Clinical, microbiologic, and immunologic factors of orthodontic treatment-induced gingival enlargement

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Introduction: The aim of this study was to investigate the microbiologic and immunologic factors related to orthodontic treatment-induced gingival enlargement (GE). Methods: Our study included 12 patients with GE undergoing fixed orthodontic treatment and 12 periodontally healthy controls. At baseline, periodontal variables, subgingival plaque samples, and gingival crevicular fluid (GCF) samples were taken from 2 preselected sites in both the GE and the control groups. The levels of Porphyromonas gingivalis, Aggregatibacter actinomycetemcomitans, Prevotella intermedia, Treponema denticola and Tannerella forsythia were determined by real-time polymerase chain reaction. GCF interleukin (IL)-1β and transforming growth factor-beta 1 (TGF-β1) were detected by enzyme-linked immunosorbent assay (Invitrogen, Camarillo, Calif). Periodontal therapy was given to the GE group, and all parameters were reassessed after 4 weeks. Results: At baseline, the GE group showed higher prevalences of the 5 periodontal pathogens than did the control group (P < 0.05). IL-1β and TGF-β1 levels at the GE sites were also significantly higher than those at the control sites (P < 0.05). Four weeks after periodontal therapy, the GE group showed significant improvements in the clinical parameters associated with significant reductions of P gingivalis, A actinomycetemcomitans, and T denticola. The levels of IL-1β decreased significantly compared with the baseline (P < 0.05), whereas there was no significant change in TGF-β1 levels (P > 0.05). Conclusions: Periodontal pathogens might have a relationship with the initiation and development of orthodontic treatment-induced GE. Inflammatory cytokines (IL-1β and TGF-β1) can also be considered as contributing factors. (Am J Orthod Dentofacial Orthop 2011;140:58-64)

Gingival enlargement (GE) is a common complication of orthodontic treatment1; it can begin within 1 to 2 months after placement of appliances.2 The presence of fixed appliances influences plaque accumulation and the colonization of important periodontopathic bacteria.3,4 When gingival tissues are enlarged, the tooth surfaces become difficult to access, inhibiting good oral hygiene and resulting in more inflammation and bleeding. Since periodontal pathosis at a young age could eventually impose a burden over the long term, the interruption of orthodontic treatment is often advised when GE is diagnosed. Thus, temporary removal of irritating factors,5 such as attachments and appliances, periodontal debridement, chlorhexidine prophylaxis,6 and surgical intervention such as flap surgery7 can be used in some patients to restore the contour of the enlarged gingival tissues and facilitate adequate oral hygiene during subsequent orthodontic treatment.

To date, only a few studies have focused attention on the pathogenesis and management of orthodontic treatment–induced GE. Kloehn and Pfeifer8 indicated that mechanical irritation by bands, chemical irritation by cements used for banding, food impaction, and less efficient oral-hygiene maintenance are etiologic factors of orthodontic treatment-induced GE. Alexander9 characterized this phenomenon as an inflammatory response to the plaque microbiota and related by-products, because enlarged gingival tissues can influence the subgingival ecosystem by creating an appropriate anaerobic environment, leading to a shift in the composition of the microflora.10 In a recent study, Gursoy et al11 suggested that a continuing low dose of nickel released to the

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epithelium was the initiating factor in gingival overgrowth from orthodontic treatment.

The initiation and development of periodontal disease depend on a dynamic equilibrium between the microbial challenge and the host’s immune-inflammatory responses. Periodontal pathogens express multiple virulence factors, such as lipopolysaccharide and peptidoglycan, stimulating host cells to release several kinds of inflammatory cytokines. There is substantial evidence that cellular and molecular changes in the gingival extracellular matrix leading to GE are regulated via various cytokines and growth factors, including interleukin (IL)-1β, IL-6, and transforming growth factor–beta 1 (TGF–β1). The aim of our study was to evaluate (1) the microbiologic and immunologic factors (IL-1β and TGF–β1) related to orthodontic treatment-induced GE and (2) the clinical, microbiologic, and immunologic effects of periodontal therapy on orthodontic treatment–induced GE.

MATERIAL AND METHODS

Twenty-four adolescent subjects, 15 boys and 9 girls, aged 12 to 18 years, participated in this study; the GE group consisted of 12 subjects undergoing fixed orthodontic therapy for at least 6 months (mean duration, 9.4 ± 2.5 months) at the Department of Stomatology, Zhongshan Hospital, Fudan University, Shanghai, China. Each patient had at least 2 papillae covering one third to two thirds of the clinical crown, diagnosed as orthodontic treatment–induced GE. Before orthodontic treatment, all subjects had no clinical signs of gingivitis or periodontitis and received oral-hygiene instructions by the same clinician (Y.G.). The control group consisted of 12 periodontally healthy subjects, matched for age and sex.

All subjects fulfilled the following criteria for participation in this study: (1) good general health with no history of systemic disease, (2) no alveolar bone loss visible on x-rays, (3) no periodontal treatment within the last 6 months, (4) no antibiotic therapy in the last 6 months, (5) no medication known to cause GE, including phenytoin, cyclosporine, nifedipine, verapamil, diltiazem, felodipine, or nitrendipine. Informed consent was obtained from the patients or their parents before the study. The research protocol was approved by the ethics committee of Fudan University.

The clinical parameters were taken at baseline by the same doctor. In the GE group, measurements were made at 2 preselected GE sites in the anterior mandibular region where orthodontic patients normally exhibit severe GE. Two periodontally healthy sites from the same region were also measured in the control group.

The degree of GE was assessed in 4 categories on the basis of the hyperplastic index (HI) as developed by Angelopoulos and Goaz and further modified by Pernu et al: 0, no gingival overgrowth; 1, mild overgrowth, blunting of the marginal gingiva; 2, moderate overgrowth, extending to the middle of the tooth crown; and 3, severe overgrowth, covering two thirds of the tooth crown or affecting the whole of the attached gingiva (Fig 1). Probing depth (PD) in millimeters was recorded by using a periodontal probe (PCP UNC15, Hu-Friedy, Chicago, Ill) at 3 sites around the interdental area (mesiobuccal, midbuccal, and distobuccal sites). The plaque index (PI) was also evaluated according to the method of Silness and Loe, and the papillary bleeding index (PBI) was evaluated according to the method of Saxer and Muhlemann.

Subgingival plaque and gingival crevicular fluid (GCF) samples were also collected from the same sites after measuring the PI and before measuring the PBI and the PD in both groups. After careful removal of all supragingival plaque, the areas were washed with a water spray, isolated with cotton rolls, and gently dried (30 seconds). A sterile paper point (#30) was placed into the bottom of the periodontal pocket for 30 seconds. Points with marks of blood were discarded. The paper points were placed in sterile polypropylene tubes containing 1.5 mL of phosphate-buffered solution.

The teeth were washed again; the area was isolated and then gently dried. GCF was collected with paper strips (Whatman International, Maidstone, United Kingdom) placed into the gingival crevice and maintained for 30 seconds. Strips with marks of blood were discarded. The paper strips were placed in sterile polypropylene tubes. One examiner performed all microbial and GCF sampling. All samples (subgingival plaque and GCF) were stored at −20°C.

After recording the parameters, the archwires and brackets at the GE sites were temporarily removed to allow maintenance of adequate plaque control. Then, 1 section of full-mouth periodontal debridement was performed with an ultrasonic scaler with a time limit of 45 minutes. Chlorhexidine prophylaxis (0.12% chlorhexidine gluconate; South China Pharmaceutical Co, Ltd, Shenzhen, China) was also administered twice a day for 2 weeks after the periodontal treatment. Oral-hygiene instructions were reinforced again.

Four weeks after the periodontal treatment, the clinical parameters and the collected samples were reassessed at the same sites in the GE group.

The real-time polymerase chain reaction (PCR) method used in this study was based on the amplification of variable regions of the 16S rRNA genes of Porphyromonas gingivalis (Pg), Aggregatibacter actinomycetemcomitans (Aa), Prevotella intermedia (Pi), Treponema denticola (Td), and Tannerella forsythia.
Briefly, species-specific primers were selected by using software (Primer Premier version 5.0; Premier Biosoft International, Palo Alto, Calif) based on the published 16S rRNA sequences. The primers used in this study are listed in Table I.

Bacterial DNA was extracted as described previously. A primer concentration of 0.25 μmol/L was ultimately used for the 5 species. Real-time PCR reaction was carried out by using a Mastercycler system (Eppendorf, Wesseling-Berzdorf, Germany) with SYBR Green Mix (Ruicheng Biotech, Shanghai, China). Samples were assayed in duplicate in 25-μL reaction mixtures containing 2 μL of template DNA, 0.5 μL of forward primer and reverse primer, and 12.5 μL of SYBR Green Mix. The cycling conditions used were as follows: 95°C for 15 minutes, 95°C for 30 seconds, 53°C for 30 seconds, 72°C for 30 seconds, and 40 cycles for Pg and Tf; and 95°C for 15 minutes, 95°C for 30 seconds, 54°C for 30 seconds, 72°C for 30 seconds, and 40 cycles for Aa, Pi, and Td. Melting peaks were used to determine the specificity of the PCR.

Absolute quantification of target bacteria in the subgingival samples was performed by using Pg (ATCC 33277), Aa (Y4), Pi (ATCC 23256), Td (ATCC 33520), and Tf (ATCC 43037) as controls. Standard curves were established with the controls that could be used to convert cycle threshold values into the number of bacterial cells by using controls with known amounts of bacterial-specific DNA. The level of detection was set at 103 bacteria per subgingival plaque sample for real-time PCR. The determination of the DNA content in the controls was based on the genome size of each bacteria and the mean weight of 1 nucleotide pair.

GCF IL-1β and TGF-β1 levels were analyzed with ELISA by using the relevant ELISA kit (enzyme-linked immunosorbent assay; Invitrogen, Camarillo, Calif) according to the manufacturer’s instructions. GCF samples were eluted from the strips by placing them in 250 μL of phosphate-buffered solution. The dilution was considered to calculate the concentration of each GCF substance. The results were expressed as picograms per site.

### Statistical analysis

The data were analyzed by using the SPSS statistical package (version 13.0, SPSS, Chicago, Ill). Individual sites were analyzed for clinical, subgingival microbiologic, and immunologic parameters. The Mann-Whitney U test and the Wilcoxon signed rank test were used to detect significant intragroup and intergroup differences with regard to clinical periodontal parameters and GCF cytokine levels at the test sites. For the microbiologic parameters, the frequencies of periodontal pathogens were compared by using the Fisher exact test. Logarithmic transformations were performed for the amounts of bacteria to improve normality; the amounts of bacteria were compared with the Student t test. For all tests used, values of P <0.05 were considered statistically significant.
RESULTS

At baseline, all evaluated clinical parameters (including PI, PBI, PD, and HI) of the test sites in the GE group were significantly higher than those in the control group (P <0.001). Four weeks after periodontal treatment, these clinical parameters decreased significantly compared with those at baseline (P <0.001). There was no significant difference for PI when compared with the control group (Table II).

At baseline, 96% of the test sites in the GE group scored 2, and 4% scored 3 for HI. After 4 weeks, the HI of the test sites decreased; only 21% of the sites scored 2, 46% of the sites scored 1, and 33% of the sites scored 0.

Overall, 10 of 12 patients in the GE group showed marked improvements in gingival condition 4 weeks after periodontal therapy. However, 2 patients had severe GE with moderate reductions in PI and PBI at the test sites (data not shown). Therefore, these 2 patients had surgical gingivectomy to restore the physiologic gingival contour and to facilitate good oral-hygiene maintenance during the subsequent orthodontic treatments.

At baseline, the frequencies of Pg, Aa, Pi, Tf, and Td in the GE group were significantly higher compared with those in the control group (P <0.05). Four weeks after periodontal treatment, the frequencies of Pg, Aa, and Td decreased significantly (P <0.05). Compared with the control group, no significant differences were found for the 5 bacteria (P >0.05) (Table III).

The mean amounts of Pg, Aa, and Td in the GE group decreased significantly from baseline to 4 weeks (P <0.05). There were no significant changes in the amounts of Pi and Tf between the 2 periods (P >0.05) (Table IV).

At baseline, the mean levels of GCF IL-1β and TGF-β1 in the GE group were significantly higher than those in the control group (P <0.001 and P = 0.001, respectively). After 4 weeks, the levels of IL-1β decreased significantly compared with those at baseline (P <0.001). Although the mean levels of GCF TGF-β1 decreased slightly, no significant difference was found between baseline and 4 weeks (P = 0.162) (Fig 2).

DISCUSSION

In our study, the severity of GE was closely associated with gingival inflammation observed at the GE sites, where PI and PBI were significantly higher than in the controls. These results suggest that poor oral hygiene is the main reason for the orthodontic treatment-induced GE. The increase in PD at the GE sites might reflect the presence of pseudo-pockets, which create an ecologic environment that favors a qualitative shift from a predominance of aerobic gram-positive cocci to more putative periodontal pathogens, anaerobic gram-negative species.

At baseline, the frequencies of the 5 periodontopathic bacteria detected at the GE sites were

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Table II. Clinical parameters (mean ± SD) in the GE (n = 24) and control (n = 24) groups

<table>
<thead>
<tr>
<th>Periodontal index</th>
<th>GE group</th>
<th>Control group</th>
<th>P value</th>
<th>4 weeks vs control</th>
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<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>4 weeks</td>
<td></td>
<td></td>
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<td></td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>PI (mm)</td>
<td>1.58 ± 0.65</td>
<td>0.29 ± 0.46</td>
<td>0.25 ± 0.44</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>PBI (mm)</td>
<td>1.54 ± 0.78</td>
<td>0.42 ± 0.58</td>
<td>0.08 ± 0.28</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>PD (mm)</td>
<td>3.18 ± 0.44</td>
<td>2.42 ± 0.56</td>
<td>1.86 ± 0.28</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>HI</td>
<td>2.04 ± 0.20</td>
<td>0.88 ± 0.74</td>
<td>0.13 ± 0.34</td>
<td>&lt;0.001*</td>
</tr>
</tbody>
</table>

*Significant differences (Mann-Whitney U test or Wilcoxon signed rank test).

Table III. Frequencies for 5 periodontal pathogens in the GE (n = 24) and control (n = 24) groups

<table>
<thead>
<tr>
<th>Species</th>
<th>GE group</th>
<th>P value</th>
<th>4 weeks vs control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>4 weeks</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pg</td>
<td>19 (79%)</td>
<td>7 (29%)</td>
<td>6 (25%)</td>
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<tr>
<td>Aa</td>
<td>18 (75%)</td>
<td>8 (33%)</td>
<td>3 (13%)</td>
</tr>
<tr>
<td>Pi</td>
<td>14 (58%)</td>
<td>9 (38%)</td>
<td>4 (17%)</td>
</tr>
<tr>
<td>Td</td>
<td>19 (79%)</td>
<td>4 (17%)</td>
<td>1 (4%)</td>
</tr>
<tr>
<td>Tf</td>
<td>11 (46%)</td>
<td>7 (29%)</td>
<td>3 (13%)</td>
</tr>
</tbody>
</table>

*Significant differences (Fisher exact test).
significantly higher than those at the control sites. Moreover, we found improvements in gingival conditions after periodontal therapy. These improvements were associated with significant reductions in the frequencies and amounts of \( \text{Pg}, \text{Aa}, \) and \( \text{Td} \) at the GE sites, suggesting that these reductions in clinical parameters could contribute to the qualitative changes observed in the subgingival plaque.

\( \text{Pg} \) and \( \text{Td} \) have been found to correlate significantly with the development and deterioration of phenytoin-induced gingival overgrowth.\(^\text{24}\) Paolantonio et al\(^\text{25}\) demonstrated that young subjects wearing fixed orthodontic appliances harbor \( \text{Aa} \) with a remarkable frequency of detection compared with controls without orthodontic appliances. These periodontal pathogens are capable of stimulating host cells to produce excessive inflammatory cytokines such as \( \text{IL-1}\beta, \text{IL-6}, \text{IL-8}, \) and tumor necrosis factor-\( \alpha \), which exert their effects to modulate inflammatory and immune responses of gingival tissues.\(^\text{13}\)

The results of our study suggest that the mechanisms of GE could involve changes in the immune response induced by significant alterations in microbial flora.

The frequencies and amounts of \( \text{Pi} \) and \( \text{TF} \) did not show significant changes between baseline and 4 weeks after periodontal treatment; however, their contributory factors to the pathogenesis of orthodontic treatment-induced GE could not be ruled out. With the limitation of a small sample of this study, further research is needed with well-organized methods and a larger sample.

\( \text{IL-1}\beta \) has a central role in the regulation of immunologic reactions. It has been confirmed that mean levels of GCF \( \text{IL-1}\beta \) are associated with bleeding sites and gingival overgrowth in children and adolescents with and without fixed orthodontic appliances.\(^\text{26}\) Furthermore, \( \text{IL-1} \) is known to stimulate the proliferation of fibroblasts, keratinocytes, and endothelial cells and to enhance fibroblast synthesis of type I procollagen, hyaluronate, and fibronectin.\(^\text{27}\)

The immunologic results showed that the \( \text{IL-1}\beta \) levels of the test sites in the GE group were significantly higher than those in the control group. Moreover, there was a significant reduction in GCF \( \text{IL-1}\beta \) levels associated with the improvement of periodontal variables after 4 weeks of periodontal therapy, demonstrating a positive relationship between \( \text{IL-1}\beta \) level and gingival inflammation. Some studies have reported that the concentrations of \( \text{IL-1}\beta, \text{IL-6}, \) and tumor necrosis factor-\( \alpha \) in GCF were elevated during orthodontic tooth movement.\(^\text{28,29}\)

However, the elevations of these cytokines were rapid and transient, occurring only at 24 hours after mechanical stress. Thus, we propose that the elevation of the \( \text{IL-1}\beta \) level might be a causative factor for the development of orthodontic treatment-induced GE, and a diagnostic test of \( \text{IL-1}\beta \) level in GCF can be available to identify susceptible cohorts.

TGF-\( \beta \) is a multifunctional cytokine with pleiotropic properties, exhibiting both proinflammatory and anti-inflammatory effects, which affect cell proliferation and differentiation by inhibiting the destruction of extracellular matrix proteins through the suppression of matrix-degrading proteinases.\(^\text{30}\)

In this study, the GCF TGF-\( \beta \) levels in the GE group at baseline were significantly higher than those of the control group. However, periodontal treatment did not significantly reduce the GCF TGF-\( \beta \) levels, as we expected, after 4 weeks. This finding contrasts with an experimental gingivitis study, which demonstrated that total GCF TGF-\( \beta \) dropped nearly to healthy levels 14 days after prophylaxis, although there were some differences between the nature of GE and gingivitis.\(^\text{31}\) This finding suggests that these insignificant changes in GCF TGF-\( \beta \) levels after periodontal therapy cannot be solely explained by the resolution of gingival inflammation, and it might be hard to explain with the limitations of our data.

Wright et al\(^\text{15}\) suggested that subjects with high levels of baseline TGF-\( \beta \) might be at greater risk for cyclosporine-induced gingival overgrowth. An association between higher GCF TGF-\( \beta \) levels and the prevalence of GE among orthodontic patients was also observed in our study. It is known that a genetic predisposition might be associated with the initiation and progression of periodontal diseases,\(^\text{32}\) and some variants of TGF-\( \beta \) polymorphisms have been reported to increase serum TGF-\( \beta \) levels.\(^\text{16}\) Individual genetic variability with regard to GCF TGF-\( \beta \) expression might be a plausible explanation for these results, but further research is needed to investigate the possible correlation between TGF-\( \beta \) and orthodontic treatment-induced GE.

Kloehn and Pfeifer\(^\text{8}\) reported that orthodontic treatment-induced GE diminished dramatically within 48 hours after appliance removal and continued to decrease during the first 4 months of retention. However,
2 of the 12 patients in our study still had severe GE associated with moderate reductions of PI and PBI 4 weeks after periodontal therapy. These results agree with findings by Kouraki et al. That complete resolution of orthodontic treatment-induced GE is not always attained. The reasons for these results can be attributed to fibrotic changes in gingival connective tissues. Periodontal treatments could eliminate inflammatory components of enlarged gingival tissues by reducing populations of supragingival and subgingival bacteria. However, once fibrotic changes have taken place, removing the irritating factors, such as bacterial plaque, archwires, and brackets, could not return the gingival tissues to their normal physiologic contour. In such cases, surgical intervention is indicated if there is incomplete resolution after nonsurgical periodontal treatment.

CONCLUSIONS

1. The pathogenesis of orthodontic treatment-induced GE is multifactorial. Periodontal pathogens and inflammatory cytokines (IL-1β and TGF-β1) can be considered to be risk factors for orthodontic treatment-induced GE.
2. Periodontal therapy with temporary appliance removal can induce significant improvement of GE associated with significant reductions of Pg, Aa, and Td.
3. In some cases, surgical intervention is indicated if there is incomplete resolution after nonsurgical periodontal treatment.

REFERENCES


