

BIOMARKER: RE-DESIGNED, RE-INVENTED, RE-ENGINEERED- A REVIEW

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ABSTRACT

The current clinical diagnostic criteria which were introduced almost half a century ago continue to function as the basis of oral diagnosis in today's clinical practice. Evolvement with time has now brought us to the era of biomarkers. It's a new paradigm for periodontal diagnosis which is of immense benefit in managing periodontitis patients. Biomarkers are tell – tale molecules that can be used to monitor health status, disease onset, treatment response and outcome. These biomarkers can be obtained from blood components such as serum or plasma. However because of it being an invasive procedure other body fluids such as saliva and GCF are being considered for potential source of biomarkers. The various methods of collection and their high sensitivity assay development have led to the biomarkers being a promising future for periodontal diagnosis

Keywords: *Biomarkers, Saliva, GCF, periodontal diagnosis*

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INTRODUCTION

The current clinical diagnostic criteria which were introduced almost half a century ago continue to function as the basis of oral diagnosis in today's clinical practice. Evolvement with time is now brought us to the era of biomarkers. It's a new paradigm for periodontal diagnosis which is of immense benefit in managing periodontitis patients. Biomarkers are tell – tale molecules that can be used to monitor health status, disease onset, treatment response and outcome. Till date, the mere presence of a biomarker has been used to diagnose a disease, but this very life of a biomarker has led curious minds to predict the occurrence of the disease. Thus, re designing, re engineering and reinvention techniques for biomarkers have been increasingly used to enhance the predictability of periodontal disease. Need For Periodontal Disease Indicator The diagnosis of active phases of periodontal disease and the identification of patients at risk for active disease are challenges for clinical investigators and practitioners alike. Researchers are confronted with the need for innovative diagnostic tests that focus on the early recognition of the microbial challenge to the host. Optimal innovative approaches would correctly determine the presence of current disease activity, predict sites vulnerable for future breakdown and assess the response to periodontal interventions (Balwant et al 2008). A new paradigm for periodontal diagnosis would ultimately improve the clinical management of periodontal patients. Biomarkers can be categorized into five broad categories. Gingival crevicular fluid

biomarkers.; Salivary biomarkers.; Bonebiomarkers.; Proteomicbiomarker.; Genomic marker Gingival crevicular fluid Biomarkers Gingival crevicular fluid provides a non-invasive means of studying the host response factor by change of constituents in the fluid. The inflammatory exudate from gingival microcirculation cross inflamed periodontal tissue and en route collects molecules of potential interest from the local inflammatory reaction (McLaughlin et al 1996). Such factors are now finding value as potential diagnostic or prognostic markers of the Periodontium in health and disease (**Table.1**)

Salivary Biomarkers

Saliva is a mirror of oral and systemic health .Saliva is a secretion of the salivary and mucous glands and is of major importance in the maintenance of oral health. The fluid is readily accessible via a very non-invasive collection method, and contains locally produced microbial and host response mediators like GCF (Miller et al 2006) (**Table.2**).

Biomarkers of Bone Metabolism

Out of 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. . With mounting evidence for a relationship between osteoporosis and oral bone loss, investigators have sought to develop better biologic markers to determine and predict oral bone loss (Ma J et al 2000) (**Table.3**)

Proteomics - The New Era of Periodontics

Proteins are vital parts of living organisms, as they are the main components of the physiological metabolic pathways of cells.

The word “proteome” is a blend of “protein” and “genome”, and was coined by Marc Wilkins in 1996. The term “proteomics” was first coined in 1997 (James, 1997). Recent progress in tissue isolation, protein separation, quantification, sequence analysis, and structural and interaction proteomics offers great promise for bringing periodontal physiology and pathology into the modern era. Yet remarkably few applications of proteomics to the analysis of periodontal tissues have been reported

Genomic markers

The diagnostic and therapeutic benefits of deciphering the genetic basis of periodontal disease susceptibility and identifying allelic variants of genes have been widely realized during the last 10 years to be substantial. Consequently, reports of genetic polymorphisms associated with periodontal disease are increasing, and strong evidence supports the proposal that genes play a role in the predisposition to and progression of periodontal disease. . A number of studies have examined links between polymorphisms within host response factors and aggressive periodontitis. These include examination of genes encoding inflammatory cytokines such as IL-1 and TNF- α , the anti-inflammatory cytokine IL-10 and the Fc-gamma receptors (Morgan et al 1987).

Potential genomic markers are:

Cytokines Interleukin-1: Polymorphisms of the interleukin (IL)-1 gene have been proposed as potential genetic markers for periodontal diseases. Many investigators have reported a positive association between periodontitis and the presence of specific polymorphism of the IL-1 gene.

Tumor necrosis factor- α : Polymorphisms in the promoter region of the TNF-agent at positions 238 (G to A) and 308 (G to A) have been reported. Human leukocyte antigens: The human leukocyte antigen (HLA) complex plays an important role in immune responsiveness and may be involved in antigen recognition of periodontal pathogens. Polymorphisms of HLA-DR molecules in patients with periodontitis found a significant association between several DRB1 alleles and the disease (Hernandez et al 2006).

Matrix metalloproteinase: It is difficult to relate single nucleotide polymorphisms of matrix metalloproteinase genes with periodontitis. Matrix metalloproteinases are one of the most important groups of enzymes involved in periodontal connective tissue destruction (Kivela-Rajamaki et al 2003). **Cathepsin C:** Whether the pathogenetic role of cathepsin C gene variants also relates to types of periodontitis other than syndrome-associated periodontitis remains to be confirmed. Interestingly, Hewitt et al. have recently reported a decreased cathepsin C activity associated with the development of chronic periodontitis in patients who do not suffer from any syndrome such as Papillon-Lefevre syndrome. Genetic analyses for diagnosis of periodontitis. Various stages in the progression of periodontitis may be under genetic control, predisposing an individual not only to the initiation and progression of periodontitis, but also to the outcomes of treatment. Recognizing this role for genetic control means that risk assessment could well be served and targeted through genetic analyses. For a multifactorial disease such as

periodontitis, genetic diagnosis must be combined with an assessment of environmental factors.

Biochemical markers and their tests

Aspartate aminotransferase:

- Periogard™ periodontal tissue monitor system [Xytronyx Inc, San Diego, LA, USA]
- Pocket watch™ [Steri Oss Inc., Yorba Linda, CA, USA].

Alkaline phosphatase:

Para nitrophenyl phosphate method (PNP)

1. Neutral proteases :Periocheck (Bowers and Zahradnik 1989).
2. C- reactive protein : Measured by:

ELISA

- Immunoturbidometry
- Rapid immune diffusion
- Visual agglutination
- β - glucuronidase: Commercial available kits: Is being developed by ABBOTT laboratories, North Chicago, USA.
- Osteocalcin and pyridolone cross linked carboxy terminal telopeptide type I collagen (ICTP): ELISA

Evalusite (KODAK): commercially available to detect antigens for *P. gingivalis*, P intermediary .Matrix Metallo proteinases (MMP): Site specific Dipstick test TOPAS (Toxicity Prescreening Assay): Detects the indirect presence of bacteria by 2 markers of gingival infection:

- Bacterial toxin
- Bacterial proteins

BANA: 4 bacterial species (*T. denticola*, *P. gingivalis*, *B. forsythus*, *Capnocytophaga*) have a trypsin like enzyme Perioscan (Oral B laboratories) : chair- side test kit which uses BANA test for bacterial trypsin like proteases.

CONCLUSION

Despite advancements made in the areas of periodontal disease diagnosis, only limited research has been conducted to identify biomarkers that predict disease progression (PDP) prior to radiographic and clinical manifestations. The use of proteomics and gene expression will advance the diagnosis and treatment of various oral pathological conditions. Current proteomics analyses have the capacity to provide new insights into the repertoire of expressed proteins and some inkling of their interactions ,at a more global level than previously considered. Moreover, new diagnostic technologies such as nucleic acid and protein microarrays and micro fluids 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.

Table 1: Gingival crevicular fluid Biomarkers

Products and mediators of inflammation	Host derived enzymes	Tissue breakdown products
Matrix metalloproteinases	Aspartate aminotransferase	Glycosaminoglycans
Prostaglandins	Neutral protease	Hydroxyproline
Cytokines	Collagenase	Fibronectin
Antibacterial antibodies	Glucoronidase	Connective tissue proteins
Total protein and acute phase protein	Lactate dehydrogenase	

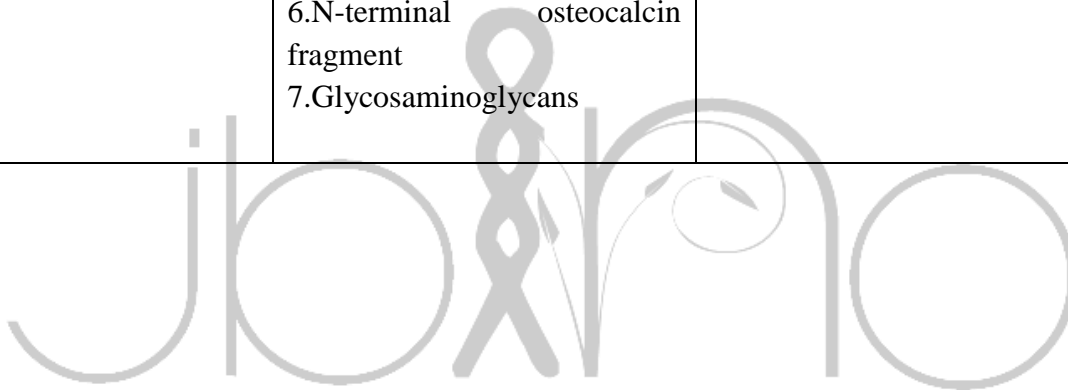
Table 2: Salivary Biomarkers

Marker	Relationship with periodontal disease	Type of periodontal disease
Immunoglobulins(IgA, IgM, IgG)	Interfere in adherence and bacterial metabolism / increased concentration in saliva of periodontal patients	Chronic and aggressive
Mucins	Interfere with the colonization of Aggregatibacter actinomycetemcomitans	Aggressive
Lysozyme	Regulates biofilm accumulation	Chronic
Lactoferrin	Inhibits microbial growth / increased correlation with A. actinomycetemcomitans	Aggressive
Histatin	Neutralizes lipopolysaccharide and enzymes known to affect the periodontium	Chronic and aggressive
Peroxidase	Interferes with biofilm accumulation / increased correlation with periodontal patients	Chronic
C-reactive protein	Increased concentration found in serum and saliva of periodontal patients	Chronic and aggressive

Table 3: Biomarkers of bone metabolism

Bone formation markers	Bone resorption markers	Bone growth factors
Type I procollagen propeptide proliferation -C terminal	Pyridinium cross link -Urine Pyridinoline (PYP), deoxypyridoniline (DPD) HPLC method	1. Bone morphogenetic proteins (BMPs 1-15) 2. Fibroblast growth factors (FGFs 1-9)

propeptide fragment (PICP) -N terminal propeptide fragment (PINP) 2. Alkaline phosphatase -Total alkaline phosphatase (AL-P) -Bone alkaline phosphatase (BAI-P) 3. Osteocalcin bone Gla protein mineralization -C terminal fragment -Mid portion -Intact	-Urine free deoxypyridinoline (FDPD) 2. Pyridinium crosslink collagen peptide fragment -Serum C-terminal telopeptide (ICTP) -Urine C-terminal telopeptide (CTX, crosslaps) -Urine N-terminal telopeptide (NTx, osteomark) 3. Tartrate resistant acid phosphatase (TRAP) 4. Galactosyl hydroxylysine (GHYL) 5. Hydroxyproline 6. N-terminal osteocalcin fragment 7. Glycosaminoglycans	3. Insulin like growth factors (IGF-I and II) 4. Platelet derived growth factor (PDGF) 5. Transforming growth factor- β (TGF- β) 6. Vascular endothelial growth factor (VEGF)
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