



**RESEARCH ARTICLE**

**CRITICAL ANALYSIS OF BIOMARKERS IN PERIODONTAL PRACTICE : A BRIEF REVIEW**

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**ABSTRACT**

**Background:** Early diagnosis and treatment of progressive periodontitis is important because of the irreversible nature of periodontal disease. . In the field of periodontology, traditional clinical criteria are often insufficient for determining sites of active disease, for monitoring the response to therapy, or for measuring the degree of susceptibility to future disease progression. Patients with periodontitis may have elevated circulating levels of specific inflammatory markers that can be correlated to the severity and present state of the disease. This review highlights the various potentials of oral biomarkers as non-invasive method of periodontal disease diagnosis.

**Methods:** The Google scholar and Medline search was conducted and the relevant literature concerning the applications of oral biomarkers for periodontal diagnosis was reviewed.

**Results:** Based on the literature, salivary markers that have been studied as potential diagnostic tests for periodontal disease include proteins of host origin (i.e., enzymes, immunoglobulins), phenotypic markers, host cells, hormones (cortisol), bacteria and bacterial products, ions and volatile compounds.

**Conclusions :** A number of markers show promise as sensitive measures of disease and the effectiveness of therapy. Longer-term longitudinal studies, however, are required to establish the relationship between specific markers and progression of periodontal disease. Furthermore, analysis of oral biomarkers may offer a cost-effective approach to assessment of periodontal disease in large populations.

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**INTRODUCTION**

One of the liveliest areas of current periodontal research is concerned with the search of diagnostic tests of periodontal disease activity. The goal of periodontal diagnostic procedure is to provide useful information to the clinician regarding the present periodontal disease type, location, and severity. (Taba *et al.*, 2005) One such diagnostic procedure is the identification of Biomarkers. The challenge for biomarkers is to allow earlier detection of disease evolution and more robust therapy efficacy measurements.

**Biomarker**

A substance that is measured objectively and evaluated as an indicator of normal biologic and pathogenic processes or pharmacologic responses to a therapeutic intervention. (Strimbu *et al.*, 2010). The diagnosis of active phases of periodontal disease and the identification of patients at risk for active disease represent challenges for clinical investigators and practitioners. Can biomarkers help to overcome this challenge is still an unanswered query.

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**Source of Biomarkers (Reddy *et al.*, 2011)**

Four potential sources are basically used for assessing biomarkers:

- Blood or serum
- Saliva
- Subgingival plaque sample
- Gingival crevicular fluid (GCF)

Markers from the first two sources (Blood or serum and saliva) relate to either the whole patient or the whole mouth rather than a local site. At best they could give some information on the patients overall periodontal condition. However, markers from the latter two sources i.e. factors from subgingival plaque samples and GCF, relate to the condition of the local periodontal site.

**Candidates for biomarkers (Reddy *et al.*, 2011)**

**Microbes and their co-products-** endotoxins, enzymes, metabolic end products, DNA probes, enzymes etc.

**Inflammatory products-** complement, cytokines, interleukins, TNF, interferon- $\alpha$ , prostaglandins, antibacterial antibodies, c-reactive protein, substance -p etc.

**Connective tissue degradation products-** collagen-telopeptides, osteocalcin, proteoglycans, breakdown products, fibronectin etc.

**Enzymes-** aminotransferase, aspartate, transferase, lactoferrin, lysozyme etc.

**Bone resorption products-** osteocalcin, osteopontin etc.

### Microbes and their coproducts

Bacterial plaque plays a primary role in the initiation and progression of periodontal diseases. Bacteria may be present in the gingival crevice or periodontal pocket, within saliva, or on surface of the oral mucosa in various parts of the mouth. There is no evidence for any specific pathogen in chronic periodontitis, therefore it may be considered as a non specific bacterial disease.

Bacteria associated with periodontal diseases are *Porphyromonas gingivalis*, *Prevotella intermedia*, *Bacteroides forsythus*, *Aggregatibacter actinomycetemcomitans*, *Capnocytophaga ochracea*, *Eikenella corrodens*, *Campylobacter recta*, *Fusobacterium nucleatum*, *Treponema denticola* etc.

These bacteria are present in higher numbers at active disease sites, and in some cases produce products capable of damaging the tissues either directly or indirectly. (Loesche *et al.*, 2001) Samples from oral mucosa or from saliva are usually obtained with sterile paper points or swabs and then transferred directly into an appropriate anaerobic transport medium. Subgingival plaque samples are obtained only after complete supragingival plaque removal either with a fresh, clean and sterile curette or with a sterile paper point. This is then transferred to anaerobic transport media for microbial analysis.

### Bacterial species can be determined using (Giannobile *et al.*, 2009)

- Darkground or phase contrast microscopy
- Culture techniques
- Immunological assays
- DNA probes
- Enzyme based assays like BANA assays and qualitative fluorescence polarization

### Bacterial products

like bacterial enzymes and volatile sulphur compounds play an important role as biomarkers and can be detected in saliva and GCF. Whole, non stimulated saliva sample is required for the detection of bacterial enzymes in this situation. (Patil and Patil, 2011) A trypsin-like protease can be detected in saliva, and the level of this enzyme correlates with disease severity and also reduces following periodontal treatment. Bacterial proteases are released into the pocket and can be detected in GCF also. Selective biochemical assays have been developed for both bacterial dipeptidylpeptidase (DPP) and trypsin-like proteases, and can distinguish them from interfering tissue derived proteases.

The trypsin-like protease detected by this assay is a cysteine proteinase, and has characteristics of enzyme now called arg-gingivain or arg-gingipain. GCF arg-gingivain/arg-gingipain appears to be an excellent predictor and GCF DPP a moderately good predictor of future progressive attachment loss. (Patil and Patil, 2011) *Perioscan* is a chairside test kit that utilizes BANA test for bacterial trypsin like proteases. (Hemmings *et al.*, 1997) Volatile sulphur compounds such as hydrogen sulphide H<sub>2</sub>S, methyl mercaptan, CH<sub>3</sub>SH, dimethyl sulphide are all toxic by-products of Gram negative anaerobic bacterial metabolism of sulphur-containing amino acids. *P. gingivalis*, *P. intermedia*, *P. melaninogenica*, *B. forsythus*, *T. denticola* and *F. nucleatum* have all been shown to be capable of producing them through their metabolic pathways and hence these sulphur compounds can be detected in chronic periodontitis patients. (Li *et al.*, 2008)

A recently developed commercially available instrument, *Diamond Probe/Perio 2000 System*® has been designed so that it combines the features of a periodontal probe with the detection of volatile sulphur compounds in the periodontal pocket. (Ramachandra *et al.*, 2011) New strategies that combine microbial identification with the host response or tissue breakdown factors using discriminant analysis may better improve the ability of microbial analysis to predict future periodontal disease.

### Host response and inflammatory mediators

Various mediators released by inflammatory and immune cells during the disease process include antibodies (IgG), complement proteins, inflammatory mediators such as prostaglandins, inflammatory cytokines such as various interleukins and tumour necrosis factor etc can be used as biomarkers. These can be assayed using ELISA techniques, which could be developed into chairside kits. (Yucel-Lindberga and Bage, 2013) Hyperaemia associated with inflammation increases the local temperature of the part affected and can be detected by a device called *Periotemp* as increased subgingival temperature has been positively correlated with increased probing depth, decreased attachment levels, gingival inflammation, higher proportions of putative pathogens and GCF enzymes. (Ramachandra *et al.*, 2011)

### Antibodies

Patients with various forms of periodontal disease produce antibodies to antigens from periodontopathic bacteria and can be detected in serum, saliva, gingival tissues and GCF. These mainly include IgG, IgM and IgA. The relationship of antibodies has been studied in various ways. These include measuring the total amount of Ig, the relative amounts of IgG subclasses and specific antibody titres to antigens from various periodontal bacteria.

These relationships are complex and are difficult to understand. The relationship of specific antibodies in serum and GCF has been shown to be higher in some patients and lower in others, with considerable variation from patient to patient and site to site. (Tabata *et al.*, 2005)

## Complement proteins

Complement proteins present at the site of inflammation, and the split fragment C3 and Factor B have been detected during experimental gingivitis and has been shown to be associated with disease activity. (Taba *et al.*, 2005) Neopterin is a well known marker of immune system, and its concentrations in body fluids have been used as degree of activation and also significantly correlates with the number of teeth with deep pockets in mouth. (Ozmeriç *et al.*, 2002) Salivary platelet aggregating factor (PAF) is significantly raised in chronic periodontitis. Cytokines like IL-1, IL-2, tumor necrosis factor (TNF) and prostaglandins also act as inflammatory markers in periodontal disease. (Rasch *et al.*, 1995)

## Connective tissue degradation products

The detection of the breakdown products of macromolecules of connective tissue (collagens I, III, V; proteoglycans, hyaluron and fibronectin) and basement membrane (which include collagen IV and laminin) could be an indicative of tissue breakdown. Huynh *et al.* in 2002 (Huynh *et al.*, 2002) suggested that fibronectin (FN) plays a role in a variety of cellular activities and have also been thought to have a role in inflammation. Hydroxyproline, collagen cross links, N-propeptide are the breakdown products of collagen. These breakdown products can also be used as biomarkers in periodontal diseases.

## Destructive enzymes

The inflammatory cells contain destructive enzymes within their lysosomes, which are normally used to degrade phagocytosed material. These enzymes are also capable of degrading gingival tissue components if released. Such enzymes may be released by inflammatory cells during their function, or when they degenerate or die and can be used as inflammatory marker. These include various *proteolytic enzymes* like collagenase, elastase, Cathepsin B, G and D, dipeptidylpeptidases, and tryptase. They also include various *hydrolytic enzymes* like aryl sulphatase,  $\beta$ -glucuronidase, alkaline phosphatase, acid phosphatase, myeloperoxidase, lysozyme and lactoferrin. Eley and Cox (Eley and Cox, 1996) in 1996 studied cathepsin B and evaluated its use as a predictor of attachment loss. Teng YT *et al.* in 1992 (Teng *et al.*, 1992) showed a twofold increase in mean MMP-9 levels in patients with recurrent attachment loss. Queiroz AC *et al.* in 2000 (Queiroz *et al.*, 2008) suggested that MMP-13 is expressed during bone formation and gingival wound healing. Commercial diagnostic kits are available like *Peiocheck* and *Prognostik* that are used to detect neutral proteinases (like collagenase) and serine proteinases (like elastase) in GCF samples respectively. (Hemmings *et al.*, 1997) Certain enzymes are released from dead cells and include aspartate amino transferase (AST) and lactate dehydrogenase (LDH). These are basically cytosolic enzymes that are confined to cell cytoplasm and are released by dead or dying cells. Since cell death is an integral and essential component of periodontal tissue destruction, they are released during the process and pass with the inflammatory exudates into GCF.

Gibert P *et al.* in 2003 (Gibert *et al.*, 2003) predicted alkaline phosphatase (ALP) as an indicator for the future periodontal breakdown. *Periogard* is a kit available for the diagnosis of AST levels in GCF. (Persson *et al.*, 1995)

## Bone markers

Several bone morphogenic proteins are involved in bone mineralization, and some connective tissue proteins also play an important role in this process. They are considered as possible markers of bone resorption and hence periodontal disease activity. These proteins include osteonectin, osteopontin, calprotectin, bone phosphoproteins (N-propeptide), osteocalcin, telopeptides of type I collagen, collagen I and proteoglycans (Kinney *et al.*, 2007). Kido *et al.* in 1999 (Kido *et al.*, 2000) suggested that calprotectin plays a role in immune regulation and of particular interest, its role as a proinflammatory protein for neutrophil recruitment and activation. Bowers *et al.* in 1989 (Bowers *et al.*, 1989) suggested that osteonectin is a single-chain polypeptide that binds strongly to hydroxyapatite and other extracellular matrix proteins including collagens.

## Genetic markers

Various genetic markers also exist nowadays. Genes encoding inflammatory cytokines like IL-1, TNF- $\alpha$ , the anti-inflammatory cytokine IL-10 and Fc-gamma were examined. 8-hydroxy-deoxyguanosine, a product of oxidative DNA damage, has been explored as biomarker for detecting periodontitis and also with periodontopathic bacterial species.<sup>22</sup> Development of *microchips, microfluidic and microelectronic system* has shown big improvement in detection of biomarkers. These *Oral Fluid Nanosensor tests*, handheld, automated, easy-to-use, integrated systems rapidly detect various salivary proteins and nucleic acid targets and other biomarkers in salivary and GCF samples. (Wong, 2006)

## Systemic biomarkers

Cellular components in peripheral blood are White Blood Cells, Red Blood Cells and Thrombocytes. Blood plasma components are Acute-Phase Proteins, Plasma Cytokine Levels, Fibrinogen and Von Willebrand factor. (Patil and Patil, 2011)

## Clinical use of a predictive diagnostic marker (Oswal *et al.*, 2010)

- To prevent destructive disease
- To prevent progression of the disease
- To identify high risk patients
- To target treatment to specific sites
- To monitor the effects of periodontal treatment

## Efficacy of diagnostic tests (Patil and Patil, 2011; Zia *et al.*, 2011)

There are a few principal concepts that must be understood by practitioners when they use diagnostic tests. The most basic of these concepts are:

- Gold standard
- Accuracy
- Sensitivity
- Specificity
- Positive predictive value
- Negative predictive value

A reliable predictive test used to assess biomarkers can predict future periodontal disease activity and thus enable site specific treatment to be given before irreversible damage had occurred. Test should have high positive and negative predictive values in diagnostic testing. They identify high risk patients and target treatment to specific sites. They are also helpful in monitoring the effects of periodontal treatment. (Zia *et al.*, 2011)

- Most of the biomarkers have low predictive value
- Very few biomarkers have been proved by human longitudinal studies
- Association of biomarkers with disease activity is weak independent

### Future directions

#### HIV diagnosis

OraSure oral specimen collection device that is placed between the buccal mucosa and buccal gingiva for 2 to 5 minutes to collect HIV-1 antibodies from the tissues of the cheek and gingiva. For different fluids (oral fluid, finger-stick or venipuncture whole blood or plasma specimens), the alternative test OraQuick (OraSure Technologies) provides accurate results for HIV-1 and HIV-2 in 20 minutes. (Patil and Patil, 2011)

#### Genetic susceptibility test

This system works by detection of two types of IL-1 genetic alleles, IL-1 $\alpha$  +4845 and IL-1 $\beta$  +3954 Potential use of genomics in the development of salivary diagnostics - RNA profiling and potential of salivary IL-8 levels to predict patients affected with squamous cell carcinoma. (Wong, 2006) Epithelial keratins occult blood, salivary ions such as calcium and phosphates, and serum markers such as cortisol.

#### Potential diagnostic tests worthy of development (Patil and Patil, 2011)

Test System	Type	Sample
Prototek	Bacteril Proteases	GCF
PGE <sub>2</sub> Assay	ARG-Gingipains	GCF
$\beta$ -Glucuronidase Assay	Enzyme Detection System	GCF

## DISCUSSION

In the field of periodontal diagnosis, there has been a steady growing trend during the last 2 decades to develop tools to monitor periodontitis. Currently, diagnosis of periodontal disease relies primarily on clinical and radiographic parameters. These measures are useful in detecting evidence of past disease, or verifying periodontal health, but provide only limited information about patients and sites at risk for future periodontal breakdown. (Kaufman *et al.*, 2000)

Ideally, diagnostic tests should demonstrate high specificity and sensitivity. Given the complex nature of periodontal disease, it is unlikely that a single marker will prove to be both sensitive and specific. A combination of two or more markers may provide a more accurate assessment of the periodontal patient. (Taba *et al.*, 2005) Novel technologies such as lab-on-a-chip and microfluidic devices have the potential to manage complex oral fluids, such as saliva and GCF, and to provide a determination of a patient's periodontal disease-risk profile, current disease activity, and response to therapeutic interventions. This approach should accelerate clinical decision-making and monitoring of episodic disease progression in a chronic infectious disease such as periodontitis. (Wong, 2006)

## Conclusion

Periodontal diagnosis and treatment planning always are very challenging which demands sound clinical judgement rather than the use of diagnostic biomarkers. Diagnostic testing should be considered an aid to the diagnostic process—not a device or procedure that provides the diagnosis.

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