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Role of Salivary Biomarkers for Early Oral Cancer Detection: A Review

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1. Abstract

1.1. Background: Oral cancer is one of the most common diseases and is a growing health problem in many countries around the world and the present stage, there is a steady increase in the incidence of malignant neoplasms.

For early detection of oral cancer, many non-invasive diagnostic index tests have been proposed as adjuncts to traditional screening examination to improve diagnostic test accuracy. Recent data suggest that the inclusion of biomarkers for early detection in several original research investigations for the diagnosis and management of patients with oral neoplasms, as it improves prognosis, therapy, and follow-up. However, there is no clear evidence about the most informative marker for early diagnosis oral cancer.

1.2. Objectives: Evaluate the diagnostic significance of salivary biomarkers for early diagnosis of squamous cell cancer of the oral cavity.

1.3. Methods: This analysis presents the role of potential saliva biomarkers for the early diagnosis of precancerous and cancerous oral lesions and monitoring disease activity. The search was conducted in PubMed, Scopus и Web of Scienc,Google Scholar, EBSCO host from 2015 to 2024 to determine the screening potential of salivary biomarkers. According to the review method used, the PRISMA. Conducted a preliminary search and reviewed 132 titles and abstracts in this review and 64 full-text articles were selected of high methodological quality.

1.4. Inclusion criteria: Included clinical trials, considered randomized controlled trials, cross-sectional studies, case-control studies, and cohort studies in human subjects that evaluated the current literature on the "Oral cancer", "oral squamous cell carcinoma, "head and neck carcinoma", "Biomarker", "Diagnostics", "Saliva", "salivary biomarker" written in English articles.There was no limitation on minimal quality, minimal sample size, or the number of patients.

1.5. Exclusion Criteria Were: original primary studies, due to language limitations, abstracts, letters to the editor, book chapters, case reports, conference abstracts, duplicate publications, and in vitro and in vivo animal experimental studies.

1.6. Main Outcome: The primary outcomes were sensitivity (SEN), specificity (SPE), positive predictive value (PPV), negative predictive value (NPV). Secondary outcomes were the relative diagnostic assessments of the different biomarkers.

1.7. Results: The total sample size for this analysis included 64 studies. Diagnostic accuracy (sensitivity, specificity 95%) The sensitivity and specificity of the biomarkers varied widely from 48 to 96% and from 34 to 100%, respectively.

The current systematic review demonstrated high sensitivity and specificity values for TNF-α, IL-1β, IL-6, IL-8, LDH and MMP-9 and are the most promising salivary biomarkers. Cytokines IL-6, IL-8 and TNF-α are found in higher concentrations in the saliva of patients with oral cancer than in healthy individuals and may therefore serve as candidate biomarkers for oral cancer.

1.8. Conclusion: Salivary biomarkers promise to have a significant impact on the earliest identification of oral carcinoma, cancer screening, and significant improvement in oral cancer treatment

outcomes. However, more studies are needed before applying these biomarkers in clinical settings.

2. Introduction

The trend of oral cancer incidence makes this pathology one of the urgent problems of public health in the whole world. According to Singh et al., 2020, Ho et al., 2019, oral cancer (OR) ranks 11th among the most frequently diagnosed types of cancer and represents a serious health problem [1]. More than 90% of oral cancer arise from the epithelium of the mucous membrane [2,3]. The lower lip, tongue and bottom of the mouth are the main sites of primary tumor localization in more than 75% of patients. The main risk factors are smoking and alcohol consumption, so prevention of these risk factors is important.

At an early stage, oral cavity cancer is often asymptomatic and mimics benign diseases, which reduces the patient's access to cancer treatment.

Early detection strategies include early diagnosis and screening [4].

1. early diagnosis, i.e. detection of symptomatic cancer in patients.

2. screening, which is the detection of an asymptomatic disease among the practically healthy target group of the population.

Early diagnosis implies activity in asymptomatic populations, aimed at revealing clinical signs and symptoms in the early stages of the disease, while delayed diagnosis consists in detecting the lesion on the basis of diagnostic tests. The purpose of screening and early detection of cancer is to treat cancer by detecting malignant neoplasms at an early stage, before symptoms appear [5]. Since oral cancer significantly reduces the quality of life of patients, affects their health and social adaptation, the problem of early diagnosis of oral cancer and its differential diagnosis with precancerous diseases turns from a medical to a socio-economic one, which requires special solutions to reduce mortality. The difficulty of early detection of early mouth cancer, in late diagnosis, leads to the survival rate being stagnant at around 50% for several decades. A sufficiently high percentage of diagnostic errors and the lack of uniform diagnostic approaches modernize the formation of oncological awareness in the professional environment of dentists, the strengthening of the oncological component in the preventive work of dentists.

Monitoring of the patient is very important for safe complex antitumor treatment, as it helps to identify side effects at an early stage, prevents serious complications and possible hospitalization. Based on the above, the most promising direction is the improvement of non-invasive and simple, accessible diagnostic methods when visiting the dentist and oncology to reduce both false-positive and false-negative results, which affect the diagnosis and subsequent treatment. For the early detection of oral cancer, many non-invasive tests have been proposed as a supplement to the traditional screening examination (COE) to increase the accuracy of diagnostic tests [6,7].

•Vital staining (toluidine blue, tolonium chloride)

•Oral cytology (for example, brush biopsy OralCDx)

•Light detection (for example, ViziLite, Microlux/DL, VELscope, Orascoptic DK, Identafi 3000) and spectroscopy of the oral cavity

•Analysis of blood and saliva.

The presence of various diagnostic methods of newly formed mucosa of the oral cavity indicates the absence of a clear algorithm for their application, objective assessment of the importance and sequence of each stage of diagnosis [8]. Many screening programs include visual oral examination (VOE), which can reduce oral cancer mortality among high-risk adult groups through early diagnosis and treatment. Visual screening for oral cancer (VOE) includes visual examination of the oral cavity, palpation when oral cancer is suspected, and assessment of lymph node enlargement followed by diagnostic testing, including tissue staining with toluidine blue, autofluorescence spectroscopy, biopsy, and final histopathological diagnosis if necessary [9].

The "gold standard" for the diagnosis of OSCC is visual examination of the oral cavity of suspicious lesions and histological examination of tissue biopsy of suspicious lesions [10]. However, biopsy can have negative psychological consequences for patients. Among the alternative diagnostic methods used for the diagnosis of oral cavity cancer, immunohistochemistry, oncomarkers, immunohistopathological diagnostics, and chemiluminescence are distinguished[10-13]. The possibilities of clinical assessment have been extended by using methods of polymerase chain reaction (PCR), magnetic resonance imaging (MRT) and computer tomography (CT), such as single-photon emission computer tomography (SPECT)[14-16]. Other new noninvasive detection tools include positron emission tomography (PET) and nuclear medicine imaging techniques, multispectral narrowband imaging, Raman spectroscopy, confocal laser endomicroscopy (CLE), and infrared thermal imaging [17-19]. Tumor markers are molecules that indicate the presence of cancer and can be used to detect early malignant neoplasms, can help in differential diagnosis of benign and malignant diseases, assessment of prognosis and postoperative monitoring[20-21]. One of the most proven screening markers is the use of fecal occult blood test (FOBT) in colorectal cancer (CRC), prostate-specific antigen (PSA) in prostate cancer screening, CA 125 in ovarian cancer screening [22-24].Tumor markers include various substances, such as cell surface antigens, cytoplasmic proteins, enzymes, hormones, oncofetal antigens, receptors, oncogenes and their products [25].

Different markers are also used to diagnose oral cancer,classification of oral cancer biomarkers shown in the diagram (Figure 1).

Oncogenes, anti-oncogenes, cytokines, growth factors, markers of epithelial-mesenchymal transition, epithelial tumor factors, cytokeratins, etc. are used for diagnosis of newly formed oral cavity, as

well as stages of development [26-28]. Currently, research is being conducted to find less invasive and cost-effective methods that will allow easier monitoring of its progression; many non-invasive methods, such as liquid biopsy, have been proposed [29,30]. In light of the above, this review aims to evaluate the latest data on the use of various saliva biomarkers for OSCC diagnosis, prognosis, therapy monitoring. The current challenges and prospects for the use of saliva biomarkers in the early diagnosis and treatment monitoring of OSCC are also discussed.

Saliva is the safest, least invasive, non-coagulable and cost-effective biofluid used in clinical diagnostics. Collecting saliva is easy for both the patient and the doctor and the resulting sample allows for easy monitoring of various biomarkers in oncological patients. Saliva is involved in many physiological and pathological processes and on the basis of its unique and informative properties in recent decades, saliva has been widely studied as a promising biomarker of OSCC for liquid biopsy. More than 100 salivary biomarkers (DNA, RNA, mRNA, protein markers) have already been identified, including cytokines (IL-8, IL-1b, TNF-α), defensin-1, P53, Cyfra 21-1, profilin, cofilin-1, transferrin and many others [31]. Saliva has advantages over other samples that include the following: (i) it reflects any physiological and pathological changes at local and distant sites of the body; (ii) it is a simpler, faster and more accessible screening tool; and (iii) saliva can be used to collect large volumes of samples for testing, perform an unlimited number of repeat tests and monitor OSCC over time [32]. It is known that cancer develops as a result of different types of DNA changes, the analysis of liquid biopsy allows to determine the molecular profile of DNA of cancer patients, the inclusion of which in the staging system of tumors, nodes and metastases can help to develop more personalized treatment and reduce the risks of inappropriate treatment [33].

In OSCC patients, ctDNA is released from cancer cells into the bloodstream; however, it can also be detected in other body fluids including saliva[34,35]. Studies have also shown that ctDNA concentrations in cancer patients reflect many characteristics of the cancer (size, cell turnover, stage, vascularization, and drug response [36,37].

ctDNA can easily reach saliva from the local site and bloodstream, carrying information about primary tumors and/or metastases[32,37].ctDNA analysis in saliva is much more sensitive than in the bloodstream due to less dilution and contamination [38]. ctDNA analysis in saliva is much more sensitive than in the bloodstream due to less dilution and contamination [38]. Study in saliva of patients with oral cavity, oropharynx tumors at early stages (I and II) ctDNA was detected in 100% of patients and in 95% of patients included in the study at advanced stages; this result is highly specific for the detection of OSCC [39,40]. The use of ctDNA as salivary biomarkers will help develop early diagnosis strategies in cancer patients, facilitate early prevention, and facilitate the development of targeted treatments. Saliva in combination with blood reflects the levels of hormonal, immunological, toxicological and infectious markers of diseases. Saliva biomarkers can be used for oral cancer screening, as they are non-invasive and administered on an outpatient basis [41-45]. Over the past few decades, exosome-based liquid biopsies have become increasingly popular due to their convenience, non-invasiveness, time-saving and reliability, reproducibility, ease of early detection, low cost, and high utility, which allows continuous sampling to obtain tumor information [46]. Salivary exosomes in patients with oral cancer as potential biomarkers for the diagnosis and ideal method for early screening of oral cancer. (Figure 2) provides a summary of salivary exosomes as a source of biomarkers for the diagnosis of oral cancer. In the study of salivary biomarker expression in patients with oral neoplasms, statistically significant differences were expressed and the diagnostic characteristics of a non-invasive saliva test: sensitivity, specificity, efficiency were determined. Conducted a systematic review of the literature evaluating salivary cytokines (SC) as potential diagnostic biomarkers for oral cancer.

10 different salivary cytokines are described, of which IL-8 and IL-6 are the most studied. Meta-analysis showed that salivary levels of IL-8, IL-6, TNF-α, IL-1β, and IL-1α cytokines were significantly higher in OC patients compared to healthy controls.47-50

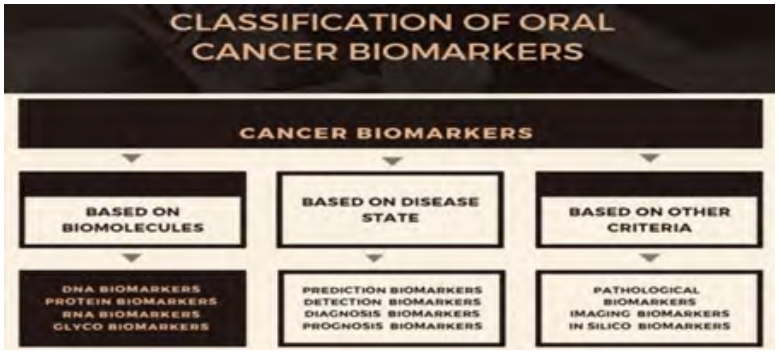

Figure 1: Classification of oral cancer biomarkers

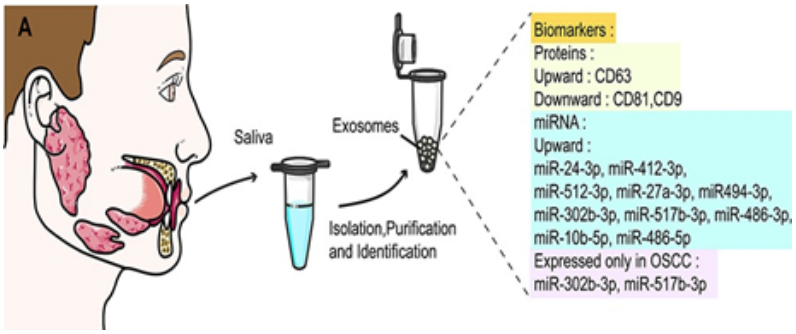

Figure 2: Salivary exosomes in patients with oral cancer

3. Methods

This analysis presents the role of potential saliva biomarkers for the early diagnosis of precancerous and cancerous oral lesions and monitoring disease activity. The search was conducted in PubMed, Scopus и Web of Scienc,Google Scholar, EBSCO host from 2000 to 2024 to determine the screening potential of salivary biomarkers.

3.1. Types of Studies

The selection of articles is demonstrated in the PRISMA flow chart (Table 1).

Inclusion criteria: included clinical trials, considered randomized controlled trials, cross-sectional studies, case-control studies, and cohort studies in human subjects that evaluated the current literature on the "Oral cancer", "oral squamous cell carcinoma, "head and neck carcinoma", "Biomarker", "Diagnostics", "Saliva", "salivary biomarker" written in English articles.There was no limitation on minimal quality, minimal sample size, or the number of patients.

Exclusion criteria were: original primary studies, due to language limitations , abstracts, letters to the editor, book chapters, case reports, conference abstracts, duplicate publications, and in vitro and in vivo animal experimental studies.

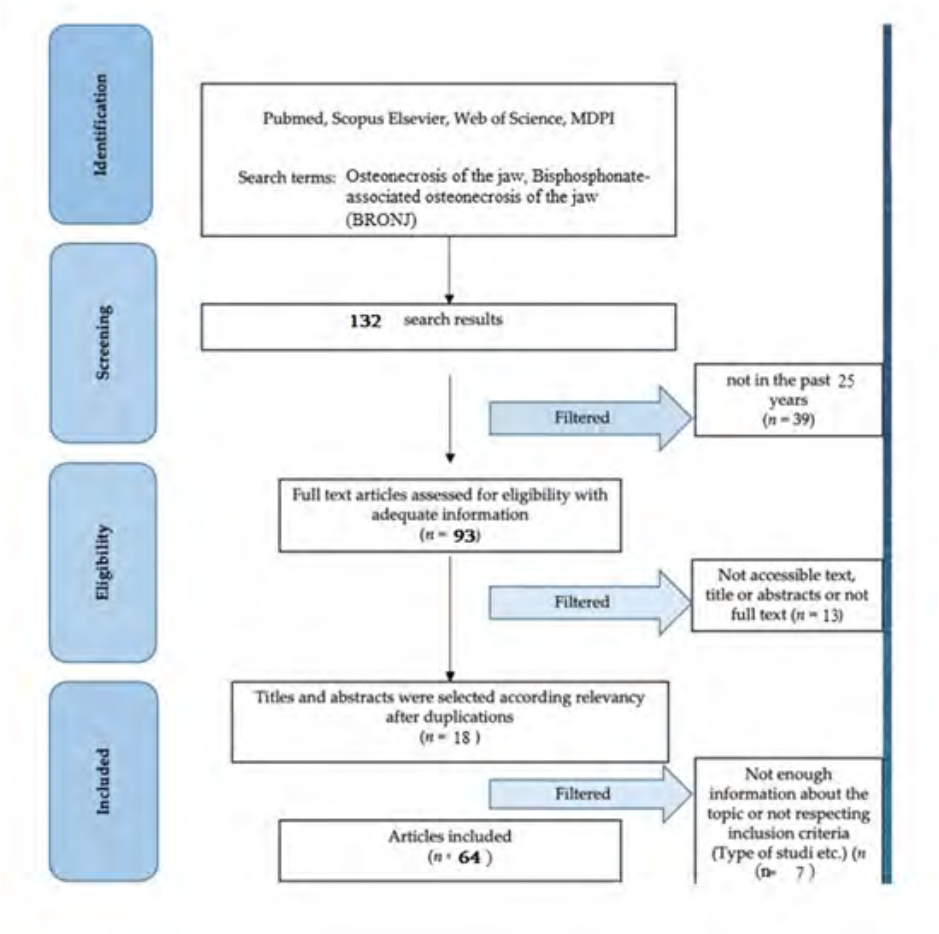

Table 1: The PRISMA flow chart

3.2. Participants

The review included study both healthy subjects and adults no limitations for age or ethnicity with suspected oral cancer based on clinical symptoms and oral examination have been included. All participants received one or several index tests.

3.3. Intervention

The index test can be one salivary biomarker for tumor-specific biomarkers, or one biomarker combines with other tumor-specific biomarkers. Blood and urine biomarkers were excluded as it was not the objective of this review.

3.4. Control

The reference standard included was placebo, control or other salivary biomarker of interest or standard of care with or without histological confirmation.

3.5. Main Outcome

The primary outcomes were sensitivity (SEN), specificity (SPE), positive predictive value (PPV), negative predictive value (NPV). Secondary outcomes were the relative diagnostic assessments of the different biomarkers.

3.6. Statistical Analysis

Statistical analyses were performed using SPSS software, p values < 0.05 were considered statistically significant. Statistical analyses were performed using SPSS software. P-value was considered significant at <0.05 and <0.001 for highly significant results.

4. Results

Conducted a preliminary search and reviewed 132 titles and abstracts in this review and 64 full-text articles were selected of high methodological quality. Characteristics of the various studies included in (Table 2). Thus, it is important that the diagnostic performance of OC is assessed in an early-stage group of patients. The current systematic review demonstrated, all studies reported high sensitivity and specificity values for TNF-α, IL-1β, IL-6, IL-8, LDH and MMP-9 and are the most promising salivary biomarkers. Cytokines IL-6, IL-8 and TNF-α are found in higher concentrations in the saliva of patients with oral cancer than in healthy individuals and may therefore serve as candidate biomarkers for oral cancer. The relationship between oral neoplastic cells and saliva makes it a good candidate for non-invasive and highly accurate diagnostic tests. Salivary biomarkers may be a good diagnostic test for early detection, monitoring and prognosis of malignant tumors and metastasis of OPMD and OSCC.

Table 2: Table summarizing key findings from each reviewed study

Author/year	Type of study/study design	Study objective	Methodology	Key findings or outcomes
Yan Li et. al2024	Systematic review	The purpose of this study is to evaluate the diagnostic value of this new approach by using oral squamous cell carcinoma (OSCC) as the proof-of-principle disease.	Unstimulated saliva was collected from patients (n = 32) with primary T1/T2 OSCC and normal subjects (n = 32) with matched age, gender, and smoking history. The predictive power of these salivary mRNA biomarkers was analyzed by receiver operating characteristic curve and classification models.	combinations of salivary biomarkers A IL8, IL1B, DUSP1, HA3, OAZ1, S100P, and SAT showed sensitivity (91%) and specificity (91%) in distinguishing OSCC from controls
Bastías D et al., 2024	Systematic Review	The aim of this study was to perform a scoping review about salivary molecules that have been assessed as possible biomarkers for the diagnosis of oral squamous cell carcinoma (OSCC).	A search was conducted using EBSCO, PubMed (MEDLINE), Scopus, and Web of Science. The research question was as follows: which molecules present in saliva have utility to be used as biomarkers for the early detection of oral cancer? Sixty-two studies were included. Over 100 molecules were assessed.	Most of the markers were oriented towards the early diagnosis of OSCC and were classified based on their ability for detecting OSCC and oral potentially malignant disorders (OPMDs), OSCC outcome prediction, and the prediction of the malignant transformation of OPMDs. TNF-α, IL-1β, IL-6 IL-8, LDH, and MMP-9 were the most studied, with almost all studies reporting high sensitivity and specificity values. TNF-α, IL-1β, IL-6 IL-8, LDH, and MMP-9 are the most promising salivary biomarkers. However, more studies with larger cohorts are needed before translating the use of these biomarkers to clinical settings.

Brinkmann O, et al., 2011	Systematic Review	study diagnostic biomarkers in cohorts of different ethnic backgrounds.	Six transcriptome (DUSP1, IL8, IL1B, OAZ1, SAT1, and S100P) and three proteome (IL1B, IL8, and M2BP) biomarkers were tested on 18 early and 17 late stage OSCC patients and 51 healthy controls with quantitative PCR and ELISA.	seven of the nine salivary biomarkers (three proteins and four mRNAs) were validated and performed strongest in late stage cancer. Patient-based salivary diagnostics is a highly promising approach for OSCC detection. This study shows that previously discovered and validated salivary OSCC biomarkers are discriminatory and reproducible in a different ethnic cohort. These findings support the feasibility to implement multi-center, multi-ethnicity clinical trials towards the pivotal validation of salivary biomarkers for OSCC detection.
Gleber-Netto FOet al., 2016	Research articale	This study evaluated the discriminatory power of salivary transcriptomic and proteomic biomarkers in distinguishing oral squamous cell carcinoma cases from controls and potentially malignant oral disorders (PMOD).	A total of 180 samples (60 OSCC patients, 60 controls, and 60 PMOD patients) were used in the study. Seven transcriptomic markers (IL8, IL1β, SAT1, OAZ1, DUSP1, S100P, and H3F3A) were measured using qPCR, and two proteomic markers (IL8 and IL1β) were evaluated by ELISA.	The combination of transcriptomic and proteomic salivary markers is of great value for oral cancer detection and differentiation from PMOD patients and controls. Clin Cancer Res; 22(13); 3340-7. ©2016 AACR.
Singh P, et al.2020	Research articale	Validate previously evaluated salivary biomarkers in Indian population.	The study enrolled 117 patients. These were grouped into subcatergories of 31 early (TNMstage I-II) and 27 late-stage OSCC (TNM stage III-IV), 30 PMOD and 29 post-treatment patients. There were 42 control subjects. We evaluated 3 protein markers, IL-1β, IL-8 and LGALS3BP using ELISA, from unstimulated saliva samples. Statistical analysis was done to calculate p-value, ROC, AUC, sensitivity, and specificity. Protein markers IL-1β and IL-8 were significantly elevated ($p < 0.05$) in OSCC patients.	lthough LGALS3BP was not found to be significantly elevated in late stage OSCC patients, but it was a significant discriminator of early stage OSCC (stage I-II) with p-value = 0.0008 and AUC = 0.7296. These salivary biomarkers have been discovered and validated in other ethnic groups earlier. Hence, the fact that these markers were discriminatory in Indian population too, strengthens the possibility of using these salivary biomarkers as screening tools in different ethnic cohorts. Such trials would potentiate use of a non-invasive tool, like saliva for diagnosis and follow-up of oral cancer.

5. Discussion

5.1. This review was designed to answer the question: is there evidence to support the role of salivary markers in the diagnosis of OC

Saliva is a truly unique biofluid with additional advantages such as non-invasiveness, pain relief, simplicity and ease of use. Salivary biomarkers have enormous diagnostic potential and are expected to play a role in the diagnosis and pathogenesis of oral cancer. Less dilution and contamination, the analysis of the ctDNA in saliva is much more sensitive than that in the bloodstream. The SC levels confirmed in this work varied widely between studies, suggesting that further technical refinement and standardization of SC measurement by ELISA are needed to successfully use these biomarkers in clinical practice. Therefore, comparison of results from different studies is limited, as differences between brands or even between brands may lead to differences in results. To further validate these SC as OC biomarkers, studies need to be designed that take into account the variability of disease manifestations, measure cytokines under controlled conditions, and use reagents developed for clinical use.

There is no consensus on whether each of these cytokines can discriminate between precancerous and cancerous and other inflammatory diseases of the oral cavity [51,52]. Most studies did not stratify patients by disease stage, limiting these results to early cancer diagnosis. According to Yan Li et al combinations of salivary biomarkers A IL8, IL1B, DUSP1, HA3, OAZ1, S100P, and SAT showed sensitivity (91%) and specificity (91%) in distinguishing OSCC from controls [53]. According to Brinkmann O et al sali-

vary biomarkers IL8, IL1B, SAT1 and S100P and all proteomic biomarkers were significantly increased ($p<0.05$) in patients with OSCC. Sensitivity/specificity for OSCC overall was 0.89/0.78, for T1-T2 0.67/0.96 and for T3-T4 0.82/0.84 [54].

Gleber-Netto FO et al in study seven transcriptomic markers (IL8, IL1β, SAT1, OAZ1, DUSP1, S100P and H3F3A) were measured by qPCR and two proteomic markers (IL8 and IL1β) were assessed by ELISA. Among the 7 transcriptomic markers, salivary IL8 protein (IL8p) had the highest AUC value between OSCC patients and controls (0.74) and between OSCC and PMOD patients (0.72). Applying the 2-marker FP model, salivary IL8p combined with IL1β gave the best AUC value to discriminate between OSCC patients and controls, and IL8p combined with H3F3A mRNA gave the best AUC value to discriminate between OSCC and PMOD patients.55

Prerana Singh et al evaluated 3 protein markers, IL-1β, IL-8 and LGALS3BP, using ELISA from unstimulated saliva samples. Protein markers IL-1β and IL-8 were significantly elevated ($p < 0.05$) in patients with OSCC [56].

Maie A R St John evaluated 2 markers IL-6 and/or IL-8 could saliva. Interleukin IL8 was detected at higher concentrations in saliva ($P<.01$) and IL-6 was detected at higher concentrations in serum of patients with OSCC ($P<.01$) [57]. Salman Aziz evaluated the immunosuppressive cytokines including IL-4, IL-10, IL-13 and IL-1RA. The results showed that all the studied salivary cytokines were elevated in OSCC patients compared to the control group, where the salivary IL-10 and IL-13 levels showed statistically significant difference ($p = .004$ and $p = .010$, respectively). They concluded that the salivary immunosuppressive cytokine levels, IL-4, IL-10, IL-13 and IL-1RA, may prove to be potential biomarkers of OSCC and can be further investigated as markers of early detection in OSCC patients [58]. Rani NAJ et al evaluated the expression of salivary interleukin-6 (IL-6) in patients with OSCC and oral potentially malignant diseases (OPMDs): IL-6 concentration values were found to be higher in the OSCC group[59]. Karolina Babiuch et al. evaluated interleukin-1alpha (IL-1α), interleukin-6 (IL-6), interleukin-8 (IL-8), and tumor necrosis factor alpha (TNF-α) in tissue samples and saliva from patients with OSCC and OPMDs. The results showed increased salivary IL-6, IL-8, and TNF-α concentrations in patients with OSCC compared to patients with OPMDs without dysplasia. The study confirmed that among all assessed cytokines, the most important biomarker in the diagnosis of malignant transformation in the oral mucosa seems to be IL-8, however, further studies on a larger sample size are needed to confirm these results [60].

Daniel Bastiat's study found that TNF-α, IL-1β, IL-6 IL-8, LDH, and MMP-9 are the most promising salivary biomarkers [61]. However, at present, molecular biomarkers or additional tests have not been shown to be useful for the accurate detection of asymptomatic oral malignancies or precancerous lesions in the context of a screening programme[62]. The literature analysis showed that all studies on the diagnostic and prognostic value biomarkers during early diagnosis of oral cancer showed positive results, however, these studies also showed differences in the sensitivity and specificity diagnostic markers. Based on the comparing the findings of this article with the findings of other authors, our results are consistent with the work of Karolina Babiukh et al. [60]. (Table 3) summarizing key findings from each reviewed study. Before a test can be used in clinical practice, its performance must be determined in experimental programs that allow calculation of the sensitivity and specificity of the test. There is no ideal value for sensitivity and specificity, although it is desirable to have a small number of false positive results to avoid unnecessary investigations and anxiety, it is also important not to miss the disease, so false negative results should also be as few as possible. In general, tests aim to have a sensitivity and specificity of about 80% or more. It should be noted that, despite numerous studies, there are currently no additional screening methods that have been prospectively tested in primary diagnostic studies of oral cancer [63.64].

This highlights the enormous potential of new tests for the primary diagnosis of oral cancer, and one of the goals of our study is to develop salivary biomarker tests.The results of the literature review indicate the development of new screening diagnostic tests that will allow detection of the neoplastic process at an early stage, as well as optimize treatment protocols for the management and medical screening of cancer patients. Currently, the widespread use of salivary biomarkers is limited by the standardization of sample collection, improvement of sample processing and storage, and reduction of the wide variability between cancer patients and non-cancer patients. Large-scale studies are currently underway to explore the potential effectiveness of salivary biomarkers for screening and early diagnosis of OSCC, and it is expected that they will be used in routine cancer screening practice in the near future. Screening with application of salivary biomarkers can potentially be used for non-invasive diagnosis of early oral cavity cancer. To obtain reliable information, standardized protocols for collecting, storing, and processing saliva samples and validating these protocols in different patient groups are important.

Table 3: Analysis of saliva testing for cancer diagnosis based on different covariates

Number of study units	Sensitivity (SEN), (95% CI)	Specificity(SPE) (95% CI)	negative predictive value (NPV)	true positives (TP)
64	0.78(0.74–0.79)	0.79 (0.78–0.80)	4.22 (3.91–5.57)	0.38 (0.29–0.37)

6. Limitations of the Review

The database searches used to search were comprehensive, however, some studies may have been missed, and large variability was observed in the estimates of the effectiveness of salivary biomarkers among studies making comparisons difficult. An important limitation of these studies is the lack of a cohort of early stage OC patients.

7. Conclusion

Salivary biomarkers promise to have a significant impact on the earliest identification of oral carcinoma, cancer screening, and significant improvement in oral cancer treatment outcomes. However, more studies are needed before applying these biomarkers in clinical settings.

8. Declaration

8.1. Funding

No Funding

8.2. Ethics Approval and Consent to Participate

Not applicable.

8.3. Consent for Publication

Not applicable.

8.4. Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. None of the authors have any relevant financial relationship(s) with a commercial interest.

8.5. Acknowledgements

Not applicable

8.6. Data Availability

No/Not applicable (this manuscript does not report data generation or analysis).

References

1. Singh S, Halder A, Sinha O, Chakrabarty N, Chatterjee T, Adhikari A, et al. Spectroscopic studies on the biomolecular recognition of toluidine blue: key information towards development of a non-contact, non-invasive device for oral cancer detection. Front
2. Mascitti M, Orsini G, Tosco V, Monterubbianesi R, Balercia A, Putignano A, et al. An overview on current non-invasive diagnostic devices in oral oncology. Front Physiol. 2018; 9: 1510.
3. Rivera C. Essentials of oral cancer. Int J Clin Exp Pathol. 2015; 8: 11884–11894.
4. Cancer control: early detection. WHO Guide for effective programmes. Geneva: World Health Organization; 2007 (http://apps.who.int/iris/bitstream/10665/43743/1/9241547338_eng.pdf, accessed 1 October 2016).
5. Loud JT, Murphy J. Cancer Screening and Early Detection in the 21st Century. Semin Oncol Nurs. 2017; 33(2): 121-128.
6. Seoane Lestón J, Diz Dios P. Diagnostic clinical aids in oral cancer. Oral Oncol. 2010; 46(6): 418-22.
7. Wong HM. Oral complications and management strategies for patients undergoing cancer therapy. ScientificWorldJournal. 2014; 2014: 581795.
8. Walsh T, Warnakulasuriya S, Lingen MW, Kerr AR, Ogden GR, Glenny AM, et al. Clinical assessment for the detection of oral cavity cancer and potentially malignant disorders in apparently healthy adults. Cochrane Database Syst Rev. 2021; 12(12).
9. Warnakulasuriya S, Kerr AR. Oral Cancer Screening: Past, Present, and Future. J Dent Res. 2021; 100(12): 1313-1320.
10. Fuller C, Camilon R, Nguyen S, Jennings J, Day T, Gillespie MB. Adjunctive diagnostic techniques for oral lesions of unknown malignant potential: systematic review with meta-analysis. Head Neck. 2015; 37: 755–762.
11. Rebaudi F, De Rosa A, Greppi M, Pistilli R, Pucci R, Govoni FA, et al. A new method for oral cancer biomarkers detection with a non-invasive cyto-salivary sampling and rapid-highly sensitive ELISA immunoassay: a pilot study in humans. Front Immunol. 2023;
12. Cheng YS, Rees T, Wright J. A review of research on salivary biomarkers for oral cancer detection. Clin Transl Med. 2014; 3(1):3.
13. Mehrotra R, Hullmann M, Smeets R, Reichert TE, Driemel O. Oral cytology revisited. J Oral Pathol Med. 2009; 38: 161–6.
14. Siasios I, Valotassiou V, Kapsalaki E, Tsougos I, Georgoulias P, Fotiadou A, et al. Magnetic Resonance Spectroscopy and Single-Photon Emission Computed Tomography in the Evaluation of Cerebral Tumors: A Case Report. J Clin Med Res. 2017; 9(1): 74-78.
15. Abdul NS. Role of Advanced Diagnostic Aids in the Detection of Potentially Malignant Disorders and Oral Cancer at an Early Stage. Cureus. 2023;15(1).
16. Yang EC, Tan MT, Schwarz RA, Richards-Kortum RR, Gillenwater AM, Vigneswaran N. Noninvasive diagnostic adjuncts for the evaluation of potentially premalignant oral epithelial lesions: current limitations and future directions. Oral Surg Oral Med Oral Path
17. Avery EW, Joshi K, Mehra S, Mahajan A. Role of PET/CT in Oropharyngeal Cancers. Cancers (Basel). 2023; 15(9): 2651.
18. Mahmood H, Shaban M, Rajpoot N, Khurram SA. Artificial Intelligence-based methods in head and neck cancer diagnosis: an overview. Br J Cancer. 2021; 124(12): 1934-1940.
19. Leclère JC, Clément C, Le Pennec R, Maheo C, Gujral DM, Schick U, et al. An Intensive 18F-Fludeoxyglucose-Positron Emission Tomography With Computed Tomography-Based Strategy of Follow-Up in Patients Treated for Head and Neck Squamous Cell Carcinoma Who A
20. Tumor Markers. Available from: https://www.cancer.gov/about-cancer/diagnosis-staging/diagnosis/tumor-markers-fact-sheet.
21. Sharma S. Tumor markers in clinical practice: General principles and guidelines. Indian J Med Paediatr Oncol. 2009; 30(1): 1-8.
22. Sharma S. Tumor markers in clinical practice: General principles and guidelines. Indian J Med Paediatr Oncol. 2009; 30(1): 1-8.

23. Brawley OW, Kramer BS. Cancer screening in theory and in practice. J Clin Oncol. 2005; 23(2): 293-300.
24. Duffy MJ. Tumor markers in clinical practice: a review focusing on common solid cancers. Med Princ Pract. 2013; 22(1): 4-11.
25. Diamandis EP. Tumor markers: Past, present, and future. In: Diamandis EP, Fritsche EP H Jr, Lilja H, Chan D, Schwartz M, editors. Tumor markers: Physiology, pathobiology, technology, and clinical applications. Washington DC: AACC Press; 2002. p. 3–8.
26. Pawlicka M, Gumbarewicz E, Błaszczak E, Stepulak A. Transcription Factors and Markers Related to Epithelial-Mesenchymal Transition and Their Role in Resistance to Therapies in Head and Neck Cancers. Cancers (Basel). 2024; 16(7): 1354.
27. Ferrari E, Pezzi ME, Cassi D, Pertinhez TA, Spisni A, Meleti M. Salivary Cytokines as Biomarkers for Oral Squamous Cell Carcinoma: A Systematic Review. Int J Mol Sci. 2021; 22(13): 6795.
28. Manzano-Moreno FJ, Costela-Ruiz VJ, García-Recio E, Olmedo-Gaya MV, Ruiz C, Reyes-Botella C. Role of Salivary MicroRNA and Cytokines in the Diagnosis and Prognosis of Oral Squamous Cell Carcinoma. Int J Mol Sci. 2021; 22(22): 12215.
29. Siravegna G, Marsoni S, Siena S, Bardelli A. Integrating liquid biopsies into the management of cancer. Nat Rev Clin Oncol. 2017; 14: 531–548.
30. Dionne KR, Warnakulasuriya S, Zain RB, Cheong SC. Potentially malignant disorders of the oral cavity: current practice and future directions in the clinic and laboratory. Int J Cancer. 2015; 136: 503–515.
31. Xu L, Li JH, Ye JM, Duan XN, Cheng YJ, Xin L, et al. A retrospective survival analysis of anatomic and prognostic stage group based on the American Joint committee on cancer 8th edition cancer staging manual in luminal B human epidermal growth factor rece
32. Hirahata T, Ul Quraish R, Quraish AU, Ul Quraish S, Naz M, Razzaq MA. Liquid Biopsy: A Distinctive Approach to the Diagnosis and Prognosis of Cancer. Cancer Inform. 2022; 21: 11769351221076062.
33. Diehl F, Schmidt K, Choti MA, Romans K, Goodman S, Li M, et al. Circulating mutant DNA to assess tumor dynamics. Nat Med. 2008; 14: 985–990.
34. Khijmatgar S, Yong J, Rübsamen N, Lorusso F, Rai P, Cenzato N, Gaffuri F, Del Fabbro M, Tartaglia GM. Salivary biomarkers for early detection of oral squamous cell carcinoma (OSCC) and head/neck squamous cell carcinoma (HNSCC): A systematic review and net
35. Chang Y, Tolani B, Nie X, Zhi X, Hu M, He B. Review of the clinical applications and technological advances of circulating tumor DNA in cancer monitoring. Ther Clin Risk Manag. 2017; 13: 1363–1374.
36. Wang Y, Springer S, Mulvey CL, Silliman N, Schaefer J, Sausen M, et al. Detection of somatic mutations and HPV in the saliva and plasma of patients with head and neck squamous cell carcinomas. Sci Transl Med. 2015; 7: 293ra104.
37. Peng M, Chen C, Hulbert A, Brock MV, Yu F. Non-blood circulating tumor DNA detection in cancer. Oncotarget. 2017; 8: 69162–69173.
38. Patel A, Patel S, Patel P, Tanavde V. Saliva Based Liquid Biopsies in Head and Neck Cancer: How Far Are We From the Clinic? Front Oncol. 2022; 12: 828434.
39. Cabezas-Camarero S, Pérez-Segura P. Liquid Biopsy in Head and Neck Cancer: Current Evidence and Future Perspective on Squamous Cell, Salivary Gland, Paranasal Sinus and Nasopharyngeal Cancers. Cancers (Basel). 2022; 14(12): 2858.
40. Shaw AK, Garcha V, Shetty V, Vinay V, Bhor K, Ambildhok K, Karande P. 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): 1483-1495.
41. Prasad G, McCullough M. Chemokines and Cytokines as Salivary Biomarkers for the Early Diagnosis of Oral Cancer. Int J Dent. 2013; 9: 1–7.
42. Prestiyanti NMI. The role of salivary biomarker as a diagnostic tool in oral cancer: a literature review. Intisari Sains Medis. 2020; 11: 112–7.
43. Rapado-González Ó, Martínez-Reglero C, Salgado-Barreira Á, et al. Salivary biomarkers for cancer diagnosis: a meta-analysis. Ann Med. 2020; 52: 131–44.
44. Ferrari E, Pezzi ME, Cassi D, Pertinhez TA, Spisni A, Meleti M. Salivary Cytokines as Biomarkers for Oral Squamous Cell Carcinoma: A Systematic Review. Int J Mol Sci. 2021; 22(13): 6795.
45. Yu W, Hurley J, Roberts D, et al. Exosome-based liquid biopsies in cancer: opportunities and challenges. Ann Oncol. 2021; 32: 466–477.
46. Nguyen TTH, Sodnom-Ish B, Choi SW, Jung HI, Cho J, Hwang I, Kim SM. Salivary biomarkers in oral squamous cell carcinoma. J Korean Assoc Oral Maxillofac Surg. 2020;4 6(5): 301-312.
47. Khurshid Z, Zafar MS, Khan RS, Najeeb S, Slowey PD, Rehman IU. Role of Salivary Biomarkers in Oral Cancer Detection. Adv Clin Chem. 2018; 86: 23-70.
48. Markopoulos AK, Michailidou EZ, Tzimagiorgis G. Salivary markers for oral cancer detection. Open Dent J. 2010; 4: 172-8.
49. Rajkumar K, Ramesh Kumar A, Ramyamalini V, Nandhini G, Dinesh Kumar T, Ashwini BK, et al. Estimation of serological and salivary biomarkers in patients with oral squamous cell carcinoma, premalignant lesions & conditions. SRM J Res Dent Sci. 2010; 1: 14–9
50. Morris RM, Mortimer TO, O'Neill KL. Cytokines: Can Cancer Get the Message? Cancers (Basel). 2022; 14(9): 2178.
51. Bastías D, Maturana A, Marín C, Martínez R, Niklander SE. Salivary Biomarkers for Oral Cancer Detection: An Exploratory Systematic Review. Int J Mol Sci. 2024; 25(5): 2634.
52. Li Y, St John MA, Zhou X, et al. Salivary transcriptome diagnostics for oral cancer detection. Clin Cancer Res. 2004; 10(24): 8442-50.
53. Brinkmann O, Kastratovic DA, Dimitrijevic MV, et al. Oral squamous cell carcinoma detection by salivary biomarkers in a Serbian population. Oral Oncol. 2011; 47(1): 51-5.

54. Gleber-Netto FO, Yakob M, Li F, Feng Z, Dai J, Kao HK, Chang YL, Chang KP, Wong DT. Salivary Biomarkers for Detection of Oral Squamous Cell Carcinoma in a Taiwanese Population. Clin Cancer Res. 2016; 22(13): 3340-7.
55. Singh P, Verma JK, Singh JK. Validation of Salivary Markers, IL-1β, IL-8 and Lgals3bp for Detection of Oral Squamous Cell Carcinoma in an Indian Population. Sci Rep. 2020; 10(1): 7365.
56. St John MA, Li Y, Zhou X, Denny P, Ho CM, Montemagno C, Shi W, Qi F, Wu B, Sinha U, Jordan R, Wolinsky L, Park NH, Liu H, Abemayor E, Wong DT. Interleukin 6 and interleukin 8 as potential biomarkers for oral cavity and oropharyngeal squamous cell carcinom
57. Aziz S, Ahmed SS, Ali A, Khan FA, Zulfiqar G, Iqbal J, Khan AA, Shoaib M. Salivary Immunosuppressive Cytokines IL-10 and IL-13 Are Significantly Elevated in Oral Squamous Cell Carcinoma Patients. Cancer Invest. 2015; 33(7): 318-28.
58. Rani NAJ, Vardhan BGH, Srinivasan S, Gopal SK. Evaluation of Salivary Interleukin-6 in Patients with Oral Squamous Cell Carcinoma, Oral Potentially Malignant Disorders, Chronic Periodontitis and in Healthy Controls - A Cross-Sectional Comparative Study. A
59. Babiuch K, Kuśnierz-Cabala B, Kęsek B, Okoń K, Darczuk D, Chomyszyn-Gajewska M. Evaluation of Proinflammatory, NF-kappaB Dependent Cytokines: IL-1α, IL-6, IL-8, and TNF-α in Tissue Specimens and Saliva of Patients with Oral Squamous Cell Carcinoma and Ora
60. Bastías D, Maturana A, Marín C, Martínez R, Niklander SE. Salivary Biomarkers for Oral Cancer Detection: An Exploratory Systematic Review. Int J Mol Sci. 2024; 25(5): 2634.
61. Macey R, Walsh T, Brocklehurst P, et al. Diagnostic tests for oral cancer and potentially malignant disorders in patients presenting with clinically evident lesions. Cochrane Database Syst Rev. 2015; CD010173.
62. Warnakulasuriya S, Kerr AR. Oral Cancer Screening: Past, Present, and Future. J Dent Res. 2021; 100(12): 1313-1320.
63. Walsh T, Warnakulasuriya S, Lingen MW, Kerr AR, Ogden GR, Glenny AM, Macey R. Clinical assessment for the detection of oral cavity cancer and potentially malignant disorders in apparently healthy adults. Cochrane Database Syst Rev. 2021;12.