## REVIEW ARTICLE

# Salivary Biomarker in Diagnosis of Oral Squamous Cell Carcinoma: A Scoping Review

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**Abstract:**

*Oral saliva is an extracellular fluid produced and secreted by salivary glands which contains 99% of water and 1% of organic and inorganic substances. Inorganic elements consist of minerals such as sodium, potassium, calcium, magnesium, fluoride, and phosphates. Organic elements comprise enzymes, hormones, immunoglobulins, antioxidants are present in the saliva. The recent findings suggest that saliva could be used as a biomarker in diagnosing oral cancer and pre cancer. Most oral cancers are oral squamous cell carcinomas. Using saliva for detection of early oral squamous cell carcinomas in the search for new clinical markers is a promising approach because of its noninvasive sampling and easy collection method.*

*Biomarkers are the molecular signatures and indicators of normal biological, pathological process, and pharmacological response to treatment hence may provide useful information for detection, diagnosis, and prognosis of the disease. This review article provides the up-to-date list of known and emerging potential salivary biomarkers for diagnosis of oral cancer and pre cancer. Salivary biomarkers will help to differentiate patients who clinically have no detectable disease but are potential candidates for oral cancer.*

*Key Words: Salivary Bio Marker, Oral Cancer, Squamous Cell Carcinoma*

## Introduction:

Oral cancer is one of the non-communicable diseases of which more than 90% are oral squamous cell carcinoma (OSCC); usually arises from a pre-existing oral potentially malignant disorder (OPMD)[1]. Globally, oral cancer accounts for 2-4% of all cases while it is regarded as the 6th most common cancer in Asia[1,2]. Early detection of OSCC can not only significantly reduce mortality and morbidity but also enable effective intervention and therapy[3]. At present, no specific early detection techniques are available other than conventional clinical oral examination and biopsy for histopathological evaluation[4]. The use of saliva as a diagnostic fluid has become matter of interest and became a roaring success in translational research in the recent years. Saliva is a good medium because it is noninvasive to collect and the donation process is largely stress-free, allowing many collections to be made without causing undue discomfort to the donor individuals. Moreover, it is simple to collect, store, and transport; for its processing it does not require highly skilled health professionals[5]. It is safer to handle than blood and other bodily fluids as well. From the late 1990 until the present, more than 40 research studies have been published and more than 100 different salivary constituents have been identified as potential OSCC salivary biomarkers[5,6]. Several excellent reviews have been previously published concerning the basis for salivary diagnostics, and the history and evolution of salivary proteomic, transcriptomic, genomic, and metabolic research for oral cancer detection[5].

Oral cancer cells or their microenvironment produce several proteins which may eventually enter the circulation system and quantitative proteomics test can assess the expression pattern of these proteins. Saliva examination shows the greatest benefit for detection of oral cancer because of its direct contact with oral cancer lesion. Using salivary fluids as a medium for biomarker

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generation and assessment helps alleviate subject/ patient discomfort as a noninvasive method to disease diagnosis and prognosis[6].

**Suitable saliva for diagnosis of oral cancer**

Saliva is a watery substance which is secreted from three major and minor salivary glands of lips, buccal mucosa and palate. Saliva plays a significant role in the protection of the intraoral structures against injuries caused by mechanical or chemical irritants and various pathogenic microbes. About 1-1.5L saliva are produced and secreted daily in healthy individual. Different factors such as hormones, enzymes, growth factors, immunoglobulins, and antibacterial components, which gain entry to saliva through blood by paracellular or transcellular routes. Functions of saliva includes lubrication, digestion, antimicrobial activity, facilitating re mineralization of tooth enamel, and maintenance of normal taste sensation. These important functions are achieved by the various chemical components of saliva including water (99%), inorganic compounds (ions), organic compounds (non-proteins and lipids), protein/ polypeptides, and hormones where proteins and polypeptides carry the main function. So far, more than 2300 proteins and peptides have been found in human saliva. The most abundant proteins are á-amylase, albumin, cystatins, hystatins, secretory-IgA, lactoferrin, mucins, lysozymes, proline rich proteins, statherin and transferrin—which together account for more than 98% of the total salivary proteins[6]. Most of the potential biomarkers for OSCC are salivary proteins. However, except for three, á-amylase, statherin, and transferrin, are non-protein OSCC salivary biomarker candidates, which are very low concentration in saliva and require methods and technologies with high sensitivity for detection and quantification[6].

**Saliva Collection**

There exist different types of methods for collection of saliva. Saliva personnel-assisted or self-collection can be performed. Stimulated saliva which affects the original salivary consistency and $P^h$ can be collected by promoting mitigatory action through chewing inert materials such as cotton or polystyrene salivate or gustatory stimulation by using sugar-free sour lemon drop, chewing gum and paraffin wax. Stimulation is given to those who cannot produce sufficient amount of saliva normally. Other method is without stimulation,

unstimulated whole saliva can be obtained by 'passive drooling' or 'spitting' directly into a sterile container. It is recommended to advice mouth rinse thoroughly prior to collection of saliva to avoid contamination by foreign bodies and organisms.

Potential salivary biomarker for oral cancer detection

By several case control studies, more than 100 potential OSCC salivary biomarkers have been reported in the literature, based mainly on comparing the levels found in OSCC patients to the levels found in non-OSCC normal control[5]. These potential OSCC salivary biomarkers can be grouped according to the types of biomarkers, as follows[7,8,9]:

1. Non-organic compound biomarkers
   Flame photometry, atomic absorption, and spectrophotometry

2. Peptide or protein biomarkers
   High performance liquid chromatography (HPLC)
   Enzyme-linked immunosorbent assay (ELISA)
   Radio-immunoassay
   Two-dimensional gel electrophoresis (2DE), followed by mass spectrometry (MS) 2DE and reverse-phase liquid chromatography (LC), followed by LC-tandem MS
   Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS)
   2DE followed by MALDI-TOF MS

3. DNA, mRNA or microRNA biomarkers
   Polymerase chain reaction (PCR) Quantitative PCR (qPCR) [Microarrays followed by qPCR

4. Metabolomic biomarkers
   Capillary electrophoresis TOF MS
   HPLC with quadrupole/TOF MS

5. Miscellaneous biomarkers (chemical and enzyme activity)
   HPLC Colorimetric (mostly commercially available) assays

**Comprehensive analysis of saliva in OSCC**

Many researchers analyzed the human saliva and found various components such as calcium, inorganic phosphate, sodium, potassium, magnesium, albumin, total protein content of saliva, insulin growth Factor I, lactate dehydrogenase (LDH), amylase, total

immunoglobulin content of saliva, salivary immuno-globulin A (IgA), and matrix metalloproteinases-2 and 9 (MMP-2 and MMP-9) in subjects having oral cancer and healthy controls. Moreover, MMP1 and MMP 3 were 6-15 times higher in patients with OSCC than in healthy people, reported by Stott-Miller et al. MMP 9 also important for early diagnosis of oral cancer. Results where oral cancer patients showed a higher salivary protein content compared with the healthy controls. Not only protein content but also various inorganic constituents (Sodium, phosphate, calcium, magnesium) were also significantly higher than healthy person whereas Amylase, potassium, and salivary IgA levels were significantly lower. Albumin, LDH, MMP-2, and MMP-9 levels showed 8–10-fold rise in oral cancer patients when compared with healthy controls[5,7,8,9].

Thus, comprehensive analysis of saliva could serve as a valuable tool for diagnosis of oral cancer.

### Different biomarkers of upregulated and downregulated in OSCC[9,10,11]

A. Up regulated biomarker
   CEA
   Cancer antigen 125 (CA125)
   p53
   TNF-$\alpha$
   IL-1$\beta$
   CD 44
   IL-6
   IL-8
   IAP
   ROS
   RNS
   MMP-2
   MMP-9
   LDH

B. Down regulated biomarker
   Salivary antioxidants
   Amylase
   Potassium
   IgA

### Methods for analyzing salivary proteomic in OSCC
For the analysis of salivary proteomics, various methods are used which include capillary reversed phase liquid chromatography with quadrupole–time-of-flight mass spectrometry, multi analytic profiling technology shotgun proteomics on the basis of reverse-phase liquid chromatography, and human genome-microarrays to prepare a profile of salivary transcriptome[9,12].

Moreover, a unique diagnostic modality can identify a large group of mRNAs in the saliva which can be utilized popularly known as salivary transcriptome diagnostics[13]. The mRNA markers in saliva are the transcripts of interleukin-1, interleukin-8, and dual specificity protein phosphate 1. These significant protein biomarkers distinguish between OSCC patient and healthy controls [6,9,10].

### ELISA
ELISA analysis is done to evaluate the total levels of proteins present in the saliva among oral cancer patients in duplicating using a sandwich ELISA kit specific for each enzyme. The total amount of each enzyme is determined using its standard curve, which is plotted using Professional software[14].

### RT- PCR
To generate templates for in vitro transcription for the salivary oral cancer RNA markers, conventional real-time PCR (RT-PCR) is carried out using total RNA from human OSCC cell line as templates. The cDNA is synthesized in of reverse transcription reaction mix with reverse transcriptase. Therefore, it optimizes a preamplification procedure for salivary mRNA for oral cancer target detection, and detailed methods for primer design, one-step RT and PCR, excess primer removal, and quantitative PCR programming[9,14].

### LC-MS/MS
High-resolution, high-mass accuracy nanoflow LC"MS/MS is performed with a combined linear quadrupole ion trap (LTQ) and Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometer equipped with a nano-electrospray ion source. The most abundant ions detected in the scanning is subjected to MS/MS analysis performed in the LTQ mass spectrometer[10,11]. The identified protein data obtained via mass spectrometry is exported to a table form with information on the molecular weights, intensities and analyzed scores of the protein candidates[15,16].

### Conclusion
Salivary screening can be best choice for screening test for oral squamous cell carcinoma. If the lesion can be identified by early detection methods, can lead to reduction of early death of oral cancer patient and also giving the patient a chance for a better quality of life. Fortunately, current improvements in salivary

proteomics technology have led to a hopeful future for salivary biomarkers in oral cancer care. As a result, saliva is expected to be used more frequently as a diagnostic fluid through identification of salivary biomarkers as diagnostic and prognostic tools for oral cancer and pre-cancer patients. The use of salivary biomarkers for screening of OSCC invokes extensive research in the future. This review suggests that pursuing of saliva as a tool to detect the different types of cancer can pave the way for improved outcome of future noninvasive investigation in this field.

**Conflict of interest:**
Nothing to declare

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