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Oral Premalignant Conditions And Oral Squamous Cell Carcinoma: A Review Of Salivary Biomarkers As A Diagnosis Tool

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Abstract:
The term "biomarker" refers to measurable and quantifiable biological parameters that can be used as indicators for assessments of health and physiology, such as pathogenic processes, environmental exposure, disease diagnosis and prognosis, or pharmacologic reactions to a therapeutic intervention. Saliva is a complex bodily fluid that, like blood, is known to contain a number of cellular and molecular components. Saliva collection is easy, safe, non-invasive, and economical, which are all benefits of using saliva as a diagnostic tool. It also has the advantages of being simple to sample, handle, and process. Due to these factors, saliva evaluation could be thought of as a potential replacement for blood and/or tissue analyses to investigate certain molecules (DNA, RNA, proteins, and metabolites) connected to the occurrence of systemic illnesses and malignancies. Changes in the expression of proteins and mRNA markers in saliva can result from mutational activities that turn healthy cells malignant. Researchers have looked into the clinical importance of salivary biomarkers in breast cancer, ovarian cancer, salivary gland cancers, gastric cancer, and pancreatic cancer. The significance of salivary biomarkers, even for the detection of OSCC, has grown as a result of further studies. In recent years, there has been a substantial increase in the use of saliva as a diagnostic tool for the early detection of oral cancers including oral squamous cell carcinoma. MMP-9 (specificity 100%) and Chemerin (specificity 100%) were highly specific indicators for oral squamous cell carcinoma. PUBMED, Google, manual searches, and 20 publications back references from the previous five years were included in the search approach.
Key words:Saliva, biomarkers, oral squamous cell carcinoma, potentially malignant diseases, and early detection

Introduction:

Different diagnostic methods are used to identify diseases. The foundation of modern testing algorithms is tissue biopsy and bodily fluids such blood, serum, urine, and saliva (6). Saliva from the mouth is a readily accessible non-invasive sample, making it a compelling choice for the diagnosis, monitoring, and prognosis of many human illnesses. The noted benefit of salivary samples is that they can be used in paediatric settings, with people with disabilities, and in routine follow-up operations. When the biopsy specimen is insufficient for further processing, it would be a great backup plan (7). Many oral diseases have the possibility of transforming into malignancy. One of the most common types of oral cancer is oral squamous cell carcinoma (OSCC). The histological analysis of a biopsy is used to make the diagnosis of OSCC in the majority of instances. The diagnosis and prognosis of this oral condition can be determined through the study of saliva, which doesn't require for any intrusive procedures. Samples are more stable than other sources and are easily and non invasively collected. They also require little processing and have a simpler makeup. saliva contains metabolites, proteins, mRNA, DNA, enzymes, hormones, antibodies, antibacterial components, and growth factors that may be linked to the disease phenotype and aid in diagnosis and prognosis. It should be highlighted that several biomarkers found in saliva can be utilized to diagnose a range of diseases and are not specific to one disease. Therefore, it is essential to take into account the various biomarkers that are impacted by each disease in order to arrive at a diagnosis and prognosis that are much more precise (8). Generic, proteomic, or metabolomic expression can be used to classify the underlying tissue changes and conditions associated with it. More than 100 possible saliva biomarkers for OSCC have been described in the literatures as a result of extensive study into salivaomics. Researchers and physicians have found salivary diagnostic to be a promising potential modality for screening, early diagnosis, and prognostic evaluation (9).

Oral premalignant conditions:

A malignant transformation (MT), which could be an epithelial lesion or another condition, is more likely to occur in cases of oral potentially malignant disorders (OPMDs). They are thought to be the initial sign of oral squamous cell carcinoma (OSCC). Leucoplakia, lichen planus, oral submucous fibrosis, erythroplakia, and erythroleukoplakia. The estimated prevalence of OPMD worldwide is 4.5%, while a Taiwanese cohort study found that the MT in OPMD was 4.32% with a follow-up of between 6 and 67 months. Patients with a history of alcohol use, betel quid chewing, or a family history of oral cancer are at an elevated risk for malignancy, according to clinical characteristics.

Compared to other lesions, the risk is higher when verrucous hyperplastic leucoplakia, erythroplakia, many sites of occurrence, and large size are present. The chance of developing malignancy is also predicted by the histopathology grade of OPMD dysplasia. Significantly more severe dysplastic lesions are likely to develop into cancer. Currently, this is the approved diagnostic method for detecting MT in OPMDs (7).

Salivary biomarkers in oral potentially malignant disorder (OPMD) :

Many studies have established association of various salivary biomarkers with OPMD.

Table 1. Salivary Biomarkers involved in OPMD

BIOMARKER	INFERENCE	REFERENCE
MMP-9	Compared to controls and patients with premalignant lesions, OSCC patients had MMP-9 levels that	(1)

	were considerably greater.	
IL-8 ,IL-6, & TNF-α	**Patients with oral leucoplakia, submucous fibrosis, and lichen planus had higher levels of these cytokines than did healthy controls.**	(1)
IL-1-Ra	**Expression of IL-1-Ra steadily declines as oral dysplasia progresses, and it may help distinguish between premalignant oral lesions and OSCC.**	(1)
IL-6	**The majority of researchers discovered noticeably higher salivary IL-6 protein concentrations in OPML patients compared to controls.**	(2)
IL-6	**Patients with oral neoplasia and premalignant lesions had significantly greater serum and salivary IL-6 concentrations than healthy controls.**	(3)
IL-6	IL-6 is a biomarker for premalignant differentiation that is primarily detected in the saliva of individuals with oral leukoplakia.	(4)
Salivary actin and myosin	Premalignant lesions	(5)
Salivary actin and myosin	Other intriguing salivary biomarkers for premalignant differentiation and malignant oral lesions include actin and myosin.	(4)
Salivary actin and myosin.	Premalignant lesions	(6)
Epstein-Barr virus (EBV) DNA	Detected in potentially malignant oral disorders, OSCC, controls	(7)
MicroRNA-21, MicroRNA-31	820-day follow-up on OPMD with healthy controls is the average	(7)
Salivary exosomal miRNA-4484 miRNA-1246 and miRNA-1290	OLP/healthy controls (NS)	(7)
miR-203	OLP/healthy controls/human whole saliva	(7)
miRNA-21, miRNA-184 and miRNA-145	OPMD/OSCC/RAS and also in healthy controls	(7)
E-cadherin-160C/A (CDH1-160 polymorphism)	Leucoplakia /oral submucous fibrosis/ controls with tobacco related habits	(7)
L8, IL-1β, SAT1, OAZ1, DUSP1, S100p and H3F3A mRNA and	OSCC, OPMDs with dysplasia and healthy control	(7)

IL8 and IL1 β proteins		
Endothelin-1	Oral Sub Mucous Fibrosis/ Oral Leukoplakia/ OSCC/healthy individual	(7)
Chemerin and MMP-9	Oral premalignant lesions/OSCC/healthy control	(7)
IgA, Adiponectin and cortisol	Healthy control /Oral Lichen Planus	(7)
Epidermal growth factor	Leukoplakia	(7)
P53 (wild type)	OLP/OSCC	(7)
Ornithine, Arginine, Carnitine, O-Hydroxybenzene, N-Acetylglucosamine 1-phosphate and Ribose 5 -phosphate	OSCC/Persistent suspicious oral mucosal lesions /Oral Epithelial Dysplasia	(7)
Lipid peroxidation (TBARS) Glutathione S transferase,uric acid and nitrite	Leukoplakia	(7)
Lactate dehydrogenase	Oral submucous fibrosis	(7)
Cortisol	OSCC, PMD's	(7)
Aldehyde dehydrogenase 1	Lichen planus	(7)
8 -hydroxy -2-deoxyguanosine (8-OHdG), Vitamin C,Vitamin E, malondialdehyde (MDA)	Oral lichenplanus,oral leukoplakia, Osmf, oral squamous cell carcinoma	(7)
8-OHdG, MDA, Uric acid, GPx, CTX, TAC, MMP-8	helpful indicator for detecting oral precancerous lesions	(7)
Ornithine+Ohydroxybenzoate+ R5F	Screening to diffrentiate OSCC/OED from PSOML	(7)
MDA, Vitamin C Vitamin E and 8-OHdG	Useful marker in diagnosing oral precancerous lesions	(7)
cystatins, keratin 10, Alpha amylase, lysozyme precursor,	Oral leukoplakia	(6)

and CK10		
IL10, IL1α & IL1β, TNF- α, IL4, IL6, IL8	IL8 is a reliable salivary biomarker for the severity of OLPs despite its uncertain relationship with premalignant oral lesions.	(8)

Sensitivity, Specificity and AUC values of biomarkers in OPMDs. OPMD: Oral potentially malignant disorder, OSCC: Oral squamous cell carcinoma, PMOD: Potentially malignant oral disorder, OPML: Oral potentially malignant lesion, OED: Oral epithelial dysplasia, PSOML: Persistent suspicious oral mucosal lesion, 8-OHdG: 8-hydroxy-2-deoxyguanosine, MDA: Malondialdehyde, MMP: Matrix metalloproteinase, AUC: Area under the curve, TNF-α: Tumor necrosis factor α, IL-6: Interleukin-6, MMP-9:Matrix metallopeptidase 9

Oral squamous cell carcinoma :

Ninety percent of oral potentially malignant disorders are oral squamous cell carcinomas (OSCC). The tongue, the oral floor, and the lower lip are the most common sites where oral cancer begins. of the oral cavity, metastases in the cervical lymph nodes are common in this situation. OSCC is an invasive epithelial neoplasia formed histologically by squamous cells that may have undergone various degrees of differentiation. The upper and lower gingiva, the palate, and the buccal mucosa are additional, less typical sites. The malignancy has the capacity to extend much further infect nearby tissues like muscles and bone. Due to extensive lymphatic vascularization of the oral cavity, metastases in the cervical lymph nodes are common in this situation.

Indeed, a chronic stimulus acting on keratinocytes can result in hyperplasia, different degrees of dysplasia and progress to carcinoma in situ and invasive carcinoma (9) .

Human pancreatic alpha-amylase (HPA), keratin-10 (K-10), human salivary amylase (SAA), and human serum albumin (GA-HSA), four promising proteins, have lately been suggested as salivary biomarkers for OSCC, but more validation is needed (11).

Table 2. Salivary Biomarkers involved in OSCC

BIOMARKER	INFERENCE	REFERENCE
Salivary cathepsin B	Patients with OSCC had significantly higher salivary Cathepsin B levels.	(10)
IL-1β and IL-8	considerably higher in patients with OSCC at all phases	(11)
LGALS3BP	significantly increased, especially in OSCCs in the early stages	(11)
miRNA	OSCC has considerably higher levels of miRNA than healthy controls.	(9)
IL-6, 8, 1α, 1β	Strong salivary indicators in diagnosis of OSCC	(9)

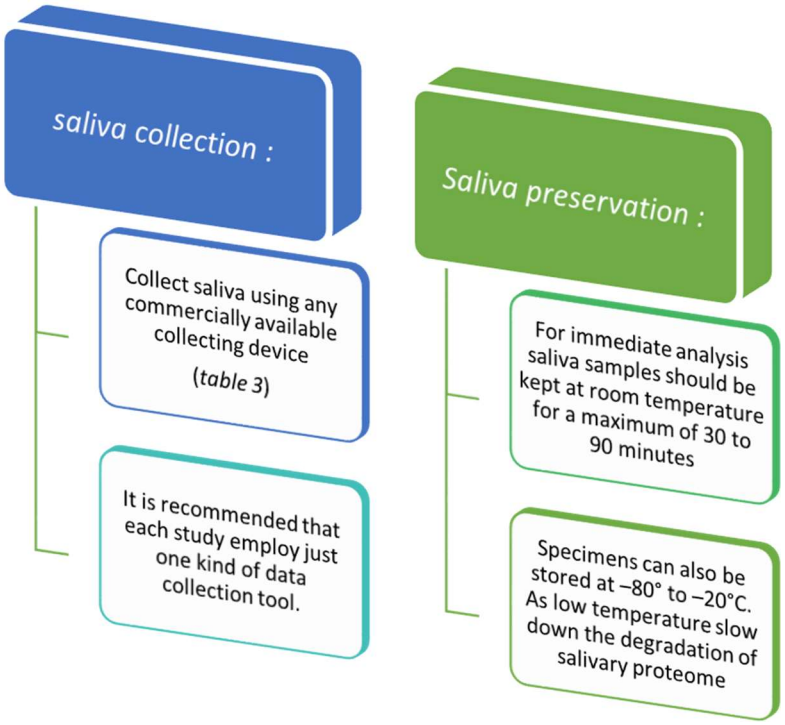
Cyfra-21-1, IL8 and IL1β	Expression of IL8 and IL1 was elevated in OSCC patients' saliva, suggesting that they could be potential biomarkers for oral cancer.	(12)
IL-1β, IL-6, IL-8, IL-1-Ra & TNF- α, MMP-9	MMP-9 is a vital diagnostic and prognostic biomarker for OSCC and may be used to indicate the progression of OSCC.	(1)
8-OHdG	8-OHdG is a DNA damage biomarker that can be used to measure disease progression.	(1)
IL-6, IL-6 mRNA	salivary IL-6 mRNA is a robust biomarker in OSCC	(2)
IL-6, IL-8, IL-1α, IL-1β, TNF-α, TPA, Cyfra 21- 1, CA 125, M2BP, telomerase	Pro-inflammatory and proangiogenic cytokines have been found to be indicators of the carcinogenic transformation from oral precancerous lesions to oral cancer.	(5)
α-1-antitrypsin (AAT)	AAT is helpful for forecasting and assessing OSCC aggression.	(5)
miRNA-21 miRNA-184 miRNA-145	Significantly increased miRNA-21, miRNA-184 OPMD vs normal, OSCC vs normal	(7)
Endothelin-1	Significantly increased expression of salivary endothelin OSCC	(7)
Cyfra 21-1, cancer antigen 125 (CA 125), telomerase, Mac-2 binding protein (M2BP)	TPA markers, CA 125, and Cyfra 21- 1 are employed as diagnostic tools, and tumours exhibit telomerase activity.	(6)
a-1-antitrypsin (AAT)	A1-antitrypsin (AAT) is beneficial for prediction of aggressive OSCC	(6)
miR-345, miR-31-5p, and miR-424- 3p	Overexpression of miR-31-5p and miR-345 appears to be correlated, and miR-345 upregulation is highly specific to OSCC	(13)

LDH	Patients with oral leukoplakia have elevated salivary levels of the enzyme LDH, whose expression is directly associated to cell necrosis, and those with OSCC have even greater amounts.	(8)
TNF-α	TNF-α acts as a prognostic marker of OSCC	(8)

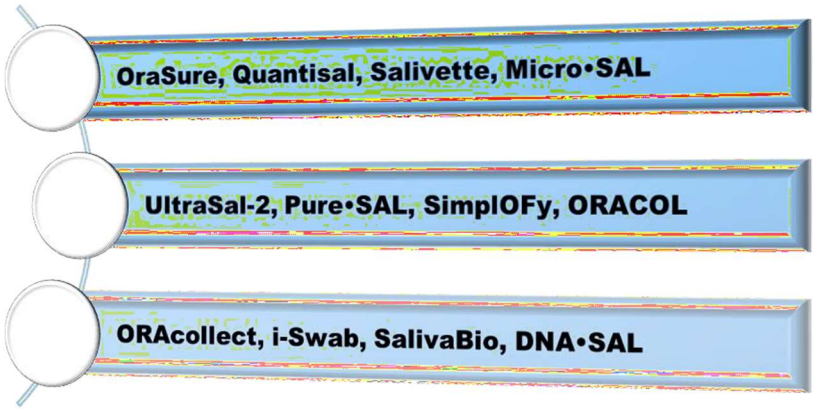
OSCC: Oral squamous cell carcinoma, 8-OHdG: 8-Oxo-20 –deoxyguanosine, TNF-α: Tumor necrosis factor alpha, MMP-9: Matrix metallopeptidase 9, IL: Interleukine, IL-1-Ra: IL-1 receptor antagonist, TPA: tissue polypeptide antigen, TPA: Tissue polypeptide antigen, CA 125: Cancer antigen 125, M2BP: Mac-2 binding protein, miRNA: MicroRNA, LDH: lactate dehydrogenase

Further manipulations:

It is essential to take into account the various biomarkers that are impacted by each disease in order to produce a diagnosis and prognosis that are much more precise. For Cushing disease or stress disorders, salivary biomarkers may include cortisol; for cardiovascular disease, C-reactive protein (CRP), creatine kinase isoform MB, and myoglobin; for infectious processes, pathogens, nucleic acids, and antibodies; for diabetes, -2-macroglobulin and glycosylated haemoglobin (HbA1c); and for cancers, gut disorders, and muscle or joint disorders. Salivary biomarkers and proteome analysis can both be used in the field of oral health and dental sciences to help understand a variety of oral disorders, such caries, periodontitis (aggressive/chronic), Sjogren's syndrome, Behcet syndrome, oral leucoplakia, and carcinomas particularly in the head and neck region (6) .



Commercially available saliva collection devices



Downsides:

Despite several advantages, the use of saliva sampling as a diagnostic tool is still under controversy. Firstly, salivary molecular identification and evaluation might vary from one experiment to another. A standardized system for saliva collection and analysis is therefore indispensable. In addition, salivary proteins are vulnerable to environmental factors like proteolytic enzymes, oral micro- organisms, and circadian patterns. Immediate processing, the use of freezers and protease inhibitors are recommended to tackle this problem. As we all know, many informative proteins are generally present in lower amounts in saliva than in serum, so highly sensitive tools and methods are necessary for salivary proteomic analysis. Moreover, it is very necessary to combine salivary proteomic analysis with conventional oral examinations (14) .Many studies suggest that the method of collecting saliva also plays a major role when comes to the accuracy of the analysis. For example, the concentration of complexes present in saliva varies with samples collected by active stimulation and that of passive. Also the preservation of the collected samples should be taken in account as, the salivary proteome is temperature sensitive. A part from the above, genomic and transcriptomic studies demands futuristic methods and techniques for analysis.

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