



“GINGIVAL CREVICULAR FLUID AS A BIOMARKER OF PERIODONTAL DISEASE”- A NARRATIVE REVIEW

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ABSTRACT

Periodontitis is one of the most common oral diseases in the world. Periodontitis when diagnosed early have the better prognosis for the individual to save the tooth. Diagnostic markers are useful to indicate the presence of a disease process before excessive clinical damage occurs. One such main source of biomarker is gingival crevicular fluid. This review article deals with Gingival crevicular fluid(GCF) as a source of biomarkers for periodontal disease progression and severity which helps in diagnosis and prognosis in the field of Periodontology and also details about available various chairside diagnostic kits for the biomarkers.

KEYWORDS: Biomarkers, Gingival crevicular Fluid, Periodontal Disease

INTRODUCTION:

Periodontitis is one of the most prevalent oral diseases in the world. Approximately 5–20% of adults worldwide suffer from severe periodontitis which may lead to tooth loss¹. Thus, the complex interaction between the pathogens and the host response, in addition to environmental and genetic factors is considered as the common bacterial infection in the world². Even though disease initiation is brought about by specific bacteria, tissue destruction subsequent to disease progression is caused by an imbalance between the protective and destructive host mechanisms that are triggered with the infection. Further, periodontitis is thought to be a multifactorial disease; risk factors such as uncontrolled systemic diseases (e.g. diabetes), smoking and genetic predisposition can contribute to the progression of periodontal disease³. Currently, most medical fields are searching for useful biological diagnostic markers that can indicate the presence of a disease process before extensive clinical damage has occurred. As far Dentistry is concerned, various fluids in the oral cavity are being considered as a source of diagnostic biomarkers in predicting disease and its progression.

GCF AS A DIAGNOSTIC TOOL:

Early diagnosis and treatment of progressive Periodontitis is important because of the irreversible nature of this disease⁴. One of the goal of periodontal diagnostic procedures is to provide useful information to the clinician regarding the current 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.

There is a need for the 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.

The collection of GCF is a minimally invasive procedure and the analysis of specific constituents in the GCF provides a quantitative biochemical indicator for the evaluation of the local cellular metabolism that reflects a person's periodontal health status. Since GCF is an inflammatory exudate that reflects ongoing events in the periodontal tissues that produce it, an extensive search has been made for GCF components that might serve as potential diagnostic or prognostic markers for the progression of periodontal disease. (Fig 1)

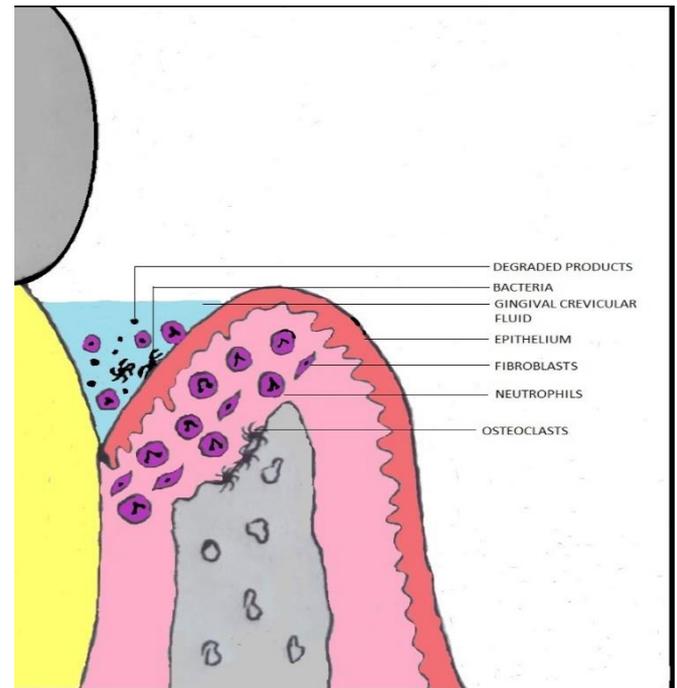


Fig 1: Gingival crevice fluid – a window to periodontal disease. Gingival crevice fluid is composed of substances derived from serum, leukocytes, bacteria, activated epithelial cells, connective tissue cells, and bone cells.

Several techniques have been employed for the collection of GCF and the technique chosen depends upon the objectives of the study, as each technique has its own advantages and disadvantages. The methods of collection may be broadly divided into the intracrevicular and the extra crevicular techniques. The former depends on the strip being inserted into the gingival crevice, whereas in the latter, the strips are overlaid on the gingival crevice region in an attempt to minimize trauma.

BIOMARKERS IN GCF:

HOST-DERIVED ENZYMES AND THEIR INHIBITORS

- **Aspartate aminotransferase**

It is a cytoplasmic enzyme that is released upon cell death and elevated levels of total enzyme activity were found to be strongly associated with active disease sites⁵. Sites with severe gingival inflammation and progressive attachment loss demonstrate marked elevation in AST levels in GCF samples⁶.

- **Alkaline Phosphatase**

It is a membrane-based glycoprotein produced by many cells within the area of the periodontium and gingival crevice. Alkaline phosphatase is thought to play a role in

bone metabolism and mineralization and collagen formation. The activity of alkaline phosphatase has shown to be correlated with pocket depth and the percentage of bone loss⁷, this activity was found to be 20 times greater in GCF from active sites than in serum.

- **Acid phosphatase**

It has been widely investigated amongst the lysosomal enzymes and has often been used as a lysosomal marker. Quantitative analysis confirmed that gingival fluid contains 10-20 times more acid phosphatase than serum. The host sources are the PMNs and desquamating epithelial cells⁸. About 60% of the total acid phosphatase in whole gingival fluid originates from bacteria. The levels of acid phosphatase do not correlate with measurements of disease severity or activity.

- **β -Glucuronidase**

It is one of the hydrolases found in the azurophilic or primary granules of PMNs. The enzyme is liberated from macrophages, fibroblasts and endothelial cells of healthy or chronically inflamed gingival tissues. The level of β -glucuronidase correlates significantly with attachment loss that may subsequently occur in individuals with adult periodontitis⁹.

- **Elastase**

Neutrophil elastase is a serine proteinase confined to the azurophil granules of PMNs which are analogous to lysozymes. It acts upon elastin, proteoglycans, hemoglobin, fibrinogen and collagen. Leukocyte elastase degrades mature collagen fibers. Amounts of GCF elastase are greater in periodontitis patients than healthy controls¹⁰.

- **Macroglobulins (Alpha 2, Beta 2):**

Macroglobulins play a major role in the immune system. They have the ability to inactivate varied microorganisms, thereby protecting oral tissues. Alpha 2M has been associated with tissue destruction whereas Beta 2M plays a role in T and B lymphocytes response. Inflamed tissues release Alpha 2M and Beta 2M to stall inflammation and negate proliferation of micro-organisms.

- **Cathepsins:**

It is an enzyme belonging to the class of cysteine proteinases. In GCF, macrophages are the main producers of cathepsin B¹¹. GCF concentrations of cathepsin B were found to be elevated in patients with periodontal disease, but

lower in patients with gingivitis¹². Cathepsin D, a carboxy endopeptidase, is present at high concentration in inflamed tissues. Its concentration is found to be 10 times higher in GCF during periodontal destruction. Cathepsin G is serine endopeptidase contained in the azurophil granules of PMNs. It hydrolyzes hemoglobin and fibrinogen, casein, collagen and proteoglycans. Measurements of cathepsin and neutral proteases, have also shown a relationship to the severity of inflammation but no association with disease activity has been demonstrated.

- **Dipeptidyl Peptidases (DPP)**

They are derived from lymphocytes, macrophages, and fibroblasts. DPP II has been localised to macrophages and fibroblasts in gingival tissue and in cells in GCF¹³. DPP Eley and Cox, monitored GCF levels of DPP II and IV and reported higher levels of both enzymes in sites with rapid and gradual attachment loss than in paired sites without attachment loss¹⁴.

- **Collagenase-2 (MMP-8)**

MMP-8 is also called collagenase-2. It is predominant collagen in GCF. Increased levels of MMP-8 in GCF is associated with severity of periodontitis. It is released from PMNs during maturation. Increased levels of MMP-8 are signify conversion of gingivitis into periodontitis. No associations are found between MMP-8 levels and a bone loss. It was found that 18-fold increase of MMP-8 in patients experiencing active periodontal tissue breakdown as compared with patients under stable condition¹⁵.

- **Gelatinase (MMP-9)**

Gelatinase (MMP-9), another member of the collagenase family, is produced by neutrophils and degrades collagen extracellular ground substance. There is a twofold increase in mean MMP-9 levels is reported in patients with recurrent attachment loss.

- **Collagenase-3 (MMP-13)**

Collagenase-3, referred to as MMP-13, is another collagenolytic MMP with exceptionally wide substrate specificity. The expression of MMP13 is specifically induced in undifferentiated epithelial cells during chronic inflammation due to exposure to cytokines and collagen. MMP-13 has also been implicated in peri-implantitis. Elevated levels of both MMP-13 and MMP-8 are correlate with irreversible peri-implant vertical bone loss around loosening dental implants¹⁶.

- **Tissue inhibitors of matrix metalloproteinases (TIMPs)**

They are locally produced and their main role is defending connective tissues in the very local area around the cell from which metalloproteinases are secreted. The tissue degradation is further thought to be induced by an imbalance between MMPs and TIMP¹⁷. The mean amounts of SL and TIMP in diseased sites (gingivitis and periodontitis) is significantly higher than the mean amount of these GCF components in healthy sites¹⁸.

TISSUE BREAKDOWN PRODUCTS

- **Glycosaminoglycans**

Proteoglycans have a core protein on which one or more heteropolysaccharides (called glycosaminoglycans) are bound covalently. In general, chondroitin-4-sulfate is the most common glycosaminoglycan in the periodontium. Chondroitin-4-sulfate appears to be the major glycosaminoglycan in untreated chronic periodontitis sites, as shown in both animal and human studies. Elevated glycosaminoglycan concentrations were also found in aggressive periodontitis, and associations have been made with periodontal pathogens such as *P. gingivalis*¹⁹. It is elevated in peri implantitis patient.

- **Hydroxyproline**

It is a characteristic amino acid and is a major component of the collagen. Hydroxyproline and proline play key role for maintaining collagen stability. They permit the sharp twisting of the collagen helix. Thus it is a major breakdown product of collagen present in the GCF²⁰.

- **Fibronectin fragments**

It is one of the components of the extracellular matrix (ECM) of periodontal tissue²¹ its main role is in cell adhesion and proliferation, which explains its potential use in regenerative strategies. The presence in GCF would indicate fibronectin fragmentation due to tissue destruction and not simply inflammation²².

CONNECTIVE TISSUE AND BONE PROTEINS

- **Osteonectin**

Also referred to as secreted protein acidic and rich in cysteine and basement membrane protein (BM-40), osteonectin is a single-chain polypeptide that binds strongly to hydroxyapatite and other extracellular matrix proteins including collagens. Because of its affinity for collagen and hydroxylapatite, osteonectin has been implicated in the

early phases of tissue mineralization²³.

- **Osteocalcin**

It is a small calcium-binding protein of bone and is the most abundant non collagenous protein of mineralized tissues²⁴. Osteocalcin is predominantly synthesized by osteoblasts and it has an important role in both bone resorption and mineralization²⁵. Elevated serum osteocalcin levels have been shown in periods of rapid bone turnover. Serum osteocalcin is presently considered a valid marker of bone turnover when resorption and formation are coupled, and a specific marker of bone formation when formation and resorption are uncoupled.

- **Type I collagen peptides:**

The most common extracellular matrix component is collagen, which is synthesized in a pro-form containing a terminal propeptide. Collagen I carboxy-terminal propeptide and collagen III amino-terminal propeptide were detectable in the GCF of patients with periodontitis, but not in healthy subjects, suggesting that turnover is higher in inflamed sites. The GCF levels of these collagens are increased after nonsurgical periodontal treatment and return to baseline levels after a few days.

- **Receptor activated nuclear factor-kappa B ligand (RANKL):**

Receptor activated nuclear factor-kappa B ligand (RANKL) was another frequently reported biomarker that was reported in six studies. These studies, except (Inanc et al., 2014)²⁶ reported an increased concentration of RANKL in GCF of patients with periodontitis versus that of control patients.

- **Osteopontin (OPN):**

It is a single-chain polypeptide. In bone matrix, OPN is highly concentrated at sites where osteoclasts are attached to the underlying mineral surface, that is, the clear zone attachment areas of the plasma membrane. In 2006, Sharma et al published findings from an investigation of GCF OPN that its concentrations increased proportionally with the progression of disease and when nonsurgical periodontal treatment was provided, its levels were significantly reduced.

- **Laminin:**

It is a 900-kDa glycoprotein found in all basement membranes. During gingival inflammation, neutrophils

leave the blood vessels and migrate through the connective tissue towards the inflammatory lesion, and some of them invade the gingival crevice.

Higher amounts of laminin in GCF from patients with periodontitis suggest the presence of hyperactive neutrophils during the transmigration process through the endothelium/epithelium²⁷.

- **Calprotectin**

It is a 36-kDa protein composed of a dimeric complex of 8- and 14-kDa subunits. It also plays a role in immune regulation through its ability to inhibit immunoglobulin production and acts as a proinflammatory protein for neutrophil recruitment and activation.

The expression of calprotectin from inflammatory cells appears to offer protection of the epithelial cells against binding and invasion by *P. gingivalis*. In periodontal disease, it appears to improve resistance to *P. gingivalis* by boosting the barrier protection and innate immune functions of the gingival epithelium²⁸.

- **Hemoglobin β -chain peptides**

Two peptides derived from the hemoglobin (Hb) are β -chain decapeptide and a dodecapeptide. They are pharmacologically and physiologically active, and act as inflammatory mediators²⁹. Both peptides may also act as substrates of proline-specific peptidases studied in treponemes isolated from the human subgingival dental plaque. These two particular Hb β -chain sequences were present in GCF and successful periodontal therapy will reduce the levels of these peptides³⁰.

- **Pyridinoline crosslinks (ICTP)**

They represent a class of collagen-degrading molecules that include pyridinoline, deoxypyridinoline, N-telopeptides, and C-telopeptides³¹. Subsequent to osteoclastic bone resorption and collagen matrix degradation these molecules are released into the circulation. Given their specificity for bone resorption, pyridinoline cross-links represent a potentially valuable diagnostic aid in periodontics, because biochemical markers specific for bone degradation may be useful in differentiating the presence of gingival inflammation from active periodontal and peri-implant bone destruction³².

INFLAMMATORY MEDIATORS AND HOST RESPONSE MODIFIERS:

- **Interleukin 1 β :**

Studies reported a presence of IL-1 β in diseased sites, low levels of IL-1 β in healthy peri-implant sites,

significantly high levels of IL-1 β in periodontal disease patients as compared to controls and a decrease in the level of IL-1 β with an improvement in clinical parameters following treatment.

- **Prostaglandin E2:**

Arachidonic acid metabolites like PGE2 play a key role in the progression of periodontal destruction. PGE2, a metabolite of cyclooxygenase pathway, is considered to induce fibroblasts and osteoblasts activity for the synthesis of MMPs, IL-1 β and other cytokines. PGE2 levels are enhanced and have been correlated with the severity of periodontal disease.

PERI IMPLANT CREVICULAR FLUID (PICF) IN PERI IMPLANTITIS:

- **Bone Markers: (RANK, RANKL)**

Soluble RANKL and OPG levels were evaluated in 84 samples of PICF from implants showing different peri-implant tissue clinical conditions without demonstrating any correlation between these levels and the studied clinical outcomes³³.

However, one study demonstrated the presence of OPG in 79% of the PICF samples and showed a positive correlation between BOP, while RANKL was only detected in 12% of PICF samples and did not show any positive correlation with clinical inflammation.

- **Enzymes: (MMP, CATHEPSIN K)**

CatK is a known marker of bone turnover due to its key role in remodeling and cartilage breakdown in bone by hydrolyzing extracellular bone matrix proteins. CatK is highly and quite selectively expressed in active, resorbing osteoclasts. This suggests its role in the pathogenesis of PI. CatK levels were evaluated in PICF to assess the levels of CatK in healthy implants and PI in order to correlate these findings with clinical parameters³⁴. The authors concluded that increased levels of MMP-8 and IL-1 β in PICF or GCF may be associated with inflammation around teeth and implants while lower levels of MMP-1/TIMP1 may be an indicator of disease progression around implants.

CHAIRSIDE DIAGNOSTIC KITS: -

Biochemical tests: (Fig-2)

KIT	INDICATOR	MARKER
Periogard	Fast red-color intensity	Aspartate aminotransferase (AST)
Pocket watch	Malachite green to pink	AST
Periocheck	Blue color	Neutral Proteases
Prognostik	Fluorescent light	Matrix MetalloProteinases (MMP)
MMP Dipstick	Immunofluorometric Assay	MMP8

Microbial tests: (Fig-3)

KIT	INDICATOR	MARKER
BANA/Perioscan	Orange (color marker)	Porphyromonas Gingivalis (Pg), Treponema Denticola (Td), Capnocytophaga
EvaluSite	Pink spot	Aggregatibacter Actinomycetum Comitans (Aa), Pg, Prevotella Intermedia (Pi)
Perioscan/Diamond Probe/Probe 2000	Titre	Pg, Pi, Volatile Sulphur Compounds (VSC)
TOPAS	Levels	Bacterial toxins and proteins

Genetic Test

The Periodontitis Susceptibility Trait test (PST) is the test which identifies the genetic predisposition of the patient for periodontitis by detecting the polymorphism in IL-1 gene. Polymorphism in two positions of IL-1 i.e position - 889 and + 3953 has been associated with periodontal disease³⁵. Within both polymorphisms allele 1 harbors a cytidin (C), whereas allele 2 carries a thymidin (T) at the respective position. In particular, when both genes carry allele 2 a strong over-production of the local inflammatory mediator, interleukin-1 will occur.

Implant Safe Rapid Test Kit

The ImplantSafe® device may be useful in differentiating active from inactive periodontal and peri-implant sites easily, quickly and inexpensively with high sensitivity and specificity (sensitivity of 90% and a

specificity of 70%–85%)³⁶. Elevated levels of MMP-8 in PICF are associated with peri-implant inflammation while low MMP-8 levels (<20 ng/mL) indicate healthy peri-implant tissues. Pathologically elevated levels of MMP-8 (>20 ng/mL) can be detected by a quantitative MMP-8 chair-side device, ImplantSafe®³⁷.

CONCLUSION:

GCF as a diagnostic and prognostic tool has been explored since the initial studies on GCF which aimed to demonstrate that the flow of gingival fluid was sufficiently indicative of the inflammatory state of the periodontal tissues. Over time, research methods have evolved to enable the assessment of the transition phase between health and inflammation at the gingival level to disease progression. More recently, metabolomic analysis that measure small degradation molecules associated with host and bacterial metabolism show promise. There may be many different cytokine inflammatory pathways or microbial stimuli that are associated with the causal pathway of periodontal pathogenesis.

Prospective healthcare is a new approach that incorporates all the power of current disease-oriented medicine but is based on the concept of strategic health planning, a proactive, prospective approach to care. In this system, individuals are evaluated to determine their baseline risk for a specific disease, their current health status, and their likelihood of developing specific clinical problems given their risks. As mentioned before, allocation of resources to prevent periodontitis/peri-implantitis would be optimized and may help to reduce costs if diagnostic information would assist in identifying susceptible patients and help, providing more specific prevention/treatment strategies for high-risk and low-risk patients.

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There are no conflicts of interest.

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