DOI: 10.7860/JCDR/2015/11268.5438

Review Article

Dentistry Section

Bonebiomarkers in Periodontal Disease: A Review Article

V. SHANKAR RAM[1], PARTHIBAN[2], UMA SUDHAKAR[3], NIMISHA MITHRADAS[4], RAMACHANDRA PRABHAKAR[5]

ABSTRACT

Periodontitis, is an inflammatory state of the tooth supporting structures and if left untreated, the disease continues to progressive bone destruction and subsequent tooth loss. The increasing prevelance of periodontal disease paved way to the development of new diagnostic tests that could detect the presence of active disease,the course of the disease and its response to treatment. Bone is a metabolically active tissue and undergo continuous remodelling, a process that largely relies on the activity of osteoclasts to remove bone and of osteoblasts to form bone. In health ,bone resorption and formation are coupled to each other and its long term maintenance of skeletal balance is brought about by the systemic hormones and local mediators.In contrasts,during disease there is a pronounced in balance in bone turnover. Bone biomarkers which are produced either in health or in disease state are tell tale markers which would be used to monitor the health status. This review highlights, the recent advances in the use of biomarkers of bone remodelling,that could facilitate the screening, diagnosis and management of periodontal diseases.

Keywords: ALP, Biomarkers, ICTP, Osteocalcin, Osteonectin, Osteopontin

INTRODUCTION

Periodontitis is "an inflammatory lesion, mediated by complex host-parasite interactions, that leads to the loss of connective tissue attachment to root surface cementum and adjacent alveolar bone [1,2]. Post its initiation, the disease progresses with the loss of collagen fibers and attachment to the cemental surface, apical migration of the pocket epithelium, formation of deepened periodontal pockets, and the resorption of alveolar bone [3].

Bone is constantly undergoing the process of remodelling. In bone remodelling, the bone is constantly resorbed on a particular bony surface, followed by a phase of bone formation. In normal adults, there is a balance between the amount of bone resorbed by osteoclasts and the amount of bone formed by osteoblasts through coupling mechanism [4,5]. The coupling process ensures that the amount of bone removed is equivalent to the amount of bone laid down during the subsequent bone formation phase. The concept of bone remodelling is based on the hypothesis that, the osteoclastic precursors become activated and differentiate into osteoclasts, and this starts the process of bone resorption. This phase is followed by a bone formation phase. These two phases together determines the tissue turn over rate. The number of sites entering the bone formation phase is called the activation frequency [6].

To reach the current objective, the need for periodontal diagnostic tool which would provide adequate information for differential diagnosis, localization of disease, and severity of infection becomes all the more indispensable. These diagnostics should not only aim at mere diagnosis of the underlying disease but should also, serve as a basis for planning treatment and provide the means for assessing the effectiveness of periodontal therapy. In affected tissues, biochemical signaling involving three biological phases-inflammation, connective tissue degradation, and alveolar bone turnover contributes to the clinical morbidity. Circulating molecules in these biological phases have been detected at elevated levels in the gingival crevicular fluid, serum and whole saliva of patients who have periodontal disease making them putative biomarkers of the disease [7].

Many biomarkers in oral fluid represent inflammatory mediators, collagen degradation and bone turnover related molecules and therefore emerged as a possible disease activity. Information on bone remodelling status could be an early indicator of bone destruction which leads to progressive periodontal disease. Biomarkers of bone remodelling can be of two types,bone resorption markers and bone formation markers which reflect osteoclastic activity(degradation products of type1 collagen and osteoblastic activity(products of collagen synthesis,matrixproteins or osteoblastic enzyme). Bone resorption and bone formation are coupled processes and therefore in most situations any of these markers will reflect a change in bone turnover.

A biomarker is a substance used to indicate a biologic state. It can be 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 [8]. Non invasive means of detection of disease activity and treatment outcome could be the most desirable goal in health cause promotion and delivery.

Biochemical markers of bone turnover have proved to be a useful, non invasive and relatively an inexpensive tools for studying bone metabolism in population studies and are gradually becoming established in clinical practice. Their main use, however, is in monitoring response to treatment. The continued development of new markers of bone turnover will increase the knowledge of the pathophysiology of periodontitis.

Bone Markers [Table/Fig-1]

Parallel with better understanding of biochemical processes in bone and isolation and characterization of cellular components of skeletal matrix, the number of new potential biochemical markers of bone formation and resorption is increasing. Generally, markers are classified into the following groups [9]:

a. Enzyme activity markers of bone formation (connected with osteoblast activity) and of bone resorption (connected with osteoclast activity);

b. Bone matrix proteins and resorption products of organic skeletal matrix, which are released into circulation during bone formation and resorption;

c. Inorganic skeletal matrix markers (calcium, phosphorus which, above all, reflect calcium-phosphorus homeostasis).

Important criteria in critical judgement on whether to measure some bone formation or bone resorption markers are the following:

Biological factors like tissue specificity, effect of change in liver or kidney function on marker clearance, biological rhythm of the marker due to standardization of physiological sampling time, immobilization, etc.

Pre-analytical factors sample storage procedures, i. e. time and temperature, sample freezing and thawing, anticoagulant effect, etc.

Analytical specificity and accuracy microheterogeneity of markers as, e. g. , degree of ALP glycosylation, possibility of marker resorption into several different fragments as in case of osteocalcin, *bias* in methods due to non-harmonized calibrations, specificity of antibodies and, enzyme activity inhibitors.

Diagnostic validity the question of differences between markers considering their diagnostic sensitivity and specificity [9].

Alkaline Phosphatase

ALP is a non-specific hydrolase enzyme present in all bodily tissues, but is particularly concentrated in the liver, kidney and bone. It is a glycoprotein and membrane bound enzyme and hydrolyzes monophosphate ester bonds at alkaline pH, increasing local concentrations of phosphate ions. In the periodontium, alkaline phosphatase is a part of the normal turnover of periodontal ligament, root cementum formation and maintenance, and bone homeostasis. It is associated with the calcification process and an elevated ALP level commensurate with active bone remodelling. ALP and periodontal disease in an experimental gingivitis model showed a significant correlation between ALP and pocket depth and between ALP and inflammation [10]. As a predictive indicator for future periodontal breakdown, ALP therefore might serve as a marker in periodontal treatment planning and monitoring.

Osteocalcin

Osteocalcin, also called bone Gla-protein is a small (5. 4kDa) calcium-binding protein of bone, and is the most abundant noncollagenous protein of mineralized tissues [11]. It is a protein with a molecular mass of approximately 6kd, containing 49 aminoacids. Osteocalcin is predominantly synthesized by osteoblasts, odontoblasts and hypertrophic chondrocytes and it has an important role in both bone resorption and mineralization. Serum osteocalcin is presently considered a valid marker of bone turnover when resorption and formation are coupled, and a specific marker of bone formation when formation and resorption are uncoupled. It may be involved in recruiting osteoclasts to sites of newly formed bone and thus may function as a negative regulator. Elevated serum osteocalcin levels have been found during periods of rapid bone turnover, such as osteoporosis, multiple myeloma, and fracture repair.

Pyridinoline Cross-linked Carboxyterminal Telopeptide of Type I Collagen (ICTP)

Type I collagen comprises 90% of the organic matrix of bone and is the most abundant collagen in osseous tissue. Collagen degradation products have emerged as valuable markers of bone turnover in a multitude of osteolytic and osseous metabolic diseases. Pyridinoline crosslinks represent a class of collagen-degrading molecules that include pyridinoline, deoxypyridinoline, N-telopeptides,and C-telopeptides [12]. Pyridinoline and deoxypyridinoline are mature intermolecular cross-links of collagen. Subsequent to osteoclastic bone resorption and collagen matrix degradation, pyridinoline, deoxypyridinoline and amino-, and carboxy-terminal cross-linked telopeptides of type I collagen are released into the circulation. Because the cross-links and cross-linked telopeptides result from post-translational modification of collagen molecules, they cannot be reused during collagen synthesis, and are therefore considered to be specific to bone resorption. Given their specificity for bone resorption, pyridinoline cross-links represent a potentially valuable diagnostic aid in periodontics, because biochemical markers

Bone Formation Markers			
Total Alkaline Phosphatase (ALP); specific for bone formation only in patients with no liver or bile duct disease	Bone, liver	serum	colomitery
Bone Alkaline Phosphatase(B-ALP);specific osteoblast product; some procedures show cross reactivity with ALP liver isoenzyme	bone	serum	Colomitery, electrophoresis, precipitation, IRMA, EIA
Osteocalcin (OC, BGP); Specific osteoblast product; there are several reactive forms in blood; some can NASTATI during bone resorption	Bone, trombocytes	serum	RIA, ELISA, IRMA, ECLIA
C-terminal propeptide of type I procollagen (PICP); specific proliferating osteoblast and fibroblast product	Bone, skin, soft tissues	serum	RIA, ELISA
N-terminal propeptide of type I procollagen (PINP); specific proliferating osteoblast and fibroblast product; partially incorporated into skeletal matrix	Bone, skin	serum	RIA, ELISA
Bone Resorption Markers			
Marker	**Tissue Origin**	**Analytical Sample**	**Analytical Method**
Hydroxyproline, total and dialyzable (OH-Pro,OHP); specific for all fibrilar collagens and a part of collagen proteins, including Ciq, elastin;present in newly synthesized and mature collagen	Bone, skin, cartilage, soft tissues	urine	Colorimetry, HPLC
Pyridinoline (PYD, Pyr); high concentrations in cartilage and collagen: not present in skin;present only in mature collagen	Bone, tendon, cartilage	urine	HPLC,ELISA
Deoxypyrindoline (DPD,d-Pyr); high concentrations only in bone collagen: not present in cartilage or in skin; present only in mature collagen	Bone, dentine	urine	HPLC, ELISA
Cross-linked C-terminal of type I collagen(ICTP); high proportion from bone collagen in type I collagen; can partly originate from newly synthesized collagen	Bone, skin	serum	RIA
Cross-linked C-termianltelopeptide of type I collagen (fragments alpha-CTX, beta-CTX); in type I collagen; probably high proportion from bone collagen	All tissue containing type I collagen	Urine, serum	ELISA, RIA, ECLIA
Cross-linked N-terminal telopeptide of type I collagen (fragments NTX); in type I collagen; big proportion from bone	All tissues containing type I collagen	Urine (alpha/ beta), serum(only beta)	ELISA, RIA, ICMA
Hydroxylysine-glycosides (Hyl-Glyc); collagens and collagen proteins; glucogalactosyl-hydroxilysine is highly represented in soft tissue collagens and C1q; galactosil OHLys is highly represented in bone collagen	Bone, skin, soft tissue, serum complement	urine	HPLC, ELISA
Bone sialoprotein (BSP); synthesized by active osteoblasts and lay in extracellular bone matrix; it seems to express osteoclast activity	Bone, dentine, hypertrophic cartilage	Serum	RIA, ELISA
Tartarat-resistant acid phosphatase (TR-ACP); osteoclasts, thrombocytes, erthryocytes	Bone, blood	Plasma/ serum	Colorimetry, RIA, ELISA
Free gamma carboxyglutaminacid (GLA); resulted from bone proteins (eg. Osteocalcin, matrix Gla protein) and from coagulation factor	Blood, bone	Serum/ urine	HPLC

[Table/Fig-1]: Bone Remodelling Biomarkers

specific for bone degradation may be useful in differentiating the presence of gingival inflammation from active periodontal and peri-implant bone destruction.

Osteonectin

Secreted protein, acidic, rich in cysteine (SPARC)/osteonectin is a non structural matricellular glycoprotein secreted by osteoblasts, which binds calcium in bone and has an affinity for collagen. It is a normal component of bone matrix and is involved in cell-matrix interaction during tissue remodeling. Osteonectin is secreted as Ca-binding glycoprotein found in different cells, including osteoblasts, endothelial cells and fibroblasts. 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 osteogenetic bone cells indicating bone formation [13]. 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. SPARC has been characterized as a counter adhesive protein that modulates interactions of cells with the extracellular matrix.

Osteopontin

Osteopontin is a glycosylated phosphoprotein with a molecular mass of 41.5kd and is unusual because of its high content of serine, asparagine and glutamate. A stretch of aspartate residues in osteopontin are implicated in hydroxyapatite binding. Although osteopontin was considered to be a principle component of bone, osteopontin is found in several nonmineralized tissues including kidney, arterial smooth muscle cells, and at the luminal surface of epithelial cells of ductal tissues [14]. Highest levels of osteopontin expression are observed in preosteoblastic cells early in bone formation and by mature osteoblasts at sites of bone remodelling. Osteopontin is considered to play a significant role in both mineralization and resorption of bone . Secreted osteopontin is present in concentrated amount in areas of bone formation, and it has been implicated in the recruitment and stimulation of macrophages and lymphocytes in response to non-specific infections.

Pyridinoline (PYD); Deoxypyridinoline (DPD)

DPD is formed by reaction of side-chains of two hydroxylysine molecules and one lysine molecule, and pyridinoline (PYD) is formed by reaction of side-chains of three hydroxylysine molecules (both compounds have inborn immunogenetics and fluorescence). DPD is found mostly in bones, not so much in dentine, while PYD is located in bone collagen fibrils and cartilage and to a lesser extent in other tissues (tendons, ligaments, blood vessel walls). Since the bones have the most intensive remodeling, they are considered as the most important source. Deoxypyridinoline is considered a specific resorption marker because it is formed during collagen maturation (not during biosynthesis and therefore it appears only as a resorption product of the mature matrix).

Tartarate-Resistant Acid Phosphatase (TR-ACP)

Total TR-ACP activity measurement in serum, classified as a bone resorption marker, has many disadvantages. It is not used in diagnosis because of its low activity and stability. Osteoclasts secrete TRACP-5b into the blood circulation as a catalytically active enzyme that is inactivated and degraded to fragments in the circulation. Thus, all catalytically active TRACP-5b molecules measured in the serum are freshly liberated from the osteoclasts, providing a sensitive resorptive index.

DISCUSSION

Early diagnosis and treatment of progressive periodontits is important because of the irreversible nature of this disease. It should provide useful information to the clinician regarding the present periodontal disease type, location and severity. Optimal innovative approaches would correctly determine the presence of current disease activity, predict sites for future breakdown and assess the response to periodontal intervention.

Bone biomarkers of disease play an important role in life sciences and have begun to assume a greater role in diagnosis, monitoring of the ray outcomes and drug discovery [15]. The ability to monitor health status, disease onset & progression and treatment outcome through non invasive means is a desirable goal in health care promotion and delivery. The isolation and characterisation of cellular and extracellular components of the skeletal matrix have resulted in the development of the molecular markers that are considered to reflect either bone formation or bone resorption [16]. These are helpful tools in the diagnostic and therapeutic assessment of metabolic bone disease.

Alkaline phosphatase – a membrane bound glycoprotein is involved in the maintenance of alveolar bone and renewal of periodontal ligament. Gibert et al., [17] analysed serum levels of ALP from patients with chronic periodontal disease and compared with control patients. There was a positive relationship between attachment loss in the periodontal group and a drop in ALP activity in serum. ALP might serve as a marker in periodontal treatment planning and monitoring.

Elevated serum osteocalcin levels have been found during periods of rapid bone turnover, such as osteoporosis, multiple myeloma, and fracture repair. Kunimatsu et al., [18] reported a positive correlation between GCF osteocalcin, N-terminal peptide levels and clinical parameters in a cross-sectional study of patients with periodontitis and gingivitis. The authors also reported that osteocalcin could not be detected in patients with gingivitis, Nakashima et al., [19] reported significant GCF osteocalcin levels from both periodontitis and gingivitis patients. Osteocalcin levels were also significantly correlated with pocket depth and gingival index scores, as well as GCF levels of ALP and PGE2.

Osteonectin is a single-chain polypeptide that binds strongly to hydroxyapatite and other extracellular matrix proteins. Osteonectin and N-propeptide alpha I type I collagen were significantly increased in patients with periodontal disease. Osteonectin appeared to be the more sensitive marker for detection of periodontal disease status, when compared with N-propeptide alpha I type I collagen.

Osteopontin (OPN) a single chain polypeptide,is highly concentrated at sites where osteoclasts are attached to the underlying mineral surface. OPN is produced by both osteoclasts and osteoblasts. It holds a dual function in bone maturation and mineralization as well as bone resorption . GCF OPN secretion increased proportionally with the progression of disease and with non-surgical treatment it was significantly reduced [19].

Given the specificity and sensitivity for bone resorption, ICTP represent a potentially valuable diagnostic aid for periodontal disease. It is useful in differentiating between the presence of gingival inflammation and active periodontal or perimplant bone destruction. ICTP has been shown to be a promising predictor of both future alveolar bone and attachment loss [20]. ICTP levels strongly correlated with whole subject level of several periodontal pathogens including T for sythensis, P gingivalis and T denticola. Golub et al., found that treatment of chronic periodontitis patients with non surgical periodontal therapy and local drug delivery resulted in a 70% reduction in GCF, ICTP levels after one month, concomitant with a 30% reduction in collagenase levels.

A number of times diagnosis is based solely on periodontal probing measurement which may lead to improper diagnosis and inappropriate therapeutic intervention.Risk factors as a modifiers of disease activity may influence the initiation and progression of periodontitis and cause successive change on biomarkers.Thus additional diagnostic and prognostic tests have developed to address this problem.

Recent advances in diagnostic research are moving towards methods whereby periodontal risk can be identified and quantified by objective measures such as bone biomarkers [21].

Biochemical markers of bone turn over have proved to be useful, non invasive and a relatively inexpensive tool for studying bone metabolism in population studies and are gradually becoming established in clinical practice [22]. The continued development of new markers of bone turn over will increase the knowledge of pathophysiology of periodontitis and metabolic bone diseases like osteoporosis. After further evaluation these markers may find a place in the clinical case of post-menopausal women.

New diagnostic technologies such as microarray and microfluidics are now currently available for risk assessment and comprehensive screening of biomarkers. Electrochemical biosensors coupled to Magnetic Beads are used for the detection of clinical biomarkers. These recent advances in biomarkers and diagnostic tools could prove a way for better treatment approaches to periodontal disease.

CONCLUSION

There has been an steady increase to develop newer diagnostic tools to detect periodontitis, right from probing measurement to genetic analysis are extensively used to detect the disease process. A number of improvements have been made on the understanding of the mediators implicated on the initiation and progression of periodontitis,however it is also clear that no single marker has been able to fullfill all the criteria necessary for assessment of the clinical state of the periodontium and future research should be directed possibly at the production of these marker packages.

REFERENCES

[1] Ellison SA. Oral bacteria and periodontal disease. *J Dent Res*. 1970;49:198–99.
[2] Genco RJ. A Review of Dental Reasearch in microbiology with emphasis on periodontal disease. *J Am Dent Assoc*. 1969;78:1016–36.
[3] Armitage GC. Periodontal diagnoses and classification of periodontal diseases. *Periodontol. 2000*. 2004;34:9–21.
[4] Ericksen EF. Trabecular bone remodelling and balance in primary hyperparathyroidism. *Bone*. 1986;7:213-21.
[5] Frost HM. Dynamics of bone remodeling. *Bone biodynamics.* 1964; Boston 315-33.
[6] Parfitt AM. The coupling of bone formation to bone resorption: a critical analysis of the concept and of its relevance to the pathogenesis of osteoporosis. *Metab Bone Dis Re1 Res*. 1982;4:1-6.
[7] Taba M Jr, Kinney J, Kim AS, Giannobile WV. Diagnostic Biomarkers for Oral and Periodontal disease. *Dent Clin N Am*. 2005;49:551–71.
[8] AJ Atkinson, et al. Biomarkers Definitions Working Group. Biomarkers and surrogate endpoints: preferred definitions and conceptual framework. *Clin Pharmacol Ther*. 2001;69(3):89–95.
[9] Seibel MJ, Woitge WH. Biochemical markers of bone metabolism Part I: Basic principles. *Clin Lab*. 1999;45:237-56.
[10] Nakamura M, Slots J. Salivary enzymes. Origin and relationship to periodontal disease. *J Periodontal Res*. 1983;18:559–69.
[11] Lian JB, Gundberg CM. Osteocalcin. Biochemical considerations and clinical applications. *Clin Orthop.* 1988;226:267–91.
[12] Calvo MS, Eyre DR, Gundberg CM. Molecular basis and clinical application of biological markers of bone turnover. *Endocrine Review*. 1996;17(4):333-68.
[13] Zung P, Domenicucci C, Wasi S, Kuwata F, Sodek J. Osteonectin is a minor component of mineralized connective tissues in rat. *Biochem Cell Biol.* 1986;64(4):356–62.
[14] Denhardt DT, Guo X. Osteopontin: a protein with diverse functions. *FASEB J*. 1993;7:1475–82.
[15] Sheetal Sanikop, Suvarna patel, Pallavi agarwal. gingival crevicular fluid alkaline phosphatase as a potential diagnostic marker of periodontal. *JISP*. 2010;16(4):513-88.
[16] J isfahan, Saura dabra, Preetandee singh. Evaluating the levels of salivary alkaline and acid phosphotase activities as biochemical markers for periodontal disease. *DENTRES*. 2012;9(1):41-45.
[17] Gibert P, Tramini P, Sieso V, Piva MT. Alkaline phosphatase isozyme activity in serum from patients with chronic periodontitis. *Journal of periodontal research*. 2003;38(4):362-65.
[18] Kunimatsu K, Mataki S, Tanaka H, Mine N, Kiyoki M, Hosoda K, et al. A cross-sectional study on osteocalcin levels in gingival crevicular fluid from periodontal patients. *JOP*. 1993;64(9):865-69.
[19] Nakashima K, Giannopoulou C, Andersen E, Roehrich N, Brochut P, Dubrez B, et al. A longitudinal study of various crevicular fluid components as markers of periodontal disease activity. *J Clinical Periodontology*. 1996;23(9):832-38.
[20] Charles P, Mosekilde L, Risteli L, Risteli J, Eriksen EF. Assessment of bone remodeling using biochemical indicators of type I collagen synthesis and degradation: relation to calcium kinetics. *Bone and Mineral*. 1994; 37(6):805–10.
[21] Gloria A. Florez-Moreno, Lina M Marin-Restrepo, Dianom, Isazo –Guzman and Sergio I. Tobon–Aeroyavao; screening for salivary levels of deoxypyridinoline and bone specific alkaline phosphatase during orthodontic tooth movement a pilot study,Eur. *JOrthod.* 2013,35(3);361-68.
[22] Sakira Dabra, Kamalpreet China, and Alka Kaushik. Salivary Enzymes as diagnostic markers for detection of gingival /periodontal disease and their correlation with the severity of disease. *Jisp*. 2012;16(3);358-64.

PARTICULARS OF CONTRIBUTORS:
1. Reader, Department of Periodontics, Thai Moogambagi Dental College, Chennai, Tamil Nadu, India.
2. Senior Lecturer, Department of Periodontics, Thai Moogambagi Dental College, Chennai, Tamil Nadu, India.
3. Professor and HOD, Department of Periodontics, Thai Moogambagi Dental College, Chennai, Tamil Nadu, India.
4. Post Graduate Student, Department of Periodontics, Thai Moogambagi Dental College, Chennai, Tamil Nadu, India.
5. Dean, Professor and HOD, Department of Orthodontics, Thai Moogambigai Dental College, Chennai, Tamil Nadu, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Ramachandra Prabhakar,
DEAN and Head, Department of Orthodontics, Thai Moogambigai Dental College and Hospital,
Maduravoil, Chennai, Tamil Nadu, India.
Email:dr.ramachandra.prabhakar@gmail.com

FINANCIAL OR OTHER COMPETING INTERESTS: None.

Date of Submission: **Sep 15, 2014**
Date of Peer Review: **Oct 15, 2014**
Date of Acceptance: **Nov 08, 2014**
Date of Publishing: **Jan 01, 2015**