Oral cancer: An overview on the role of salivary biomarkers in diagnosis and follow-up

Amal Al-Mabrok Hossin Edawadi[1] Jbireal J M[2]

Department of oral biology, Faculty of dentistry, Sabratha university, Libya[1].

Department of Physiology, Faculty of Medicine; Director of Training& Department, Research-Consultation &Training Center, Sabratha University, Libya[2].

j.jbireal@sabu.edu.ly

تاريخ الاستلام 2024-08-18

Abstract

Furthermore, the process of carcinogenesis is complicated and involves both genetics and phenotype. The accumulation of genetic and epigenetic alterations that upset the normal balance between cell division and apoptosis is what propels the development of cancer. In the medical field, early cancer diagnosis is currently of the highest priority. Promising salivary biomarkers as a tool for early detection of oral cancer have been found by recent systematic reviews and meta-analyses in a number of original research studies. This review aimed to perform methodological and reporting quality evaluation, reevaluate the meta-analysis results, and use previously published meta-analyses to rank the biomarkers according to their diagnostic outcomes in oral cancer.

Different scientific databases including Google Scholar, Scopus, National Institutes of Health (NIH), PubMed, and Web of Science were used to view the systematic review articles and meta-analysis conducted on oral squamous cell carcinoma (OSCC) and oral potentially malignant disorders (OPMD). It was only suggested twenty years ago to employ saliva analysis as a diagnostic tool for systemic disorders, but due to its revolutionary potential as a liquid biopsy, there has been a recent rise in interest in this sector. It is possible to use salivary biomarkers such as metabolites, lipids, carbohydrates, proteins, nucleic acids, peptides, transforming enzyme activity, and antibodies. Salivary indicators can therefore be helpful for the detection, tracking, and even prognosis of all oral malignancies.

Keywords: Oral cancer; Salivary biomarkers; Oral squamous cell carcinoma; oral potentially malignant disorders; Saliva.

Introduction

An overview of oral cancer

Oral cancer is one of the most common cancers in the world, representing a serious problem for global health (1). Moreover, carcinogenesis is a complex process that occurs at the phenotype and genotype levels. Cancer development is driven by the accumulation of genetic and epigenetic changes that disturb the homeostatic equilibrium between cell proliferation and cell death (2). The main obstacle to lowering the disease's mortality and morbidity rates is to create methods for early oral cancer detection and identification, which will allow for efficient treatment and intervention.

Currently, specialist clinical examination and histological investigation of suspect spots are used to detect new oral cancer cases in early stages, although it may remain undetected in hidden spots. Therefore, screening for high-risk patients may benefit from the use of sensitive and specific biomarkers for oral cancer.

According to the estimated number of new cancer cases and deaths by sex, USA, 2024, an estimated 58,450 new cases of oral cavity cancer and pharynx will be diagnosed in the US and about 12,230 people will die from this cancer (3). Additionally, many diseases of the oral cavity can undergo malignant transformation. Oral squamous cell carcinoma (OSCC) is one of the most frequent oral cancers and still has a five-year survival rate of only 50–65% despite diagnostic and therapeutic advances, in part attributable to diagnostic delay (4).

Globally, in 2018 cancer statistics have mentioned cancers of lip and oral cavity as the most frequent type of cancer in South Asian countries (5). Many of risk factors such as alcohol consumption, tobacco use, nutritional deficiency, etc. could be implicated in oral cancer. Apart from these, human papillomavirus infection and chronic trauma from sharp teeth or broken appliances may eventually lead to cancer (6).

Oral cancer is therefore expected to rise within Southeast Asia, in line with population growth (7). As a consequence, over the next 20 years, it is expected that the majority of head and neck cancer (HNC) will be Human papillomavirus (HPV)-positive, with projections that in some European countries, such as the UK, oropharyngeal cancer incidence will overtake cancer of the oral cavity (8).

In a cord with these results, estimates for 2012 showed that oral and oropharyngeal cancers would account for approximately 442,760 new cases, of which 314,106 cases would affect males and 128,654 females, representing the sixth most common malignancy worldwide (9, 10, 11).

For South America, Globocan estimated 22,773 new cases of oral cavity and other pharynx cancers for 2012 (15,695 in males with an age-standardized rate (ASR) of 8.2/100,000 and 7078 new cases in females with an ASR of 3.0/100,000) (11).

Salivary Biomarkers

Predominantly, there are many significant challenges for diagnosing oral cancer in early stages. Using the analysis of saliva in a diagnostic approach for systemic diseases was proposed just two decades ago, but great interest in the field has emerged recently because of its revolutionary potential as a liquid biopsy. Saliva is called the mirror of the body (12), as it is considered an ultra-filtrate of the blood (13).

Extremely important to know, that today, there are about five diagnostic alphabets have been characterised in saliva: proteome, transcriptome, microRNA (miRNA), metabolome and microbiome (14). More recently, piwi-interacting RNAs (piRNAs), circular RNAs (circRNAs) and other non-coding RNAs (ncRNAs) have also been described as a new landscape of salivary RNAs (15). Another multi molecules isolated from saliva can be used as an oral communication battery (OCB) for detection, prognosis, treatment, drug monitoring and pharmacogenetic studies (16).

Currently, besides biomarkers analysis the diagnosis and detection of oral cancer, clinical oral examination combined with histopathological procedures of biopsies can be used. Actually, for early detection of oral cancer, several diagnostic adjuncts have been developed, including the use of salivary biomarkers (17, 18).

Scientifically, salivary biomarkers have been identified as “a specific novel, or structurally altered cellular macromolecules or temporarily, spatially, or quantitatively altered normal molecules that are associated with malignant (and in some cases benign) neoplastic cell” (19). Biomarkers such as nucleic acids, proteins, peptides, alterations in enzyme activity, antibodies, metabolites, lipids, and carbs can be used. Therefore, salivary biomarkers can be useful for the diagnosis, monitoring, and even prognosis of all of oral cancers (Table 1).

Data sources

Using "AND" and "OR," a search was conducted using the databases Google Scholar, Scopus, National Institutes of Health (NIH), PubMed, and Web of Science with the following keywords: Oral cancer; Salivary biomarkers; Oral squamous cell carcinoma; oral potentially malignant disorders; Saliva. The search was restricted to studies published in the recent years, more precisely between January 2016 and May 2024, to ensure that the topic under evaluation remained current. Only English-language papers pertaining to human saliva research were included in the search.

Table 1: Salivary Biomarkers involved in main oral cancers (OSCC/OPMD) diagnosis and follow up.

Biomarkers	Sub-types	OSCC	OPMDs	Function
Enzymes	Metalloproteinases -MMP-9 -MMP-2 -Other MMPs	-High levels of MMP-9 (20).	Significant differences in salivary MMP-9 and MMP-2 concentrations between OPMD patients compared to controls (21).	-To differentiate between patients with OSCC and an OPMD (22).

				-other MMPs altered between healthy and OSCC patients (23, 24).
	-LDH, 1-fructose, cathepsin V, AKR1b10, kallikrein	- Increased (25, 26).	-Unknown	In diagnostic and prognostic purposes (27, 28).
Glycoproteins	-CEA -CD44	-Increased CEA levels (29, 30). -High CD44 levels (31).	Undetermined	Diagnostic and prognostic value.
Cytokines	IL-4, IL-6, IL-8, IL-10, IL-13, IL-1β, IL-1RA, IL-17A, IL-17F, IFN-γ, TNF-α, HGF, CRP, VEGF.	-High IL-8, IL-1 β. (33). -High TNF-α (34). High HGF and VEGF (35).	- High TNF-α (33, 34).	In an improvement in diagnostic accuracy and staging (36).
MicroRNAs	miR-21, miR-184, miR-let-7-5p, miR-412-3p, miR-512-3p, miR-302-3p, miR-517-3p, miR-30c-5p, miR-SAT, miR-OAZ, miR-H3F3A, and miR-24-3p.	All shown significant differences as compared with control.	All shown significant differences as compared with control. - less specificity in identifying epithelial dysplasia (37, 38).	-Diagnostic value. -In differentiation between OSCC/OMPD (39).
Metabolites	ornithine, ohydroxyvenzonate, TSA and R5P.	-significant increase in less differentiated tumor (40, 41).	to improve therapeutic outcomes [40].	-in staging. -In differentiation.
Other biomarkers	ANG,ANG2,NUS1,transgelin, FSA, PBSA, KPNA2, LGALS3BP and RCN1.	-Increased (35, 42, 43). KPNA2 increased in advanced stages (39).	LGALS3BP increased in early stages (33).	In disease progression (39).

Role of salivary biomarkers in diagnosis and follow up of oral cancer

Human gingival and saliva fluids would close the gap that results from utilizing blood and urine as markers, speeding up the diagnosing procedure and improving accuracy. Saliva is a complex natural reservoir that includes ions, glycoproteins, immune modulators, cytokines, hormones, and amylases (44). From all other diseases of oral cavity, this review focusing on OSCC and OPMDs to understand the role of different salivary biomarkers in early detection of oral cancer.

Significantly, saliva is a useful bodily fluid with a wealth of analytes (DNA, mRNA, and protein) that can be exploited as biomarkers for clinical and translational research (45). Moreover, as a clinical tool, saliva offers numerous advantages over serum and tissues. These advantages include ease of collection, cost-effectiveness, easy availability of large sample volume for analysis, and repeated sampling for long-term monitoring (46). Therefore, using salivary samples as a tool for early detection and diagnosis of oral cancer shows great benefit.

The primary reason saliva is chosen as a diagnostic tool is that it contains cells that have fallen out of mouth cavities. This makes saliva the first option for screening and identifying possible biomarkers in cases of oral cancer (47). Additionally, a hypotonic, multipurpose biological fluid, saliva is a great tool for detecting biomolecules linked to different types of oral cancer. Saliva is a favored screening medium for intraoral cavity and systemic disorders, as well as prognostic surveillance, due to its non-invasiveness, relative ease of collection, low cost, convenience of handling, and minimal knowledge required.

Marginally, saliva is a frothy, watery, somewhat acidic (pH:6-7) material made up of the secretions from the lips, palate, and buccal mucosa's three major and minor salivary glands (48, 49). Saliva production ranges from 1 to 1.5 liters per day, and it comprises a complex mixture of different salts, proteins, and minerals. Because of the constant

changes in its content and consistency that occur in both health and illness, it is commonly referred to as the mouth's mirror. Its non-invasive collection process has the potential to aid in the identification of biomarkers for oral cancer. Saliva has become increasingly important in the surveillance of oral cancer due to its advantages as a diagnostic medium (50).

For oral cancer outcome prediction, a number of markers, including Epidermal Growth Factor Receptor (EGFR), Cytokeratin Fragment 21-1(CYFRA-21), miR-139-5p, and IL-5, have been proposed (51-55). Salivary microRNAs; miR-139-5p levels were able to distinguish between patients with OSCC and healthy individuals before to surgical treatment, as well as between patients with OSCC and those who had surgery. Moreover, in patients having tumor excision, concentrations of this marker reverted to normal within 4-6 weeks postoperative period (53). In this context, after the initial surgery, a higher amount of salivary IL-5 was discovered by another investigation. In patients with a second primary, this was followed by a drop in IL-5, which was then followed by an increase in IL-5 saliva concentrations following tumor removal (51).

On the other hand, it has been determined that some salivary biomarkers may be used to predict the malignant transformation of OPMDs. In comparison to healthy and other disease control patients, Zaharan et al. (56), observed an increase in salivary miRNA-21 and miRNA-184 in OPMDs (with and without dysplasia) and OSCC. Similar to this, Sabarathinam et al. found that as OPMDs progressed to OSCC, salivary glutathione peroxidase (GPx), Malondialdehyde (MDA), Tumor necrosis factor α (TNF-α), and Alpha-Fetoprotein (AFP) steadily increased (57). According to a different study, miRNA-31 was upregulated in OPMDs, Oral lichen planus (OLP) with dysplasia, and in cases where an OMPD had undergone malignant transformation (58).

Actually, a single biomarker is unlikely to detect OSCC in all cases because of the variety of carcinogenic pathways, heterogeneity in tumors, and substantial variation in risk factors. As a result, biomarker combinations are more likely to increase diagnostic

validity (59). Molecular, transcriptomic, genomic, proteomic, metabolomic, and phenotypic methods were used to identify these biomarkers.

Clinically, in order to assess the efficacy of chemiluminescence and tissue autofluorescence-based adjunctive devices in the identification of OSCC and OPMD, Nagi et al. (2016) carried out a systematic review (60). The selection criteria were satisfied by twenty published primary research. Ten made use of tissue autofluorescence and ten of chemiluminescence. Chemiluminescence was assessed using ViziLite, with a sensitivity range of 0.771 to 1.00 and a specificity range of 0.00 to 0.278. With enhanced oral cancer assessment system (VELscope), tissue autofluorescence was assessed. The sensitivity and specificity of this method were assessed at 0.22–1.00 and 0.16–1.00, respectively. The study came to the conclusion that additional clinical studies should be carried out in the future to prove optical imaging's effectiveness as an auxiliary tool in the early diagnosis.

According to Singh et al. (61), they observed increased IL-1β and IL-8 levels at every stage of OSCC. Other investigators who have noted that IL-6 is increased in instances of stages T3 and T4, as well as those in whom there is cervical metastasis, confirm this idea by suggesting that IL-6 and IL-8 may have diagnostic utility to discriminate between OSCC and healthy individuals (62).

Related to the role of glycoproteins, Cluster of Differentiation 44 **(**CD44), and carcino-embryonic antigen (CEA) as salivary biomarkers in oral cancer detection, three investigations looked at CEA, and they all found that patients with OSCC had much more saliva than control patients ($p < 0.005$) (29, 30, 41). When CEA salivary levels were examined in combination with the marker Naa10p, the sensitivity and specificity increased significantly to 92.5% and 85%, respectively (63). Additionally, it was discovered that OSCC patients' saliva had more CD44 variations than those of control patients. Accordingly, CD44v6 may play a role in the early stages of cancer, and both

CD44v6 and CD44v10 may play a role in locoregional aggression and histological conditions (31).

More important, that patients with OSCC have also demonstrated substantial amounts of other potential biomarkers, including matrix metalloproteinases 1 and 3, (MMP1, MMP3), peptidyl arginine deiminase type 1 (PADI1), tenascin-C (TNC), and cystatin-A (CSTA) (64). MMP1 levels were found to be higher in patients with OSCC by Hsiao et al. and Chang et al., but were not found in healthy people, suggesting the possibility of a biomarker to distinguish patients from healthy subjects (65, 66).

Remarkably, volatile organic compounds (VOCs), which are found in saliva, are another set of biomarkers that have been proposed for the investigation of oral cancer; nevertheless, the literature on this topic is lacking. One of the most important studies in this context is the study of Aro et al. (67). This study has conducted in Poland on 30 subjects of which five patients had oral cancer. Despite the fact that Aro et al. anxiety that the sample size was small, they observed a significant increase in five VOCs that are derived from oleic, palmitoleic, and linoleic acid (E-2-octenal, heptanoic acid, octanoic acid, E-2-nonenal, nonanoic acid, and 9-undecenoic acid) in patients with oral cancer (67).

Salivary enzymes such as MMP levels were measured in more than eight studies, with MMP-9 being the most often studied. According to one study, there were statistically significant changes in the salivary MMP-9 and MMP-2 concentrations between OPMD patients and control groups (68). An additional investigation revealed that patients with OSCC had considerably higher salivary MMP9 levels than those with OPMD (69). Other studies evaluated the capacity of Salivary metalloproteinase (MMP) , with MMP-9. According to one study, there were statistically significant differences ($p = 0.05$ and $p = 0.02$) in the salivary MMP-9 level and MMP-2 concentrations between OPMD patients and controls. Significantly increased salivary MMP9 levels ($p < 0.001$) were seen in another investigation (68).

On the other hand, according to the results of the study (26), there was a noteworthy increase in Lactate Dehydrogenase (LDH) levels in high-risk premalignant lesions and OSCC compared to control groups. The increases were 2.5 and 3.9 times higher, respectively. Similarly, when compared to control groups, salivary concentrations of various enzymes, including kallikrein, cathepsin V, l-fructose, AKR1b10, and LDH, have been found to be enhanced in OSCC patients (26, 25). Significantly, LDH is the most researched enzyme among these six enzymes belonging to salivary biomarkers.

Other researchers have also obtained similar results (29). Patients with OSCC had substantially higher average salivary levels of Aldo-Keto Reductase Family 1 Member B10 **(**AKR1b10); (646.47 ± 402.43 pg/mL) than others. Comparing OSCC patients and high-risk premalignant lesions to control groups, it was shown that LDH levels increased significantly (26), by 3.9 and 2.5 times respectively.

Currently, the development of novel approaches for the early diagnosis and precise treatment of numerous illnesses, including oral cancer, has significantly advanced with the recent advances in nanotechnology (70). Biosensors (BS) based on nanomaterials (NMs) have become a promising platform for the development of cancer therapeutic applications, i.e., simultaneous cancer diagnosis and treatment. The aforementioned constraints are addressed by the latest advancements in biosensing via the application of nanotechnology, which may enhance future methods for the identification and diagnosis of different oral cancers (71). Saliva is directly in contact with lesions related to oral cancer, making it a potentially more sensitive and specific screening tool than other approaches. Since the nanostructure and nanoparticles facilitate communication between the electrodes and the enzyme, they demonstrated encouraging results at a high surface volume ratio. Tests for the rapid, simple, robust, and reliable detection of oral cancer have been made possible by the development of nano BS-based screening and diagnostic techniques.

Salivary biomarkers appearance in saliva

The identification of molecular patterns in saliva that correspond to systemic disorders has led to the development of a novel non-invasive diagnostic approach known as "salivary diagnostics". Though the precise process behind the existence of these tumor markers in saliva is unclear, it's possible that they originate locally or are extracted from serum (72).

Moreover, saliva is comprised of hundreds of micro salivary glands, gingival crevice fluid, and secretions from the parotid, sublingual, and mandibular glands. Food digestion, bolus generation, lubrication, taste facilitation, and immune-related processes mediated by released antimicrobial peptides (73) and immunoglobulins (74) are among the functions of saliva. But these are only a few of the ingredients that make up saliva. Despite the discovery of several molecular species, little is known about how they act in the oral cavity.

Messenger RNA (mRNA) and microRNA (miRNA) transcripts (75, 76), metabolites (77, 78), and over a thousand proteins that are assumed to be involved in a broad range of biological functions have all been discovered to be present in cell-free saliva (CFS) (79). Changes in salivary concentrations of these molecules have made it possible to identify systemic and oral illnesses. Advances in genomic, proteomic, and metabolomic methodologies have enabled sensitive and high-throughput study of saliva and are showing growing value in the diagnostic domain.

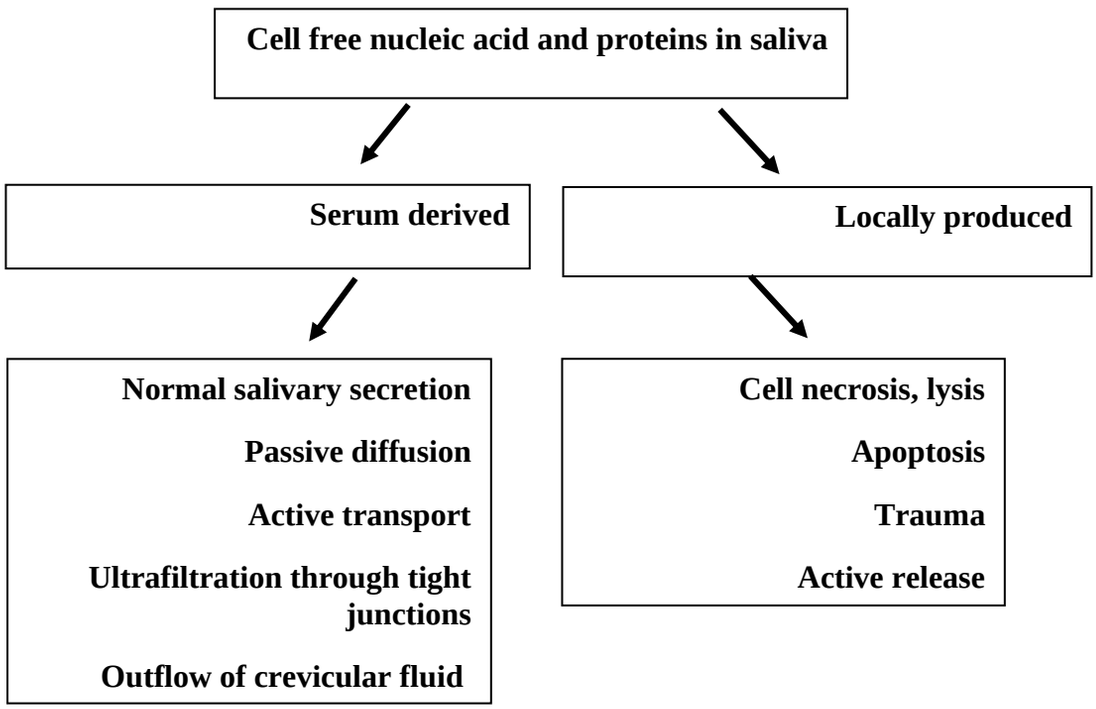

Figure 1: The possible leading mechanism for the presence of molecular markers in the saliva (80).

The genetic markers found in saliva

The development of special genetic abnormalities drives the onset and advancement of malignant tumors, tumor-specific genomic markers, comprising DNA and RNA markers, can be identified in saliva and are useful for the identification of oral cancer. Research has indicated that frequent loss of heterozygosity (LOH) occurs early in the development of oral cancer in chromosomes 3 p , 9 q , 13 q , and 17 p (81-84) .

It has also been possible to identify the exfoliated OSCC cells in saliva by looking for alterations in mitochondrial DNA. Direct sequencing has identified these mutations in 67% of the saliva samples from OSCC patients (85).

Conclusion

A special kind of biofluid, human saliva has enormous therapeutic and diagnostic potential. It offers a singular chance for head and neck (Oral cancer) pathology medical research by combining inexpensive, non-invasive investigation. Thus, integration data analysis for the proteome, metabolome, microbiome, and exosomes is a very promising area of research for the identification of translational biomarkers. Therefore, when it comes to the early screening and diagnosis of oral and head and neck squamous cell carcinoma, salivary biomarkers can be a useful tool. It also became clear that more study was required to increase the number of possible biomarkers for these malignancies. The results of this investigation are especially crucial for public health since early identification of oral malignancies can greatly improve patient outcomes, survival and mortality rates.

Funding: This research received no external funding.

Conflicts of Interest: The authors declare no conflicts of interest.

Data availability: The included meta-analysis in this review enables direct access to the data, which was gathered from previously published systematic reviews and meta-analyses.

Abbreviations

Abbreviation	Name
AKR1b10	Aldo-Keto Reductase Family 1 Member B10
AFP	Alpha-Fetoprotein
ASR	age-standardized rate
BS	Biosensors
CD44	Cluster of Differentiation 44
CEA	Carcinoembryonic Antigen

CFS	cell-free saliva
circRNAs	circular RNAs
CSTA	Cystatin A
CYFRA-21	Cytokeratin Fragment 21-1
DNA	Deoxyribonucleic acid
EGFR	Epidermal Growth Factor Receptor
GPx	Glutathione Peroxidase
HNC	Head and Neck Cancer
HPV	Human papillomavirus
IL	Interleukin
LDH	Lactate Dehydrogenase
LOH	Loss of heterozygosity
MDA	Malondialdehyde
miRNA	MicroRNA
MMP	Matrix Metalloproteinase
mRNA	Messenger RNA
ncRNAs	non-coding RNAs
NMs	nanomaterials
OCB	oral communication battery
OLP	Oral lichen planus
OPMD	Oral potentially malignant disorders
OSCC	Oral squamous cell carcinoma
PADI1	Peptidyl Arginine Deiminase 1
piRNAs	piwi-interacting RNAs
RNAs	Ribonucleic acid
TNC	tenascin-C
TNF-α	Tumor necrosis factor α

VELscope	Enhanced Oral Cancer Assessment System
VOCs	volatile organic compounds

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