International Journal of Science and Research (IJSR)
ISSN: 2319-7064
SJIF (2019): 7.583

GCF Biomarkers in Periodontal Diseases

Dr. Maya Mhaske[1], Dr. Sushma Belkhede[2], Dr. Mithila Kakade[3], Dr. Kalyani Reddy[4]

Abstract: ***The diagnosis of periodontal disease is based on traditional diagnostic parameters such as probing depth, bleeding on probing, clinical attachment levels, assessment of plaque, calculus and alveolar bone levels. These parameters only reflects the past periodontal destruction and not current disease status. Hence various clinical trials have been attempted to identify potential biomarkers of periodontal disease activity. 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 gingival crevicular fluid (GCF) biomarker-based disease diagnostics that focus on the identification of active periodontal disease.***

Keywords: Biomarkers, Saliva, Gingival Crevicular Fluid, Periodontal disease

1. 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.[1]

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 E2. IL-1 and TNF-α have an important role in periodontal tissue destruction and PGE2 appears to partly responsible for bone loss associated with periodontal diseases[2.]

More than 600 bacterial species have been identified from subgingival plaque and only a small number has been found to play a causal role in the pathogenesis of periodontal disease.[3]A biomarker or biological marker is a substance that is objectively measured and evaluated as an indicator of normal biologic process, pathogenic process or pharmacologic responses to a therapeutic intervention. An ideal diagnostic marker should indicate the presence of a disease process before extensive clinical damage has occurred. Such a marker should have high specificity and sensitivity, and one should be able to use it easily at the chairside or as a home use device or test.

On the basis of our current understanding of the complexity of periodontitis, the identification of one single diagnostic marker for all forms of periodontitis seems illusionary. Nevertheless, researchers have been searching actively for unequivocal markers of periodontitis in gingival crevicular fluid to develop a simple test ,to be used chairside, to determine whether a patient suffers from periodontitis and needs therapy, as opposed to another patient who needs no intervention even though he has gingivitis.[4]

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

This article highlights a brief review from the literature on periodontal disease biomarker especially in gingival crevicular fluid (GCF) 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.

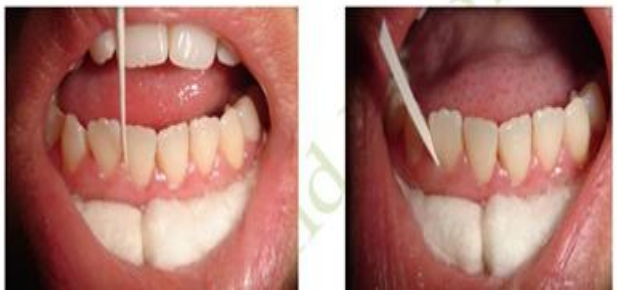
Biomarkers in GCF as follows:

1) Cathepsin B
2) Matrix metalloproteinases (MMPs)
3) Alkaline phosphatase

4) ß glucuronidase
5) C-reactive protein
6) Neutrophil elastase
7) Aspartate aminotransferase enzyme (AST)
8) Osteopontin (OPN)
9) Osteocalcin
10) Osteonectin
11) PGE2

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 .[5]

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).



Cathepsin B is a cysteine protease involved in proteolysis. Kunimatsu et al (1990) [6] 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)[7] 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.

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 [8].

ß glucuronidase ,alysosomal enzyme that degrades proteoglycans and ground substance and serves as a marker for primary grade release from PMNs in response to stimuli such as Nformyl-methionyl-leucylphenylalanine, 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.[9]

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.[10]

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)[11] 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.[12,13]

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[14]. Pyridinoline cross linked carboxyterminaltelopeptide 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)[15], GCF ICTP levels were related to subgingivalmicroflora of periodontal diseases.

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)[16] 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.

Osteocalcin: Osteocalcin is a 5.4 kDa calcium binding protein of bone and is the most abundant non collagenous protein of mineralized tissues. It chemotactically attracts osteoclast progenitor cells and blood monocytes . In addition, it is stimulated by vitamin D3producing concentration that inhibit collagen synthesis in osteoblast, promote bone resorption. Due to these reasons osteocalcin has been suggested as a possible marker of bone resorption and hence periodontal disease progression.[17]
A number of investigators studied relationship between GCF osteocalcin levels and periodontal diseases. Kunimatsu et al (1993)[18] demonstrated a positive correlation between GCF osteocalcin and clinical parameters of periodontitis and gingivitis patients. Treatment of chronic periodontitis patients with subantimicrobial dose of doxycycline failed to reduce GCF osteocalcin levels[19]. . In addition, no difference in GCF osteocalcin levels between deep and shallow sites in periodontitis patients.

Osteonectin and bone phosphoprotein (N-propeptide): Osteonectin is a normal component of bone matrix which is thought to play an important role in the initial phase of 4 mineralization. Bone phosphoprotein which is an amino propeptide extension of α-1 chains of Type I collagen appears to be involved in the attachment of connective tissue cells to 19 the substratum. Both proteins have been detected in GCF of periodontitis patients. The total amount of both proteins have shown to increase with increasing probing depth.[20]

PGE2 involved in the pathogenesis of periodontal diseases, was originally identified in GCF in mid 1970s and subsequently studied in relation to periodontal diseases. Offenbacheret al[21] (1986) showed that there were differences in the GCF concentration of PGE2 in patients with gingivitis compared with periodontitis. Subsequently, it was found that there was a correlation between increased PGE2 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[22] (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.

2. Controversies

Though GCF analysis can provide site-specific status of inflammation without necessitating histopathological evaluation, it is still rife with controversy, like GCF quantity outflow along with inflammatory status could also be affected by extent of sulcular epithelium ulceration.

There is a wide variation in the methods of collection, storage and analysis along with elusion protocols making the values alter based on this variation. Also, timing of fluid sample collection could alter results. Another important consideration is dry or buffer based storage of samples. Though recommended temperature of sample storage is −80 degrees centigrade, whether, all samples collected for various studies strictly adhere to this protocol is questionable.

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.

3. Conclusion

None of the markers discussed so far have the ability to predict periodontal disease activity. The search for that specific marker of periodontal disease activity is still progressing. More longitudinal studies are needed to testify the usefulness of a particular marker. The tests for these biomarkers should be simple and easy to perform.

References

[1] Champagne CM, Buchanan W, Reddy MS, Preisser JS, Beck JD, Offenbacher S, 2003. Potential for gingival crevice fluid markers as predictors of risk for periodontal diseases.Periodontology 2000, 31: 167–80.
[2] Miyasaki KT, Nisengard RJ, Haake SK. 2004 Immunity and inflammation; basic concepts. 9th edition. In: MG Newman, HH Takei, FA Carranza, editors. Carranza's Clinical Periodontology,
[3] Maria TabaJr, Janet Kinney, Amy S.Kim, William V. Giannobile. Diagnostic Biomarkers for oral and periodontal diserases. Dent Clin North Am 2005;49:551-571.
[4] Bruno G Loos, Stanley Tjoa. Host derived diagnostic markers for periodontitis : do they exist in gingival crevice fluid. Periodontol 2000 2005 : 39 : 53-72.
[5] Lamster IB, Oshrain IL, Harder DS, Bader HI, Boyd RL. 1988. A comparison of 4 methods of data presentation for lysosomal enzyme activity in gingival crevicular fluid. Journal of Clinical Periodontology, 15: 347–352.
[6] Kunimatsu K, Ichimaru E, Kato I. 1990. Granulocyte medullas in levels in gingival crevicular fluid from chronic adult periodontitis patients and experimental gingivitis subjects. Journal of Periodontal Research, 25: 352–357.
[7] Chen HY, Cox SW, Eley BM, Mantyla P, Ronka H, Sorsa T, 2000. Matrix metalloproteinase-8 levels and elastase activities in gingival crevicular fluid from chronic adult periodontitis patients. Journal of Clinical Periodontology, 27: 366–9.
[8] Nakashima K, Giannopoulou C, Adersen E, Roehric N, Brochut P, 1996. A longitudinal study of various crevicular fluid components as markers of periodontal disease activity. Journal of Clinical Periodontology, 23: 832–838.

[9] Lamster IB, Osliran RL, Fiorello LA, Celenti RS, Gordon JM 1988. Enzyme activity in crevicular fluid for detection and prediction of clinical attachment loss in patients with chronic adult periodontitis.Six month results. Journal of Periodontology, 59: 516–523.
[10] D_Aiuto F, Ready D, Tonetti MS, 2004. Periodontal disease and creactive protein-associated cardiovascular risk. Journal of Periodontal Research, 39: 236–241.
[11] Eley BM, Cox SW, 1996. A 2-year longitudinal study of elastase in human gingival crevicular fluid and periodontal attachment loss. Journal of Clinical Periodontology, 23: 681– 692.s
[12] Persson GR, DeRouen TA, Page RC, 1990a. Relationship between levels of aspartate aminotransferase in gingival crevicular fluid and gingival inflammation. Journal of Periodontal Research, 25: 17–24.
[13] Persson GR, DeRouen TA, Page RC, 1990b. Relationship between gingival crevicular fluid levels of aspartate aminotransferase and active tissue destruction in treated chronic periodontitis patients. Journal of Periodontal Research, 25: 81–87.
[14] Eriksen EF, Charles P, Melsen F, Mosekilde L, Risteli L, Risteli J, 1993. Serum markers of type I collagen formation and degradation in metabolic bone disease: correlation with bone histomorphometry. Journal of Bone and Mineral Research, 8(2): 127–32.
[15] Palys MD, Haffajee AD, Socransky SS, Oringer RJ, Iranmanesh A, Fiorellini JP, Giannobile WV. 1998. Relationship between C-telopeptidepyridinoline cross-links (ICTP) and putative periodontal pathogens in periodontitis. Journal of Clinical Periodontology, 25(11 Pt 1): 865–7.
[16] Kido J, Nakamura T, Asahara Y, Sawa T, Kohri K, Nagata T, 2001. Osteopontin in gingival crevicular fluid. Journal of Periodontal Research, 36: 328–33.
[17] B.M. Eley, M.Soory, J.D. Manson. Diagnosis Tests of th Periodontal disease activity. Periodontics 6 edition; 178-205.
[18] Kunimatsu K, Mataki S, Tanaka H, Mine N, Kiyoki M, Hasoda K, 1993. A cross-sectional study on osteocalcin levels in gingival crevicular fluid from periodontal patients. Journal of Periodontology, 64(9): 865–9.
[19] Golub LM, Lee HM, Greenwald RA, Ryan ME, Sora T, 1997. A matrix metalloproteinase inhibitor reduces bone-type collagen degradation fragments and specific collagenases in gingival crevicular fluid during adult periodontitis. Inflammation Research, 46(8): 310–9.
[20] B.M. Eley, M.Soory, J.D. Manson. Diagnosis Tests of th Periodontal disease activity. Periodontics 6 edition; 178-205
[21] Offenbacher SB, Odle M, Van Dyke TE, 1986. The use of crevicular fluid prostaglandin E2 levels as a predictor of periodontal attachment loss. Journal of Periodontal Research, 21: 101– 112.
[22] Liu CM, Hou LT, Wong MY, Rossomando EF. 1996. Relationships between clinical parameters, interleukin 1B and histopathologic findings of gingival tissue in periodontitis patients. Cytokine, 8: 161–167.