Effect of Orthodontic Tooth Movement on Gingival Crevicular Fluid Infiltration; a Preliminary Investigation

A. Dannan 1, MA. Darwish 2, MN. Sawan 3

1 Assistant Clinical Specialist, Department of Periodontology, School of Dentistry, Witten/Herdecke University, Witten, Germany
2 Professor, Department of Periodontology, School of Dentistry, Damascus University, Damascus, Syria
3 Professor, Department of Orthodontics, School of Dentistry, Damascus University, Damascus, Syria

Abstract:
Objective: The gingival crevicular fluid (GCF) is an inflammatory exudate found in the gingival sulcus. The forces exerted during orthodontic treatment cause distortion of the periodontal ligament (PDL) extra-cellular matrix, resulting in some biological features that can lead to modification of both GCF volume and its components. The present study investigated the effect of orthodontic tooth movements, specifically canine retraction, on the volume of GCF exudate.

Materials and Methods: Fourteen upper and lower canines of patients with different Angle classifications were selected for the study. After extraction of the first premolars, the canines were subjected to orthodontic distal retraction. GCF was sampled from mesial and distal gingival crevices of each canine separately at baseline, 1 hour, 7 days, 14 days, 21 days, and 28 days after the application of the orthodontic distal retraction. GCF volume was determined by means of an electronic device.

Results: GCF volume at tension sites was slightly greater after 21 and 28 days compared to other observation time points. At pressure sites, GCF volume was slightly greater after 28 days compared to other observation time points. None of the observed differences, however, was statistically significant (P>0.05).

Conclusion: Orthodontic tooth movement, namely canine retraction, does not significantly increase the volume of GCF exudate. The slight increase in GCF volume could be due to a slight degree of gingival inflammation.

Key Words: Gingival Crevicular Fluid; Periodontal Ligament; Tooth Movement

INTRODUCTION
The gingival crevicular fluid (GCF) is an osmotically mediated inflammatory exudate found in the gingival sulcus. As an exudate, it tends to increase in volume with inflammation and capillary permeability.

Serum is the main source of the aqueous component of the GCF. The composition of GCF, however, can be modified by the gingival tissue through which the fluid passes, as well as bacteria both in tissues and the gingival crevice [1,2]. Consequently, the constituents of this fluid vary according to the condition of the periodontal tissues. Generally, cells, immunoglobulins, microorganisms, toxins, and lysosomal enzymes can all be found in the GCF.

It has been shown that GCF flow rate is a reliable indicator of gingivitis development in experimentally induced gingivitis [3]. Moreover, the levels of some of the GCF components like alkaline phosphatase, β-glucuronidase, aspartate aminotransferase, prostaglandins, immunoglobulin G4, interleukin-1 (IL-1) and others correlate specifically with the actual clinical
measurements of periodontal disease progression [4-13].

The forces exerted during orthodontic treatment cause distortion of the periodontal ligament (PDL) extra-cellular matrix, resulting in some biological features that can lead to cellular activation by changing membrane polarity and ion channel activity. In addition, as the capillaries are stretched or compressed excessively, tissue damage may occur. Such events and interactions lead to the synthesis and secretion of extracellular matrix components, tissue-degrading enzymes, acids, and local factors; induce cellular proliferation and differentiation; and promote wound healing and tissue remodeling. In vivo studies suggest that as biologic reactions progress at varying rates and intensities during different periods of treatment, alternate combinations of biochemical molecules come into play. These combinations are dependent on alveolar remodeling dynamics, the cycles of injury and healing, and the composition of the PDL cell population at each period [14-16].

More recently, Rhee et al [17] conducted a study to provide a better understanding of both the dynamics and the metabolic stages of orthodontic tooth movement in terms of Cystatins and Cathepsin B relationships in GCF. They showed that the balance between enzyme and inhibitor might reflect the clinical status of orthodontic tooth movement and provide valuable information for the assessment of recall intervals and retention procedures [17].

Such changes in the deeper periodontal tissues during orthodontic treatment can lead to modification of both the GCF volume and its components.

The present study was undertaken to investigate the effects of one type of orthodontic tooth movements, specifically canine retraction, on the secretion of GCF from retracted teeth, and to consider any implications of such findings for clinical orthodontic procedures.

MATERIALS AND METHODS

Study Population

The samples were selected from the patients referred to Department of Orthodontics, Faculty of Dental Medicine, Damascus University. To be eligible for the study, the patients had to meet the following criteria: good general health; lack of antibiotic therapy during the previous six months; absence of anti-inflammatory drug administration during the month preceding the study; and periodontal health with generalized probing depths $\leq 3$ mm and no radiographic evidence of periodontal bone loss. They also required upper and/or lower first premolars extraction and canine distal tooth movement as part of orthodontic treatment plan. Based on these criteria seven orthodontic patients comprising four females, (mean age 16.5 years, SD=1.5) and three males (mean age 17.6 years SD=2.5) were selected.

An oral approval to be subjected to the study was obtained from the patients or from the parents of patients under 18 years of age, prior to the commencement of the study. One week before the baseline examination, all patients underwent a session of supra- and sub-gingival ultrasonic scaling.

Experimental Design

Based on complete orthodontic treatment

| Table 1. Mean and standard deviation (SD) of the clinical parameters used in the study. |
|---------------------------------|---------------------------------|------------------|
|                                 | **Baseline**                   | **Day 28**       | **ANOVA Test** |
| Plaque Index                    | Mean (SD)                      | Mean (SD)        |                 |
| 0.24 (0.13)                     | 0.19 (0.10)                    | NS               |
| Probing Depth (mm)              | 1.6 (0.51)                     | 1.5 (0.45)       | NS               |
| Gingival Index                  | 0.23 (0.20)                    | 0.30 (0.28)      | NS               |

NS= Not statistically Significant
plans, six upper and eight lower first premolars were extracted for all of the patients as the first step. Three weeks after the extraction of the first premolars, the canines next to the extraction areas were moved in a distal direction using 90g forces for mandibular and 115g forces for maxillary canines. These teeth comprised six upper canines (four canines in two female patients and two canines in one male patient) and eight lower canines (four canines in two female patients and four canines in two male patients). The forces were exerted by means of an archwire using a retraction spring. The amount of forces was verified using a calibrated orthodontic force gauge, which was adjusted for each case separately.

Clinical Monitoring
The status of the periodontal tissues was determined by clinical periodontal assessments including plaque index (PI) [18], gingival index (GI) [19] and probing depth (PD). These clinical parameters were assessed twice: at baseline (prior to orthodontic appliance placement) and on day 28.

GCF Samples Collection
GCF samples from each tooth was collected separately from the mesial and distal gingival crevices of each canine where the orthodontic forces were applied at baseline (before the application of the orthodontic force) and one hour, 7 days, 14 days, 21 days and 28 days after the application of the orthodontic force. Gingival crevicular fluid was sampled from the mesial and distal gingival crevices of each canine, where the orthodontic forces were applied, using the method described by Offenbacher et al [20]. The area was isolated with cotton rolls and the teeth and adjacent marginal gingival were dried with air to minimize saliva contamination. Then a paper strip (Roeko Inc®, Germany) was inserted into the crevice to a level of one millimeter below the gingival margin for 60 seconds. After removing the first strip and waiting for one minute, a second strip was placed at the same site for another 60 seconds in order to obtain a sufficient (measurable) amount of GCF since using one strip might not be sufficient. Strips contaminated by saliva or blood were excluded. GCF volume was measured with a calibrated electronic device (Periotron® 8000, Ora Flow, USA). We then used a software program (MLconvert.exe, Ora Flow, USA) to convert the measurements to microliters [21].

Data Processing
The data was analyzed using SPSS (version 11, SPSS Inc., USA). One-way ANOVA test served for statistical analysis. The measurements of GCF volume were expressed as the overall volume for each experimental group considering the tooth itself as the statistical

Table 2. Mean and standard deviation of GCF volumes at mesial (tension) and distal (pressure) sites of the 14 retracted canines.

<table>
<thead>
<tr>
<th>Time</th>
<th>at Mesial Sites Mean (SD)</th>
<th>at Distal Sites Mean (SD)</th>
<th>ANOVA Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>0.46 (0.15)</td>
<td>0.50 (0.15)</td>
<td>NS</td>
</tr>
<tr>
<td>One-hour</td>
<td>0.50 (0.15)</td>
<td>0.50 (0.08)</td>
<td>NS</td>
</tr>
<tr>
<td>Day 7</td>
<td>0.47 (0.14)</td>
<td>0.44 (0.09)</td>
<td>NS</td>
</tr>
<tr>
<td>Day 14</td>
<td>0.46 (0.10)</td>
<td>0.47 (0.10)</td>
<td>NS</td>
</tr>
<tr>
<td>Day 21</td>
<td>0.53 (0.12)</td>
<td>0.50 (0.11)</td>
<td>NS</td>
</tr>
<tr>
<td>Day 28</td>
<td>0.55 (0.15)</td>
<td>0.56 (0.17)</td>
<td>NS</td>
</tr>
</tbody>
</table>

NS = Not statistically Significant
unit. A probability of $P<0.05$ was accepted for rejection of the null hypothesis and to state that with a 95% level of confidence that the two parameters are not the same.

**RESULTS**

At the baseline, the clinical indices, expressed as the mean, were recorded as follows: 0.24 mm (SD=0.13), 1.6 mm (SD=0.51), and 0.23 (SD=0.2) for PI, PD and GI, respectively. The corresponding figures after 28 days were 0.19 mm (SD=0.1), 1.5 mm (SD=0.45) and 0.30 (SD=0.28), respectively. No statistically significant difference of pairwise comparisons over the two time points was found (Table 1). No sign of periodontal destruction was observed in any subject.

GCF volume at tension sites was slightly greater after 21 and 28 days compared to other observation time points (Table 2). At pressure sites, GCF volume was slightly greater after 28 days compared to other observation time points. None of the observed differences, however, was statistically significant ($P>0.05$).

**DISCUSSION**

The present study investigated the effect of orthodontic canine retraction on GCF volume in a sample of seven orthodontic patients. According to the results, no statistically significant increase occurred in GCF volume during orthodontic tooth movement.

In periodontal tissues, orthodontic tooth movement produces a biological process previously described as a continuous phenomenon, leading to bone resorption in pressure sites and bone deposition in tension sites [22-25]. Histological animal research has shown that both bone deposition and resorption occur in both tension and pressure sites in the alveolar bone undergoing mechanical stress through tooth movement [26]. Based on these data, first a wave of resorption occurs in 3 to 5 days. This early wave is followed by its reversal in 5 to 7 days. Then a late wave of bone formation lasting for 7 to 14 days occurs. This process can be detected on both compression and tension sides of the alveolar wall [26]. This model is marked out by a preliminary asynchronous period in which bone resorption is greater than bone deposition, while, at later times, resorption and deposition may become synchronous. It is supposed that all those histological actions, which take place during orthodontic treatments, may affect the flow rate of the GCF of the related teeth.

It has been shown that the placement of fixed orthodontic appliances has a significant impact on microbial and periodontal clinical variables including GCF’s flow and contents [27-29]. Baldwin et al [30] reported that the increase in GCF flow induced by orthodontic tooth movement begins earlier than the pronounced changes in GCF components. This finding suggests an immediate effect of orthodontic force on the blood vessels, rather than induction of biochemical changes in the extracellular matrix. In contrast, Uematsu et al [31] reported that the volume of fluid around the experimental tooth during orthodontic movement was similar to the fluid around healthy teeth. Corroborating evidence was produced by Miyajima et al [32] who found no significant differences in GCF volume between treatment, retention, and control groups, although the mean value of the retention group was smaller than that of the other groups.

Tersin [33] reported an increase in GCF production during orthodontic treatment, in both a group receiving oral hygiene instruction and supervision, and a group not receiving these interventions, while a recent study [34] found no significant difference between teeth undergoing orthodontic treatment and untreated contralateral teeth.

In the present study, a slight increase in the volume of GCF occurred at the mesial sites one hour after the force had been started. After one week, all the values of GCF volume decreased. These values increased gradually after
21 and 28 days. However, none of these changes was statistically significant. Many studies have reported a significant correlation between plaque accumulation, gingival inflammation, and the volume of gingival exudate [1]. This may account for the contradictory evidence, as the additional effect of orthodontic treatment itself on gingival fluid flow rate cannot be determined unless such influences are eliminated. However, some studies considering gingival status reported that significant increase in GCF flow rate during orthodontic treatment is partly unrelated to the presence of significantly more severe gingival inflammation [35-37]. Later, Pender et al [38] reported that the GCF tended to increase at both non-inflamed and moderately inflamed sites, compared to similar sites before treatment. Last et al [36] demonstrated an increased GCF flow rate at early stages of retention compared to an untreated control group. The slight insignificant increase in GCF volumes in the present study might be due to a slight gingival inflammation existed after 28 days. However, this gingival inflammation did not reach – at any time – destructive values.

CONCLUSION
According to the results obtained, and within the limitations of the study, it could be stated that GCF volume was not significantly affected by the orthodontic movements and the forces applied. However, the slight increase in GCF volume, although not statistically significant, could be due to slight degree of gingival inflammation, which is normally found during any orthodontic treatment. Further studies are needed to establish procedures useful for clinical monitoring of biologic processes in the deeper periodontal tissues and the GCF during orthodontic tooth movement in human beings. The association between clinical parameters and tissue remodeling represented by GCF alterations can be clinically useful to biologically monitor and predict orthodontic treatment. For future studies in this field, we suggest the use of cephalometric analysis in order to control the tooth movement more precisely.

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