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SALIVARY BIOMARKERS IN ORAL CANCER SCREENING

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ABSTRACT

Oral squamous cell carcinoma (OSCC) is among the most common cancers worldwide. Late diagnosis precludes to poor prognosis. A major barrier in early detection of cancer is the lack of early detection protocols. This has warranted the need for novel methods of cancer detection. The non-invasiveness and ease of handling saliva as a diagnostic tool can be considered as a useful adjuvant in oral cancer detection. The analysis of salivary biomarkers (proteomics, genomics, & metabolomics) will be a promising tool in early detection and management of OSCC. This literature review aims to categorize the various salivary biomarkers that have been identified in oral squamous cell carcinoma. A PubMed literature search was conducted and relevant articles were reviewed.

Keywords: Oral squamous carcinoma; saliva; salivary biomarkers; cancer detection

INTRODUCTION:

Oral pharyngeal cancers are the most common types of cancers with Oral squamous cell carcinoma (OSCC) being the 6th most common cancer worldwide. 90% of oral

cancers are OSCC. It represents 35-40% of all malignant cancers in India. It has an average survival rate of 5 years. It is not diagnosed until the advanced stage even when the oral cavity is easily visualized. This has resulted in the low survival rate of the patients **[1]**. Since the collection of saliva is effortless and non-invasive, it can be utilized in the early diagnosis of oral cancer. The clinical applications include estimation of disease risk, screening for primary cancers, differentiating benign from malignant tumours, distinguishing different malignancies, determination of prognosis, screening and monitoring of disease status and also in identification of therapeutic targets (effect of therapy) **[2, 3]**.

METHODOLOGY:

A PubMed literature search was conducted and relevant articles were reviewed and their findings have been tabulated.

RISK FACTORS FOR ORAL CANCER

The most common cause of oral cancer is tobacco and alcohol consumption which is a modifiable risk factor. The other predisposing factors for oral cancer are Human papillomavirus (HPV) infection, use of smokeless tobacco, poor oral hygiene, dentures, use of mouthwash, diet, low socio economic status, co-morbidities, and genetic disorders.

BIOMARKERS

Biomarkers play a vital role in identifying the presence or absence of disease. Biomarkers are present in body fluids such as blood serum, plasma, saliva, sputum, urine, stool and tissues. They consist of proteins, peptides, nucleic acid, enzymes, antibodies, metabolites, lipids and carbohydrates.

Saliva as a biomarker:

Saliva is a biological fluid that is secreted by major and minor salivary glands. It contains various organic and inorganic substances along with oral micro-organisms and their products. Saliva plays a vital role in digestion, deglutition, lubrication, antimicrobial action, taste perception, mineralization of enamel, buffering. The human saliva has abundant electrolytes (Na^+, K^+, Cl^-, $H_2PO_4^-$, Ca^{2+}, HCO_3^-, I^-, Mg^{2+}, SCN^-), proteins (mucin, enzymes, immunoglobulins (IgA), exosomes, proline-rich proteins, statherins), lipids, antioxidants (salivary peroxidase, superoxide dismutase, catalase, glutathione peroxidase, myeloperoxidase), hormones, water, uric acid, ascorbic acid, and albumin. Saliva exhibits anti-bacterial, anti-viral, and anti-fungal properties due to secretion of amylases cystatin, histatin, lactoferrin, lysozyme and agglutinin. Saliva serves as the first line of defense against oxidative stress (OE), reactive oxygen species (ROS) and free radicals.

Along with the secretions of the major salivary glands (Parotid, Submandibular, Sublingual) and minor salivary glands, saliva also comprises of oral mucosal transudate, gingival crevicular fluid, nasal and pharyngeal mucosal secretions, non-adherent bacteria, desquamated oral epithelial cells, keratin debris, food debris, and blood cells **[4]**.

Classification of salivary Biomarkers:

Biomarkers are biological molecules found in tissues and body fluids and are indicators of normal or abnormal process or condition of disease. Types of biomarkers are Genome, Epigenome, Transcriptome, Proteome, Metabolome and Microbiome. Salivary genome contains the DNA of the individual, oral microbiome and any DNA viruses affecting the individual. Epigenome is a group of chemical compounds that change the genome. It is the set of all epigenetic changes in the DNA of a cell. Epigenetic markers help in predicting the nature of disease (prognostic markers) and the response to treatment (predictive markers) **[5]**. Proteome is the collection of proteins that are produced by an organism **[6]**. Transcriptome is the collection of all RNA transcripts including coding and non-coding RNA in an organism **[7] (Table 1)**.

Biomarkers are also classified based on the disease state, biomolecules and other variables **(Table 2)**.

The techniques used for analyzing biomarkers vary depending on the type of biomarker **(Table 3)**.

Sensitivity and specificity of salivary biomarkers in detection of oral squamous cell carcinoma have been reported by various studies as mentioned in **Table 4**.

Table 1: Types of Salivary Biomarkers [1, 8, 9]

TYPE OF BIOMARKER	SALIVARY BIOMARKERS
Genome	p53, Promoter hypermethylation of DAPK, TIMP3, p16, and MGMT genes, Cyclin D1 gene amplification, Maspin (Mammary serine protease inhibitor)
Epigenome	DAPK, DCC, MINT-31, TIMP-3, p16, MGMT, CCNA1, KIF1A, EDNRB, HOXA9, NID2
Transcriptome	DUSP 1, H3F3A, IL1B, IL8, OAZ1, SAT, S100P
Proteome	α amylase, Proline-rich proteins (PRPs), Statherins, Cystatins, Cyfra 21.1, tissue polypeptide antigen (TPA), Defensin-1, P53 autoantibody, cancer antigen (CA125), IL-1, IL-6 IL-8, TNF- α , Haptoglobin, complement C3, transthyretin, α1-antitrypsin, Lactate dehydrogenase, Transferrin (MRP14, M2BP, MRP14, CD59, Catalase)
Metabolome	Cadaverine, Alanine, Choline, Piperidine, Taurine piperidine, Pipercolic acid, Betaine, Carnitine, alpha-aminobutyric acid, Serine, Glutamine, Leucine, Isoleucine, Tyrosine, Histidine, Tryptophan, Glutamic acid, Threonine
Microbiome	*Bifidobacteriales*, *Lactobacillaceae*, *Firmicutes, Lactobacillus*, *Haemopilus*, *Neisseria*, *Gemellaceae*, *Aggregatibacter*[9], *Streptococcus mitis, Capnocytophaga gingivalis, Prevotella melaninogenica*
Inorganic compounds	Sodium, Magnesium, Fluoride, Calcium

(DAPK: Death associated Protein Kinase, TIMP3:Tissue Inhibitor of Metalloproteinase 3, MGMT: Methylguanine methylytransferase, DCC: Deleted in Colorectal carcinoma, MINT-31:, CCNA1: Cyclin A1, KIF1AKinesin Family Member 1A, EDNRB: Endothelin Receptor type B, HOXA9: Homeobox protein A9, NID2: Nidogen 2, DUSP: Dual-specificity phosphates, H3F3A: H3.3 Histone A, IL1B: Interleukin 1 beta, OAZ1: Ornithine decarboxylase antizyme 1, SAT: Spermidine/spermine N1 Acetyltransferase, S100P: S100 calcium binding Protein, TNF-α: Tumour necrosis factor, MRP: multi drug resistance associated protein, M2BP: Mac-2 binding protein.)

Table 2: Classification based on factors associated with carcinogenesis [10]

Factors	Salivary Biomarkers
Angiogenesis	CD31 EFNB2 ANGPT1, ANGPT2 VEGF miR125
Inflammation	IL-6 IL-8 IL-1β
Metastasis	CD44 Maspin S100P
Oxidative stress	8-OHdG DNA damage marker Glutathione
Metabolism	Non-organic compounds – Na, Ca, F, Mg Fucose Albumin Actin and myosin L-phenylalanine

(CD31: Cluster of Differentiation 31, EFNB2: Ephrin B2, ANGPT: Angiopoietin, VEGF: Vascular endothelial growth factor, miR125:microRNA , IL: Interleukin, S100P: S100 calcium binding Protein, 8-OHdG DNA: 8- Hydroxyoxyguanosine DNA)

Table 3: Techniques for analyzing Biomarkers [2]

Genomics	Proteomics	Metabolomics
DNA: DNA arrays PCR Southern blot analysis	Proteins: Liquid chromatography Western blot analysis Protein sequencing	Lipids: Liquid chromatography
Mt DNA: PCR Restricted Fragment length polymorphism Southern blot analysis	Peptides : Protein arrays High performance liquid chromatography (HPLC) Enzyme-linked immunosorbant assay (ELISA) Radio-immunoassay 2-dimensional gel electrophoresis followed by mass spectrometry 2DE and reverse-phase liquid chromatography followed by LC-tandem Matrix-assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF MS) 2DE followed by MALDI-TOF MS	Carbohydrates: Nuclear magnetic resonance
RNA: DNA arrays Cross linking Immunoprecipitation	Antibodies: Immunofluorescence Protein arrays	Enzymes: Enzyme assays HPLC Colorimetric assays
mRNA: DNA arrays RIP-Chip		Metabolites: Mass spectrometry Capillary electrophoresis TOF MS HPLC with quadrupole/ TOF MS
miRNA: DNA arrays RIP-Chip		
Polymerase chain reaction (PCR) Quantitative PCR (qPCR) Microarrays followed by qPCR		

Table 4: Sensitivity and Specificity of salivary biomarkers in OSCC detection:

SALIVARY BIOMARKER	SENSITIVITY (%)	SPECIFICITY (%)
IL8, IL1B, DUSP1, HA3, OAZ1, S100P, and SAT [7]	91	91
Valine, Lactic acid [11]	86.5	82.5
miR-31 [12]	80	68
M2BP, Profilin, CD59, MRP14, Catalase [6]	90	83
HOXA9, NID2 [13]	94	97

(IL: Interleukin, DUSP1: Dual-specificity phosphate 1, HA3: Hemagglutinin 3, OAZ1: Ornithine decarboxylase antizyme 1, S100P: S100 Calcium binding protein, SAT: Spermidine/spermine N1 Acetyltransferase, miR-31: MicroRNA-31, M2BP: Mac-2 binding protein, MRP14: multi drug resistance associated protein-14, HOXA9: Homeobox protein A9, NID2: Nidogen 2.)

The higher expression of p-m TOR protein and GF 15 in OSCC can be used as a therapeutic target **[3, 14]**.

Interleukins (IL-6, IL-8, IL-1, TNF-α) are the most studied biomarkers. The high level of this protein is associated with decreasing prognosis of OSCC and suggests that pro-angiogenesis and pro-inflammatory compounds play a key role in carcinogenesis **[11]**. Therefore interleukin is a suitable biomarker that can be used for diagnosing OSCC and oral potentially malignant disorders (OPMD). The increased level of immunoglobulins such as IgG also shows the role of angiogenesis in cancer proliferation **[15]**.

The serum level of Carcinoembryonic antigen (CEA) has been reported to increase in malignancy especially in carcinomas of colon or Gastro-intestinal tract. CEA is a glycoprotein that plays a role in cellular adhesion **[16]**.

There is an increased expression of cell-surface glycoproteins (CD44, CD59, CEA) in OSCC **[11]**. The salivary biomarker ZNF510 has a greater sensitivity and specificity in early stages (T1, T2) than in advanced stages (T3, T4) of oral cancer **[3]**.

A study found that 5 proteins –M2BP, MRP14, CD59, catalase and prolifin have 90% sensitivity and 83% specificity for the diagnosis of OSCC **[13]**. 24-mer ZNF510 peptide can be used for early diagnosis of OSCC **[17]**. Another study found that MMP1, KNG1, ANXA2 and HSPA5 proteins were useful in the diagnosis of OSCC **[18, 19]**.

Chu *et al.,* conducted a study comparing the salivary proteomes of healthy volunteers, patients with oral potentially malignant disorders and patients with OSCC. The result showed an increase in salivary level of CFH (complement factor H), FGA (fibrinogen alpha chain) and alpha-1-antitrypsin (ERPIA1) in advanced stages of OSCC **[7]**.

Cathepsin V, kallikrein5 and ADAM9 have high sensitivity and specificity, increased expression of MMP-1, MMP-2, MMP-10, MMP-12, ADAM9 (a disintegrin and metalloprotease 9), ADAMST13 in OSCC **[20]**.

The association of inflammatory cytokines and chemokines with cancer was studied and found that TNF-308 polymorphism was related to high plasma levels of the cytokine at presentation of disease. IL-1, IL-6 and TNF are produced excessively in multiple myeloma **[21]**.

CONCLUSION:

Early diagnosis of OSCC has the potential to increase the survival rate and thus save lives. Inappropriate site of biopsy may result in a false negative diagnosis. Salivary biomarkers can be used to observe disease progression and response of the patient to treatment. Therefore, detection of salivary biomarkers for OSCC can help in early diagnosis and treatment.

COMPLIANCE WITH ETHICAL STANDARDS:

This article complies with ethical standards

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Informed consent was not required for this article.

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