Periodontal pathogen levels in adolescents before, during, and after fixed orthodontic appliance therapy

Michelle J. Thornberg, a Christopher S. Riolo, b Burcu Bayirli, c Michael L. Riolo, d Elizabeth A. Van Tubergen, e and Richard Kulbersh f
Detroit, Ypsilanti, and Ann Arbor, Mich

Introduction: This purpose of this study was to document and investigate changes in periodontal pathogen levels before, during, and after orthodontic treatment in adolescents. Methods: DNA gene probe analysis was used to quantify the levels of 8 periodontal pathogens before, during, and after treatment with fixed orthodontic appliances in 190 concurrently treated adolescent orthodontic patients. The 8 pathogens examined were Actinobacillus actinomycetemcomitans (AA), Porphyromonas gingivalis (PG), Prevotella intermedia (PI), Tannerella forsythia (TF), Eikenella corrodens (EC), Fusobacterium nucleatum (FN), Treponema denticola (TD), and Campylobacter rectus (CR). Chi-square tests were used to determine whether the percentages of subjects with high counts significantly changed over time. Logistic regression analyses were also performed to derive the relative risk of higher counts of pathogenic bacteria with fixed appliances at the various time intervals studied. Results: For 6 (PI, TF, EC, FN, TD, CR) of the 8 pathogens, the percentages of subjects with high pathogen counts increased significantly after 6 months of fixed appliance treatment, but these returned to pretreatment levels by 12 months of orthodontic treatment. No pathogen level was significantly higher after 12 months of orthodontic treatment, and orthodontic treatment was found to be significantly protective for half of the pathogens (EC, FN, TD, CR) posttreatment. Conclusions: Orthodontic treatment with fixed appliances does not increase the risk of high levels of these periodontal pathogens. (Am J Orthod Dentofacial Orthop 2009;135:95-8)

Basic science advances have made possible the transfer of technologically advanced diagnostic decision-making methods from the laboratory to the dental office. Clinical dentistry has yet to take full advantage of these advances or their diagnostic possibilities. Oral conditions such as periodontal diseases can be diagnosed and evaluated with greater precision. Various bacteria, particularly certain anaerobic strains, have been associated with periodontal diseases for decades. In addition, the detection of putative periodontal pathogens in subgingival specimens by DNA amplification has been developed for both suspect anaerobic species and their various strains. Few studies have been conducted on the bacterial flora of early onset periodontitis or juvenile patients. A study by Albander et al used the OmniGene probe to analyze detectable levels of red group bacteria or bacteria commonly associated with the etiology of periodontitis over 6 years. Bacterial strains of interest included Porphyromonas gingivalis (PG), Prevotella intermedia (PI), Fusobacterium nucleatum (FN), Campylobacter rectus (CR), and Treponema denticola (TD). It was concluded that patients with early onset periodontitis had greater percentages of bacteria than did the control groups. This study is one of the few long-term bacterial analyses of juvenile patients. Studies have shown that loss of attachment and supporting bone of at least 1 site are seen in 1% to 9% of patients 5 to 11 years old and in 1% to 46% of those 12 to 15 years old. Epidemiologic studies clearly indicate that destructive forms of periodontal disease can occur in children and adolescents. Investigating the potential for disease in these patients during orthodontic treatment would be beneficial, because fixed
orthodontic appliance therapy might increase the putative periodontal pathogens.\textsuperscript{12,13}

DNA tests can identify putative periodontal pathogens. The tests are noninvasive and relatively inexpensive as a screening tool, easy to administer, and extremely accurate in sensitivity and specificity.\textsuperscript{14,15} For these reasons, DNA tests are valuable research tools in evaluating periodontal flora in all orthodontic patients. A diagnostic test should ideally lead to more information for choices of treatment for the patient.

The specific aims of this research were to describe the distribution of various levels of 8 putative periodontal pathogens in child and adolescent populations by using the OmniGene (OmniGene Bioproducts, Inc, Cambridge, Mass) DNA probe analysis, and to document changes in the levels of these pathogens in patients during and after fixed orthodontic appliance therapy.

MATERIAL AND METHODS

The study sample included 190 adolescent orthodontic patients who were consecutively treated at the University of Detroit Mercy graduate orthodontic clinic in Detroit, Michigan, from 1993 to 1995. Of these patients, 47\% were boys, and 53\% were girls. The racial composition was 62\% white, 37\% black, and 1\% either Asian or Hispanic. Their average age was 13 years 6 months. The inclusion criteria were no systemic disease, no antibiotic use for 3 months before data collection, and no previous orthodontic treatment.

Sterile single paper points were used to collect the subgingival plaque samples. Because of the dehydrating effect of these paper points on the genetic material, plaque enzymes were inhibited from degrading the target DNA strands or the DNA probe itself until the DNA analysis. These subgingival plaque samples were collected at the following sites for each patient: (1) mesial aspect of the maxillary right first molar, (2) distal aspect of the maxillary right central incisor, (3) mesial aspect of the maxillary left first premolar, (4) mesial aspect of the mandibular left first molar, (5) distal aspect of the mandibular left central incisor, and (6) mesial aspect of the mandibular right first premolar. If the first premolars were extracted during orthodontic treatment, the mesial aspect of the second premolars adjacent to the extraction site was used. These 6 samples were pooled so that the results were species specific and not site specific. The data were collected before starting fixed orthodontic treatment (T1), at 6 months of orthodontic treatment (T2), at 12 months of orthodontic treatment (T3), at more than 12 months of orthodontic treatment (T4), and about 3 months after all appliances were removed (T5).

The subgingival microflora were analyzed for 8 anaerobic or facultative anaerobic bacteria by using the DNA probe technique.\textsuperscript{16} The 8 pathogens analyzed were Actinobacillus actinomycetemcomitans (FDC strain Y4) (AA), PG (ATCC strain 33277), PI (FD
c

Table I. Categories of pathogen cell counts

<table>
<thead>
<tr>
<th>Category</th>
<th>Pathogen cell counts</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>&lt;6000</td>
</tr>
<tr>
<td>Low</td>
<td>6000 to &lt;600,000</td>
</tr>
<tr>
<td>Medium</td>
<td>60,000 to &lt;600,000</td>
</tr>
<tr>
<td>High</td>
<td>≥600,000</td>
</tr>
</tbody>
</table>

DNA probe analysis tested for the presence of bacterial DNA fragments based on cross reactivity patterns with known bacterial types. This technique entailed preparing single-strand pieces of DNA by releasing the genetic material from the cell. The DNA sample was then denatured into single-strand segments. These single strands of DNA were inhibited from interaction by controlling the temperature and salinity of the medium.

Then the single-strand sample of DNA was fixed to a solid nitrocellulose surface. The DNA probe, consisting of a complement strand radioactively marked with a phosphorus isotope (P32), was added to the single-strand DNA segment. The DNA segment was hydrolyzed by the probe. The excess probe was washed from the nitrocellulose surface. A radiographic plate was placed near the nitrocellulose surface that contained the sample DNA hydrolyzed with radioactive probes. Afterward, the radiographic plate was exposed. After exposure, the plates were digitally read to quantify the specific bacterial DNA. Pathogen cell counts for each patient were divided into 4 categories described in Table I.

Statistical analysis

Chi-square tests were performed to determine whether the percentages of subjects with medium and high counts significantly changed over time. A logistic regression analysis was also performed to calculate the relative risk of having a higher pathogen count when exposed to fixed appliances for each time interval.
RESULTS

At T3, no subjects had high counts of AA (Table II). For 6 of the 8 pathogens, the percentages of subjects with high pathogen counts increased significantly after 6 months of fixed appliance treatment (Tables II and III). There was a decrease in the percentages of subjects with high counts for each of these 6 pathogens by 12 months of orthodontic treatment, so that none was significantly different from the distribution of the subjects with high and low counts before treatment. At time T3, 3 of the 8 pathogens, EC, FN, and TD, had significantly lower percentages of subjects with high counts than at T1 (Tables II and III). At time T4, a significantly fewer subjects had high counts of EC, FN, TD, and CR compared with the counts at time T1 (Table II).

The relative risks were calculated with a logistic regression analysis. The null cells in Table IV indicate that no subjects had high pathogen counts (ie, treatment was protective).

Table II. Percentages of subjects with high pathogen counts

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Treatment categories</th>
<th>T1 (%)</th>
<th>T2 (%)</th>
<th>T3 (%)</th>
<th>T4 (%)</th>
<th>T5 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA</td>
<td></td>
<td>5.5</td>
<td>5.3</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>PG</td>
<td></td>
<td>14.2</td>
<td>8.0</td>
<td>12.0</td>
<td>7.3</td>
<td>7.7</td>
</tr>
<tr>
<td>PI</td>
<td></td>
<td>27.6</td>
<td>50.7*</td>
<td>16.0</td>
<td>14.6</td>
<td>15.4</td>
</tr>
<tr>
<td>TF</td>
<td></td>
<td>12.8</td>
<td>22.7*</td>
<td>16.0</td>
<td>9.8</td>
<td>7.7</td>
</tr>
<tr>
<td>EC</td>
<td></td>
<td>32.7</td>
<td>68.0*</td>
<td>32.0</td>
<td>9.8*</td>
<td>11.5*</td>
</tr>
<tr>
<td>FN</td>
<td></td>
<td>32.9</td>
<td>54.7*</td>
<td>32.0</td>
<td>14.6*</td>
<td>11.5*</td>
</tr>
<tr>
<td>TD</td>
<td></td>
<td>24.2</td>
<td>45.3*</td>
<td>26.0</td>
<td>9.8*</td>
<td>7.7*</td>
</tr>
<tr>
<td>CR</td>
<td></td>
<td>20.1</td>
<td>32.0*</td>
<td>28.0</td>
<td>19.5</td>
<td>0.0*</td>
</tr>
</tbody>
</table>

*Significantly different from pretreatment percentage, \( P < 0.05 \).

Table III. Comparison of high pathogen counts at T1 to T2, T3, T4, and T5

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Chi-square</th>
<th>T1 vs T2</th>
<th>T1 vs T3</th>
<th>T1 vs T4</th>
<th>T1 vs T5</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA</td>
<td>0.959</td>
<td>0.964</td>
<td>0.968</td>
<td>0.974</td>
<td></td>
</tr>
<tr>
<td>PG</td>
<td>0.153</td>
<td>0.152</td>
<td>0.231</td>
<td>0.362</td>
<td></td>
</tr>
<tr>
<td>PI</td>
<td>0.0001*</td>
<td>0.825</td>
<td>0.078</td>
<td>0.181</td>
<td></td>
</tr>
<tr>
<td>TF</td>
<td>0.0258*</td>
<td>0.524</td>
<td>0.578</td>
<td>0.451</td>
<td></td>
</tr>
<tr>
<td>EC</td>
<td>0.0001*</td>
<td>0.926</td>
<td>0.0051*</td>
<td>0.0349*</td>
<td></td>
</tr>
<tr>
<td>FN</td>
<td>0.0044*</td>
<td>0.900</td>
<td>0.0206*</td>
<td>0.0335*</td>
<td></td>
</tr>
<tr>
<td>TD</td>
<td>0.0002*</td>
<td>0.779</td>
<td>0.0441*</td>
<td>0.0712</td>
<td></td>
</tr>
<tr>
<td>CR</td>
<td>0.0225*</td>
<td>0.195</td>
<td>0.929</td>
<td>0.0349*</td>
<td></td>
</tr>
</tbody>
</table>

*\( P < 0.05 \).

Table IV. Relative risk of high pathogen counts at T2, T3, T4, and T5 compared with T1

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Relative risks</th>
<th>T1 vs T2</th>
<th>T1 vs T3</th>
<th>T1 vs T4</th>
<th>T1 vs T5</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA</td>
<td>0.972</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>PG</td>
<td>0.527</td>
<td>0.827</td>
<td>0.479</td>
<td>0.505</td>
<td></td>
</tr>
<tr>
<td>PI</td>
<td>2.691*</td>
<td>0.499</td>
<td>0.449</td>
<td>0.476</td>
<td></td>
</tr>
<tr>
<td>TF</td>
<td>1.999*</td>
<td>1.299</td>
<td>0.737</td>
<td>0.568</td>
<td></td>
</tr>
<tr>
<td>EC</td>
<td>4.384*</td>
<td>0.971</td>
<td>0.223*</td>
<td>0.269*</td>
<td></td>
</tr>
<tr>
<td>FN</td>
<td>2.462*</td>
<td>0.961</td>
<td>0.350*</td>
<td>0.266*</td>
<td></td>
</tr>
<tr>
<td>TD</td>
<td>2.597*</td>
<td>1.100</td>
<td>0.339*</td>
<td>0.261*</td>
<td></td>
</tr>
<tr>
<td>CR</td>
<td>1.872*</td>
<td>1.547</td>
<td>0.964</td>
<td>—</td>
<td></td>
</tr>
</tbody>
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*\( P < 0.05 \); null cells indicate that no subjects had high pathogen counts (ie, treatment was protective).

DISCUSSION

These periodontal pathogens were prevalent before orthodontic treatment in this adolescent sample. During the first 6 months of fixed appliance therapy, the levels of certain pathogens increased or remained the same. During this time, gross tooth movements took place, which might have been more conducive to periodontal pathogenicity. Also, fixed appliances might induce changes in the local environment that could be a transient advantage for these periodontal pathogens. As the ratio of bacteria in the oral environment returned to a new equilibrium, bacteria that are more suited to this new environment might flourish, resulting in increased competition in the environment and decreased levels of periodontal pathogens by 12 months of orthodontic treatment. In addition, this decrease in the periodontal pathogens by 12 months of orthodontic therapy is significant with respect to the risk of high counts of these pathogens. Table IV indicates that, after 12 months of orthodontic treatment, the risk of having high counts of EC, FN, TD, and CR was significantly greater before the initiation of orthodontic treatment.

Table IV. Relative risk of high pathogen counts at T2, T3, T4, and T5 compared with T1

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Relative risks</th>
<th>T1 vs T2</th>
<th>T1 vs T3</th>
<th>T1 vs T4</th>
<th>T1 vs T5</th>
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*\( P < 0.05 \); null cells indicate that no subjects had high pathogen counts (ie, treatment was protective).
determine whether there is a permanent change in the levels of these periodontal pathogens after treatment.

CONCLUSIONS

1. Fixed appliance therapy induced changes in the levels of periodontal pathogens during and after treatment.
2. There is no increased risk for high levels of periodontal pathogens from fixed appliance treatment.
3. The apparent reduced risk of high counts of periodontal pathogen levels deserves further investigation, because identifying the factors that produce these protective changes could be useful in treating periodontal diseases associated with the 8 putative periodontal pathogens.

REFERENCES
