

Salivary biomarkers in oral and systemic health: A comprehensive review of diagnostic potential

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Abstract:

Saliva act as a diagnostic fluid that enables non- invasive sampling and diagnosis of diseases. Saliva reflects the composition of blood or serum due to its physiological exchange mechanisms between the systemic circulation and the salivary glands. This review comprehensively discusses about the various biomarkers of saliva which is used to diagnose, plan and monitor the treatment outcome or the progression of a disease. The review also highlights the importance of biomarkers in diagnosis of oral diseases like periodontitis, dental caries, Sjogren's syndrome, orofacial pain, premalignant conditions and malignancy and also the systemic diseases like diseases of lung, cardiovascular diseases, viral and fungal infections, neurodegenerative disorders and stress related disorders. By use of salivary biomarkers, it is possible to diagnose diseases at an early stage thereby enhancing the overall health status of the patients, thus a hopeful way for better diagnosis and a great tool for future research.

Keywords: Biomarkers, Diagnosis, Investigations, Saliva

Introduction Clinical examination is the gold standard for the identification and diagnosis of oral diseases which is then followed by the further investigations like histopathological investigations for confirmation of diseases.¹⁷ saliva has many proteins and peptides that take part in various biological processes like digestion, enamel formation, lubrication., etc. Besides all these functions saliva also contains biomarkers for the various oral and systemic diseases. In many diseases, saliva undergoes changes in its composition which act as an indicator of diseases .¹⁶

Biomarker- definition Biomarkers are biological identifiers which provide vital information on development of disease, diagnosis, and progression.¹⁰

Saliva is a biological fluid which contains plasma exudates, cell debris, inorganic ions cytokines, enzymes, chemokines, immunoglobulins.³

Biomarkers may be of three types

- Diagnostic markers
- Predictive markers
- Prognostic markers

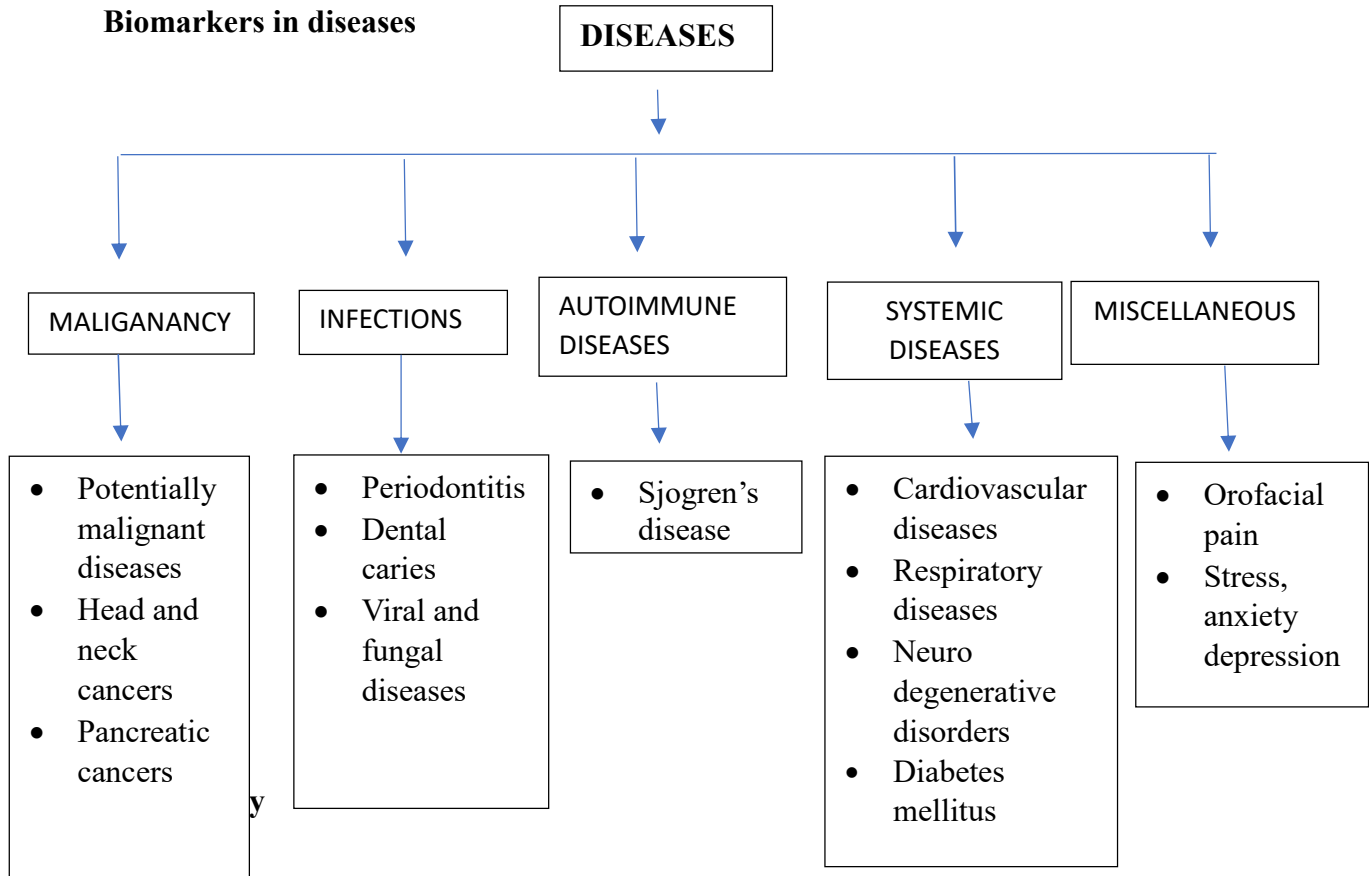
Diagnostic markers are used in differentiating various lesions. Predictive markers are used to identify the risk of a disease. Prognostic markers are used to find or predict the outcome of a disease.¹⁰

Salivary biomarkers

Blood biomarkers

Tissue biomarkers³

Biomarkers in diseases



Potentially malignant disorders the most effective and an economical way of reducing the incidence morbidity and mortality due to cancers is by early detection and management of premalignant lesions and conditions, thereby reducing the treatment related complications including pain, disfigurement and impaired functions.¹⁰

Oral submucous fibrosis It is a chronic inflammatory disease that with a high potential to progress into oral cancer. This is associated with the habit of betel nut chewing. The clinical features of oral submucous fibrosis includes loss of elasticity of oral mucosa, altered taste perception, high sensitivity to hot and spicy foods, xerostomia and ulceration, that significantly affects the daily life by causing difficulty in eating and speaking. Patients may report with dental-related problems such as dental caries and periodontal disorders in addition to the clinical symptoms listed above due to difficulty maintaining oral hygiene. The contents of betel nut include arecoline, polyphenols, crude fibres, and adipose elements. Betel nut ingredients not only cause excessive collagen production but also hinder or interrupt the cell's ability to break down abnormal collagen, because of which the abnormal collagen accumulates at an alarming rate in individuals with oral submucous fibrosis.

. Arecoline also involved in genetic mutations which induces cytotoxic transformation of fibroblasts.⁴

Salivary biomarkers are elevated lactate dehydrogenase, miRNA-21, miRNA-31. Downregulated glutathione peroxidases (GPX), superoxide dismutase (SOD) are also found as a biomarker of oral submucous fibrosis.⁴ Also upregulated 8-hydroxy-2-deoxyguanosine (8-OHdG) is associated with OSMF. The levels of miRNA-21 vary with progression of the condition.⁴ Metalloproteinase inhibitor 2, keratin, type I cytoskeletal 13 are related to this condition, thereby used as biomarker for detection and monitoring the progression of the condition.¹⁷

Oral leukoplakia there is very high association of p53 in transformation of leukoplakia into squamous cell carcinoma. The tumour suppressor p16 loss found in progressive lesions and also in non-progressive lesions, but there is an increased association with progression lesions when compared with non progressive lesions.¹⁰ Stem cells and cancer cells share the common features or characteristics like self-renewal ,differentiating property,etc., CA9, Ki-67, p16, p53,β-catenin, IMP3, c-Met, c-Jun,PDPN,COX-2are involved in renewal of stem cells and thereby they are very much useful in identifying and monitoring the progression of a leukoplakia. TGM3 is another tumour suppressor, that takes part in progression of leukoplakia into malignancy hence they are utilised in monitoring the progression.¹⁰ Higher expression of MAGE-A has been identified more in leukoplakia undergoing transformation into malignant lesion on comparing to static lesions.¹⁰

Oral lichen planusIt is a chronic insidious disease affecting oral cavity, including oral mucosa, tongue and gingival tissues. It is also considered as a potentially malignant disorder that has a higher potential of developing into oral cancer. Cortisol is one of the glucocorticoids that participates in immunoregulation. Elevated levels of cortisol are linked with stress that leads to immune mediated diseases. Elevated levels of salivary cortisol are found in oral lichen planus than those without the disease. Hence cortisol can be an ideal marker for diagnosing lichen planus.¹⁷

Higher salivary nitric oxide (NO) and reactive oxygen species (ROS) are noted in individuals with lichen planus than healthy individuals. The increased levels demonstrate the severity and advancement of the disease. The C reactive protein is a marker of inflammation and are detected in increased levels in saliva of those with oral lichen planus. In cytokines the elevated levels of salivary tumour necrosis factor α (TNF- α), IL1, IL4, IL6, and IL8 are the diagnosis and monitoring markers of oral lichen planus.¹⁷

Head and neck cancers P53 is an important factor in deciding the effect or outcome of genomic damage to the cells.¹⁰ One protein that is seen in malignant tissue rather than healthy tissue is MAGE-A.¹⁰ Common inflammatory cytokines, such as IL-6, IL-8, and TNF- α , are utilized to diagnose oral squamous cell cancer early. Chemerin and MMP-9 are most promising molecules in saliva, to be utilised as a diagnostic tool for oral carcinoma because of its high specificity.³ Levels of H3 histone family 3A, ornithine decarboxylase antizyme 1, S100 calcium binding protein, dual specificity phosphatase 1 are indicators or the markers of oral cancer.²² Pilocarpic acid (increased in OSCC), Choline, betaine, and L-carnitine (decreased in OSCC) levels also vary in saliva.²⁰ Cytokeratin 19 fragment (Cyfra 21-1),²⁰ cancer antigen 125, tissue polypeptide antigen, levels are also elevated in squamous cell carcinoma.²⁷ Oral squamous cell cancer was also associated with salivary transferrin.²⁷

Saliva contains both miRNA and RNA. Salivary RNA biomarkers for oral squamous cell cancer include DUSP1, SAT, S100P, HA3, IL8, OAZ1, and IL1B. The salivary microRNAs miR-200a and miR-125a can be used to distinguish between healthy people and those who have oral squamous cell cancer. This demonstrates how well salivary miRNA may be utilized to detect the presence of cancer.²⁴ SRY-box transcription factor 2 (SOX2) is connected to the onset and spread of head and neck malignancies.¹⁰ Mutations of PI3KCA oncogene was commonly found in head and neck cancers. Some of the biomarkers of cancers were circulating tumour cells (CTCs), ctDNA/RNA and exosome. Cell free tumour DNA (cfDNA) is found in both healthy as well as tumour cells. Circulating tumour DNA is a part of tumour that is released into the circulation. The concentration of ctDNA reflects the staging and type of the tumour.⁵ Endothelin 1 (ET-1) is a vasoactive peptide that is present in excess and linked to malignancies.²⁸

Pancreatic cancer They have the ability to rapidly disseminate to the distant organs through lymphatic system. About 15-20% of the patients reporting with pancreatic cancers ends up in

surgical treatment due to resistance to targeted therapeutic agents and lack of early detection by use of biomarkers. The salivary extracellular RNAs are used in early detection of pancreatic cancers. Salivary transcriptome profile may be applied or utilized to detect the pancreatic cancers at an early stage. The biomarkers that can be detected by transcriptome are acrosomal vesicle protein 1, dolichyl-phosphate mannosyltransferase polypeptide 1, methyl-CpG binding domain protein 3-like 2, v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog.²² Pancreatic cancers may be diagnosed using salivary exosomal RNA. KRAS, MBD3L2, ACRV1, and DPM1 are the four messenger RNA biomarkers that may be used to distinguish pancreatic cancer from non-cancer conditions.²⁰

Infections

Periodontitis It is a multifactorial inflammatory disease leading to disruption of supporting tissues of tooth resulting in tooth loss.¹² Periodontitis is an infection of the gingiva that leads to the destruction of periodontal ligament and alveolar bone. IL1 β , TNF- α , IL6, and the receptor activator of nuclear factor κ B ligand (RANKL) are found to be the immune regulators in progression of periodontal disease. Monocyte chemoattractant protein 1 levels in saliva are also linked to the disease's progression.¹⁷

Patients who have periodontitis are at a higher risk of developing diabetes, respiratory conditions, and cardiovascular problems.³ The decreases level of tissue inhibitor metalloproteinase-1 (TIMP-1) are found to be linked with periodontitis.¹⁷ The salivary interleukins and metalloproteinases that are used as biomarkers with their specificity (values in parentheses) include: IL-6 with MMP-8 (86.2%/80.5%); IL-1 β with IL-6 (83.0%/83.7%); IL-1 β with MMP-8 (82.7%/80.8%); MIP-1 α with MMP-8 (71.0%/75.6%); IL-1 β , IL-6, and MMP-8 (81.8%/84.3%); and IL-1 β , IL-6, MIP-1 α , and MMP-8 (76.6%/79.7%).³ Salivary RANKL, osteoprotegerin (OPG), and osteocalcin (OSC) are also associated with

periodontitis.¹⁷ The levels of cell activity markers like alkaline phosphatase (ALP), lactate dehydrogenase (LDH), aspartate aminotransferase (AST), alanine aminotransferase (ALT), and matrix metalloproteases (MMPs) are linked to the progression of periodontitis.¹⁷ Other biomarkers include *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, *Tannerella forsythia*, *Treponema denticola*, and Interleukin-1.²²

Dental caries the common way of identifying dental caries is by routine clinical (visual and tactile) examination and radiographic investigation, but it is possible to identify only in case of complete demineralization or established caries. So early detection offers opportunity to prevent the establishment of caries by stabilizing the disease or by even remineralization. The biomarkers include mucin, histatin, proline-rich proteins (PRP), lactoperoxidase, C-reactive proteins, cathelicidin (LL-37), immunoglobulin (IgA), albumin, statherin, salivary total protein, SOD, copper, zinc, proteinase 3 (PR3), alpha-amylase, and carbonic anhydrase. Salivary pH, buffering capacity, and flow rate are additional caries risk prediction markers that can be measured chairside.³ There is an increase in salivary *n*-butyrate, lactate, FAs and acetate levels in children with dental caries when compared to healthy subjects.⁸ There is a correlation of salivary enzymatic markers like alpha-amylase, proteinase-3 and carbonic anhydrase (CA VI) with increased incidence of dental caries.⁹ Some of the other salivary markers are antimicrobial peptides (AMPs) like cathelicidin peptide LL-37, beta-defensins, statherin and histatins and these enzyme based biomarkers in saliva plays a vast role in dental caries.⁹

Viral and fungal diseases Saliva contains HIV and anti-HIV antibodies, which can be used as a marker to identify the illness. This provides an alternative for the invasive investigations like collection of blood samples. “OraSure” is a collecting device for collection of saliva for HIV antibody test. “OraSure” is commercially available in united states for salivary testing of HIV. Saliva collection has several benefits over blood collection, such as being painless, easy,

quick, cheap, and having a lower chance of infection than blood. Saliva can be used to diagnose a number of infectious disorders, including dengue, Ebola, Mycobacterium tuberculosis, malaria, and Herpes Simplex virus. In malaria the IgG antibodies in saliva is strongly correlated with that of serum.¹Saliva can potentially include viral antigens in addition to viral antibodies. Saliva contains the viruses listed below: HIV, HPV, VZV, EBV, HSV-1, HSV-2, CMV, and HCV. Hence, they can be used to diagnose the viral infections. Thereby saliva act as a non-invasive tool. Fungal infections like candidiasis can also be detected by saliva.¹

Autoimmune diseases

Sjogren's syndrome Sjogren's syndrome is a lymphoproliferative disease that has an autoimmune background which are characterized by mononuclear infiltration of exocrine glands like lacrimal and salivary glands. The clinical manifestations include mainly dryness of eyes (keratoconjunctivitis sicca), dryness of the mouth (xerostomia). This is due to production of autoantibodies. There exists no single clinical, laboratory, or radiographic characteristic to be considered as the "gold standard" for diagnosis. Sjogren's syndrome's most commonly recognized biomarkers are found in serum such as autoantibodies and cytokines, DNA (found through gene profiling and/or genome-wide association studies), cells or at the cellular level (found based on the various phenotypes or properties/functions of cells).¹⁹

Serum contains autoantibodies against the autoantigens Ro/SSA and La/SSB, which are frequently employed as biomarkers in the diagnosis and follow-up of Sjogren's syndrome [anti-Ro/SSA and anti-La/SSB]. Anti-nuclear antibodies (ANA) (21) and rheumatoid factor (RF) are also routinely used in monitoring the diseases.^{19,30} Muscarinic type 3 receptor (M3R) is a new biomarker for diagnosing Sjogren's syndrome as it is linked with exocrine secretion. Anti-M3R is found associated with Sjogren's syndrome in some studies. salivary Calprotectin

has found association with Sjogren's syndrome.¹⁹ According to certain research, people with Sjogren's syndrome have higher levels of IgA, IgG, IgM, lactoferrin, albumin, β_2 microglobulin, cystatin S and C, lipid, prostaglandin E2, and thromboxane B2. Primary Sjogren's syndrome is identified by three mRNA biomarkers: low-affinity IIIb receptor for the Fc fraction of IgG, guanylate binding protein 2 [GBP-2], myeloid cell nuclear differentiation antigen [MNDA]. Protein biomarkers include cathepsin D [CPD], α -enolase, and β_2 -microglobulin [β_2m].²⁰ PGE2 and thromboxane B2 levels are elevated in Sjogren's syndrome.⁶ There is a significant rise in the protein biomarkers α -enolase, β_2 -microglobulin (B2M), and cathepsin D in patients experiencing primary Sjogren's syndrome.²¹ Few biomarkers used to distinguish healthy individuals from Sjogren's syndrome patients are carbonic anhydrase VI (CA-VI), B2M, glyceraldehydes-3-phosphate dehydrogenase (G3PDH), and immunoglobulin k light chain (IGK-light chain), epidermal fatty acid binding protein (E-FABP).²¹

Systemic diseases

Cardiovascular diseases Salivary levels of IL-1 β , IL-6, TNF- α & prostaglandin E2 increases in atherosclerosis.¹ Acute C reactive protein (CRP) and cardiac troponin T levels in saliva may be considered to diagnose and track myocardial infarction. Salivary NT-proBNP levels rise and α -2-HS glycoprotein levels fall, and these changes can be utilized to detect heart failure early. MMP-8 is a key player in the process of coronary artery disease. It has been discovered that MMP-8 and TIMP-1 (tissue inhibitor of matrix metalloproteinase-1) are related to ischemia and infarction.¹ Salivary levels of troponin I, C reactive protein is increased 2.0 to 3.5 folds indicating heart diseases.²⁶ Salivary α amylase is used as an important and an individual marker for myocardial infarction.²⁰

Respiratory diseases One of the leading causes of death worldwide is lung cancer. The three important markers for lung cancers are calprotectin, zinc- α -2-glycoprotein and haptoglobin. The levels of the biomarkers in saliva differ from those of patients without the disease, the transcriptomic biomarkers of lung cancer, such as the fibroblast growth factor receptor substrate 2, the epidermal growth factor receptor, B-Raf gene, cyclin I, fibroblast growth factor 19 are some of the biomarkers to diagnose lung cancer.²² Chronic respiratory infections, mainly in children shows lower levels of IgA in saliva.²⁹ Cys-leukotriene levels have been observed to be higher in asthmatic patients.⁶

Neurodegenerative diseases Neuronal loss in neurodegeneration is a complicated process that is both progressive and irreversible.¹⁵ Neuro degenerative disorders like dementia, Alzheimer's disease, Parkinson's disease. The salivary levels of taurine, succinate, glycerol and dimethyl sulphone were found reduced in patients with dementia.⁸

Parkinson's disease (PD) The loss of dopamine-producing neurons in the substantia nigra is a hallmark of Parkinson's disease (PD), a neurodegenerative illness. According to recent studies, various neurodegenerative conditions may change the salivary exosomes. The phosphorylated α -synuclein in salivary exosomes are found increased in patients with Parkinson's disease. The level of concentration of extracellular vesicles are also increased in Parkinson's disease. Whereas lower concentrations of total α -synuclein in salivary extracellular vesicles are found in multiple system atrophy parkinsonism. This is used to differentiate Parkinson's disease with multiple system atrophy parkinsonism, thereby its high specificity is used for potential diagnosis of the condition.

studies have found that Alzheimer's disease has increased levels of salivary A β ₄₂. Reduced levels of salivary acetylcholinesterase seen in Alzheimer's disease. When compared to

healthy individuals, patients with Alzheimer's disease found to have reduced salivary lactoferrin levels. There is a strong correlation between Alzheimer and salivary lactoferrin.¹⁵

Diabetes mellitus Most widely used test for noninsulin-dependent diabetes is by blood sample collection to measure hba1c levels. Saliva is superior to blood samples in several ways.²³In Type-2 Diabetes Mellitus, Alpha-2 macroglobulin increases in saliva.Indicators of diabetes mellitus also include salivary glucose levels.¹Individuals experiencing diabetes mellitus also have alterations in their alpha hydroxybutyrate levels.¹0.5 to 1 mg/dl of glucose is present in saliva. This increases after intake or ingestion of food that strongly correlates with blood.¹³ Salivary amylase levels are higher in diabetes mellitus patients.¹³When compared to individuals with good health, those with higher blood glucose levels (diabetic) had higher concentrations of salivary lactate, acetate, and sucrose and lower amounts of succinate.¹³

Miscellaneous

Orofacial pain One of the primary causes of patients seeking dental care is thought to be pain. Pain may be acute or chronic. It is found that chronic orofacial pain affects around 15% of the population mainly due to temporomandibular disorders. Salivary cortisol levels are observed to be higher in patients experiencing orofacial pain.²⁵Burning mouth syndrome is another persistent type of orofacial discomfort (BMS).Patients with burning mouth syndrome have lower levels of uric acid, although salivary alpha amylase (sAA), IgA, and macrophage inflammatory protein-4 (MIP4) were higher.²⁴

Stress anxiety and depression Prolonged emotional pressure and chronic stress may lead to a wide variety of physical and psychological diseases.¹⁴The influence of childhood trauma and emotional instability affects the immune system regulation evidenced by shedding of DNA by Epstein Barr virus (EBV). In previously infected adolescents, the childhood

traumatic events resulted in 100% increase in EBV DNA shedding. Thus, it indicates the salivary biomarkers of EBV may be the indicators of childhood trauma.⁷ Cortisol is considered as the gold standard for indication of stress. The cortisol level in saliva is directly proportional to its level in blood. High cortisol affects the concentration of testosterone in blood.¹⁴ Alpha-amylase (sAA) and chromogranin A (sCgA) are also some of the markers of stress activity in body. It has been discovered that both physical and psychological stress raise chromogranin A levels. Numerous investigations have identified salivary cortisol, salivary chromogranin A, and alpha amylase as stress markers.¹¹ Another stress related protein is immunoglobulin A whose level alters in stressful situations.¹⁴

Advantages of saliva as a biomarker

- A cost-effective approach.
- Multiple sample collection can be done easily.
- A non-invasive collection method with a minimal risk of infections.

Disadvantages of saliva as a biomarker

- Lower accuracy
- No standardization in collection of samples and analysis
- Lack of reference ranges
- Affected by oral status
- Low concentration of analytes.⁶

Conclusion Since sampling or collection of saliva is less invasive compared to serum or blood for clinical analysis, it has gained attention as an attractive diagnostic fluid for diagnosis and monitoring of a disease. Unlike serum or blood samples that is prone for

clotting, salivary samples are very much easier to handle and needs less pre analysis and manipulation. In the future, we highly anticipate that salivary diagnostics will be used as a supplemental tool or aid in health monitoring, early diagnosis, and prevention of possible health risks.

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