32 Healthy control: 32		7 transcripts were confirmed to be elevated in OSCC with statistical significance *(P<0.05)* Combinations of these biomarkers displayed a sensitivity and specificity of up to 91% (ROC=0.95) in distinguishing patients from controls placing them amongst the most discriminatory panels of cancer biomarkers from a body fluid.
23)	2014, F. Momen-Heravi[28] - The United States of America.	Salivary biomarker - miRNA	18 OSCC: 09 Healthy control: 09		13 miRNA's were significantly deregulated in saliva of OSCC patients compared to healthy controls. miRNA-27b levels were significantly higher in saliva of OSCC patients compared to healthy controls. miRNA-136 was underexpressed in both OSCC patients as well as the healthy control group. 1). At the optimal cutoff value of 14.73 for miRNA-27b in OSCC patients vs. HCs where the values of sensitivity and specificity were considered to be maximal for miRNA- 27b.
24)	2015, F Zahran[29] - Jeddah, Saudi Arabia.	Salivary biomarker - miRNA	40 OSCC: 20 Healthy controls: 20		The results showed a highly significant increase *(P>0.001)* in salivary miRNA-184 in cases with OSCC when compared to healthy controls, There was a non-significant difference *(P>0.05)* between OSCC, PMD's with dysplasia regarding levels of salivary miRNA-21 and miRNA-145. Thus, miRNA-184 was the only tested miRNA that registered significant differences between OSCC and healthy controls.
25)	2021, Nikolay Mehterov[30] - Plovdiv, Bulgaria	Salivary biomarker - miRNA	45 OSCC: 33 Healthy controls: 12		Among the screened miRNAs, miR-30c-5p *(P < 0.04)* was significantly decreased in OSCC saliva. miR-30c-5p showed a significant statistical difference between cases and controls. In conclusion, these findings show that downregulated miR-30c-5p has the potential to serve as a novel, non-invasive biomarker for early OSCC detection.

Table 1 : Summary of the studies included.

ISSN: 0975-3583,0976-2833 VOL 16, ISSUE 11, 2025

Conclusion

Findings of the study provide supporting evidence on diagnostic ability of salivary biomarkers for early cancer screening and diagnosis. The findings of the study demonstrate that these biomarkers overall have high sensitivity and specificity to be used as a non-invasive method for early oral squamous cell carcinoma diagnosis. Thus, we can conclude salivary biomarkers for secondary level of prevention for early OSCC under early diagnosis and prompt treatment.

Funding statement - NIL

Conflicts of interest - NIL

Author contribution statement

Dr. Aditi Vaidya; Contribution towards the manuscript: Concepts, design, definition of intellectual content, literature search, data acquisition, data analysis, statistical analysis, manuscript preparation, manuscript editing. Dr.Treville Pereira; Contribution towards the manuscript: Manuscript preparation, manuscript editing, manuscript review. Dr. Mayura Mahajan; Contribution towards the manuscript: Manuscript editing, manuscript review, manuscript proof reading. Dr.Pooja Kamath; Contribution towards the manuscript: Design, data analysis, statistical analysis, manuscript preparation, manuscript editing, manuscript review. Dr. Swati Gotmare; Contribution towards the manuscript: Definition of intellectual content, manuscript preparation, manuscript editing, manuscript review. Dr.Nasreen Akhai; Contribution towards the manuscript: Manuscript preparation, manuscript editing, manuscript review.

ISSN: 0975-3583,0976-2833 VOL 16, ISSUE 11, 2025

References

1. Arellano-Garcia, M.E., Hu, S., Wang, J., Henson, B., Zhou, H., Chia, D. and Wong, D.T., 2008. Multiplexed immunobead-based assay for detection of oral cancer protein biomarkers in saliva. *Oral diseases*, *14*(8), pp.705-712.
2. Cohen JF, Korevaar DA, Altman DG, et al (2016). STARD 2015 guidelines for reporting diagnostic accuracy studies: explanation and elaboration. *BMJ Open*, **6**, 1-17.
3. Deepthi, G., Nandan, S.R.K. and Kulkarni, P.G., 2019. Salivary tumour necrosis factor-α as a biomarker in oral leukoplakia and oral squamous cell carcinoma. *Asian Pacific journal of cancer prevention: APJCP*, *20*(7), p.2087.
4. Ghallab, N.A. and Shaker, O.G., 2017. Serum and salivary levels of chemerin and MMP-9 in oral squamous cell carcinoma and oral premalignant lesions. *Clinical oral investigations*, *21*, pp.937-947.
5. Grimes DA, Schulz KF (2005). Refining clinical diagnosis with likelihood ratios. *The Lancet*, **365**, 1500–5.
6. Hilden J (2005). What properties should an overall measure of test performance possess?. *Clin Chem,* **51**, 471-72
7. Horváth, J., Szabó, A., Tar, I., Dezső, B., Kiss, C., Márton, I. and Scholtz, B., 2018. Oral health may affect the performance of mRNA-based saliva biomarkers for oral squamous cell cancer. *Pathology & Oncology Research*, *24*, pp.833-842.
8. Hu, S., Arellano, M., Boontheung, P., Wang, J., Zhou, H., Jiang, J., Elashoff, D., Wei, R., Loo, J.A. and Wong, D.T., 2008. Salivary proteomics for oral cancer biomarker discovery. *Clinical cancer research*, *14*(19), pp.6246-6252.
9. John, M.A.S., Li, Y., Zhou, X., Denny, P., Ho, C.M., Montemagno, C., Shi, W., Qi, F., Wu, B., Sinha, U. and Jordan, R., 2004. Interleukin 6 and interleukin 8 as potential biomarkers for oral cavity and oropharyngeal squamous cell carcinoma. *Archives of Otolaryngology–Head & Neck Surgery*, *130*(8), pp.929-935.
10. Jones CM, Athanasiou T (2005). Summary Receiver Operating Characteristic Curve Analysis Techniques in the Evaluation of Diagnostic Tests. *Ann Thorac Surg*, **79**, 16–20.
11. Lee, L.T., Wong, Y.K., Hsiao, H.Y., Wang, Y.W., Chan, M.Y. and Chang, K.W., 2018. Evaluation of saliva and plasma cytokine biomarkers in patients with oral squamous cell carcinoma. *International Journal of Oral and Maxillofacial Surgery*, *47*(6), pp.699-707.

12. Li, Y., St. John, M.A., Zhou, X., Kim, Y., Sinha, U., Jordan, R.C., Eisele, D., Abemayor, E., Elashoff, D., Park, N.H. and Wong, D.T., 2004. Salivary transcriptome diagnostics for oral cancer detection. *Clinical Cancer Research*, *10*(24), pp.8442-8450.
13. Lijmer JG, Bossuyt PMM, Heisterkamp SH (2002). Exploring sources of heterogeneity in systematic reviews of diagnostic tests. *Stat Med*, **21**, 1525-37
14. Mehterov, N., Vladimirov, B., Sacconi, A., Pulito, C., Rucinski, M., Blandino, G. and Sarafian, V., 2021. Salivary miR-30c-5p as potential biomarker for detection of oral squamous cell carcinoma. *Biomedicines*, *9*(9), p.1079.
15. Momen-Heravi, F., Trachtenberg, A.J., Kuo, W.P. and Cheng, Y.S., 2014. Genomewide study of salivary microRNAs for detection of oral cancer. *Journal of dental research*, *93*(7_suppl), pp.86S-93S.
16. Oh, S.Y., Kang, S.M., Kang, S.H., Lee, H.J., Kwon, T.G., Kim, J.W., Lee, S.T., Choi, S.Y. and Hong, S.H., 2020. Potential salivary mRNA biomarkers for early detection of oral cancer. *Journal of clinical medicine*, *9*(1), p.243.
17. Peisker, A., Raschke, G.F., Fahmy, M.D., Guentsch, A., Roshanghias, K., Hennings, J. and Schultze-Mosgau, S., 2017. Salivary MMP-9 in the detection of oral squamous cell carcinoma. *Medicina oral, patologia oral y cirugia bucal*, *22*(3), p.e270.
18. Rajkumar K, Nandini G, Ramya R, Rajashree P et al (2014). Validation of diagnostic utility of salivary interleukin-8 in differentiation of potentially malignant oral lesion and malignant oral squamous cell carcinoma in high endemic setting region. Oral Surg. Oral Med. Oral Radiol, ***14***, 15-21.
19. Salameh J-P, Bossuyt PM, McGrath TA, et al (2020). Preferred reporting items for systematic review and meta-analysis of diagnostic test accuracy studies (PRISMA-DTA): explanation, elaboration, and checklist. *BMJ*, **10**, 1-17.
20. Saleem, Z., Shaikh, A.H., Zaman, U., Ahmed, S., Majeed, M.M., Kazmi, A. and Farooqui, W.A., 2021. Estimation of salivary matrix metalloproteinases-12 (MMP-12) levels among patients presenting with oral submucous fibrosis and oral squamous cell carcinoma. *BMC Oral Health*, *21*(1), p.205.
21. Shaw, A.K., Garcha, V., Shetty, V., Vinay, V., Bhor, K., Ambildhok, K. and Karande, P., 2022. Diagnostic accuracy of salivary biomarkers in detecting early oral squamous cell carcinoma: a systematic review and meta-analysis. *Asian Pacific Journal of Cancer Prevention: APJCP*, *23*(5), p.1483.

ISSN: 0975-3583,0976-2833 VOL 16, ISSUE 11, 2025

22. SHAW, A.K., MAHAJAN, M., RSHNEY, S., JENA, M., ROHATGI, L., BASHIR, S., TEWARI, S. and ROY, J., 2022. Diagnostic Accuracy of Chemiluminescence for Oral Potentially Malignant Disorders: A Systematic Review and Meta-analysis. *Journal of Clinical & Diagnostic Research*, *16*(7).
23. Shpitzer, T., Hamzany, Y., Bahar, G., Feinmesser, R., Savulescu, D., Borovoi, I., Gavish, M. and Nagler, R.M., 2009. Salivary analysis of oral cancer biomarkers. *British journal of cancer*, *101*(7), pp.1194-1198.
24. Singh, P., Verma, J.K. and Singh, J.K., 2020. Validation of salivary markers, IL-1β, IL-8 and Lgals3bp for detection of oral squamous cell carcinoma in an Indian population. *Scientific reports*, *10*(1), p.7365.
25. Smriti, K., Ray, M., Chatterjee, T., Shenoy, R.P., Gadicherla, S., Pentapati, K.C. and Rustaqi, N., 2020. Salivary MMP-9 as a biomarker for the diagnosis of oral potentially malignant disorders and oral squamous cell carcinoma. *Asian Pacific journal of cancer prevention: APJCP*, *21*(1), p.233.
26. Spielmann, N. and Wong, D.T., 2011. Saliva: diagnostics and therapeutic perspectives. *Oral diseases*, *17*(4), pp.345-354.
27. Whiting PF (2011). QUADAS-2: A Revised Tool for the Quality Assessment of Diagnostic Accuracy Studies. *Ann Intern Med*, **155**, 529-36.
28. Yu, J.S., Chen, Y.T., Chiang, W.F., Hsiao, Y.C., Chu, L.J., See, L.C., Wu, C.S., Tu, H.T., Chen, H.W., Chen, C.C. and Liao, W.C., 2016. Saliva protein biomarkers to detect oral squamous cell carcinoma in a high-risk population in Taiwan. *Proceedings of the National Academy of Sciences*, *113*(41), pp.11549-11554.
29. Zahran, F., Ghalwash, D., Shaker, O., Al-Johani, K. and Scully, C., 2015. Salivary micro RNA s in oral cancer. *Oral diseases*, *21*(6), pp.739-747.
30. Zimmermann, B.G. and Wong, D.T., 2008. Salivary mRNA targets for cancer diagnostics. *Oral oncology*, *44*(5), pp.425-429.