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Review Article

Oral Fluid-Based Biomarkers in Periodontal Disease – Part 2. Gingival Crevicular Fluid

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***Abstract*:**

Periodontal diagnosis and treatment plan are based on the assessment of probing depth, clinical attachment level, plaque index, gingival index, bleeding on probing, suppuration, furcation involvement, mobility, and radiographic findings. However, these clinical parameters are not sufficiently sensitive and specific to identify disease activity in individual sites or to predict future attachment loss. Hence, attention is focused on the development of diagnostic tools that could screen and differentiate the active inflamed sites and predict future tissue destruction. Gingival crevicular fluid (GCF), has gained great interest on possible diagnostic value in periodontal disease. It contains a large number of proteins and peptides derived from inflamed host tissues. The analysis of the GCF components can reflect the disease status of individual sites and thus, identify potential biomarkers of periodontitis. A literature search was carried out to find out all the available tests that indicate periodontal disease markers in GCF. All major databases were searched to compile the information on published reports between 1999 and 2014. The list of GCF-biomarkers available to date is compiled and presented in a table format. Based on the available literature on GCF biomarkers, it can be concluded that several sensitive and reliable markers are present to detect the presence, severity, and response to treatment. Further studies are warranted to analyze the sensitivity and reliability of these indicators which might help in developing noninvasive tests that could help in the diagnosis of periodontal disease.

***Key Words*:** Biological markers, gingival crevicular fluid, periodontal disease

Introduction

Periodontitis is characterized by the destruction of connective tissue, loss of periodontal attachment, and resorption of alveolar bone. The tissue destruction in periodontal disease appears as a result from the interplay between the pathogenic bacteria and the host's immune and inflammatory responses. The immune system is activated in order to protect against local microbial attack and their damaging products from spreading or invading the gingival tissues. This defense mechanism might be harmful to the host, by destroying surrounding cells and connective tissue structures.[1] Diagnosis of the diseases affecting the periodontium and assessing its outcomes are based on clinical signs such as tissue color and contour, the presence or absence of bleeding on probing, gingival recession, probing pocket depths, attachment levels, suppuration, and tooth mobility.[2] Radiographs are used as an additional tool to visualize the loss of periodontal tissue, by determining the amount of bone loss around the teeth.[3] However, these methods are only useful to assess the past disease activity. Reliable diagnostic methods are essential to assess the active disease status and for monitoring the response to periodontal therapy.[4]

Gingival crevicular fluid (GCF) is a complex mixture of substances derived from serum, host inflammatory cells, structural cells of the periodontium, and oral bacteria. GCF originates from the vessels of the gingival plexus of blood vessels and flows through the external basement membrane and the junctional epithelium to reach the gingival sulcus. GCF can be isolated from healthy sulcus, although only in small amounts. In the healthy periodontium, GCF represents the transudate of gingival tissue interstitial fluid produced by an osmotic gradient.[5] The products of the inflammatory response which occur during the disease process can be found in the GCF. Monitoring of the presence of such components can be of potential value in evaluating periodontal disease status or outcomes of periodontal therapy.[6]

Current understanding of the pathogenesis of periodontal disease showed a wide variation in the magnitude of the inflammatory response suggesting a high risk subgroup of periodontitis with rapid progression. Factors such as smoking, diabetes, psychological stress, reduced serum antibodies, or biochemical mediators of inflammation may also contribute to disease progression. Hence, the severity and progression

of diseases have been linked to a combination of genetic, host response, microbial challenge, and the local environmental factors.

Biomarkers of disease have gained considerable attention during the past decade. These markers generally fall into three categories:

1. Indicators of current disease activity;
2. Predictors of future disease progression;
3. Predictors of future disease initiation at currently healthy sites.

The potential biomarkers in the GCF have been grouped into three general categories (Table 1):

- Host-derived enzymes
- Inflammatory mediators and products
- Tissue-breakdown products.

Since the GCF accumulates at the gingival margin, it contains potential markers derived not only from the host tissues and serum, but also from the subgingival microbial plaque. The changes in the GCF constituents could be used as a potential marker in the periodontitis progression.

Table 1: Host-derived enzymes in GCF.

Host-derived enzymes	References
MMP-1 MMP-2 MMP-3 MMP-8 MMP-9 MMP-13	Chen *et al.*;[7] Tervahartiala *et al.*;[8] Kiili *et al.*;[9] Kinane *et al.*;[10] Mäntylä *et al.*;[11] Emingil *et al.*;[12] Emingil *et al.*;[13] Hernandez *et al.*;[14] Ilgenli *et al.*;[15] Hernández *et al.*;[16] Maeso *et al.*;[17] Alfant *et al.*;[18] Pirhan *et al.*;[19] Sorsa *et al.*;[20] Tüter *et al.*;[21] Kushlinskii *et al.*;[22] Konopka *et al.*;[23] Khongkhunthian *et al.*[24]
TIMP-1	Alpagot *et al.*;[25] Tüter *et al.*;[26] Choi *et al.*;[27] Hernández *et al.*;[16] Biyikoglu *et al.*;[28] Kardesler *et al.*;[29] Marcaccini *et al.*[30]
Elastase	Chen *et al.*;[7] Jin *et al.*;[31] Yamalik *et al.*;[32] Alpagot *et al.*;[33] Buchmann *et al.*;[34] Jin *et al.*;[35] Jin *et al.*;[36] Cox *et al.*[37]
Cathepsin G, D, B	Soell *et al.*;[38] Cox *et al.*;[37] Mogi and Otogoto;[39] Garg *et al.*[40]
Beta-N-acetyl-hexosaminidase	Buchmann *et al.*[34,41]
α1-proteinase inhibitor	Nakamura-Minami *et al.*[42]
α2-macroglobulin	Knöfler *et al.*[43]
AST	Shimada *et al.*[44]
Gingipain	Guentsch *et al.*[45]
Plasminogen	Yin *et al.*[46]
Glycosidases	Söder *et al.*[47]
Myeloperoxidases	Buchmann *et al.*[34,41]
Creatinine kinase	Nomura *et al.*[48]
Neutral protease	Bader and Boyd.[49]
Dipeptidyl peptidases, ALP, BG, stromyelysins, lactate dehydrogenase Arylsulfatase, lysozyme, dipeptidylpeptidase, creatine kinase Immunoglobulin-degrading enzymes BG, trypsin-like enzymes	Lamster and Ahlo;[50] Buduneli and Kinane[2]

GCF: Gingival crevicular fluid, MMP: Matrix metalloproteinase, TIMP: Tissue inhibitor of matrix metalloproteinase-1, BG: Beta-glucuronidase, ALP: Alkaline phosphatase, AST: Aspartate aminotransferase

The objective of the current review is to critically analyze the current understanding of the various constituents in the GCF that are used as biomarkers and the future developments that could help to establish noninvasive diagnostic aid in this area.

Materials and Methods

The review was conducted in January 2014. All relevant studies published between, January 1999 to January 2014 were identified and included in the article. Two authors independently searched the Medline, EMBASE, Cochrane Library, Web of Science, Google Scholar, and Scopus databases for relevant studies. The search was carried out using a combined text and the MeSH search strategies: Using the key words; "periodontitis" and "GCF," "biomarkers" and "early detection of periodontitis." We also examined the bibliographies from identified studies, reviews, and gray literature. The last search was conducted on January 31, 2014. Studies reporting the use of any biomarkers in the GCF for diagnostic or prognostic aspect of periodontitis is included and summarized in the article. The scope of using these biomarkers as chair side test is critically analyzed.

Discussion

Host-derived enzymes in GCF

Enzymes, especially proteinases, play a central part in the control of periodontal tissue turnover in health and tissue destruction in periodontitis.

Aspartate aminotransferase (AST)

AST is a soluble enzyme that is released to the extracellular environment upon cell death.[51] The level of AST is elevated at sites with active periodontitis.[52] Clinical attachment loss and inflammation have shown a marked elevation of AST in GCF.[53]

Alkaline phosphatase (ALP)

ALP is a membrane-bound glycoprotein produced by many cells, such as leukocytes, osteoblasts, macrophages, and fibroblasts. Bacteria present in the sulcus or pocket also produce ALP and contribute to ALP levels in GCF. ALP in GCF has been suggested as a potential diagnostic marker for periodontitis.[54,55]

Nakashima *et al.*[54] found a positive correlation between crevicular fluid ALP levels from active sites than in the inactive sites with periodontitis. However, ALP levels in GCF as a marker of periodontal disease diagnosis are limited.[4]

Beta-glucuronidase (BG)

BG is one of the enzymes involved in the destruction of noncollagenous components of the extracellular matrix and is considered as an indicator or predictor of periodontal disease activity.[56] BG contributes to noncollagenous matrix degradation in periodontal disease, and its activity might be a good indicator or predictor of periodontal disease.[57,58]

Elastase

Elastase is released from polymorphonuclears (PMNs) to the gingival crevice as a result of host-microbial interactions. It is considered as a risk factor for the development of periodontitis. Elevated levels of GCF elastase enzyme have been reported in periodontitis.[33]

Cathepsin B

Cathepsin B is a cysteine proteinases enzyme and in GCF, it originates specifically from macrophages. The level of cathepsin B in the GCF was found to be elevated in patients with periodontitis.[59] It has shown a direct correlation to the severity of periodontitis.[60] The level of cathepsin in the GCF can be used as an indicator of the attachment loss and also as a prognostic indicator in periodontal disease.[61]

Matrix metalloproteinases (MMPs)

MMPs are a family of enzymes that are responsible for the degradation of extracellular matrix components such as collagen, proteoglycans, laminin, elastin, and fibronectin. They play a central role in the periodontal ligament (PDL) remodeling, both in physiological and pathological conditions. MMP-8, in conjunction with MMP-9 and functional granulocyte elastase, is involved in tissue destruction in subjects with periodontal disease.[47] The bacterial plaque induces the initial infiltrate of inflammatory cells in the gingival crevice including macrophages and lymphocytes.

These activated inflammatory cells produce inflammatory mediators which stimulate the production of MMPs from fibroblasts, epithelial cells, and PMNs.

Leptin

Leptin is involved in the host response, which stimulates the immune system by enhancing pro inflammatory cytokine production and phagocytosis by macrophages. Hence, during infection and inflammation, leptin expression is modulated in a manner similar to the cytokine response to infection and injury.[62] During gingival inflammation, the concentration of leptin is decreased as a result of the expansion of the vascular network caused by vascular endothelial growth factor, which may increase the net rate of leptin removal from the gingival tissues.[63] Studies have shown that the leptin levels in GCF decreased with the progression of periodontal disease and could be used as an indicator.[64,65]

Hepatocyte growth factor (HGF)

HGF is known to be a multi-functional cytokine involved in a variety of physiological processes, including tissue development, regeneration, and wound healing.[66] HGF plays an important role in the progression of periodontitis, by stimulating intense growth of epithelial cells and preventing regeneration of the connective tissue attachment. HGF is well-known as a serum marker indicating disease activity in various diseases, including periodontitis.[67] An association between HGF and periodontitis has been reported earlier.[68,69] A higher level of HGF in GCF was expressed at periodontally compromised sites.[70,71]

Inflammatory mediators and host response modifiers (Table 2)

Cytokines

Cytokines are important modulators of both normal and pathologic processes within the periodontium. The analysis of cytokine production levels has been also used as a tool for studying the local host response to bacterial challenge.

Cytokines present in the GCF have been proposed as potentially useful diagnostic or prognostic markers of periodontal destruction. Of these, interleukin-1b (IL-1b), IL-4, and IL-8 have been shown to function in concert with

Table 2: Inflammatory mediators and host response modifiers.

Inflammatory mediators and host response modifiers	References
Prostaglandin E2	Preshaw and Heasman;[72] Söder *et al.*;[47] Yalçn *et al.*;[73] Biyikoglu *et al.*;[74] Mizrak *et al.*;[75] Kurtis *et al.*;[76] Zhong *et al.*;[77] Buduneli *et al.*[78]
PA	Yin *et al.*;[46] Kinnby;[79] Olofsson *et al.*;[80] Ullbro *et al.*;[81] Buduneli *et al.*;[82] Biyikoglu *et al.*;[74] Buduneli *et al.*;[83] Kardesler *et al.*;[84] Tüter *et al.*[85]
Platelet-activating factor	Emingil *et al.*;[86] Keles *et al.*;[87] Zheng *et al.*;[88] Chen *et al.*[89]
SP	Hanioka *et al.*;[90] Lundy *et al.*;[91] Pradeep *et al.*;[92] Ozturk *et al.* [93]
PAI-2	Ullbro *et al.*;[81] Biyikoglu *et al.*;[74] Kardesler *et al.*;[84] Tüter *et al.*[85]
Calgranulin A (MRP-8)	Kojima *et al.*;[94] Lundy *et al.*;[95,96] Andersen *et al.* [97]
Neopterin	Ozmeriç *et al.*;[98] Pradeep *et al.*[99]
Vasoactive intestinal peptide	Linden *et al.*[100]
Neurokinin A	Lundy *et al.*[91]
CD14	Jin and Darveau.[101]
Cystatins	Sharma *et al.*[102]
TNF-α, interferon-α	Erdemir *et al.*;[103] Schierano *et al.*;[104] Ulker *et al.*;[105] Bastos *et al.*[106]
MCP-1	Anil *et al.*;[107] Gupta *et al.*;[108] Kumari *et al.*[109]
Antibacterial antibodies: IgG1, IgG2, IgG3, IgG4, IgM, IgA	Grbic *et al.*;[110] Stefanovic *et al.*;[111] Brajovic *et al.*;[112] Guentsch *et al.* [113]
RANTES (chemoattractant and activator of macrophages and lymphocytes)	Gamonal *et al.*;[114] Gamonal *et al.* 2001;[115] Emingil *et al.*[116]
Leukotriene B4	Emingil *et al.*;[117] Pradeep *et al.*[99]
Acute-phase proteins: Lactoferrin, transferrin, α2-macroglobulin, α1-proteinase inhibitor, C-reactive protein	Tüter *et al.*;[118] Pradeep *et al.*;[119] Fujita *et al.*;[120] Kumar *et al.*;[121] Keles *et al.*;[122] Kinney *et al.*;[123] Kumari *et al.*[109]
Cytokines: IL-1α, IL-1β, IL-1ra, IL-2, IL-6, IL-8	de Campos *et al.*;[124] Shaker and Ghallab; [125] Darabi *et al.*;[126] Fu *et al.*;[127] Lagdive *et al.*;[128] Shimada *et al.*;[129] Shivaprasad and Pradeep;[130] Keles *et al.*[122]

IL: Interleukin, MCP-1: Monocyte chemoattractant protein-1, TNF: Tumor necrosis factor-α, PAI-2: Plasminogen activator inhibitor-2, PA: Plasminogen activator, SP: Substance P

other members of the cytokine network to regulate the cellular inflammatory response in the periodontium.

Substance P (SP)

SP is localized in sensory nerves that innervate blood vessels. SP is a member of the tachykinin family of these neuropeptides, and it is stored in the secretory granules of sensory neurons and their peripheral branches. Release of SP in human gingiva was related to periodontal inflammation. The SP level in GCF is correlated with the degree of periodontal inflammation, and it has been shown that a reduction in inflammation as a result of effective periodontal treatment is associated with a reduction in the levels of undecapeptide SP in GCF.[90-92]

Tumor necrosis factor-α (TNF-α)

TNF-α is a proinflammatory cytokine that is often over expressed in periodontitis and is responsible for alveolar bone resorption during periodontitis.[131]

Monocyte chemo-attractant protein (MCP)

MCP-1, a potent mediator of both monocyte recruitment and activation. MCP-1 is expressed by monocytes, endothelial cells, fibroblasts, and T-cells, primarily on the basal layer of epithelial tissues. MCP-1 is related to the stages of oral infection by means of its monocyte chemotactic ability, which has been known to increase with increasing inflammation. High levels of MCP-1 have been reported in the GCF of both aggressive and chronic periodontitis patients.[107,116,132]

The MCP activity in GCF increases with the advancement of periodontitis and could be involved in the mechanism of monocyte recruitment from the circulating pool into periodontal tissues.

Tissue breakdown products (Table 3)

Laminin

In the oral cavity, laminin is expressed mainly in epithelial cells which stimulates the migration of epithelial cells and, in the formation of periodontal pockets in the progression of periodontitis, is believed to be one of the key factors in the apical migration of epithelial cells.[133] PDL fibroblasts demonstrate enhanced expression of some other laminin isoforms.[134] Several laminin isoforms are involved in the procession of periodontal inflammatory diseases and tissue remodeling.[135]

Osteopontin (OPN)

OPN is a major glycosylated phosphoprotein in bone matrix and is produced by several cells including osteoblasts, osteoclasts, and macrophages. Studies have reported an increased level of GCF OPN in periodontitis and its reduction after periodontal therapy.[138-140]

Table 3: Tissue breakdown products.

Tissue breakdown products	References
Laminin	Figueredo and Gustafsson; [135] Kivelä-Rajamäki *et al.*;[136] Emingil *et al.*;[137] Emingil *et al.* [12]
Osteopontin	Kido *et al.*;[138] Sharma and Pradeep[139,140]
Osteocalcin	Bullon *et al.*;[141] Bullon *et al.*;[142] Becerik *et al.* [143]
Calprotectin	Nakamura *et al.*;[144] Kaner *et al.*;[145] Becerik *et al.*;[143] Kaner *et al.* [146]
Fibronectin fragments	Huynh *et al.*;[147] Brajovic *et al.*;[112] Feghali and Grenier[148]
Hemoglobin β-chain peptides	Ngo *et al.*;[149] Kido *et al.* [150]
Chondroitin 4-sulfate	Khongkhunthian *et al.* [151]
Chondroitin 6-sulfate	Khongkhunthian *et al.* [151]
Pyridinoline crosslinks (ICTP)	Al-Shammari *et al.*;[152] Jepsen *et al.* [153]
GAG's	Yan *et al.* [154]
Osteonectin, hyaluronic acid, hydroxyproline	Lamster and Ahlo; [50] Buduneli and Kinane[2]

GAC's: Glycosaminoglycans

Osteocalcin (OC)

OC is a noncollagenous matrix protein of calcifying and calcified tissue.[155] It is produced by osteoblasts and has been described as the most specific marker of osteoblast function.[156] Structurally, it binds to both major bone components (collagen and apatite) and is believed to play a role in both bone resorption and mineralization.

OC has been found in GCF from patients with periodontal disease, and increases in GCF OC concentration were associated with high rates of bone turnover.

Raised levels of GCF OC are reported in adult periodontitis may be related to the severity of breakdown and/or repair of alveolar bone. During active bone resorption, OC and OC fragments are likely to be released from the extracellular matrix into the GCF. Nakashima *et al.*[54] reported the mean concentration of OC in GCF was ten-fold than that found in serum and speculated that OC was produced locally by periodontal tissues. Several investigations on OC levels in GCF from patients with periodontitis have been reported, suggesting that OC levels in GCF may reflect inflammation at diseased sites and there has been recent interest in OC as a potential marker of bone turnover in periodontal disease.[141,142]

Conclusion

GCF, which is an exudate that can be harvested from the sulcus or periodontal pocket, has been regarded as a promising medium for the detection of periodontal disease activity. The composition of this fluid resembles that of serum, and the intensity of its flow has been shown to vary as a function of gingival inflammation. GCF has been used as a medium to measure a variety of molecules and bacteria present in both the oral cavity and the PDL space. GCF contains a variety of substances including immunoglobulins, microorganisms, toxins, cells, and lysosomal enzymes and markers the immune and inflammatory reactions arising from periodontitis.

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