Role of GCF As Potential Biomarker in the Diagnosis of Periodontal Disease

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Abstract: Diagnosis of periodontal disease is very critical in the phases of its treatment. At present diagnostic methods for periodontal disease are not precisely accurate and only allow retrospective diagnosis of attachment loss. We are handicapped in making precise diagnosis and prognosis by two important limitations ie no reliable markers for disease activity and no reliable criteria for identifying the risk individuals. Therefore it's necessary to have a knowledge on the present available information regarding the advanced diagnostic Biomarkers in Gingival crevicular fluid (GCF) for the better understanding of the onset of disease pathogenesis, course of disease progression so that the treatment will be successful.

Key words: Periodontitis, Dental Plaque, GCF, Biomarker.

INTRODUCTION

Periodontitis is a group of inflammatory diseases that affect the connective tissue attachment and supporting bone around the teeth. The initiation and the progression of periodontitis are dependent on the presence of virulent microorganisms capable of causing disease. Although the bacteria are initiating agents in periodontitis, the host response to the pathogenic infection is critical to disease progression.¹ After its initiation, the disease progresses with the loss of collagen fibers and attachment to the cemental surface, apical migration of the junctional epithelium, formation of deepened periodontal pockets, and resorption of alveolar bone.² If left untreated, the disease continues with progressive bone destruction, leading to tooth mobility and subsequent tooth loss.³

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 periodontal diagnostic parameters used clinically include probing depths, bleeding on probing, clinical attachment levels, plaque index, and radiographs assessing alveolar bone level.⁴ Under diagnosis of periodontal disease results in significant amounts of untreated disease and low rates of appropriate therapeutic intervention. Researchers created biomarkers that indicated the presence or absence of periodontal pathogens, gingival and periodontal inflammation, the host inflammatory-immune response to certain pathogenic species, and host tissue destruction. The biological media of choice included saliva, serum, sub gingival plaque, tissue biopsies, and gingival crevicular fluid. As a result, and after many biomarkers and diagnostic tests were developed.

BIOMARKER

A biomarker is a substance used as an indicator of a biologic state. It may be measured and evaluated as an indicator of normal or pathogenic biologic processes, or pharmacologic responses to a therapeutic intervention.⁵ Since periodontitis is a multifactorial disease that includes initiation by bacteria and host interaction, it’s unlikely that a single biomarker will be able to predict periodontal disease activity. A combination of biomarkers may emerge eventually, and in the meantime, risk assessment is more meaningful than simple clinical measures such as periodontal probing. Gingival crevicular fluid (GCF) is a fluid occurring in minute amounts in the gingival crevice. Gingival crevicular fluid is a complex mixture of substances derived from serum, leukocytes, structural cells of the periodontium and oral bacteria. These substances possess a great potential for serving as indicators of periodontal disease⁶. In health GCF represents the transudate of gingival tissue interstitial fluid but in the course of gingivitis and periodontitis GCF is transformed into true inflammatory exudates.⁷ The flow rate of GCF may increase about 30 fold in periodontitis compared to the healthy sulcus. However, its resting volume also increases at the same time with the formation of gingival pocket⁸.

POTENTIAL MICROBIAL FACTORS

Bacterial plaque plays a primary role in the initiation and progression of periodontal disease but the composition of the sub gingival flora is a complex and vary from patient to patient and site to site. Despite these differences and the complex interactions that exist between bacteria and the host a number of possible pathogens have been suggested on the basis of their association with disease progression and heir possession of virulence factors which could damage the tissue⁹.¹⁰,¹¹,¹²

MAIN BACTERIA ASSOCIATED WITH PERIODONTAL DISEASE

•  Phorphyromonas gingivalis
•  Prevotella intermedia
•  Bacteroides forsythus
•  Actinobacillus actinomycetemcomitans
•  Capnocytophaga ochracea
•  Eikenella corrodens
•  Campylobacter recta
•  Fusobacterium nucleatum
•  Treponema denticola

BACTERIAL PROTEASES IN GCF

Bacterial proteases are released into the pocket by the subgingival flora and can be detected in GCF.¹³,¹⁴,¹⁵ Selective biochemical assays have been developed for two bacterial proteases ie dipetidyl peptidase (DPP) and trypsin like proteases. The trypsin like protease detected by this assay is a cysteine protease and has the characteristics of the enzyme now called arg-gingivain or arg-
Main advantages of periodontal diagnostic test system using bacterial markers:

- Some appears to be predictive of disease activity in longitudinal study
- Simple to use
- Chair side test kits available e.g. Evalusite, omnigene, perioccan.
- Chair side test kits produce visual results which can shown to patient

**POTENTIAL INFLAMMATORY AND IMMUNE MARKERS**

The primary cause for the periodontitis is no doubt dental plaque and sub gingival flora. But the bacteria triggers the local inflammatory response and general and local specific immune response which, along with the direct effects of bacteria, causes most of the tissue destruction. Most of the substances which are released from inflammatory and immune cells in the tissue pass into the GCF. GCF is easy to sample and therefore these substances are easily available for the analysis.

**POTENTIAL IMMUNE AND INFLAMMATORY MEDIATORS**

The substances released by the inflammatory and immune cells during the disease process include antibodies (immunoglobulin, Ig), complement proteins, inflammatory mediators such as prostaglandins (PG) and the pro-inflammatory cytokines such as the various interleukins (IL) and tumour necrosis factor (TNF). The potential immune and inflammatory mediators relevant to periodontal pathology are:

**Immune response**
- Antibody: total immunoglobulin and IgG sub groups
- Complement

**Inflammatory response**
- Arachidonic acid derivatives, e.g. prostaglandin E2 (PGE2)
- Cytokines, e.g. IL-1, IL-2, IL-4, IL-6, TNF-α.

**DIAGNOSTIC TEST**

GCF PGE2 has considerable potential as a screening test for periodontal activity strangely no commercial efforts are currently underway to develop one. Therefore it is now possible to assay GCF PGE2 with an ELISA assay using a monoclonal rabbit anti PGE2 antibody.

**POTENTIAL PROTEOLYTIC AND HYDROLYTIC ENZYMES OF INFLAMMATORY CELL ORIGIN**

Inflammation leads to accumulation of polymorphonuclear neutrophil leucocytes (PMNs), macrophages, lymphocytes and mast cell which are very important in protecting the body against infection. The inflammatory cell contains destructive enzymes within their lysosomes which are normally used to degrade phagocytosed material. These enzymes are, however, capable of degrading gingival tissue components if released. Such enzymes may be released by the inflammatory cells during their function or when they degenerate or die. Cells and tissues in the vicinity of these cells will be damaged and this process is known as bystander damage. The main tissue damage in this process are the connective tissue components and the breakdown of these tissues around the inflammatory cells helps the spread of these cells through the tissues.

Inflammatory and connective tissue cells and the proteolytic enzymes and inhibitors which they contain within their cytoplasmic bodies.

<table>
<thead>
<tr>
<th>Polymorphonuclear neutrophil leucocytes (PMNs)</th>
<th>Collagenase, Gelatinase, Tissue inhibiting metaloproteinase (TIMP), Plasminogen, Elastase, Cathepsin G, Cathepsin B, Cathepsin D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Macrophages</td>
<td>Cathepsin G, Cathepsin B, Cathepsin D, TIMP, α1 antiproteinase inhibitor, α2macroglobulin, plasminogen activator, elastase, gelatinase</td>
</tr>
<tr>
<td>Mast cell</td>
<td>Heparin enzyme complexes, tryptase, chymase, histamine</td>
</tr>
<tr>
<td>Fibroblast</td>
<td>Cathepsin B, Cathepsin L, DPP IV, TIMP, α1 antiproteinase inhibitor, α2macroglobulin, collagenase</td>
</tr>
</tbody>
</table>

Biomarkers of periodontal disease activity may be obtained from potential proteolytic and hydrolytic enzymes of inflammatory cells.

**COLLAGENASE AND RELATED METALLOPROTEINASE**

Collagenases are members of a family of metalloproteinase which degrade collagen. They are synthesized by macrophages, neutrophils, fibroblasts and keratinocytes and are secreted by these cells as latent enzymes when stimulated by the appropriate cytokines and some bacterial products. These cells also produce inhibitors known as tissue inhibitors of metalloproteinase. In periodontitis, GCF collagenase activity has been shown to increase with increasing severity of gingival inflammation and increasing pocket depth and alveolar bone loss.

**PROTEOLYTIC AND HYDROLYTIC ENZYMES IN INFLAMMATORY CELLS**

**Proteolytic enzymes**
- Collagenase
- Elastase
- Cathepsin G
- Cathepsin B
- Cathepsin D
- Dipetidylpeptidase
- Tryptase

**Hydrolitic enzymes**
- Aryl sulphatase
- β-glucoronidase
- Alkaline phosphatase
- Acid phosphatase
- Myeloperoxidase
- Lysozyme
- Lactoferrin

There are some test kits based on some of the GCF factors are currently available. For example, Periocheck to detect the presence of neutral proteinases such as collagenase in GCF, Prognostik to detect the presence of the serine proteinase, elastase, in GCF samples.

Advantages of diagnostic test systems based on proteolytic and hydrolytic enzymes are:
- Some are predictive of disease activity in longitudinal studies e.g.; cathepsin B, elastase, dipetidylpeptidase II and IV
- Since it is a colour detection system, simple to use
- Short chair side time
- Can be shown to the patient related to the areas.
All enzymes released from inflammatory cells are likely to be associated with gingival inflammation. Since gingival inflammation is often present in the absence of disease activity this association with inflammation could produce a false association with disease activity. It is therefore very important to show that a potential marker has a true association with periodontal disease activity which is independent of and stronger than any association it may have with gingival inflammation.

**POTENTIAL MARKERS OF CELL DEATH AND TISSUE DEGRADATION**

Periodontal disease activity involves both damage to the epithelial cells of the pocket lining and to the connective tissue cells in the sites of connective tissue degradation. Active periodontal tissues are densely infiltrated with inflammatory cells most of these cells may be damaged. The damaged cells release their cytosolic enzymes (enzymes within the cytoplasms of the cells) and the concentration of these may well reflect the amount of cell death within the lesion. Two of these enzymes are Aspartate aminotransferase (AST) and lactate dehydrogenase (LDH), have been widely used in medicine for several decades as diagnostic aids to assess cell death and tissue destruction. These enzymes would be expected to pass from the periodontal tissues in the inflammatory exudates into the gingival crevicular fluid (GCF). Therefore, GCF levels of these enzymes, should provide evidence of cell death within the periodontal tissues and hence, possibly disease activity. For these reasons they have been studied as potential marker of disease activity.

**CONNECTIVE TISSUE DEGRADATION MARKERS**

The degradation of connective tissue by inflammatory cells and possibly bacterial enzymes during active periodontitis can release components of these tissues. These components could be cleaved sections of the major molecules of the periodontal connective tissue and basement membrane such as collagens and proteoglycans. The components that could be degraded during periodontitis are listed in table 1.

**Table 1. The connective tissue components that could be degraded during periodontitis**

<table>
<thead>
<tr>
<th>Soft tissue components</th>
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</thead>
<tbody>
<tr>
<td>Collagen I, III, V</td>
</tr>
<tr>
<td>Proteoglycans</td>
</tr>
<tr>
<td>Hyaluronan</td>
</tr>
<tr>
<td>Fibronectin</td>
</tr>
<tr>
<td>Collagen IV</td>
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<tr>
<td>Laminin</td>
</tr>
</tbody>
</table>

The detection of the breakdown products of the components of connective tissue and bone in GCF could be indicative of tissue breakdown associated with periodontal disease activity. The breakdown products of these components that have been found in GCF are listed in table 2.

**Table 2. Breakdown products of connective tissue and bone in GCF**

<table>
<thead>
<tr>
<th>Components</th>
<th>Breakdown products</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibronectin Collag</td>
<td>Cleaved components of fibronectin</td>
</tr>
<tr>
<td>Proteoglycans GAGs</td>
<td>Hydroxylproline</td>
</tr>
<tr>
<td></td>
<td>Collagen cross link peptides</td>
</tr>
<tr>
<td></td>
<td>Terminal peptides</td>
</tr>
<tr>
<td></td>
<td>Glycosaminoglycans (GAGS)</td>
</tr>
<tr>
<td></td>
<td>Heparin sulphate</td>
</tr>
<tr>
<td></td>
<td>Chondroitin-4-sulphate</td>
</tr>
<tr>
<td></td>
<td>Chondroitin-6-sulphate</td>
</tr>
</tbody>
</table>

**POTENTIAL MARKERS OF BONE RESORPTION**

Several bone morphogenic proteins are involved in bone mineralisation and some connective tissue proteins also play an important role in this process. Some of these proteins are Osteonectin, Bone phosphoprotein (N-propeptide), Osteocalcin, Telopeptides of type I collagen have been considered for possible markers of bone resorption and hence periodontal disease activity.

**OSTEONECTIN AND BONE PHOSPHOPROTEIN (N-PEPTIDE)**

Osteonectin is a normal component of bone matrix which is thought to play an important role in the initial phase of mineralisation. Bone phosphoprotein, which is an amino propeptide part of type I collagen, appears to be involved in the attachment of connective tissue cells to the substratum. Both of these proteins have been detected in GCF from patients with periodontitis. The total amount of both component is increased in GCF at the site of increased probing depth.

**OSTEOCALCIN**

Osteocalcin is a calcium-binding proteins of bone and is the most abundant non-collagenous protein of the mineralised tissues. It chemotactically attracts osteoclast progenitor cells and blood monocytes. In addition, it is stimulated by vitamin D₃, producing concentration that inhibit collagen synthesis in osteoblasts, promote bone resorption. Further elevated levels of osteocalcin are found in the blood during periods of rapid bone turnover such as osteoporosis and fracture repair. Therefore osteocalcin has been suggested as a possible marker for bone resorption and hence periodontal disease progression, it is present in GCF.

**CONCLUSION**

Periodontal practice ranges from the detection, diagnosis and treatment of attachment loss due to periodontitis. The new diagnostic technologies may be capable of providing the clinician with effective tools that can assist in the early identification of periodontal disease that can result in expidated treatment. The newer diagnostic technique are still at an adolescent stages of development and much work remains to performed to fully validate this utility such that they become important and cost effective for the successful periodontal management.

**REFERENCES**


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