ACTA ODONTOLOGICA SCANDINAVICA, 2016
VOL. 74, NO. 3, 170–177
http://dx.doi.org/10.3109/00016357.2015.1110249

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REVIEW ARTICLE

Biomarkers in saliva for the detection of oral squamous cell carcinoma and their potential use for early diagnosis: a systematic review

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ABSTRACT

Objective To determine the capacity of salivary biomarkers in the early diagnosis of oral squamous cell carcinoma. **Study design** A systematic review of the literature was performed based on the English titles listed in the PubMed, EBSCO, Cochrane, Science Direct, ISI web Science and SciELO databases using the following search descriptors: Oral cancer, diagnosis, biomarkers, saliva and oral squamous cell carcinoma. Abstracts and full-text articles were assessed independently by two reviewers. International checklists for assessment of methodological quality were used. Levels of evidence and grades of recommendation through the Scottish Intercollegiate Guidelines Network (SIGN) template were recognized. The units of analysis were identified through a reference matrix. **Results** Through the research strategy and after application of different filters and considering choosing criteria, six studies were obtained for analysis. Salivary biomarkers for oral cancer most frequently found were mRNA and proteins for IL-8, CD44, MMP-1 and MMP-3. New peptide-biomarkers such as Cyfra 21-1 and ZNF510 were found. ZNF 510 was the only biomarker which increased in the population with tumour stage T1 + T2 and T3 + T4. Only one study showed a sensitivity and specificity of 96% when the biomarker ZNF 510 is employed to discriminate early and late tumour stages. **Conclusions** There is no sufficient scientific evidence to support the capacity of the identified salivary biomarkers for the early diagnosis of oral cancer (sub-clinical stages of the pathogenic period before cancer phenotypes are manifested). Salivary biomarkers, however, may be employed to discriminate between healthy and cancer patients.

ARTICLE HISTORY
Received 22 June 2015
Revised 10 September 2015
Accepted 15 October 2015
Published online
17 November 2015

KEYWORDS
Biomarkers; diagnosis; oral cancer; saliva

Introduction

Oral cancer is the sixth most common form of human cancer. At the same time, oral squamous cell carcinoma (OSCC) is the commonest form of head and neck cancer (HNC) and its mortality rate at 5 years is ~60%.[1] Regrettably, OSCC is usually diagnosed in advanced stages of malignant development. Despite improvements in therapeutic strategies, its poor survival rate has not ameliorated over the last 30 years. The challenge of this decade, therefore, is to reduce both mortality and morbidity of this disease through the development of strategies that detect OSCC in its early stages. In addition to the conventional clinical oral examination, currently there are no scientifically validated techniques for the early detection of OSCC.[1,2]

The application of advanced biochemical methods and molecular biology techniques to detect biomarkers may contribute, but they must progress to the identification of potential biomarkers and their eventual application in clinical trials to improve early diagnosis, intervention and treatment.[3,4] One critical point is the absence of routine oral cancer screening, which is neither invasive nor expensive.[5] According to some studies, identification and diagnosis can take as long as 6 months.[3,6] Due to its relatively low incidence (4.6–6.9; ASR per 100 000 individuals age standardized rate), it is difficult to educate the public on oral cancer, which would reduce the time of diagnosis.[7]

Early detection of pre-malignant lesions is critical for prognosis and survival rates. In the case of OSCC, if the malignancy is detected in the T1 stage, the survival rate at 5 years is 80%, while if it is detected in T3 and T4 stage it is 20–40%.[8] The literature suggests various methods for such detection. Self-fluorescent visualization has been used as an adjunct to white light visualization in monitoring oral cancer. However, said methods are sensitive but not specific enough to detect pre-malignant lesions in patients at high risk of oral cancer.[9] The sensitivity and specificity of oral cytology is weak, but has improved with the advent of molecular tools.[10]

Recently, saliva has taken an important role as a diagnostic fluid because sampling is inexpensive, easy and not invasive. Moreover, potential biomarkers for diseases such as periodontitis, breast cancer, oral cancer and Sjögren Syndrome have been identified in saliva.[11–13] Salivary biomarkers for OSCC include biomolecules such as DNA, RNA and proteins that can

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be quantified from saliva samples, cancer cells, inflammatory cells and correlate with OSCC.[14–16] Cytokines and chemokines are important biomarkers in inflammation and carcinogenesis and are detected in the saliva of patients with OSCC stages I–IV.[17,18] Transferrin levels have been linked to the size and stage of the tumour, according to the Tumour, Node, Metastasis (TNM) staging system adopted by the International Union Against Cancer (UICC).[19]

Nevertheless, the early detection of salivary biomarkers in subjects with Oral Potentially Malignant Disorder (OPMD) leukoplakia and dysplasia is an important aspect before cancer phenotypes manifest.[20,21] In 2011, Hoffmann et al. [22] did not find any differences in level of saliva of endothelin-1 between patients with OSCC and leukoplakia. Therefore, it is necessary to find a good marker of malignant transformation to validate salivary biomarkers for early OSSC diagnosis. This study aims at identifying potential salivary biomarkers for the early diagnosis of oral cancer through a systematic review of the literature.

Materials and methods

Study design: Systematic review.
Object of study: Salivary biomarkers for early oral cancer diagnosis.
Material to study: Primary sources of research (scientific articles).

Units of analysis

- Clinical stages of the natural history of the disease in which oral cancer biomarkers are expressed in saliva.
- Expression of the different types of biomarkers related to oral cancer.
- Molecular identification methods for the diagnosis of oral cancer.
- Parameters of specificity, sensitivity, Area Under Curve (AUC) or Receiver Operating Characteristic (ROC), Curve Analysis of the Diagnostic Tests.

Eligibility criteria

Inclusion criteria

Scientific papers written in English using human beings that do not discriminate age ranges published between 2000–2013. Descriptive observational, analytical observational designs, diagnostic tests, intervention studies (experimental), systematic reviews and meta-analysis were selected and included in this review.

Exclusion criteria

Articles with patients having systemic diseases, whose study population had one or more diagnosed cancerous lesions different from OSCC, whose study population was under medical treatment for oral cancer and pathologies involving immune and inflammatory processes and papers describing the use of indirect (oral rinse) saliva sampling and processing. A double crosscheck was performed on citations and full-text articles by two investigators independently to review all manuscripts and perform a comprehensive quality assessment. Any disagreement about inclusion or exclusion of an article was solved by consensus

Search strategy

A systematic review of the literature was performed based on the English titles listed in the PubMed, EBSCO, Cochrane, Science Direct, ISI web Science and SciELO databases. Search descriptors were: Oral cancer, diagnosis, biomarkers, saliv, and OSCC. A combination of different Boolean operators was employed. After delimitation of the search, we proceeded to read the titles. Duplicated articles were discarded. Abstract reading was performed and those meeting the defined eligibility criteria were selected. Quality assessment of papers was performed using Strobe (cross-sectional and analytical studies), Consort (clinical trials), QUADAS (diagnostic tests) and Prisma (systematic reviews). Levels of evidence and grades of recommendation suggested by the Scottish Intercollegiate Guidelines Network (SIGN) of selected articles were identified and a matrix analysis of results was performed. Finally, with the analyzed literature, a response was given to the research question posed by structured way PICO: Patients (subject with oral cancer), Intervention (salivary biomarkers), Comparative (methods of salivary biomarkers detection), Outcome (detection of salivary biomarkers in sub-clinical stages of the disease).

Results

Six thousand and eighty-three titles were identified and 10 met the inclusion criteria. Four of them were excluded [23–26] due to methodological limitations related with sample size being evident, the method used for the identification of biomarkers not having enough validation to reproduce the results or the conclusions were not consistent regarding the results of the study. The six selected studies were analytical cross-sectional [27–30] and case-control [17,31] studies (Figure 1). Such publications sought to answer the research question regarding the utility of salivary biomarkers to diagnose OSCC. The identified literature in the final filter was classified according to the units of analysis in order to answer the PICO question of this research. The articles were stratified according to their level of evidence as defined by SIGN (Table 1).

Before saliva collection, subjects could not eat, drink alcohol, smoke or brush teeth. The oral cavity was rinsed with Normal Saline Solution 0.9% (NSS). Collected saliva was centrifuged at 4 °C and the supernatant treated with proteases inhibitors or RNase, depending on whether the molecular analysis was for proteins or RNA. Samples were frozen at −20 °C or −80 °C until analysis (Table 1).

Regarding the clinical stages of the natural history of the disease related to the expression of salivary biomarkers, none of them were identified in the early stages of the disease (Table 2). Only the study by Jou et al. [29] found an increase in the salivary biomarker ZNF510 in T1 + T2 and T3 + T4 patients, while in the control population its expression was low, with

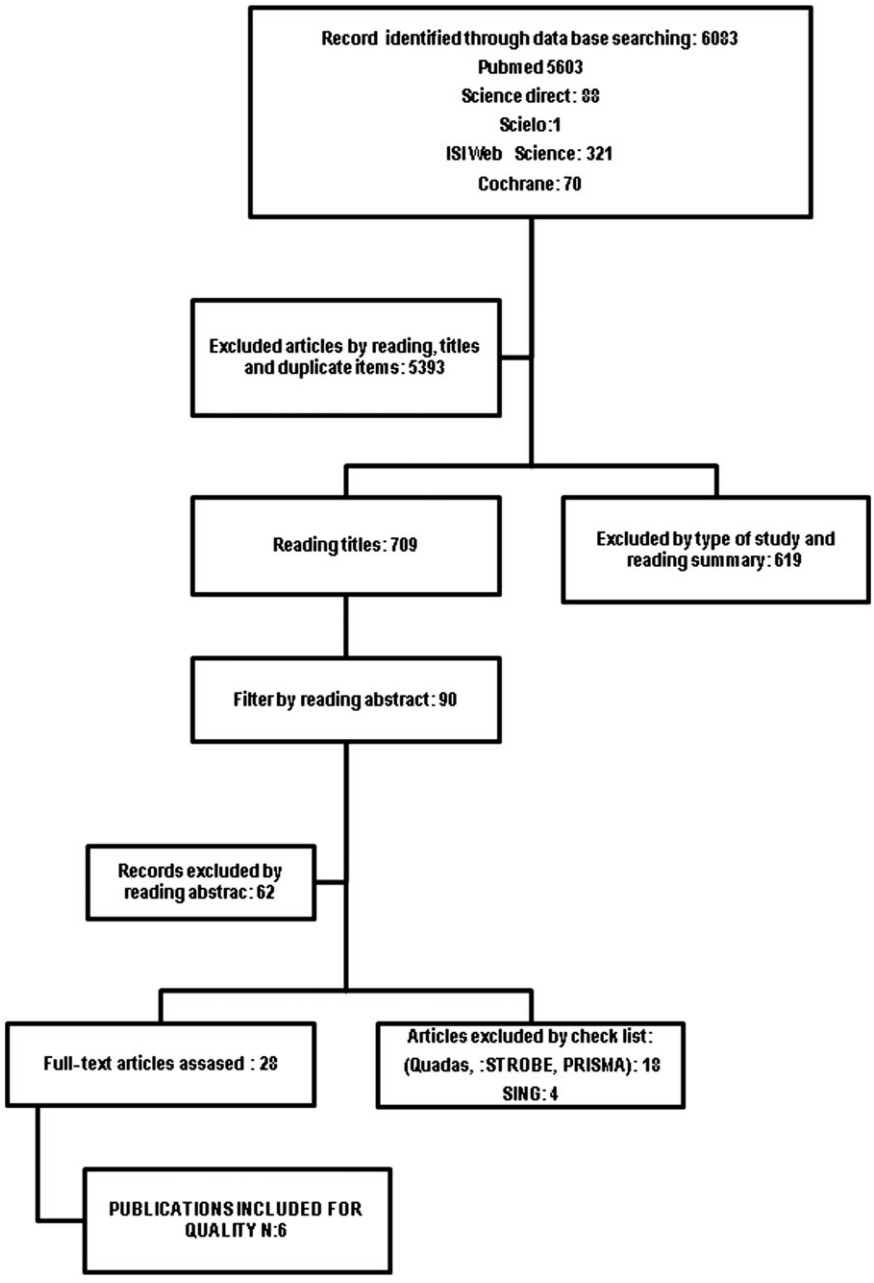

Figure 1. Flow-chart outlining the search strategy and results along various steps.

$p < 0.0001$. Concerning biomarkers and molecular methods for their identification in saliva, mainly mRNA and proteins for cytokines, chemoattractants and metalloproteinases were detected using techniques of proteomic analysis, ELISA and qPCR (Table 2). IL-8, CD44, MMP-1 and MMP-3 were the most frequently found biomarkers. Some new ones, i.e., Cyfra 21-1 and ZNF510, were found to have a good level of evidence.

In the methodology implemented by Elashoff et al.,[17] qPCR protocols were validated in laboratories from the National Cancer Institute-Early Detection Research Network (NCI-EDRN) obtaining reproducible results. They determined that mRNA for interleukin-8 (IL-8) and SAT showed the highest predictive values in five cohorts, a group of individuals diagnosed with OSCC ($n = 196$) and a control group ($n = 226$). In the study of Li et al. [28] it was found that mRNA for IL-8, IL-1B, OAZ1, S100P, H3F3A and SAT were expressed differentially among people with OSCC ($n = 32$) and healthy controls ($n = 32$). IL-8 showed a 24.3-times more increased expression, being the best predictor biomarker for the presence of OSCC.

Stott-Miller et al. [30] found by qPCR and ELISA that the presence of MMP1 was 6.2-times (33 clinical stage I/II, 26 stage III/IV) higher in OSCC patients than in healthy controls (25, 95% CI = 3.32–11.73, $p < 0.001$). MMP-3 was 14.8-times higher than the control population (95% CI = 6.75–32.56, $p < 0.001$). The expression values for these genes in tissues correlated well with the values of MMP1 in saliva (Pearson correlation coefficient = 0.63, $p < 0.001$) and MMP3 (0.67, $p < 0.001$). In the analysis by category of disease (controls, dysplasia, stage I/II OSCC and stage III/IV OSCC) an increase in salivary MMP1 and MMP3 was observed in the later stages.

Franzmann et al.,[31] using ELISA, encountered differences between the two populations in the expression of soluble

Table 1. Level of evidence for selected studies.

Reference	Type and population of study	Technique of saliva sampling	Limitations of study	Level of scientific evidence (SIGN)
Zhong et al. [27]	Observational Analytic Patients OSCC ($n = 30$) Healthy ($n = 30$)	Unstimulated saliva was taken before treatment. Supernatant was used for Cyfra 21-1 protein analysis.	Sample size of the study group and the control group.	2 + C
Elashoff et al. [17]	Cases ($n = 169$) and controls ($n = 226$). Five independent cohorts	Unstimulated whole saliva. The supernatant of saliva was stored with protease inhibitor and RNase for mRNA analysis (IL-8, IL-1B, SAT, OAZ1) and protein (IL-8, M2BP, IL1B).	It does not specify whether there was loss of participants during the course of the study.	2++B
Li et al. [28]	Observational Analytic Patients OSCC ($n = 32$) Healthy ($n = 32$)	Unstimulated saliva supernatant were treated with RNase inhibitor for molecular analysis by Microarray and mRNA (IL8, IL1B, USP1, HA3, OAZ1, S100P and SAT).	Sample size of the study group and the control group. Not specify whether participants were lost during the course of the study.	2 + C
Jou et al. [29]	Observational Analytic Patients OSCC ($n = 47$) Healthy ($n = 30$)	Sampling by expectoration, with pre-rinse with saline solution. The supernatant of unstimulated saliva was treated with protease inhibitor and stored at −80 °C until proteomic analysis and ELISA.	Not specify all strategies used to control bias.	2−
Stott-Miller et al. [30]	Observational Analytic Patients OSCC ($n = 59$) Healthy ($n = 25$)	Whole saliva supernatants were stored at −80 °C until analysis of MMP1 and MMP3 protein.	Not specify whether participants were lost during the course of the study.	2++B
Franzmann et al. [31]	Cases and controls Patients HNSCC ($n = 102$) Control ($n = 84$)	Sample collection by oral rinse with saline solution. Saliva was stored at −80 °C until analysis of total protein and solCD44.	The authors recommend increasing the size of the sample and develop a matched pair case-controls design.	2++B

The level of evidence and grade of recommendation of the selected studies was performed through the SIGN guideline (Scottish Intercollegiate Guidelines Network) available in: http://www.sign.ac.uk/about/index.html.

CD44 in saliva and total protein (controls, $n = 84$: total protein concentration of 0.55 mg/dL and soluble CD44: 9.31 cases, $n = 102$: 1.07 concentration of total protein and soluble CD44 = 24.44). Category assessment revealed that higher protein levels were significantly associated with head and neck squamous cell carcinoma (HNSCC) when the value of soluble CD44 was > 14.56 ng/dL and protein > 0.4325 (OR = 24.09, 95% CI = 9.04–68.57, $p = 0.0001$). Univariate analysis for soluble CD44 showed an increase of 2.2-fold risk (1.66–2.93) for HNSCC, whereas for protein it was 5.82-fold (2.80–12.13). In bivariate analysis the adjusted OR for soluble CD44 and protein was 1.86 (95% CI = 1.38–2.50) and 3.10 (95% CI = 1.47–6.52), respectively.

Using ELISA, Zhong et al. [27] reported that Cyfra 21-1 was higher in the OSCC group than in the control group ($p = 0.048$). Jou et al. [29] found through a proteomic study that ZNF510 peptide in saliva was spread differentially among the population diagnosed with OSSC ($n = 47$) and a control group ($n = 30$). The peptide showed higher levels in saliva samples from patients with neoplasms T1 + T2 and T3 + T4, which was higher than in control patients, such differences being statistically significant ($p = 0.001$).

Regarding the parameters of specificity, sensitivity and Receiver Operating Characteristic Curve (ROC), analysis of diagnostic tests related with biomarkers in relation to biopsy, which is considered the Gold Standard (Table 2). The analyzed studies did not mention the biopsy as the standard. Therefore, it is difficult to perform comparative analysis. The authors mention only the behaviour of biomarkers in relation to their ability to detect OSSC already established, expressing the values of sensitivity and specificity. Zhong et al. [27] mentioned sensitivity for the diagnosis of Cyfra 21 of 0.500 and a specificity of 0.86. AUC was 0.64 with $p = 0.048$ (95% CI = 0.501–0.797).

Elashoff et al. [17] reported individual analysis for IL-8 with sensitivity = 0.68 and specificity = 0.64; SAT: sensitivity = 0.66 and specificity = 0.63. The combined model improved the AUC by an average of 0.03; SAT and IL-8 have the most consistent predictive ability. Li et al. [28] demonstrated that IL-8 was the best predictor biomarker, taking into account the AUC value (0.85). The combination of three biomarkers (IL-8, SAT and H3F3A) increased the sensitivity and specificity to predict the presence of OSCC at 90.6%. The AUC for the combined biomarkers was 0.95.

Jou et al. [29] demonstrated that the peptide ZNF 510 showed the ability to discriminate between healthy subjects and patients, with an AUC value of 0.95, whereas for T3 and T4 stages and healthy individuals it was 0.98 (95% CI = 0.2–0.51). Stott-Miller et al. [30] reported that the AUC was 84.5% for MMP1 (95% CI = 76–92.95%), whereas MMP3 was 87.66% (95% CI = 80.16–95.17%). Franzmann et al. [31] reported for sCD44 an AUC of 0.796. The best efficiency was obtained using a cut-off of 0.47 for predictive probability, with values of sensitivity and specificity of 80.4% and 65.5%, respectively.

Discussion

The aim of this study was to establish potential use of salivary biomarkers in the early diagnosis of OSCC. In order to achieve said goal, we implemented a systematic review of the literature listed in different databases using descriptors such as oral cancer, diagnosis, biomarkers, saliva and oral squamous cell carcinoma. The six studies used for this review put in evidence that mRNA and proteins for IL-8, CD44, MMP-1 and MMP-3 are the

Table 2. Efficacy of salivary biomarkers and detection methods for OSCC.

Reference	Biomarker type	Classification TNM/clinical	Expression level	Methodology	Diagnostic efficacy
Zhong et al. [27]	Cyfra 21-1 protein fragment	No relation to clinical stage	OSCC (85.9 μg/L), control group (42.2 μg/L). ($p = 0.048$).	Commercial ELISA BioKeyTM Cyfra 21-1.	Sensibility = 0.50 Specificity = 0.86. AUC = 0.64; $p = 0.048$ (95% CI = 0.501–0.797)
Franzmann et al. [31]	Total protein and soluble CD44	Relation to T1, N0, N1–N2	CD44 (> 14.56 ng/dL); protein (> 0.4325) (OR 24.09; 95% CI = 9.04–68.57)	Commercial ELISA (Bender MedSystems). The total protein (BioRad, Hercules, CA)	By bivariate analysis (categorical) OR = 24.9 (95% CI = 9.04–68.57), AUC = 0.786. Sensibility = 68.6% Specificity = 74.4%
Elashoff et al. [17]	mRNA for IL-8, SAT, S100P, OAZ1	Not report data supporting that relation	The IL-8 and SAT biomarkers showed the highest predictive values in 5 cohorts	RT-PCR, qPCR. IL-8, IL1B y M2BP in saliva was quantified with commercial ELISA (Pierce)	For IL-8: Sensibility = 0.68 Specificity = 0.64 IL1B: Sensibility = 0.65 Specificity = 0.60 SAT: Sensibility = 0.66 Specificity = 0.63 AUC = 0.76–0.85
Li et al. [28]	mRNA for IL-8, IL-1B, OAZ1, S100P, H3F3A and SAT	Not determined	IL-8 was expressed 24.3-times more than the others biomarkers	qPCR	IL-8: AUC = 0.85. IL-8 + SAT + H3F3A: AUC = 0.95 Sensibility = 90.6% Specificity = 90.6
Jou et al. [29]	ZNF510 (peptide)	T1 + T2, T3 + T4 ($p < 0.0001$)	T1 + T2 (Abs 405 nm: 0.368), T3 + T4 (Abs = 0.445), Control group (Abs = 0.208); $p = 0.001$	Indirect and competitive ELISA	T1 + T2: AUC = 0.95 T3 + T4: AUC = 0.98 (95% CI = 0.2–0.51).
Stott-Miller et al. [30]	mRNA and protein for MMP1 and MMP3	Relation of MMP-1 to I–IV clinical stage but not to progression of diseases	MMP1 was 6.2-times higher in OSCC patients that healthy controls (95% CI = 3.32–11.73; $p < 0.001$). MMP-3 was 14.8-times higher than control population (95% CI = 6.75–32.56; $p < 0.001$).	qPCR in tissue samples. Salivary proteins were quantified by multiplex protein array for ELISA.	MMP1: AUC = 84.5% (95% CI = 76–92.95%) MMP3: AUC = 87.66% (95% CI = 80.16–95.17%)

salivary biomarkers most frequently found for OSSC. ZNF 510 was the only biomarker that increased in the population with tumor stage T1 + T2 and T3 + T4. Only one study showed a sensitivity and specificity of 96% when the biomarker ZNF 510 is employed to discriminate early and late tumour stages.

The biomarkers analysed in this systematic review were not detected in the pathogenic sub-clinic stage (asymptomatic) of the disease's natural history. Nevertheless, they were able to discriminate between the healthy population and those affected by the pathology. In our selection process (Figure 1), selected studies included patients with pre-malignant lesions (leukoplakia, erytroplakia and lichen planus). However, these studies did not meet the parameters established in the checklists of methodological evaluation and, therefore, were excluded. It must be noted that this kind of patient with pre-malignant lesions should be taken into account in future studies with the purpose of evaluating potential salivary biomarkers predictive for oral cancer.

IL-8 has been frequently assayed as a biomarker for the detection of oral cancer, observing increased levels in saliva unlike the control population.[32–34] In these studies, IL-8 did not correlate with the early detection of oral cancer. These results are consistent with the study of Elashoff et al.,[17] who found that the levels of IL-8 at mRNA level are not associated with tumour stage (I–IV). The authors, nevertheless, indicate that biomarkers IL-8 and SAT are associated with oral cancer and the levels of these biomarkers in saliva are induced by tumour response. Therefore, it could not be used to assess disease progression.

IL-8 seems not to be a good biomarker for the assessment of the progression of the disease. Nevertheless, it could be considered a good 'surrogate marker' which measures aspects of the disease without being directly involved in the disease process.[15] In this sense, it was recently demonstrated that salivary IL-8 could distinguish between healthy individuals, those with potentially malignant lesions and patients with OSCC [35]. Since IL-8 is an inflammatory cytokine increased in individuals with inflammatory processes, future studies should evaluate the periodontal status of patients with OSCC. A recent study showed that IL-8 and IL-6 salivary levels could detect OSCC and discriminate between healthy patients, those with periodontitis and oral lichen planus.[36]

Matrix metalloproteinases (MMP) have been used as targets for use as biomarkers of OSCC and have been associated with tumour invasion and metastasis.[37] mRNA levels of MMP-1 in the saliva have demonstrated differences between OSCC patients and healthy controls and over-expression of these genes of MMP1. MMP9 has been linked to the progression from dysplasia to cancer.[38,39] Of note, such studies were not focused on determining the potential diagnostic value of MMPs in OSCC and did not take into account the expression of MMPs in saliva to be a biomolecule directly related to biological function at the cellular level. In a study by Stott-Miller et al. [30] it was found that both mRNA present in tumour tissues and proteins such as MMP-1 and MMP-3 present in saliva were increased. These salivary biomarkers did not have the potential to discriminate between neoplasm and dysplasia.

CD44 is a receptor expressed on the surface of the basal epithelium of the aerodigestive tract. When a dysplasia arises, this receptor is expressed in all layers of the epithelium. This is also true for invasive HNSCC cancer.[40] Several studies have evaluated the potential of soluble CD44 in saliva as a biomarker for OSCC.[23,31,41] Although CD44 is a tumour marker, current evidence does not support its use as a soluble salivary biomarker for early diagnosis of HNSCC.

New biomarkers like those belonging to the zinc finger proteins family (ZNF), such as ZNF510 and Cyfra 21-1, are evident. The latter is a soluble fragment of cytokeratin 19 (CK19), a protein of the cytoskeleton, which correlates well to OSCC. Zhong et al. [27] noted a good correlation between Cyfra 21-1 levels present in saliva and CK19 present in OSCC tissues. ZNF proteins contain binding dominions to DNA and they are involved with cellular growing, proliferation, differentiation and apoptosis. ZNF510 is a tumour-derived protein initially described in the early detection of breast cancer.[42] Despite the frequency of expression of these two biomarkers, levels evidenced not demonstrate their ability to early detection of OSCC, given its presence from the early stages, but symptomatic (T1, T2).[27,29]

With the exception of the paper by Jou et al.,[29] which showed sensitivity and specificity of 96% when the ZNF 510 biomarker is employed to discriminate early and late tumour stages. The literature review does not demonstrate conclusive data regarding sensitivity and specificity. The study by Jou et al.,[29] showed sensitivity and specificity of 96% when biomarker ZNF 510 was employed to discriminate both early and late tumor stages. Some biomarkers such as Cyfra 21-1 showed increased sensitivity and specificity when combined with other biomarkers, although the authors do not recommend their use as a clinical instrument for disease screening.[27] In this sense, some research groups support the concept that using the combination of two or three biomarkers for OSCC are related to an increase in diagnostic accuracy.[28,31]

Currently, the salivary test is being widely employed for the diagnosis and monitoring of these pathologies because it is safe, non-invasive and affordable.[43] However, it should be used with precaution because it is unstable. Certain parameters, such as standardization of the freezing process, avoid both protein fragmentation and peptide increase among the collected sample.[44] Athough the use of protease inhibitors reduces proteolysis, some protein degradation still occurs, demonstrating the complexity of this fluid for biomarkers analysis.[45] Salivary sample should be centrifuged and stored under low temperature conditions. Some researchers believe that storing the samples at –80 °C does not affect the proteomic analysis of the saliva.[46] In cases where the presence of inhibitors is not a critical factor for biomarkers detection, it is recommended to add protease-inhibitors of bacterial origin in order to avoid degradation of potential biomarkers due to the presence of bacteria related to health and disease.[47]

The analysis of salivary RNA as potential biomarkers seems to be favoured by the presence of exosomas that protect and diminish nucleic acid degradation.[48] Recently, a RNAProSAL collection system was standardized in order to keep saliva in optimal conditions for its posterior RNA or protein biomarker analysis.[49] The six studies selected for this revision employed

a salivary treatment method that kept the samples in optimal conditions for biomarker analysis, thus reducing biases related to sample taking and management.

Emerging biomarkers, like salivary microRNAs, are being assessed for the diagnosis of OSCC.[25,50,51] Although said biomolecules are stable in saliva, the studies so far are exploratory and have not been validated as biomarkers for early detection of OSCC. To employ a biomarker for the diagnosis of OSCC, an integrated approach bringing robust methodologies and highly efficient techniques is required.[4] However, perhaps the most important factor is the validation of methodologies and techniques to correlate basic research to clinical practice.[52] In this sense, only Elashoff et al. [17] bring the parameters required to validate methodologies for the analysis of potential salivary biomarkers for the diagnosis of OSCC. Pre-validation of salivary biomarkers in this study were the employment of independent study cohorts, standardization of sample taking and automatized RNA analysis by real time PCR, therefore lowering the inter-laboratory variance. Independent validation was achieved with the National Cancer Institute's Early Detection Research Network (NCIEDRN)'s Biomarker Reference Laboratory (BRL) to confirm result reproducibility.

Studies that used enzyme-linked immunosorbent assays (ELISA) for the detection of biomarkers did not describe methods to validate their identification in saliva. Although most ELISA tests have been validated for analysis of plasma, serum, urine or culture samples, they work well with saliva.[53] However, issues such as inter- and intra-assay variation in such methodologies should be considered for the detection of biomarkers in saliva.

Currently new technologies based on biosensors are under development. Their aim is to identify proteic salivary biomarkers.[54,55] Such biosensors will identify low concentrations of biomarkers in small amounts of saliva, which will help in the early diagnosis of diseases such as oral cancer.[56,57] Therefore, it is required that future studies advance to validate potential salivary biomarkers related to their methods, sample size and clinical applications.

Detection of biomarkers before the clinical manifestations and malignancy could be relevant to monitor the progression into OSCC and its treatment. Taking that into account, it is important to identify the biomarkers during the sub-clinical phase, which will improve the prognosis. The possibility of detecting oral cancer in an asymptomatic stage is related to the growth rate of the malignant cells and the detection method employed. Early diagnosis during primary tumour formation and the onset of symptoms is important for patient survival.[3] Therefore, predictive biomarkers that could be detected during this period are of great importance for the treatment and follow-up of oral cancer.

Conclusion

This systematic review demonstrates that there is not enough scientific evidence to support the use of salivary biomarkers for early diagnosis of OSCC, although salivary biomarkers may be employed to discriminate between healthy and con cancer oral patients. The great majority of salivary biomarkers identified for this study are under development and only one study showed a pre-validation of the salivary biomarkers, but it is not available for clinical use. Therefore, it is required that future studies provide validation methods for the use of salivary biomarkers that could be used for the early detection of oral cancer. Such studies should take into account the use of standardized protocols, reliable detection methods and greater population including patients with risk factors for oral cancer and patients with potentially malignant lesions in different clinical stages, so the specificity of salivary biomarkers for the early detection of oral cancer can be assessed and implemented in clinical practice as a diagnostic tool.

Acknowledgements

The authors express their gratitude to Dr Jaime Castro-Núñez, oral and maxillofacial surgeon and researcher at Institución Universitaria Colegios de Colombia, for his assistance during the preparation of the English version of this manuscript.

Disclosure statement

The authors report no conflicts of interest. This work was supported in full by the Institución Universitaria Colegios de Colombia, Research Division, Bogotá, Colombia.

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