

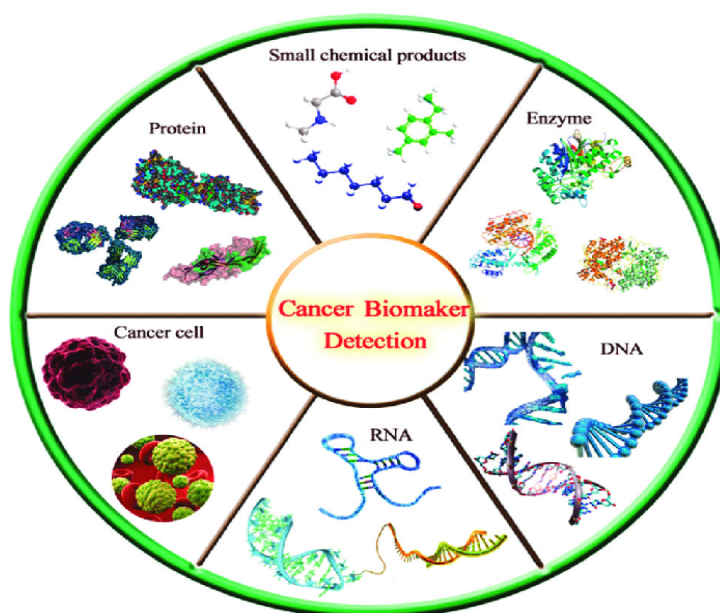
SALIVAOMICS:**An emerging diagnostic tool for biomarkers in oscc..... Review**

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SALIVAOMICS:**An emerging diagnostic tool for biomarkers in oscc**

Introduction

- Oral cancers are the sixth most frequent cancer with a high mortality rate. Oral squamous cell carcinoma accounts for more than 90% of all oral cancer¹
- Human whole-mouth saliva contains proteins, peptides, electrolytes, organic, and inorganic salts secreted by salivary glands and complimentary contributions from gingival crevicular fluids and mucosal transudates which can be of diagnostic importance and are called salivary biomarkers.²
- Salivary biomarkers represent a promising non-invasive approach for oral cancer detection, and an area of strong research interest³
- **This review presentation is aimed to enlighten on the different salivary biomarkers that will help in diagnosis and early management of OSCC. And to include salivaomics in routine investigations for both early diagnosis and also to improve the treatment modalities, determine the prognosis.**

Eligibility criteria:

Inclusion criteria: Scientific papers written in e PubMed, Science Direct, and SciELO databases. Descriptive observational, analytical observational designs, intervention studies (experimental), were included in this review article.

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 process.

- **Oral squamous cell carcinoma accounts for more than 90% of all oral cancer**
- The distribution of oral cancer is approximately 32% in buccal mucosa, 22% in tongue, 11% in lower lip, 11% in palate, 8% in vestibule, 5% in alveolus, 5% in floor of the mouth, and 3% in gingiva⁽⁴⁾.
- The risk factors are many including smoking, betel nut, tobacco chewing, drinking, poor nutrition, HPV virus, mouth washes with a high alcohol content, poor oral hygiene, immune system suppression, age, gender, etc. Diagnosis at the earliest stage is therefore very important for increasing the rates of patient survival. The survival rates are approximately 80–90% when detected at the earliest⁽⁵⁾.

• Saliva:

- The definition of biofluid is “a liquid or mixture of different molecules produced by living organisms which are of biological importance. e.g. saliva, urine, serum, blood, interstitial fluid, plasma, cytosol, etc.”
- Saliva collection is non-invasive, simple, and rapid method. Saliva can be collected in two ways:
- 1) Unstimulated saliva is collected by draining or drool, spitting, suction.
- 2) stimulated whole saliva is collected by providing the patients with a stimulant agent, such as citric acid, paraffin, or a gum base⁽⁶⁾.
- When stimulated, saliva is obtained primarily from the parotid gland (active secretion), whereas unstimulated (resting) saliva is produced primarily by the submandibular gland, with minor contributions from the parotid and sublingual glands⁽⁷⁾.
- Normal salivary flow is 0.3 -0.4 ml /min for unstimulated flow and 1.5 -2.0 ml/min for stimulated flow.
- Unstimulated saliva flow rate of less than 0.1 ml /min and less than 0.7 for stimulated flow rate is considered as hypo function.

For sialometry...the patient is instructed to refrain from eating, drinking, smoking, chewing gum, and oral hygiene practice or any other stimulation for at least 90 min before the test.

And before 10 min prior to test patient is asked to rinse with water to remove debris.

The main methods of whole saliva collection are

- Active drainage methods
- Passive drainage method
- Suction and absorption method

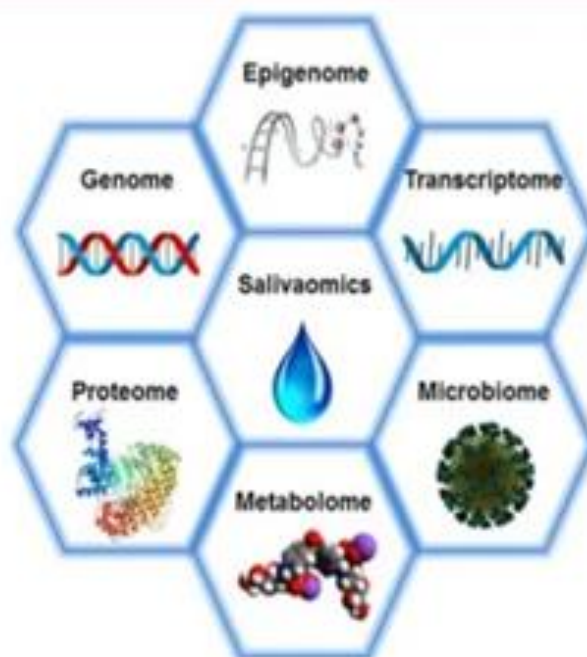


Various types of saliva collection devices; (A) RNAPro, SAL (Oasis Diagnostics), (B) SimplOFy (Oasis Diagnostics), (C) OraGene (DNA Genotek), (D) Salivette (Sarstedt), (E) Saliva DNA Collection Device (Norgen Biotek), (F) SalivaBio (Salimetrics) devices

Biomarker:

- According to World Health Organization (WHO), biomarkers are any substance, structure or process that can be measured in the body or its products and influences or predicts the incidence of outcome or disease and are considered as molecular markers or signature molecules that are exclusive for a particular disease (e.g.oral cancer)
- saliva contains a large number of proteins, bioactive peptides, nucleic acids, and electrolytes originating from the parotid glands, submandibular gland, sublingual glands, and other minor glands.
- Saliva's direct contact with oral cancer lesions makes it more specific and potentially sensitive screening tool, where as more than 100 salivary biomarkers (DNA, RNA, mRNA, protein markers) have already been identified, including cytokines (IL-8, IL-1b, TNF-a), defensin-1, P53, Cyfra 21-1, tissue polypeptide specific antigen, dual specificity phosphatase, spermidine/spermineN1-acetyltransferase , profilin, cofilin-1, transferrin, and many more.
- **Applications of biomarkers⁽⁹⁾**
 1. Biomarkers help in predicting the preventive measures that could be formulated.
 2. Aids in detection of various stages of oral malignant transformation.
 3. Evaluates the molecular changes related to oral carcinogenesis. Enhances the prognosis, diagnosis, and treatment of oral carcinomas.
 4. Helps in manipulating the drugs used for the treatment of cancer.

Salivary biomarkers for tumors



Genomic biomarkers identified in OSCC---

- DNA (promoter hyper methylation)⁽¹⁰⁾
- Histone family 3 (H3) which signifies DNA binding capacity⁽¹¹⁾
- S100 calcium-binding protein P (S100) for Protein and calcium ion binding⁽¹¹⁾
- P53 gene codon 63 gives fast, accurate, and sensitive diagnosis of OSCC.⁽¹²⁾

Transcriptomic Biomarkers Identified in Unstimulated Whole Saliva (USWS):

- IL-1b, IL-8 signifies Angiogenesis, cell adhesion, chemotaxis, immune response, replication, signal transduction, proliferation, inflammation, and apoptosis,⁽¹³⁾
- H3 histone family 3A(H3F3A) for DNA binding activity⁽¹¹⁾
- miR-125a, miR-200a, miR-31 signifying Posttranscriptional regulation by
- RNA silencing complex, cellular growth, and proliferation in elevated levels in OSCC.⁽¹⁴⁾

Metabolomics Biomarkers identified in OSCC:

- Cadaverine, alanine, serine, glutamine, piperdine, taurine piperdine, choline, pyroline hydroxyl carboxylic acid, beta-alanine, alpha-aminobutyric acid betaine, tyrosine, leucine β isoleucine, histidine, tryptophan, glutamic acid, threonine, carnitine, pipercolic acid, lactic acid, phenylalanine and valine^(15,16)

Miscellaneous Biomarkers Identified in OSCC:

- Oxidative stress related molecules like, Glutathione S-transferase (GST), peroxidase, malondialdehyde (MDA), 8-hydroxy-2- deoxyguanosine (8-OHdG), glutathione S-transferase (GST), reactive nitrogen species (RNS) such as nitric oxide (NO), nitrites (NO₂) and nitrates (NO₃), superoxide dismutase⁽¹⁷⁾
- Nonorganic compounds like Mg, Ca, F, and Na because Mineral composition is helpful in checking the oral dehydration and hence the severity of OSCC in communication with other biomarkers⁽¹⁸⁾

• Evidence from the literature for salivary biomarkers in OSCC

<i>Year</i>	<i>Authors</i>	<i>Biomarker studied</i>	<i>Observations</i>
1998	Mizukawa et al. ⁽²¹⁾	Defensin-1	Higher concentrations were found
1999	Mizukawa et al. ⁽²¹⁾	Intermediate filament protein (Cyfra 21-1), TPS	Protiens Found in OSCC
2007	Franzmann et al. ⁽²³⁾	CD44	Increased CD44 levels in saliva (oral rinse) of OSCC patients (n = 102) compared to controls (n = 69)
2004	St John et al. ⁽²⁴⁾	IL-8, IL-1b	Increased concentration of IL-8, IL-1b in OSCC patients
2008	Boyle et al. ⁽²⁵⁾	P53	p53 mutations were identified in 71% of saliva samples of OSCC patient
2013	Khor et al. ⁽²⁶⁾	DUSP1	Significantly elevated in OSCC patients
2012	Liu et al. ⁽²⁷⁾	miR-125a, miR-200a, miR-31	miR-125a, miR-200a levels are significantly reduced in patients with OSCC. miR-31 levels are increased in patients with OSCC compared to controls.
2011	Lajer et al. ⁽²⁸⁾	miR-31	Was considered as a potential biomarker in OSCC.
2021	Kiran B. Jadhav MDS, Vandana Shah MDS ⁽²⁹⁾	microRNA-21	Showed high sensitivity and specificity in oscc metastasis
2021	Jiajia Li, MD, and Xiufa Tang, PhD ⁽³⁰⁾	PFKFB3	PFKFB3 as a predictor of LNM in OSCC
	Jade silva kozlowski de oliveira, tainã marques siqueira, monarko nunes de azevedo, rita de cássia gonçalves alencar, eneida franco vêncio ⁽³¹⁾	Differential expression of CD44 and CD133	CD44 in the invasive behavior of conventional SCC. CD133 seems to be associated in the metastatic process.
2020	M.S. Lekshmy, T.T. Sivakumar, Anna P. Joseph, B.R. Varun, Vinod Mony, and A. Reshmi ⁽³²⁾	Aquaporin-3, a transmembranous protein	AQP3 as a potential biomarker for predicting the malignant transformation of OED and tumor progression

Merits and demerits of salivary biomarkers⁸

Merits:

- Salivary biomarkers have many advantages over serum because they are inexpensive, non-invasive, cost effective and easily accessible media and plays a vital role in diagnosis, prediction of prognosis and monitoring of patient's health.
- It can be used for early detection of carcinomas.
- It is a convenient medium for multisampling and safe for health care professionals compared to blood and is also used for detection of other types of cancers.

Demerits

- Lack of standardization procedures like sample collection, processing and storage.
- Variability in the levels of salivary biomarkers.
- Validation in oral inflammatory conditions.

Need for further validation and future work

- The oral cavity is subjected to several inflammatory conditions such as infections, periodontal, and pulpal diseases.
- These non neoplastic conditions may affect the potential OSCC biomarkers in saliva. Ultimately such conditions may lead to false positive due to rise in the level of inflammatory salivary biomarkers and ultimately may overshadow or reduce the potential of salivary biomarkers in OSCC detection^{33,34}.
- In addition to oral cancers, salivary biomarkers have been identified in other malignant lesions such as breast and lung cancers, i.e., CA125, profiling, haptoglobin, transferring, S100 calcium-binding protein and these biomarkers are also found to be associated with OSCC detection.
- Hence further refinery work is needed to establish a set of biomarkers than a single, for the determination of specific disease or type of tumour, which could overcome false positive results.

Conclusion

- Even though biopsy is still considered as a gold standard for validation of several forms of carcinoma, it is often considered the most laborious, painful, and time-consuming screening technique.
- The human WMS as a non-invasive specimen for liquid biopsy is expected to play a significant role to uncover the secrets of diagnosis and pathogenesis of oral cancers.
- It will have additional benefits such as non-invasive, painless, simple, and easier method to administer.
- The emergence of new technologies with higher sensitivity for detection purposes can be expected in near future. Accessibility of these highly sensitive techniques will help in next generation sequencing, will enable even smaller quantities of salivary analysis for accurate diagnosis.
- **Early detection of premalignant lesions and early stage OSCC is associated with improved survival rates.**
- **Salivary biomarkers in OSCC clinical routine, will certainly help to establish consistent strategies for early diagnosis of cancer lesions, facilitate advance prevention, and support the development of targeted therapies; this will improve treatment outcomes of OSCC patients, also reducing chemotherapy/radiotherapy side effects.**
- **Their use can enable the clinician to alter the treatment modalities for better results.**
- **Therefore the review study was taken to enlighten on the advanced methods of diagnosis of OSCC.**

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