

CRITICAL BIOMARKERS IN PERIODONTICS

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Abstract:

Traditional periodontal diagnostic parameters used clinically include probing depths, bleeding on probing, clinical attachment levels, plaque index, and radiographs assessing alveolar bone level. Traditional diagnostic procedures are inherently limited, in that only disease history, not current disease status, can be assessed. Researchers in the biotechnology and medical realm are currently investigating the use of oral fluids for the diagnosis of oral and systemic diseases and for drug development. In the pharmaceutical industry, the use of biomarkers is avidly being developed for use in tailored dosing and drug metabolism studies. Moreover, new diagnostic technologies such as nucleic acid and protein microarrays and microfluids are under development for risk assessment and comprehensive screening of biomarkers. These recent advances are leading to the development of more powerful diagnostic tools for practitioners to optimize their treatment predictability.

Introduction

Periodontitis is a group of inflammatory diseases that affect the connective tissue attachment and supporting bone around the teeth. Although the bacteria are initiating agents in periodontitis, the host response to the pathogenic infection is critical to disease progression. If left untreated, the disease continues with progressive bone destruction, leading to tooth mobility and subsequent tooth loss.

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Biomarkers of disease in succession play an important role in life sciences and have begun to assume a greater

role in diagnosis, monitoring of therapy outcomes, and drug discovery. The challenge for biomarkers is to allow earlier detection of disease evolution and more robust therapy efficacy measurements. [1]

Review of Literature

BIOMARKER: A substance that is measured objectively and evaluated as an indicator of normal biologic processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention. [2]

MICROBIAL FACTORS FOR THE DIAGNOSIS OF PERIODONTAL DISEASES

Of more than 600 bacterial species that have been identified from subgingival plaque, only a small number have been suggested to play a causal role in the pathogenesis of destructive periodontal diseases in the susceptible host. [3]

Furthermore, technologic advances in methodologies such as analysis of 16S ribosomal RNA bacterial genes indicate that as many as several hundred additional species of not-yet-identified bacteria may exist. The presence of bacteria adjacent to the gingival crevice and

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the intimate contact of bacterial lipopolysaccharide with the host cells trigger monocytes, polymorphonuclear leukocytes (neutrophils), macrophages, and other cells to release inflammatory mediators such as interleukin (IL)-1, tumor necrosis factor (TNF)- α , and prostaglandin E₂. [1]

A number of specific periodontal pathogens have been implicated in periodontal diseases, including *Tannerella forsythensis*, *Porphyromonas gingivalis*, and *Treponema denticola*. These three organisms are members of the “red complex” of bacteria (and exhibit benzoyl-DL-arginine-naphthylamide, or BANA, activity) that are highly implicated in the progression of periodontal diseases. [1]

Although it is considered that microbial identification is a valuable adjunct to the management of patients with periodontal diseases, there is a lack of strong evidence to support this practice. It is also possible that as-yet-identified, uncultivable microbial species are essential to disease initiation and progression. If so, microbial-based tests for these species are unavailable.[1]

HOST RESPONSE AND INFLAMMATORY MEDIATORS AS POTENTIAL BIOMARKERS

Periodontal inflammation occurs in the gingival tissue in response to plaque bacteria biofilms.[4],[5] Gingivitis is characterized by an initial increase in blood flow, enhanced vascular permeability, and the influx of cells (neutrophils and monocyte-macrophages) from the peripheral blood to the gingival crevice.[6] Subsequently, T cells and B cells appear at the infection site.

After they appear at the lesion, these cells produce a myriad of cytokines such as IL-1, IL-6, TNF- α , and immunoglobulins as an antigen specific response. Initially, tissue degradation is limited to epithelial cells and collagen fibers from the connective tissue. Later on, the inflammatory process may reach periodontal supportive tissue, leading to bone resorption.[1]

Increased tissue levels of PMNs have been associated with active (destructive) periodontal lesions, whereas salivary or gingival crevicular fluid levels of neutrophil proteolytic enzymes such as collagenase and elastase

correlate with disease activity or clinical indices of disease.

Elastase (a neutrophil enzyme that has indirect antibacterial properties) and collagenase can degrade several components of the extracellular matrix (eg, various collagens and elastin), thus destroying the three-dimensional scaffolding necessary for tissue organization.

The significance of collagenase in periodontitis tissue damage has been clinically exploited by the introduction of nonantimicrobial-dose doxycycline therapy; low-dose doxycycline inhibits host collagenase without having any discernible effect on the microflora present. [2]

The contributions of IL-1 α and IL-1 β (two distinct but related molecules, collectively referred to as IL-1 here) to alveolar bone loss and periodontal disease have received considerable attention. IL-1, produced by monocytic, epithelial, osteoblastic, and other cells is a potent stimulator of bone resorption and inhibitor of bone formation.

TNF- α is a molecularly distinct cytokine that shares many biologic activities with IL-1. TNF- α has been implicated in the periodontal disease process because of its ability to stimulate bone resorption and other catabolic processes. The use of TNF inhibitors (in combination with IL-1 inhibitors) results in decreased inflammation and tissue destruction in nonhuman primates.

Prostaglandin E₂ and thromboxane B₂ are lipid molecules produced by many host cells through the cyclooxygenase pathway, one of the two major paths of arachidonic acid metabolism.

Host cell-derived enzymes such as matrix metalloproteinases (MMPs) are an important group of neutral proteinases implicated in the destructive process of periodontal disease that can be measured in GCF. [1] The neutrophils are the major cells responsible for MMP release at the infected site, specifically MMP-8 (collagenase-2) and MMP-9 (gelatinase-B).[7]

Kinane et al. (2003) [8] & Mantyla et al. (2003) [7] presented the use of a rapid chairside test based on the immunologic detection of elevated MMP-8 in GCF to diagnose and monitor the course and treatment of periodontitis.

Advanced stages of periodontal lesions are populated by a large proportion of B lymphocytes and plasma cells and increased levels of immunoglobulins in GCF.[1] **Plombas et al. (2002)** [9] investigated GCF and whole saliva from periodontitis patients and periodontally healthy adults for the presence of IgA and IgG antibodies to *Actinobacillus actinomycetemcomitans*, *Porphyromonas gingivalis*, *Prevotella intermedia*, and *Fusobacterium nucleatum*. Compared with healthy patients, the GCF of periodontitis patients contained significantly higher levels of IgA and IgG antibodies to the four microorganisms tested.

Aspartate aminotransferase, a tissue destruction biomarker released from necrotic cells in GCF, is associated with periodontitis severity. Aspartate aminotransferase–positive sites are positively correlated with higher prevalence of *Porphyromonas gingivalis*, *Streptococcus intermedius*, *Peptostreptococcus micros*, *Campylobacter concisus*, *Bacteroides forsythus*, *Campylobacter gracilis*, *Campylobacter rectus*, and *Selenomonas sputigena*. [10]

Table 1[1] - Examples of biomarkers of periodontal disease identified from plaque biofilm, gingival crevicular fluid, or saliva

Category mediator	Examples
Microbial factors	DNA probes or culturing of putative periodontal pathogens (eg, <i>Porphyromonas gingivalis</i> , <i>Tanerella forsythensis</i> , <i>Treponema denticola</i>)
Host response factors	IL 1 \hat{a} ; TNF- \acute{a} ; aspartate aminotransferase; elastase
Connective tissue breakdown products	Collagen telopeptides; osteocalcin; proteoglycans; fibronectin fragments

BONE-SPECIFIC MARKERS OF TISSUE DESTRUCTION FOR PERIODONTAL DIAGNOSIS

Of the 50 or more different components in GCF and saliva evaluated to date for periodontal diagnosis, most lack specificity to alveolar bone destruction and essentially constitute soft tissue inflammatory events. When examining the destruction of alveolar bone that is preceded by microbial infection and inflammatory response, the measurement of connective tissue–derived molecules may lead to a more accurate assessment of tissue breakdown due to the tremendous variability of the host response among individuals.[1]

Pyridinoline cross-linked carboxyterminal telopeptide of type I collagen

Type I collagen composes 90% of the organic matrix of bone and is the most abundant collagen in osseous tissue. The cross-linked telopeptides result from post-translational modification of collagen molecules, they cannot be reused during collagen synthesis and are therefore considered specific biomarkers for bone resorption. [1]

In addition, the value of pyridinoline cross-links as potential markers of bone turnover relates to their specificity for bone. The pyridinoline cross-linked carboxyterminal telopeptide of type I collagen (ICTP) is a 12- to 20-kd fragment of bone type I collagen released by digestion with trypsin or bacterial collagenase. [11]

Elevated serum ICTP and other pyridinoline cross linked components have been shown to be correlated with the bone resorptive rate in several bone metabolic diseases including osteoporosis, rheumatoid arthritis, and Paget’s disease. [1]

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 between the presence of gingival inflammation and active periodontal or peri-implant bone destruction.[12]

Palys et al. (1998) [13] related ICTP levels to the subgingival microflora of various disease states on GCF. **Oringer et al. (1998)** [14] examined the relationship between ICTP levels and subgingival species around

implants and teeth in 20 partially edentulous and 2 fully edentulous patients.

Diagnostic tools have also been applied to evaluate the response to active periodontal therapy. An investigation of periodontitis patients by **Golub et al (1997)** [15] treated with SRP also demonstrated significant correlations between GCF ICTP level and clinical periodontal disease parameters, including attachment loss, pocket depth, and bleeding on probing. In addition, elevated GCF ICTP levels at baseline, especially at shallow sites, were found to be predictive for future attachment loss as early as 1 month after sampling.

Osteocalcin

Osteocalcin is synthesized predominantly by osteoblasts and has an important role in bone formation and turnover. Elevated serum osteocalcin levels have been shown during periods of rapid bone turnover (eg, osteoporosis, multiple myeloma, and fracture repair). [1]

Serum osteocalcin is presently a valid marker of bone turnover when resorption and formation are coupled and is a specific marker of bone formation when formation and resorption are uncoupled. Several studies have investigated the relationship between GCF osteocalcin levels and periodontal disease. [1]

Kunimatsu et al. (1993) [16] reported a positive correlation between GCF osteocalcin aminoterminal peptide levels and clinical parameters in a cross-sectional study of periodontitis and gingivitis patients. The investigators also reported that osteocalcin could not be detected in patients with gingivitis.

In contrast, **Nakashima et al. (1994)** [17] reported significant GCF osteocalcin levels from periodontitis and gingivitis patients. Osteocalcin levels were also significantly correlated with pocket depth, gingival index scores, and GCF levels of alkaline phosphatase and prostaglandin E₂.

The results of these studies show a potential role for intact osteocalcin as a bone-specific marker of bone turnover but not as a predictive indicator for periodontal disease. Additional longitudinal studies may be warranted to more fully elucidate the utility of osteocalcin as a periodontal disease activity diagnostic aid. [1]

Role of oral fluid biomarkers in periodontal diagnosis

Because saliva and GCF are fluids easily collected and contain 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. Due to the noninvasive and simple nature of their collection, analysis of saliva and GCF may be especially beneficial in the determination of current periodontal status and a means of monitoring response to treatment.

Oral fluid biomarkers that have been studied for periodontal diagnosis include proteins of host origin (i.e. enzymes and immunoglobulins), phenotypic markers, host cells, hormones, bacteria and bacterial products, ions, and volatile compounds. [1]

Conclusion

Researchers in the biotechnology and medical realm are currently investigating the use of oral fluids for the diagnosis of oral and systemic diseases and for drug development. In the pharmaceutical industry, the use of biomarkers is avidly being developed for use in tailored dosing and drug metabolism studies. Moreover, new diagnostic technologies such as nucleic acid and protein microarrays and microfluids are under development for risk assessment and comprehensive screening of biomarkers. These recent advances are leading to the development of more powerful diagnostic tools for practitioners to optimize their treatment predictability. [1]

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