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REVIEW ARTICLE

Salivary Biomarkers: A Periodontal Overview

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ABSTRACT

The current clinical diagnostic criterias which were introduced almost half a century ago continue to function as the basis of oral diagnosis in today's clinical practice. Evolvement with time is now brought us to the era of biomarkers. It's a new paradigm for periodontal diagnosis which is of immense benefit in managing periodontitis patients. Biomarkers are tell – tale molecules that can be used to monitor health status, disease onset, treatment response and outcome.

These biomarkers can be obtained from blood components such as: serum or plasma. However because of it's being an invasive procedure other body fluids such as saliva and GCF are being considered for potential source of biomarkers. The simple and non- invasive nature of saliva collection and its high sensitivity assay development has led to the salivary biomarkers being a promising future for periodontal diagnosis.

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INTRODUCTION

The second most common oral diseases next to dental caries are the periodontal diseases which are considered to be inflammatory disorder that damages tissue through the complex interaction between periopathogens and the host defence systems (1).

Because of the increasing prevalence and associated co morbidities (2), screening and diagnostic modalities for the early identification of oral disease initiation and progression, as well as objective measures for response to therapy, are being sought.

A biomarker, or biological marker, is in general a substance used as an indicator of a biological state

In oral diagnostics, it has been a great challenge to determine biomarkers for screening, prognosis and evaluating the disease activity and the efficacy of therapy (diagnostic tests). An oral diagnostic tool, in general, should provide pertinent information for differential diagnosis, localization of disease and severity of infection. Traditional diagnostic measures, such as visual examination, tactile appreciation, periodontal pocket depth, attachment level, and plaque index, bleeding on probing and radiographic assessment of alveolar bone loss are still popular and universally used, however they are almost 50 years old and still act as basis for oral diagnosis till date.

Saliva is simple, non-invasive, readily available and easily collected without specialized equipment or personnel. For the past two decades, saliva has been increasingly evaluated as a diagnostic fluid for detecting breast cancer (3), oral cancer (4), caries risk (5), salivary gland diseases (6), periodontitis (7), and systemic disorders such as hepatitis C and the presence of human immunodeficiency virus (HIV) (8). It may reflect levels of therapeutic, hormonal, and immunologic molecules and can yield diagnostic markers for infectious and neoplastic diseases. Various mediators of chronic inflammation and tissue destruction have been detected in whole saliva of patient with oral diseases.

Also, for some diagnostic purposes, salivary biomarkers proved more useful than serum analysis. Salivary biomarkers have also been used to examine the effect of

lifestyle factors, including smoking, on periodontal health.

Why is a periodontal disease indicator needed???

The diagnosis of active phases of periodontal disease and the identification of patients at risk for active disease are challenges for clinical investigators and practitioners alike. Researchers are confronted with the need for innovative diagnostic tests that focus on the early recognition of the microbial challenge to the host (9). Optimal innovative approaches would correctly determine the presence of current disease activity, predict sites vulnerable for future breakdown and assess the response to periodontal interventions. A new paradigm for periodontal diagnosis would ultimately improve the clinical management of periodontal patients.

POSSIBLE SALIVARY BIOMARKER

- **Enzymes**: Alkaline phosphatise, Amino peptidase, Trypsin, á glucosidase, â galactosidase, â glucosidase, â glucoronidase, Gelatinase, Esterase, Collagenase, Kininase
- **Immunoglobulin:** Ig A, Ig G, Ig M, sIg A
- **Protein:** Cystatin, Fibronectin, Lactoferrin, Vascular endothelial growth factors, Platelet activating factors, Epidermal growth factors
- **Phenotypic marker:** Epithelial keratin
- **Host cell:** Leukocytes (PMN'S)
- **Ion:** Calcium
- **Hormones:** Cortisol
- **Bacteria:** A.a, P. gingivalis, P. intermedia, P. micros, C. rectus, T. denticola, B. forsythus, P. micros, mycoplasma
- **Volatile compounds:** Hydrogen sulphide, Methyl mercaptan, Picolines, pyridines

SALIVARY PROTEOMICS FOR EXISTING PERIODONTAL DISEASES

Proteomics is the study of proteins. Salivary proteomics stands for the study of protein in saliva, salivary proteomics can be used as a method to evaluate the disease process.

Variable amounts of blood, serum, serum products, GCF, electrolytes, epithelial and immune cells, microorganisms, bacterial degradation products, lipopolysaccharides, bronchial products and other foreign substances are present in whole saliva. This makes saliva, the best periodontal diagnostic tool. Saliva contains biomarkers specific for the unique physiological aspects of periodontitis, and qualitative changes in the composition of these biomarkers could be diagnostic. It contains a wide variety of periodontal proteomic markers from immunoglobulins to bone remodelling proteins.

- **Interleukin (IL) 1β** is a proinflammatory cytokine that stimulates the induction of adhesion molecules and other mediators which in turn facilitate and amplify the inflammatory response. Its levels correlated significantly with periodontal parameters after adjusting for the confounders. Moreover, combined levels of Il-1â and matrix metalloproteinase (MMP)-8 increased the risk of experiencing periodontal disease by 45 folds (10).
- **MMPs**: MMP-8 a key enzyme in extracellular collagen matrix degradation, derived predominantly from PMNs during acute stages of periodontal disease also correlated significantly with periodontal activity even after adjusting for the confounders, Moreover, its presence significantly increased the risk of periodontal disease (odds ratios in the 11.3-15.4 range)(10) . Strong correlations between MMP-8 and traditional periodontal diagnostic methods further support the contention that MMP-8 is not only an indicator of disease severity, but also disease activity. MMP-1 (interstitial collagenase) also appeared to be activated in periodontitis (10). Higher levels of other MMPs, including MMP-2, MMP-3 and MMP-9, were also reported in the saliva of patients affected by periodontitis.
- **Immunoglobulin (Ig)**: 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 (11).Also the values decrease significantly post treatment.
- **Acid Phosphatase (ACP) and Alkaline Phosphatase (ALP):** A significant positive correlation between salivary ACP and calculus formation has been found (12). It was found that mixed whole saliva of adult periodontitis patients revealed the highest enzyme activities with ALP than that of healthy individuals who revealed lowest enzyme activities (13). The increase in salivary ALP activity in periodontitis can be associated with alveolar bone loss, a key feature of periodontal disease.
- **Esterase, Lysozyme, Lactoferrin**: A statistically significant, positive correlation between salivary esterase and calculus formation (12) was found. It was found that the esterase activity of whole saliva was higher in individuals with periodontal disease than in periodontaly healthy subjects (13). 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 (14). Patients with low levels of lysozyme in saliva are more susceptible to plaque accumulation, which is considered a risk factor for periodontal disease.

  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).

Epithelial Keratins

Epithelial cells from the lining of the oral cavity are found in saliva, but the contribution of crevicular or pocket epithelial cells to the total number of salivary epithelial cells is not known (16).

To study epithelial cell function in periodontal disease and periodontal diagnosis, specific keratin antigens in saliva may be evaluated. Furthermore, detection of keratins by monoclonal antibodies may have diagnostic value in detection of epi-

thelial dysplasia, oral cancer, odontogenic cysts and tumours (17). 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 (18). Similar findings were not observed in saliva.

Inflammatory Cells

] The number of leukocytes in saliva varies from person to person, and cell counts vary for an individual during the course of the day. The majority of salivary leukocytes enter the oral cavity via the gingival crevice (19). Studies in the late 1960's and the early 1970's examined the presence of leukocytes (orogranulocytes) in saliva. Klinkhammer et al. standardized collection and counting of leukocytes in saliva and developed the orogranulocytes migratory rate (OMR). The OMR was found to be correlated with gingival index. In an experimental gingivitis model, the number of granulocytes in saliva increased before the appearance of clinical gingivitis (20).

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. However conflicting results were reported by Cox et al. (1974). Occult blood in saliva in relation to gingival inflammation has also been examined using a home-screening test (21). According to the authors, this method demonstrated sensitivity of 75.9% and specificity of 90.5% for detection of gingival inflammation.

Salivary Ions

Calcium (Ca) is the ion that has been most intensely studied as a potential marker for periodontal disease in saliva. 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 (22). In another study, salivary Ca, and the saliva Ca to phosphate ratio were higher in periodontitis- affected subjects in comparison to healthy controls (23). No differences between groups were found for flow rate and buffering capacity of saliva.

Another study from the same investigators examined differences in salivary calcium levels and found higher concentration of Ca in whole stimulated saliva from the periodontitis patients (24). The authors concluded that an elevated Ca concentration in saliva was characteristic of patients with periodontitis.

SERUM MARKERS IN SALIVA WITH POTENTIAL IMPORTANCE FOR PERIODONTAL DISEASE

Studies have suggested that emotional stress is a risk factor for periodontitis (25). 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 (26). 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 (27).

Higher salivary cortisol levels were detected in individuals exhibiting severe periodontitis, a high level of financial strain, and high emotion-focused coping, as compared to individuals with little or no periodontal disease, low financial strain, and low levels of emotion-focused coping (27).

Bacteria

Specific species of bacteria colonizing the subgingival environment have been implicated in the pathogenesis of periodontal disease (28). Consequently, studies have evaluated levels of bacteria in saliva in relation to periodontal status. It has been suggested that microorganisms in dental plaque can survive in saliva, and can utilize salivary components as a substrate. It was shown that saliva could serve as a growth medium for oral *Streptococcus* species and *A. Viscous*. Bowden (1997) suggested that the number of bacterial cells for a given species in unstimulated saliva may indicate whether that microorganism is actively growing in plaque.

De Jong et al. (1986) studied that, microorganisms from supragingival plaque were grown on saliva agar. When supragingival plaque was placed on saliva and blood agar plates, the composition of the microflora isolated from the plates were similar. The authors concluded that the supragingival microflora could utilize saliva as a complete nutrient source.

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. These 3 micro-organisms, in addition to *Prevotella nigrescens*, were detected more often in saliva than in the subgingival samples. It was also stated that accurate detection of *A. actinomycetemcomitans* and *B forsythus* in the oral cavity requires analysis of both whole saliva and periodontal pocket samples.

The effect of plaque accumulation on the salivary counts of some dental plaque microorganisms was examined in 20 subjects who refrained from oral hygiene for 7 days. The large increase in the number of bacteria on the teeth was reflected by an increase in salivary counts of *Actinomyces* species.

A highly significant correlation was found between *S. Mutans* level in dental plaque and the salivary level of this microorganism. Furthermore, in a study of 60 children aged 5–9 years, isolation, frequency and numbers of *Mycoplasma* species in saliva were consistently related to gingivitis scores, which supports an association of Mycoplasma and gingivitis in children (29). Salivary levels of periodontal pathogens were found to vary with periodontal status and as a result of treatment.

Salivary levels of *A. actinomycetemcomitans*, *P. gingivalis*, *P. intermedia*, *Campylobacter rectus*, and *Peptostreptococcus micros* were determined by bacterial culture and related to clinical periodontal status in 40 subjects with varying degrees of periodontitis (30). In a study of 15 patients with moderate to severe periodontitis, a decrease in the number of patients harbouring *A. actinomycetemcomitans* and *P. gingivalis* in saliva was noted after Scaling and root planing and surgery. 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. In a companion study (Tal & Rosenberg 1990), Oratest results were correlated with plaque index and gingival index scores, and the authors stated that this test provides a reliable estimate of gingival inflammation.

Volatiles

Volatile sulphur compounds, primarily hydrogen sulfide and methylmercaptan, are associated with oral malodour (31). 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. In a study of self estimation of oral malodour, estimation of malodour based on saliva yielded a significant correlation with objective parameters (32). However, no specific association between levels of volatiles and periodontal status has been reported.

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, interleukin-6 and tumour necrosis factor-alpha 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 polymorphonuclear leukocytes and osteoclasts, leading to the degradation of connective tissue collagen and alveolar bone. During the inflammatory process, intercellular products are synthesized, released and diffuse towards the gingival sulcus or periodontal pocket.

Prostaglandins are arachidonic acid metabolites composed of 10 classes, of which D, E, F, G, H and I are of main importance. Of this group, prostaglandin E2 is one of the most extensively studied mediators of periodontal disease activity, Prostaglandin 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 (33).

Markers of Alveolar Bone Loss

Many different biomarkers associated with bone formation, resorption and turnover, such as alkaline Phosphatase, osteocalcin, osteonectin and collagen telopeptidases, have been evaluated in gingival Crevicular fluid and saliva (34). These mediators are associated with local bone metabolism (in the case of periodontitis) as well as with systemic conditions (such as osteoporosis or metastatic bone cancers).

During progressive periodontal breakdown, gingival and periodontal ligament collagens are cleaved by host cell-derived interstitial collagenases. Also 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 (35). The MMP-8 level is also elevated in peri-implant sulcular fluid from peri implantitis lesions (36). Collectively, these results show promise for the use of MMP-8 as a biomarker in the active phase of peri-implant disease.

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 (37). When analyzed, a twofold increase in mean MMP-9 levels was reported in patients with progressive attachment loss. These results show that the future use of MMP-9 in oral fluid diagnostics may serve as a guide in periodontal treatment monitoring.

Collagenase-3 or MMP-13 is another collagenolytic MMP with exceptionally wide substrate specificity. MMP-13 has also been implicated in peri-implantitis. It was concluded that elevated levels of both MMP-13 and MMP-8 correlated with irreversible perio-implant vertical bone loss around loosening dental implants (38). MMP- 13 may be useful for diagnosing, monitoring the course of periodontal disease and for tracking the efficacy of therapy (39).

In brief, the studies assessing the role of gingival Crevicular fluid carboxyterminal telopeptide of type I collagen levels as a diagnostic marker of periodontal disease activity have produced promising results to date. Carboxyterminal telopeptide of type I collagen has been shown to be a promising predictor of both future alveolar bone and attachment loss.

Also, the levels of carboxyterminal telopeptide of type I collagen were strongly correlated with clinical parameters and putative periodontal pathogens, and demonstrated significant reductions after periodontal therapy.

Elevated serum osteocalcin levels have been found during periods of rapid bone turnover, such as in osteoporosis and multiple myeloma and during fracture repair. Therefore, studies have investigated the relationship between gingival Crevicular fluid osteocalcin levels and periodontal disease. When a combination of the biochemical markers osteocalcin, collagenase, prostaglandin E2, alpha-2 macroglobulin, elastases and alkaline phosphatise was evaluated, increased diagnostic sensitivity and specificity values of 80 and 91%, respectively, were reported (40).

Table 1: Specific and nonspecific markers in salivary glands

Marker	Relationship with periodontal disease	Type of periodontal disease
SPECIFIC		
Immunoglobulin (Ig A, Ig M, Ig G)	Interferes with adherence and bacterial metabolism/ increased concentration in saliva of periodontal patients	Chronic and aggressive
NON SPECIFIC		
Mucins	Interferes with the colonisation of A.a	Aggressive
Lysozyme	Regulate biofilm accumulation	Chronic
Lactoferrin	Inhibit microbial growth/increased correlation with A.a	Aggressive
Histatin	Neutralizes lipopolysaccharide and enzymes known to affect the periodontium	Chronic and aggressive
Peroxidise	Interferes with biofilm accumulation/ increased correlation with periodontal patients	Chronic

CONCLUSION

Diagnostic tests are routinely used in evaluation of many systemic disorders. In contrast, diagnosis of periodontal disease relies primarily on clinical and radiographic parameters. These measures are useful in detecting evidence of past disease, or verifying periodontal health, but provide only limited information about patients and sites at risk for future periodontal breakdown.

This review of the literature concerning the use of saliva for periodontal diagnosis allows some conclusions to be drawn regarding the types of biochemical indicators that appear to hold promise as useful tests for periodontal disease. Longer studies examining the relationship of the identified markers to the natural history of periodontal disease is the obvious next step in this line of investigation.

Saliva (oral fluid) is a mirror of the body. It could be used to monitor the general health and the onset of specific diseases. 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. Informative biomarkers can further serve as early sentinels of disease, and this has been considered as the most promising alternative to classic environmental epidemiology.

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