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Biomarkers in Gingival Crevicular Fluid

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Abstract: *Conventional clinical and radiographical methods of periodontal diagnosis are only capable of retrospective diagnosis of attachment and bone loss. These are unable to either detect or predict periodontal disease activity (PDA). For these reasons a large proportion of recent periodontal research has been concerned with finding and testing potential markers of PDA. The existence of gingival crevice fluid (GCF), which emerges between the surface of the tooth and the epithelial integument, has been recognized for over 100years. Since it can be easily collected and contains locally and systemically derived markers of periodontal disease, they may offer the basis for a patient-specific biomarker assessment for periodontitis and other systemic diseases. This article reviews the various biomarkers in GCF used for assessing periodontal disease activity.*

Keywords: *Biomarkers, Exudate, GCF.*

I. Introduction

A biomarker is defined as a "Parameter that is objectively measured and evaluated as an indicator of normal biological or pathological processes, or pharmacological responses to a therapeutic intervention" (NIH 1998).

Gingival crevice fluid (GCF) is an inflammatory exudate that seeps into gingival crevices or periodontal pockets around teeth with inflamed gingiva [1]. The diagnostic potential of GCF has been studied over 50 years. In 1960, it was first suggested that analysis of GCF might be a way to quantitatively evaluate the inflammatory status of gingival and periodontal tissues [2].

Presence and function of proteins especially enzymes in GCF were first explored by Seleda, Bang and Cimasoni [3]. It was soon understood that enzymes released from damaged periodontal tissue possessed an enormous potential for periodontal diagnosis. Ohlsson [4], Golub [5] & Uitto [6] discovered that collagenase and elastase in GCF are derived primarily from human cells most notably neutrophils and that their activity is correlated with gingival inflammation and gingival pocket depth.

GCF contains a rich array of cellular and biochemical factors which have been shown to indicate the metabolic status of various tissue components of the periodontium (Fig 1). Such factors are now finding value as potential diagnostic or prognostic markers of the periodontium in health and disease [7]. The identification of high-risk and low-risk patient groups in relationship to current disease activity and progression and the initiation of preventive measures are at the heart of much current study. Over 65 GCF components have been preliminarily examined as possible markers for the progression of periodontitis [2] (Table- 1, 2, 3). These components fall into three general categories:

1.1 Host-derived enzymes and their inhibitors
1.2 Inflammatory mediators and host-response modifiers
1.3 Tissue breakdown products

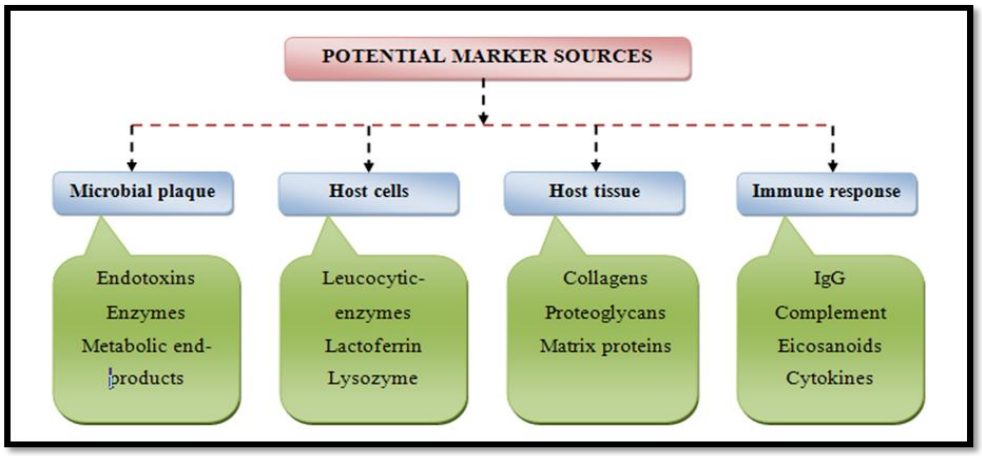

Fig 1: Potential marker sources [7]

HOST DERIVED ENZYMES AND THEIR INHIBITORS IN GCF [2]	
Aspartate aminotransferase	Glucosidase
Alkaline phosphatase	Dipeptidylpeptidase
Acid phosphate	Nonspecific neutral proteinases
ß- Glucoronidase	Collagenases Matrix metalloproteinase-1 (MMP-1) Matrix metalloproteinase-3 (MMP-3) Matrix metalloproteinase-8 (MMP-8) Matrix metalloproteinase-13 (MMP-13)
Elastase	Gelatinases Matrix metalloproteinase-2 (MMP-2) Matrix metalloproteinase- 9 (MMP-9)
Elastase inhibitors α2 -macroglobulin α1- proteinase inhibitor	Tissue inhibitor of MMP-1 (TIMP-1)
Cathepsins Cysteine proteinases Serine proteinase Cathepsin D	Stromyelysins & Myeloperoxidases
Trypsin like enzymes	Lactate dehydrogenases & ß-N-acetyl-hexosaminidase
Immunoglobulin- degrading enzymes	Acrylsulfatases & Creatinine kinase

Table –1: Host derived enzymes and their inhibitors in GCF

INFLAMMATORY MEDIATORS AND HOST RESPONSE MODIFIERS IN GCF [2]	
CYTOKINES IN-1α, IL-1ß, IL-1ra, IL-2, IL-6, IL-8, Tumor necrosis factor α Interferon α	Substance P
RANTES (chemoattractant & activator of macrophages and lymphocytes)	Vasoactive intestinal peptide
PROSTAGLANDIN E2	Neurokinin A
LEUCOTRIENE B4	Neopterin
ACUTE PHASE REACTANTS Lactoferrin, transferring, α2- macroglobulin, α1- proteinase inhibitor, C- reactive protein	Platelet-activating factor
Auto antibodies Anti-desmosomal antibodies	CD-14
Antibacterial antibodies IgG1, IgG2, IgG3, IgG4 IgM, IgA	Cystatins
Plasminogen activator (PA)	Calgranulin A (MRP-8)

Table- 2: Inflammatory mediators and host response modifiers in GCF

TISSUE BREAKDOWN IN GCF [2]	
Glycosaminoglycan Hyaluronic acid Chondroitin-4-sulfate Chondroitin-6-sulfate Dermatan sulfate	Laminin
Hydroxyproline	Calprotectin
Fibronectin fragments	Hemoglobin ß- chain peptides
Connective tissue and bone proteins Osteonectin, Osteocalcin, Type 1 collagen peptides, Osteopontin	Pyridinoline crosslinks

Table -3: Tissue breakdown in GCF

The advantage of collecting GCF as a biomarker is that it is noninvasive, site-specific about teeth, comparatively easy to perform, and offers one of the most accessible entrees of any tissue in the body as a means of assessing the disease state.

II. Biomarkers from microbial plaque

Presence of lipopolysaccharides (endotoxins) has been positively correlated with gingival inflammation [8]. Patients with localized aggressive periodontitis have increased antibody levels to the lipopolysaccharide of *Actinobacillus actinomycetemcomitans* and interleukin-1 inhibitor. The bacterial proteinases are usually of the serine endopeptidase type and have formed the basis of diagnostic L-BANA test. Similar trypsin-like enzymes are also associated with *Treponema denticola*.

The metabolic end products of carbohydrates, lipids, and proteins which include H_2S, butyrate and proprionate show a positive correlation exists among the degree of inflammation, GCF volume and H_2S generating potential of GCF fluid.

III. Biomarkers from host cells

Alkaline phosphatase correlates with increasing inflammation in an experimental gingivitis model. In serum, the enzyme is associated with bone disease, and its elevation in GCF could well reflect changes of alveolar bone in localized area [7].

Extensive studies reviewed by Lamster *et al.* (1991) [9] on glycogen-degrading enzymes like β-glucuronidase have indicated a significant increase above baseline values some two to four weeks following initiation of inflammation.

Wide range of proteolytic enzymes has been detected in GCF; many of host cell origin. Ishikawa *et al.* (1972) [10] demonstrated 10-fold increase in cathepsin D levels in GCF by comparison with serum, an observation correlated with pocket depth. Also elastase has demonstrated a significant promise as a marker of periodontal disease. Lactoferrin showed better correlation with clinical indices than PMNs and was found to be increased twofold in GCF in sites showing gingivitis, periodontitis, and localized aggressive periodontitis.

Glycosaminoglycan concentrations were found to be elevated in aggressive periodontal diseases, and associations have been made with periodontal pathogens such as *P. gingivalis.*

IV. Biomarkers from host tissue damage host tissue damage

Collagen represents the most important structural protein of periodontium. Collagen is usually measured by the hydroxyproline assay. Hara and Takahashi (1975) [11] assayed the hydroxyproline levels of serum and GCF in patients before, one month after, and six months after periodontal surgery and found the levels to be significantly lower in both serum and GCF after six months.

Collagenase-3 (MMP-13) rather than collagenase-2 (MMP-8) secreted by neutrophils, is a significant enzyme because it appears to be released from fibroblasts and pocket epithelial cells during tissue destruction. Gelatinases are prevalent enzymes in periodontitis and they are released from fibroblasts (MMP-2), epithelial cells (MMP-9 and MMP-2) and neutrophils (MMP-9).

Proteoglycans have the ability to bind most collagens as well as fibronectin. Upon degradation of periodontal tissues, glycosaminoglycans are released, making their way into the GCF. Chondroitin-4-sulfate appears to be the major glycosaminoglycan in untreated chronic periodontitis sites, as shown in both animal [12,13] and human [14,15] studies.

V. Host factors: immune response and associated inflammatory mediators

The work of Reinhardt *et al.* (1989) [16] showed higher levels of IgG, IgG1 and IgG4 in GCF from active sites by comparison with stable or healthy sites. The presence of IL-1 in GCF has been shown by Charon *et al.* (1982) [17], to be higher in inflamed sites than in normal sites. Reports have been noted of raised levels of TNF-α in GCF from diseased sites and of an order similar to IL-1β.

Prostaglandin E_2 has been found in elevated quantities in GCF from patients with periodontitis by comparison with gingivitis patients only. PGE_2 levels were also higher in aggressive periodontitis patients by comparison with those in adult periodontitis patients.

A review by Offenbacher *et al.* (1991) indicated the diagnostic potential of PGE2 in GCF. They found that the GCF levels in humans, dogs, and monkeys increased 2- and 3- fold in inflammation and 5- to 6-fold during periods of active attachment loss and bone resorption. In addition, patients with high PGE2 levels are at high risk of attachment loss. He reported higher levels of LTB_4 in GCF obtained from clinical sites associated with adult periodontitis, alveolar bone loss, and aggressive periodontitis.

VI. Proteolysis host cell enzymes and their inhibitors in GCF

MMP-8 and MMP-9 are the main collagen-degrading enzymes in GCF and they are believed to be mainly responsible for collagen degradation in inflamed tissue during gingivitis and adult periodontitis. MMP-8 and MMP-9 are considered as good indicators for periodontal inflammation.

Cathepsin B levels are reduced after periodontal therapy. Both before and after therapy total amounts of cathepsin B in gingival crevice fluid correlate significantly with clinical parameters making cathepsin B a promising factor and potential chair-side diagnostic marker.

VII. Matrix molecules and growth factors as indicators of periodontal disease

Collagen degradation products have emerged as valuable markers of bone turnover in a multitude of osteolytic and osseous metabolic diseases (Tables 4 & 5)).

BONE FORMATION MARKERS [18]
1) Type I protocollagen propeptide proliferation a) C- terminal propeptide fragment (PICP) b) N- terminal propeptide fragment (PINP)
2) Alkaline phosphatase matrix formation a) Total alkaline phosphatase (Al-p) b) Bone alkaline phosphatase (BAL-p)
3) Osteocalcin, bone Gla proteinmineralization (BGP) a) C-terminal fragment b) Mid portion c) Intact

Table-4 Bone formation markers

BONE RESORPTION MARKERS [18]
1) Pyridinium cross-link a) Urine pyridinoline (PYP), deoxypyridinoline (DPD), HPLC method b) Urine free deoxypyridinoline
2) Pyridinium cross-linked collagen peptide fragment a) Serum C- terminal telopeptide (ICTP) b) Urine C-terminal telopeptide (CTx, crosslaps) c) Urine N-terminal telopeptide (NTx, osteomark)
3) Tartrate- resistant acid phosphatase (TRAP)
4) Galactosyl hydroxylysine (GHYL)
5) Hydroxyproline
6) N-terminal osteocalcin fragment
7) Glycosaminoglycans (GAG`s)

Table –5 Bone resorption markers

Pyridinium cross-link collagen peptide fragment (ICTP) has been shown by various studies to be a good predictor of future alveolar bone and attachment loss. A longitudinal study using an experimental periodontitis model in beagle dogs reported strong correlation between GCF osteocalcin levels and active bone turnover [19].

Diagnostic tools have also been applied to evaluate the response to active periodontal therapy. Golub *et al.* (1997) [20] found that treatment of 18 chronic periodontitis patients with scaling and root planing and matrix metalloproteinase inhibitor (sub-antimicrobial doxycycline hyclate) resulted in a 70% reduction in GCF ICTP levels after 1month, concomitant with a 30% reduction in collagenase levels. Rapid reductions in GCF ICTP levels in periodontitis subjects after scaling root planing and local delivery of minocycline were also found.

The future suggests that bone-specific biomarkers in GCF will become sensitive enough to give accurate and predictable diagnoses, thus enabling practitioners to serve their periodontal patients better.

Furthermore, the elucidation of biomarkers of active periodontitis will greatly add to our understanding of the molecular mechanisms of the progression of periodontal diseases to give 'real-time' measures of bone metabolism.

VIII. Futuristic chairside diagnostic test based on GCF sampling:

Considering the GCF fluid as a potential analyte for the screening of multiple biomarkers, a rapid, chairside diagnostic tool or a ''mini-lab'' could be used by clinicians for risk assessment and decision making on treatment planning.

The advantages of such a tool would be enhanced predictability of clinical outcomes and well-informed patients regarding personalized treatment needs. A simple clinical procedure for GCF collection could be used, followed by extraction of analytes from the test strip. The fluid present on the test strip would be subjected to volumetric quantification.

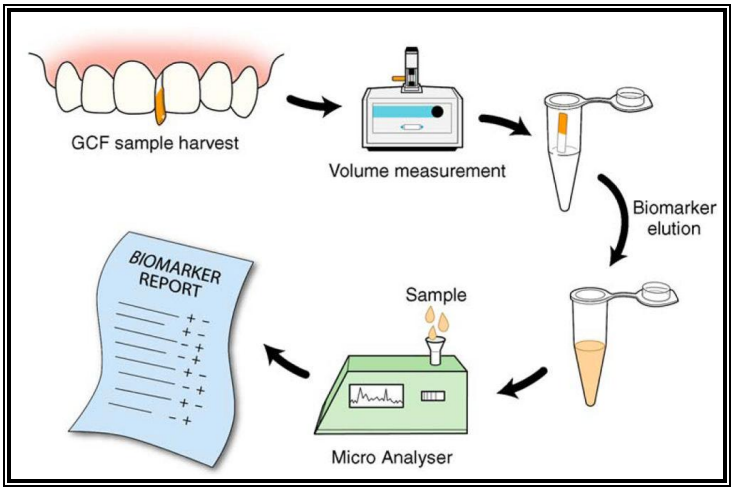

Fig- 2: Micro-analyser or Mini-lab

There is a plethora of possibilities for the future use of oral fluids in biotechnology and health care applications, especially in the field of diagnostics. A tremendous amount of research activity is currently under way to explore the role of oral fluids as a possible medium in a variety of applications. Recent advances in HIV diagnosis have been made using oral fluids.

IX. Conclusion

The search for markers of periodontal disease activity will progress with the refinement and application of specific detection techniques for selected factors. GCF will continue to be a vehicle for monitoring tissue and cell products and allows a degree of non-invasive access to the periodontium, unlike the majority of other tissues in the body.

Several products show potential benefit, particularly those directly from specific regions of the periodontium which give a clue as to which tissue components are at risk. It is clear that no single marker will fulfill all the criteria necessary for assessment of the clinical state of the periodontium, and future research should be directed at the production of "marker packages".

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