Microbiologic Findings in Relation to Risk Assessment for Periodontal Disease: A Cross-Sectional Study

Yong Hur,* Seung Kee Choi,† Yumi Ogata,* Paul C. Stark,‡ and Paul A. Levi*

Background: In this study, an association between a computerized risk calculator and microbiologic testing is examined in patients with periodontitis.

Methods: Seventy-four patients with moderate and severe periodontitis were selected from patients receiving treatment at Tufts University School of Dental Medicine. Their periodontal risk was analyzed with a periodontitis risk assessment tool, and microbiologic testing was performed. Periodontitis risk assessment and microbiologic testing were examined for a possible association. The data were evaluated by the χ^2 test at $P < 0.05$ levels.

Results: Forty-six patients scored as having a "very high" risk of periodontitis and 22 patients scored as having a "high" risk of periodontitis by the risk assessment tool. Patients with a risk score of very high risk showed a higher detection of each bacterium except *Capnocytophaga* species than the rest of the study population. *Treponema denticola* and *Prevotella intermedia* ($P = 0.01$ and $P = 0.02$, respectively) were two bacteria that showed a statistically significant difference between patients at very high risk and those at high risk.

Conclusions: Patients with periodontitis were identified as high risk and very high risk compared with the rest of the risk categories by the risk assessment tool. The study population, categorized mostly as very high risk, showed high detection of putative periodontal bacteria. *J Periodontol 2016;87:21-26.*

KEY WORDS

Microbiology; periodontal diseases; risk assessment; risk factors.

* Department of Periodontology, Tufts University School of Dental Medicine, Boston, MA.
† Private practice, Boston, MA.
‡ Department of Public Health and Community Service, Tufts University School of Dental Medicine.

Periodontitis, considered in the past as a result of bacterial infection, is now regarded as an outcome of a complex interaction between an individual's inflammatory immune response and the bacterial infection.[1-3] Various factors that affect this interaction have been identified as risk factors, which may increase the likelihood of developing periodontitis or its progression.[4] Smoking, diabetes, and other systemic diseases, as well as genetic factors and periodontal microbes, have been associated with increased risks of periodontitis.[5-10]

The American Academy of Periodontology guidelines state that analyzing risk factors is "increasingly important in periodontal treatment planning and should be part of every comprehensive dental and periodontal evaluation."[11] Risk assessment may improve clinical decision-making and the prediction of disease progression.[11] Despite this clear need for risk assessment of periodontitis, the multifactorial nature of the disease etiology and various subjective assessment methods make it challenging to determine the disease risk accurately.[12]

A periodontitis risk assessment tool[§] is a computerized risk assessment tool using the history of periodontal disease.[13,14] Its purpose is to produce a more consistent and accurate risk analysis than subjective periodontal clinical assessment by an individual dentist. The validity of the calculator was reassessed by tooth loss

§ Periodontal Risk Calculator, PreViser, Mount Vernon, WA.

doi: 10.1902/jop.2015.140237

and radiographic bone loss over 15 years.[15] However, its effect on periodontal patient management is still unknown.

Ideal treatment of periodontal disease should include elimination or reduction of periodontal pathogens.[16] The target species for periodontal therapy have been established.[17-20] Microbiologic analysis for periodontally compromised individuals provides a guide to a treatment decision, which leads to comprehensive/targeted therapy for superior therapeutic outcomes.

This study examines patients with moderate to severe periodontitis using a risk assessment tool and a polymerase chain reaction (PCR)-based microbiologic test. A periodontits risk assessment tool produces a risk score (on a scale of 1 to 5, with 5 being the highest risk) based on mathematic algorithms, whereas the microbiologic test helps analyze subgingival periodontopathogenic species, given that periodontitis is a bacterial infection. Combining these two may aid clinicians in assessing patients' disease status, which in turn may help determine appropriate therapeutic strategy with increased accuracy.

The purpose of this study is to investigate the association between risk analysis according to the risk assessment tool and detection of putative periodontal bacteria.

MATERIALS AND METHODS

Study Population

In this cross-sectional study, 74 consecutively enrolled patients were selected from the patient population attending the clinic at Tufts University School of Dental Medicine from September 2007 to July 2008. The study protocol was approved by the Institutional Review Board at Tufts Medical Center and Tufts University Health Sciences Campus before initiation (Protocol 8308). A written informed consent was signed by all patients before enrollment.

The inclusion criteria were the following: 1) patients in good general health; 2) patients who were willing to comply with all study-related procedures; and 3) patients diagnosed with moderate to severe periodontitis with ≥30% teeth manifesting ≥5 mm periodontal pockets based on a comprehensive periodontal evaluation and complete mouth series of radiographs. The exclusion criteria was defined as the following: 1) pregnancy or nursing; 2) periodontal therapy within the past 12 months; 3) antibiotic therapy in the previous 3 months; and 4) any systemic condition other than diabetes that might influence the course of periodontal disease or treatment (e.g., AIDS, heart conditions, and joint replacements).

Clinical Measurement

All comprehensive periodontal evaluations were performed by one investigator (YH). The evaluation included obtaining the following clinical parameters of periodontal disease: 1) probing depth (PD); 2) clinical attachment loss (AL); 3) bleeding on probing (BOP); 4) plaque score at six sites per tooth (mesio-buccal, buccal, disto-buccal, disto-lingual, lingual, and mesio-lingual);[21] and 5) furcation involvements. All patients received the evaluation with a complete-mouth series of radiographs.

Intraexaminer Calibration

An intraexaminer calibration exercise was performed before the study enrollment. PD and AL were measured twice on the same side of four patients within a 1-week period. The calibration was accepted if the difference in measurements was no greater than 1 mm at 90% of the time. Based on this criterion, the examiner (YH) showed an agreement of 100%.[22]

Microbiologic Testing

The molecular biologic test‖ used for microbial analysis was based on DNA-strip technology with a molecular biologic PCR DNA probe method.[23] Eleven bacteria previously considered associated with periodontal disease (*Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, *Tannerella forsythia*, *Treponema denticola*, *Prevotella intermedia*, *Parvimonas micra*, *Fusobacterium nucleatum*, *Campylobacter rectus*, *Eubacterium nodatum*, *Eikenella corrodens*, and *Capnocytophaga* species) were tested using this probe from individual pooled samples of subgingival plaque obtained from the deepest pocket in each sextant.[16,20]

To obtain the bacteria samples, a pair of sterile cotton pliers was used to insert a single paper point into the base of the deepest pocket in each of the patient's sextants. If there was more than one pocket of the same depth (deepest), then the selected teeth were allocated randomly to be a sampling site.

After the 10 seconds of insertion, the paper point was retrieved and inserted into its respective individual tube for the DNA PCR detection.

Periodontitis Risk Assessment

Dental practitioners seeking proper periodontal risk assessment may use the risk assessment tool to develop a more comprehensive diagnosis and treatment plan for improved treatment outcomes.

After completion of the microbiologic sampling, data were collected for all patients to determine a risk score calculation for each individual. The collection was done as per the system protocol of the manufacturer, which required the following parameters to be obtained: 1) dental care frequency; 2) oral hygiene level; 3) past history of periodontal surgery; 4) BOP; 5) calculus on radiographs or clinically assessed below the gingival margin; 6) furcation involvement;

‖ micro-IDent plus, Hain Lifescience, Nehren, Germany.

Table 1.

Demographics and Clinical Parameters of the Study Population (N = 74)

Variables	Mean ± SD or n (%)
Age (years)	44.1 ± 14.2
Sex	
Females	30 (40.5)
Males	44 (59.5)
AL (mm)	4.1 ± 1.3
PD (mm)	3.5 ± 1.1

7) subgingival restorations; 8) smoking history; 9) diabetic state; 10) vertical bone lesions; 11) deepest pocket per sextant; and 12) radiographic bone height from the cemento-enamel junction to the alveolar crest.[24]

Using these risk factors, the risk assessment tool produced a risk score (1, very low risk; 2, low risk; 3, moderate risk; 4, high risk; or 5, very high risk) for each patient.[13]

Statistical Analyses

Patients were categorized by the presence of specific bacteria. Because of the small number of patients between risk scores 1 and 3, only the patients with risk scores 4 and 5 were analyzed against the bacteria distribution. Risk score groups and the presence of specific bacteria were compared statistically using the χ^2 test¶ for the ratio of categorical variables between patients with and without a bacterial detection at $P<0.05$.

RESULTS

Forty-four males and 34 females were recruited in this study, aged 18 to 71 years (mean age: 44.08 years). Table 1 describes the demographic and clinical attributes of the study population.

Periodontitis Risk Assessment and Microbial Analyses

Periodontitis risk assessment. Forty-six patients (62.2%; 28 males and 18 females) scored 5 ("very high" risk of periodontitis), whereas 22 patients (29.7%; 10 males and 12 females) scored 4 ("high" risk). The rest of the study population (8.1%) scored 2 or 3 (low or moderate risk, respectively) (Table 2). The mean ages of the very high risk group and high risk group were 39.52 ± 12.84 and 52.40 ± 12.46 years, respectively ($P>0.05$).

Microbial analyses. The three most prevalent microbes were *T. forsythia*, *T. denticola*, and *P. gingivalis* (86.5%, 82.4%, and 67.6%, respectively). Very high risk patients (score 5) showed a higher detection of all bacteria except *Capnocytophaga* species compared with lower risk patients. *E. corrodens* showed no detection in any of the risk scores (Table 2).

The Association of Periodontitis Risk Assessment and Microbial Analyses

Because of the small number of patients among risk scores 1 to 3, only those with scores 4 and 5 obtained from the risk assessment tool were compared with microbial data from the microbiologic testing.

χ^2 test revealed significant differences between these two score groups, very high and high, for *T. denticola* and *P. intermedia* ($P=0.01$ and $P=0.02$, respectively) (Fig. 1).

Pilot Model to Use Microbiologic Testing With Other Risk Factors in Relation to Clinical Parameters

The purpose of this pilot model was to evaluate the association of the microbiologic testing of 11 periodontal pathogens and clinical parameters (AL and PD) in the presence of known risk factors (age, diabetes, sex, oral hygiene, and smoking) in patients with periodontitis. The multiple linear regression model# was used for multiple independent variables that included the individual risk factors (i.e., smoking, diabetes, oral hygiene, and sex) and microbiologic testing. For the dependent variables, AL and PD were used. Any variables without a significant P value (<0.05) were omitted from the results except for age and sex.

AL (Table 3). Among the results from the microbiologic testing and individual risk factors, it was found that oral hygiene and *P. gingivalis* had significant associations to AL. Patients with poor oral hygiene (plaque free score <80%) were associated with more AL than those with good oral hygiene (plaque free score ≥80%) ($P=0.01$). Patients with the presence of *P. gingivalis* were also associated with more AL than those without the presence of *P. gingivalis* ($P<0.01$).

PD (Table 4). Oral hygiene and *A. actinomycetemcomitans*, *T. forsythia*, age, and male sex were risk factors that were statistically significant in relation to PD. Patients with poor oral hygiene were associated with greater PD than those with good oral hygiene ($P<0.01$). Patients with the presence of *A. actinomycetemcomitans* or *T. forsythia* were associated with greater mean PD than those without the presence of *A. actinomycetemcomitans* or *T. forsythia* ($P<0.01$ and $P=0.01$, respectively). Older age and male sex showed greater mean PD than female patients ($P=0.01$ and $P=0.02$, respectively).

DISCUSSION

Risk assessment, unlike diagnosis of a current clinical condition, helps determine the likelihood of periodontitis or its progression by considering

¶ SPSS v.10 for Windows, IBM, Chicago, IL.
# SPSS v.10 for Windows, IBM.

Table 2.

Distribution of Risk Scores and Bacteria in the Study Population (N = 74)

Risk Scores	Patients (n)	*Aa*	*Pg*	*Tf*	*Td**	*Pi**	*Pm*	*Fn*	*Cr*	*En*	*Ec*	*Cs*
1	0	0	0	0	0	0	0	0	0	0	0	0
2	4	1	0	0	1	0	2	0	0	0	0	0
3	2	0	0	2	2	0	0	0	0	0	0	0
4	22	8	14	20	15	6	0	0	3	0	0	2
5	46	21	26	42	43	28	1	9	8	5	0	1
Total	74	30	40	64	61	34	3	9	11	5	0	3

Aa = *A. actinomycetemcomitans*; *Pg* = *P. gingivalis*; *Tf* = *T. forsythia*; *Td* = *T. denticola*; *Pi* = *P. intermedia*; *Pm* = *P. micra*; *Fn* = *F. nucleatum*; *Cr* = *C. rectus*; *En* = *E. nodatum*; *Ec* = *E. corrodens*; *Cs* = *Capnocytophaga* species.
* $P < 0.05$ between scores 4 and 5.

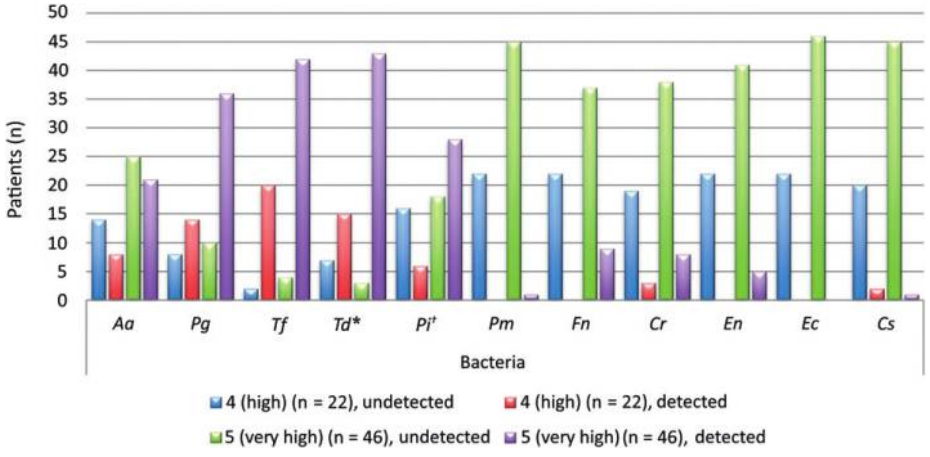

Figure 1.
Risk scores versus bacteria: χ^2 *test (P <0.05). Prevalence of two bacteria,* T. denticola *(Td) and* P. intermedia *(Pi), was found to be significantly different between the two risk score groups.* *P = *0.01*; †P = *0.02*. Aa = A. actinomycetemcomitans; Pg = P. gingivalis; Tf = T. forsythia; Pm = P. micra; Fn = F. nucleatum; Cr = C. rectus; En = E. nodatum; Ec = E. corrodens; Cs = Capnocytophaga species.*

various risk factors. The use of risk assessment tools can be a valuable addition to periodontal therapy because it allows the clinicians to apply information derived from the risk assessment objectively.[12] A recent review identified two risk assessment tools that have been assessed with longitudinal studies and were well-suited with patient-based risk assessment.[25] Understanding the concepts behind risk assessment could help clinicians properly apply various relevant information and assess risks accurately.

Although the risk assessment tool can quantitatively assess risk of periodontal disease, this tool has strengths and weaknesses. For example, the risk scores derived from the risk assessment tool were found to be strong predictors of alveolar bone and tooth loss in periodontal patients.[14] However, because microbiology is an aspect of the disease that risk assessment does not address, the risk assessment tool cannot provide any information on periodontal pathogens involved in patients' disease progression. Clinicians wanting to obtain the microbiologic profiles of their patients may use microbiologic testing to form a microbiology-based therapy.[26]

PCR-based microbiologic testing, like the one used in this study, is highly sensitive and high yielding because PCR can synthesize and amplify even when a small amount of a particular bacterium is detected in the collected sample. So far, studies on microbiologic tests seem to agree that patients with chronic or aggressive periodontitis in which conventional mechanical therapy (e.g., scaling and root planing [SRP]) is not effective have shown improvement with antibiotic therapy that was based on microbiologic testing.[26,27] Microbiologic testing is useful for periodontally compromised patients as an aid to risk assessment and treatment planning and is necessary for patients with a history of smoking and/or systemic conditions.

Because of the multifactorial nature of periodontal disease, the authors speculated that it would be more efficacious to apply microbiologic analysis against the risk assessment tool to provide a more comprehensive risk analysis and therapeutic strategies. The types and levels of subgingival microbiota are different for each individual.[16] Adding microbiologic testing to understand individual bacterial composition may provide

Table 3.

AL Versus Risk Factors (N = 74)

Risk Factors	Standardized Coefficients (β)	*t*	*P*
Oral hygiene	0.28	2.57	0.01*
Pg	0.35	0.31	<0.01*
Age	0.03	0.14	0.78
Sex	0.07	0.30	0.56

Pg = P. gingivalis.
Multiple linear regression was used to determine AL in the presence of various risk factors. Age and male sex were adjusted for the comparison.
* *P* <0.05.

Table 4.

PDs Versus Risk Factors (N = 74)

Risk Factors	Standardized Coefficients (β)	*t*	*P*
Oral hygiene	0.39	4.22	<0.01*
Aa	0.38	3.77	<0.01*
Tf	0.26	2.90	0.01*
Age	0.27	2.73	0.01*
Sex	0.21	2.30	0.02*

Aa = A. actinomycetemcomitans; *Tf = T. forsythia.*
Multiple linear regression was used to determine PDs in the presence of various risk factors. Age and male sex were adjusted for the comparison.
* *P* <0.05.

additional evidence supporting appropriate treatment strategies.[16]

Having examined a population of patients with periodontitis using a combination of the risk assessment tool and a microbiologic test, this study reveals that there was an association between the two: patients who scored 5 and considered as very high risk for periodontitis by the risk assessment tool showed higher detection of all bacteria except *Capnocytophaga* species than patients with lower scores. When very high risk and high risk patients were compared, it was found that *T. denticola* and *P. intermedia* showed statistical significance. Nevertheless, the findings only support the current notion that the presence of these bacteria in diseased sites of patients' dentition cannot lead to a definitive basis for risk assessment or disease prediction because the bacterial presence alone does not mean it initiated or caused periodontal inflammation.[26] Still, understanding and determining the bacterial profile of this study population is clinically relevant and worthy of additional investigation.

A pilot model was developed to examine a potential combination effect of risk factors (age, diabetes, sex, oral hygiene, and smoking) and microbiologic testing to the current clinical parameters (AL and PD). It is important to note that assessing individual risk factors and/or bacteria profile by no means implies causality or direct relation to the current disease status.[28] However, the AL and PD data obtained during cross-sectional studies could be used as a baseline for future studies. Data obtained from subsequent studies can be compared with these baseline data, and this comparison could be analyzed further in the context of risk assessment based on individual factors. The proposed model could be developed as a beneficial tool, which can include various risk factors and microbiologic findings.

Although there is an association among microbiologic findings, risk assessment, and periodontitis, periodontal disease is a complex multifactorial disease that cannot simply be defined by the combination of the variables described above. The results of the current investigation contribute to understanding part of this complex disease and its etiology. Additional studies to amplify the findings and determine the effect of therapy on the above studied parameters are suggested.

CONCLUSIONS

Patients with periodontitis were identified as high and very high risk compared to the rest of risk categories by a risk assessment tool. Patients who were identified as very high risk showed higher detection of putative periodontal bacteria (except *Capnocytophaga* species) than the rest of the study population. A pilot model was suggested to evaluate the association of the microbiologic testing and known risk factors to clinical parameters in patients with periodontitis. Future studies with large sample sizes will be required to further explore the hypothesis.

ACKNOWLEDGMENTS

The materials used in this research study were provided by PreViser (Mount Vernon, WA) and Hain Lifescience (Nehren, Germany). This project was partially supported by the National Center for Advancing Translational Sciences, National Institutes of Health Award UL1TR001064. The authors report no conflicts of interest related to this study.

REFERENCES

1. Burt B; Research, Science and Therapy Committee of the American Academy of Periodontology. Position paper: Epidemiology of periodontal diseases. *J Periodontol* 2005;76:1406-1419.
2. Page RC, Offenbacher S, Schroeder HE, Seymour GJ, Kornman KS. Advances in the pathogenesis of periodontitis: Summary of developments, clinical implications and future directions. *Periodontol 2000* 1997;14: 216-248.
3. Page RC, Kornman KS. The pathogenesis of human periodontitis: An introduction. *Periodontol 2000* 1997; 14:9-11.

4. Albandar JM. Global risk factors and risk indicators for periodontal diseases. *Periodontol 2000* 2002;29:177-206.
5. Bergström J, Preber H. Tobacco use as a risk factor. *J Periodontol* 1994;65(Suppl. 5):545-550.
6. Genco RJ, Jeffcoat M, Caton J. Consensus report. Periodontal diseases: Epidemiology and diagnosis. *Ann Periodontol* 1996;1:216-222.
7. Kornman KS, Crane A, Wang HY, et al. The interleukin-1 genotype as a severity factor in adult periodontal disease. *J Clin Periodontol* 1997;24:72-77.
8. Page RC, Beck JD. Risk assessment for periodontal diseases. *Int Dent J* 1997;47:61-87.
9. Beck JD. Risk revisited. *Community Dent Oral Epidemiol* 1998;26:220-225.
10. Mealey B. Diabetes and periodontal diseases. *J Periodontol* 1999;70:935-949.
11. American Academy of Periodontology. American Academy of Periodontology statement on risk assessment. *J Periodontol* 2008;79:202.
12. Persson GR, Mancl LA, Martin J, Page RC. Assessing periodontal disease risk: A comparison of clinicians' assessment versus a computerized tool. *J Am Dent Assoc* 2003;134:575-582.
13. Page RC, Krall EA, Martin J, Mancl L, Garcia RI. Validity and accuracy of a risk calculator in predicting periodontal disease. *J Am Dent Assoc* 2002;133:569-576.
14. Page RC, Martin J, Krall EA, Mancl L, Garcia R. Longitudinal validation of a risk calculator for periodontal disease. *J Clin Periodontol* 2003;30:819-827.
15. Martin JA, Page RC, Loeb CF, Levi PA Jr. Tooth loss in 776 treated periodontal patients. *J Periodontol* 2010; 81:244-250.
16. Teles RP, Haffajee AD, Socransky SS. Microbiological goals of periodontal therapy. *Periodontol 2000* 2006;42:180-218.
17. Genco RJ, Kornman K, Williams R. Consensus report. Periodontal diseases: Pathogenesis and microbial factors. *Ann Periodontol* 1996;1:926-932.
18. Haffajee AD, Socransky SS. Microbial etiological agents of destructive periodontal diseases. *Periodontol 2000* 1994;5:78-111.
19. Zambon JJ. Periodontal diseases: Microbial factors. *Ann Periodontol* 1996;1:879-925.
20. Socransky SS, Haffajee AD, Cugini MA, Smith C, Kent RL Jr. Microbial complexes in subgingival plaque. *J Clin Periodontol* 1998;25:134-144.
21. Silness J, Löe H. Periodontal disease in pregnancy. II. Correlation between oral hygiene and periodontal condition. *Acta Odontol Scand* 1964;22:121-135.
22. Polson AM. The research team, calibration, and quality assurance in clinical trials in periodontics. *Ann Periodontol* 1997;2:75-82.
23. Eick S, Straube A, Guentsch A, Pfister W, Jentsch H. Comparison of real-time polymerase chain reaction and DNA-strip technology in microbiological evaluation of periodontitis treatment. *Diagn Microbiol Infect Dis* 2011;69:12-20.
24. Garcia RI, Nunn ME, Dietrich T. Risk calculation and periodontal outcomes. *Periodontol 2000* 2009;50:65-77.
25. Lang NP, Suvan JE, Tonetti MS. Risk factor assessment tools for the prevention of periodontitis progression a systematic review. *J Clin Periodontol* 2015;42(Suppl. 16):S59-S70.
26. Loomer PM. Microbiological diagnostic testing in the treatment of periodontal diseases. *Periodontol 2000* 2004;34:49-56.
27. Haffajee AD, Socransky SS, Gunsolley JC. Systemic anti-infective periodontal therapy. A systematic review. *Ann Periodontol* 2003;8:115-181.
28. Beck JD. Methods of assessing risk for periodontitis and developing multifactorial models. *J Periodontol* 1994;65(Suppl. 5):468-478.

Correspondence: Dr. Yong Hur, Tufts University School of Dental Medicine, Department of Periodontology, One Kneeland St., MA, Boston 02111. Fax: 617/636-0911; e-mail: Yong.Hur@tufts.edu.

Submitted April 23, 2014; accepted for publication August 8, 2015.