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BONE TURNOVER BIOMARKERS OF ORAL FLUIDS: A REVIEW

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ABSTRACT

Diagnostic techniques conventionally used to assess periodontitis are clinical and radiographic measurements. These methods are inherently limited only to a historical perspective and lacks to identify and provide knowledge about the highly susceptible individuals who are at a greater risk for future bone loss.[1] Biomarkers not only diagnose the disease but also predicts the risk of future disease activity by simple and affordable means.[2] Bone remodeling is a process of bone formation and resorption. Bone turnover markers (BTMs) assess the rate of bone turnover. BTMs detected in GCF (gingival crevicular fluid) or saliva of patients with periodontitis provides information about the extent of alveolar bone involvement and predicts the susceptibility of individuals towards risk of bone loss. Hence, detection of these biomarkers, aids in diagnosis of periodontitis at an early stage and also reduces the disease severity and progression.[1]

KEYWORDS: Bone turnover biomarkers, periodontal disease, peri-implant disease.

INTRODUCTION

Periodontal diagnostic procedures provide useful data to clinician regarding present periodontal disease and severity, based on which treatment is planned. **Biomarkers** play a

significant role in biologic sciences and have begun to assume a prominent role in diagnosis, monitoring of treatment outcomes, and drug discovery. It also provides information on the future risk of bone loss.[3]

Bone constantly undergoes remodeling, which is an inevitable process, in which the rate of deposition of bone is faster than resorption, to maintain bone homeostasis. Alveolar bone loss is a critical aspect of periodontitis. Based on this fact, various studies have identified the bone turnover biomarkers in GCF and saliva of periodontitis patients, that were closely related to disease progression and severity.[1]

Osteoporosis is a bone disease, characterized by reduced bone mass and bone mineral density, which increases the risk factor of bone fracture. Proper management of osteoporosis involves early diagnosis of the disease, which could be possible by the specific biomarkers of bone resorption.[4]

BIOMARKERS

Biomarker is defined as **"A characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention.[5]**

Classification

Table 1: General classification of biomarkers.[6]

Proteomic	Genetic	Microbial	Others
Cystatins **αglucosidase** Acid phosphatase **Alkaline phosphatase** Aminopeptidase **Lactoferrin, Gelatinase** Translactoferin **IgM, IgG, IgA** MMP-13, MMP-8, MMP-9 **Cathepsin B, Osteocalcin** Osteopontin, Osteonectin **Elastase** Platelet-activating factor **Epidermal growth factor** Platelet-derived growth factor, Trypsin **Esterase** Pyridinoline crosslinked carboxy	Cathepsin **C-gene mutation** Collagen gene mutation **IL-1 polymorphisms** IL-10 polymorphisms **Tumor necrosis factor** Polymorphisms	Aggregatibacter actinomycetem comitans **Campylobacter rectus** Mycoplasmas **Porphyromonas gingivalis** Prevotella intermedia **Pepto streptococcus Micros** Prevotella nigrescens **Treponema denticola** Tannerella forsythia	Calcium **Cortisol** Hydrogen sulphide **Methyl mercaptan** Pyridine

terminal telopeptide **Fibronectin** Vascular endothelial growth factor		**Treponema socransky**	

Markers from microbial plaque

a) Lipopolysaccharides (Endotoxin)
b) Bacterial Enzymes
c) Metabolic End-products

Markers from host cells

a) Acid and Alkaline Phosphatases
b) Glycoprotein-degrading Enzymes
c) Proteinases
d) Aspartate aminotransferase
e) Alkaline phosphatase
f) Lactate dehydrogenase
g) Matrix metalloproteinases (MMPs)
h) Cathepsin B
i) Leukotrienes B4
j) Prostaglandins E2
k) Substance P
l) Platelet activating factor
m) Cystatins
n) CD14

Markers of connective tissue degradation and cell death

a) Lysozyme
b) Lactoferrin
c) Myeloperoxidase
d) Glycosaminoglycans
e) Lactate dehydrogenases
f) Fibronectins
g) Hydroxyproline-containing peptides.[7,8,3]

Bone turnover biomarkers

A) Enzyme activity markers of bone formation (connected with osteoblast activity):

a) Alkaline Phosphatase (ALP)
b) Osteocalcin (OC)
c) C-terminal pro-peptide of type I procollagen (PICP)
d) N-terminal pro-peptide of type I procollagen (PINP)
e) Osteoprotegerin (OPG)
f) Osteopontin
g) Osteonectin

B) Enzyme activity markers of bone resorption (connected with osteoclast activity):

a) Hydroxyproline
b) Pyridinoline (PYD)
c) Bone sialoprotein (BSP)
d) Tartarat-resistant acid phosphatase (TR-ACP)
e) Free gamma carboxy-glutamin acid (GLA)
f) Deoxypyrindoline (DPD, d-Pyr)
g) Cross-linked C-terminal of type I collagen (ICTP)
h) Cross-linked C-terminal telopeptide of type I collagen (fragments alpha-CTX, beta-CTX)
i) Cross-linked N-terminal telopeptide of type I collagen (fragments NTX)
j) RANKL.[9,10,1]

Bone Turnover Biomarkers of Oral Fluids

Advances in bone cell biology over the past decade have resulted in several new BTMs for measurement of bone homeostasis. Having recognized a relationship between osteoporosis and oral bone loss, investigators have sought to develop better biochemical markers to determine oral bone loss.[11]

Osteoclasts, which are tartrate-resistant acid phosphatase (TRAP)-positive multinucleated giant cells, play a central role in bone destruction.[12] Bone resorption is mediated by osteoclasts that exhibit specific abilities to degrade organic and inorganic components of bone.[13]

Alkaline phosphatase (ALP)

Alkaline phosphatase, a biomarker of osteoblast activity and bone formation, is a glycoprotein and hydrolase enzyme, mainly present in the hepatic, renal and osteogenic cells.[14,1] Ester bonds are hydrolyzed by ALP, at an alkaline pH, due to which levels of phosphate ions are raised in serum/plasma. ALP plays a prominent role in cementogenesis, maintenance of bone homeostasis and calcification process. It also participates actively in the bone remodeling phase.[1]

Osteoprotegerin (OPG): It plays an important role in osteogenesis and bone homeostasis. It is a glycoprotein having a high affinity for receptor activator of nuclear factor kappa-B ligand (RANKL) and suppresses its activity by inhibiting osteoclasts formation.

Osteocalcin: Most abundant protein found in extracellular matrix of bone, it contains glutamic acid residues.[1] Predominantly synthesized by osteoblasts, osteocalcin plays an important role in bone formation and turnover. It exhibits chemo-attractive activity for osteoclast progenitor cells and monocytes, and its synthesis in vitro is stimulated by 1,25-dihydroxyvitamin D3.[3]

It actively binds to Ca^{++} ions present in hydroxyapatite crystal lattice of bone and moves freely as decarboxylated form in serum/plasma. In bone, it is present as inactive carboxylate form. Osteocalcin is a marker of bone formation, but due to its role in recruiting osteoclasts to site of bone resorption, it is now accepted widely as a bone turnover marker.[1] Its increased levels are associated with rapid bone remodelling.[6]

Osteonectin: Bowers (1989) suggested that osteonectin is a single-chain polypeptide that binds strongly to hydroxyapatite and other extracellular matrix proteins including collagen.[3] Osteonectin/ Secreted protein, acidic, rich in cysteine **(SPARC),** is a protein that is secreted by fibroblasts, osteoblasts and red blood cells. It is chemo-attractant to Ca^{++} ions present in bone and its levels in bone matrix corresponds to the bone formation process during bone remodeling. It is present in active osteoblasts and young osteocytes (but not in inactive osteocytes) and therefore it is considered suitable as a marker for differentiation of osteogenic bone cells indicating bone formation.

Although the function of SPARC has not yet been determined, its ubiquitous expression and association with rapidly remodelling tissues is indicative of a fundamental biological role. By

recruiting the osteoblasts to site of active bone deposition, SPARC upregulates bone formation. Hence, osteonectin is considered as a predictive marker of bone formation.[9,1] It also plays a vital role in early phase of mineralization.[6]

Osteopontin/ Bone Sialoprotein -I(BSP-I): Osteopontin (OPN) is a glycoprotein found in non-mineralized tissues.[1] In bone matrix, it is highly concentrated at sites where osteoclasts are attached to the underlying mineral surface. OPN is released by both osteoblasts and osteoclasts.[6] Hence, plays a dual function in both bone mineralization and resorption.[1] It has a high content of serine, asparagine and glutamate. A stretch of aspartate residues in osteopontin are implicated in hydroxyapatite binding.[9] It is a single-chain polypeptide with a molecular weight of approximately 32,600.[10]

It enhances dentine formation during bone remodeling process.[1]Highest levels of osteopontin expression are observed in pre-osteoblastic cells early in bone formation and by mature osteoblasts at sites of bone remodelling.

C-terminal propeptide of type I procollagen (PICP)

It is a specific proliferating osteoblast and fibroblast product, is found in the bone, skin and soft tissues, associated with bone formation.

N-terminal propeptide of type I procollagen (PINP)

It is found in the bone and skin and is a specific proliferating osteoblast and fibroblast product, as PICP and is also partially incorporated into skeletal matrix. PINP is associated with bone formation.[9]

Receptor activator of nuclear factor kappa-β ligand (RANKL): RANKL is a cytokine, which acts as a gene regulator and ligand for the receptor RANK. RANK results in alveolar bone resorption, as it controls the activation, proliferation, and differentiation of osteoclasts.[1]

Pyridinoline cross-links: They represent a class of collagen degradative molecules that include deoxypyridinoline, pyridinoline, N-telopeptides, and C-telopeptides. Pyridinoline and deoxypyridinoline are mature inter-molecular cross-links of collagen. Subsequent to osteoclastic bone resorption and collagen matrix degradation, pyridinoline, deoxypyridinoline, and amino- and carboxyterminal cross linked telopeptides of type I collagen are released into the circulation.

Elevated serum 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.[3]

Carboxyterminal telopeptide of type I collagen or pyridinoline (ICTP): ICTP is a 12- to 20-kDa fragment of bone type I collagen that is released into the circulation subsequent to osteoclastic bone resorption and collagen matrix degradation by proteases or bacterial collagenase.[15] Increased ICTP levels are directly proportional to increased collagen degradation, during periodontal disease progression.[3]

Deoxypyridinoline (DPD)

Deoxypyridinoline is a specific marker of bone resorption. It is formed by the reaction of side-chains of a lysine molecule and two hydroxylysine molecules. DPD is found mostly in bones, not so much in dentine.

Tartarate – resistant acid phosphatase (TR-ACP)

Osteoclasts secrete TRACP-5β into the blood circulation as a catalytically active enzyme that is inactivated and degraded to fragments in the circulation. Thus, all catalytically active TRACP-5 β molecules measured in the serum are freshly liberated from the osteoclasts, providing a sensitive resorptive index.[9]

N-terminal telopeptide type I collagen (NTx): NTx is a short telopeptide, released as a byproduct of bone resorption, whose increased levels in GCF predicts the severity of alveolar bone destruction.[1]

C-terminal telopeptide of type I collagen (BETA-CTX)

Beta-CTX is generated from the organic matrix of type I collagen, by lysosomal cathepsin K attack. Later in the resorption process, matrix metalloproteinases break down cross-link peptides from the carboxy-terminal telopeptide areas of type I collagen into ICTP.[13]

Matrix metalloproteinases (MMPs): MMPs are the zinc-dependent endopeptidases derived predominantly from polymorphonuclear leukocytes during acute stage of periodontal disease.[16]

MMPs can be divided into five major groups

A) Collagenases (MMP-1, -8, -13)

B) Gelatinases (MMP-2, MMP-9)
C) Stromelysins (MMP-3, -10, -11)
D) Membrane-type MMPs (MMP-14, -15, -16, - 17) and
E) Others.

MMPs can degrade almost all components of extra-cellular matrix and basement membrane and their excess activity leads to periodontal tissue destruction. Tissue inhibitors of matrix metalloproteinases (TIMPs) regulate the activities of these enzymes and TIMP-1 is more effective on interstitial collagenases. An imbalance between TIMPs and MMPs result in the pathological tissue destruction observed in periodontitis.

Cathepsin K: a cysteine protease, is capable of hydrolysing extracellular bone matrix proteins, is highly expressed in osteoclasts, and it is a well-known marker of osteoclast activity.[17]

Cytokines

Interleukin -1β (IL-1β): is associated with higher alveolar bone loss.[18]
Salivary levels of IL-1β reflects periodontal disease severity.[19]

Interleukin-6 (IL-6): is a pleiotropic cytokine that communicates inflammatory signals with a number of cell types and can elicit bone resorptive processes.[20]

Interleukin-17 (IL-17): is a pro-inflammatory cytokine produced by activated Th17 cells that induce a distinct profile of effector cytokines, which may exacerbate inflammation and tissue damage. Th17 cells are found in human periodontal disease (PD) as well as IL-17 expression in the alveolar bone of subjects with periodontitis, suggests a causal link between IL-17 and bone destruction.[21]

Macrophage inflammatory protein-1α (MIP-1α): is a chemokine that is frequently recruited into the sites of inflammation and they induce bone resorption.[20]

Salivary MIP-1α levels are significantly elevated in periodontal disease and demonstrate strongest correlation with clinical parameters of periodontal disease.[13]

Members of the interleukin-1 (IL- 1) family are thought to be the key mediator when the host responds to microbial invasion, inflammation, tissue injury and immunological reactions.

Both directly and indirectly, IL-1 plays a role in the T cell activation, induction of fever, degradation of cartilage, and healing of wounds. The arachidonic acid metabolite **Prostaglandin E2 (PGE2)** is an inflammatory mediator released from various cell membranes by action of the enzyme cyclo-oxygenase. Among inflammatory mediators, interleukin-1β and PGE2 are actively involved in alveolar bone resorption.[22]

Oncostatin-M(OSM): is a member of the interleukin (IL)-6 family of cytokines. In the cascade of periodontal inflammation, human T cells and monocyte lineages can synthesize and secrete OSM and IL-6 in response to bacterial products, which play a key role in regulating periodontal bone resorption, by acting on both osteoblast and osteoclast receptor activator of nuclear factor-κ B ligand (RANKL) regulation.[23]

Chemokine CXCL10: also known as IP-10, is a 10 kDa protein, that is functionally categorized as an "inflammatory" chemokine. It is secreted by various cell types, such as monocytes, neutrophils, endothelial cells etc., and appears to have a direct influence on osteoclastogenesis.[24]

Hydroxyproline: It is present in newly synthesized and mature collagen. Bone, skin, cartilage and soft tissues are the tissues of origin of hydroxyproline, which are bone resorptive biomarkers.

Free gamma carboxyglutamin acid (GLA)

GLA originates from bone proteins (e.g. Osteocalcin, matrix Gla protein) and coagulation factors. They are recognized as bone resorptive biomarkers.[9]

Sclerostin

Sclerostin, a protein expressed by cells involved bone metabolism, such as osteocytes, hypertrophic mineralized chondrocytes and cementocytes, is considered a biomarker that negatively regulates bone formation by reducing the mineral content of bone, both the amount of trabecular bone, as the thickness of the cortex, in a way antagonizing with bone morphogenic proteins. Sclerostin is reported to be up-regulated by pro-inflammatory cytokines as well as by excessive load. Based on these facts, this bioprotein may also serve as an indicator of peri-implantitis.[25]

Role of Bone Turnover Biomarkers in Periodontal Disease

Pathogenesis

Innate host defence responses are triggered by bacterial lipopolysaccharide and other microbial components and products. As a result, polymorphonuclear leukocytes, monocytes and activated macrophages are recruited to the site, and release numerous cytokines, such as prostaglandin E2, tumour necrosis factor (TNF) and interleukins, which direct further inflammatory processes. As a consequence, matrix metalloproteinases (MMPs), are produced by alveolar bone and polymorphonuclear leukocytes. Later, pyridinoline cross-linked carboxyterminal telopeptide and osteocalcin are released into the surrounding area and transported through gingival crevicular fluid into the periodontal pocket.[26]

Table 2: Bone-related biomarkers of oral fluids associated with periodontal disease.

Biomarker	Detection Medium	Role in Periodontitis	Human Studies
Alkaline phosphatase (ALP)	Gingival crevicular fluid (GCF), saliva	✓ Associated with the treatment planning and outcome.[27] ✓ Remarkably increased activity in the acute phase of periodontal disease, and after periodontal therapy.[28] ✓ There exists a significant positive correlation between gingivitis, probing pocket depth (PPD), and elevated ALP levels.[1]	➢ **Koss et al.** conducted a case-control study, investigating the ALP levels in normal and periodontitis patients. They observed the levels of ALP to be increased in saliva of periodontitis patients, compared to controls.[1] ➢ **Sarita Dabra** and **Preetinder Singh** conducted a prospective study, in which, they examined the activities of salivary ALP in patients with periodontal disease, before and after periodontal treatment. The experimental groups consisted of twenty gingivitis and twenty periodontitis patients. Control group had healthy subjects. Results found that salivary ALP levels are significantly increased in patients with periodontal disease and the salivary enzyme levels decrease in concomitance with the periodontal treatment.[28]
Receptor activator of nuclear factor kappa-β ligand (RANKL)	Gingival crevicular fluid, saliva	✓ Responsible for increased osteoclastic activity.[1]	➢ **LS Branco et al**. investigated GCF samples of aggressive periodontitis patients and found probing pocket depth (PPD) and clinical attachment loss (CAL) to be significantly correlated with RANKL. ➢ In a study done by **Ochanji et al**., the values of RANKL / OPG ratio were

			positively correlated with periodontal disease severity.[1]
Deoxy pyridinoline (DPD)	Gingival crevicular fluid, serum, saliva	✓ Specific for bone degradation.[26]	➢ **Suhail Syed et al**. conducted a cross-sectional study in 15 periodontally healthy subjects and 15 chronic periodontitis patients, estimated GCF and blood samples for DPD. Significantly higher GCF DPD levels were detected in chronic periodontitis patients when compared to periodontally healthy group.[29]
Carboxy terminal telopeptide of type i collagen or pyridinoline (ICTP)	Gingival crevicular fluid, saliva	✓ Actively participates in collagen degradation.[1]	➢ Salivary levels of ICTP, were evaluated by **Mishra et al,** in patients with chronic periodontitis and gingivitis. Results depicted a much higher ICTP levels in patients with periodontitis. ➢ **Quesada JG** and **Alvarez SR** observed an increased GCF level of ICTP in chronic periodontitis patients.[1]
Cross-linked n-terminal telopeptide of type I Collagen(NTX)	Gingival crevicular fluid, saliva	✓ Promotes bone resorption.[1]	➢ **Aruna G** detected that the GCF levels of NTX were higher in chronic periodontitis patients and positively correlated with clinical periodontal parameters.[1]
Osteocalcin	Gingival crevicular fluid, saliva	✓ Stimulates bone formation and is a potential diagnostic bone turnover marker in periodontitis.[1]	➢ **Kunimatsu et al** reported a positive correlation between GCF osteocalcin amino terminal peptide levels and clinical parameters in a cross-sectional study of periodontitis and gingivitis patients. ➢ **Nakashima et al** reported significant GCF osteocalcin levels from periodontitis and gingivitis patients. Osteocalcin levels were also significantly correlated with pocket depth and gingival index scores.[3]
Osteonectin	Gingival crevicular fluid	✓ bone-regulating protein and maintains periodontal ligament (PDL) hemostasis.[1]	➢ **M Baeza et al**. analyzed osteonectin levels in GCF of 106 chronic periodontitis patients and found significantly raised concentrations of osteonectin.[1]
Osteopontin (OPN)	Gingival crevicular fluid	✓ Significant role in bone mineralization and resorption.[26]	➢ **Kido et al**. reported in his case-control study that increased levels of GCF osteopontin coincided with probing pocket depth values ➢ **Sharma** reported that GCF OPN concentrations increased proportionally with the progression

			of disease and GCF OPN levels were significantly reduced when non-surgical periodontal treatment was provided.[3]
CXCL10	Gingival crevicular fluid, saliva	✓ plays a role in progenitor osteoclast recruitment and activation of RANKL expression leading to bone resorption in periodontitis.[24]	➢ **Salwa Aldahlawi, Abdel-Rahman Youssef and Syed Shahabuddin** determined the concentration of CXCL10 in saliva, serum, and GCF samples between chronic periodontitis patients (n=31) with a periodontal probing depth (PD) of ≥4 mm and clinical attachment level (CAL) of ≥3 mm in >30% of the teeth and control group (n=25) having PD ≤3 mm and/or CAL ≤2 mm. CXCL10 level in GCF was higher in the periodontitis group as compared with the control group.[24]
Osteo-protegerin (OPG)	Gingival crevicular fluid, saliva	✓ Inhibits osteoclastic activity ✓ Increased in disease progression.[1]	➢ **Buduneli N** observed that OPG correlate positively with probing depth, bleeding on probing and clinical attachment level. It has also been reported to be present at lower levels in smokers than non-smokers who have chronic periodontitis.[13]
Matrix metalloproteinases (MMPs)	Saliva	✓ Increased osteoclastic activity.[16]	➢ **Balwant Rai et al,** in a study conducted among 20 patients with periodontitis, 18 patients with gingivitis and 15 healthy controls, observed elevated MMP levels patients with periodontitis. Salivary MMP-8 levels and the crevicular MMP-9 levels were higher in patients with gingivitis and periodontitis than the healthy controls. Reduced levels of MMP-2 were highly corelated with the clinical loss of attachment and bleeding on probing.[16]
Cathepsin K	Gingival crevicular fluid	✓ Increases with periodontal severity.[17]	➢ **Otogoto (2007)** investigated GCF levels of cathepsin K in 20 mild, 24 moderate, and 22 severe chronic periodontitis patients in comparison with 19 healthy control subjects. Cathepsin K was below the detection limit in the healthy control group, whereas it was detectable in all periodontitis samples.[17]
Interleukin-17 (IL-17)	Gingival crevicular fluid	✓ Exacerbates inflammation and tissue damage.[21]	➢ **Thiago Alvares et al**., collected biopsy samples by osteotomy in 18 patients with advanced periodontal

			disease (PD) and in 17 controls during third molar extraction, in which alveoloplasty was necessary after extraction of teeth with previous extensive periodontal damage. Patients presented increased accumulation of IL-17 in connective tissue next to bone; number of IL-17 was higher in PD regions than in control healthy tissues.[21]
Macrophage inflammatory protein-1α (MIP-1α)	Saliva	✓ Synthesis and distribution of pro-inflammatory cytokines.[30]	➢ **Nisha et al** designed a cross sectional study to estimate MIP-1 α levels in saliva of 75 patients [healthy (n = 25), gingivitis (n = 25) and chronic generalized periodontitis (n = 25)]. Results showed a substantial increase in the concentration of MIP-1 α with increasing severity of periodontal disease.[31]

Bone Turnover Biomarkers Associated With Peri-Implant Diseases

Table 3: Categories of biomarkers associated with peri-implant diseases.[32]

Interleukins (ILs)	Matrix metalloproteinases (MMPs)	Others
IL- 6,8,10,12 and 1β	MMP- 1, 3, 8 and 13	Alkaline phosphatase, Elastase, Prostaglandins E2, Cathepsin-K, Osteoprotegerin, Receptor activator of nuclear factor kappa-β ligand

Plagnat D et al conducted a cross-sectional study on 15 subjects, selected from a group of patients treated with implants. Pregnant women, as well as patients who required premedication, were not included. Eight subjects carried one or several implants with clinical signs of periimplantitis, and seven subjects carried one or several implants with clinically healthy peri-implant tissues. Significantly higher amounts of alkaline phosphatase (6 times) and elastase (13 times) were found in peri-implant sulcus fluid (PISF) around implants with peri-implantitis compared with healthy controls.[33]

Nomura et al collected PISF at 1, 2, 4, and 12 weeks after implantation, from 10 Osseo-integrated implants in 6 subjects and at first appointment from 6 subjects with 10 implants affected with peri-implantitis. They observed an increase in MMP-8 levels in peri-implantitis.[34]

Candel Martí E et al conducted a PubMed literature search of articles, using the key words "cytokine and dental implants". Fourteen articles were found and classified into two groups relating interleukin levels to:

a) Peri-implant disease
b) Their influence upon dental implant osteointegration without peri-implant disease.

It was concluded that the IL-6, 8, 10, and 12 levels are increased in peri-implantitis.[35]

ADVANTAGES OF BIOMARKERS

- Non-invasive nature of obtaining samples for analysis.[32]
- Reflects current periodontal disease activity as well as severity.[36]
- Beneficial in monitoring the response to treatment.
- Possibilities for the future applications in biotechnology and health care, especially in the field of diagnostics.[3]
- Accuracy
- Rapid chair-side diagnostic kit, providing enhanced patient assessment, that will allow oral health-care providers to improve prevention and treatment of periodontal diseases.[6,8]
- As salivary testing becomes more commonplace, the costs of biomarker detection could drop below.[10]
- Biomarkers can also be measured in peri-implant sulcus fluid.[32]

Future Directions of Oral Fluid Biomarkers

In the field of oral disease diagnosis, there has been a growing trend towards developing tools to monitor periodontitis. From periodontal probing to genetic susceptibility analysis and molecular assays for detection of biomarkers associated with the disease, substantial improvements have been made. Simultaneously, this evolution has promoted the discovery of new biomarkers and new therapeutic strategies, such as host modulation.

Development of a wide spectrum of marker factors will be a primary goal of periodontal research.[6] Personalized medicine is a medical model that uses genetic, genomic, environmental and clinical diagnostic testing to individualize patient care. A combined analysis is required to identify the set of biomarkers with the most favorable combination of sensitivity, specificity, reproducibility and correlations with established disease diagnostic criteria. Utilization of this model in oral health care, specifically in periodontology, has the

potential to provide discriminating patient stratification models to develop highly individualized diagnosis, prognosis and personalized treatment.

A medical testing that is not performed in a laboratory, yet at the patient's home, or the doctor's office, is referred to as Point-of-care (POC) diagnostics. By a POC device using saliva, patients could easily diagnose periodontitis at home and visit their periodontist accordingly. These self-performed tests should accelerate clinical decision-making and monitoring of periodontal disease progression. Currently, the activity MMP-8 lateral-flow POC immune-tests is a recently developed commercially available mouth-rinse that is practical, convenient and inexpensive test that takes just 5 minutes to predict, detect and monitor the course and treatment of periodontitis.

The value of biomarkers has been recognized and extensively explored using proteomic methods. These approaches are anticipated to be a useful biochip array amenable to low-cost POC devices. Advances in proteomics technology based on analysis of GCF proteome might have conclusive prognostic and diagnostic value leading to the identification of novel biomarkers of periodontal health or disease. Salivary proteomics detect even low levels of a specific biomarkers.

Folz, found a total of 327 GCF proteins in periodontally healthy individuals using a gel-free method that were analyzed directly by liquid chromatography–tandem, suggesting that they may be used as a reference in future proteomic studies on GCF biomarkers of periodontal disease. Later on, ***Baliban*** et al. proposed a large-scale proteomic analysis and mixed-integer linear optimization that provided a new insight into the identification of novel combinations of GCF-derived sets of biomarkers which can accurately discriminate between periodontal health or disease with greater than 95% predictive accuracy.[8]

However, additional observational and longitudinal studies are required to target the prognostic value and diagnostic accuracy of these techniques.[1]

CONCLUSION

The time is growing to a point where oral health-care providers will be able to utilize high-throughput biomarker validation tools for rapid chair-side testing. The focus has been on the development of miniature-sized 'chemical processing units' that process fluids and provide information that is relevant to the inflammatory, connective tissue-degradation and bone-loss

phases of periodontitis. As the specific biomarkers for periodontal disease and progression are determined through longitudinal analysis, it appears that the technology is prepared to meet the scientific discovery.[30]

Hence in this revolutionary era of oral-fluid based diagnostics, biomarkers are opted as better diagnostic tools for monitoring, diagnosis, and management of periodontitis, as they serve as an adjunctive method for the clinical parameters, which are still considered the most reliable methods for diagnosis of periodontal disease progression.[1,8]

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