Diagnostic accuracy of serum biomarkers in early oral squamous cell carcinoma: A systematic review and metanalysis

Dr. Ashwini Nerkar Rajbhoj[1*], Dr. Priyanka T Rathod[2], Dr. Nayana Anasane[3], Dr. Mrinal Shete[4], Dr. Ujwala Rohan Newadkar[5], Dr. Rashmi Sapkal[6]

[1*]MDS, PhD, Associate professor, Department of Oral Medicine and Radiology, Sinhgad Dental College and Hospital, Pune, Maharashtra, India

[2]Assistant Professor, MGV KBH Dental College and Hospital, Nasik, Maharashtra, India

[3]MDS, Professor, Department of Prosthodontics, Dr. DY Patil Dental College and Hospital, Dr. DY Patil Vidyapeeth, Pimpri, Pune, Maharashtra, India

[4]MDS, Associate Professor, Department of Oral Pathology and Microbiology, DY Patil Dental School, Pune, Maharashtra, India

[5]MDS Department of oral medicine and radiology, Associate professor and Head, ACPM dental college and hospital, Dhule, India.

[6]MDS Department of oral medicine and radiology, Professor, M.A Rangoonwala college of dental sciences and research center, Pune, India.

***Corresponding Author:**

Dr. Ashwini Nerkar Rajbhoj

MDS, PhD, Associate professor, Department of Oral Medicine and Radiology, Sinhgad Dental College and Hospital, Pune, Maharashtra, India

Email ID: dr.ashwini.nerkar@gmail.com

Abstract

Objective: Evaluate the diagnostic ability of various serum biomarkers for early oral squamous cell carcinoma (OSCC) diagnosis compared to histopathological investigation

Method: Review was performed in accordance to Preferred Reporting Items for Systematic Reviews and Meta-Analysis – Diagnostic Test Accuracy (PRISMA- DTA) checklist and the review protocol is registered under PROSPERO(CRD42024625802). Databases were searched from January 2000 to September 2024 to identify the diagnostic potential of various serum biomarkers. Quality of selected studies was evaluated based on QUADAS (Quality assessment of diagnostic accuracy studies)- 2 tool. Meta-analysis was performed in Meta-Disc 1.4 software and Review Manager 5.3 using a bivariate model parameter for the sensitivity and specificity and summary points, summary receiver operating curve (SROC), confidence region, and area under curve (AUC) were calculated.

Results: 23studies were included in review with data evaluated from 3309 subjects (diseased - 2069 and 1240 – controls). Included studies had presence of moderate to high risk of bias. Various biomarkers like CYFRA 21 -1, E – CADHERIN, Interleukins, (IL-6, IL-8), Protein Peaks, Matrix Metalloproteinases (MMP ,9), Vascular Endothelial Growth Factor (VEGF) and various RNA biomarkers were evaluated belonging to protein and microRNA class of biomarkers. It was found that these biomarkers had sensitivity and specificity ranging from 16% to 94% and 37% to 100% while highest accuracy was shown by IL-8 with mean sensitivity and specificity of 86.5% and 98% with highest AUC seen for CYFRA 21 -1 (0.53) suggesting that the overall diagnostic accuracy of these serum biomarkers is being moderate to good in diagnosing the desired condition.

Conclusion

serum biomarkers is a valid tool and overall has good diagnostic potentiality in diagnosing the target condition and can be used as an alternative adjunct to histopathology. Serum biomarkers can be undertaken for early diagnosis and prompt treatment under secondary level of prevention.

Keywords: biopsy, diagnosis, histopathology, prognosis

How to cite this article: Rajbhoj AN, Rathod PT, Anasane N, Shete M, Newadkar UR, Sapkal R. Diagnostic accuracy of serum biomarkers in early oral squamous cell carcinoma: A systematic review and metanalysis. Int J Drug Deliv Technol. 2026;16(64s):1124-1147. DOI: 10.25258/ijddt.16.64s.121

Introduction

Oral cancer is the sixth most common malignancy worldwide [1]. Approximately, 90% of cancer located in the oral cavity are oral squamous cell carcinoma (OSCC) [1]. Most oral cancers are superficial and easily detected, but deeply located tumours may not be noted until they have grown large and reached an advanced stage [2].

Oral cancer ranks among the top 10 most common cancers globally, with over 500,000 new cases annually [3]. Approximately 90-95% of oral cancers originate from mouth-lining cells [3]. This disease

poses significant global health concerns, responsible for over 200,000 deaths in 2012 and projected to rise to 856,000 cases by 2035 [4].

Despite easy accessibility for examination, effective screening mechanisms for early lesions remain elusive [5]. Most lesions go untreated until advanced or metastasized. A technique distinguishing between normal, inflammatory, premalignant, and malignant cases rapidly would enhance detection and treatment efforts [6].

Adjuvant diagnostic techniques have been suggested, but evidence supporting their use is limited [7]. Biopsy, the gold standard for oral cancer diagnosis, is often impractical for screening due to its invasive nature and limited expertise [8]. Disappointingly, survival has not markedly improved in recent decades because patients still frequently develop local and regional recurrences, distant metastases and second primary tumours [9].

At present, several therapeutic approaches are used in the management of OSCC, but they are typically aggressive and associated with numerous side effects that significantly hamper patient quality of life [10].

The main treatments for OSCC are surgery, radiotherapy, chemotherapy, or a combination of two or more of these techniques [10]. The search for new OSCC therapies should consider both the ability of patients to tolerate the treatment's side effects and the toxicity associated with that treatment [11]. To reduce the side effects and toxicity caused by these conventional treatments, researchers have invested great efforts to develop effective and less invasive diagnostic methods capable of identifying OSCC at early stages [11].

Biomarkers have emerged as critically important tools to detect diseases in their various clinical stages by increasing the accuracy to precisely characterize the disease in a diagnostic or prognostic level. Serum biomarkers are defined as substances changing quantitatively in the serum during tumour development [12]. Classically, a marker is synthesized by the tumour and released into circulation or expressed at the cell surface in large quantity by malignant cells [13]. These markers can been used in the prognosis of tumour recurrence or metastasis because the development of the malignant tumour changing their concentrations [14].

The mortality rate in OSCC can be effectively reduced through the prevention, early detection, and treatment, thus leading to better outcomes in OSCC patients [14]. Diagnostic accuracy, including sensitivity, specificity, and summary receiver operating characteristics (SROC) analysis, is crucial for clinicians to reach correct diagnoses and choose effective treatments [14,15].

Till date, no studies have provided a comprehensive, quantitative and diagnostic accuracy analysis of various serum biomarkers for OSCC diagnosis. Therefore, we updated our research for existing scientific evidences and conducted this review with the aim to assess and evaluate the diagnostic accuracy of serum biomarkers compared to histopathological investigation in patients with oral squamous cell carcinoma (OSCC).

Methodology

Protocol and Registration

The review was adhered in accordance to PRISMA-DTA checklist and registered in PROSPERO (CRD42024625802).

Study Design

The 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 serum biomarkers (Index Test) compared to biopsy/histopathological investigations (gold standard) for oral squamous cell carcinoma (OSCC) diagnosis?

Eligibility Criteria:

Inclusion Criteria:

(1) Cross-sectional and analytical studies comparing the diagnostic accuracy of serum biomarkers compared to biopsy followed by histopathological investigation were included

(2) Studies involving the diagnostic ability of various serum biomarkers for OSCC

(3) Outcome measure reported in terms of sensitivity, specificity, accuracy, predictive values (PV) using different methods irrespective of the methods of quantifying the outcomes.

(4) Articles written in English language and from open access journals

(5) Articles from January 2000 – September 2024 and available as free available full text articles

Exclusion Criteria:

(1) In-vitro studies, animal studies, case reports and case series were excluded
(2) Patients with presence of any systemic complication
(3) Patients with oral potentially malignant disorders (OPMD) or oral epithelial dysplasia (OED) were excluded
(4) Studies not reporting primary outcomes of accuracy, sensitivity, and specificity

Search protocol and study selection

An electronic database search was performed till September 2024 for the studies published within the last 24 years (from 2000 to 2024) using the following

databases: PubMed and EBSCOhost. The searches in the clinical trials database, cross-referencing and grey literature were conducted using Google Scholar, Greylist, and OpenGrey.

Search Strategy

Proper keywords and Medical Subject Heading (MeSH) terms were selected and combined with Boolean operators like AND/OR. The search strategy used was as follows: (serum biomarkers AND oral squamous cell carcinoma AND histopathology AND sensitivity AND specificity AND diagnosis)

Search Strategy according to PIRT Format:

	Strategy
Population	("oral cancer diagnosis"[MeSH Terms] OR "oral cancer prediction" OR "malignant transformation" OR ("oral squamous cell carcinoma"[MeSH Terms] OR "head and neck cancer") OR ("diagnosis"[MeSH Terms] OR ("prognosis").
Index test	("serum biomarkers"[MeSH Terms] OR "diagnosis" AND "oral cancer" AND "oral squamous cell carcinoma" OR "diagnostic accuracy" OR "tissue fluids" OR "tissue biopsy" AND "treatment/surgery" OR "oral tissue" OR ("diagnostic accuracy" AND "sensitivity" AND "specificity"
Reference standard	"histopathology" OR "oral biopsy"[MeSH Terms] OR "tissue biopsy" AND "treatment/surgery" OR "oral tissue" OR ("diagnostic accuracy" AND "sensitivity" AND "specificity"
Target condition	((("sensitivity AND specificity"[MeSH Terms] OR "head and neck cancer" OR ("oral cancer"[MeSH Terms] OR ("diagnostic accuracy") AND "oral potentially malignant disorder" OR "oral squamous cell carcinoma" OR ("comparative study" AND "randomized controlled trial" AND "cross-sectional study" OR "prospective study".

Screening process

A rigorous two-phase screening process was conducted by two authors to select relevant articles. Initially, titles and abstracts were reviewed, and non-relevant articles were excluded. In phase two, full-text reviews were performed independently by the same reviewers, with disputes resolved through discussion. A third reviewer was consulted when necessary to ensure consensus.

Data extraction

For included studies, study details were extracted under following headings: authors, study year, sample size (diseased cases/controls), study design, biomarkers evaluated, class of biomarker and method of estimation. Data like sensitivity and specificity were compiled and values like true positive (TP), true negative (TN), false positive (FP) and false negatives (FN) were calculated for the studies using the below formula's where the data was not provided by authors. The corresponding authors were also 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

Risk of bias was assessed through quality assessment of diagnostic accuracy studies - 2 (QUADAS-2) tool[17] through its four domains: patient selection, index test, reference standard, flow and timing of patient. Every domain had flagging inquiries with choices of "Yes", "No" or "Unclear". The general risk of bias was evaluated as high: whenever responded to 'No' to any question, Low: whenever addressed 'Yes' to all inquiries and Unclear: whenever addressed 'Unclear' to all inquiries or joined by any 'Yes' using Review Manager (RevMan) software version 5.3.

Statistical analysis

Crude information was utilized to work out responsiveness and explicitness for each biomarker with their assessment technique. For by and large exactness, we determined pooled responsiveness, pooled explicitness with 95% certainty stretch, region under outline recipient working trademark. (Understanding of AUC values were as per the following: esteem above 80% were considered as brilliant, somewhere in the range of 70% and 80% as great, somewhere in the range of 60% and 69% as fair

and beneath 60% as unfortunate results for a symptomatic test.[18]

Data synthesis

To evaluate the effect of heterogeneity, Higgins I^2 test was utilized. This test addresses the extent of fluctuation because of heterogeneity instead of because of inspecting blunder.[19] As per I2 test measurement the heterogeneity could be low (I2 <50%) or high (I2 >50%).

Additional analysis

Additional analysis was performed with positive likelihood ratio (PLR) and negative likelihood ratio (NLR) using DerSimonian-Laird's estimator considering 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.[20]

Results

Study Selection

After copies evaluation, references of all included studies were screened. Of which 30 records were barred. After this full text articles were evaluated for qualification and articles that didn't meet consideration rules were barred. Twenty three studies fitted into inclusion criteria and were subjected to qualitative analysis and twenty one studies for meta-analysis as shown in Figure 1.

Study Characteristics

A summary of descriptive characteristics of all included studies is shown in **Table 1.** Data was evaluated from twenty-three studies[21-43] from an aggregate sample size of 3309 patients, of which diseased cases were 2069 and 1240 were controls. All the included studies were cross-sectional in nature. Among the included studies, five studies were from India [33,35,36,39,41], four studies were from USA [23,27,30,34] and China [22,28,38,42] three studies each from Spain [21,26,31], Taiwan [25,32,40] and one study each from France [24], Belgium [29], Germany [37] and Iran [43]. All the included studies, assessed and evaluated the diagnostic accuracy of various biomarkers like CYFRA 21 -1, E – Cadherin, interleukins, (IL-6, IL-8), protein peaks, matrix metalloproteinases (MMP ,9), vascular endothelial growth factor (VEGF), galectin (1,3) and various RNA biomarkers. All these biomarkers belong to protein and microRNA class of biomarkers.

As shown in below **table 2,** various serum biomarkers (index test) were compared with the reference standard. It was found that all these biomarkers had sensitivity and specificity ranging from 16% to 94% and 37% to 100%. CYFRA 21-1 had mean sensitivity and specificity of 60.5% and 93.5%. E-CADHERIN mean sensitivity and specificity of 56% and 71%. Protein peaks had mean sensitivity and specificity of 80.3% and 77%. MMP-2 had mean sensitivity and specificity of 32.5% and 67% while MMP-9 had mean sensitivity and specificity of 50% and 74.67%. VEGF had mean sensitivity and specificity of 76% and 88.33%. GALECTIN 1 had mean sensitivity and specificity of 56% and 80% while GALECTIN 3 had mean sensitivity and specificity of 63% and 75%. IL -6 had mean sensitivity and specificity of 73% and 99% while IL-8 had mean sensitivity and specificity of 86.5% and 98% and miRNA biomarkers had mean sensitivity and specificity of 77.75% and 81.5%.

Risk of Bias within Studies

For risk of bias domain, the patient selection and index test criteria were at the highest risk while reference standard and flow and timing were at lowest risk while for applicability concern, the patient selection and index test were at highest risk while reference standard at lowest risk. The flow and timing and reference standard had low risk due to absence of insufficient details reported as to whether results of index test was interpreted without prior knowledge of reference standard results, lack of pre-specification of a test-positive threshold. Patient selection had high risk, which was mainly due to use of case-control design, different ways of patient enrollment, types of study design and implementing inappropriate exclusion as depicted in **Figure 2 and 3.**

Synthesis of Results

Summary statistics measure was calculated in terms of pooled sensitivity, specificity, positive and negative likelihood ratio (PLR & NLR), diagnostic odd's ratio (DOR) and area under the curve (AUC) for determining the overall diagnostic accuracy of various serum biomarkers.

A) CYFRA 21 -1

As shown in **Figure 4. (a), (b),** data was evaluated from four studies [21,22,24,41] investigating the overall diagnostic accuracy. The pooled sensitivity was 0.37 (CI 0.03- 0.87) and pooled specificity was 0.42 (CI 0.03- 0.91) with I^2 being 0%.

Figure 4 (a). Pooled sensitivity of CYFRA 21 -1 for OSCC

Figure 4 (b). Pooled sensitivity of CYFRA 21 -1 for OSCC

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 5 (a), (b),** pooled positive likelihood ratio (PLR) 0.65 (0.16 – 2.53) and negative likelihood ratio (NLR) 1.33 (0.41

– 41.26) was estimated. Pooled +PLR suggested that CYFRA 21-1 is 0.65 times more likely to have a positive detection of target condition than someone without while pooled -NLR suggested that CYFRA 21-1 is 1.33 times as likely to have a negative OSCC condition detection as someone without the presence of OSCC.
As shown in **figure 6.** the pooled Diagnostic Odds Ratio (DOR) is 0.36 (0.01 – 9.26) suggesting that overall ability of index test in correctly diagnosing the target condition is moderate to good.

B) E CADHERIN

As shown in **Figure 7. (a), (b),** data was evaluated from two studies [27,31] investigating the overall diagnostic accuracy. The pooled sensitivity was 0.46 (CI 0.01- 0.98) and pooled specificity was 0.14 (CI 0.01- 0.98) with I^2 being 0%.

As shown in **figure 8. (a), (b),** pooled positive likelihood ratio (PLR) 0.53 (0.10 – 2.81) and negative likelihood ratio (NLR) 3.93 (0.03 – 441.65) was estimated. Pooled +PLR suggested that E-CADHERIN is 0.53 times more likely to have a positive detection of target condition than someone without while pooled -NLR suggested that E-CADHERIN is 1.14 times as likely to have a negative OSCC condition detection as someone without the presence of OSCC.

As shown in **figure 9.** the pooled Diagnostic Odds Ratio (DOR) is 0.14 (0.00 – 52.20) suggesting that overall ability of index test in correctly diagnosing the target condition is moderate to good.

C) PROTEIN PEAK

As shown in **Figure 10. (a), (b),** data was evaluated from three studies [25,28,30] investigating the overall diagnostic accuracy. The pooled sensitivity was 0.46 (CI 0.03- 0.95) and pooled specificity was 0.31 (CI 0.00- 0.96) with I^2 being 0%.

As shown in **figure 11. (a), (b),** pooled positive likelihood ratio (PLR) 0.75 (0.18 – 3.15) and negative likelihood ratio (NLR) 1.55 (0.16 – 14.83) was estimated. Pooled +PLR suggested that protein peak is 0.75 times more likely to have a positive detection of target condition than someone without while pooled -NLR suggested that protein peak is 1.44 times as likely to have a negative OSCC condition detection as someone without the presence of OSCC.
As shown in **figure 12.** the pooled Diagnostic Odds Ratio (DOR) is 0.44 (0.01 – 22.55) suggesting that overall ability of index test in correctly diagnosing the target condition is moderate to good.

D) MMP 2

As shown in **Figure 13 (a), (b),** data was evaluated from two studies [31,33] investigating the overall diagnostic accuracy. The pooled sensitivity was 0.31 (CI 0.00- 0.98) and pooled specificity was 0.25 (CI 0.00- 0.97) with I^2 being 0%.

As shown in **figure 14. (a), (b),** pooled positive likelihood ratio (PLR) 0.37 (0.04 – 3.84) and negative likelihood ratio (NLR) 2.18 (0.14 – 34.28) was estimated. Pooled +PLR suggested that MMP-2 is 0.37 times more likely to have a positive detection of target condition than someone without while pooled -NLR suggested that MMP-2 is 2.18 times as likely to have a negative OSCC condition detection as someone without the presence of OSCC.

As shown in **figure 15.** the pooled Diagnostic Odds Ratio (DOR) is 0.10 (0.00 – 210.08) suggesting that overall ability of index test in correctly diagnosing the target condition is moderate to good.

E) MMP 9

As shown in **Figure 16. (a), (b),** data was evaluated from three studies [26,31,33] investigating the overall diagnostic accuracy. The pooled sensitivity was 0.30 (CI 0.00- 0.89) and pooled specificity was 0.26 (CI 0.00- 0.90) with I^2 being 0%.

As shown in **figure 18. (a), (b),** pooled positive likelihood ratio (PLR) 0.43 (0.06 – 2.92) and negative likelihood ratio (NLR) 2.39 (0.26 – 21.98) was estimated. Pooled +PLR suggested that MMP-9 is 0.43 times more likely to have a positive detection of target condition than someone without while pooled -NLR suggested that MMP-9 is 2.39 times as likely to have a negative OSCC condition detection as someone without the presence of OSCC.

As shown in **figure 19.** the pooled Diagnostic Odds Ratio (DOR) is 0.63 (0.08 – 5.20) suggesting that overall ability of index test in correctly diagnosing the target condition is moderate to good.

F) VEGF

As shown in **Figure 20. (a), (b),** data was evaluated from three studies [34-36] investigating the overall diagnostic accuracy. The pooled sensitivity was 0.54 (CI 0.05- 0.97) and pooled specificity was 0.32 (CI 0.00- 0.93) with I^2 being 0%.
As shown in **figure 21. (a), (b),** pooled positive likelihood ratio (PLR) 0.81 (0.21 – 3.14) and negative likelihood ratio (NLR) 1.38 (0.15 – 12.74) was estimated. Pooled +PLR suggested that VEGF is 0.81 times more likely to have a positive detection of target condition than someone without while pooled -NLR suggested that VEGF is 1.38 times as likely to have a

negative OSCC condition detection as someone without the presence of OSCC.
As shown in **figure 22.** the pooled Diagnostic Odds Ratio (DOR) is 0.58 (0.02 – 20.48) suggesting that overall ability of index test in correctly diagnosing the target condition is moderate to good.

G) GALECTIN 1

As shown in **Figure 23. (a), (b),** data was evaluated from two studies [29,36] investigating the overall diagnostic accuracy. The pooled sensitivity was 0.37 (CI 0.00- 0.97) and pooled specificity was 0.26 (CI 0.00- 0.96) with I^2 being 0%.
As shown in **figure 24. (a), (b),** pooled positive likelihood ratio (PLR) 0.61 (0.08 – 4.39) and negative likelihood ratio (NLR) 2.55 (0.17 – 38.66) was estimated. Pooled +PLR suggested that Galectin -1 is 0.61 times more likely to have a positive detection of target condition than someone without while pooled -NLR suggested that Galectin-1 is 2.55 times as likely to have a negative OSCC condition detection as someone without the presence of OSCC.
As shown in **figure 6.** the pooled Diagnostic Odds Ratio (DOR) is 0.18 (0.00 – 22.8) suggesting that overall ability of index test in correctly diagnosing the target condition is moderate to good.

H) GALECTIN 3

As shown in **Figure 26. (a), (b),** data was evaluated from two studies [29,26] investigating the overall diagnostic accuracy. The pooled sensitivity was 0.42 (CI 0.01- 0.98) and pooled specificity was 0.23 (CI 0.00- 0.97) with I^2 being 0%.

As shown in **figure 27. (a), (b),** pooled positive likelihood ratio (PLR) 0.61 (0.10 – 3.89) and negative likelihood ratio (NLR) 2.48 (0.10 – 62.00) was estimated. Pooled +PLR suggested that galectin -3 is 0.61 times more likely to have a positive detection of target condition than someone without while pooled -NLR suggested that galectin -3 is 2.48 times as likely to have a negative OSCC condition detection as someone without the presence of OSCC.

As shown in **figure 28.** the pooled Diagnostic Odds Ratio (DOR) is 0.21 (0.00 – 28.1) suggesting that overall ability of index test in correctly diagnosing the target condition is moderate to good.

I) IL – 6

As shown in **Figure 29 (a), (b),** data was evaluated from two studies [23.34] investigating the overall diagnostic accuracy. The pooled sensitivity was 0.38 (CI 0.00- 0.97) and pooled specificity was 0.51 (CI 0.01- 0.99) with I^2 being 0%.
As shown in **figure 30. (a), (b),** pooled positive likelihood ratio (PLR) 0.81 (0.10 – 6.62) and negative likelihood ratio (NLR) 1.24 (0.25 – 6.11) was estimated. Pooled +PLR suggested that IL-6 is 0.81 times more likely to have a positive detection of target condition than someone without while pooled -NLR suggested that IL-6 is 1.24 times as likely to have a negative OSCC condition detection as someone without the presence of OSCC.
As shown in **figure 31.** the pooled Diagnostic Odds Ratio (DOR) is 0.60 (0.01 – 41.30) suggesting that overall ability of index test in correctly diagnosing the target condition is moderate to good.

J) IL -8

As shown in **Figure 32. (a), (b),** data was evaluated from two studies [27,34] investigating the overall diagnostic accuracy. The pooled sensitivity was 0.49 (CI 0.01- 0.99) and pooled specificity was 0.43 (CI 0.01- 0.98) with I^2 being 0%.

As shown in **figure 33. (a), (b),** pooled positive likelihood ratio (PLR) 0.86 (0.13 – 5.50) and negative likelihood ratio (NLR) 1.19 (0.15 – 9.46) was estimated. Pooled +PLR suggested that IL-8 is 0.86 times more likely to have a positive detection of target condition than someone without while pooled -NLR suggested that IL-8 is 1.19 times as likely to have a negative OSCC condition detection as someone without the presence of OSCC.

As shown in **figure 34.** the pooled Diagnostic Odds Ratio (DOR) is 0.71 (0.01 – 38.80) suggesting that overall ability of index test in correctly diagnosing the target condition is moderate to good.

K) miRNA

As shown in **Figure 35. (a), (b),** data was evaluated from four studies [32,37,38,40] investigating the overall diagnostic accuracy. The pooled sensitivity was 0.45 (CI 0.05- 0.91) and pooled specificity was 0.44 (CI 0.02- 0.95) with I^2 being 0%.

As shown in **figure 36. (a), (b),** pooled positive likelihood ratio (PLR) 0.81 (0.19 – 3.48) and negative likelihood ratio (NLR) 1.20 (0.23 – 6.14) was estimated. Pooled +PLR suggested that miRNA biomarker is 0.81 times more likely to have a positive detection of target condition than someone without while pooled -NLR suggested that Serum biomarkers is 1.20 times as likely to have a negative OSCC condition detection as someone without the presence of OSCC.
As shown in **figure 37.** the pooled Diagnostic Odds Ratio (DOR) is 0.67 (0.03 – 16.74) suggesting that overall ability of index test in correctly diagnosing the target condition is moderate to good.

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 38. (a) & (b).** The highest and lowest AUC was seen was MMP -9 (0.53) and CYFRA 21 -1 (0.33) respectively.

Discussion

Early disease diagnosis is the hallmark for any planned treatment in order to reduce patient's mortality and morbidity [5]. Presence of OSCC is a major public health concern worldwide. Biopsy is the gold standard for oral lesion diagnosis but its several disadvantages has limited its use [6].

Fernández-Olavarría et al., 2016 [44] conducted a systematic review to assess the role of serum biomarkers in diagnosis and prognosis of oral cancer (OC). Databases were searched case control, retrospective and prospective studies which yielded 29 studies. It was found that among the various biomarkers, cancerous phenotype was determined by 2 biomarkers, worse prognosis was shown by 11 biomarkers and overall survival, 4 biomarkers had shown association between biomarker concentration with clinical stage of disease, 4 studies found that these biomarkers could be helpful in disease diagnosis while 8 studies did not explain exact diagnostic mechanism of serum biomarkers. It was found and concluded that sufficient scientific evidences should be present to support the role of serum biomarkers as a therapeutic agent in oral cancer diagnosis and prognosis.

Guerra et al., 2016 [45] carried out systematic review and meta-analysis to evaluate the diagnostic accuracy of serum biomarkers for head and neck cancer when used in combination with each other or individually. Databases were searched till April 2015, yielding sixty-five studies which were included in analysis with data evaluated form 9098 subjects. It was found that combined biomarkers had shown greater overall better accuracy than individual biomarkers. 12.8% of individual biomarkers and 34.3% of biomarkers differentiated patients with head and neck cancer (HNC) with controls. It was concluded that serum biomarkers has the ability to be used as a potential biomarker for HNC diagnosis.

Rezaei et al., 2019 [46] conducted a systematic review and meta-analysis to assess and evaluate the serum concentrations of interleukin biomarkers (IL-6, IL-8) in patients with oral squamous cell carcinoma (OSCC). Databases were searched till 2019 yielding 26 studies included in final analysis. Meta-analysis showed that OSCC patients had high serum concentration of IL-6 (Mean difference (MD) = 19.06, 95% CI 14. 78 – 23.33) and IL-8 (MD = 199.14, 95% CI 47.39 – 350.89) compared to healthy controls. Serum concentration of IL-6 and IL-8 were significantly elevated in OSCC patients. Hence, it was concluded that these biomarkers could be considered as a potent biomarker in overall OSCC disease diagnosis and prognosis.

Marakala et al., 2024 [47] conducted systematic review and meta-analysis to evaluate the diagnostic ability of various biomarkers present in plasma, serum, tissue and saliva of patients with head and neck cancer (HNC) compared to healthy controls. Databases were searched till October 2022 yielding 17 comparative studies. From the results of the study, it was found that sensitivity and specificity of biomarkers ranged from 29.5% to 100% and 57.1% to 100%. It was concluded that theses biomarkers have good therapeutic applications and may help in HNC diagnosis.

There have been few systematic reviews and meta-analysis [44-47] published in past but due to presence of data heterogeneity, none of them actually could provide a comprehensive qualitative and quantitative analysis on overall diagnostic accuracy of serum biomarkers compared to histopathology for OSCC diagnosis. According to our knowledge, this is the first systematic review and meta-analysis which assessed and evaluated the overall diagnostic ability between various serum biomarkers for early OSCC diagnosis.

Twenty-three studies fulfilled the eligibility criteria's and were included in review, for which the diagnostic accuracy values of various serum biomarkers. Included studies, assessed and evaluated the diagnostic accuracy of various biomarkers like CYFRA 21 -1, E – Cadherin, interleukins, (IL-6, IL-8), protein peaks, matrix metalloproteinases (MMP ,9), vascular endothelial growth factor (VEGF), galectin (1,3) and various RNA biomarkers. All these biomarkers belong to protein and microRNA class of biomarkers. From the results of the study, it was found that these biomarkers had sensitivity and specificity ranging from 16% to 94% and 37% to 100% while highest accuracy was shown by IL-8 with mean sensitivity and specificity of 86.5% and 98% suggesting that the overall diagnostic accuracy of these serum biomarkers is being moderate to good in diagnosing the desired condition.

Although the findings of the study, confirmed serum biomarkers overall has a good diagnostic accuracy in diagnosing the underlying desired target condition as a whole, however the study is limited by the fact that high risk of bias was reported by many included studies. Therefore, furthermore diagnostic or analytical cross-sectional studies should be conducted with strict and vigorous reporting through "standards for reporting diagnostic accuracy studies" (STARD) checklist [48], so as to validate the study findings.

The systematic review adhered to PRISMA guidelines, employing a comprehensive literature search and rigorous methodology, including QUADAS-2 tool

ROB assessment. This resulted in high-quality studies with minimal bias, providing a robust evidence base for therapeutic recommendations on optimizing the usage of serum biomarkers for diagnosing the target condition.

Systematic reviews and meta-analyses are considered the highest level of evidence, offering transparency and reproducibility in addressing specific research questions. However, the quality of included studies impacts the strength of evidence. This review included sufficient studies with brief observation periods and known risk of bias.

Acknowledgements:

We Acknowledge Dr. Amar Shaw for helping with the statistical analysis of this review. I appreciate his insightful input throughout the research process. His expertise greatly enriched the content of this article.

Funding Statement: Nil

Conflict of Interest: There are no conflicts of interest.

Authors, year of study	**Country**	**Sample size (Diseases/total)**	**Study Design**	**Biomarkers evaluated**	**Class of biomarker**	**Estimation method**
Ayude et al., 2003 [21]	Spain	40/101	Cross-sectional	CYFRA 21-1	protein	Colorimetry assay, biuret method
Deng et al., 2003 [22]	China	142/50	Cross-sectional	CYFRA 21-1	protein	ECLIA
St. John et al., 2004 [23]	USA	19/31	Cross-sectional	IL-6	Protein, mRNA	RNA isolation, RT-PCR, real time PCR
Cerusee et al., 2005 [24]	France	300/71	Cross-sectional	CYFRA 21-1	protein	IRMA
Cheng et al., 2005 [25]	Taiwan	57/29	Cross-sectional	Protein peaks	protein	MALDI-TOFMS
Al Kassam et al., 2007 [26]	Spain	39/10	Cross-sectional	MMP-2, MMP-9	protein	ELISA
Linkov et al., 2007 [27]	USA	116/117	Cross-sectional	MMP- 2	protein	Multiplex immuno bead-based serum analysis
Cheng et al., 2008 [28]	China	252/110	Cross-sectional	Protein peaks	protein	SELDI – TOF MS
Saussez et al., 2008 [29]	Belgium	102/38	Cross-sectional	Galectin-1, Galectin-3	protein	ELISA, immunohistochemistry
Gourin et al.,	USA	46/97	Cross-sec	Protein peaks	protein	SELDI – TOF MS

2009 [30]			tional			
Marcos et al., 2009 [31]	Spain	39/10	Cross-sectional	E-Cadherin, MMP-2, MMP-9	Protein	ELISA
Liu et al., 2010 [32]	Taiwan	43/21	Cross-sectional	miR-31	microRNA	RT-qPCR
Singh et al., 2011 [33]	India	75/50	Cross-sectional	MMP-2, MMP-9	protein	ELISA
Malhotra et al., 2012 [34]	USA	78/49	Cross-sectional	IL-6, IL-8, VEGF	protein	ELISA, immune-array
Naik et al., 2012 [35]	India	60/20	Cross-sectional	VEGF-A	mRNA	RT-qPCR, ELISA
Aggarwal et al., 2014 [36]	India	70/30	Cross-sectional	VEGF	protein	ELISA
Ries et al., 2014 [37]	Germany	57/33	Cross-sectional	microRNA	microRNA	RT-PCR
Wang et al., 2014 [38]	China	52/49	Cross-sectional	miR-21	microRNA	qRT-PCR
Aggarwal et al., 2015 [39]	India	60/30	Cross-sectional	Galectin 1, Galectin 3	protein	ELISA
Lu et al., 2015 [40]	Taiwan	90/53	Cross-sectional	miR-196a and miR-196b	microRNA	RT-qPCR
Rajkumar et al., 2015 [41]	India	100/100	Cross-sectional	CYFRA 21	Protein	ELISA
Fan et al., 2020 [42]	China	212/121	Cross-sectional	LOC284454	microRNA	RT-qPCR
Karimi et al., 2020 [43]	Iran	20/20	Cross-sectional	miR-21, miR-24, and miR-29a	microRNA	RT-qPCR

CYFRA: cytokeratin fragment; ELISA: enzyme linked immunosorbent assay; IL: interleukin; IRMA: immunoradiometric assay; MALDI – TOF MS: matrix-assisted laser desorption ionization time -of-flight mass spectrometry; MMP: matrix

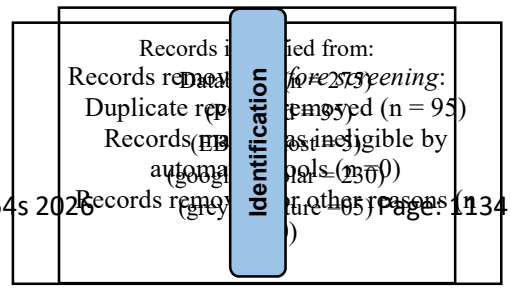
metalloproteinase; RNA: ribonucleic acid; RT-PCR: reverse transcriptase polymerase chain reaction; SELDI – TOF MS: surface enhanced laser desorption/ionization time-of-flight mass spectrometry; VEGF: vascular endothelial growth factor

Table 1: descriptive study details of included studies

Authors, year of study	True positive (TP)	True negative (TN)	False positive (FP)	False negative (FN)	Sensitivity (%)	Specificity (%)
CYFRA 21 -1						
Ayude et al., 2003 [21]	0.14	0.67	0.24	0.86	50	96
Deng et al., 2003 [22]	0.44	0.20	0.68	0.56	60	94
Cruse et al., 2005 [24]	0.58	0.13	0.75	0.42	72	94
Rajkumar et al.,2015 [41]	0.30	0.40	0.40	0.70	60	90
E CADHERIN						
Al Kassam et al., 2007 [26]	0.44	0.08	0.50	0.55	56	71
Marcos et al.,2009 [31]	0.44	0.08	0.50	0.55	56	71
PROTEIN PEAK						
Cheng et al.,2005 [25]	0.54	0.29	0.62	0.45	82	96
Cheng et al.,2008 [28]	0.60	0.15	0.55	0.39	87	85
Gourin et al.,2009 [30]	0.23	0.18	0.18	0.77	72	50
MMP -2						
Marcos et al.,2009 [31]	0.38	0.01	0.57	0.62	49	39
Singh et al., 2011 [33]	0.09	0.35	0.55	0.43	16	95
MMP -9						
Al Kassam et al., 2007 [26]	0.24	0.16	0.67	0.76	49	37
Marcos et al.,2009 [31]	0.24	0.16	0.67	0.76	32	92
Singh et al., 2011 [33]	0.09	0.35	0.55	0.43	69	95
VEGF						

Malhotra et al.,2012 [34]	0.54	0.36	0.59	0.39	89	98
Nayak et al.,2012 [35]	0.55	0.25	0.75	0.40	73	100
Aggarwal et al.,2014 [36]	0.46	0.20	0.37	0.54	66	67
GALECTIN -1						
Saussez et al.,2008 [29]	0.16	0.27	0.73	0.84	22	100
Aggarwal et al.,2015 [40]	0.57	0.17	0.51	0.43	90	60
GALECTIN -3						
Saussez et al.,2008 [29]	0.26	0.17	0.63	0.74	36	90
Aggarwal et al.,2015 [39]	0.56	0.17	0.51	0.40	90	60
IL-6						
St. John et al.,2004 [23]	0.21	0.63	0.37	0.78	57	100
Malhotra et al.,2012 [34]	0.55	0.36	0.59	0.45	89	98
IL-8						
Malhotra et al.,2012 [34]	0.55	0.36	0.59	0.45	89	98
Linkov et al.,2014 [27]	0.42	0.48	0.51	0.58	84	98
miRNA						
Liu et al.,2010 [32]	0.50	0.14	0.48	0.50	74	81
Ries et al.,2014 [37]	0.27	0.31	0.29	0.73	55	80
Wang et al.,2014 [38]	0.48	0.21	0.24	0.50	94	73
Lu et al.,2015 [40]	0.55	0.58	0.55	0.45	88	92

Table 2: showing descriptive diagnostic accuracy values



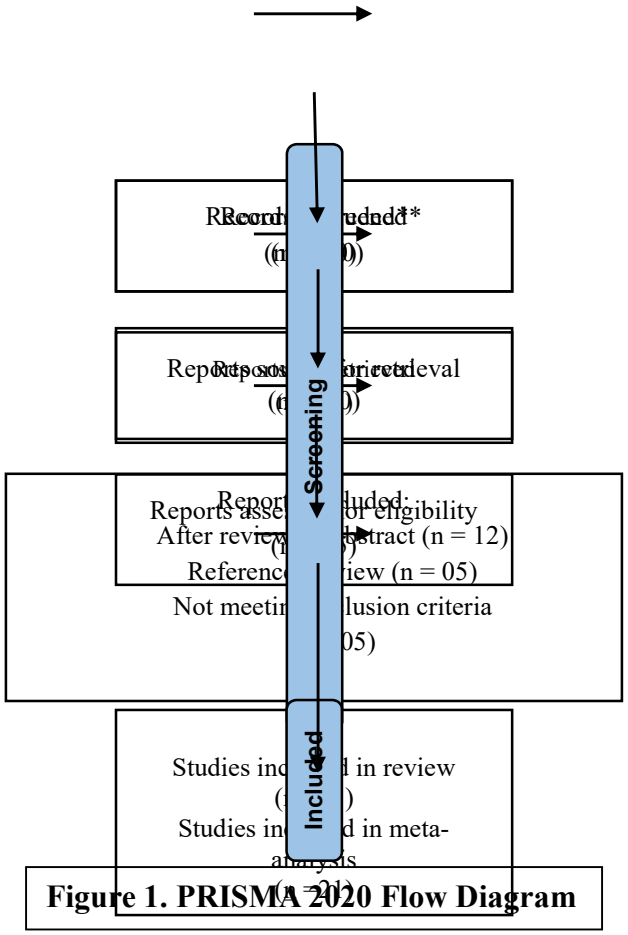


Figure 1. PRISMA 2020 Flow Diagram

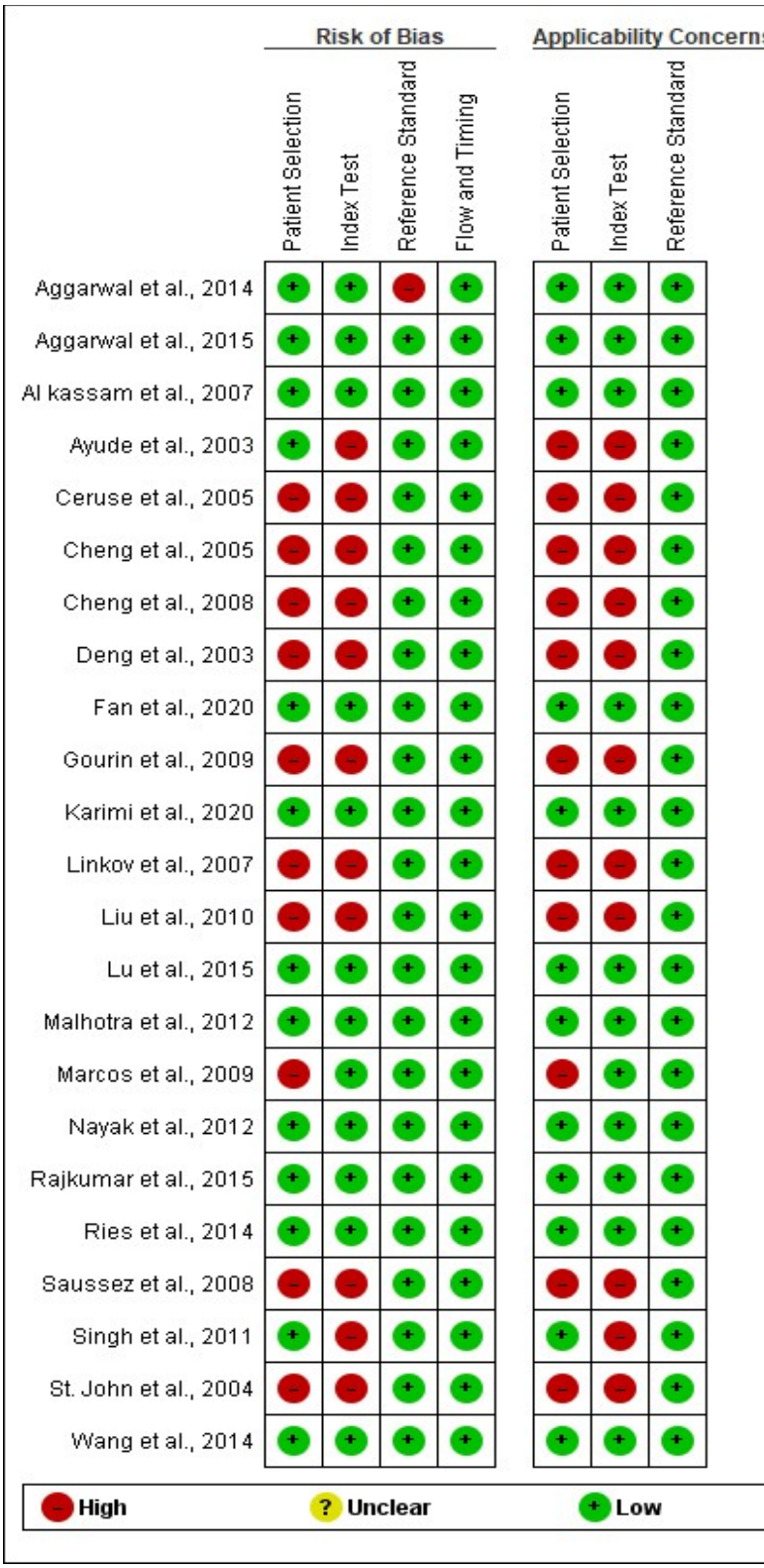


Figure 2. risk of bias graph: presented as percentages across all included studies.

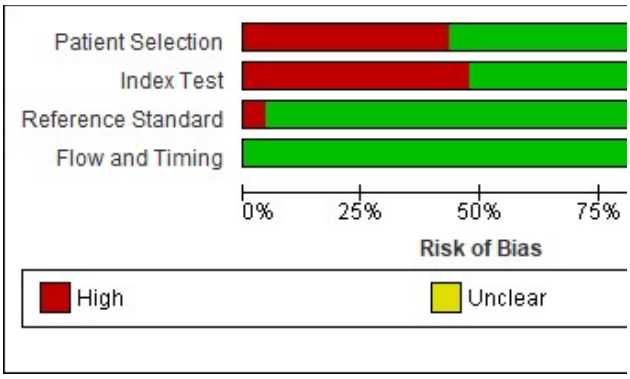


Figure 3. risk of bias summary: for each included study

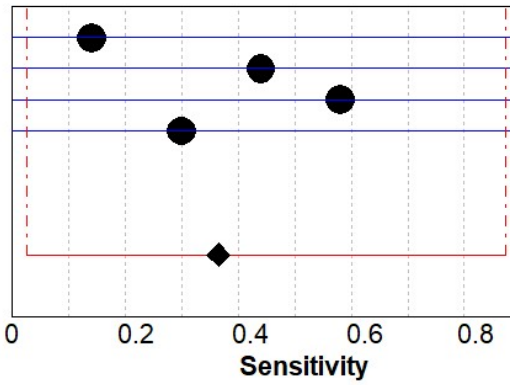


Figure 4 (a). Pooled sensitivity of CYFRA 21 -1 for OSCC

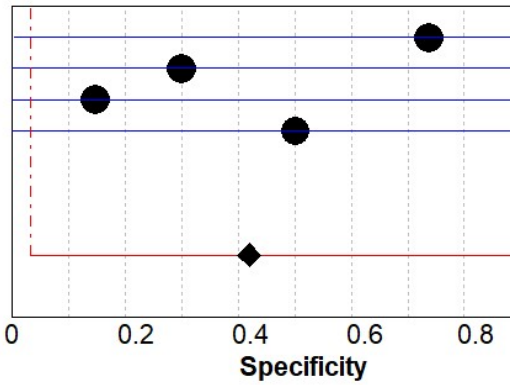


Figure 4 (b). Pooled specificity of CYFRA 21 -1 for OSCC

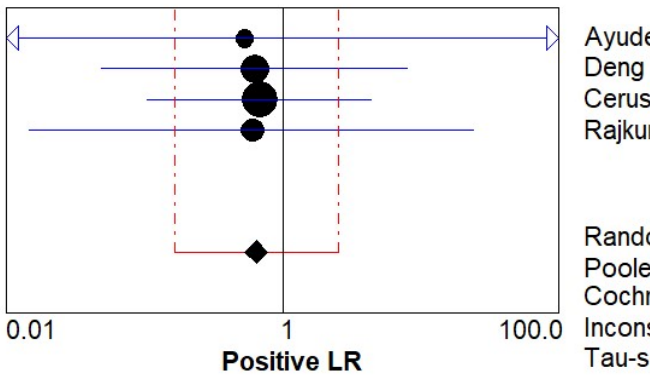


Figure 5 (a). Pooled +LR of CYFRA 21 -1 for OSCC

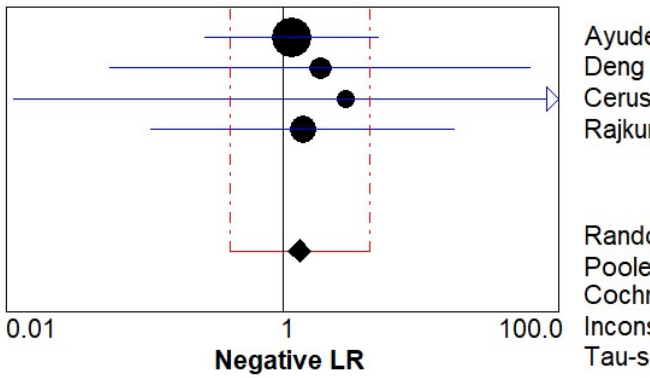


Figure 5 (b). Pooled -LR of CYFRA 21 -1 for OSCC

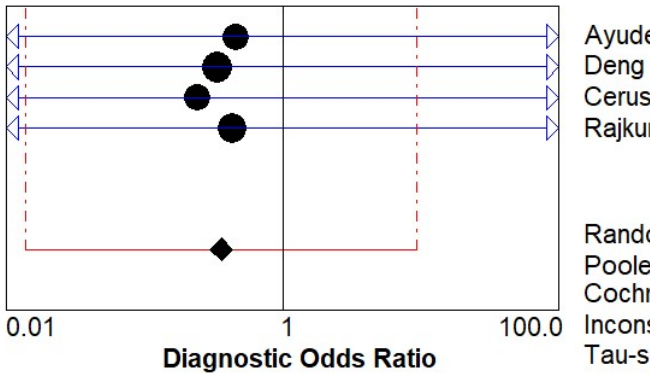


Figure 6. Pooled (DOR) of CYFRA 21 -1 for OSCC

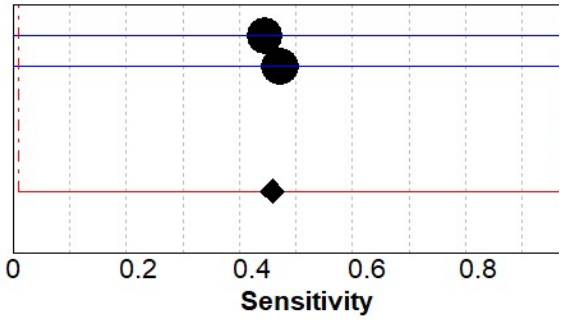


Figure 7 (a). Pooled sensitivity of E-CADHERIN for OSCC

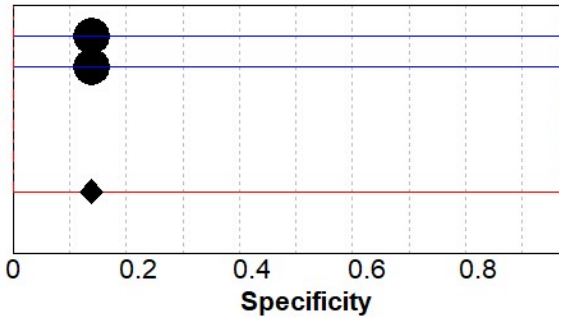


Figure 7 (b). Pooled specificity of E-CADHERIN for OSCC

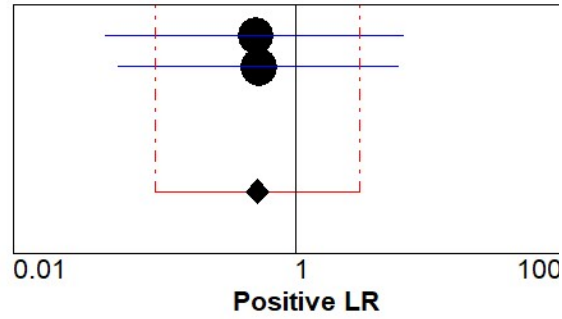


Figure 8 (a). Pooled +LR of E-CADHERIN for OSCC

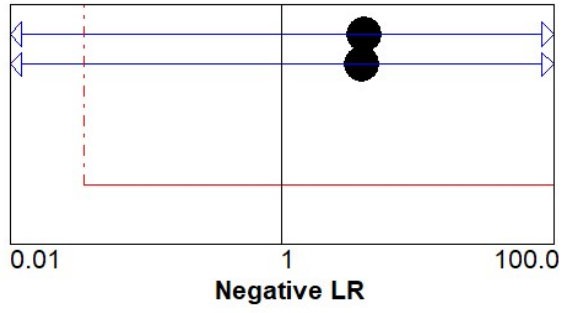


Figure 8 (b). Pooled -LR of E-CADHERIN for OSCC

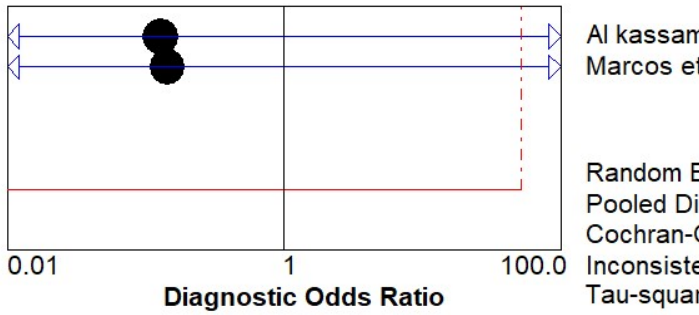


Figure 9. Pooled (DOR) of E-CADHERIN for OSCC

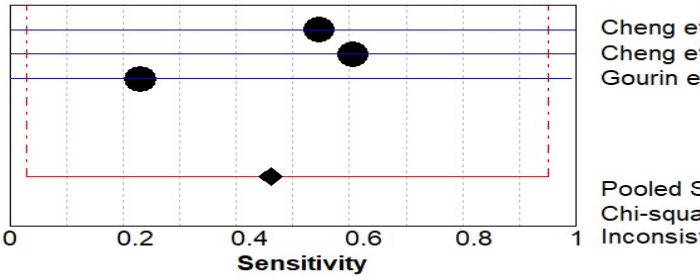


Figure 10 (a). Pooled sensitivity of protein peaks for OSCC

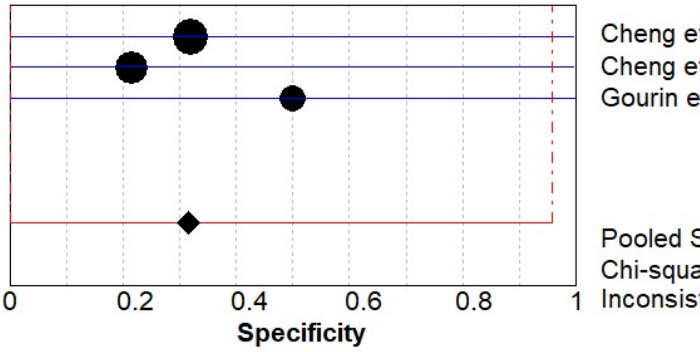


Figure 10 (b). Pooled specificity of protein peaks for OSCC

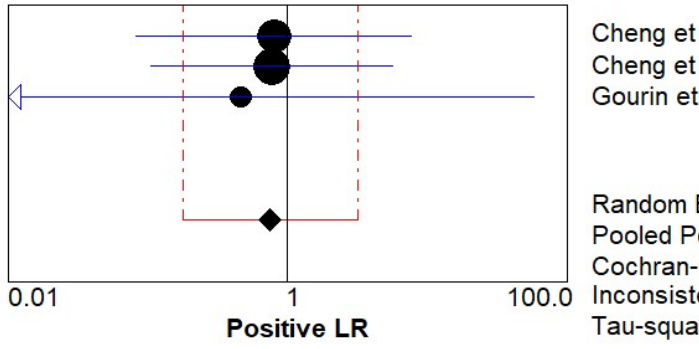


Figure 11 (a). Pooled +LR of protein peaks for OSCC

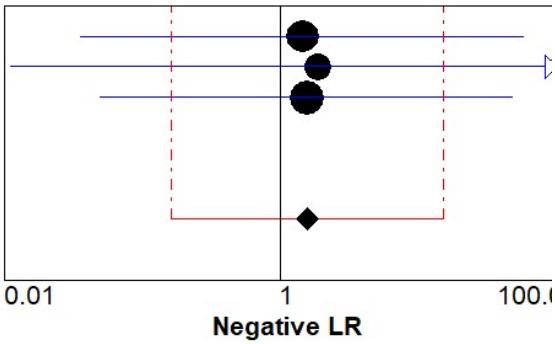


Figure 11 (b). Pooled -LR of protein peaks for OSCC

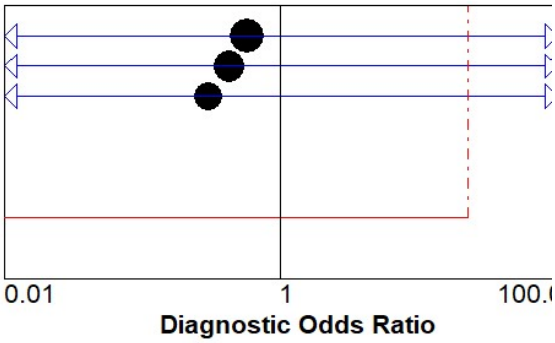


Figure 12. Pooled (DOR) of protein peaks for OSCC

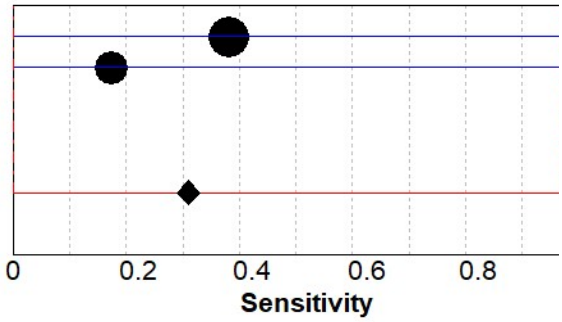


Figure 13 (a). Pooled sensitivity of MMP-2 for OSCC

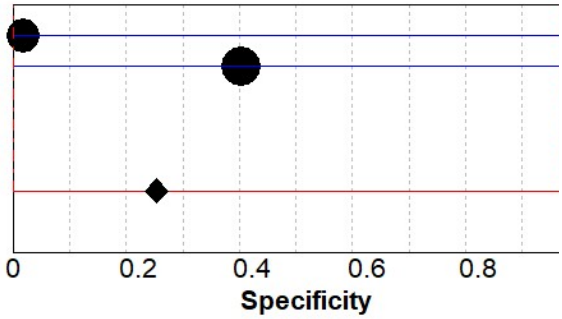


Figure 13 (b). Pooled specificity of MMP-2 for OSCC

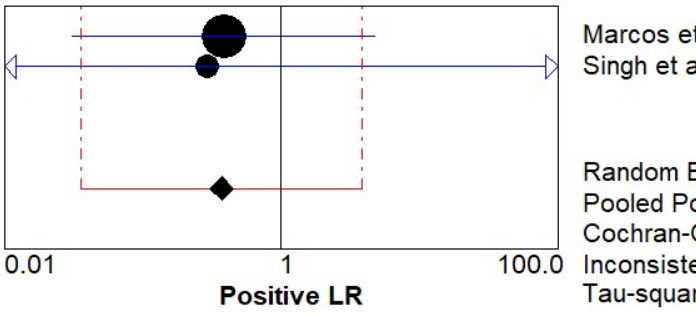


Figure 14 (a). Pooled +LR of MMP-2 for OSCC

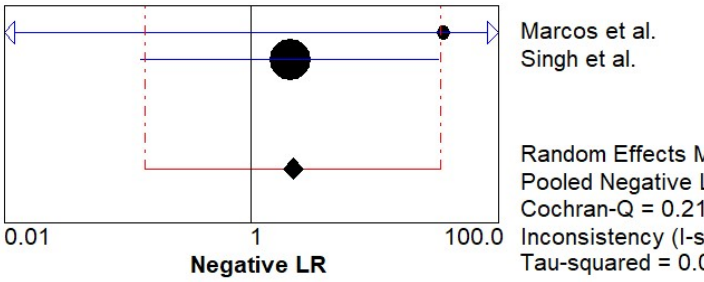


Figure 14 (b). Pooled -LR of MMP-2 for OSCC

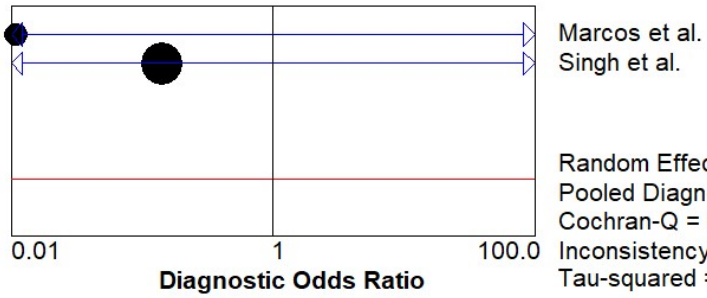


Figure 15. Pooled (DOR) of MMP-2 for OSCC

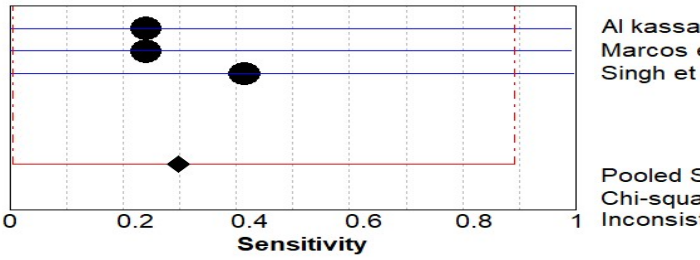


Figure 16 (a). Pooled sensitivity of MMP-9 for OSCC

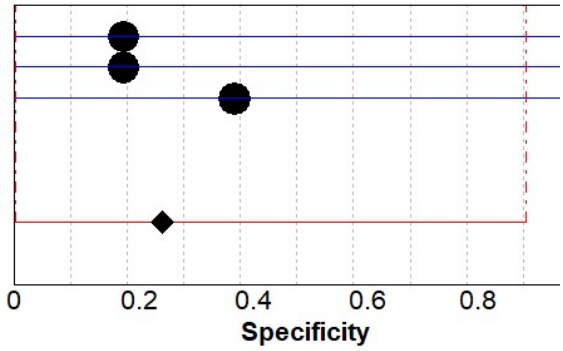


Figure 17 (b). Pooled specificity of MMP-9 for OSCC

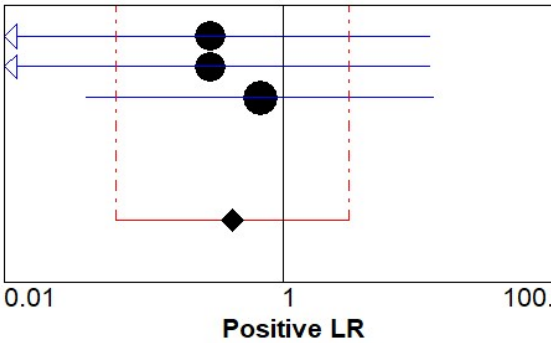


Figure 18 (a). Pooled +LR of MMP-9 for OSCC

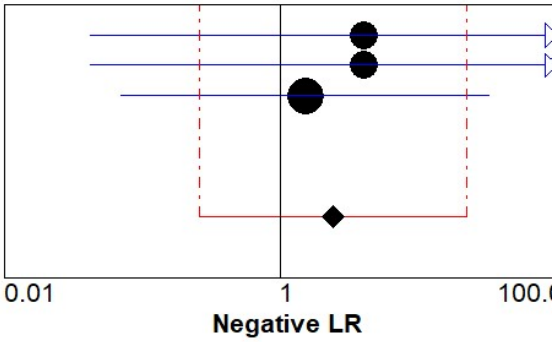


Figure 18 (b). Pooled -LR of MMP-9 for OSCC

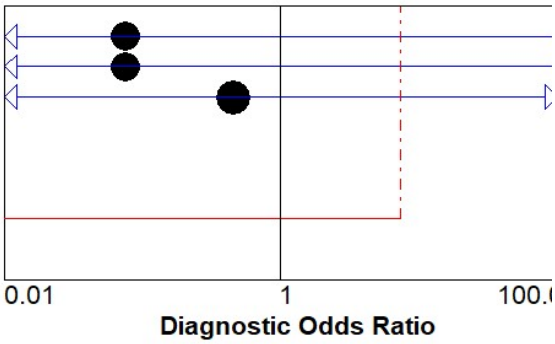


Figure 19. Pooled (DOR) of Serum biomarkers for OSCC

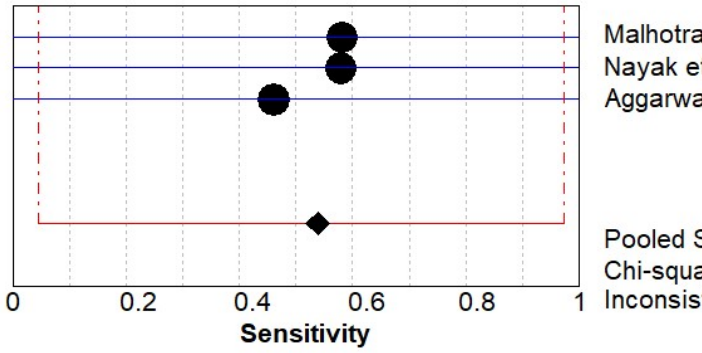


Figure 20 (a). Pooled sensitivity of VEGF for OSCC

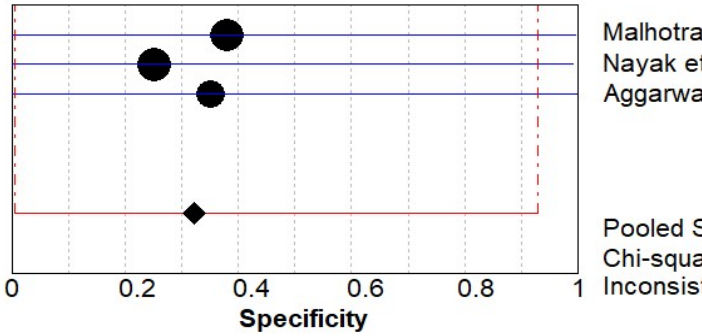


Figure 20 (b). Pooled specificity of VEGF for OSCC

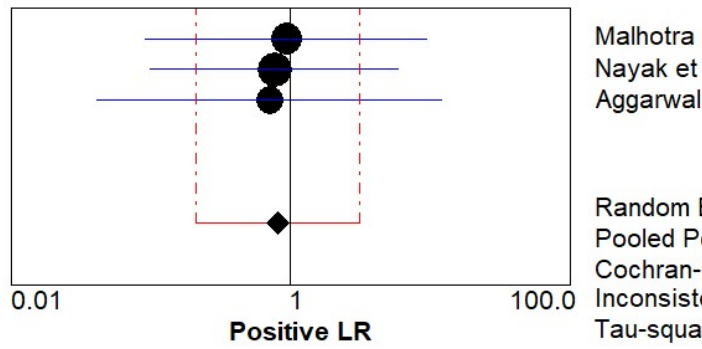


Figure 21 (a). Pooled +LR of VEGF for OSCC

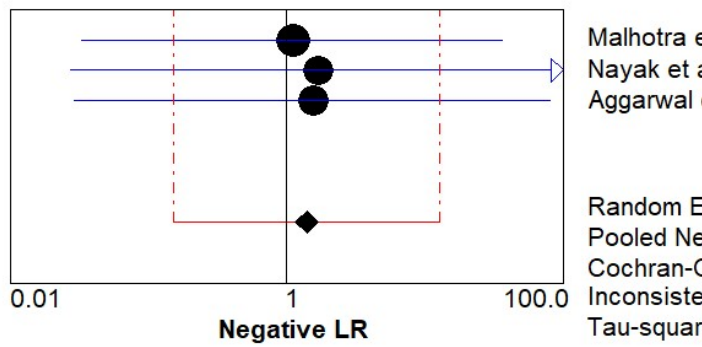


Figure 21 (b). Pooled -LR of VEGF for OSCC

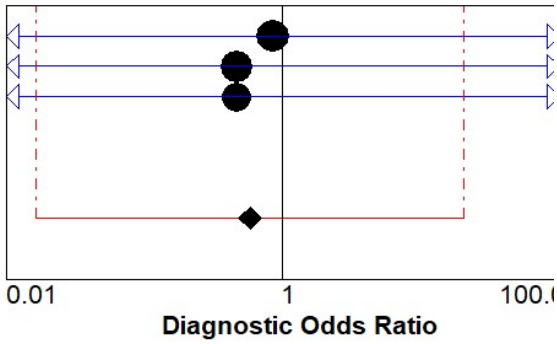


Figure 22. Pooled (DOR) of VEGF for OSCC

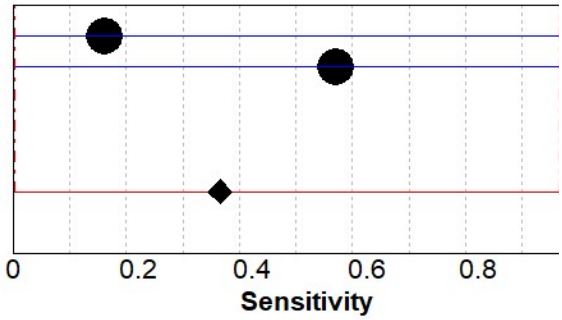


Figure 23 (a). Pooled sensitivity of GALECTIN 1 for OSCC

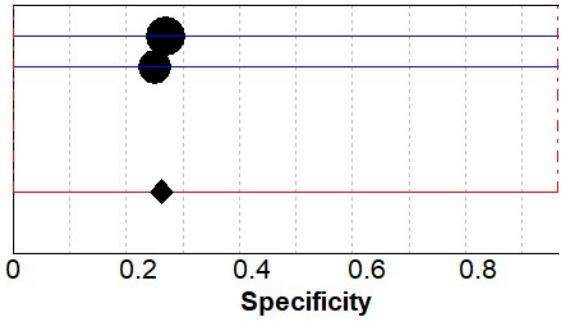


Figure 23 (b). Pooled specificity of GLECTIN 1 for OSCC

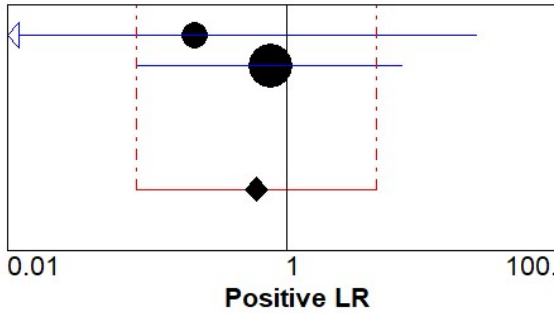


Figure 24 (a). Pooled +LR of GALECTIN 1 for OSCC

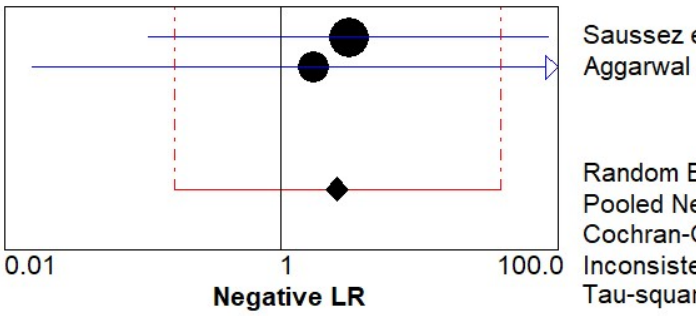


Figure 24 (a). Pooled -LR of GALECTIN 1 for OSCC

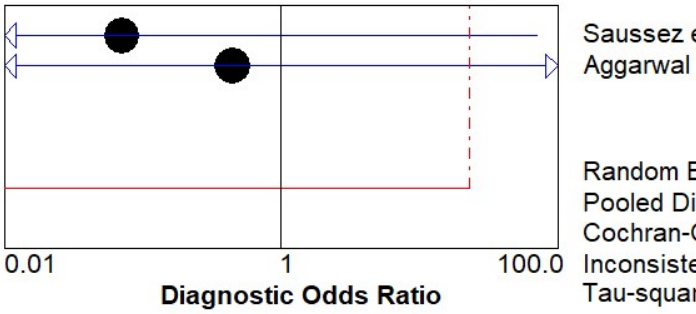


Figure 25. Pooled (DOR) of GALECTIN 1 for OSCC

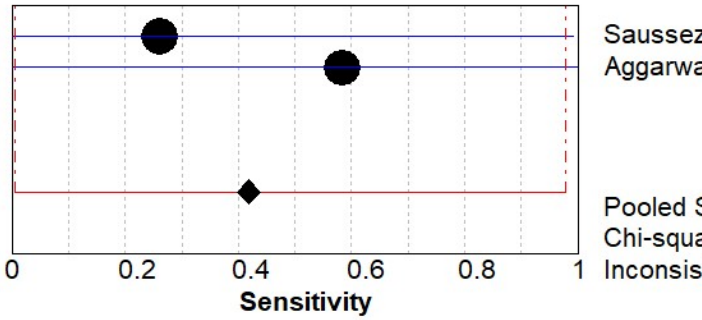


Figure 26 (a). Pooled sensitivity of GALECTIN 3 for OSCC

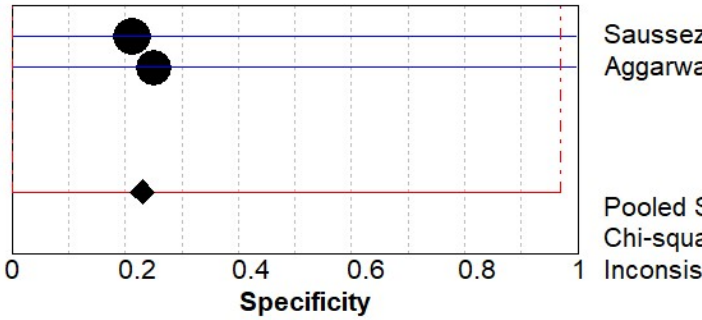


Figure 26 (b). Pooled specificity of GALECTIN 3 for OSCC

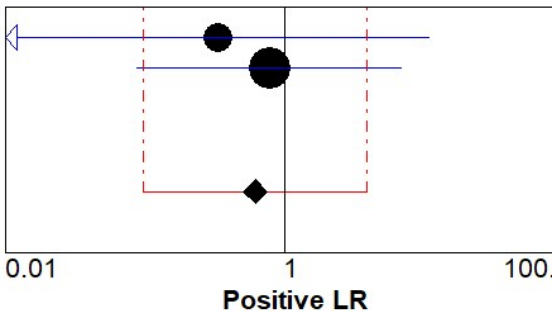


Figure 27 (a). Pooled +LR of GALECTIN 3 for OSCC

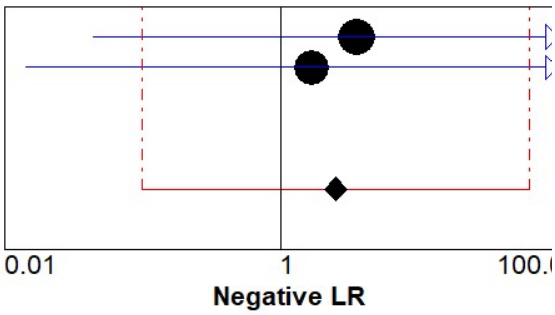


Figure 27 (b). Pooled -LR of GALECTIN 3 for OSCC

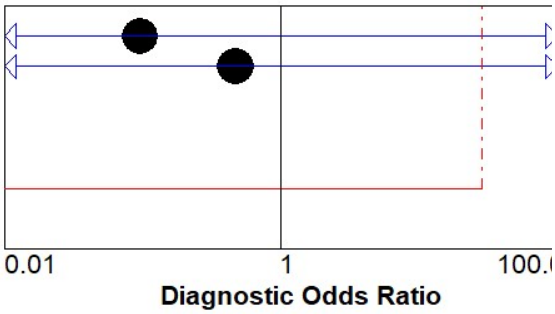


Figure 28. Pooled (DOR) of GALECTIN 3 for OSCC

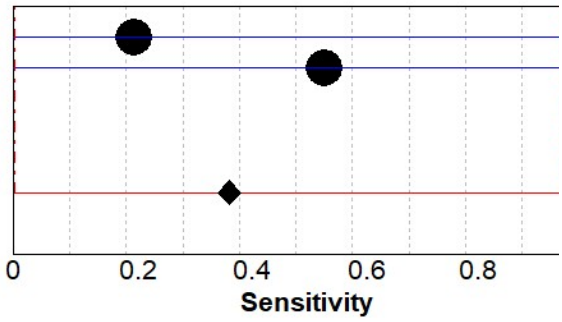


Figure 29 (a). Pooled sensitivity of IL-6 for OSCC

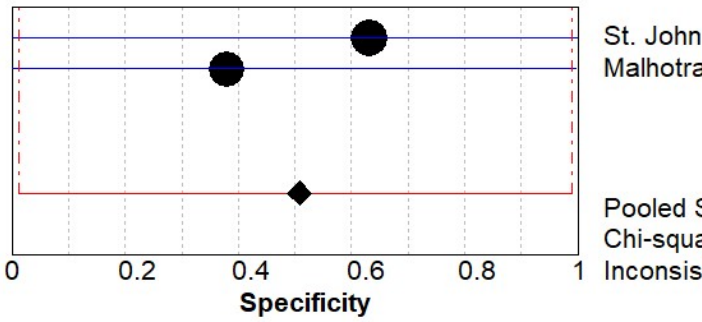


Figure 29 (b). Pooled specificity of IL-6 for OSCC

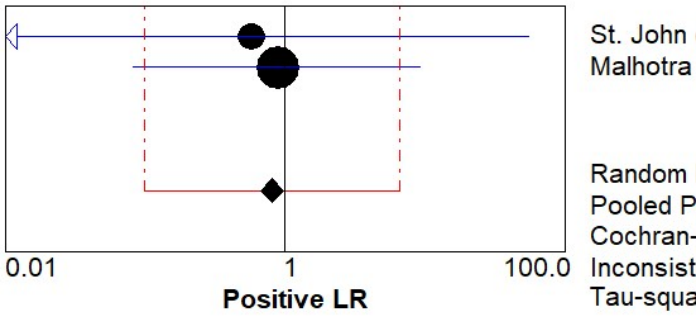


Figure 30 (a). Pooled +LR of IL-6 for OSCC

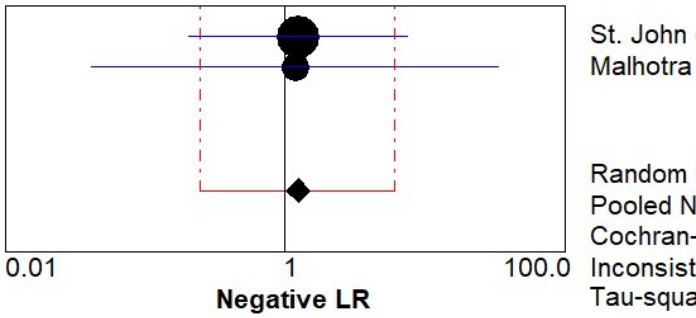


Figure 30 (b). Pooled -LR of IL-6 for OSCC

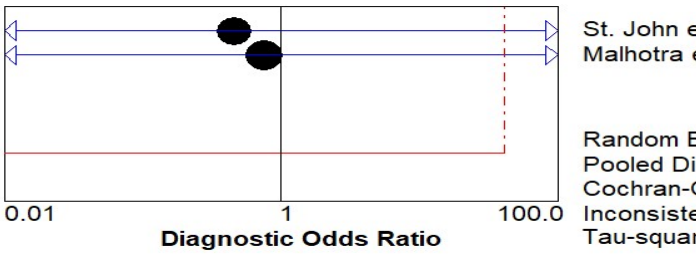


Figure 31. Pooled (DOR) of IL-6 for OSCC

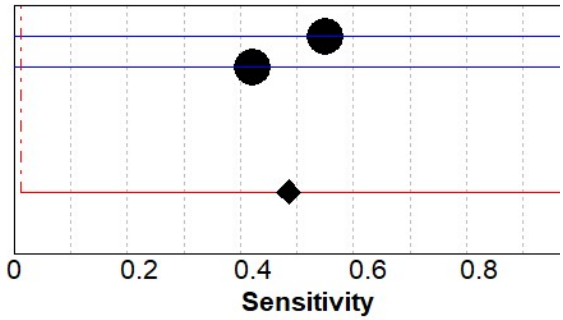


Figure 32 (a). Pooled sensitivity of IL-8for OSCC

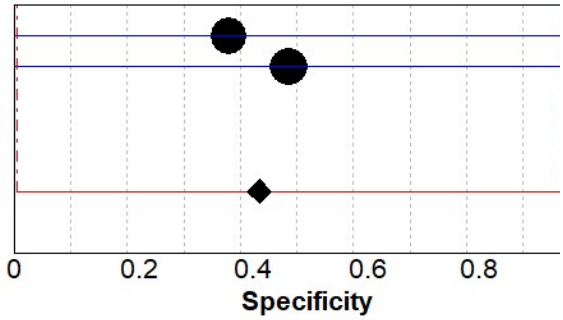


Figure 32 (b). Pooled specificity of IL-8 for OSCC

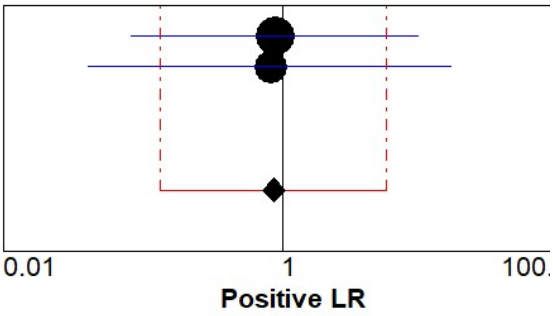


Figure 33 (a). Pooled +LR of IL-8 for OSCC

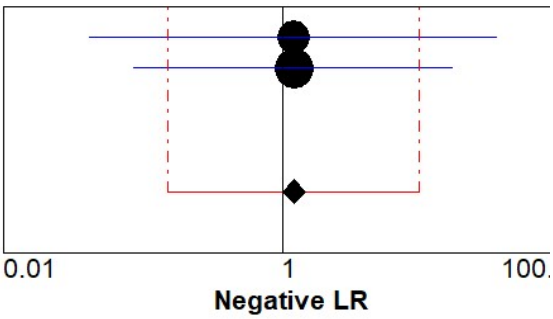


Figure 33 (b). Pooled -LR of IL-8 for OSCC

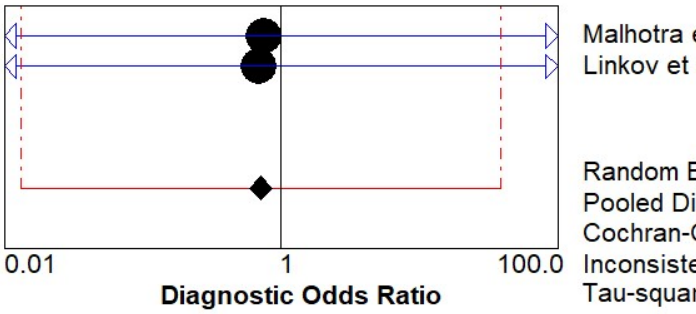


Figure 34. Pooled (DOR) of IL-8 for OSCC

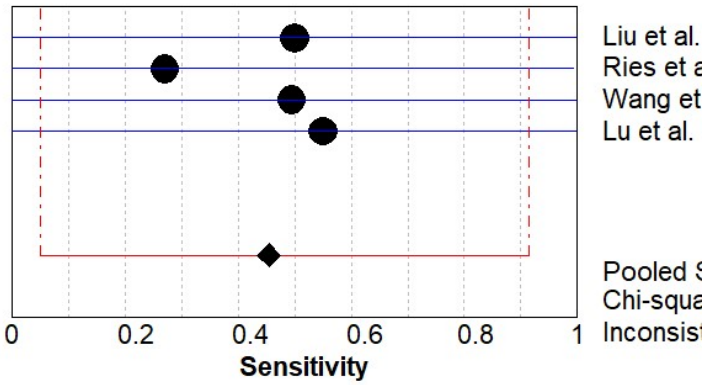


Figure 35 (a). Pooled sensitivity of miRNA for OSCC

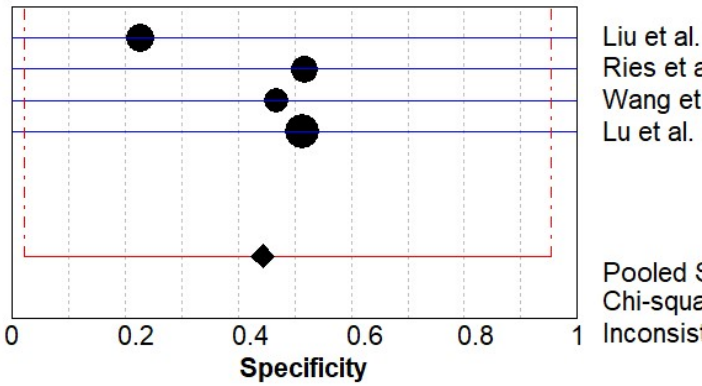


Figure 35 (b). Pooled specificity of miRNA for OSCC

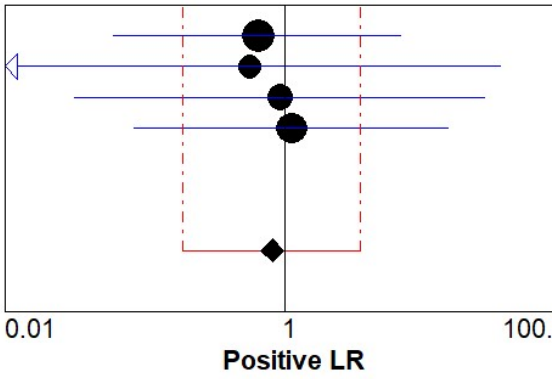


Figure 36 (a). Pooled +LR of miRNA for OSCC

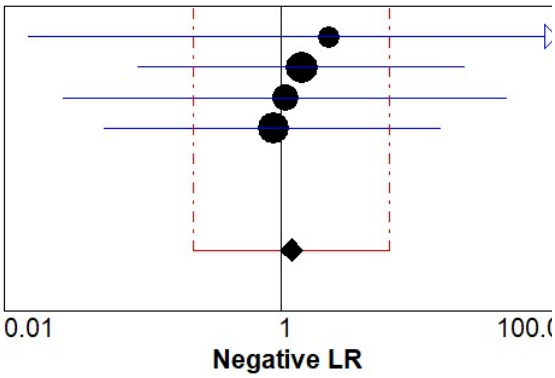


Figure 36 (b). Pooled -LR of miRNA for OSCC

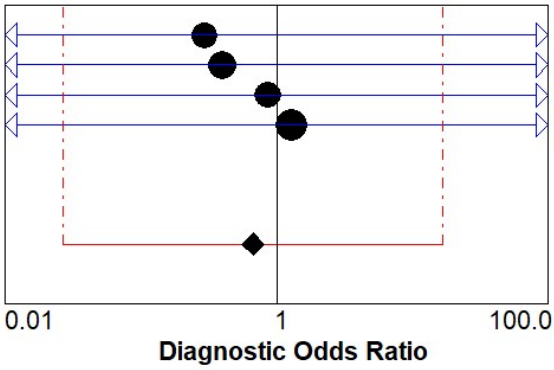


Figure 37. Pooled (DOR) of miRNA for OSCC

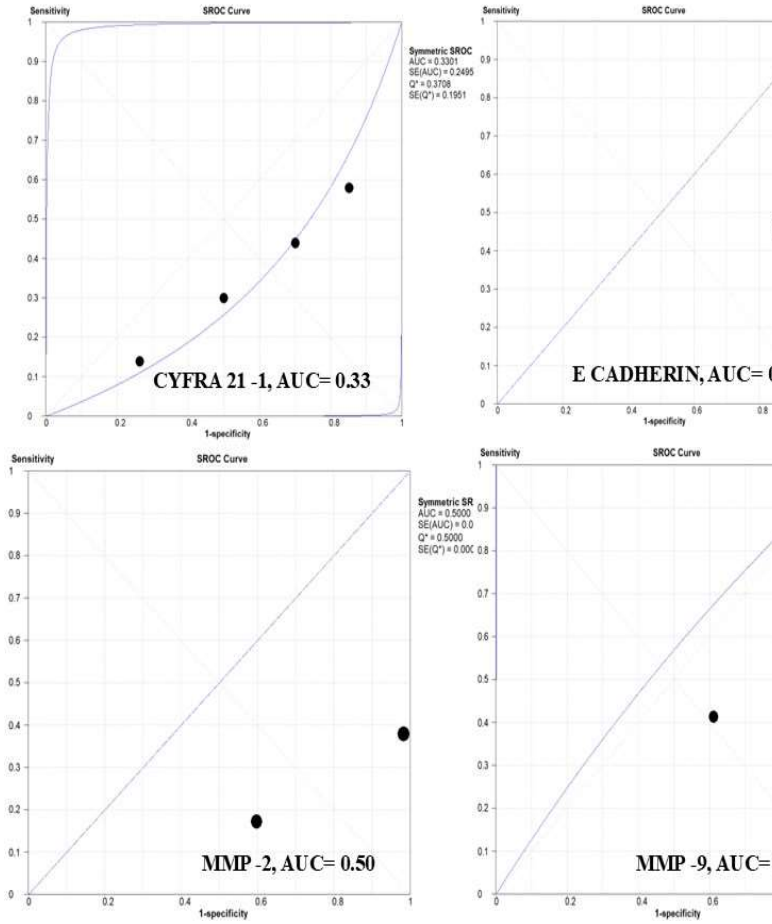


Figure 38 (a). overall accuracy of biomarkers evaluated

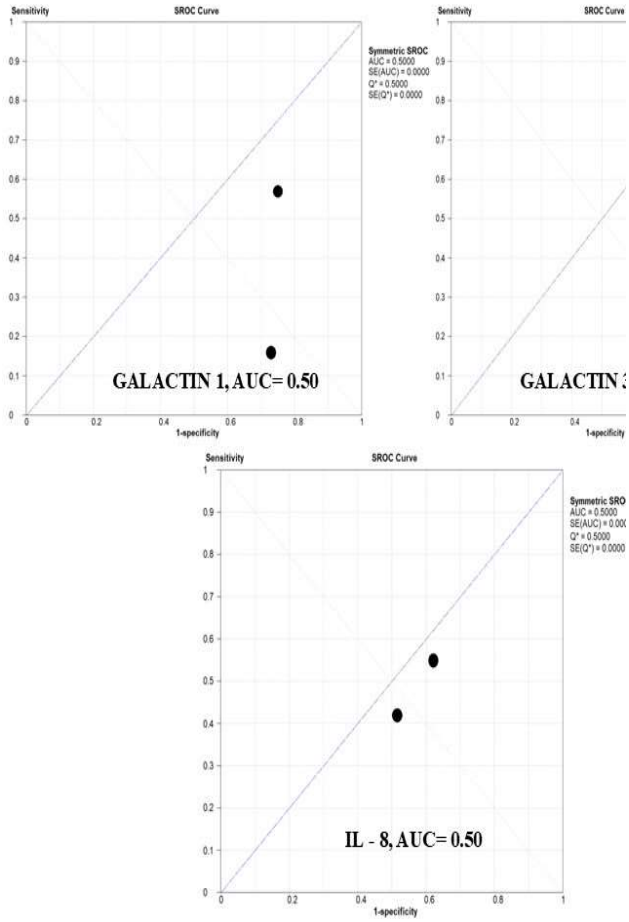


Figure 38 (b). overall accuracy of biomarkers evaluated

Conclusion

From the results of the study, it was concluded that serum biomarkers is a valid tool and overall has good diagnostic potentiality in diagnosing the target condition and can be used as an alternative adjunct to histopathology. It has great potential in predicting and diagnosing disease outcome and can improve the quality and reach of OSCC early screening and detection, Serum biomarkers can be undertaken for early diagnosis and prompt treatment under secondary level of prevention.

References

1. Jemal A, Siegel R, Ward E, Hao Y, Xu J, Thun MJ. Cancer statistics, 2009. CA Cancer J Clin. 2009; 59:225-49.
2. Tanaka T, Ishigamori R. Understanding carcinogenesis for fighting oral cancer. J Oncol. 2011;2011:603740.
3. Mishra R. Biomarkers of oral premalignant epithelial lesions for clinical application. Oral Oncol. 2012;48:578-84.
4. Gupta PC, Murti PR, Bhonsle RB, Mehta FS, Pindborg JJ. Effect of cessation of tobacco use on the incidence of oral mucosal lesions in a 10-yr follow-up study of 12,212 users. Oral Dis. 1995;1:54-8.
5. Bundgaard T, Bentzen SM, Wildt J. The prognostic effect of tobacco and alcohol consumption in intra-oral squamous cell carcinoma. Eur J Cancer B Oral Oncol. 1994;30B:323-8.
6. Sawant SS, Zingde SM, Vaidya MM. Cytokeratin fragments in the serum: Their utility for the management of oral cancer. Oral Oncol. 2008;44:722-32.
7. Bijian K, Mlynarek AM, Balys RL, Jie S, Xu Y, Hier MP, et al. Serum proteomic approach for the identification of serum biomarkers contributed by oral squamous cell carcinoma and host tissue micro-environment. J Proteome Res. 2009;8:2173-85.
8. Cordes C, Von Lingen J, Gorogh T, Ambrosch P, Gottschlich S, Hoffmann M. Molecular and immunological aspects of p53 and p53- autoantibodies in head and neck squamous cell carcinoma. Oncol Rep. 2009;22:1299-303.
9. Kato H, Torigoe T. Radioimmunoassay for tumor antigen of human cervical squamous cell carcinoma. Cancer. 1977;40:1621-8.

10. Hoffmann-Fazel A, Hoffmann M, Gottschlich S, Maass JD, Rudert H, Maune S. Cyfra 21-1 in diagnosis of distant metastases of head and neck carcinoma. Anticancer Res. 2003;23:917-20.
11. Joshi M, Patil R. Estimation and comparative study of serum total sialic acid levels as tumor markers in oral cancer and precancer. J Cancer Res Ther. 2010;6:263-6.
12. Doweck I, Barak M, Uri N, Greenberg E. The prognostic value of the tumour marker cyfra 21-1 in carcinoma of head and neck and its role in early detection of recurrent disease. Br J Cancer. 2000;83:1696- 701.
13. Malhotra M, Shaw AK, Priyadarshini SR, Metha SS, Sahoo PK, Gachake A. Diagnostic Accuracy of Artificial Intelligence Compared to Biopsy in Detecting Early Oral Squamous Cell Carcinoma: A Systematic Review and Meta Analysis. Asian Pacific Journal of Cancer Prevention: APJCP. 2024;25(8):2593.
14. Shaw AK, Garcha V, Shetty V, Vinay V, Bhor K, Ambildhok K, et al. Diagnostic accuracy of salivary biomarkers in detecting early oral squamous cell carcinoma: A systematic review and meta-analysis. Asian Pac J Cancer Prev. 2022;23(5):1483–95.
15. Shaw AK, Mahajan M, Varshney S, Jena M, Rohatgi L, Bashir S, Tewari S, Roy J. Diagnostic accuracy of chemiluminescence for oral potentially malignant disorders: A systematic review and meta-analysis. J Clin Diagn Res. 2022;16:7.
16. Salameh JP, Bossuyt PM, McGrath TA, Thombs BD, Hyde CJ, Macaskill P, et al. Preferred reporting items for systematic review and meta-analysis of diagnostic test accuracy studies (prisma-dta): Explanation, elaboration, and checklist. BMJ. 2020;370:m2632.
17. Whiting PF, Rutjes AW, Westwood ME, Mallett S, Deeks JJ, Reitsma JB, et al. Quadas-2: A revised tool for the quality assessment of diagnostic accuracy studies. Ann Intern Med. 2011;155(8):529–36.
18. Jones CM, Athanasiou T. Summary receiver operating characteristic curve analysis techniques in the evaluation of diagnostic tests. Ann Thorac Surg. 2005;79(1):16–20
19. Lijmer JG, Bossuyt PM, Heisterkamp SH. Exploring sources of heterogeneity in systematic reviews of diagnostic tests. Stat Med. 2002;21(11):1525–37.
20. Grimes DA, Schulz KF. Refining clinical diagnosis with likelihood ratios. Lancet. 2005;365(9469):1500–5.
21. Ayude D, Gacio G, Páez De La Cadena M, Pallas E, Martínez-Zorzano VS, De Carlos A, Rodríguez-Berrocal FJ. Combined use of established and novel tumour markers in the diagnosis of head and neck squamous cell carcinoma. Oncology reports. 2003 Sep 1;10(5):1345-50.
22. Deng YF, Chen P, Lin YZ, Le JZ, Wu XL, Yu MQ, Zhuang PY, Gao MH. Analytical and clinical evaluation of CYFRA 21-1 by electrochemiluminescent immunoassay in head and neck squamous cell carcinoma. The Journal of Laryngology & Otology. 2003 Mar;117(3):190-4.
23. St. John MA, Li Y, Zhou X, Denny P, Ho CM, Montemagno C, Shi W, Qi F, Wu B, Sinha U, Jordan R. Interleukin 6 and interleukin 8 as potential biomarkers for oral cavity and oropharyngeal squamous cell carcinoma. Archives of Otolaryngology–Head & Neck Surgery. 2004 Aug 1;130(8):929-35.
24. Céruse P, Rabilloud M, Charrié A, Dubreuil C, Disant F. Study of Cyfra 21–1, a tumor marker, in head and neck squamous cell carcinoma. Annals of Otology, Rhinology & Laryngology. 2005 Oct;114(10):768-76.
25. Cheng AJ, Chen LC, Chien KY, Chen YJ, Chang JT, Wang HM, Liao CT, Chen IH. Oral cancer plasma tumor marker identified with bead-based affinity-fractionated proteomic technology. Clinical Chemistry. 2005 Dec 1;51(12):2236-44.
26. Al Kassam D, Blanco I, de Los Toyos JR, Llorente JL. Diagnostic value of E-cadherin, MMP-9, activated MMP-13 and anti-p53 antibodies in squamous cell carcinomas of head and neck. Medicina Clinica. 2007 Dec 1;129(20):761-5.
27. Linkov F, Lisovich A, Yurkovetsky Z, Marrangoni A, Velikokhatnaya L, Nolen B, Winans M, Bigbee W, Siegfried J, Lokshin A, Ferris RL. Early detection of head and neck cancer: development of a novel screening tool using multiplexed immunobead-based biomarker profiling. Cancer Epidemiology Biomarkers & Prevention. 2007 Jan
28. Cheng S, Low I, Ng D, Brasch H, Sullivan M, Davis P, Tan ST. Epidermal growth factor receptor: a novel biomarker for aggressive

head and neck cutaneous squamous cell carcinoma. Human pathology. 2008 Mar 1;39(3):344-9.

29. Saussez S, Lorfevre F, Lequeux T, Laurent G, Chantrain G, Vertongen F, Toubeau G, Decaestecker C, Kiss R. The determination of the levels of circulating galectin-1 and-3 in HNSCC patients could be used to monitor tumor progression and/or responses to therapy. Oral oncology. 2008 Jan 1;44(1):86-93.
30. Gourin CG, Zhi W, Adam BL. Proteomic identification of serum biomarkers for head and neck cancer surveillance. The Laryngoscope. 2009 Jul;119(7):1291-302.
31. Marcos CÁ, Martínez DA, de Los Toyos JR, Domínguez Iglesias F, Hermsen M, Guervós MA, Pendás JL. The usefulness of new serum tumor markers in head and neck squamous cell carcinoma. Otolaryngology—Head and Neck Surgery. 2009 Mar;140(3):375-80.
32. Liu CJ, Kao SY, Tu HF, Tsai MM, Chang KW, Lin SC. Increase of microRNA miR-31 level in plasma could be a potential marker of oral cancer. Oral diseases. 2010 May;16(4):360-4.
33. Singh RD, Nilayangode H, Patel JB, Shah FD, Shukla SN, Shah PM, Patel PS. Combined evaluation of matrix metalloproteinases and their inhibitors has better clinical utility in oral cancer. The International journal of biological markers. 2011 Jan;26(1):27-36.
34. Malhotra R, Patel V, Chikkaveeraiah BV, Munge BS, Cheong SC, Zain RB, Abraham MT, Dey DK, Gutkind JS, Rusling JF. Ultrasensitive detection of cancer biomarkers in the clinic by use of a nanostructured microfluidic array. Analytical chemistry. 2012 Jul 17;84(14):6249-55.
35. Nayak S, Goel MM, Chandra S, Bhatia V, Mehrotra D, Kumar S, Makker A, Rath SK, Agarwal SP. VEGF-A immunohistochemical and mRNA expression in tissues and its serum levels in potentially malignant oral lesions and oral squamous cell carcinomas. Oral oncology. 2012 Mar 1;48(3):233-9.
36. Rajkumar K, Ramya R, Nandhini G, Rajashree P, Ramesh Kumar A, Nirmala Anandan S. Salivary and serum level of CYFRA 21-1 in oral precancer and oral squamous cell carcinoma. Oral diseases. 2015 Jan;21(1):90-6.
37. Aggarwal S, Devaraja K, Sharma SC, Das SN. Expression of vascular endothelial growth factor (VEGF) in patients with oral squamous cell carcinoma and its clinical significance. Clinica Chimica Acta. 2014 Sep 25;436:35-40.
38. Ries J, Vairaktaris E, Agaimy A, Kintopp R, Baran C, Neukam FW, Nkenke E. miR-186, miR-3651 and miR-494: potential biomarkers for oral squamous cell carcinoma extracted from whole blood. Oncology reports. 2014 Mar 1;31(3):1429-36.
39. Wang J, Zhou Y, Lu J, Sun Y, Xiao H, Liu M, Tian L. Combined detection of serum exosomal miR-21 and HOTAIR as diagnostic and prognostic biomarkers for laryngeal squamous cell carcinoma. Medical oncology. 2014 Sep;31:1-8.
40. Aggarwal S, Sharma SC, Das SN. Galectin-1 and galectin-3: Plausible tumour markers for oral squamous cell carcinoma and suitable targets for screening high-risk population. Clinica Chimica Acta. 2015 Mar 10;442:13-21.
41. Lu YC, Chang JT, Huang YC, Huang CC, Chen WH, Lee LY, Huang BS, Chen YJ, Li HF, Cheng AJ. Combined determination of circulating miR-196a and miR-196b levels produces high sensitivity and specificity for early detection of oral cancer. Clinical biochemistry. 2015 Feb 1;48(3):115-21.
42. Fan C, Wang J, Tang Y, Zhang S, Xiong F, Guo C, Zhou Y, Li Z, Li X, Li Y, Li G. Upregulation of long non-coding RNA LOC284454 may serve as a new serum diagnostic biomarker for head and neck cancers. BMC cancer. 2020 Dec;20:1-9.
43. Karimi A, Bahrami N, Sayedyahossein A, Derakhshan S. Evaluation of circulating serum 3 types of microRNA as biomarkers of oral squamous cell carcinoma; A pilot study. Journal of Oral Pathology & Medicine. 2020 Jan;49(1):43-8.
44. Fernández-Olavarría A, Mosquera-Pérez R, Díaz-Sánchez RM, Serrera-Figallo MA, Gutiérrez-Pérez JL, Torres-Lagares D. The role of serum biomarkers in the diagnosis and prognosis of oral cancer: A systematic review. Journal of clinical and experimental dentistry. 2016 Apr;8(2):e184.
45. Guerra EN, Rego DF, Elias ST, Coletta RD, Mezzomo LA, Gozal D, Canto GD. Diagnostic accuracy of serum biomarkers for head and neck cancer: A systematic review

and meta-analysis. Critical reviews in oncology/hematology. 2016 May 1;101:93-118.

46. Rezaei F, Mozaffari HR, Tavasoli J, Zavattaro E, Imani MM, Sadeghi M. Evaluation of serum and salivary interleukin-6 and interleukin-8 levels in oral squamous cell carcinoma patients: systematic review and meta-analysis. Journal of Interferon & Cytokine Research. 2019 Dec 1;39(12):727-39.
47. Marakala V. Head and neck cancer biomarkers: Systematic review and meta-analysis. Clinica Chimica Acta. 2023 Mar 1;542:117280.
48. Smidt N, Rutjes AW, Van Der Windt DA, Ostelo RW, Bossuyt PM, Reitsma JB, Bouter LM, De Vet HC. Reproducibility of the STARD checklist: an instrument to assess the quality of reporting of diagnostic accuracy studies. BMC Medical Research Methodology. 2006 Dec;6:1-0.
49. Singh N, Sharma S, Varshney SR, Bhatnagar D, Tomer G, Sethy P, et al. Oral health-related quality of life among adults undergoing orthodontic procedure with ceramic braces versus aligners. Bioinformation. 2025 May 31;21(5):1128–1134. doi:10.6026/973206300211128. PMID: 40822804; PMCID: PMC12357681.
50. Singh R, Singh N, Chauhan S, Siddiqui LT, Bhola L, Sale MS, et al. Correlation between endo-perio lesions and systemic diseases: A cross-sectional study. Bioinformation. 2025 May 31;21(5):1144–1148. doi:10.6026/973206300211144. PMID: 40822794; PMCID: PMC12357700.
51. Shah A, Sikarwar V, Joshi A, Singh N, Singh A, Kardam S. Pediatric facial fracture in Garhwal Himalayan region of Uttarakhand state: A retrospective study. J Pharm Bioallied Sci. 2024 Feb;16(Suppl 1):S912–S914. doi:10.4103/jpbs.jpbs_1098_23.
52. Chandra H, Singh N, Shah A. Correlation between periodontitis and hyperlipidemia: A cross sectional study. Neuroquantology. 2022;20(11):7510–7518.