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SIGNIFICANCE OF SALIVA AS A SENSOR DETECTOR IN MARKING ORAL CANCER

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Abstract
One of the most deadly forms of cancer is oral cancer. Since the screening methods currently in use are poor, a consistent portion of oral health issues are diagnosed at any late stage. Because existing diagnostic processes are complex and imprecise, it would be imperative to enhance them. The possibility of diagnosis by the discovery of biomarkers from biological fluids. The salivary glands secrete protein, peptides, electrolytes, organic and inorganic salts, and gingival crevicular fluids and mucosal transudates, which contribute in a complementary manner to human whole mouth saliva. This study focuses on biosensors that work at the molecular level, such as DNA, RNA, and protein biosensors, and it discusses tactics that use diverse biosensors to target different types of biomarkers. We also looked at non-invasive electrochemical techniques, optical techniques, and nanobiosensors for analyzing the cancer biomarkers found in bodily fluids including serum and saliva. Consequently, this review clarifies the advancements made in the creation of novel biosensors for the early identification and diagnosis of oral cancer. To clearly clarify the clinical situation, an introduction to oral cancer diagnosis, prognosis, and treatment is provided. Saliva is then offered as an alternative biofluid, along with its benefits, drawbacks, and methods of collection. It is suggested that salivary biomarkers be used as non-invasive diagnostic instruments.

Keywords: Biosensors, Biomarkers, saliva, oral cancer.

Introduction

Biosensors are receptor–transducer devices that use biological material to interact with an analyte (1) and deliver quantitative or semi-quantitative information utilising a biological recognition element. Biosensors are popping up all over the place in the medical profession. They are employed as diagnostic instruments for identifying infections, monitoring and detecting hazardous metabolites, glucose monitoring, cholesterol testing, and vitamin and other nutrient measurements (2) Oral fluid-based biosensors, for example, are used in dentistry to diagnose caries, periodontitis, and oral cancer by detecting saliva GCF samples. We also explain the methods used by biosensors to target different types of biomarkers and focused on biosensors that function at the molecular level. Early tumors often appear in the form of erytholeuko-plastic lesions, but they can also primarily manifest as white patches (leukoplakia) or red patches (erythroplakia) and they are usually asymptomatic, thus there is a high risk that they go unnoticed. OSCC, in its advanced stages, may appear like an ulcerated lesion fixed to underlying tissues, with raised borders and hard on palpation, but it can also be exophytic with a papillary or fungating surface [9 10 11]. At these stages, patients usually suffer from mild or severe pain, depending on the case, that can even spread to the ear. Other signs and symptoms are frequent bleeding, teeth mobility, dysphagia, paresthesi, or even cachexia and severe anemia. Growth of malignant tissue in the oral cavity, which usually affects the tongue, floor of the mouth, cheek, gingiva, lips, or palate, is recognized as oral cancer. OC accounts for one-third of all cancer cases worldwide and India accounts for roughly 30% of all cases. The most frequent indicators of OC are a non-healing sore in the mouth and discomfort that is difficult to ease. Other symptoms include a lump or thickening in the cheek; white or red spots on the gums, tongue, and other regions of the oral cavity; as well as a persistent sore throat and difficulty eating or swallowing. OC can be avoided by reducing the risk factors such as consumption of tobacco (both smokeless and chewable) and alcohol, as well as raising awareness about these issues. The oral etiology begins with a burning feeling in the mouth, some restriction while opening the mouth, and any red or white lesions in the oral cavity; however, a lesion's histology may show dysplastic characteristics.

Such conditions are categorized under oral potentially malignant disorders. Early identification is critical for the disease's prognosis and the sufferer's survival if not detected in the very early stage of OPMD, which can be life-threatening and associated with increased morbidity. In fact, several risk factors have been identified for etiopathogenesis of oral cancer and, among these, tobacco and alcohol consumption seem to have a synergistic effect. Male sex, old age, high-risk human papillomavirus (HPV), dietary habits, oral bacteria, ultraviolet radiation, as well as betel- quid chewing, have also proved to play a major role in the appearance of the disease Moreover, several premalignant lesions, such as erythroplakia, leukoplakia, oral submucous fibrosis and oral lichen planus, have been associated with the onset of the neoplasia. Indeed, a chronic stimulus acting on keratinocytes can lead to hyperplasia, different degrees of dysplasia and then to carcinoma in situ and invasive carcinoma.

Types of oral cancer:

1. Tumors originate from surface epithelium:
   A.squamous cell carcinoma most common type (90-95) *m*elanoma
2. Tumors originate from glandular tissues (salivary glands, metastatic cancer from breast, prostate, lung)
   Adenocarcinoma
   adenocystic carcinoma
   mucoepidermoid carcinoma
3. tumors originate from mesenchymal tissues:
   sarcoma (osteosarcoma, chondrosarcoma. fibrosarcoma, Ewing sarcoma) lymphoma.

Etiology:

Tobacco and alcohol use. Tobacco use of any kind, including cigarette, pipe and cigar, and electronic cigarette smoking, as well as chewing tobacco and snuff puts you at risk for developing oral cancers. Heavy alcohol use also increases the risk. Using both tobacco and alcohol increases the risk even further.

- HPV. Infection with the sexually transmitted human papillomavirus (specifically the HPV 16 type) has been linked to oral cancers.
- Age. Risk increases with age. Oral cancers most often occur in people over the age of 40.
- Sun Exposure. Cancer of the lip can be caused by sun exposure.
- Poor Nutrition. A diet low in fruits and vegetables has been linked with increased risk of oral cancer.
- Genetics. People with inherited defects in certain genes have a high risk of mouth and middle throat cancer.

Symptoms:

- A sore irritation, lump or thick patch in your mouth, lip, or throat.
- A white or red patch in your mouth.
- Persistent sore throat, a feeling that something is caught in your throat, or hoarseness or loss of your voice.
- lump in the neck.
- Difficulty chewing, swallowing, or speaking.
- Difficulty moving your jaw or tongue.
- Swelling of your jaw that causes dentures to fit poorly or become uncomfortable.
- Pain or bleeding in the mouth.
- Numbness in your tongue or other areas of your mouth.

Organs involved in oral cancer:

- Buccal Mucosa Cancer (Inner Cheek Cancer).
- Floor of Mouth Cancer.
- Gum Cancer.
- Hard Palate Cancer
- Lip Cancer.
- Tongue Cancer

Various types of biosensors used in detection of oral cancer:

Biosensors are used for identifying cancer indicators are being designed and developed by researchers and scientists in order to detect early cancer. OC may be detected effectively and early using biosensors. DNA, RNA, and protein biosensors have all been proven in studies for their efficiency in detecting OC and providing useful information to allow for non-invasive OC detection. The use of biosensors for protein biomarker analysis has emerged as a promising and cost-effective method for developing point-of-care devices. Electrochemical biosensors have been used in detecting cancer markers. Surface plasmon resonance sensors

(SPR), which are based on spectroscopy of surface plasmons, are being employed for the label-free detection of cancer markers (3). Because of their light weight, great sensitivity, and low power requirements, piezoelectric biosensors have also been used to detect cancer markers. Tan et al. developed a surface-immobilized optical protein sensor to detect an IL-8 marker for the diagnosis of OC. Yuan et al. created an SPR-based biosensor for detecting cancer markers in ovarian cancer patients (4). Kumeria et al. created a microchip biosensor for detecting circulating tumour cells based on nanoporous aluminia [23]. Malima et al. have developed a very sensitive microscale in vivo sensor for various biomarker detections that is enabled by the electrophoretic assembly of nanoparticles [24]. Because of their excellent sensitivity, specificity, compact size, rapidity, and cost-efficiency, optical biosensors are currently gaining popularity for biomarker Detection. Nanotechnology, MEMS (micro electromechanical systems), NEMS, biotechnology, and other multidisciplinary techniques have been applied in the development of novel optical biosensors

DNA Biosensor:

DNA is the genetic information carrier and the building block of biological heredity. Following the identification of DNA, DNA-based diagnostics such as the RAPD, RFLP, and PCR methods emerged. About 5–10% of malignancies are inherited and caused by single-gene mutations. Several hereditary cancer syndromes and their causative genes have been identified. To address these issues, DNA sensors were included in high through put analysis, implying a significant reduction in effort, time and cost. biosensors and biorecognition elements that, when combined with various transduction mechanisms, have aided the rapid expansion in the domain of bioanalysis and its associated technologies. These characteristics, as well as additional benefits such as the ease of manufacturing and operation and the cheap costs, make it an attractive option for the non- invasive early detection of OC in saliva.

RNA Biosensor:

Multiple cancer-causing defects, such as the inactivation of the anti-tumour gene, chromosomal degradation, and gene hypermethylation, change the signature of normal cells. These cancer-causing aberrations, such as microRNAs (Mir), are considered RNA- based cancer biomarkers. RNA biomarkers allow cancer to be diagnosed even when no physical signs are present. Wang et al. produced a POC adaptable magnetic-controllable electrochemical-based biosensor with great sensitivity (5).

Protein Biosensor:

For POC and clinical analysis, electrochemical biosensors provide a sensitive, fast, and low-cost diagnostic framework for detecting protein cancer biomarkers. Because the interactions between an antibody and an antigen operate similar to a lock-and-key binding mechanism, immunosensors are very selective. Protein biomarkers for cancer detection can be used to measure components that are thought to be indications of aberrant biological processes, disease processes, or treatment intermediation responses. These biomarkers are frequently collected from biofluids and their expression level usually indicates disease status. Since many tumour markers are identified in saliva, the salivary samples are of great interest for the non-invasive screening of OC. New carbon or metal nanomaterials such as graphene and its derivatives, have been developed with unique qualities, such as high electron transfer rate and compatibility, and a result, numerous studies have combined nanomaterials with immunosensors (6).

Salivary Metabolomics:

Saliva has advantages over other biofluids, such as blood and urine, since its collection is non- invasive and relatively fast. Some salivary metabolites have been successfully identified using 1 H NMR and inter-subject variability has been studied but to date, chemometrics has not been applied to saliva metabolite datasets to find the potential markers for disease. As a first step in understanding the human saliva metabolome, we employed high-resolution 1 H NMR spectroscopy to determine if the salivary metabolite composition differs due to gender, stimulation, or smoking status.

Salivary Proteomics:

A global quantitative analysis of human salivary proteins without resources using a mass spectrometer can be easily obtained using a 2-DE strategy, which is widely used for biomarker discovery, namely for oral diseases, dental caries and periodontitis and also for other

pathophysiological conditions such as Sjögren syndrome (SS) and non-Hodgkin's lymphoma. In addition to biological variability, technical bias induced by protein migration during the focusing step or by gel staining could make it difficult to spot detection and their boundaries, making 2-DE gel analysis a hard task technology.

Electrochemical biosensor:

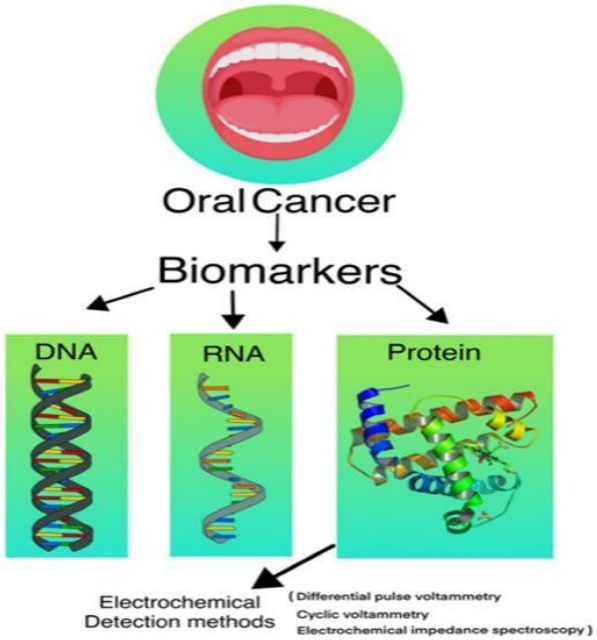


Fig.1: Electrochemical Detection method

Electrochemical biosensors have a higher rate of implementation than other biosensor because they can detect practically any type of biomarker and are simple to combine with typical laboratory benchtop equipment. Furthermore, because they are easily downsized, they are likely to be integrated into wearable and portable devices. The integration of electrochemical sensors into compact devices must meet strict requirements for convenience, comfort, ease of operation, and flexibility, making the creation of dependable, wearable, and portable POC ultrasensitive devices difficult. (7)

Saliva based Biosensor:

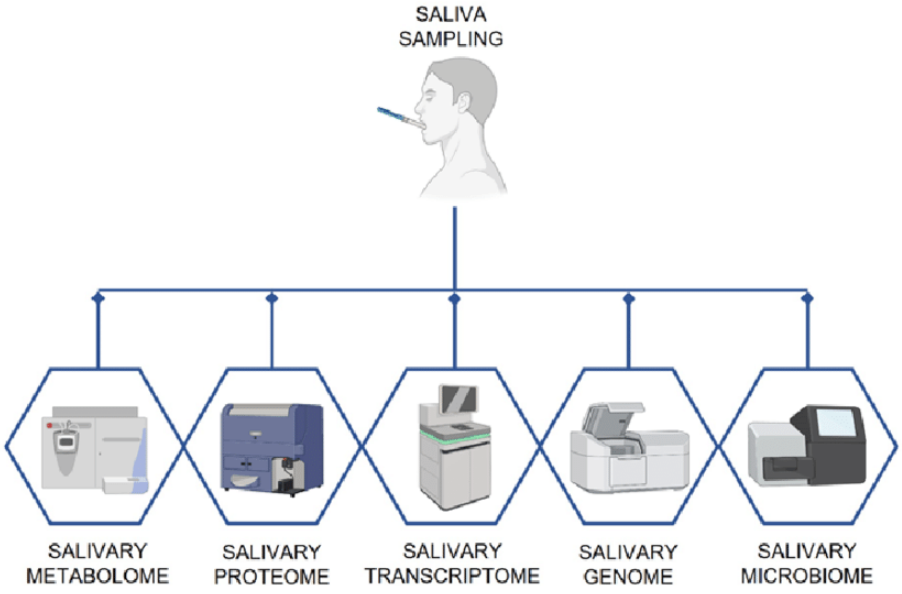


Fig2: Saliva based Biosensor

Saliva is a thin watery liquid secreted by the salivary glands into the mouth. Active transport or passive diffusion can bring salivary components from salivary glands or the associated vasculature. Proteomic, microbiome, immunologic, genomic (transcriptomic and epigenome), and metabolomics biomarkers have been identified as components that correlate with specific disorders. Three groups of researchers in the United States have successfully discovered 1166 proteins in human saliva. Matrix metalloproteinases (MMP1, MMP3, and MMP9), cytokines (IL-6, IL-8), and vascular endothelial growth factor were among the biomarkers they discoveredAs a result of these indicators, saliva appears to be a viable diagnostic fluid

However, there are a number of downsides to using saliva as a diagnostic fluid, the most significant of which is its low specificity and sensitivity .Microfluidics and microelectromechanical devices for DNA, gene transcripts (mRNA), proteins, electrolytes, and tiny chemicals in saliva, as well as the overall profile, correlate to a disease state. These technologies identify diseases using a mix of many biomarkers rather than a single biomarker, addressing the limitations of sensitivity and specificity in single-marker

location. Genetic variations in patients can be found by examining micro -Namaskars. As a result of these indicators, saliva appears to be a viable diagnostic fluid (8).

Saliva as a diagnostic and monitoring tool:

Saliva, which consists for more than 99% of water, contains a variety of electrolytes, including potassium, magnesium, calcium, sodium, bicarbonate and phosphates, as well as biological elements, such as immunoglobulins, mucins, enzymes, proteins and nitrogenous products . The main functions of this biofluid are lubrification, antibacterial activity, buffering action, digestion and tooth protection and clearance. Saliva is produced by two types of salivary glands: the major, which include parotid, submandibular and sublingual glands and are responsible for more than 90% of saliva secretion, and the minor, which are located in the oral mucosa and in the palate

While blood still remains the gold standard for most examinations, the collection of salivary samples has several advantages: it is non-invasive, simple, stress-free, easily repeatable and it does not require special storage condition or skilled clinicians. Moreover, saliva is stable over time, it does not clot, and it ensures availability of large sample quantities. Traditional methods of collecting saliva include burr collection or stimulation methods (e.g., mechanical, gustatory, and olfactory stimuli), which are meant to obtain a larger sample volume. Salivary flow rate is variable, but values greater than 0.1 mL/min are generally considered physiological for unstimulated saliva, while these values increase to 0.2 mL/min for stimulated saliva. Proteomics is a branch of salivaomics that deals with the study of salivary proteins, including those coming from microbial species. More specifically, α-amylases, statherin, P-B peptide, histatins, salivary ("S-type") cystatins and mucins are the most common markers that can be found in saliva, along with a few proteins, which are mainly secreted by minor glands and gingival crevicular fluid, including α-defensins, b- thymosins and lipocalin (9).

Techniques used for diagnosis of oral cancer:

Early detection is very important to reduce the mortality rate of patients suffering from oral cancer. Thus, there is a huge demand for oral cancer diagnostic techniques that are non- invasive, rapid, and easy-to-use. For oral cancer diagnosis, traces of oral lesions in the mouth are first observed properly by a physician. Upon suspected malignancy, it is further referred to an oral or maxillofacial surgeon who conducts the specific tests. In the case of oral cancer, a dentist plays a pivotal role in the early examination of occurrence.

Physical examination:

The primary and the most crucial assessment for oral cancer is the physical examination, which usually consists of two steps – systematic visual examination and palpation. Primarily, the external parts such as lymph nodes, salivary glands, lips, etc. are inspected, and subsequently, an internal examination of the buccal cavity is performed. Abnormalities, irregularities, swelling, and fluctuance in superficial anatomy are recorded. Soft tissue thickening, lumps, soreness, trouble in jaw movement, chewing and swallowing, ear pain, etc. are some of the common observations. The parotid gland (largest salivary gland) is

palpated [65] both intra-orally as well as externally and further, the submandibular and sublingual glands are palpated. The examiner compares physical observations with the patient's clinical presentations. The pathological changes are recorded along with abnormalities in the texture and color.

Histopathological examination:

By the histopathological standpoint, the OSCC varies from indolent tumors to very aggressive tumors with high invasive potential. The gradual development of the carcinoma in the oral cavity starting from simple dysplasia to highly invasive tumors is revealed by the histological assays. The histopathological analysis is essential to verify the proliferation of cells and maturation abnormalities, cellular and cytoplasmic atypia, and alteration of the surface epithelium or deep tissue cytoarchitecture. Identification and adequate sampling of the oral lesion is an important step in a histological investigation. Sometimes, histopathological changes may occur in the areas that do not show any evidence of oral lesions during physical examination. Molecular and genetic changes may take place in benign tissues before microscopic and clinical morphological changes occur. Thus, a procedure that detects both, histopathological and molecular changes, is preferred to diagnose benign or malignant tumors (10).

Vital staining techniques:

Visual tissue staining is an adjunct technique used in the diagnosis of cancer .Tol onium chloride (also known as toluidine blue) staining is used to detect the mucosal abnormalities in the oral cavity. Toluidine blue is a type of acidophilic metachromatic dye that stains acidic components of tissue such as sulphate, phosphate, and carboxylic moieties (i.e. DNA and RNA), selectively. Because of the ease of use and cost-effectiveness, this technique is suitable in developing countries like India. Lugol's iodine in combination with toluidine blue aids in the distinction of the inflammatory lesions. This combination predicts the degree of differentiation of malignant lesions, which makes it an important visual staining technique for the pre-therapeutic assessment of oral cancer . In a study from Maharashtra, self-examination and clinical examination were carried out initially, after which, screening methods using toluidine blue and Lugol's iodine stains were performed followed by a biopsy. Lugol's iodine was found to be more sensitive in comparison to toluidine blue.

Biopsy:

A tissue sample is removed surgically from the suspected region and sent to the pathological laboratory for the detailed microscopic examination. This is the only way to ascertain the presence of an oral cavity or oropharyngeal cancer . For a biopsy, careful handling of the tissue is very critical for confident histological diagnosis. Improper handling of the sample may result in a defective biopsy and the procedure needs repetition. Depending on the specific requirement, biopsies such as exfoliative cytology and incisional biopsy are carried out.

Brush biopsy:

In brush biopsy, the transepithelial cells from the oral lesion are obtained by scraping the surface mucosa. A brush biopsy is a simple, painless, chair-side, inexpensive, highly sensitive, and risk-free method for oral cancer screening. It is useful in identifying any suspicious lesion, which includes small red and white oral lesions, to rule out any dysplastic features. Brush biopsy has higher sensitivity and specificity of around 90% in comparison to other biopsy techniques . The brush biopsy has been coupled with portable tablet-based microscopes for digital detection of abnormalities in stained tissues.

Imaging technique:

Several advanced imaging techniques are used for the diagnosis of oral cancer. Among these, the most routinely used scanning techniques are magnetic resonance imaging (MRI), computed tomography (CT), and positron emission tomography (PET).

Magnetic resonance imaging:

MRI provides the details of the structures in the oral cavity along with adjacent parts. The soft-tissue discrimination by MRI aids in assessing the extent of local and regional spread of the tumour, invasion depth, and extent of its lymphadenopathy. MRI is an accurate technique for determining the spread of oral cancer to its surrounding soft tissues as it has a very high-contrast resolution and multiplanar views. Thus, MRI is critical for the pre- treatment analysis of advanced oral and oropharyngeal cancer MRI also assists in recognizing the source, position, and margins of lesions. Thus, MRI may be considered as a supportive technique to biopsy for routine screening of oral cancer cases.

Computed tomography:

The CT scan uses the x-ray radiations and a computer to create pictures of the body to locate the malignant lesion and determine its spread to the other parts of the body. CT scan

is a widely accessible and comparatively less expensive procedure and thus, is considered as a standard imaging technique for the detection of head and neck tumors. But it is observed that lesions in their early stages cannot be recognized using a CT scan. Minor early-stage lumps existing in the buccal cavity can be detected by CT scan only upon enhancement using an intravenous contrast medium.

Positron emission tomography:

The PET scan is used to determine the spread of tumour cells to the lymph nodes or other parts of the body. A radioactive dye is administered orally or intravenously and the gamma rays emitted from the positron decays are scanned. It is an accurate method that determines the staging of the lymph nodes. It does not implicate any change in the treatment approach even if additional lymph node metastasis is detected if the nodes are detected apart from the affected site . The status of lymph nodes is determined before surgery by PET scan using fluorodeoxyglucose (FDG). Thus, PET scanning is crucial for the early detection of oral malignancy .

Optical and radiological techniques

:X-ray is used to determine the spread of cancer to the other organs outside of the mouth and oropharynx. The x-ray for oral cancer is known as an orthopantomogram also known as the panorex scan . X-ray images of the area surrounding the upper jawbone (maxilla) and the lower jawbone (mandible) demonstrate the presence of cancerous cells around these bones. Other radiological techniques that are used for the detection of cancer and staging of the cancer cells are fluoroscopy, ultrasound, etc. Various optical spectroscopy techniques such as laser-induced tissue autofluorescence and diffuse reflectance are recently recognized for the diagnosis of oral malignancy.

Biosensors and bioelectronic platforms for the detection of oral cancer biomarkers in saliva:

The techniques and methods for cancer diagnosis employed in clinical practice have not substantially changed in the last couple of decades, while many advanced technology- gies are constantly being developed in laboratory settings. The traditional diagnostic pro- cadre involves two separate stages. At first, an invasives tissue biopsy of the affected region is performed. This is usually followed by medical imaging, including computed tomography (CT), magnetic resonance (MRI) or positron emission tomography (PET) these methods have been validated extensively and are thus widely accepted, but they also suffer from inherent disadvantages such as invasiveness, cumbersome procedures, and high costs. Blood tests have opened new avenues for rapid screen-Ing of the population and early diagnosis, but they still present a certain degree of invar- siveness, especially for fragile individuals. Commercially available ELISA kits provide good sensitivity, but they suffer from an inherently long assay time. Biosensors in this regard offer substantial advantages such as rapid time to response, and easier protocols. The main components of a biosensing system are presented Components of a biosensor and its working principle.

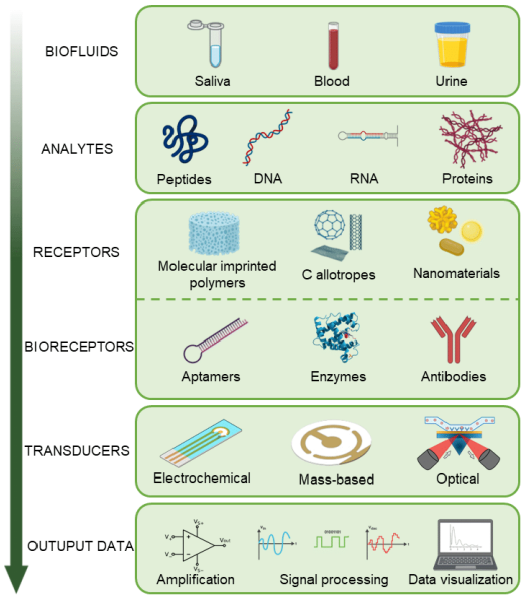


Fig 3: Components of a biosensor and its working principle.

A biomarker, or a set of biomarkers, is selected based on the criteria of sensitivity and specificity and then researched in the body fluid of interest. The recognition element serves as the binding site for the selected biomarker, and it can be of natural (bioreceptor) or synthetic (receptor) nature, or a combination of both. The transducer then operates the conversion of the biological signal generated by the binding event into a measurable sig- nal, through a specific detection mechanism (electrochemical, mass-based, optical, etc.) that also depends on the biomarker. The signal obtained is then amplified and undergoes several steps of signal conditioning and processing, either on the device itself (on-board) or after the signal has been transmitted to a remote device.

The possibility to non-invasively analyze body fluids, including serum and saliva, expanded the library of available biofluids for earlier diagnosis and timely treatment of : malignancies The field of biomarker discovery has largely relied on serum or plasma as the biofluid of choice. Saliva is a readily available biofluid that, as a biomarker resource, has been relatively unexplored until about a decade ago. Then D.T. Wong and his research group pioneered the use of saliva as biofluid for oral cancer bi- markers detection and since that moment the field has seen a marked increase in interest from the scientific community

The rapid advances in flexible electronics and miniaturized technologies now allow the development of versatile and scalable bioelectronic platforms that could be employed for early cancer screening, from readily available body fluids the identification of changes in saliva indicating disease progression underlines the utility of saliva as a non- invasive source of informative biomarkers reflecting disease burden and progression. Sa- live also possesses drawbacks, including rapid biofouling on the surface of biosensors the effect of interferon's that are present in saliva in different concentrations and the presence of a highly dynamic environment orally. Nevertheless, the development of advanced bioelectronic tools have enabled researchers to overcome most of the bottle- necks that are present today in salivary diagnostics. Biosensor summarizes the most recent efforts in the development of biosensors for early diagnosis of oral cancer. The year of publication has been included to present the reader with the evidence of an increasing interest among the scientific community toward oral cancer detection through biosensors targeting saliva. Quantitative performance parame- ters such as limit of detection (LOD) and response/incubation time have been reported as a metric of comparison between the studies. In most cases, raw saliva is the only biofluid that has been analyzed to clinically validate the developed biosensor. However, there are studies where the authors decided to evaluate the device performance in both artificial and real saliva, in order to estimate the matrix effect of a real biofluid as opposed to the artificial one

Summary of recent studies of biosensors and bioelectronic systems for early diagnosis of oral cancer

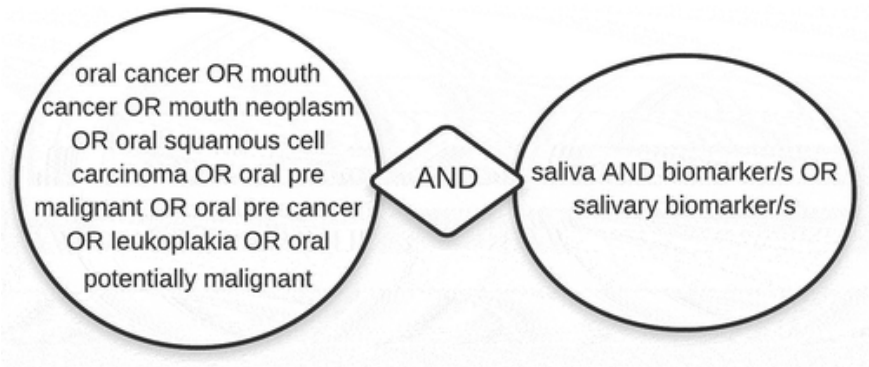
Methodology:

Data sources:

A systematic electronic literature search was conducted to identify relevant published studies using Medline (Ovid), Web of Science, Embase and Scopus databases. The original literature search was conducted in May 2018, and the search was updated in September 2020

Search strategy:

The keywords (Figure 1) were combined with AND/OR Boolean to generate the search syntax. The search was conducted without time or language restrictions. Search syntax used for Web of Science database was as follows: 'oral cancer*' Mesh terms (mouth cancer, mouth neoplasm, mouth carcinoma, squamous cell carcinoma) OR 'oral premalignant' MeSH terms (pre-cancerous) AND 'saliva* biomarkers*' MeSH terms (saliva, biomarkers).



Screening and study selection:

Abstracts retrieved from the search were exported to RefWorks library. Following title and abstract screening 346 articles were selected for the full-text screening. These were screened by two blinded reviewers with four pairs of reviewers (NP+SR, NP+PC, NP+RA, NP+RMSR) assessing approximately 65 papers per pair. Study selection at all stages was conducted using the following eligibility criteria.

Inclusion criteria:

Original research articles containing primary data.

Studies including patients with head and neck cancer including oral cavity, OSCC or OPMD aged 18 and above

Exclusion criteria:

Reviews, systematic reviews, meta-analysis, conference proceedings, case reports and case series.

Full text articles published in languages other than English.

Studies using non-human subjects.

Studies that did not analyze biomarkers in saliva or salivary rinse of participants.

Study selection is summarized using PRISMA (preferred reporting systems for systematic reviews and meta-analysis) flow chart

Reviewer calibration:

All reviewers extracted and analyzed data from five randomly selected papers for training and calibration. Once calibration was achieved two reviewers extracted data from each paper independently and blinded to one another's scores. Four pairs of reviewers (NP+SR, NP+PC, NP+RA, NP+RMSR) conducted the data extraction. Disagreements were resolved through discussion and when necessary, with the involvement of a third reviewer

Data extraction:

The variables extracted from included articles were: first author, published year, country where the study sample was obtained, study design, age, gender, sample size, biomarkers, method used to analyse the biomarkers, relationships between salivary biomarkers and risk factors and main conclusions. The data were recorded and summarized using bespoke Microsoft Excel spreadsheet and descriptive data analyses were performed

Quality assessment:

Risk of bias assessment of the studies was conducted using Newcastle Ottawa scale. A star (*) was awarded to the feature of the study that minimized risk of bias in each category. Studies with 6–9 stars were graded with high quality

A most important result found in this study is that all eight salivary parameters analyzed in the cancer patients were altered in a highly significant manner and were characterized by relatively high sensitivity and specificity values moreover all markers talked with each other that is this alteration significantly correlated among themselves indicating that

Conclusion:

The development of biosensors for the effective and rapid non-invasive detection of cancer markers is a major priority for scientists and cancer researchers due to the shortcomings of current cancer detection approaches. Chemicals known as cancer markers indicate that the body contains cancer cells. Saliva, blood, and other physiological fluids contain these markers. A technical developmental approach with a small number of individuals and hundreds of variables forms the basis of most earlier studies. More transitional and translational studies are required to turn this fundamental knowledge into clinical practice for the detection and prevention of oral cancer, as well as for different clinical applications. The way for the integration of biosensors into routine clinical practice will be paved by carefully thought out and structured clinical trials that evaluate the diagnostic, prognostic, and predictive capabilities of these devices. Future research endeavors ought to examine the biosensors included in the evaluation to determine their suitability for everyday clinical settings. Researchers are always looking for new biomarkers for different types of cancer, and some of these have already been used in clinical settings for cancer monitoring and screening. A fresh and creative method for the quick early detection of cancer is made possible by the invention of biosensors.

Author contributions

All authors are contributed equally.

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Declaration of Competing Interest
The authors have no conflicts of interest to declare.
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