https://doi.org/10.46389/rjd-2024-0446
www.jrdiabet.ro

Rom J Diabetes Nutr Metab Dis
2024; Volume 31, Issue 1, pages 446-458

ASSESSING BONE TURNOVER BIOMARKERS IN PERIODONTITIS: A LITERATURE REVIEW

Hussein Alaa Alhusseini[1*] and Firas Bashir AL-Taweel[2]

[1*] MSc student, Department of Periodontology, College of Dentistry, University of Baghdad, Baghdad, Iraq.

[2] PhD, Department of Periodontology, College of Dentistry, University of Baghdad, Baghdad, Iraq.

* Corresponding author: Hussein Alaa Alhusseini

email: hussein.alaa1205a@codental.uobaghdad.edu.iq

email: Firas.basheer@codental.uobaghdad.edu.iq

Corresponding author ORCID: Hussein Alaa Hasan Alhusseini

https://orcid.org/0009-0004-8115-7604

Corresponding author Scopus ID: Hussein Alhusseini

https://www.scopus.com/home.uri?zone=header&origin=searchauthorfreelookup

Abstract

Background: Chronic periodontitis is a prevalent inflammatory condition affecting the supporting structures of teeth, including alveolar bone. Bone turnover markers (BTMs) have gained recognition as potential indicators of the dynamic interplay between bone resorption and formation in chronic periodontitis. This literature review aims to provide a comprehensive analysis of the current state of knowledge regarding the use of BTMs in understanding the pathogenesis, diagnosis, and monitoring of chronic periodontitis. The review begins by elucidating the pathophysiology of chronic periodontitis, highlighting the intricate interactions between microbial agents, host immune response, and local inflammation that contribute to alveolar bone loss. Subsequently, the biochemical basis of bone turnover is explored, with a focus on the roles of osteoclasts, osteoblasts, and their signalling pathways in bone metabolism. The main section of the review discusses various BTMs that have been investigated in the context of chronic periodontitis. These markers include both bone resorption markers (e.g., Cathepsin K, Receptor activator of nuclear factor Kappa-B ligand, NTx and ICTP) and bone formation markers (e.g., osteocalcin, Alkaline phosphatase, Osteopontin (OPN) and Periostn). The potential of BTMs to provide insights into disease severity, treatment response, and risk assessment for bone loss is critically evaluated. In conclusion, this literature review underscores the significance of bone turnover markers in advancing our understanding of the complex relationship between chronic periodontitis and alveolar bone metabolism. A comprehensive grasp of the role of BTMs in this context could have far-reaching implications, ranging from improved diagnostic approaches to the development of novel therapeutic interventions targeting bone health in chronic periodontitis.

Keywords: bone turnover biomarkers, chronic periodontitis, oral-fluid diagnostics, bone resorption, bone formation

Introduction

The primary objective of this comprehensive review is to thoroughly elucidate the multifaceted role played by bone turnover biomarkers within the context of chronic periodontitis. To accomplish this objective, an extensive search was undertaken across various electronic databases, namely Science Direct, PubMed, Scopus, and Google Scholar. A meticulously crafted combination of pertinent keywords, including "saliva" and "GCF" in conjunction with "bone turnover biomarkers," "oral diagnostics," "periodontitis diagnosis," and "salivary biomarkers" alongside "GCF biomarkers," was employed to ensure the retrieval of relevant and pertinent literature. The scope of our investigation encompassed a meticulous examination of full-text articles published in dental journals from the span of 1990 to 2022. The encompassed literature spanned a diverse array of research methodologies, including case-control studies, cross-sectional studies, clinical trials, systematic reviews, as well as concise reviews.

Periodontal disease (PD) constitutes a group of infectious oral inflammatory disorders that exert their influence on the intricate periodontal apparatus surrounding the tooth. This condition emanates from the intricate disruption of the harmonious interplay between the oral microbial flora and the host's immune defense system. This intricate and dynamic interrelationship is characterized by repetitive episodes of microbial provocation followed by subsequent intervals of abatement, ultimately culminating in a gradual and insidious progression of tooth degradation and, inevitably, its eventual loss (Kinane et al., 2017).

In a comprehensive classification scheme, periodontal disorders are categorized into two primary overarching groups: the initial phase being gingivitis, which is typified by transient and readily reversible inflammation confined to the gingival tissues; and in instances where timely intervention is not undertaken, this initial stage may advance into the more advanced and irreversible manifestation known as periodontitis (Caton et al., 2018). The clinical manifestations that signify the presence of periodontitis encompass a spectrum of discernible changes within the oral cavity. These alterations encompass the progressive emergence of periodontal pockets with increased depth, the detachment of the periodontal ligament and cementum from the tooth's root surface, as well as the gradual erosion and resorption of the alveolar bone that provides structural support to the teeth. This intricate cascade of pathological changes ultimately culminates in the irreversible outcome of tooth loss, which serves as a critical indicator of the advanced stage of periodontitis (Papapanou and Susin, 2017). Individuals afflicted by chronic periodontitis may also experience additional adverse effects on their quality of life, including difficulties in chewing and swallowing, speech impediments, aesthetic worries, and more (Ferreira et al., 2017). Furthermore, chronic periodontitis has been linked to diverse systemic conditions such as cardiovascular disorders, renal irregularities, diabetes mellitus, asthma, unfavourable birth outcomes, and obesity (Nazir, 2017, Zeng et al., 2016, Keller et al., 2015).

The origin of chronic periodontitis is multifaceted. The oral cavity is inhabited by over 700

bacterial species, with nearly 400 found in subgingival regions. Notably, three bacterial microorganisms—*Aggregatibacter actinomycetemcomitans*, *Tannerella forsythia*, and *Porphyromonas gingivalis*—are recognized as the principal microbial agents responsible (GonÇAlves et al., 2016, Hajishengallis, 2015). Additional environmental and acquired factors also contribute to the development of periodontitis. These encompass stress, suboptimal oral hygiene, unsatisfactory dietary practices, alcohol and tobacco use, smoking, and genetic predisposition (Bouchard et al., 2017). The development of chronic periodontitis encompasses a series of interconnected processes that result in the activation of host immune responses due to the release of harmful substances from bacterial microorganisms. These microbial byproducts initiate a chain reaction involving the activation of cytokines, chemokines, pro-inflammatory agents, and macrophages, which collectively contribute to the gradual deterioration of underlying gingival tissues and eventual tooth loss (Meyle and Chapple, 2015, Bartold and Van Dyke, 2019).

Review

Role of oral fluid biomarkers in chronic periodontitis

Traditionally utilized diagnostic methods for periodontitis assessment involve clinical and radiographic evaluations. Common clinical indicators comprise probing pocket depth (PPD), bleeding on probing (BOP), and clinical attachment loss (CAL). However, these techniques are retrospective and lack insight into current disease status and activity. Moreover, they do not pinpoint individuals at high risk of future bone loss (Javaid et al., 2016). In recent decades, substantial research has focused on oral fluid diagnostics, revealing various biomarkers in periodontitis patients' oral fluids like saliva and gingival crevicular fluid (GCF) (Korte and Kinney, 2016, Barros et al., 2016). These biomarkers encompass enzymes, proteins, hormones, host-derived molecules, DNA, RNA, bacterial elements, and volatile products. Given their noninvasive and easily accessible characteristics, these biomarkers emerge as ideal diagnostic instruments, offering early detection, improved diagnosis, and timely management of chronic periodontitis (Jaedicke et al., 2016). In the context of its definition, a biomarker refers to a measurable quantitative variable used to indicate regular biological processes, pathological changes, or responses to therapeutic interventions (Buduneli, 2019).

Bone turnover biomarkers in chronic periodontitis

Biomarkers associated with periodontal disease are commonly characterized as molecules linked to three distinct pathological stages, namely inflammation, collagen degradation, and turnover of the alveolar bone. The process of bone turnover is a constant occurrence within the body, involving a necessary balance where bone deposition exceeds bone resorption rates to maintain bone stability. Nonetheless, in instances of pathological conditions like periodontitis, this equilibrium is disrupted, leading to a noticeable alteration in the overall rate of alveolar bone turnover. The measurement of bone turnover rate relies on bone turnover markers (BTMs), which are categorized into markers for bone formation and markers for bone resorption. The loss of alveolar bone is a pivotal component of periodontitis. Consequently, several studies have evaluated the levels of bone turnover biomarkers in both saliva and serum of periodontitis patients, revealing a close

correlation with disease advancement and severity (Gursoy et al., 2016, Betsy et al., 2019, Miricescu et al., 2014, Wilson et al., 2003). In the context of this review, various types of bone turnover markers were considered. However, the review specifically concentrated on the examination of two markers associated with bone resorption, namely RANKL and cathepsin K. Additionally, periosin was highlighted as a marker indicative of bone formation. Table 1 enumerates the distinct biomarkers of bone turnover in periodontitis.

Table 1: distinct biomarkers of bone turnover in periodontitis.

Biomarker	**Role in Periodontitis**	**Detection Medium**
Bone formation markers		
Alkaline phosphatase (ALP)	Crucial function in the process of calcification amplifies as the severity of PD advances.	GCF, saliva
Osteocalcin	Induces bone generation and holds promise as a potential biomarker for assessing bone turnover in cases of periodontitis.	GCF, saliva
Osteopontin (OPN)	steopontin serves a dual role in both bone mineralization and resorption.	GCF, saliva
Periostn	Periostin likely contributes to tissue turnover by boosting adhesion, cellular differentiation, viability, and fibrogenesis.	GCF, saliva
Bone resorption markers		
RANKL	Accountable for heightened osteoclastic activity in the context of periodontitis.	GCF, saliva
NTx	Facilitates bone breakdown and exhibits an elevation in cases of periodontitis.	GCF, saliva
ICTP	Engages in the active degradation of collagen and experiences augmentation in the context of periodontitis.	GCF, saliva
Cathepsin K	Enhanced CTSK levels in individuals with chronic periodontitis effectively degrade type I collagen.	GCF, saliva

RANKL= Receptor activator of nuclear factor Kappa-B ligand; NTx= Cross-linked N-terminal telopeptide of type I collagen; ICTP= Cross-linked C-terminal of type I collagen; GCF= Gingival crevicular fluid; PD= Periodontal disease

Alkaline phosphatase (ALP)

Alkaline phosphatase (ALP), an enzyme characterized by its glycoprotein nature and hydrolase activity, is primarily found in renal, hepatic, and osteogenic cell types. Its enzymatic action involves breaking ester bonds under alkaline pH conditions, resulting in an elevation of phosphate ion levels in the serum and plasma. Within the periodontium, ALP assumes a critical function in

cementogenesis and the preservation of bone equilibrium. It triggers the process of calcification and significantly engages in the phase of bone turnover (Gul and Phil, 2019), Several studies have observed that in individuals with untreated chronic periodontitis, the amount of alkaline phosphatase in whole saliva is increased when compared to healthy individuals. Furthermore, researchers have established a direct correlation between pocket depth and levels of alkaline phosphatase (AP) in individuals affected by periodontitis. (Patel et al., 2016, Kumar and Sharma, 2011). Some studies reported increased activity of AP in the acute phase of periodontal disease, and also observed that the enzyme level was restored to its normal range after periodontal therapy (Jeyasree et al., 2018, Yan et al., 1995).

In a 2006 study conducted by Todorovic et al., it was noted that elevated activity levels of specific salivary enzymes such as creatine kinase, lactate dehydrogenase, aspartate aminotransferase, alanine transaminase, and gamma-glutamyl transferase suggest a confined pathogenic impact on soft tissues. Conversely, the heightened alkaline phosphatase (AP) activity indicates the involvement of the alveolar bone in the pathological destructive process, implying an advanced stage of periodontal disease (Todorovic et al., 2006). A study conducted in 2012 investigated the salivary activities of alkaline phosphatase in individuals with periodontal disease, both prior to and following periodontal treatment. The findings from this study demonstrated noteworthy and statistically significant elevations in the activities of ALP in the saliva of patients afflicted with periodontal disease when compared to the control group. Additionally, a marked reduction in the levels of these enzymes was observed subsequent to conventional periodontal therapy (Dabra and Singh, 2012).

Osteocalcin

Osteocalcin stands as the predominant protein within the extracellular matrix of bone. Comprising glutamic acid residues, this protein is synthesized by both bone and cartilage cells. Notably, it engages in active binding to the calcium ions within the hydroxyapatite crystal lattice of bone. While in serum/plasma, it circulates in a decarboxylated state, whereas within bone, it exists in the inert carboxylated form (Rodan, 1992). Osteocalcin is predominantly produced and released by osteoblasts, odontoblasts, and chondrocytes (Hienz et al., 2015). Osteocalcin holds a significant function in the process of bone turnover and is typically acknowledged as a biomarker for bone formation. However, in the context of periodontitis, where bone equilibrium is disrupted due to heightened resorption rates, osteocalcin assumes the role of attracting osteoclasts to areas of bone degradation, thereby facilitating their transformation into active osteoclasts. Elevated osteocalcin levels in bodily fluids indicate irregular bone turnover activity observed during periodontitis. Consequently, it is now widely recognized as an indicator of bone turnover rather than exclusive bone formation (Becerik et al., 2011). A noteworthy association was established between salivary osteocalcin levels and the clinical attachment level. Likewise, various other findings demonstrated elevated osteocalcin concentrations in the saliva of individuals with chronic periodontitis. In summary, it can be affirmed that osteocalcin not only possesses substantial diagnostic capabilities

but can also function as a prognostic marker, offering insight into the potential disease progression (Hienz et al., 2015, Miricescu et al., 2014, Giannobile et al., 2009, Ram et al., 2015).

Osteopontin (OPN)

Osteopontin (OPN) is a glycosylated phosphoprotein that lacks collagen content, featuring extensive phosphorylation and a notable presence of sialic acid. This calcium-binding protein includes an arginine-glycine-aspartic acid (RGD) sequence, Functioning as an extracellular matrix cell-adhesion protein, osteopontin is prominently found in bone tissue and is primarily produced by preosteoblasts, osteoblasts, and osteoclastic cells situated within the mineralized segment of the bone matrix (Sharma and Pradeep, 2006b). Moreover, osteopontin (OPN) plays a crucial role in governing both the normal mineralization processes and the aberrant mineralization seen in pathological conditions (Mazzali et al., 2002). During the inflammatory cascade, osteopontin (OPN) functions as a versatile cytokine with the capacity to attract diverse cell types, including monocytes/macrophages, through chemotactic mechanisms. It assumes a crucial role in facilitating cell-mediated immunity and maintaining the appropriate Th-1 cytokine response in the context of granuloma formation (Denhardt et al., 2001). Research indicates that the OPN molecule has been identified within gingival crevicular fluid (GCF), with its concentrations showing a proportional rise as periodontal disease advances. This trend implies that the levels of OPN in GCF could potentially serve as an indicator of the extent of alveolar bone deterioration (Kido et al., 2001). In a separate investigation, the levels of osteopontin in gingival crevicular fluid (GCF) exhibited significant elevation in concurrence with the severity of the disease. After a period of six to eight weeks of nonsurgical treatment administered to individuals with chronic periodontitis, these osteopontin levels experienced a notable reduction (Sharma and Pradeep, 2006a).

Periostin

Periostin is a matricellular protein that is secreted into the extracellular matrix and contains glutamate residues. Initially identified in a mouse osteoblastic cell line, it was initially labeled as osteoblast-specific factor 2 (OSF-2), the protein was later given the name “Periostin” because of its location in the periosteum and periodontal ligament (PDL) (Takeshita et al., 1993). Periostin demonstrates elevated levels of expression within fibrous connective tissues that possess a substantial collagen content and are subjected to persistent mechanical stresses. These tissues encompass the periosteum, periodontal ligaments (PDLs), tendons, heart valves, and skin. Periostin likely assumes a function in tissue turnover by enhancing adhesion, encouraging cellular differentiation and viability, and instigating fibrogenesis (Kim et al., 2000). Prior investigations involving osteoblasts in long bones have shown that a mutation in periostin resulted in compromised adhesion of osteoblasts to the bone matrix. Additionally, this mutation led to a notable reduction in the expression levels of key factors including type I collagen, osteocalcin, osteopontin, and alkaline phosphatase. These alterations in functionality impede the progression of osteoblast differentiation into mature cells and subsequently diminish the ability to carry out mineralization processes in vitro (Bonnet et al., 2012, Litvin et al., 2004). Numerous research

studies have revealed significant discrepancies in periostin levels between individuals in good health and those affected by periodontal diseases. Specifically, the concentration of periostin was markedly diminished in subjects exhibiting healthy periodontal tissue when contrasted with those grappling with periodontitis (Arslan et al., 2021, Al-Rihaymee and Sh. Mahmood, 2023).

Receptor Activator of Nuclear Factor Kappa-B Ligand (RANKL)

Receptor Activator of Nuclear Factor Kappa-B Ligand (RANKL), a homotrimeric transmembrane protein, exists in both membrane-bound and secreted forms. The soluble variant results from proteolytic cleavage or alternative splicing of the membrane-bound form. As a member of the Tumor Necrosis Factor (TNF) ligand superfamily, RANKL is a cell-bound factor that stimulates osteoclast differentiation and bone resorption (Abdullameer and Abdulkareem, 2023, Al-Mufti et al., 2017). Upon binding of RANKL to its corresponding RANK receptor on pre-osteoclast surfaces, it initiates their fusion and transformation into fully developed osteoclasts, consequently inducing bone resorption (Teitelbaum and Ross, 2003). Research has revealed elevated expression of RANKL in B and T lymphocytes within periodontal tissues, particularly in patients afflicted with chronic periodontitis, as opposed to those with a healthy periodontium (Kawai et al., 2006). In a cross-sectional investigation, the RANKL levels were assessed in the saliva of individuals with chronic periodontitis. The study demonstrated a notable correlation ($p<0.05$) between RANKL levels and clinical periodontal parameters (periodontal pocket depth and clinical attachment loss) (Al-Ghurabi and Mohssen, 2015, Bostanci et al., 2011).

Cross-linked N-terminal telopeptide Type I collagen (NTx)

The N-terminal cross-linked telopeptide of type I collagen (NTx) constitutes the amino-terminal fragments of fully formed type I collagen, featuring linked cross-links, and is liberated during the process of bone resorption.(Hanson and Eyre, 1996). Multiple research studies have indicated that heightened levels of N-terminal cross-linked telopeptide of type I collagen (NTx) detected in gingival crevicular fluid (GCF) could potentially function as predictive indicators of alveolar bone deterioration. Notably, the concentrations of NTx in GCF were found to be elevated among individuals with chronic periodontitis, and these levels exhibited a positive correlation with various clinical periodontal parameters (Almehmadi and Alghamdi, 2018, Aruna, 2016, Wilson et al., 2003).

Pyridinoline (ICTP)

Pyridinoline (ICTP) also known as carboxyterminal telopeptide of type I collagen is a constituent of collagen breakdown that, subsequent to bone resorption and degradation of the collagen matrix, is liberated into the bloodstream. It cannot undergo anabolic pathways or be recycled during collagen synthesis, thus rendering it a distinct biomarker indicative of bone resorption (Quesada and Alvarez, 2016). The occurrence of ICTP within gingival crevicular fluid (GCF) has been linked to both bone loss and attachment loss in both experimental and naturally occurring periodontitis (Giannobile et al., 1995). As periodontal disease advances, the concentrations of ICTP exhibit a direct correlation with escalated collagen degradation. In a study by Mishra et al.,

the salivary ICTP levels were assessed among individuals with chronic periodontitis and gingivitis. The findings indicated significantly elevated levels of ICTP in the chronic periodontitis group compared to the gingivitis group, a difference that was statistically significant. Hence, ICTP is proposed as a potential marker for predicting subsequent alveolar bone loss (Mishra et al., 2015, Quesada and Alvarez, 2016).

Cathepsin K

Lysosomes hold numerous hydrolases, notably cathepsins, key proteases linked to diverse biological actions (Goto et al., 2003). Cathepsin K (CTSK), an acidic cysteine endoproteinase mainly found in osteoclasts, acts as a potent extracellular matrix degrading enzyme, crucial in osteoclast-driven bone resorption (Costa et al., 2011). The RANKL-RANK signaling pathway regulates CTSK expression, initiating the transcription of CTSK (Balkan et al., 2009). Cathepsin K can proficiently degrade type I collagen, constituting approximately 90% of the organic matrix within the bone's extracellular matrix, including the glycoprotein osteonectin (Bossard et al., 1996). Moreover, earlier investigations have established that CTSK possesses the capacity to cleave and activate matrix-metalloproteinase-9 (MMP-9), an additional protease essential for bone matrix degradation (Christensen and Shastri, 2015). Numerous investigations have indicated an increased concentration of CTSK in individuals with chronic periodontitis in comparison to those who are in a healthy state (Mogi and Otogoto, 2007, Gajendran et al., 2018).

Conclusions

In this era of innovative oral-fluid diagnostics, biomarkers serve as potential tools for improved monitoring, diagnosis, and clinical management of periodontitis. Various quantitative and qualitative approaches like genomic profiling, proteomic analysis, and transcriptomics help researchers comprehensively screen biomarkers, defining human physiology and pathology. Detecting bone biomarkers in periodontitis patients' gingival crevicular fluid (GCF) or saliva reveals alveolar bone involvement extent and predicts susceptible individuals for bone loss. These biomarkers not only detect periodontal disease early but also mitigate severity and progression. However, further longitudinal and observational studies are necessary to assess the prognostic and diagnostic value of these bone turnover markers in periodontitis.

Conflict of interest

The authors have no conflicts of interest to declare.

Author contributions

HAA; study conception and design. HAA; data collection. HAA; Methodology HAA; statistical analysis and interpretation of results. HAA; original draft manuscript preparation. HAA; Writing

- review & editing. Supervision; FBA. All authors reviewed the results and approved the final version of the manuscript to be published.

Acknoledgement and funding

No grant or financial support was recieved from any governmental or private sector for this study.

References

ABDULLAMEER, M. A. & ABDULKAREEM, A. A. 2023. Diagnostic potential of salivary interleukin-17, RANKL, and OPG to differentiate between periodontal health and disease and discriminate stable and unstable periodontitis: A case-control study. *Health Science Reports,* 6**,** e1103.

AL-GHURABI, B. H. & MOHSSEN, S. M. 2015. Salivary level of RANKL and OPG in chronic periodontitis. *Journal of Baghdad College of Dentistry,* 27**,** 189-194.

AL-MUFTI, S. M. T., SALIEM, S. S. & ABDULBAQI, H. R. 2017. The association between receptor activator of nuclear factor kappa-β ligand and clinical attachment level among waterpipe smoker. *Journal of Indian Society of Periodontology,* 21**,** 376.

AL-RIHAYMEE, S. & SH. MAHMOOD, M. 2023. The efficacy of non-surgical platelet-rich fibrin application on clinical periodontal parameters and periostin level in periodontitis: Clinical trial. *Journal of Cellular and Molecular Medicine,* 27**,** 529-537.

ALMEHMADI, A. H. & ALGHAMDI, F. 2018. Biomarkers of alveolar bone resorption in gingival crevicular fluid: A systematic review. *Archives of oral biology,* 93**,** 12-21.

ARSLAN, R., KARSIYAKA HENDEK, M., KISA, U. & OLGUN, E. 2021. The effect of non-surgical periodontal treatment on gingival crevicular fluid periostin levels in patients with gingivitis and periodontitis. *Oral Diseases,* 27**,** 1478-1486.

ARUNA, G. 2016. Plasma levels of N-telopeptide of Type I collagen in periodontal health, disease and after treatment. *Dental research journal,* 13**,** 18.

BALKAN, W., MARTINEZ, A. F., FERNANDEZ, I., RODRIGUEZ, M. A., PANG, M. & TROEN, B. R. 2009. Identification of NFAT binding sites that mediate stimulation of cathepsin K promoter activity by RANK ligand. *Gene,* 446**,** 90-98.

BARROS, S. P., WILLIAMS, R., OFFENBACHER, S. & MORELLI, T. 2016. Gingival crevicular fluid as a source of biomarkers for periodontitis. *Periodontology 2000,* 70**,** 53-64.

BARTOLD, P. M. & VAN DYKE, T. E. 2019. An appraisal of the role of specific bacteria in the initial pathogenesis of periodontitis. *Journal of Clinical Periodontology,* 46**,** 6-11.

BECERIK, S., AFACAN, B., ÖZTÜRK, V. Ö., ATMACA, H. & EMINGIL, G. 2011. Gingival crevicular fluid calprotectin, osteocalcin and cross-linked N-terminal telopeptid levels in health and different periodontal diseases. *Disease markers,* 31**,** 343-352.

BETSY, J., AHMED, J. M., MOHASIN, A. K., MOHAMMED, A. & NABEEH A, A. 2019. Diagnostic accuracy of salivary biomarkers of bone turnover in identifying patients with periodontitis in a Saudi Arabian population. *Journal of Dental Sciences,* 14**,** 269-276.

BONNET, N., CONWAY, S. J. & FERRARI, S. L. 2012. Regulation of beta catenin signaling and parathyroid hormone anabolic effects in bone by the matricellular protein periostin. *Proceedings of the National Academy of Sciences,* 109**,** 15048-15053.
BOSSARD, M. J., TOMASZEK, T. A., THOMPSON, S. K., AMEGADZIE, B. Y., HANNING, C. R., JONES, C., KURDYLA, J. T., MCNULTY, D. E., DRAKE, F. H. & GOWEN, M. 1996. Proteolytic Activity of Human Osteoclast Cathepsin K: EXPRESSION, PURIFICATION, ACTIVATION, AND SUBSTRATE IDENTIFICATION (∗). *Journal of Biological Chemistry,* 271**,** 12517-12524.
BOSTANCI, N., SAYGAN, B., EMINGIL, G., ATILLA, G. & BELIBASAKIS, G. N. 2011. Effect of periodontal treatment on receptor activator of NF-κB ligand and osteoprotegerin levels and relative ratio in gingival crevicular fluid. *Journal of Clinical Periodontology,* 38**,** 428-433.
BOUCHARD, P., CARRA, M. C., BOILLOT, A., MORA, F. & RANGÉ, H. 2017. Risk factors in periodontology: a conceptual framework. *Journal of Clinical Periodontology,* 44**,** 125-131.
BUDUNELI, N. 2019. Biomarkers in Saliva and Serum Samples for Periodontal Disease and Interactions with Systemic Health. *Current Oral Health Reports,* 6**,** 31-36.
CATON, J. G., ARMITAGE, G., BERGLUNDH, T., CHAPPLE, I. L. C., JEPSEN, S., KORNMAN, K. S., MEALEY, B. L., PAPAPANOU, P. N., SANZ, M. & TONETTI, M. S. 2018. A new classification scheme for periodontal and peri-implant diseases and conditions – Introduction and key changes from the 1999 classification. *Journal of Periodontology,* 89**,** S1-S8.
CHRISTENSEN, J. & SHASTRI, V. P. 2015. Matrix-metalloproteinase-9 is cleaved and activated by cathepsin K. *BMC research notes,* 8**,** 1-8.
COSTA, A. G., CUSANO, N. E., SILVA, B. C., CREMERS, S. & BILEZIKIAN, J. P. 2011. Cathepsin K: its skeletal actions and role as a therapeutic target in osteoporosis. *Nature Reviews Rheumatology,* 7**,** 447-456.
DABRA, S. & SINGH, P. 2012. Evaluating the levels of salivary alkaline and acid phosphatase activities as biochemical markers for periodontal disease: A case series. *Dental research journal,* 9**,** 41.
DENHARDT, D. T., NODA, M., O'REGAN, A. W., PAVLIN, D. & BERMAN, J. S. 2001. Osteopontin as a means to cope with environmental insults: regulation of inflammation, tissue remodeling, and cell survival. *The Journal of clinical investigation,* 107**,** 1055-1061.
FERREIRA, M. C., DIAS-PEREIRA, A. C., BRANCO-DE-ALMEIDA, L. S., MARTINS, C. C. & PAIVA, S. M. 2017. Impact of periodontal disease on quality of life: a systematic review. *Journal of Periodontal Research,* 52**,** 651-665.
GAJENDRAN, P. L., PARTHASARATHY, H. & TADEPALLI, A. 2018. Comparative evaluation of cathepsin K levels in gingival crevicular fluid among smoking and nonsmoking patients with chronic periodontitis. *Indian Journal of Dental Research,* 29**,** 588.
GIANNOBILE, W. V., BEIKLER, T., KINNEY, J. S., RAMSEIER, C. A., MORELLI, T. & WONG, D. T. 2009. Saliva as a diagnostic tool for periodontal disease: current state and future directions. *Periodontology 2000,* 50**,** 52.

GIANNOBILE, W. V., LYNCH, S. E., DENMARK, R. G., PAQUETTE, D. W., FIORELLINI, J. P. & WILLIAMS, R. C. 1995. Crevicular fluid osteocalcin and pyridinoline cross-linked carboxyterminal telopeptide of type I collagen (ICTP) as markers of rapid bone turnover in periodontitis: A pilot study in beagle dogs. *Journal of clinical periodontology,* 22**,** 903-910.

GONÇALVES, C., SOARES, G. M. S., FAVERI, M., PÉREZ-CHAPARRO, P. J., LOBÃO, E., FIGUEIREDO, L. C., BACCELLI, G. T. & FERES, M. 2016. Association of three putative periodontal pathogens with chronic periodontitis in Brazilian subjects. *Journal of Applied Oral Science,* 24.

GOTO, T., YAMAZA, T. & TANAKA, T. 2003. Cathepsins in the osteoclast. *Microscopy,* 52**,** 551-558.

GUL, S. & PHIL, M. 2019. Cross Sectional Analysis of Biomarkers In Chronic Periodontitis Patients. *JPDA,* 28**,** 23.

GURSOY, U. K., LIUKKONEN, J., JULA, A., HUUMONEN, S., SUOMINEN, A. L., PUUKKA, P. & KÖNÖNEN, E. 2016. Associations Between Salivary Bone Metabolism Markers and Periodontal Breakdown. *Journal of Periodontology,* 87**,** 367-375.

HAJISHENGALLIS, G. 2015. Periodontitis: from microbial immune subversion to systemic inflammation. *Nature Reviews Immunology,* 15**,** 30-44.

HANSON, D. A. & EYRE, D. R. 1996. Molecular site specificity of pyridinoline and pyrrole cross-links in type I collagen of human bone. *Journal of Biological Chemistry,* 271**,** 26508-26516.

HIENZ, S. A., PALIWAL, S. & IVANOVSKI, S. 2015. Mechanisms of bone resorption in periodontitis. *Journal of immunology research,* 2015.

JAEDICKE, K. M., PRESHAW, P. M. & TAYLOR, J. J. 2016. Salivary cytokines as biomarkers of periodontal diseases. *Periodontology 2000,* 70**,** 164-183.

JAVAID, M. A., AHMED, A. S., DURAND, R. & TRAN, S. D. 2016. Saliva as a diagnostic tool for oral and systemic diseases. *Journal of Oral Biology and Craniofacial Research,* 6**,** 67-76.

JEYASREE, R. M., THEYAGARAJAN, R., SEKHAR, V., NAVAKUMAR, M., MANI, E. & SANTHAMURTHY, C. 2018. Evaluation of serum and salivary alkaline phosphatase levels in chronic periodontitis patients before and after nonsurgical periodontal therapy. *Journal of Indian Society of Periodontology,* 22**,** 487.

KAWAI, T., MATSUYAMA, T., HOSOKAWA, Y., MAKIHIRA, S., SEKI, M., KARIMBUX, N. Y., GONCALVES, R. B., VALVERDE, P., DIBART, S. & LI, Y.-P. 2006. B and T lymphocytes are the primary sources of RANKL in the bone resorptive lesion of periodontal disease. *The American journal of pathology,* 169**,** 987-998.

KELLER, A., ROHDE, J. F., RAYMOND, K. & HEITMANN, B. L. 2015. Association Between Periodontal Disease and Overweight and Obesity: A Systematic Review. *Journal of Periodontology,* 86**,** 766-776.

KIDO, J. I., NAKAMURA, T., ASAHARA, Y., SAWA, T., KOHRI, K. & NAGATA, T. 2001. Osteopontin in gingival crevicular fluid. *Journal of periodontal research,* 36**,** 328-333.

KIM, J.-E., KIM, S.-J., LEE, B.-H., PARK, R.-W., KIM, K.-S. & KIM, I.-S. 2000. Identification of motifs for cell adhesion within the repeated domains of transforming growth factor-β-induced gene, βig-h3. *Journal of Biological Chemistry,* 275**,** 30907-30915.
KINANE, D. F., STATHOPOULOU, P. G. & PAPAPANOU, P. N. 2017. Periodontal diseases. *Nature Reviews Disease Primers,* 3**,** 17038.
KORTE, D. L. & KINNEY, J. 2016. Personalized medicine: an update of salivary biomarkers for periodontal diseases. *Periodontology 2000,* 70**,** 26-37.
KUMAR, R. & SHARMA, G. 2011. Salivary Alkaline Phosphatase level as Diagnostic marker for periodontal disease. *Journal of International Oral Health,* 3.
LITVIN, J., SELIM, A. H., MONTGOMERY, M. O., LEHMANN, K., RICO, M. C., DEVLIN, H., BEDNARIK, D. P. & SAFADI, F. F. 2004. Expression and function of periostin-isoforms in bone. *Journal of cellular biochemistry,* 92**,** 1044-1061.
MAZZALI, M., KIPARI, T., OPHASCHAROENSUK, V., WESSON, J., JOHNSON, R. & HUGHES, J. 2002. Osteopontin—a molecule for all seasons. *Qjm,* 95**,** 3-13.
MEYLE, J. & CHAPPLE, I. 2015. Molecular aspects of the pathogenesis of periodontitis. *Periodontology 2000,* 69**,** 7-17.
MIRICESCU, D., TOTAN, A., CALENIC, B., MOCANU, B., DIDILESCU, A., MOHORA, M., SPINU, T. & GREABU, M. 2014. Salivary biomarkers: Relationship between oxidative stress and alveolar bone loss in chronic periodontitis. *Acta Odontologica Scandinavica,* 72**,** 42-47.
MISHRA, D., GOPALAKRISHNAN, S., ARUN, K., KUMAR, T. S. S., DEVANATHAN, S. & MISRA, S. R. 2015. Evaluation of salivary levels of pyridinoline cross linked carboxyterminal telopeptide of type I collagen (ICTP) in periodontal health and disease. *Journal of Clinical and Diagnostic Research: JCDR,* 9**,** ZC50.
MOGI, M. & OTOGOTO, J. 2007. Expression of cathepsin-K in gingival crevicular fluid of patients with periodontitis. *Archives of oral biology,* 52**,** 894-898.
NAZIR, M. A. 2017. Prevalence of periodontal disease, its association with systemic diseases and prevention. *Int J Health Sci (Qassim),* 11**,** 72-80.
PAPAPANOU, P. N. & SUSIN, C. 2017. Periodontitis epidemiology: is periodontitis under-recognized, over-diagnosed, or both? *Periodontology 2000,* 75**,** 45-51.
PATEL, R. M., VARMA, S., SURAGIMATH, G. & ZOPE, S. 2016. Estimation and comparison of salivary calcium, phosphorous, alkaline phosphatase and pH levels in periodontal health and disease: A cross-sectional biochemical study. *Journal of clinical and diagnostic research: JCDR,* 10**,** ZC58.
QUESADA, J. G. & ALVAREZ, S. R. 2016. Pyridinoline (ICTP) levels in Gingival Crevicular fluid (GCF) in chronic periodontitis. *Odovtos-International Journal of Dental Sciences,* 18**,** 61-68.
RAM, V. S., SUDHAKAR, U., MITHRADAS, N. & PRABHAKAR, R. 2015. Bonebiomarkers in periodontal disease: a review article. *Journal of clinical and diagnostic research: JCDR,* 9**,** ZE07.
RODAN, G. A. 1992. Introduction to bone biology. *Bone,* 13**,** S3-S6.

SHARMA, C. D. & PRADEEP, A. 2006a. Gingival crevicular fluid osteopontin levels in periodontal health and disease. *Journal of periodontology,* 77**,** 1674-1680.
SHARMA, C. G. D. & PRADEEP, A. R. 2006b. Gingival Crevicular Fluid Osteopontin Levels in Periodontal Health and Disease. *Journal of Periodontology,* 77**,** 1674-1680.
TAKESHITA, S., KIKUNO, R., TEZUKA, K.-I. & AMANN, E. 1993. Osteoblast-specific factor 2: cloning of a putative bone adhesion protein with homology with the insect protein fasciclin I. *Biochemical Journal,* 294**,** 271-278.
TEITELBAUM, S. L. & ROSS, F. P. 2003. Genetic regulation of osteoclast development and function. *Nature Reviews Genetics,* 4**,** 638-649.
TODOROVIC, T., DOZIC, I., VICENTE BARRERO, M., LJUSKOVIC, B., PEJOVIC, J., MARJANOVIC, M. & KNEZEVIC, M. 2006. Salivary enzymes and periodontal disease.
WILSON, A., SCHMID, M., MARX, D. & REINHARDT, R. A. 2003. Bone turnover markers in serum and periodontal microenvironments. *Journal of periodontal research,* 38**,** 355-361.
YAN, F., CAO, C. & LI, X. 1995. Alkaline phosphatase levels in gingival crevicular fluid of periodontitis before and after periodontal treatment. *Zhonghua kou qiang yi xue za zhi= Zhonghua kouqiang yixue zazhi= Chinese journal of stomatology,* 30**,** 204-6, 255.
ZENG, X.-T., LENG, W.-D., LAM, Y.-Y., YAN, B. P., WEI, X.-M., WENG, H. & KWONG, J. S. W. 2016. Periodontal disease and carotid atherosclerosis: A meta-analysis of 17,330 participants. *International Journal of Cardiology,* 203**,** 1044-1051.

