Review article

Salivary Biomarkers for Periodontal Diseases-A Review

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Abstract

Second most common oral disease next to dental caries is periodontal disease. It is considered to be inflammatory disorder that damages tissue through the complex interaction between periopathogens and the host defense systems. Researchers involved in periodontal disease diagnostics are currently investigating the possible use of oral fluids, such as saliva, for disease assessment. Secretions from the major salivary glands, which have a large number of proteins and peptides, are responsible for maintaining the integrity of the oral cavity. Also, because of its importance in oral biofilm formation and host defense, secreted saliva with its biomarkers may have a significant role in the establishment and progression of periodontal disease.

Keywords – saliva, biomarker, periodontitis, non invasive, diagnostic fluid

Introduction

Early detection of disease plays a crucial role in successful therapy. In most cases, the earlier the disease is diagnosed, the more likely it is to be successfully cured or well controlled. However, most systemic diseases are not diagnosed until morbid symptoms become apparent in the late phase. To overcome this challenge, medical researchers are devoted to finding molecular disease biomarkers[1]. Biomarkers, whether produced by normal healthy individuals or by individuals affected by specific systemic diseases, are tell-tale molecules that could be used to monitor health status, disease onset, treatment response and outcome. Through the work of the human salivary proteome project, more than 1,000 proteins in saliva have been identified. Advancements in analytical techniques have enabled scientists to discover the specific proteins associated with human diseases. These proteins are referred to as biomarkers[2].

Saliva may reflect levels of therapeutic, hormonal and immunologic molecules & also yield diagnostic markers for infectious and neoplastic diseases[3]. Various mediators of chronic inflammation and tissue destruction have been detected in whole saliva of patient with oral diseases[4].

Advantages of saliva as a diagnostic fluid[5,6]

Noninvasive diagnosis and monitoring of disease.

Painless, patient suffers no discomfort and little anxiety in the collection process.

Simple in collection with a modest trained assistant and applicable in remote areas.

Relatively cheap technology as compared to other tests.

Cost effective applicability for screening large population.

Can be used to study special population where

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blood sampling is a problem eg: anxious/handicap/elderly patients
Safer for health professionals than blood tests.
Compared to blood and urine, saliva is also cheaper to store and transport.
In addition saliva does not clot and can be manipulated more easily than blood.

Limitations of saliva as a diagnostic fluid[7,8] Levels of certain markers in saliva are not always a reliable reflection of the levels of these markers in serum.
Salivary composition can be influenced by the method of collection and degree of stimulation of salivary flow.
Changes in salivary flow rate may affect the concentration of salivary markers and also their availability due to changes in salivary p^H.
Variability in salivary flow rate is expected between individuals and in the same individual under different conditions.
In addition, many serum markers can reach whole saliva in an unpredictable way (*i.e.* gingival crevicular fluid flow and through oral wounds). These parameters will affect the diagnostic usefulness of many salivary constituents.
Furthermore, certain systemic disorders, numerous medications and radiation may affect salivary gland function and consequently the quantity and composition of saliva.
Classification of salivary biomarkers:
Specific markers
1. Immunoglobulin (Ig)
The predominant immunoglobulin in saliva is secretory IgA (sIgA), which is derived from plasma cells in the salivary glands. Lesser amount of IgG and IgM are also found in saliva. IgA, IgG, and IgM influence the oral microbiota by interfering with the bacterial adherence or by inhibiting bacterial metabolism. Patients with periodontal disease are shown to have higher salivary concentrations of Ig A, Ig G and Ig M specific to periodontal pathogens compared with healthy patients. Also the values decrease significantly post treatment.[9, 10, 11, 12]

2. Enzymes
Alkaline Phosphatase (ALP):
ALP is a catalyzing enzyme that accelerates the removal of phosphate groups in the 5 and 3 positions from a variety of molecules, including nucleotides, proteins and alkaloids. Alkaline phosphatase is involved in maintenance of alveolar bone and renewal of the periodontal ligament. Early investigations of ALP and periodontal disease showed a significant correlation between ALP and pocket depth and between ALP and inflammation. Mixed whole saliva of adult periodontitis patients revealed highest enzyme activities with ALP than that of healthy individuals who revealed lowest enzyme activities. The increase in salivary ALP activity in periodontitis can be associated with alveolar bone loss, a key feature of periodontal disease.[11]
Esterase:
Esterase activity of whole saliva was higher in individuals with periodontal disease than in periodontally healthy subjects. Moreover periodontal treatment reduced its levels. Hence the efficacy of periodontal treatment may be readily monitored by changes in levels of activity of specific enzymes like esterase in whole saliva.[12]
Lysozyme:
Lysozyme is an antimicrobial enzyme with the ability to cleave chemical bonds in the bacterial cell wall. It can lyse some bacterial species by hydrolyzing glycosidic linkages in the cell wall peptidoglycan. This leads to destabilization of the cell membrane, probably as a result of the activation and deregulation of endogenous bacterial autolysins. Patients with low levels of lysozyme in saliva are more susceptible to plaque accumulation, which is considered a risk factor for periodontal disease.[13]

3. Salivary Ions
A high concentration of salivary Ca was correlated with good dental health in young adults, but no relationship was detected with periodontal bone loss as measured from dental radiographs. In another study, salivary Ca, and the saliva Ca to phosphate ratio were higher in periodontitis-affected subjects in comparison to healthy controls[14].

Non specific markers
1. Lactoferrin
Lactoferrin is an iron-binding glycoprotein produced by salivary glands, which inhibits microbial growth by sequestering iron from the environment, thus depriving bacteria of this essential element. Lactoferrin is strongly up-regulated in mucosal

secretions during gingival inflammation and is detected at a high concentration in saliva of patients with periodontal disease compared with healthy patients.[15]

2. Epithelial Keratins

Detection of keratins by monoclonal antibodies may have diagnostic value in detection of epithelial dysplasia, oral cancer, odontogenic cysts and tumours. It has been suggested that phenotypic markers for junctional and oral sulcular epithelia might eventually be used as indicators of periodontal disease. McLaughlin et al. demonstrated that the keratin concentration in GCF was significantly higher at sites exhibiting signs of gingivitis and periodontitis compared with healthy sites.[16,17]

3. Inflammatory Cells

Standardizated collection and counting of leukocytes in saliva leads to orogranulocyte migratory rate (OMR). The OMR was found to be correlated with gingival index. In a study by Raeste et al. (1978), the OMR was determined with sequential mouth rinse sampling in periodontitis patients and controls. The results indicated that the OMR reflects the presence of oral inflammation, and the authors suggested that this measure can be used as a laboratory test.[18,19,20]

Hormones

Studies have suggested that emotional stress is a risk factor for periodontitis. One mechanism proposed to account for the relationship is that elevated serum cortisol levels associated with emotional stress exert a strong inhibitory effect on the inflammatory process and immune response. The presence of cortisol in saliva has been recognized for more than 40 years. Recently, salivary cortisol levels were used to evaluate the role of emotional stress in periodontal disease. Higher salivary cortisol levels were detected in individuals exhibiting severe periodontitis, a high level of financial strain, and high emotion-focused coping,[21,22]

Bacteria

Umeda et al. (1998) examined the presence of periodontopathic bacteria in whole saliva in relation to occurrence of the microorganisms in subgingival plaque. Using polymerase chain reaction, a fair agreement was found between the presence of P. gingivalis, Prevotella intermedia and T. Denticola in whole saliva and in periodontal pocket samples. An oral microbial rinse test (Oratest) was described by Rosenberg et al. (1989). In this study Oratest was found to be a simple method for estimating oral microbial levels.[23,24]

Volatiles

Volatile sulphur compounds, primarily hydrogen sulfide and methylmercaptan, are associated with oral malodour. Salivary volatiles have been suggested as possible diagnostic markers and contributory factors in periodontal disease. For example, pyridine and Picolines were found only in subjects with moderate to severe periodontitis. Furthermore, saliva seems to be a useful medium to evaluate oral malodour. A significant association between the BANA scores from saliva and oral malodour was found.[25]

Group	Biomarkers
Enzymes [11,12,13,14]	Alkaline phosphatase, glucoronidase, Gelatinase, Esterase, Collagenase, Kininase
Immunoglobulin [9,10,11,12]	Ig A, Ig G, Ig M, s Ig A
Protein[15]	Cystatin, Fibronectin, Lactoferrin, Vascular endothelial growth factors, Platelet activating factors, Epidermal growth factors
Phenotypic marker[16,17]	Epithelial keratin
Host cell[18,19,20]	Leukocytes (PMN S)
Ion [14]	Calcium
Hormones[21,22]	Cortisol
Bacteria[23,24]	Actinobacillus actinomycetumcomitans, Porphyomonas gingivalis, Porphyromonas intermedia
Volatile compounds[25]	Hydrogen sulphide, Methyl mercaptan, pyridines

Systemic markers related to periodontal infection

C-reactive protein is a systemic marker released during the acute phase of an inflammatory response. Produced by the liver and is stimulated by circulating cytokines, such as TNF-a and interleukin-1, local and/or systemic inflammation such as periodontal inflammation. Circulating C-reactive protein may reach saliva via GCF or the salivary glands. High levels of C-reactive protein have been associated with chronic and aggressive periodontal diseases and with other inflammatory biomarkers.[26]

Markers of periodontal soft tissue inflammation

During the initiation of an inflammatory response

in the periodontal connective tissue, numerous cytokines, such as prostaglandin E2, interleukin-1beta and TNF-a are released from cells of the junctional epithelia and from connective tissue fibroblasts, macrophages and polymorphonuclear leukocytes. Subsequently, enzymes such as matrix metalloproteinase (MMP)-8, MMP- 9 and MMP-13 are produced by neutrophils and osteoclasts, leading to the degradation of connective tissue collagen and alveolar bone.

Prostaglandins (PG) are arachidonic acid metabolites composed of 10 classes, of which D, E, F, G, H and I are of main importance. Of this group, PG E2 is one of the most extensively studied mediators of periodontal disease activity. PG E2 acts as a potent vasodilator and increases capillary permeability, which elicits clinical signs of redness and oedema. It also stimulates fibroblasts and osteoclasts to increase the production of MMPs.[27]

Markers of alveolar bone loss

The level of MMP-8 was demonstrated to be highly elevated in saliva from patients with periodontal disease using a rapid point of- care micro fluidic device. Gelatinase (MMP-9) is produced by neutrophils and degrades collagen intercellular ground substance. In a longitudinal study patients were asked to rinse and expectorate, providing subject-based instead of site based gingival Crevicular fluid samples. When analyzed, a twofold increase in mean MMP-9 levels was reported in patients with progressive attachment loss. Collagenase-3 or MMP-13 is another collagenolytic MMP with exceptionally wide substrate specificity. MMP-13 has also been implicated in peri-implantitis. MMP-13 may be useful for diagnosing, monitoring the course of periodontal disease and for tracking the efficacy of therapy.[28,29,33]

PRODUCT	NAME PURPOSE
MyPerioID[30]	identifies the type and concentration of the specific bacteria that cause periodontal diseases.
My PerioPath[30]	determines the cause of periodontal infections.
Oral Fluid NanoSensor Test[31]	simultaneous and precise detection of multiple salivary proteins and nucleic acids.
Electronic Taste Chips[32]	detects multiple biomarkers for early diagnosis of periodontal disease
OraQuick[32]	an antibody test that provides results in 20 minutes, usually detects HIV 1 and HIV 2
Integrated Microfluidic Platform for Oral Diagnostics[29]	rapidly (3 10 min) measures the concentrations of MMP-8 and other biomarkers in small amounts (10 ml) of saliva

Conclusion

Saliva is said to be a “mirror of the body” because it is an indicator of health not just in the oral cavity but throughout the body. It is likely that the development of saliva based biomarkers will impact and expand the role of dentists. Integrating these new salivary diagnostics methods into clinical practice is important to aid dental professionals in making essential health-related decisions for patients. In the near future, taking a saliva sampling in a dental clinic will become as routine as obtaining a urine or serum sample at a physician’s office.

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