Bone Remodeling Biomarkers of Periodontal Disease in Saliva

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Background: Tumor necrosis factor-alpha (TNF-α), C-telopeptide pyridinoline cross-links of type I collagen (ICTP), and receptor activator of nuclear factor-kappa B ligand (RANKL) have been associated with bone remodeling and periodontal tissue destruction. This study evaluated the level of these biomarkers in saliva with respect to periodontal disease status.

Methods: Levels of TNF-α in unstimulated whole saliva of 74 adults (35 subjects with moderate to severe periodontal disease and 39 healthy controls) and salivary levels of RANKL and ICTP of a subset of 21 subjects and 21 matched controls were examined using enzyme immunosorbent assays in a case-control clinical study.

Results: Salivary levels of TNF-α were detected in all subjects, whereas levels of ICTP and RANKL were detected in only a minority of subjects. Mean salivary levels of TNF-α were significantly higher in individuals with periodontal disease (mean: 4.33 pg/ml) than in controls (mean: 2.03 pg/ml; $P = 0.02$), with a maximum level (27.96 pg/ml) observed in periodontitis. Subjects with salivary TNF-α levels above a threshold of 5.75 pg/ml (i.e., two standard deviations above the mean of the controls) had significantly more sites with bleeding on probing, probing depths ≥4 mm, and attachment loss ≥2 mm ($P \leq 0.01$).

Conclusion: Salivary levels of TNF-α were elevated in patients who had clinical indicators of periodontitis, suggesting that this biomarker may serve in a panel of salivary biomarkers that could facilitate the screening, diagnosis, and management of periodontal disease. *J Periodontol 2008;79:1913-1919.*

KEY WORDS

C-telopeptide pyridinoline; periodontal diseases; receptor activator of nuclear factor-kappa B ligand; tumor necrosis factor-alpha.

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Periodontal disease is a chronic microbial infection that triggers inflammation-mediated loss of the periodontal ligament and alveolar bone that supports the teeth. It affects ~45% of adults older than 50 years of age in the United States and is a major cause of tooth loss worldwide.[1,2] Periodontal disease increases with aging, and with the expanding aging United States population, longer retention of teeth, and increased incidence in obesity and diabetes, the prevalence of periodontal disease can be expected to increase, with social and economic impacts on society.[3-10] Because of the increasing prevalence and associated comorbidities,[11-14] screening and diagnostic modalities for the early identification of periodontitis initiation and progression, as well as objective measures for response to therapy, are being sought.

Whole saliva represents a promising diagnostic fluid for the screening of periodontal disease. It is a fluid that contains constituents of exocrine glands in the oral cavity and gingival crevicular fluid (GCF). Saliva is readily available and easily collected without specialized equipment or personnel. Several mediators of chronic inflammation and tissue destruction have been detected in whole saliva of periodontitis patients.[15-21] Alpha$_2$-macroglobulin, β-glucuronidase, C-reactive protein (CRP), and cathepsin G are host response indicators reported at higher levels in whole saliva of patients who had periodontal disease compared to control

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subjects.[21-23] Also, the proinflammatory cytokine interleukin (IL)-1β and matrix metalloproteinase (MMP)-8 (neutrophil collagenase) were shown to correlate with clinical indices and radiographic evidence of periodontal disease.[24-26] Other salivary biomarkers putatively associated with periodontal disease include hepatocyte growth factor, osteonectin, and osteoprotegerin (OPG).[24,26]

From a biologic perspective, periodontal disease can be considered to consist of three phases: inflammation, destruction of the connective tissue attachment apparatus followed by apical migration of the junctional epithelium, and altered alveolar bone turnover with net loss of bone density and height. Levels of several salivary biomarkers were reported to correlate with two of the phases of periodontal disease (i.e., the inflammatory and connective tissue destruction phases).[15-26] However, few analytes associated with alveolar bone turnover activity have been identified in saliva.[24,27] In this report, we tested the hypothesis that biomolecules involved in bone remodeling (i.e., tumor necrosis factor-alpha [TNF-α], receptor activator of nuclear factor-kappa B ligand [RANKL], and C-telopeptide pyridinoline cross-links of type I collagen [ICTP]) are increased in the saliva of patients with periodontal disease compared to control subjects. These biomarkers represent key aspects of bone remodeling[28] and could serve in a salivary diagnostic panel for periodontitis.

MATERIALS AND METHODS

Subjects

Subjects ≥18 years of age who were in good general health and had ≥20 erupted teeth were eligible to participate. The cases (n = 35) had existing moderate to severe periodontitis.[29,30] Cases were included for study if they had ≥30% of sites with bleeding on probing (BOP), ≥20% of sites with probing depth (PD) ≥4 mm, ≥10% of sites with interproximal clinical attachment loss (AL) >2 mm, and evidence of alveolar crestal bone loss ≥2 mm at ≥30% of sites visible in posterior vertical bitewing films. Thirty-nine healthy adults (controls) of similar age, race, and gender who had <10% sites with BOP, <2% of sites with PD ≥5 mm, no sites with PD ≥6 mm, <1% of sites with clinical AL >2 mm, and no radiographic bone loss evident in posterior vertical bitewings films, were enrolled. Patients were excluded if they had a history of alcoholism; liver, kidney, or salivary gland dysfunction; inflammatory bowel disease; granulomatous disease; immunosuppression; or were undergoing or had undergone organ transplant or cancer therapy. Pregnancy, use of antibiotics or immunosuppressant medication within the last 6 months, need for antibiotics for infective endocarditis prophylaxis during dental procedures, symptoms of acute illness (i.e., fever, sore throat, body aches, and diarrhea), or detection of an oral mucosal inflammatory condition (e.g., aphthous, lichen planus, leukoplakia, and oral cancer) were additional exclusion criteria. The study was performed at the University of Kentucky between August 2005 and January 2007 and was approved by the University Institutional Review Board. All subjects provided written informed consent and received incentives (i.e., monetary compensation and a clinical examination) as part of the study protocol.

Study Procedures

Saliva collection. Unstimulated whole expectorated saliva was collected from each subject according to a modification in the method described by Navazesh.[31] Subjects rinsed their mouth with tap water, then expectorated whole saliva into sterile tubes while seated in an upright position. Collected samples were placed immediately on ice and aliquoted prior to freezing at –80°C. Samples were thawed and analyzed within 6 months of collection.

Clinical examination. Clinical periodontal indices (PD, BOP, and AL) were recorded for each subject by one examiner after the collection of saliva. PD was measured at six locations per tooth (mesial-buccal, mid-buccal, distal-buccal, mesial-lingual, mid-lingual, and distal-lingual) using a probe.§ Clinical AL was obtained by measuring interproximal sites only.

Biomarker analysis. Concentrations of TNF-α in saliva were determined in duplicate for each subject using enzyme immunosorbent assay (EIA) kits,‖ according to the manufacturer's directions, by technologists in the Clinical Laboratory Improvements Amendments–certified General Clinical Research Core laboratory. Concentrations of salivary RANKL and ICTP were determined in duplicate using EIA kits,¶# but were limited to a subset of 21 subjects in each group because preliminary analysis showed that levels were below the limit of detection in >80% of subjects in both groups. Standards were included on all runs, and all results are reported within the linearity of the assays.

Statistical analysis. Demographic variables were compared between the cases and controls using the Fisher exact test and/or the two-sample *t* test. Mean periodontal indices and concentrations of the salivary analytes were compared between the two groups using two sample *t* tests with unequal variances, and a linear mixed model was used to analyze the covariates age, race, tobacco use, and alcohol use. Correlations between an analyte and a dental index were measured by computing the Pearson correlation coefficient and then by computing the odds ratio between the events: expression of the analyte (more than two standard

§ PUNC 15, Hu-Friedy, Chicago, IL.
‖ Human Quantikine high-sensitivity TNF-α assay kit, R&D Systems, Minneapolis, MN.
¶ Human soluble RANKL kit, Biomedica Gruppes, Vienna, Austria.
# Serum Crosslaps ELISA (ICTP kit), Nordic Bioscience Diagnostics, Herlev, Denmark.