

Dental Journal

Majalah Kedokteran Gigi

Vol. 44. No. 2 June 2011

Literature Review

Saliva as a future potential predictor for various periodontal diseases

Zahreni-Hamzah

Laboratory of Biomedic
Faculty of Dentistry, Jember University
Jember - Indonesia

ABSTRACT

Background: There are many diagnostic biomarkers have been found in saliva. Saliva contains a wide variety of proteins, including bacteria and products, enzymes, inflammatory mediators and host response modifiers, products of tissue breakdown. **Purpose:** The purpose of the study was studied current development of diagnostic biomarkers in saliva that will lead to the development of simple and accurate diagnostic tools for periodontal disease. **Reviews:** Specifically, the salivary biomarkers divided for three aspects of periodontitis i.e. inflammation, collagen degradation and bone turnover, correlated with clinical features of periodontal disease. The diagnostic biomarkers is in saliva, such as enzyme, immunoglobulin, cytokines, bacteria and bacterial products, hormones. For the past two decades, oral health researchers have been developing salivary diagnostic tools to monitor oral diseases. **Conclusion:** The indicators of acute periodontitis can detect with β -glucuronidase and AST, IL-1 β , and MMP-8, whereas indicators for chronic periodontitis can detect with ALP. The indicators for collagen degradation and bone turnover suggest ICTP, fibronectin fragments, and osteonectin. The indicators of severity of periodontitis especially can be predict by *B. forsythus*.

Key words: Saliva, diagnostic biomarker, periodontal disease

ABSTRAK

Latar belakang: Banyak biomarker telah ditemukan dalam saliva. Saliva terdiri dari berbagai protein unik meliputi bakteri dan produk bakteri, enzim, mediator inflamasi dan modifikasi respon host (immunoglobulin, sitokin), produk kerusakan jaringan (telopeptida kolagen, osteokalsin, proteoglikan, fragmen fibronectin). **Tujuan:** Menkaji biomarker dalam saliva untuk pengembangan metode diagnostik sederhana dan akurat untuk penyakit periodontal. **Tinjauan Pustaka:** Secara khusus, biomarker saliva pada periodontitis dibagi dalam tiga aspek yaitu inflamasi, dan degradasi kolagen serta pergantian tulang. Biomarker diagnostik dalam saliva, meliputi enzim, imunoglobulin, sitokin, bakteri dan produk-produk bakteri, hormon. Selama dua dekade terakhir, para peneliti kesehatan mulut telah mengembangkan alat diagnostik melalui saliva yang tepat untuk memonitor beberapa penyakit periodontal. **Kesimpulan:** Indikator periodontitis akut dapat dideteksi dengan β -glucuronidase dan AST, IL-1 β , dan MMP-8, whereas indikator untuk periodontitis kronis dapat dideteksi dengan ALP. Indikator untuk degradasi kolagen dan penggantian tulang dideteksi melalui ICTP, fibronectin fragments, dan osteonectin. Sedang indikator untuk keparahan periodontitis terutama dapat diprediksi melalui *B. forsythus*.

Kata kunci: Saliva, biomarker diagnostik, penyakit periodontal

Correspondence: Zahreni-Hamzah, c/o: Laboratorium Biomedik, Fakultas Kedokteran Gigi Universitas Jember. Jl. Kalimantan 37 Jember 68121, Indonesia. E-mail: reni61_hamzah@yahoo.co.id

INTRODUCTION

Periodontitis is a chronic destructive category of periodontal disease that progresses to the resorption of

alveolar bone, which leads to progressive bone destruction and tooth loss. As a consequence of resorption, breakdown of products are released into periodontal tissues, migrating toward the gingival sulcus and gathering from the

surrounding site in whole saliva, where several of them have been identified. Several important components of saliva that can be used as indicators of periodontal disease are microbial factors, host derived enzymes, inflammatory mediators and host response modifiers, and products of tissue breakdown.¹⁻⁸

Recently, saliva has been studied as an important biological marker for introducing new diagnostic tests that can contribute to making the accurate diagnosis to explain the pathogenesis of a disease. There was many biomarkers of periodontal diseases have found in saliva.²⁻⁵ Over the last two decades, researchers in the oral health have been trying to develop a diagnostic tool to use saliva to monitor various periodontal diseases.^{6,8} The development of salivary diagnostic technology currently leads the development of diagnostic tools that simply, quickly and accurately to facilitate the dentists in making clinical decisions and predict the outcome of treatment in the oral cavity, including the diseases related to the general health. Considering the exchange activity of extra cellular fluid and saliva is high, so its allow the compounds that important as an indicators of disease associated with general health can be found also in saliva.^{1,8,9} Furthermore, the dentist expected to be the first person to recognize the oral disease and the other general disease. The salivary biomarkers specific that correlated with clinical features of periodontal disease can determine by three aspects: inflammation, collagen degradation, and bone turnover.^{3,10}

The purpose of the study was to studied current development of diagnostic biomarkers in saliva that will lead to the development of simple and accurate diagnostic tools for periodontal disease. It can use to make clinical decisions and predict the outcome of treatment in the oral cavity, including diseases related to the general health.

Periodontal inflammation

Periodontal diseases are further divided into reversible and nonreversible categories. Gingivitis is a reversible inflammatory reaction of the marginal gingiva to dental plaque biofilms. Gingivitis is characterized by an initial increase in blood flow, enhanced vascular permeability, and influx of cells (polymorphonuclear leukocytes [PMNs] and monocyte-macrophages) from the peripheral blood into the periodontal connective tissue. Overt soft tissue alterations during the state of gingivitis include redness, edema, bleeding, and tenderness. Whereas, periodontitis is the destructive category of periodontal disease, is a nonreversible inflammatory state of the supporting structures. After 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.¹¹

Recent studies have indicated that host response factors to inflammation and periodontal diseases includes a mixture of molecules from blood, host tissue, plaque films, such as electrolytes, small molecules, proteins, cytokines, antibodies, bacterial and product bacterial

antigens and enzymes. The number and various of the materials that present in saliva differ in different types of periodontal disease. Until now, the number of markers analyzed was limited. Edentulous persons and chronic periodontitis persons have reduced salivary concentrations of host inflammatory proteins. These findings suggest that a reduction in host responsiveness might play a role in the pathogenesis of chronic periodontitis.^{3,10,12}

Salivary indicator of periodontal inflammation is cytokines. The concentrations of interleukine/IL-1 α , IL-6 and IL-8 were found in whole saliva at significantly higher than in major salivary gland secretions. In another study, transforming growth factor (TGF)- β , interleukin (IL)-1 α and tumour necrosis factor (TNF)- α were statistically significantly higher in whole saliva compared to parotid saliva. Although not statistically significant, IL-8 and IL-6 also displayed a trend towards higher levels in whole saliva. The other study examined the relationship between clinical parameters of periodontal disease and the levels of IL-1 β , matrix metalloproteinase (MMP)-8, and osteoprotegerin (OPG) in whole saliva. They reported that the mean levels of IL-1 β and MMP-8 in saliva were significantly higher in periodontitis subjects than in periodontally healthy controls. Combined elevated salivary levels of MMP-8 and IL-1 β increased the risk of experiencing periodontal disease 45-fold. Among the salivary cytokines (IL-1 β , IL-6, and TNF- α), IL-1 β was the only biomarker associated with periodontitis. They suggest that salivary IL-1 β in saliva more thoroughly as markers of periodontitis.^{3,12-17} OPG is glycoprotein that inhibits osteoclast differentiation an activity competitively by preventing osteoclast differentiatin factor receptor activator of nuclear factor kappa-beta ligand (RANKL) from binding to osteoclast precursor and promoting the formation of bone-resorbing osteoclast. OPG used as a marker of bone turnover.^{4,5}

Oncostatin M (OSM), a member of the IL-6 family of cytokines, is multifunctional unique cytokine that plays an important role in various biological systems such as inflammatory response, haematopoiesis, tissue remodelling and development.¹³ In periodontitis, OSM alone may stimulate the production of IL-6, or it may act synergistically with IL-6 or TNF- α to upregulate the production of MMPs or augment IL-6 production. IL-6 may act on both the osteoblasts and osteoclasts through autocrine and paracrine RANKL regulation causing bone resorption. OSM as an inflammatory and bone resorptive biomarker of periodontal disease.¹⁵

Salivary indicators such as intracellular enzymes are increasingly released from the damaged cells of periodontal tissues into the gingival crevicular fluid (GCF) and saliva.² The activities several enzymes of periodontal disease such as creatine kinase (CK), lactate dehydrogenase (LDH), aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma glutamil transferase (GGT), alkaline phosphatase (ALP) and acid phosphatase (ACP) enzymes were significantly increased in the saliva of patients with periodontal disease when compared to healthy subjects.

Meanwhile, other investigators reported that enzymes elastase, LDH was the only biomarker associated with periodontitis. LDH activity increased proportionally with the advance of periodontitis.^{12,18–21}

The other marker of the acute inflammatory response is immunoglobulin. Levels of immunoglobulin G (IgG) have been found not to have a consistent association with periodontal disease, although subtypes of IgG may be found in subjects at higher risk for periodontal disease progression. Levels of immunoglobulin have been found not to have a consistent association with risk for periodontal disease progression.^{4,5}

There are relationship of periodontal parameters to the presence of these periodontopathic bacteria. Most of bacteria can produce tissue destruction by two ways: directly, through invasion of the tissue and production of harmful substances that induce cell death and tissue necrosis; and indirectly, through activation of inflammatory cells that can produce and release mediators that act on effector, with potent inflammatory and catabolic activity. The pathogenesis of periodontal destruction involves the sequential activation of different components of the host immune and inflammatory response.²²

In chronic gingivitis, Gram-negative bacteria constitute $\pm 45\%$ and anaerobic organisms $\pm 45\%$ of the total recoverable subgingival flora. Predominant isolates include various species of *Actinomyces*, *Streptococcus*, *Fusobacterium*, and *Bacteroides*, as well as *Eikenella corrodens* and *Capnocytophaga gingivalis*. Acute necrotizing ulcerative gingivitis (ANUG) has been associated with high proportions of *Bacteroides intermedius* and *Treponema*. Advanced adult periodontitis lesions are characterized by approximately 75% Gram-negative and 90% anaerobic organisms. Common isolates include *Bacteroides gingivalis*, *B. intermedius*, *Actinobacillus actinomycetemcomitans*, *Prevotella intermedia* (Pi), and *Tannerella forsythensis* (Tf) and various species of *Fusobacterium*, *Wolinella*, *Capnocytophaga species*, *Campylobacter rectus*, and non-pigmenting *Bacteroides*. In recent studies of the analysis microbiota associated with identify specific periodontal diseases, identify antibiotic susceptibility of infecting organisms colonizing diseases sites, and predict diseases activity.^{2–4,22–26}

Collagen degradation and bone turnover

Initially, tissue degradation is limited to epithelial cells and collagen fibers from the connective tissue. Later on, the inflammatory process may reach periodontal supportive tissue and leading to bone resorption.²⁷

Collagen degradation products is markers of bone turnover in multitude of bone resorptive and metabolic diseases. The collagen degradative molecules is in periodontal such as pyridoline cross-links (including pyridinoline, deoxypyridinoline, N-telopeptides, and C-telopeptides). Pyridoline cross-links considered as specific biomarkers for bone resorption. The pyridoline cross-linked carboxyterminal telopeptide of type I collagen (ICTP) have been shown to be correlated with bone resorptive rate in several bone metabolic diseases. In a cross-sectional study evaluated the markers of bone turnover are ICTP, osteocalcin and osteonectin in stimulated whole saliva collected from untreated dental patients. Increased levels of salivary osteonectin were associated with less bone loss.^{4,5,28–30}

DISCUSSION

A biomarker or biologic biomarker is substance that is objectively measured and evaluated as indicator of normal biologic processes, pathogenic processes, or pharmacologic responses to therapeutic intervention.³ Whole saliva is important physiologic fluid that contain a highly complex mixture of substances, locally and systemically, derived markers of periodontal and systemic diseases that easily to collected. So, it may offer the basis for a patient-specific biomarker assessment for periodontitis and other systemic diseases.¹

Generally, there are many clinicians reported that clinical parameters of periodontal disease, such as probing depth and bleeding on probing were limited in their ability to predict the activity of periodontal disease. Therefore, saliva can be used as a non-invasive diagnostic fluid to measure biomarkers released during disease initiation and progression (Tabel 1).^{4–8}

Various assays have been used to detect the presence of specific periodontal pathogens, such as culture techniques, immunofluorescence, and DNA probe technology. Using culture techniques requires that a viable bacterial sample be obtained from the patient and transferred to the laboratory. The sampling technique for these assays is relatively non-invasive, using either endodontic paper points or a sterile curette to obtain bacteria from the subgingival environment, include *B. forsythus*, *P. gingivalis*, *T. denticola*, and *A. actinomycetemcomitans* as periodontal pathogen.^{22–26} The prevalence of *Bacteroides forsythus* in severe periodontitis patients was reported significantly higher than

Table 1. Potential predictors of periodontal disease identified from whole saliva^{3,4}

Category mediator	Biomarkers
Microbial factors	<i>B. forsythus</i> , <i>P. gingivalis</i> , <i>A. actino-mycetemcomitans</i> , <i>Treponema denticola</i>
Host-derived enzyme	ALP, AST, elastase
Inflammatory mediators & host response modifier	IL-1 β ; IL-6, TNF- α , MMP-8
Connective tissue breakdown products	Collagen telopeptides (ITCP), osteoprotegerin (OPG), osteocalcin, osteonectin, fibronectin fragments.

in those with healthy gingiva or gingivitis. This suggests that the presence of *Bacteroides forsythus* in whole saliva are possible to be risk indicators for periodontal disease, especially in elderly patients.^{1,22–26}

Evaluating utility of new laboratory-based diagnostic methods must present either the sensitivity and specificity of test or as a measure of risk periodontal disease. Sensitivity refers to the ability of a diagnostic test to detect the presence of the disease. Specificity refers to the ability of a diagnostic test to detect the absence of a disease. Sensitivity and specificity tend to be inversely related. The goal of ideal test for periodontal disease that would be able to predict which patients will experience attachment loss (“active” periodontal disease) in the near future and to predict which teeth or which sites will experience attachment loss in the near future. In addition to predicting which patients or sites are at higher risk of becoming active, diagnostic tests can be used to categorize patients into different disease categories; ie, aggressive or chronic periodontitis, or response or no response to treatment. Besides, it can also be used to determine the prognoses of treatment.²⁷ In addition, an ideal periodontal diagnostic test would have to be economically feasible and easy to use.

Analysis of the host responses to periodontal disease for diagnostic purposes has involved the quantification of specific host-derived molecules within gingival crevicular fluid, serum, or saliva. In whole saliva, AST, ALT, GGT, LDH, CK, ALP, ACP are intra cellular enzymes that have enzymatic activity reflect metabolic changes in the gingiva and periodontium inflammation.¹ Those enzyme used to measure a death cell. The elevation of AST levels was associated with a 9 to 16 times greater risk of experiencing active periodontal tissue destruction. Whereas, ALP shown a remarkably increased activity in acute phase of periodontal disease, and restored to the value in healthy person.^{18,19} The other site, elastase found in the lysosomal granules of polymorphonuclear leukocytes (PMNs). also elevated in patients with active periodontal disease.^{9,18–21}

ALP is a membrane-bound glycoprotein produced by many cells, such as PMNs leukocytes during inflammation, osteoblasts, macrophages, and periodontal ligament fibroblasts, during bone formation and periodontal regeneration respectively, within the area of the periodontium and gingival crevice. The ALP activity was found highest in osteoblasts, moderate in periodontal ligament (PDL) fibroblasts, and lowest in gingival fibroblasts. The enzyme ALP plays a role in bone metabolism and bone mineralization by releasing an organic phosphate that contributes to the deposition of calcium phosphate complexes into the osteoid matrix. ALP might also promote mineralization by hydrolyzing inorganic pyrophosphate, a potent inhibitor of hydroxyapatite crystal formation and dissolution, within the extra cellular calcifying matrix vesicles. In the periodontium, ALP is very important enzyme as it is part of normal turnover of periodontal ligament, root cementum, and maintenance of bone homeostasis. Some studies have

shown a remarkably increased activity of ALP in the acute and chronic phase of periodontal disease, and after the periodontal and orthodontic therapy. The periodontal destruction such as alveolar bone loss, periodontal pockets, gingival bleeding and suppuration are related to higher ALP and AST levels in saliva. AST is a tissue destruction biomarker released from necrotic cells in GCF, is associated with periodontitis severity.^{8,18–21}

Cytokines are molecules that modulate the function of a wide variety of cells and are involved in regulating the immune and inflammatory response. Pro-inflammatory cytokine with the most promise for diagnostic testing is known as IL-1 β , IL-6 and TNF- α . IL-1 β and TNF- α are produced by a wide variety of cell types (macrophages, fibroblasts, keratinocytes, and PMNs leukocytes), but the primary producer of IL-1 β in the gingival tissues is the macrophage.^{4,5,13–17} IL-1 β has a number of biologic effects, including initiating the acute phase response to infection. However, in the periodontium, it likely functions to mediate connective tissue destruction and osteoclastic bone resorption. Levels of IL-1 β have been found to be consistently associated with the severity of periodontal disease.²³ The acute inflammatory response in periodontal disease is MMPs. It released by inflammatory cells, polymorphonuclear leukocytes and osteoclasts, leading to the degradation of connective tissue collagen and alveolar bone that have also been shown to aid in the diagnosis of periodontal disease. The MMP-8 in GCF is very effective in evaluating the outcome of periodontal treatment. Salivary levels of IL-1 β , MMP-8, OPG, and MIP-1 α reflected disease severity and response to therapy suggesting their potential utility for monitoring periodontal disease status.^{13–17} OPG used as a marker of bone turnover.^{3–5,17}

It can be concluded that the indicators of acute periodontitis can detect with β -glucuronidase and AST, IL-1 β , and MMP-8, whereas indicators for chronic periodontitis can detect with ALP. The indicators for collagen degradation and bone turnover suggest ICTP, fibronectin fragments, and osteonectin. The indicators of severity of periodontitis especially can be predict by *B. forsythus*.

REFERENCES

1. Keles GC, Acikgoz G, Ayas B, Sakallioğlu E, Firatlı E. Determination of systemically and locally induce periodontal defect in rats. *Indiana J Med Res* 2005; 121: 176–84.
2. Khiste SV, Ranganath V, Nichani AS, Rajani V. Critical analysis of biomarkers in the current periodontal practice. *J Indian Soc Periodontol* 2011; 15: 104–10.
3. Taba M, Kinney J, Kim AS, Giannobile V. Diagnostic biomarkers for oral and periodontal diseases. *Dent Clin North Am* 2005; 49(3): 551–6.
4. Ali J, Pramod K, Tahir MA, Ansari SH. Autoimmune responses in periodontal disease. *Autoimmunity Reviews* 2011 May; 10 (issue 7): 426–31.
5. Miller CS, King CP, Langub MC, Kryscio RJ, Thomas MV. Salivary biomarkers of existing periodontal disease: A cross-sectional study. *J Am Dent Assoc* 2006; 137: 322–9.

6. Grbic JT, Steven P, Engebretson SP. The future of periodontal diagnostic testing. *Dentistry Today*. 2010. Available at: <http://www.dentistrytoday.com/periodontics/1617>. Accessed October 30th, 2010.
7. Giannobile WV, Beikler T, Kinney JS, Ramseier CA, Morelli T, Wong DT. Saliva as a diagnostic tool for periodontal disease: current state and future directions. *Periodontology* 2000, 2009; 50: 52–64.
8. Pfaffe T, Cooper-White J, Beyerlein P, Kostner K, Punyadeera C. Diagnostic potential of saliva: Current state and future applications. *Clinical Chemistry* 2011; 57: 675–87.
9. Reddy S, Kaul S, Prasad MGS, Agnihotri J, Asutkar H, Bhowmik N. Biomarkers in periodontal diagnosis: “What the future holds...”. *Int J of Clin Dent Sci* 2011; 2(1): 76–83.
10. Ansai T, Soh I, Ishisaka A, Yoshida A, Awano S, Hamasaki T, Sonoki K, Takata Y, Takehara T. Determination of cortisol and dehydroepiandrosterone levels in saliva for screening of periodontitis in older Japanese adults. *Int J Dent* 2009; 2009: 280737.
11. Kinney JS., Ramseier CA, Giannobile WV. Oral fluid–based biomarkers of alveolar bone loss in periodontitis. *Ann NY Acad Sci* 2007; 1098: 230–51.
12. Desai S, Shinde H, Mudda J, Patil V. Levels of alkaline phosphatase (ALP) in saliva of patients with chronic periodontitis; A clinical and biochemical study. *The Internet J Dental Science* 2009; 8(1): 1–19.
13. Gursoy UK, Könönen E, Uitto VJ, Pirkko J, Pussinen PJ, Kati Hyvärinen K, Liisa Suominen-Taipale L, Knuuttila M. Salivary interleukin-1 β concentration and the presence of multiple pathogens in periodontitis. *J Clin Periodontol* 2009; 36(11): 922–7.
14. Aurer A, Jorgic-Srdjak K, Plancak D, Stavljenic-Rukavina A, Aurer-Kozelj J. Proinflammatory factors in saliva as possible markers for periodontal disease. *Coll Antropol* 2005; 29(2): 435–9.
15. Pradeep AR, Manojkumar ST, Garima G, Raju A. Serum levels of oncostatin M (a gp 130 cytokine): an inflammatory biomarker in periodontal disease. *Biomarkers* 2010; 15(3): 277–82.
16. Sexton WM, Lin Y, Kryscio RJ, Dawson DR, Ebersole JL, Miller CS. Salivary biomarkers of periodontal disease in response to treatment. *J Clin Periodontol* 2011 May; 38(5): 434–41.
17. Rai B, Kharb S, Jain R, Anand SC. Biomarkers of periodontal disease in oral fluids. *J Oral Sci* 2008; 50: 53–6.
18. Totan A, Greabu M, Totan C, Spinu T. Salivary aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase: possible markers in periodontal diseases? *Clin Chemistry and Laboratory Med* 2006; 44(5): 612–5.
19. Todorovic T, Dozic I, Vicente-Barrero M, Ljuskovic B, Pejovic J, Marjanovic M. Salivary enzymes and periodontal disease. *Med Oral Patol Oral Cir Bucal* 2006; 11: E115–9.
20. Zappacosta B, Manni A, Persichilli S, Boari A, Scribano D, Minucci A, Raffaelli L, Giardina B, Sole PD. Salivary thiols and enzyme markers of cell damage in periodontal disease. *Clinical Biochemistry* 2007; 40(9–10): 661–5.
21. Kumar R, Sharma G. Salivary alkaline phosphatase level as diagnostic marker for periodontal disease. *J Int Oral Health* 2011; 3(Issue 5): 81–6.
22. Ruhl S, Hamberger S, Betz R, Sukkar T, Schmalz G, Seymour RA, Hiller KA, Thomason JM. Salivary composition in gingival overgrowth. *J Dent Res* 2004; 83(4): 322–6.
23. Bascones-Martinez A, Munoz-Corcuera M, Noronha S, Mota P, Bascones-Ilundain C, Campo-Trapero J. Host defence mechanisms against bacterial aggression in periodontal disease: Basic mechanisms. *Med Oral Pathol Oral Cir Bucal* 2009; 14(12): e680–5.
24. Bascones Martínez A, Figuero Ruiz E. Periodontal diseases as bacterial infection. *Av Periodon Implantol*. 2005; 17(3): 111–8.
25. Kestic L, Milasin J, Igic M, Obradovic R. Microbial etiology of periodontal diseases—mini review. *Medicine and Biology* 2008; 15(1): 1–6.
26. Ramseier CA, Kinney JS, Herr AE, Thomas Braun T, Sugai JV, Shelburne CA, Rayburn LA, Tran HM, Singh AK, Giannobile WV. Identification of pathogen and host-response markers correlated with periodontal disease. *J Periodontol* 2009; 80: 436–46.
27. Zia A, Khan S, Bey A, Gupta ND, Mukhtar-Un-Nisar S. Oral biomarkers in the diagnosis and progression of periodontal diseases. *Biology and Medicine* 2011 March; 3(2): 45–52.
28. Seibel MJ. Clinical application of biochemical markers of bone turnover. *Arq Bras Endocrinol Metabol* 2006; 50: 603–20.
29. McGehee Jr. JW, Johnson RB. Biomarkers of bone turnover can be assayed from human saliva. *The Journals of Gerontology: Series A* 2003; 59 (Issue 3): B196–200.
30. Frodge BD, Ebersole JL, Kryscio RJ, Thomas MV, Miller CS. Bone remodeling biomarkers of periodontal disease in saliva. *J Periodontol* 2008; 79: 1913–9.