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Oral biomarkers in the diagnosis and progression of periodontal diseases

***Zia A[1], Khan S[1], Bey A[1], Gupta ND[1], Mukhtar-Un-Nisar S[2]**

[1] Department of Periodontics and Community Dentistry, Dr. ZA Dental College and Hospital, AMU, Aligarh 202002, India.

[2] Department of Conservative Dentistry and Endodontics, Dr. ZA Dental College and Hospital, AMU, Aligarh 202002, India.

***Corresponding Author:** afafzia@gmail.com

Abstract

Periodontitis is a disease characterized by loss of connective tissue attachment and bone around the teeth in conjunction with the formation of periodontal pockets due to the apical migration of the junctional epithelium. Early diagnosis and treatment of progressive periodontitis is important because of the irreversible nature of this disease The long-term aim is that treatment and prevention of periodontal disease will be founded on diagnostic tests based on aetiopathogenic factors rather than just clinical experience. Clinical measurements used in diagnosis of periodontal diseases are often of limited usefulness in that they are indications of previous periodontal disease rather than the present disease activity. Biochemical mediators in oral fluids like saliva and gingival crevicular fluid (GCF) are highly beneficial in the determination of current periodontal status. These substances known as biomarkers help in determination of inflammatory mediator levels, as they are good indicators of inflammatory activity. This review highlights recent advances in the use of salivary and gingival crevicular fluid (GCF) biomarker-based disease diagnostics that focus on the identification of active periodontal disease.

Keywords: Periodontitis; gingival crevicular fluid; biomarkers.

Introduction

Periodontal diseases are heterogeneous and include a variety of infections and inflammatory lesions. Notably, periodontitis is a prevalent disease of man that is characterized by loss of connective tissue attachment and bone around the teeth in conjunction with the formation of periodontal pockets due to apical migration of the junctional epithelium. The microbial nature of many periodontal diseases has been recognized long ago. More recently, it has been realized that the host related factors might be the keys to understanding of the disease processes in periodontitis. Periodontal disease progression is episodic in nature on a tooth site level; however, the risk of periodontal disease is principally patient based rather than site based (Champagne et al 2003).

Bacterial virulence factors either result in degradation of host tissues or cause the release of biologic mediators from host tissue cells that lead to host tissue destruction. Mediators produced as a part of host response that contribute to tissue destruction include proteinases, cytokines and prostaglandins. Also, a variety of enzymes produced by periodontal microorganism cause tissue destruction.

Locally, presence of bacteria adjacent to gingival crevice and the intimate contact of bacterial lipopolysaccharide with host cells trigger monocytes, polymorphonucleoleukocytes, macrophages and other cells to release inflammatory mediators such as IL-1, TNF-α, and prostaglandin E_2. IL-1 and TNF-α have an important role in periodontal tissue destruction and PGE_2 appears to partly responsible for bone loss associated with periodontal diseases (Miyasaki 2004).

Early diagnosis and treatment of progressive periodontitis is important because of the irreversible nature of this disease (Kinane 2000). A goal of periodontal diagnostic procedures is to provide useful information to the clinician regarding the present periodontal disease type, location and severity. These findings serve as a basis for treatment planning and provide essential data during periodontal maintenance and disease monitoring phases of treatment.

Traditional clinical measurements (probing pocket depth, bleeding on probing, clinical attachment loss, plaque index, radiographs) used for periodontal diagnosis are often of limited usefulness in that they are indicators of previous periodontal disease rather than present disease activity. There is a need for development of new diagnostic tests that can detect the presence of active disease, predict

future disease progression and evaluate the response to periodontal therapy, thereby improving the clinical management of periodontal patients. Advances in oral and periodontal disease diagnostic research are moving toward methods whereby periodontal risk can be identified and quantified by objective measures such as biomarkers (Table 1).

Table 1. Diagnostic tools to measure periodontal diseases.

Level	Example of Process	Example of Diagnostic Tool
Molecular	Activation of receptors for endotoxin: CD-14; toll-like receptors	Polymerase chain reaction; DNA-DNA hybridization; laser-capture microdissection
Cellular	Inflammatory cell activation such as neutrophils; osteoclast activation	ELISA; immunohistochemistry
Tissue	Downgrowth of junctional epithelium; bone and connective tissue loss	Histomorphometry; immunohistochemistry
Clinical	Attachment loss; bleeding; bone loss	Periodontal probing radiographs

Biomarkers may be defined as a substance that is measured objectively and evaluated as an indicator of normal biologic processes, pathogenic processes, and pharmacologic responses to a therapeutic intervention. 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. Biomarkers of disease in succession play an important role in life sciences and have begun to assume a greater role in diagnosis, monitoring and therapy outcomes and drug discovery. The challenge for biomarkers is to allow earlier detection of disease evolution and more robust therapy efficacy measurements (Colburn 2003).

This article highlights a brief review from the literature on periodontal disease biomarker especially in gingival crevicular fluid (GCF) and saliva focusing on the identification of active periodontal disease.

Potential biomarkers in GCF for periodontal diseases

Gingival crevicular fluid is an inflammatory exudate from the gingival microcirculation that crosses inflamed periodontal tissues and en route collects molecules of potential interest from the local inflammatory reaction. The constituents of the fluid are derived from a variety of sources. GCF contains substances from the host as well as from microorganisms in the subgingival and supragingival plaque. Constituents from the host include molecules from blood, and contributions from cells and tissues of the periodontium. The latter includes the vasculature, epithelium, nonmineralized and mineralized connective tissues, as well as the inflammatory and immune cells that have infiltrated into the periodontal tissues. The cellular components of GCF are 70–80% granulocytes, 10–20% monocytes/macrophages, 5% mast cells and 5% T lymphocytes. The collection and analysis of GCF samples provides a non-invasive means to assess the pathophysiological status of the periodontium in a site-specific manner.GCF could be easily collected by means of paper strips, absorbent points and micropipettes from gingival crevices of teeth (Lamster et al 1988, 1997).

According to Armitage (2004), more than 65 GCF constituents have been evaluated as potential diagnostic markers of periodontal disease progression. These markers can be divided into three groups: host-derived enzymes and their inhibitors, inflammatory mediators and host-response modifiers, and byproducts of tissue breakdown.

Neutrophil mediators identified in GCF include leukotriene B4 (LTB4), PAF, thromboxane B2, elastase and collagenase (Schenck 1993). Monocytes/macrophages secrete mediators of inflammation such as PGE2, IL-1, IL-6, IL-8 and TNF. In addition, pro-inflammatory and anti-inflammatory cytokines are identified in GCF. PGE_2 involved in the

pathogenesis of periodontal diseases, was originally identified in GCF in mid 1970s and subsequently studied in relation to periodontal diseases. Offenbacher et al (1986) showed that there were differences in the GCF concentration of PGE_2 in patients with gingivitis compared with periodontitis. Subsequently, it was found that there was a correlation between increased PGE_2 concentration and clinical attachment loss in patients who were diagnosed with moderate to severe periodontitis. Proinflammatory cytokines in particular IL-1β, may play an integral role in the etiology of periodontal disease. Lieu et al (1996) demonstrated that with an increase in gingival index and probing, there was a corresponding increase in IL-1β in both the gingival tissue and GCF. Engebretson et al through a longitudinal study suggested that GCF IL-1β expression is genetically influenced and not solely a result of local clinical parameters. Also, a GCF level of IL8 was found to be higher in periodontal diseases and was influenced by local IL-1β activities.

Figure 1. Gingival crevicular fluid is most often collected with absorbent paper points or methylcellulose filter paper strips inserted into the crevice. Standardization is obtained with timed sampling (30 seconds).

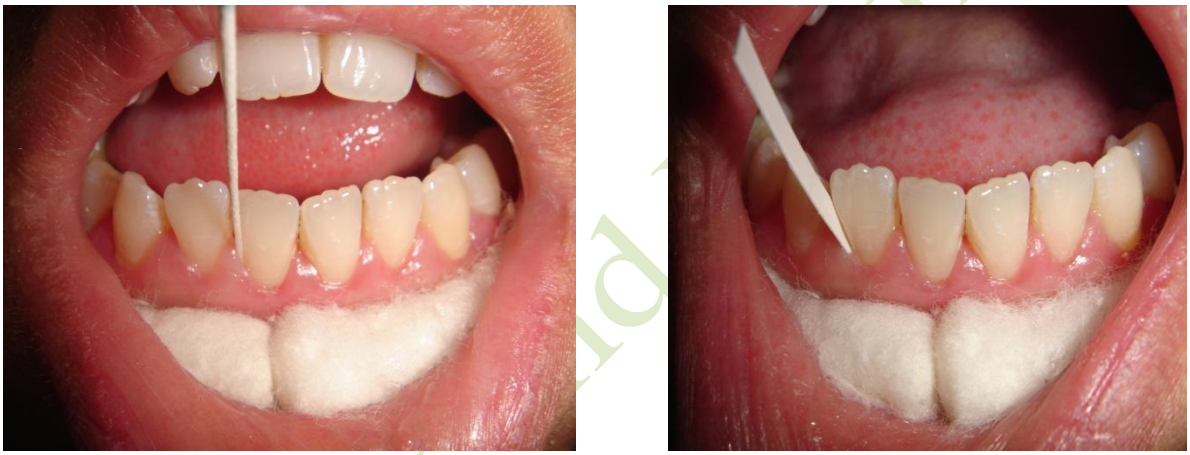

Table 2. Certain biomarkers of periodontal diseases identified from gingival crevicular fluid and saliva.

Category mediator	Examples
Microbial factors	DNA probes or culturing of putative periodontal pathogens (e.g., *Porphyromonas gingivalis*, *Tanerella forsythensis*, *Treponema denticola*)
Host-derived enzymes	Aspartate aminotransferase; elastase
Inflammatory mediators and host-response modifiers	IL-1b; TNF-α; immunoglobulins
Byproducts of tissue breakdown	Collagen telopeptides; osteocalcin; proteoglycans; fibronection fragments

Loos and Tjoa (2005) reviewed potential diagnostic markers of periodontitis present in GCF. Their review identified eight potentially valuable markers, including alkaline phosphatase, β glucuronidase, cathepsin B, collagenase-2 (matrix metalloproteinase, MMP-8), gelatinase (MMP-9), dipeptidyl peptidase (DPP) II and III, and elastase.

Alkaline phosphatase is a membrane-bound glycoprotein that is involved in maintenance of alveolar bone and renewal of the periodontal ligament. In GCF, it is believed to originate primarily from polymorphonuclear leukocytes (PMNs). Similar levels of alkaline phosphatase in GCF have been found in gingival health and experimental gingivitis, but a longitudinal study demonstrated that elevated alkaline phosphatase levels preceded clinical attachment loss and that the total amount of alkaline phosphatase in GCF was significantly higher in active sites (Nakashima 1996).

ß glucuronidase ,a lysosomal enzyme that degrades proteoglycans and ground substance and serves as a marker for primary grade release from PMNs in response to stimuli such as N-formyl-methionyl-leucyl-phenylalanine, platelet activating factor, anaphylotoxin C5a, LTB4 and IL8. ß glucuronidase is a glycoprotein of about 332,000 dalton. It is a homotetramer comprised of four identical subunits. It has high sensitivity and specifity when related to occurrence of clinical attachment loss. This enzyme also proved to be a good predictor of the response to treatment and the risk for future periodontal breakdown (Lamster et al 1998).

Cathepsin B is a cysteine protease involved in proteolysis. Kunimatsu et al (1990) observed that levels of cathepsin B were increased in periodontitis when compared to gingivitis, despite similar GCF flow. The source of cathepsin B in GCF is mainly macrophages, and analysis of cathepsin B in GCF appears to differentiate chronic gingivitis from periodontitis. Furthermore, GCF levels of cathepsin B correlate significantly with clinical parameters before and after periodontal treatment, suggesting a use for this enzyme in assessment of treatment outcomes. Cathepsin G may contribute to periodontal tissue destruction directly and indirectly, via proteolytic activation of latent neutrophil procollagenase (promatrix metalloproteinase-8).

Matrix metalloproteinases (MMPs) form the most important family of proteinases that participate in the normal turnover of periodontal tissues as well as their degradative aspects during periodontal diseases. Some of the members of the matrix metalloproteinase family and the tissue inhibitors were also identified in GCF. Chen et al (2000) reported the increased levels of active neutrophil collagenase in the GCF of periodontitis patients. The active forms of neutrophil type MMP-8 and MMP-13 in GCF were demonstrated to contribute to GCF collagenase activity. Many, rather than single, cellular sources of MMP in the diseased periodontium were identified in untreated periodontitis. Another MMP, MMP-3 and TIMP-1 in GCF were evaluated as prognostic factors for the progression of periodontitis in 40 systemically healthy subjects over a 6-month period. GCF was sampled from both healthy and diseased sites of each patient. The mean amounts of MMP-3 and TIMP-1 in diseased sites were significantly higher than in healthy sites. GCF levels of MMP-3 were highly correlated with clinical measurements taken at baseline, 3-month and 6-month visits. It was stated that sites with high GCF levels of MMP-3 and TIMP-1 were at significantly greater risk for progression of periodontitis.

Neutrophil elastase (or elastase) is a potent proteolytic enzyme found in lysosomal granules. Elastase levels in GCF increase with induction of experimental gingivitis, and decrease when plaque removal is reinsitituted. In a longitudinal study, Eley and Cox (1996) demonstrated that increased elastase in GCF was predictive of periodontal attachment loss. Long-term observation of adult patients with periodontitis undergoing supportive periodontal therapy showed a positive correlation of elastase in GCF with clinical attachment loss.

Aspartate aminotransferase enzyme (AST) is one of the components of GCF that is released and can be detected as a result of cell death. Significant associations between GCF levels of AST and clinical measurements have been published, and a test system, the PeriogardTM periodontal tissue monitors (PTM), has been developed (Persson et al 1990a, b).

Biological markers present in GCF that determine bone loss include bone collagen fragments, extracellular and matrix proteins such as osteopontin, osteonectin and osteocalcin. As the cross-linked telopeptides resulting from posttranslational modification of collagen molecules cannot be reused during collagen synthesis are considered specific biomarkers for bone resorption (Eriksen 1993). Pyridinoline cross linked carboxyterminal telopeptide of type1collagen (ICTP) is 12 to 20 Kd fragment of bone type1collagen released by digesting with trypsin or bacterial collagenase. According to Palys et al (1998), GCF ICTP levels were related to subgingival microflora of periodontal diseases. Golub et al (1997) demonstrated that GCF ICTP levels reduced after one month in chronic periodontitis patients when managed by

scaling and root planning and systemic doxycycline. GCF ICTP levels are a good predictor of future alveolar bone and attachment loss and are strongly correlated with clinical parameters and putative periodontal pathogen. Shibutani et al (1993) demonstrated an association between increase in GCF content glycosaminoglycans and periodontal disease destruction.

Osteopontin (OPN) is noncollagenous calcium binding glycosylated phosphoprotein in bone matrix and is produced by several cells including osteoblasts, osteoclasts and macrophages. Kido et al (2001) demonstrated that OPN level in GCF was increased with progression of periodontal disease. However, no significant difference was observed when OPN level was compared between diseased and healthy sites.

Another noncollagenous calcium binding protein, osteocalcin, is synthesized mainly by osteoblasts. A number of investigators studied relationship between GCF osteocalcin levels and periodontal diseases. Kunimatsu et al (1993) demonstrated a positive correlation between GCF osteocalcin and clinical parameters of periodontitis and gingivitis patients. Significant GCF osteocalcin levels from periodontitis and gingivitis patients were reported by Nakashima et al (1994). Treatment of chronic periodontitis patients with subantimicrobial dose of doxycycline failed to reduce GCF osteocalcin levels (Golub 1997). In addition, no difference in GCF osteocalcin levels between deep and shallow sites in periodontitis patients. Additional studies are needed to fully elucidate the utility of osteocalcin as a periodontal disease activity diagnostic aid.

Kido et al (1999) demonstrated that calprotectin, a major cytosol protein of leukocytes, concentration in GCF from periodontitis patients was significantly higher than that in GCF from healthy subjects. Calprotectin from inflammatory cells appear to protect epithelial cells against binding and invasion by *P. gingivalis*.

Although these GCF markers are promising as diagnostic tests, limitation to the application of a GCF based diagnostic test clearly exists. GCF collection can be technically challenging and time consuming. In addition, selection of the teeth and sites at risk for disease progression is often difficult. Besides, laboratory tests to manage periodontal disease are not routinely employed for dental disease.

Possible salivary biomarkers for periodontal diseases

Saliva is a secretion of the salivary and mucous glands and is of major importance in the maintenance of oral health. The fluid is readily accessible via a very non-invasive collection method, and contains locally produced microbial and host response mediators like GCF. Saliva is also considered to be useful for screening periodontitis and monitor response to treatment. Unlike GCF, its collection is easier technically. Salivary constituents that have been studied as potential diagnostic biomarkers for periodontal disease mainly include proteins of host origin (i.e., enzymes and immunoglobulins), phenotypic markers, host cells, hormones, bacteria and bacterial products, ions and volatile compounds.

Saliva contains both host-derived and microbial derived factors, including several enzymes that degrade proteins, proteoglycans, lipids and carbohydrates. Enzymes in saliva can originate from cells in salivary glands, microorganisms, epithelial cells, PMN and can be derived from GCF. Dipeptidylpeptidase IV (DPP IV) and alanine aminopeptidase (AAP) are proteinases that contribute in collagen degradation. In a study, DPP IV and alanine aminopeptidase (AAP) activity in whole saliva of periodontitis patients were found to be increased compared to the periodontally healthy controls (Elgun et al 2000).

Neutrophils are an important cell type in host defense against periodontopathogenic bacteria. Neutrophil granules contain hydrolytic neutral enzymes, such as elastase, cathepsin G, myeloperoxidase and lysozyme, as well as hydrolases, including cathepsin B, cathepsin D and β-glucuronidase. Lactoferrin, neutrophil collagenase (matrix metalloproteinases- 8, MMP-8) and MMP-9 are also stored in neutrophils' granules. Neutrophilic proteins such as lysozyme, myeloperoxidase and lactoferrin are elevated in periodontal disease conditions. Lactoferrin was demonstrated to interact with *A. actinomycetemcomitans*, which is a causative microorganism in aggressive periodontitis (Allugupali et al 1995). Therefore, colonization of *A. actinomycetemcomitans* may occur more readily in an environment containing lactoferrin with low iron levels and depressed level of iron found in lactoferrin may be resulted from both the iron-sequestering pathogenic bacteria and reduced capacity of lactoferrin to bind iron in the saliva of aggressive periodontitis patients. MMPs in the neutrophil granule are also

associated with tissue destruction in periodontitis MMP1 and TIMP1 are detected in the saliva of the periodontitis patients but no significant elevated levels were observed. Mean levels of IL1B and MMP8 in saliva were significantly higher in periodontal disease subjects than in controls. Salivary levels of TNFα were elevated in patients who had clinical indicators of periodontitis. MMP8 is not only an indicator of disease severity but also disease activity (Herr et al 2007). β glucuronidase in saliva is an indicator of neutrophil influx into gingival tissues and may provide as a risk factor in periodontal disease (Lamster et al 2003).

Nitric oxide (NO), which is a free radical with important cellular functions, is produced and released from human neutrophils and macrophages (Larfars et al 1999). NO is synthesized from the conversion of L-arginine to L-citrulline by nitric oxide synthase (NOS). Arginase, which is an arginine-depleting enzyme, can compete with NOS for the common substrate L-arginine, and thus inhibit NO production. The increased salivary arginase activity in periodontitis perhaps causing a decrease in NO synthesis also leads to a decrease in the antibacterial property of saliva and cause periodontal tissues to become more susceptible to existing pathogens (Ozmeric et al 2000). Chitinase plays a role in the defense against chitin containing pathogens. Studies showed that this enzyme was raised in the saliva of periodontitis patients and decreased after treatment (Van Steijn et al 2002).

A number of studies have demonstrated significant correlation between salivary platelet activating factor (PAF), a potent phospholipid inflammatory mediator, levels and the extent of periodontal disease and post treatment (Rasch et al 1995). Another nonenzymatic protein VEGF, a multifunction angiogenic cytokine was found to be higher in saliva samples of patients as compared to healthy (Booth et al 1998). Oshima et al (2002) showed significant corelation between salivary hepatocyte growth factor (HGF) levels and the number of probing depths exceeding 4 and 6 mm. HGF acts as mitogen and antiapoptic factor for various kinds of epithelial cells. Fibronectin is a glycoprotein which mediates adhesion between cells. *P. gingivalis* fimbriae bind to salivary fibronectin in periodontitis as a result salivary fibronectin is reduced in periodontitis.

Immunoglobulins have an effect on oral microbiota as they interfere in adherence and bacterial metabolism and it was shown that salivary IgA, IgG, IgM have higher concentration in patients with periodontal disease as compared with healthy patients (Hagewald 2002).

C-reactive protein is a systemic marker released during acute phase of an inflammatory response and is produced by liver. Circulating CRP reaches saliva via GCF or salivary glands. High levels of CRP are associated with chronic and aggressive periodontal diseases (Aiuto et al 2004).

It is understood from all these studies that several mediators in saliva were mentioned in the pathogenesis of periodontal diseases. Since saliva is noninvasively and repeatedly sampled, it holds considerable promise as a biologic fluid that can be analyzed for diagnosis of periodontal diseases. Further, it can be collected by individuals without special training and can offer a cost-effective approach for the screening of large populations. However, most of the studies mentioned above were cross-sectional; therefore, it is difficult to interpret the significance of those few reports that examine levels of any particular marker. Well-controlled clinical trials conducted in large populations are necessary to confirm the importance of a specific diagnostic mark.

Conclusion

In the field of oral disease diagnosis, there has been a steady growing trend during the last two decades to develop tools to monitor periodontitis. From physical measurements such as periodontal probing to sophisticated genetic susceptibility analysis and molecular assays for the detection of biomarkers on the different stages of the disease, substantial improvements have been made on the understanding of the mediators implicated on the initiation and progression of periodontitis. At the same time, this evolutionary process has promoted the discovery of new biomarkers and the development of new therapeutic approaches mainly using host modulation. Moreover, new diagnostic technologies such as nucleic acid and protein microarrays and micro fluidics are under development for risk assessment and comprehensive screening of biomarkers. These recent advances are leading to the development of more powerful diagnostic tools for practitioners to optimize their treatment predictability.

Since GCF has the chance of being closely approximated to the periodontal tissues where periodontal disease begins, it seems to

provide more information than markers in saliva. The molecules in saliva can be also originated from salivary glands which cellular and biochemical mediators in saliva may reflect the diseases and metabolic status of glands instead of periodontal diseases. Because GCF constituents reflects the host response to the periodontopathogenic bacterial antigens and the disease progression is essentially dependent upon the host response, evaluation of the markers in GCF is considered to be a good method in the determination of a person's risk for periodontal disease.

While the future of periodontal disease diagnosis using oral fluid biomarkers looks promising, obstacles to these approaches may appear in the clinical setting. Validation of novel periodontal diagnostics will need to be benchmarked with existing gold standards of disease, such as alveolar bone levels and clinical attachment levels, in large patient populations. Acceptance by dentists and treatment clinicians is also necessary and may prove difficult. If more efficient periodontal therapy can be delivered, clinicians will be more likely to utilize new diagnostic approaches. Although challenges remain ahead, the use of biomarkers based oral fluid diagnostics appear promising for future application to diagnose periodontal diseases and to prognosticate periodontal treatment outcomes.

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