

Biomarkers in periodontal disease

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Introduction

Periodontitis is a group of inflammatory diseases that affect the connective tissue attachment and supporting bone around the teeth whose initiation and progression depends on the presence of virulent microorganisms capable of causing disease [1]. Periodontitis is considered to be a multifactorial disease with no clear cut etiology, so its identification and early diagnosis becomes more difficult [2]. The current clinical diagnostic parameters were introduced more than 50 years ago. But all the methods provide disease severity rather than disease activity.

A biomarker is a substance used to indicate a biologic state and is an objective measure to evaluate the present and future disease activity. It is defined as – A substance that is measured objectively and evaluated as an indicator of normal biologic processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention [3]. Various biological media like saliva, serum and gingival crevicular fluid are used to determine biomarkers in periodontal health and disease. A single biomarker will not be able to predict periodontal disease activity and severity. So combinations of biomarkers are used to predict the disease activity [4].

Advantages of traditional diagnostic techniques

Easy to use, Cost effective, Non invasive, Measures disease severity [5].

Limitations of traditional periodontal diagnostic techniques

- Clinical and radiological measurements of attachment loss are not precisely accurate
- Full mouth recording is necessary because of the site specific nature of periodontal disease progression.
- Individual susceptibility to periodontitis varies both genetically and over time
- All clinical diagnostic techniques provide information about past disease activity and are unable to diagnose present disease activity [5].

Need for biomarker

Under diagnosis periodontal therapy leads to failure of periodontal treatment. For that researchers phrased biomarkers that indicated the presence or absence of periodontal disease [6]. The biological media of choice included saliva, serum and gingival crevicular fluid.

Proteomic biomarkers

The word “proteome” is a blend of “protein” and “genome”, and was coined by Marc Wilkins. The proteome is the entire complement

of proteins, including the modifications of a particular set of proteins. Proteomics offers a new approach to the understanding of the changes occurring as oral micro-organisms adapt to environmental change within their habitats in the mouth [8].

Pyridinoline cross-linked carboxyterminal telopeptide of type I collagen

Type I collagen is major collagen present in mineralized tissues. The degradation products of collagen act as markers for bone metabolism. The degradation products of collagen are pyridinoline, deoxypyridinoline, N-telopeptides and C-telopeptides. Increased levels of ICTP are associated with most of pathogens including *T. forsythensis*, *P. gingivalis*, *P. intermedia*, and *T. denticola* [9]. Non-surgical mechanical therapy doesn't not significantly reduce ICTP and IL-1 levels [10]. Contrary to this, SRP and local drug delivery lead to rapid reductions in GCF ICTP levels [11].

Osteocalcin

Osteocalcin is non collagenous protein. It is predominately present in mineralized tissues. It is produced by osteoblasts, help in bone remodeling. Increased levels of osteocalcin are associated with rapid bone remodeling. The levels of osteocalcin are remain unchanged in patients with gingivitis [12]. Osteocalcin levels are increased in periodontitis [13].

Alkaline phosphatase (ALP)

ALP is a catalyzing enzyme that accelerates the removal of phosphate groups in the 5 and 3 positions from a variety of molecules, including nucleotides, proteins, and alkaloids. Although it is present in all tissues, ALP is particularly concentrated in the bone, liver, bile duct, kidney and placenta. The enzyme is likely to be largely derived from the periodontal tissues [14]. The major source of ALP in early inflammation is polymorphonuclear leukocyte [15]. There is a significant correlation of ALP with pocket depth and inflammation. There is a relationship between attachment loss in the periodontitis group to a drop in ALP activity in serum [16]. Contrary to these results, the measurements of periodontal destruction (probing depth, gingival bleeding, and suppuration) are related to higher levels of ALP in saliva [17]. As a predictive indicator for the future periodontal breakdown, ALP may serve as a marker in periodontal treatment planning and monitoring.

Cathepsin B

Cathepsin B is responsible for proteolysis. Macrophages produce

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Cathepsin B in GCF. Cathepsin B levels are increased in periodontitis. It levels are increased while progression of the periodontal disease. The levels of Cathepsin B are useful for differentiating periodontitis from gingivitis [16] and also helpful for proper treatment plan [17].

Matrix metalloproteinases

Matrix metalloproteinases (MMPs) are genetically distinct but structurally related zinc dependent metalloendopeptidases. MMPs are host proteinases responsible for both tissue degradation and remodeling. MMPs degrade extracellular matrix and further potentiate proteolysis and inflammation by processing of bioactive non-matrix substrates, such as cytokines, chemokines and growth factors, and also by activating other MMPs.

The 23 MMPs expressed in humans can be classified into different subgroups based on their primary structures and substrate specificities: Collagenases (MMP-1, -8 and -13), Gelatinases (MMP-2 and -9), Membrane type MMPs (MT-MMPs, MMP-14, -15, -16, -17, -24 and -25) and other MMPs. In the healthy condition, the periodontal ligament apparatus is protected from matrix metalloproteinases mediated proteolytic attack by tissue inhibitors of metalloproteinases (TIMP) [18].

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 signify conversion of gingivitis into periodontitis. No associations are found between MMP-8 levels and bone loss [18]. MMP-8 levels reflect soft tissue destruction and periodontal response to treatment. It is believed that MMP-8 may serve as a proinflammatory marker, but not as a discriminating marker for chronic periodontitis and gingivitis [19]. It is found that 18-fold increase of MMP-8 in patients experiencing active periodontal tissue breakdown as compared with patients under stable condition [19].

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. After giving one dose of systemic metronidazole, the levels of MMP-9 in mouth rinse samples from patients with initial elevated MMP-9 concentrations markedly decreased [20]. Given these results, future use of MMP-9 in oral diagnostics may best serve as a guide in periodontal treatment monitoring [21].

Collagenase-3 (MMP-13)

Collagenase-3, referred to as MMP-13, is another collagenolytic MMP with exceptionally wide substrate specificity. MMP-13 is expressed by subular epithelial cells, endothelial cells, macrophage-like cells, fibroblasts, plasma cells and osteoblasts. The expression of MMP-13 is specifically induced in undifferentiated epithelial cells during chronic inflammation due to exposure to cytokines and collagen [17]. MMP-13 has also been implicated in peri-implantitis. Elevated levels of both MMP-13 and MMP-8 are correlated with irreversible peri-implant vertical bone loss around loosening dental implants [17]. In patients with untreated periodontal disease, collagenase present predominantly in the active form [21]. In the future, MMP-13 may be useful for diagnosing and monitoring the course of periodontal disease as well as tracking the efficacy of therapy.

Myeloperoxidase

Neutrophil-derived myeloperoxidase (MPO) is contained in primary (azurophilic) granules from neutrophils and catalyzes the formation of hypochlorous acid (HOCl), a powerful antibacterial agent, which reflects the strength of oxidative stress. MPO can inactivate pathogenic microbes by generating reactive oxygen species, oxidatively activate latent proMMP-8 and 9, as well as inactivate TIMPs. Thus, MPO can also oxidatively potentiate MMP-cascades in periodontal tissue destruction, becoming potentially deleterious. The increased MPO activity is attributed to increased infiltration and degranulation of PMNs. During therapy salivary peroxidase concentrations are declined below the control values [22].

Calprotectin

Calprotectin is released from neutrophils. It is a calcium and zinc binding protein, has both antimicrobial and antifungal activity and play a vital role in inflammation. It inhibits immunoglobulin production and act as a proinflammatory protein. Increased expression of calprotectin at the site of inflammation offer protection against bacterial invasion to epithelial cells especially *P. gingivalis* [23]. Calprotectin appears to improve resistance to *P. gingivalis* by boosting the barrier protection and innate immune functions of the gingival epithelium.

Osteonectin

It is a secreted protein. It is acidic in nature and contains cysteine; base membrane protein BM-40. It has strong avidity to hydroxyapatite and collagen. It plays a vital role in early phase of mineralization so it can act as a sensitive marker for detection of periodontitis. The sensitivity of this marker for diagnosing periodontal disease more when compared with N-propeptide of type I collagen [17].

Osteopontin (OPN)

OPN is released by both osteoblasts and osteoclasts. The concentration of OPN is higher at the clear zone where osteoclasts are attached. It helps in bone remodeling. In periodontitis, OPN levels are increased. There is a positive correlation between increased levels of OPN to probing pocket depth [24,25]. When nonsurgical periodontal treatment is provided GCF OPN levels are significantly reduced [26].

Cystatins

Cystatins act as biomarkers for periodontal disease diagnosis. Many isoforms of Cystatins are secreted into saliva and GCF in periodontitis. Cystatin C in saliva act as a biomarker for diagnosing periodontitis as its levels are increased in saliva in periodontitis. GCF Cystatins are poor biomarkers for periodontitis when compared with Cystatins in saliva [27].

Fibronectin

Fibronectin is a glycoprotein that promotes selective adhesion and colonization of certain bacterial species. It is involved in chemotaxis, migration, inflammation, wound healing and tissue repair. Changes in oral cleanliness may contribute to the rapid fluctuations in salivary proteases and epithelial cell fibronectin [28]. There are no statistically significant differences between pre- and post-treatment concentrations of fibronectin, whether expressed as micrograms fibronectin/micrograms protein or as micrograms fibronectin/ml saliva [29].

Lysozyme

Lysozyme is a proteolytic enzyme, mainly present in salivary gland

secretions. It damages bacterial cell wall. Patients with low lysozyme activity in saliva are more prone to periodontal disease. Levels of lysozyme is inversely proportional to plaque accumulation [30]. Many studies are shown that lysozyme concentrations are decreased in periodontitis [31].

Lactoferrin

Lactoferrin is mainly secreted from salivary glands. It is an antibacterial iron binding glycoprotein. Increased levels of lactoferrin in saliva are strongly associated with periodontitis [31].

Immunoglobulins

The predominant immunoglobulin in saliva is secretory IgA (sIgA) which is derived from plasma cells in the salivary glands. There are two subclasses of IgA – IgA1 and IgA2. IgA1 is predominates in serum while IgA2 is found in higher concentrations in external secretions [33]. Saliva from treated periodontitis patients has higher IgA and IgG levels than saliva from control subjects. These higher antibody levels are observed for periodontal pathogens (*P. gingivalis* and *Treponemadenticola*), but also for the normal inhabitant of the oral cavity *Streptococcus salivarius* [34]. Significantly elevated levels of IgG antibody to *A. actinomycetemcomitans* are found [35]. High level of salivary IgA is directed against bacteria in dental plaque and may protect against the development of gingivitis [36].

Platelet activating factor

Platelet activating factor, also known as PAF, is a potent phospholipid activator and mediator of many leukocyte functions including platelet aggregation, degranulation, inflammation, and anaphylaxis. It is produced by platelets, endothelial cells, neutrophils, monocytes, and macrophages. A significant positive correlation is observed between the level of PAF in saliva and measures of periodontal

inflammation [37]. Thus, initial periodontal therapy is reduced salivary PAF levels in concert with improvements in clinical estimates of marginal and sub marginal periodontal inflammation suggesting that PAF may participate in inflammatory events during periodontal tissue injury and disease [38].

Epidermal growth factor

Epidermal growth factor stimulates cell growth, proliferation and differentiation by binding to its receptor EGFR. The elevated rate of salivary EGF secretion in aggressive patients may be associated with the pathogenic mechanisms of aggressive periodontitis [39].

Platelet-derived growth factor: *In vitro* and *in vivo* studies suggest PDGF is the most thoroughly described growth factor associated with periodontal health. There are different isoforms of PDGF (PDGF-AA, -AB, -BB), and all have been shown to have a fibroblast proliferative activity [40]. PDGF is present in increased levels in the human inflamed gingiva and is mainly localized to the pocket epithelium. It is possible that expression of PDGF contributes to the inflammatory changes those occur during periodontal diseases. PDGF supports the healing. Since PDGF is chemo tactic for fibroblasts, it induces collagen synthesis, stimulates fibroblasts to synthesize the proteoglycans for extracellular matrix development [41]. Thus decrease in PDGF can be a useful marker for periodontal disease [42].

Vascular endothelial growth factor (VEGF): VEGF is a key regulator of physiological and pathological angiogenesis, because it induces endothelial cell proliferation, stimulates angiogenesis and increases vascular permeability, contribute to periodontal healing [42]. In periodontitis patients, VEGF is detected within vascular endothelial cells, neutrophils, plasma cells, and junctional, pocket and gingival epithelium [43]. Various authors reported increased VEGF expression in epithelial cells and endothelial cells in periodontitis-affected gingiva could be an useful marker for periodontal disease (Table 1 and 2).

Table 1. Classification of biomarkers [7].

Proteomic biomarkers	Genetic biomarkers	Microbial biomarkers	Other biomarkers
Cystatins, αglucosidase, Acid phosphatase, Alkalinephosphatase, Aminopeptidase, Lactoferrin, Translactoferrin, IgM, MMP-13, MMP-8, MMP-9, Cathepsin B, Osteonectin, Osteocalcin, Osteopontin, Elastase Platelet-activating factor, Epidermal growth factor, Platelet-derived growth factor, Esterase, Pyridinoline crosslinked carboxy –terminal telopeptide, Fibronectin, sIgA (secretory IgA) Gelatinase, IgA, Trypsin, Vascular endothelial growth factor, IgG	Cathepsin C gene Mutation, Collagen gene mutation, IL-1 polymorphisms, IL-10 polymorphisms, Tumor necrosis factor, Polymorphisms.	<i>Aggregatibacter actinomycetemcomitans</i> , <i>Campylobacter rectus</i> , <i>Mycoplasmas</i> , <i>Porphyromonas gingivalis</i> , <i>Prevotella intermedia</i> , <i>Peptostreptococcus Micros</i> , <i>Prevotella nigrescens</i> , <i>Treponema denticola</i> , <i>Tannerella forsythia</i> , <i>Treponema socransky</i> .	Calcium, Cortisol, Hydrogensulphide, Methylmercaptan, Pyridine.

Table 2. Chair side diagnostic kits by using various biomarkers [55].

Assay	Kit	Manufacturer/Supplier	Function
Bacterial enzymes & host enzymes	BANA periodontal test	Ora Tec Corporation Manassas (USA)	It utilizes the BANA test for bacterial trypsin like proteases
	Periocheck	CollaGenex Pharmaceuticals, Newtown, PA	Detects presence of neutral proteinases <i>i.e.</i> Collagenase
	Perioscan	Oral B Laboratories	Detects enzymatic activity of <i>Aggregatibacter actinomycetemcomitans</i> , <i>T. forsythus</i> , <i>P. gingivalis</i>
Immunological identification	Evalusite	Kodak Eastman Company (Switzerland)	Immunological detection of antigens of <i>Aggregatibacter actinomycetemcomitans</i> , <i>P. intermedia</i> , <i>P. gingivalis</i> using antibodies (ELISA)
Biochemical identification	Prognostic	Dentsply	Aids in detection of serine proteinases and elastases
	Biolise	SLT-Lab instruments, Crailsheim, Germany	Aids in detection of elastase
	Periogard	Colgate	Detects the presence of AST
	Pocket watch	SteriOss®, San Diego, CA, USA	Detects aspartate aminotransferase through colorimetric detection
	TOPAS	Affinity Labelling Technologies (USA)	Detects toxins derived from anaerobic metabolism and measures GCF protein level

Genetic biomarkers

Interleukin polymorphisms: A study reported that a “composite” IL-1 genotype consisting of at least one copy of the rarer allele at both an IL-1 α and IL-1 β loci was associated with severe periodontitis [45]. Karimbux *et al.* in their meta-analysis reported that *IL1A* and *IL1B* genetic variations are significant contributors to chronic periodontitis in Caucasians [46].

Cathepsin c polymorphisms: The underlying causation of Papillon-Lefevre syndrome has been the subject of considerable debate in the literature. Papillon-Lefevre syndrome is caused by mutation in gene coding cathepsin C. This enzyme is expressed at high levels in many immune cells including polymorphonuclear leukocytes and macrophages and their precursors. In addition, it has been found that cathepsin C is expressed in areas of epithelium often affected by hyperkeratosis lesions such as palms, soles, knees and oral keratinized gingiva. But hyperkeratosis present only in homozygous trait.

TNF α gene polymorphism: The *TNF α* gene is located on chromosome 6 within the major histocompatibility complex (MHC) gene cluster at the location 6p21.3. It is an important mediator in inflammatory reactions and appears to play a central role in the pathogenesis of severe chronic inflammatory diseases. Differences in the rate of production of TNF have been demonstrated and a familial ability to produce higher or lower cytokine levels seems to exist [47]. The TNF synthesis may be influenced by the presence of certain gene polymorphisms [48]. Some consistent results on association of *TNF α* gene polymorphisms with diseases are reported for infectious diseases particularly malaria. *TNF α* gene polymorphisms were also investigated in association with periodontitis [49].

CD14 gene polymorphism: The *CD14* gene is on the chromosome 5 at the location 5q31.1. The production of the sCD14 depend on C to T transition at position -159 (also called -260). Subjects with the homozygous TT genotype exhibited significantly higher sCD14 levels which influenced the activation of Th2- to Th1 type cells in the response to bacterial challenge. The -260 *CD14* gene polymorphism [50] has been associated with Crohn’s disease and also with periodontitis.

Microbial markers

Although there are almost 600 bacterial species present in subgingival plaque, only few of them are causing periodontal disease in a susceptible host.

A number of specific periodontal pathogens have been implicated in periodontal diseases, including *Tanarella forsythensis*, *Porphyromonas gingivalis*, and *Treponema denticola*. These three organisms are members of the “red complex” of bacteria that are highly implicated in the progression of periodontal diseases. *Actinobacillus actinomycetemcomitans* has been linked with early-onset forms of periodontal disease and aggressive periodontitis, whereas red complex bacteria are associated with Chronic Periodontitis.

A study conducted to determine whether the presence of bacterial antigens for *Porphyromonas gingivalis* (Pg), *Prevotella intermedia* (Pi), and *Actinobacillus actinomycetemcomitans* (A.a) in sub gingival plaque of periodontitis patients after periodontal treatment was associated with progressive alveolar bone loss. Progressive alveolar bone loss was determined using digital subtraction radiography with standardized radiographs taken at baseline and 6 months after treatment and concluded the presence of *P. gingivalis* in plaque after treatment was significantly associated with progressive bone loss [51].

Other biomarkers

Cortisol: A study evaluated the association of stress, distress, and coping behaviors with periodontal disease and concluded that higher salivary cortisol levels were detected in individuals exhibiting severe periodontitis [52].

Calcium: A study conducted to examine differences in salivary calcium levels in periodontitis patients in comparison to periodontally healthy subjects. The results show that subjects in the high salivary Ca-group had significantly more intact teeth than their pairs in the low salivary Ca group and concluded that an elevated calcium concentration in saliva was characteristic of patients with periodontitis [53].

Volatiles: Volatile sulphur compounds, primarily hydrogen sulfide and methylmercaptan, are associated with oral malodor. Salivary volatiles have been suggested as possible diagnostic markers and contributory factors in periodontal disease. For example, pyridine and Pico lines were found only in subjects with moderate to severe periodontitis. Furthermore, saliva seems to be a useful medium to evaluate oral malodor [54].

Conclusion

In the field of oral disease diagnosis, there has been a steady growing trend during the last two decades to develop tools to monitor periodontitis. From physical measurements such as periodontal probing to sophisticated genetic susceptibility analysis and molecular assays for the detection of biomarkers on the different stages of the disease, substantial improvements have been made on the understanding of the mediators implicated on the initiation and progression of periodontitis. At the same time, this evolutionary process has promoted the discovery of new biomarkers and the development of new therapeutic approaches mainly using host modulation. 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”. The development of a wide spectrum of marker factors will be a primary goal of periodontal research.

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