Salivary biomarkers as a diagnostic tool

Taniya Malhotra[1], Akshat Sachdeva[2], Sumit Bhateja[3,*], Geetika Arora[4]

[1,2]Dental Surgeon (BDS), [3,4]Reader (MDS), [3]Dept. of Oral Medicine and Radiology, [4]Dept. of Public Health Dentistry, [1-3]Manav Rachna Dental College Faridabad, Haryana, [4]Inderprastha Dental College Sahibabad, Uttar Pradesh, India

***Corresponding Author: Sumit Bhateja**
Email: sumit.mrdc@mrei.ac.in

Abstract
Saliva, a biological fluid is steadily emerging as a potent diagnostic tool in modern day health and disease. Advances in molecular biology have led to the invent of certain markers for diagnosis of conditions like oral cancer and dental caries to name a few. In addition, saliva based biomarkers are cost– effective, accurate and provide a noninvasive diagnostic approach. Detecting pathologies at earlier stages can significantly increase survival rates and affect treatment prognosis. The present article discusses various salivary biomarkers that can aid in the diagnosis of many such systemic conditions.

Keywords: Saliva, Biomarkers, Systemic Conditions.

Introduction
Saliva is a dilute aqueous solution that contains both inorganic & organic constituents and plays an essential role during mastication, swallowing and speech.

The term biomarker refers to any measurable and quantifiable biological entity than can serve as an indicator for health related assessments.[1] Salivary diagnostics is indeed a dynamic and emerging field that takes into consideration the concept of molecular diagnostics that aids in the diagnosis of several oral and systemic diseases using salivary biomarkers. Saliva is a readily available and easily fetched specimen, which can be collected by non – invasive procedures and contains many hormones and antibodies that have proven to be an inevitable aid in screening and diagnosis. Salivary diagnostics has undoubtedly evolved overtime and serves as a subset of the larger field of molecular diagnostics, now recognized in a wide variety of clinical areas.

Listed below are few systemic conditions and the role of salivary biomarkers in their detection:

Oral Cancer
Oral cancer is one of the globally concerned health problems. Increased incidences of tobacco consumption among young population nowadays are an alarming scenario. Delayed diagnosis is the major causative factor for high morbidity rates in oral squamous cell carcinoma.[2] Studies conducted in the field of molecular biology help in assessment of cancer risk as well as prediction of prognosis.

Tumor Marker: A tumor marker is defined as a substance found in any body fluid that may be an indication of cancer or certain noncancerous conditions.[3] They maybe unique genes or their products which are present only in cancer cells.[4] Tumor markers have been reported to be significantly increased in the saliva of patients diagnosed with oral cancer.[5]

Alterations in Host Cellular DNA: DNA markers originate from dead cells and are detected in the early stages of tumorigenesis. However, tissue specificity of DNA markers is very low.[6] Studies have suggested that premalignant lesions with aneuploidy have a higher transformation rate into malignancy as compared to lesions with normal DNA content.[7] Studies have also demonstrated that loss of heterozygosity in regions that contain a known human suppressor gene is an early predictor of malignant transformation of precancerous lesion.[8] Mitochondrial DNA mutations have proven to be useful for detection of exfoliated oral squamous cell carcinoma [OSCC] cells in saliva.[9]

RNA as a Biomarker: RNA has been found to be an informative marker for the identification of oral cancer. Scientists have earlier compared the clinical accuracy of saliva with that of blood RNA biomarker for oral cancer detection and discovered four RNA biomarkers having a sensitivity and specificity of 91% and 71% respectively.[10]

Protein Markers: Salivary protein markers have shown moderate sensitivity and specificity as prognostic markers.[9] Few studies have indicated that saliva contains specific proteins that may serve as potential biomarkers for OSCC. This can be attributed to the fact that 46 proteins were found at contrasting levels between OSCC and control groups.[11] Metalloproteinases such as MMP‑11 and MMP‑2 were found to be significantly altered in OSCC.[12] Shpitzer, *et al.* in their study found a 39% increase in MMP‑9 with a sensitivity and specificity of 100% & 79% respectively in OSCC patients.[13]

Markopoulos *et al*[9] have summarized various molecular markers useful for early diagnosis of OSCC [Table 1]

Table 1: Molecular Markers for Oral Cancer

Changes in Cellular DNA	Altered mRNA Transcript	Altered Protein Markers
Mitochondrial DNA mutations	Presence of IL-8	Elevated levels of Defensin-1
P^{53} gene mutations	Dual specificity phosphatase-1	Inhibitors of apoptosis
Cyclin D1 gene amplification	Ornithine decarboxylase antizyme 1	Serum tumor marker
Presence of HPV and EBV virus genome	S100 calcium binding protein P	Lactate dehydrogenase
Increase in Ki67 markers	Spermidine N1-acetyl transferase	Squamous cell carcinoma associated antigen

Dental Caries

Dental caries is defined as an infectious microbiologic disease of the teeth that results in localized dissolution and destruction of calcified tissues.[14] The main strain of bacteria responsible for the causation of caries is *Streptococcus mutans*. The acidic metabolites cause a local pH fall below a critical value (pH 5.5) that eventually results in demineralization of the tooth tissue.[15] In a study conducted in Colombia it was observed that in females, more proteins were found in women with a history of caries as compared to women with active caries.[16] The identification of salivary biomarkers of dental caries is important for identification of individuals with risk to develop caries and institute a preventive treatment.

Immunoglobulin A: Salivary antibodies constitute the first line of immune defense. Immunoglobulin secretory IgA (sIgA) plays an important role against the pathogenesis of dental caries by causing aggregation to specific bacterial proteins and consequently leading to agglutination and inactivation of bacterial enzymes and toxins. In addition it also promotes the inhibition of bacterial adherence by the reduction of hydrophobicity of bacteria.[17] In a previously conducted study, it was observed that the total salivary concentration of sIgA was higher in caries free children than in the other groups with active caries.[18]

Mucins: are glycoproteins produced by salivary glands which protect tooth surface from demineralization.[17] This can be demonstrated by the fact that when their levels are decreased, there is an increased incidence of dental caries.[19] Saliva contains two forms of mucins, the high-molecular-weight mucin glycoprotein-1(MG1 or MUC5b) and the low-molecular-weight mucin glycoprotein-2 (MG2 or MUC7). In a previously conducted study, a correlation between the quantity of proteins MG1, MG2 and DMFT index was observed. The results displayed that the absence of 6-13% of mucins was associated with a higher DMFT index.[19]

Defensins: are small cationic proteins with antimicrobial properties. Presence of peptides enables them to kill a variety of bacteria, fungi and enveloped viruses.[20] Defensins are divided into α-defensins and β-defensins. In a study conducted by Ouhara et al,[21] the presence of β-defensins revealed an increased incidence of antimicrobial activity against gram-negative and gram-positive bacteria, fungi as well as viruses which is an important factor for the protection of oral tissues.

Periodontal Disease

Periodontal disease (commonly referred as periodontitis) is a chronic disorder affecting the oral cavity consisting of a group of inflammatory conditions affecting the periodontium. Periodontitis is a multifactorial disease caused by both genetic as well as environmental factors. Biomarkers for diagnosis of periodontal diseases have been listed below:

Specific Markers

Immunoglobulins: Immunoglobulins (Ig) constitute major defense factors of saliva. The predominant Ig is secretory IgA (sIgA). IgG and IgM are also present but in lesser quantities as compared to IgA. IgA, IgG, and IgM collectively influence the oral microbiota by inhibiting bacterial metabolism.[22] Eggert *et al.*[23] reported that saliva from treated periodontitis patients had higher IgA and IgG levels as compared to saliva sample from control population.

Enzymes

Lysozyme: Antibacterial enzyme that can lyse bacterial species. It may also lead to disintegration of bacterial cells via interaction with monovalent anions and proteases found in saliva. Plaque accumulation, a risk factor for the causation of periodontal disease is more likely to occur in individuals with low lysozyme levels.[24]

Peroxidase: This enzyme eliminates hydrogen peroxide and reduces acid production thereby reducing plaque accumulation and disables inception of gingivitis. High peroxidase concentrations have been observed in patients of periodontal disease.[25]

Non – specific Markers

Lactoferrin: Iron binding glycoprotein which is produced by salivary glands. Lactoferrin has been detected in higher concentrations in saliva of people suffering from periodontitis as compared to healthy population.[26]

Histatin: Salivary protein secreted from parotid and submandibular glands. Inhibition of bacterial enzymes involved in the destruction of periodontium is a peculiar feature of histatin.[27]

Hormones: Studies in the past have advocated that emotional stress could probably act as a predisposing factor for periodontitis.[28] One mechanism proposed to account for this relationship is that elevated serum cortisol levels associated with emotional stress exert a strong inhibitory effect on the inflammatory process and immune response.[29] Also, higher cortisol levels were detected in individuals exhibiting severe periodontitis.

Possible HIV Testing:The use of saliva for diagnosis in clinical practice is slowly emerging and on the rise. Majority of the tests used for diagnosis of HIV utilize whole blood or plasma. A few of them have employed

nonconventional specimens like saliva and urine.[30] Oral fluid tests are based on a salivary component, the oral mucosal transudate or crevicular fluid, an interstitial transudate rich in IgG antibodies, used for diagnosing HIV infection.[30] But it cannot be ruled out that the results of oral fluid tests require do confirmatory testing with conventional tests, such as ELISA and/or western blot.

Saliva Versus Blood

Blood has been traditionally and most frequently used over the years for the purpose of analyzing measurable biomarkers. Employing salivary fluids as a medium for biomarker detection and evaluation markedly alleviates patient discomfort through the provision of a noninvasive method. Comparatively, saliva carries certain advantages over blood which include:

1. Blood collection requires trained personnel. Saliva procurement can be done by anyone including self.
2. Sample collection is painless and non – invasive thus reducing patient discomfort.
3. Samples are safer to handle.
4. Economical procedure.

Despite these favorable traits, saliva as a diagnostic tool is yet to be accepted as a mainstream idea. Both fluids are separate and unique in their own way. Hence, it is imperative that we explore saliva as a potential alternative to blood based diagnostics.

Salivaomics

Till date there have been numerous salivary biomarkers proposed for diagnosis of disorders. But it is unlikely that a single marker will prove to be both sensitive and specific. Traditional clinical criteria are insufficient for determining sites of active disease or for measuring susceptibility to future disease progression.[31] Though saliva has enormous elements with diagnostic potential, omic technology made it possible to achieve the best of the saliva's diagnostic potential into clinical practice. Omic technologies include genomics, transcriptomics, proteomics, and metabolomics. They allow for disease-specific salivary biomarker discovery and establishment of rapid and miniaturized analytical salivary assays.[32] Genomic testing could allow long-term planning for more effective dental disease prevention as well as reduce the uncertainty of diagnosis and prognosis.

Conclusion

Saliva is a potential diagnostic tool that offers an easy and noninvasive approach for disease detection like oral cancer, dental caries and periodontitis to name a few. It possesses a high potential to revolutionize the next generation of molecular diagnostics. Presence of salivary biomarkers can aid in early diagnosis. Discovering, validating and understanding saliva-based biomarkers could have a considerable role in establishing oral fluids as a credible diagnostic tool.

Conflict of Interest: None.

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How to cite this article: Malhotra T, Sachdeva A, Bhateja S, Arora G, Salivary biomarkers as a diagnostic tool. *J Surg Allied Sci* 2019;1(1):1-4