Published in final edited form as:

J Clin Immunol. 2013 January ; 33(1): 271–279. doi:10.1007/s10875-012-9771-3.

Patterns of Salivary Analytes Provide Diagnostic Capacity for Distinguishing Chronic Adult Periodontitis from Health

Jeffrey L. Ebersole^{1,2}, Julie L. Schuster², Jason Stevens¹, Dolph Dawson III^{1,2}, Richard Kryscio³, Yushin Lin³, Mark V. Thomas², and Craig S. Miller⁴

¹Center for Oral Health Research, University of Kentucky

²Division of Periodontology, Department of Oral Health Practice, University of Kentucky College of Dentistry

³Department of Biostatistics, University of Kentucky College of Public Health

⁴Division of Oral Diagnosis and Oral Medicine, Department of Oral Health Practice, University of Kentucky College of Dentistry

Abstract

Salivary biomarker discovery requires identification of analytes with high discriminatory capacity to distinguish disease from health, including day-to-day variations that occur in analyte levels. In this study, seven biomarkers associated with inflammatory and tissue destructive processes of periodontal disease were investigated. In a prospective cohort study design, analyte expression levels were determined in unstimulated whole saliva samples collected on multiple occasions from 30 healthy adults (i.e., orally and systemically) and 50 chronic adult periodontitis patients. Salivary levels of IL-1, IL-6, MMP-8, and albumin were significantly elevated (5.4 to 12.6×) and levels of IFN were consistently lower (8.7×) in periodontitis patients compared with the daily variation observed in healthy adults. ROC analyses of IL-1, IL-6 and MMP-8 yielded areas under the curves of 0.963-0.984 for discriminating periodontitis from health. These results demonstrate that levels of salivary bioanalytes of patients who have periodontitis are uniquely different from normal levels found in healthy subjects, and a panel consisting of IL-1, MMP-8 and IL-6 shows particular diagnostic potential.

Keywords

saliva; periodontitis; diagnosis; inflammation; analytes

Introduction

Periodontitis is a chronic infection and inflammation of tissues in the oral cavity and represents one of the most widely distributed and prevalent diseases of humans (1-3). The ramifications of this disease are often viewed as being localized to the oral cavity, with a permanent solution affected by removal of the teeth. However, recent data have supported that this chronic infection with continued stimulation of the inflammatory responses communicates with the systemic circulation and may contribute to systemic disease

Correspondence: Dr. Jeffrey L. Ebersole, Center for Oral Health Research, HSRB 422, College of Dentistry, University of Kentucky, Lexington, KY 40536-0297, FAX: 859-257-6566, jleber2@uky.edu.

Author Contributions: All authors have contributed to the intellectual content of this paper and meet the following 3 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; and (c) final approval of the published article.

sequelae, such as cardiovascular disease (CVD), diabetes, preterm/low birthweight infants, Alzheimer's disease and other negative aging processes (4-7) (8, 9). Of note, the prevalence of chronic oral diseases such as periodontal disease and their associated adverse outcomes increase with age, affecting approximately 45% of adults over 50 years of age in the U.S., and are disproportionately borne by persons with low socio-economic status (10, 11). Thus, oral diseases constitute a major health burden on a global scale and the health community recognizes the importance of addressing the global burden of non-communicable diseases, including periodontal disease. Furthermore, the economic impact of treatment of oral disease accounts for 3-12.5% of overall health expenditures in industrialized countries (12), making the mouth the most expensive part of the body to treat and contributing to lost economic productivity (13, 14). Expenditures on dental services in the United States in 2009 were \$102.2 billion, which represents 4.1% of the total health care expenses (15).

Due to the increasing prevalence, associated co-morbidities, and large costs associated with treating progressive disease, there is a need for screening and diagnostic modalities for early identification of periodontitis initiation and progression, as well as objective measures for response to therapy. While differentiating health from destructive periodontal disease is simple at the professional level, relying on clinical presentation to differentiate these diseases, particularly at the earliest stages of initiation and/or progression remains challenging for clinicians (16). The development and implementation of modern technologies that can provide rapid, non-invasive, screening diagnoses that can be applied in a broader health care setting, and/or put into practice for "high risk", historically underserved populations lacking well developed medical/dental support systems, would be a substantial benefit to improving oral and systemic health.

Recent strategic initiatives at the National Institutes of Health have highlighted the potential for use of biomarkers in saliva as indicators of oral or systemic health status (17-19). Specifically, studies focusing on oral health have revealed several biomarkers that have significant associations with the inflammatory, connective tissue destruction and bone remodeling phases of periodontal disease (20, 21). However, a recent report from our lab suggested that orally healthy adults demonstrate day-to-day variability in salivary biomarker levels that might affect the discriminatory capacity of these biomarkers for periodontal disease (22). Thus it was important to evaluate the potential of select salivary biomarkers of periodontal health or disease within the context of their natural daily variation in an orally and systemically healthy cohort of subjects to determine their use in clinical decision-making. Our findings from analysis of seven putative biomarkers indicate that patterns of select salivary analytes could provide clinicians important information for identification and monitoring of periodontal health in their patients.

Methods

Study Population and Clinical Assessments

Thirty volunteer healthy subjects were recruited from the faculty, staff, and students of the University of Kentucky. As we have reported previously, all of these subjects were between 18 and 45 years of age and in good general health consisting of 16 men and 14 women (22). These systemically healthy patients also demonstrated periodontal health with a minimum of 20 teeth, bleeding on probing (BOP) in less than 10% of sites, probing pocket depths (PD) of ≤ 5 mm in $<2\%$ of sites, no PD greater ≥ 6 mm, and clinical attachment loss (CAL) of ≤ 2 mm in $<1\%$ of sites. Individuals were excluded if they had a fever, infectious diseases including, but not limited to, human immunodeficiency virus (HIV), hepatitis B, and inflammatory conditions including rheumatoid arthritis (23). Additional exclusion criteria were use of medications believed to affect the inflammatory response and/or periodontal

status (*i.e.*, antibiotics, glucocorticoids, cyclooxygenase inhibitors, and bisphosphonates), as well as pregnancy and use of tobacco products.

Fifty patients with the diagnosis of chronic adult periodontitis were also included in the study (24). Participants had to have five qualifying sites in two quadrants with a minimum of two affected teeth in each quadrant with each site having PD $\geq$ 5 mm, CAL of $\geq$ 3 mm, and BOP score of $\geq$ 2 (0 = one, 1 = pinpoint, 2 = interdental bleeding, 3 = spontaneous/heavy bleeding). Subjects at least 18 years of age and were not excluded based on race, gender, or ethnicity. The study was performed at the University of Kentucky between August 2007 and October 2009 and the protocol was reviewed and approved by the Institutional Review Board (IRB) at the University of Kentucky. All subjects understood the study, provided written informed consent and received incentives (*i.e.*, monetary compensation and a clinical examination) as part of the study protocol.

Periodontal Evaluation

A comprehensive periodontal and oral examination was conducted. Potential subjects with active oral infections (*e.g.*, abscess, aphthae, lichen planus, candidiasis) or active mucosal ulceration were excluded from participation. One calibrated examiner conducted all examinations on the healthy subjects and for the periodontitis patients. Probing PD were measured at six locations per tooth (mesial-buccal, midbuccal, distal-buccal, mesial-lingual, midlingual, and distal-lingual) using a PCP-UNC 15 probe (Hu-Friedy, Chicago). Following the measurement of probing depths, all sites were observed for BOP. The degree of bleeding was estimated and recorded (0 = no bleeding, 1 = light bleeding, 2 = moderate bleeding, and 3 = heavy bleeding) for each probed site (25). CALs were measured at interproximal sites only.

Collection and Handling of Salivary Samples

Unstimulated whole saliva (5 mL) was collected from all participants between 9 and 11 a.m. according to a modification of the method described by Navazesh (26). Subjects were asked to avoid oral hygiene measures (*i.e.*, flossing, brushing, and mouth rinses), eating, drinking, or gum chewing for 1 hour before collection. Subjects rinsed their mouth with tap water, following which they expectorated at least 5 mL of whole saliva into sterile tubes containing a protease inhibitor solution (SIGMAFAST, Sigma, St. Louis, MO.). Samples were immediately placed on ice and transferred to the laboratory. Following collection of the samples, aliquots were prepared and the samples were frozen at -80°C until analysis. Samples were thawed and analyzed within six months of collection.

For the healthy subjects, a total of six saliva specimens were obtained for analysis (22). Saliva was collected on Monday and Friday for two weeks and two additional morning samples at home were collected on the interceding Wednesdays using the same protocol as was used for the clinic sample collection. The collection tubes containing these samples were placed in the home freezer and returned to the investigators at the next visit, two days later, and processed identically to the clinic samples. Unstimulated whole saliva was collected from the periodontitis patients at baseline, prior to entry into a longitudinal treatment study. Specimens were collected, processed, and stored in an identical manner to the healthy subjects.

Salivary Analysis

Salivary concentrations of interleukin-1 (IL-1), interleukin-6 (IL-6), tumor necrosis factor- α (TNF- α), and interferon- γ (IFN- γ) were measured using human Luminex[®] multiplex assays (Invitrogen Carlsbad, CA). Enzyme-linked immunosorbent assays were used to determine levels of salivary matrix metalloproteinase-8 (MMP-8) (R&D Systems,

Minneapolis, MN), prostaglandin E₂ (PGE₂) (Assay Designs, Ann Arbor, MI), and albumin (Alpco Diagnostics, Salem, NH). All assays were performed in duplicate according to the manufacturer's direction in the University of Kentucky General Clinical Research Center Core laboratory. Standards were included on all runs and all results are reported within the linearity of the assays.

Statistical Analysis

Mean levels of the analytes were compared between healthy and periodontitis patients using two sample t-statistics. Since the healthy subjects had multiple determinations of each analyte the median value was used in this and all subsequent analyses. Also, since the standard deviation of the analyte scores varied with the mean level, the two sample t-tests were based on log transformed data (variance stabilizing transformation). The ability of each analyte to distinguish disease from health was based on a logistic regression model and the optimal combination of analytes to discriminate the groups was based on a stepwise logistic regression model. For all comparisons including the logistic modeling statistical significance was set at 0.05. We determined Odds Ratios (OR; MedCalc Software 10.0.2, Mariakerke, Belgium) of effect size for each of the analytes based upon the threshold cutoff values. Measures of prediction for each classification rule were computed using area under the curve (AUC), receiver operator characteristics (ROC), sensitivity, specificity, predictive value positive (PVP), and predictive value negative (PVN). Robustness of the predictions was determined by a Random Forests analysis. All computations were done on PC-SAS Version 9.2 with the exception of the Random Forest analysis which relied on a separate statistical package (RandomForest 1.0, Salford Systems, San Diego, CA).

Results

An overview of the demographics of the fifty periodontitis patients and 30 healthy patients is shown Table 1. The periodontitis group contained significantly more men, non-Caucasians, and smokers, and was significantly older than the controls. The two groups had similar mean number of teeth. The recorded clinical indices reflected the presence of generalized chronic periodontitis in the periodontitis group, and good periodontal health in the healthy group.

Discrimination Using Individual Salivary Biomarkers

Seven salivary biomarkers associated with inflammatory and tissue destructive processes of periodontitis were evaluated. Comparative levels of these analytes in the two groups are shown in Table 2. Salivary levels of IL-1, IL-6, MMP-8 and albumin were significantly elevated (3 to 12.6×) in the periodontitis patients compared with the variation observed in healthy adults ($p < 0.001$). In contrast, IFN levels were significantly lower (8.7×) in the saliva from the periodontitis patients ($p = 0.0006$). Mean levels of PGE₂ and TNF were higher in the periodontitis group, but not significantly different from the healthy group.

Distribution plots were constructed for each analyte, and the five plots where levels were significantly different between the groups are shown in Fig. 1A and B. Within each figure, threshold levels are depicted based on a 95% upper confidence level of median values for all healthy subjects. These distribution plots demonstrate that elevated salivary levels of IL-1, MMP-8, IL-6 and albumin occurred predominately in the periodontitis group. In contrast, IFN levels were elevated mostly in the healthy participants. The data based on median values for the controls were then used to create receiver operator characteristic (ROC) curves (Table 2; Fig. 2) to estimate the sensitivity and specificity of the individual analytes. Here IL-1, MMP-8 and IL-6 demonstrated sensitivity and specificity values ranging from 80-97%, as well as positive predictive values >90%. IFN demonstrated a perfect positive

predictive value and specificity, but low sensitivity. Albumin produced intermediate values, whereas PGE₂ and TNF both had low sensitivity and only moderately high specificity (data not shown).

The discriminatory power of the three individual analytes is illustrated in Table 3. Here the ORs ranged from 3 to 5.2, for salivary IL-1, IL-6 or MMP8. These elevated levels of salivary analytes occurred in over 80% of the periodontitis group compared with the initial saliva sample from the healthy subjects. Compared with all saliva samples from the healthy group, the ORs were 2.0-2.4 for the periodontitis group. This indicated the discriminatory capacity in spite of the inherent “normal” variation in salivary analyte levels from healthy subjects. Albumin levels also were significantly different between health and periodontitis patients; however, 40% of healthy saliva samples were elevated above the threshold level. IFN levels provided a contrasting discriminatory capacity. Here we found that IFN levels were generally only elevated in the healthy participants. However, detailed inspection of the IFN levels in the healthy subjects identified that <40% of the initial saliva samples from the healthy subject's demonstrated levels of IFN greater than the threshold. Thus for IFN, the results demonstrated a high specificity, but low sensitivity. Levels of salivary PGE₂ and TNF provided minimal value in discriminating periodontal health from disease (data not shown). Based on these findings, the threshold values identified for the three analytes (*ie.* IL-1, MMP-8, IL-6) individually discriminated the majority of periodontitis patients and were, thus, evaluated in more detail.

Discrimination Using Profiles of Salivary Biomarkers

The bottom section of Table 3 provides a summary of the ROC analyses for the best discriminatory pairs of analytes, as well as a panel of three analytes for distinguishing health from periodontal disease. Table 4 provides a summary of the ROC analysis of the various combinations of these analytes to discriminate health from periodontitis. Each of the pairings of IL-1, IL-6, and MMP-8 yielded high sensitivity and specificity, ranging generally at or above 94%. The ORs calculated for the pairings ranged from 3.0-9.3 using initial or all saliva samples from healthy subjects, confirming the discriminatory power of these salivary analyte panels. These results reinforce the value of including a finite number of targeted analytes to improve the capacity of the diagnostic patterns for both the population and individual patient descriptions. The uniqueness of the two groups based on mean levels for all three analytes in saliva of each patient is shown as a 3-dimensional display in Fig. 3.

To determine the robustness of the logistic results, especially those based on pairs of analytes, we analyzed the data by Random Forest to confirm the diagnostic potential of the biomarkers that had been identified using a range of aforementioned statistical validation approaches (Fig. 4). The mean error rate for predictions by the trees in the forest was 4.7% overall, 6.0% for disease, and 3.3% for healthy indicating that the panel of analytes has high predictive capacity. The parallel coordinates plot combined with relative rankings of the biomarkers based on the rescaled Gini Importance Index demonstrated a clear value of IL-6 (Gini Index rescaled to 100%), IL-1 (90.71%) and MMP-8 (47.9%) to differentiate healthy subjects from patients with chronic adult periodontitis. Of the other mediators examined, IFN provided additional information at a level of 23.67%.

Discussion

Our previous findings regarding salivary analytes in healthy subjects demonstrated significant within-subject variation (22). However, this report extends these observations by showing that salivary levels of select analytes in healthy subjects, irrespective of this “normal” variation, are significantly different from levels in periodontitis patients.

Biological variation (intra-individual variability), which exists for all biomolecules across the human population, including ones affected by age, gender, ethnicity, and genotype fluctuate around an individual subjects “normal” set-point (27-30). This intra-individual random variation is differentiated from between-subject variation that results from different individuals having different “normal” variation. For analytes to be of diagnostic value, the differentiation of disease must exceed the natural intra-individual and between-subject variation associated with health. The magnitude of this difference beyond natural variation is critical for interpretation of laboratory results in clinical medicine. While the variability in salivary analyte levels is considered to be more than for serum analytes (31, 32), direct comparisons are not possible, since serum reference ranges are not commonly available for the salivary analytes examined in this study. Nevertheless, within this limited set of analytes we were able to identify IL-1, MMP-8, IL-6, and IFN levels in unstimulated whole saliva that differentiated chronic adult periodontitis patients from healthy subjects. We have previously reported the existence of significant within-subject variation in various salivary analytes in healthy adults (22); however, here levels of these biomarkers were significantly elevated in patients with periodontitis above this normal variation. Further, the pairing of analytes and statistical analyses performed here enabled the establishment of thresholds of specific salivary analytes that clearly discriminate health from disease. The current report also extends these findings by analyzing combinations of salivary analytes that appear to enable the potential for identification of clinical changes of periodontitis that could represent early detectable biological transitions from health.

These data are consistent with previous findings from our lab and others that IL-1 and MMP-8 concentrations are significantly elevated in the saliva of periodontitis patients (18, 33-37). Using a series of statistical analyses, we expanded our knowledge of the clinical utility of these biomarkers. Here we showed that the panel of biomarkers, specifically IL-1, IL-6, and MMP-8, provided high sensitivity and specificity for periodontitis at a population level, and even more importantly provided a high positive and negative predictive value for classification of individuals into health or disease subsets. Although our findings are somewhat limited by study size, inexact matching of the group demographics, the exclusion of individuals from both cohorts with co-morbid conditions, the requirement for generalized disease, and absence of repeated measures for the periodontitis patients as was conducted in the healthy subjects, the findings show promise for the field of salivary diagnostics. Further, the data support and extend the identification of select salivary analytes that vary in level in oral health, but appear to be biological reflections of the various inflammatory and tissue destructive processes that occur in periodontitis (38-40).

Summary

In conclusion, we found select salivary biomarkers that when used as panels provide high discriminatory capacity for distinguishing periodontal disease from health. These biomarkers are likely to provide great clinical benefit when used in conjunction with other clinical information, and will need to be further tested in larger prospective studies of periodontal disease as well as less severe forms of periodontal disease including gingivitis.

Acknowledgments

We thank Hailey Wilson, manager of the Delta Dental of Kentucky Clinical Research Center, and clinical coordinators, Dawn Dawson, Vanessa Hodges, and Brittany Fuller for their expert services in managing the patients and samples.

Research Funding: This study was supported by grant U01 DE017793, from the NIH/NIDCR, P20 RR020145 from the NIH/NCRR, and funds from the Center for Oral Health Research in the University of Kentucky College of Dentistry. The authors state no conflict of interest related to this study.

References

1. Demmer RT, Papapanou PN. Epidemiologic patterns of chronic and aggressive periodontitis. *Periodontol* 2000. 2010 Jun;53:28–44. [PubMed: 20403103]
2. Cobb CM, Williams KB, Gerkovitch MM. Is the prevalence of periodontitis in the USA in decline? *Periodontol* 2000. 2009; 50:13–24. [PubMed: 19388950]
3. Kinane D, Bouchard P. Periodontal diseases and health: Consensus Report of the Sixth European Workshop on Periodontology. *J Clin Periodontol*. 2008 Sep; 35(8 Suppl):333–7. [PubMed: 18724860]
4. Seymour GJ, Ford PJ, Cullinan MP, Leishman S, Yamazaki K. Relationship between periodontal infections and systemic disease. *Clin Microbiol Infect*. 2007 Oct; 13(Suppl 4):3–10. [PubMed: 17716290]
5. Paquette DW. Periodontal disease and the risk for adverse pregnancy outcomes. *Grand Rounds in Oral-Systemic Medicine*. 2006; 1(4):14–24.
6. Offenbacher S, Beck JD, Moss K, Mendoza L, Paquette DW, Barrow DA, et al. Results from the Periodontitis and Vascular Events (PAVE) Study: a pilot multicentered, randomized, controlled trial to study effects of periodontal therapy in a secondary prevention model of cardiovascular disease. *J Periodontol*. 2009 Feb; 80(2):190–201. [PubMed: 19186958]
7. Noble JM, Borrell LN, Papapanou PN, Elkind MS, Scarmeas N, Wright CB. Periodontitis is associated with cognitive impairment among older adults: analysis of NHANES-III. *J Neurol Neurosurg Psychiatry*. 2009 Nov; 80(11):1206–11. [PubMed: 19419981]
8. Novak KF, Taylor GW, Dawson DR, Ferguson JE 2nd, Novak MJ. Periodontitis and gestational diabetes mellitus: exploring the link in NHANES III. *J Public Health Dent*. 2006 Summer;66(3): 163–8. [PubMed: 16913241]
9. Jimenez M, Krall EA, Garcia RI, Vokonas PS, Dietrich T. Periodontitis and incidence of cerebrovascular disease in men. *Ann Neurol*. 2009 Oct; 66(4):505–12. [PubMed: 19847898]
10. Demmer RT, Kocher T, Schwahn C, Volzke H, Jacobs DR Jr, Desvarieux M. Refining exposure definitions for studies of periodontal disease and systemic disease associations. *Community Dent Oral Epidemiol*. 2008 Dec; 36(6):493–502. [PubMed: 18422705]
11. Jin LJ, Armitage GC, Klinge B, Lang NP, Tonetti M, Williams RC. Global oral health inequalities: task group--periodontal disease. *Adv Dent Res*. 2011 May; 23(2):221–6. [PubMed: 21490234]
12. Beaglehole, R.; Benzian, H.; Crail, J.; Mackay, J. Mapping a neglected global health issue. FDI World Dental Foundation; 2009. The Oral Health Atlas. <http://www.oralhealthatlas.org/Read.html>
13. Williams DM. Global oral health inequalities: the research agenda. *Adv Dent Res*. 2011 May; 23(2):198–200. Editorial Introductory. [PubMed: 21490230]
14. Casamassimo PS, Thikkurissy S, Edelstein BL, Maiorini E. Beyond the dmft: the human and economic cost of early childhood caries. *Journal of the American Dental Association*. 2009 Jun; 140(6):650–7. Review. [PubMed: 19491160]
15. Centers for Medicare & Medicaid Services. 2009. <http://www.cms.gov/NationalHealthExpendData/downloads/tablespdf>
16. Ghiabi E, Weerasinghe S. The periodontal examination profile of general dentists in Nova Scotia, Canada. *Journal of periodontology*. 2011 Jan; 82(1):33–40. Research Support, Non-U.S. Gov't. [PubMed: 20629548]
17. Zhang L, Xiao H, Wong DT. Salivary biomarkers for clinical applications. *Mol Diagn Ther*. 2009; 13(4):245–59. [PubMed: 19712004]
18. 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. *Periodontol* 2000. 2009; 50:52–64. [PubMed: 19388953]
19. Teles RP, Likhari V, Socransky SS, Haffajee AD. Salivary cytokine levels in subjects with chronic periodontitis and in periodontally healthy individuals: a cross-sectional study. *J Periodontal Res*. 2009 Jun; 44(3):411–7. [PubMed: 19210336]
20. Miller CS, Foley JD, Bailey AL, Campbell CL, Humphries RL, Christodoulides N, et al. Current developments in salivary diagnostics. *Biomark Med*. 2010 Feb; 4(1):171–89. [PubMed: 20387312]

21. Kinney JS, Morelli T, Braun T, Ramseier CA, Herr AE, Sugai JV, et al. Saliva/pathogen biomarker signatures and periodontal disease progression. *Journal of dental research*. 2011 Jun; 90(6):752–8. Clinical Trial Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't. [PubMed: 21406610]
22. Thomas MV, Branscum A, Miller CS, Ebersole J, Al-Sabbagh M, Schuster JL. Within-subject variability in repeated measures of salivary analytes in healthy adults. *Biomark Med*. 2009 Jul; 80(7):1146–53.
23. Biyikoglu B, Buduneli N, Kardesler L, Aksu K, Oder G, Kutukculer N. Evaluation of t-PA, PAI-2, IL-1beta and PGE(2) in gingival crevicular fluid of rheumatoid arthritis patients with periodontal disease. *J Clin Periodontol*. 2006 Sep; 33(9):605–11. [PubMed: 16856901]
24. Armitage GC. Periodontal diagnoses and classification of periodontal diseases. *Periodontol* 2000. 2004; 34:9–21. Review. [PubMed: 14717852]
25. Michalowicz BS, Hodges JS, DiAngelis AJ, Lupo VR, Novak MJ, Ferguson JE, et al. Treatment of periodontal disease and the risk of preterm birth. *N Engl J Med*. 2006 Nov 2; 355(18):1885–94. [PubMed: 17079762]
26. Navazesh M. Methods for collecting saliva. *Ann N Y Acad Sci*. 1993 Sep 20.694:72–7. [PubMed: 8215087]
27. Brown LF, Fraser CG. Assay validation and biological variation of serum receptor for advanced glycation end-products. *Ann Clin Biochem*. 2008 Sep; 45(Pt 5):518–9. [PubMed: 18753428]
28. Lacher DA, Hughes JP, Carroll MD. Biological variation of laboratory analytes based on the 1999-2002 National Health and Nutrition Examination Survey. *Natl HealthStat Report*. Mar; 1(21):1–7.
29. Siest G. Study of reference values and biological variation: a necessity and a model for Preventive Medicine Centers. *Clin Chem Lab Med*. 2004; 42(7):810–6. [PubMed: 15327017]
30. Williams GZ, Widdowson GM, Penton J. Individual character of variation in time-series studies of healthy people: II. Differences in values for clinical chemical analytes in serum among demographic groups, by age and sex. *Clin Chem*. 1978 Feb; 24(2):313–20. [PubMed: 627063]
31. Chiappin S, Antonelli G, Gatti R, De Palo EF. Saliva specimen: a new laboratory tool for diagnostic and basic investigation. *Clin Chim Acta*. 2007 Aug; 383(1-2):30–40. [PubMed: 17512510]
32. Niedbala RS, Feindt H, Kardos K, Vail T, Burton J, Bielska B, et al. Detection of analytes by immunoassay using up-converting phosphor technology. *Anal Biochem*. 2001 Jun 1; 293(1):22–30. [PubMed: 11373074]
33. Frodge BD, Ebersole JL, Kryscio RJ, Thomas MV, Miller CS. Bone remodeling biomarkers of periodontal disease in saliva. *J Periodontol*. 2008 Oct; 79(10):1913–9. [PubMed: 18834246]
34. Miller CS, King CP Jr, Langub MC, Kryscio RJ, Thomas MV. Salivary biomarkers of existing periodontal disease: a cross-sectional study. *J Am Dent Assoc*. 2006 Mar; 137(3):322–9. [PubMed: 16570465]
35. Scannapieco FA, Ng P, Hovey K, Hausmann E, Hutson A, Wactawski-Wende J. Salivary biomarkers associated with alveolar bone loss. *Ann N Y Acad Sci*. 2007 Mar.1098:496–7. [PubMed: 17435158]
36. Kinney JS, Ramseier CA, Giannobile WV. Oral fluid-based biomarkers of alveolar bone loss in periodontitis. *Ann N Y Acad Sci*. 2007 Mar.1098:230–51. [PubMed: 17435132]
37. Taba M Jr, Kinney J, Kim AS, Giannobile WV. Diagnostic biomarkers for oral and periodontal diseases. *Dent Clin North Am*. 2005 Jul; 49(3):551–71. vi. [PubMed: 15978241]
38. Taubman MA, Kawai T, Han X. The new concept of periodontal disease pathogenesis requires new and novel therapeutic strategies. *J Clin Periodontol*. 2007 May; 34(5):367–9. [PubMed: 17448041]
39. Kinane DF, Bartold PM. Clinical relevance of the host responses of periodontitis. *Periodontol* 2000. 2007; 43:278–93. [PubMed: 17214845]
40. Tatakis DN, Kumar PS. Etiology and pathogenesis of periodontal diseases. *Dent Clin North Am*. 2005 Jul; 49(3):491–516. v. [PubMed: 15978238]

Figure 1.

Distribution of (A) salivary IL-1, MMP-8 and IL-6 and (B) salivary PGE₂, TNF, IFN and albumin levels in healthy subjects (control) and periodontitis patients. The open circles (○) denote measures for 6 individual saliva samples from 30 healthy subjects. The 6 samples are plotted in sequence for each subject. The solid circles (●) denote one sample from each of 50 chronic adult periodontitis patients. The horizontal line describes the threshold value determined from the healthy samples, which is also detailed for each analyte.

Figure 2. Receiver Operator Characteristic (ROC) curves for IL-1 , IL-6 and MMP-8, IFN and albumin. These determinations were performed using only the initial saliva sample from the healthy subjects.

Figure 3. (A) ROC analysis for three combined analytes with AUC value. (B) 3-dimensional representation of the subject response profiles for the 3 analytes. Each red circle denotes a periodontitis patients and each blue star signifies the baseline sample for a healthy subject. The oval highlights analytes profile levels that identify healthy subjects.

Figure 4. Parallel coordinates plot for each analyte in order of importance determined from Random Forests Analysis.

Table 1

Demographics of study population.

	Healthy (n=30)	Periodontitis (n=50)	p-value
Age (years; mean $\pm$ SD)	31.4 $\pm$ 6.8	43.0 $\pm$ 10.8	<0.0001
Female (%)	46.7	28.0	NS
White (%)	93.3	44.0	<0.01
Hispanic (%)	3.3	30.0	
African American (%)	0	18.0	
Asian (%)	3.3	8.0	
Current tobacco use (%)	0	28.0	<0.01
# Teeth	28.6 (range 27-32)	24.5 (range 20-28)	NS
Periodontal Indices (% sites; mean $\pm$ SD)			
BOP Sites	4.06 $\pm$ 6.98	54.5 $\pm$ 20.8	<0.0001
PD 4mm Sites	2.64 $\pm$ 3.93	22.7 $\pm$ 12.53	<0.0001
PD 5 mm Sites	0.80 $\pm$ 2.64	14.24 $\pm$ 9.82	<0.0001

Table 2

Comparisons of salivary analytes in health and disease including ROC analyses.

Measure	IL-1 (pg/mL)	IL-6 (pg/mL)	IFN (pg/mL)	MMP-8 (ng/mL)	PGE ₂ (ng/mL)	TNF (pg/mL)	Albumin (mg/mL)
Healthy	7.24±7.69 *	3.30±2.32	27.65± 48.29	52.63±40.62	179.95± 155.31	1.85±2.11	36.15±26.29
Periodontitis	90.94±85.22	35.57±48.17	3.19±2.29	283.47±203.47	226.07±314.69	5.44±10.88	112.94±126.53
P-value	<0.0001	<0.0001	<0.0001	<0.0001	0.9110	0.0738	<0.0001
Sensitivity	0.88	0.88	0.54	0.80	0.42	0.60	0.68
Specificity	0.93	0.97	1.00	0.87	0.87	0.73	0.80
PVP	0.96	0.98	1.00	0.91	0.72	0.73	0.85
PVN	0.82	0.82	0.57	0.72	0.43	0.58	0.60
AUC	0.95	0.95	0.75	0.92	0.49	0.63	0.80
P-value	0.0004	<0.0001	0.011	<0.0001	0.46	0.09	0.001

* Mean ± SD; P values based on log transformed data

PVP = positive predictive value; PVN = negative predictive value; AUC = area under the curve

Table 3

Discriminatory power of individual and combinations of salivary analytes to identify patients with periodontal health and disease.

Elevated Analytes	N (%) of Patients Above Threshold		Odds Ratio	95% CL [†]		P-value
	Periodontitis	Healthy		Lower	Upper	
Initial Saliva Sample						
IL-1 *	44 (88.0)	8 (26.7)	3.03	1.37	7.95	0.0078
MMP-8	40 (80.0)	8 (26.7)	3.00	1.24	7.26	0.0065
IL-6	44 (88.0)	5 (16.7)	5.20	1.89	14.79	0.0015
All Saliva Samples						
IL-1	44 (88.0)	13 (43.3)	2.03	0.94	4.37	0.0701
MMP-8	40 (80.0)	11 (36.7)	2.18	0.97	4.89	0.0579
IL-6	44 (88.0)	11 (36.7)	2.40	1.07	5.35	0.0322
Initial Saliva Sample						
IL-1 + MMP-8 *	36 (72.0)	5 (16.7)	4.32	1.58	12.21	0.0058
IL-1 + IL-6	38 (76.0)	4 (13.3)	5.70	1.85	17.56	0.0024
MMP-8 + IL-6	35 (70.0)	3 (10.0)	7.00	1.98	24.75	0.0025
IL-1 +MMP-8+IL-6	31 (72.0)	2 (6.7)	9.30	2.08	41.67	0.0036
All Saliva Samples						
IL-1 + MMP-8	36 (72.0)	6 (20.0)	3.60	1.36	9.55	0.0101
IL-1 + IL-6	38 (76.0)	6 (20.0)	3.70	1.40	9.80	0.0085
MMP-8 + IL-6	35 (70.0)	4 (13.3)	4.95	1.60	15.36	0.0056
IL-1 +MMP-8+IL-6	31 (72.0)	2 (6.7)	9.00	2.01	40.39	0.0041

* Threshold values for cutoff determination were: IL-1β = 17.8, MMP-8 = 110, IL-6 = 7.5.

[†]CL – Confidence limit.

Table 4

AUC values using patterns of biomarkers and optimal cut point based on ROC curve.

Biomarkers	Variable	p-value	AUC	Sensitivity	Specificity	PVP	PVN
IL-1 + IL-6	Intercept	0.0005	0.983	0.94	0.966	0.979	0.903
	IL-1	0.0451					
	IL-6	0.0060					
IL-1 + MMP-8	Intercept	<0.0001	0.963	0.88	0.967	0.978	0.829
	IL-1	0.0062					
	MMP-8	0.0410					
IL-6 + MMP-8	Intercept	0.0004	0.975	0.94	1.000	1.000	0.906
	MMP-8	0.0275					
	IL-6	0.0029					
IL-1 + IL-6 + MMP-8	Intercept	0.0012	0.984	0.94	0.966	0.979	0.903
	IL-1	0.1604					
	IL-6	0.0604					
	MMP-8	0.3430					