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Journal: Bulletin of Tokyo Dental College, 51(3): 139-144

URL: http://hdl.handle.net/10130/1961
Investigation of Subgingival Profile of Periodontopathic Bacteria Using Polymerase Chain Reaction

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Received 18 January, 2010/Accepted for publication 12 March, 2010

Abstract

Periodontopathic bacteria such as Porphyromonas gingivalis, Aggregatibacter actinomycetemcomitans, Tannerella forsythia, Campylobacter rectus and Treponema denticola play an important role in the initiation and progression of periodontitis. The aim of this investigation was to evaluate the relationship between periodontal clinical parameters and the subgingival profile of periodontopathic bacteria. Twenty-six periodontitis patients (23–62 years of age; mean age, 40.2 ± 13.2) with no systemic disease agreed to participate in the study. Periodontal clinical parameters, including probing depth (PD) and bleeding on probing (BOP) were recorded. Subgingival plaque samples were obtained from deep (PD ≥ 4 mm) and shallow (PD ≤ 3 mm) pockets in each patient for detection of P. gingivalis, A. actinomycetemcomitans, T. forsythia, C. rectus and T. denticola by polymerase chain reaction technique. The relationship between the periodontal pathogens and clinical parameters was determined with the Fisher exact test, and a statistically significant association was found between detection of P. gingivalis, T. forsythia, C. rectus and T. denticola and PD or BOP. T. denticola was the most prevalent pathogen in both shallow PD and deep PD sites. No statistically significant association was found between detection of A. actinomycetemcomitans and the clinical parameters examined. A statistically significant association was found between detection of the red complex bacteria and the clinical parameters. These results suggest that the red complex pathogens and C. rectus play an important role in the initiation and progression of periodontitis.

Key words: Periodontopathic bacteria—Polymicrobial infection—Red complex—Periodontitis

Introduction

Periodontitis is an oral infectious disease induced mainly by periodontopathic bacteriai

Original Article
have been detected by several methods: anaerobic culture\textsuperscript{5}, microscopy\textsuperscript{11}, enzyme reaction\textsuperscript{6}, immunohistology\textsuperscript{8}, DNA probe\textsuperscript{9} and polymerase chain reaction (PCR)\textsuperscript{2}. The culture method is time-consuming and requires specific techniques. The rapid advancement of molecular biological techniques has facilitated the use of PCR for bacterial detection. Previous studies suggested that microbiological monitoring is a very important tool in the diagnosis and selection of periodontal therapy, as microflora was found to differ between active and inactive sites\textsuperscript{9}. Periodontopathic bacteria such as \textit{Porphyromonas gingivalis}, \textit{Aggregatibacter actinomycetemcomitans}, \textit{Tannerella forsythia}, \textit{Campylobacter rectus} and \textit{Treponema denticola} have been frequently detected in the subgingival pockets of patients with periodontitis. The purpose of this study was to investigate the relationship between detection of periodontopathic bacteria and periodontal clinical parameters.

### Methods

#### 1. Participants

Twenty-six periodontitis patients (23–62 years of age; mean age, 40.2±13.2) visiting Tokyo Dental College, Chiba Hospital participated in this study between 2000 and 2003. Informed consent was obtained from each patient. Table 1 shows age, sex and clinical characteristics. To be included in this study, patients had to have at least two sites with PD≥4 mm with attachment loss. Exclusion criteria included pregnancy, periodontal therapy or antibiotics in previous 3 months, any systemic condition which might have affected the progression or treatment of periodontitis and the need for pre-medication for monitoring or therapy. No patient with localized aggressive periodontitis was included in this study.

#### 2. Clinical parameters

1) Probing depth (PD)

PD was measured from the gingival margin to the bottom of the periodontal pocket at sampled sites with the Williams probe.

2) Bleeding on probing (BOP)

The dichotomous system was used to register the presence or absence of bleeding after PD measurements\textsuperscript{8}.

#### 3. Microbiological sampling

PD data were categorized into deep PD (≥4 mm) and shallow PD (≤3 mm) sites. At least two deep PD and one shallow PD sites were selected in each patient. Each sampling site was isolated with cotton rolls, and supragingival plaque and saliva carefully removed with sterile cotton pellets and dried with air. Three paper points were inserted into each selected pocket until firm resistance was felt and kept in place for 30 seconds.

#### 4. Detection of periodontal pathogens

Sedimented microorganisms were suspended in 50μl buffer (20 mM Tris-HCl, pH8.0, 2 mM EDTA, 1% tritonX-100) and boiled at 100°C for 10 min. After removal of

<table>
<thead>
<tr>
<th>Subjects (n)</th>
<th>26</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (mean±SD, range)</td>
<td>40.2±13.2 (23–62)</td>
</tr>
<tr>
<td>Sex (male/female)</td>
<td>16/10</td>
</tr>
<tr>
<td>Smoker/Nonsmoker</td>
<td>6/20</td>
</tr>
<tr>
<td>Deep sites</td>
<td>7.0±1.9</td>
</tr>
<tr>
<td>Shallow sites</td>
<td>2.5±0.6</td>
</tr>
</tbody>
</table>

PD: probing depth; BOP: bleeding on probing; SD: standard deviation.

Deep sites: PD of 4 mm or more
Shallow sites: PD of 3 mm or less

Table 1 Clinical characteristics of subjects and sampled sites
cell debris by centrifugation at 15,000×g for 15 min, the supernatant was used for detection of *P. gingivalis*, *A. actinomycetemcomitans*, *T. forsythia*, *C. rectus* and *T. denticola* by PCR according to the method of Ashimoto *et al.* Briefly, 5 μl sample was added to 45 μl reaction mixture consisting of 1× PCR buffer (Takara Biomedicals, Shiga, Japan), 12.5 μM dNTP, the specific primer pairs listed in Table 2 and 0.25 U Taq DNA polymerase (Takara Biomedicals). The PCR assay was then performed using a thermal cycler (GeneAmp PCR System 2400, Perkin Elmer Biosystem, Foster City, CA) under conditions of 32 cycles of denaturation at 95°C for 30 sec, annealing at 55°C for 30 sec, and primer extension at 72°C for 30 min. The PCR product was electrophoresed on a 2% agarose gel and visualized under UV light following staining with ethidium bromide. The detection level was expressed as the lowest number of target cells detected clearly by PCR.

5. Statistical analysis

The relationship between clinical parameters and detection of each periodontopathic bacterium was determined with the Fisher exact test.

Results

The patients were divided into two groups based on PD values. Mean percentage of bleeding sites out of 72 sites in 26 patients was 93.2% for deep sites and 14.3% for shallow sites (Table 1). The relationship between PD and detection of *P. gingivalis*, *A. actinomycetemcomitans*, *T. forsythia*, *C. rectus* or *T. denticola* by PCR and PD was significant (Table 3).

Table 2 Primers used

<table>
<thead>
<tr>
<th>Primer (5′–3′)</th>
<th>Base position (amplicon length in bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. gingivalis</em></td>
<td>AGG CAG CTT GCC ATA CTG CG 729–1,132 (404)</td>
</tr>
<tr>
<td></td>
<td>ACT GTT AGC AAC TAC OGA TGT</td>
</tr>
<tr>
<td><em>A. actinomycetemcomitans</em></td>
<td>AAA CCC ATC TCT GAG TTC TTC TTC AT TCA TGA AGT TCA TGG TAA CTG</td>
</tr>
<tr>
<td><em>T. forsythia</em></td>
<td>GCG TAT GTA ACC TGC CG CA 120–760 (641)</td>
</tr>
<tr>
<td></td>
<td>TGC TTC AGT GTC AGT TAT ACC T</td>
</tr>
<tr>
<td><em>C. rectus</em></td>
<td>TTT CGG AGC GTA AAC TCC TTT TC TTT CTG CAA GCA GAC ACT CTT</td>
</tr>
<tr>
<td><em>T. denticola</em></td>
<td>TAA TAC CGA ATG TGC TCA TTT ACA T</td>
</tr>
<tr>
<td></td>
<td>TCA AAG AAG CAT TCC TGC TCC TTC TTC TTA</td>
</tr>
</tbody>
</table>
the red complex bacteria and BOP was also statistically significant ($p<0.001$, Table 3). The detection rate of red complex bacteria was 34.7% for BOP-positive sites and 3.4% for BOP-negative sites.

We found that the relationship between co-detection of the red complex bacteria with C. rectus and the clinical parameters tested was statistically significant (Table 4).

**Discussion**

Periodontitis has been considered to be a biofilm infection\(^3,17,19\), and the polymicrobial nature of the periodontopathic bacteria in periodontal lesions has received particular attention\(^7,19\). The results of this study indicate that the relationships between co-existence of P. gingivalis, T. forsythia and T. denticola, designated the red complex, and PD or BOP were significant in periodontitis. This finding is in agreement with that of an earlier report by Socransky et al.\(^19\). In this study, no significant association was found between A. actinomycetemcomitans and the clinical parameters tested, supporting the findings of previous studies\(^9,19\). This may be due to the fact that A. actinomycetemcomitans is not an obligatory anaerobic bacterium. Our findings suggest that the red complex microbial consortium plays an important role in the progression of periodontitis. A relatively strong association was found for T. forsythia and the clinical parameters, strongly implicating it in periodontitis.

In this study, we found that the relationship between co-existence of the red complex bacteria with C. rectus and the clinical parameters tested was statistically significant (Table 4).
the pathogenesis of periodontitis\textsuperscript{10,12,14}. Further study is needed to clarify the etiology of periodontitis and its association with the co-existence of periodontopathic bacteria. Early detection of bacteria associated with the development of periodontitis by quantitative PCR would provide important information for subsequent diagnosis and treatment. Monitoring of the red complex and \textit{C. rectus} may be particularly important in periodontal therapy.

<table>
<thead>
<tr>
<th>Table 3</th>
<th>Relationship between detection of red complex bacteria and probing depth and bleeding on probing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sites</td>
<td>Red complex +</td>
</tr>
<tr>
<td>Deep</td>
<td>25 (34.2%)</td>
</tr>
<tr>
<td>*PD</td>
<td></td>
</tr>
<tr>
<td>Shallow</td>
<td>1 (3.6%)</td>
</tr>
<tr>
<td>BOP +</td>
<td>25 (34.7%)</td>
</tr>
<tr>
<td>*BOP</td>
<td></td>
</tr>
<tr>
<td>BOP −</td>
<td>1 (3.4%)</td>
</tr>
</tbody>
</table>

red complex + / − : sites with co-existence of \textit{Pg.}, \textit{T.f.} and \textit{T.d.} sites/without PD: probing depth
Deep: PD (≥4 mm) sites (n = 73)
Shallow: PD (≤3 mm) sites (n = 28)
BOP: bleeding on probing
BOP + : bleeding-positive sites on probing (n = 72)
BOP − : bleeding-negative sites on probing (n = 29)
*: statistically significant (p < 0.001) by Fisher exact test

<table>
<thead>
<tr>
<th>Table 4</th>
<th>Relationship between detection of red complex bacteria and \textit{C. rectus} and probing depth and bleeding on probing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sites</td>
<td>Red complex and \textit{C. rectus} +</td>
</tr>
<tr>
<td>Deep</td>
<td>20 (27.4%)</td>
</tr>
<tr>
<td>*PD</td>
<td></td>
</tr>
<tr>
<td>Shallow</td>
<td>1 (3.6%)</td>
</tr>
<tr>
<td>BOP +</td>
<td>20 (27.8%)</td>
</tr>
<tr>
<td>*BOP</td>
<td></td>
</tr>
<tr>
<td>BOP −</td>
<td>1 (3.4%)</td>
</tr>
</tbody>
</table>

red complex and \textit{C. rectus} + / − : sites with co-existence of \textit{Pg.}, \textit{T.f.}, \textit{T.d.} sites and \textit{C. rectus}/without PD: probing depth
Deep: PD (≥4 mm) sites (n = 73)
Shallow: PD (≤3 mm) sites (n = 28)
BOP: bleeding on probing
BOP + : bleeding-positive sites on probing (n = 72)
BOP − : bleeding-negative sites on probing (n = 29)
*: statistically significant (p < 0.01) by Fisher exact test
Acknowledgements

The authors thank Associate Professor Jeremy Williams, Tokyo Dental College, for his assistance with the English of this manuscript.

References


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