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# Effect of orthodontic force on expression levels of ten cytokines in gingival crevicular fluid

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ABSTRACT

Various types of inflammatory mediators are involved in the cascade of biological events behind tissue remodeling allowing orthodontic tooth movement. This split-mouth longitudinal study aimed to evaluate the gingival crevicular fluid (GCF) levels of ten cytokines, IL-6, IL-8, IL-10, IL-13, IL-17, IFN-γ, GM-CSF, MCP-1, MIP-1β and TNF-α, during initial orthodontic treatment. The sample comprised 15 healthy patients (9 males and 6 females, 13.9 ± 2.5 years). The lower (test) incisors were moved using fixed appliance carrying a 0.014-inch nickel titanium wire, whereas the upper (control) incisors were bonded without any force. The GCF was collected from the test and control teeth before fixed appliance mounting (baseline) and after 1, 7 and 21 days. In 6 sites per tooth, from canine to canine, periodontal conditions were defined as the percentage of sites with visible plaque and bleeding on probing. The total GCF cytokines levels were quantified using multianalysis Luminex technology. Throughout the experimental term, and for both test and control teeth, the mean percentage of sites with visible plaque and bleeding on probing were generally below 25% and 15%, respectively, although variability was also seen. In the test teeth, the GCF levels of all the cytokines remained constant throughout the experimental term. On the contrary, significant reductions were seen in the control teeth for each cytokine. Moreover, significantly greater levels of IL-6, GM-CSF, MCP-1 and TNFα were seen in the test teeth as compared to the control teeth at 7 days. The reasons for the differential behavior in the levels of all the investigated cytokines between the test and control teeth may be related to the presence of orthodontic forces and/or subclinical tissue inflammation. Further investigation is needed to elucidate potential roles for these biomarkers in the tissue remodeling incident to orthodontic tooth movement.



## 1. Introduction

Orthodontic tooth movement is a combination of force-induced periodontal ligament (PDL) and alveolar bone remodeling (Profitt et al., 2007). When exposed to orthodontic forces, fibroblasts, osteoblasts, and other cells of the periodontal ligament release signaling molecules such as neurotransmitters, arachidonic acid, and cytokine products that trigger biological processes associated with the resorption and apposition of alveolar bone (Krishnan & Davidovitch, 2006; Meikle, 2006).

These biochemical mediators are released at various stages of tooth movement and can be detected in samples of gingival crevicular fluid (GCF). The method for collecting GCF is simple and non-invasive, and the samples allow the examination of biological events throughout the observation period because they are collected at various stages of treatment (Uitto, 2003).

Cytokines are low molecular weight proteins that are involved in all phases of inflammation (Başaran, Ozer, Kaya, & Hamamci, 2006; Grant, Wilson, Rock, & Chapple, 2013). However, the huge number of mediators involved in tooth movement and the complexity of their interactions makes the comprehension of the molecular mechanisms incident to orthodontic tooth movement a difficult task (Krishnan, Nair, Ranjit, & Davidovitch, 2012; Kapoor, Kharbanda, Monga, Miglani, & Kapila, 2014).

However, there is still considerable heterogeneity in the methodologies used, particularly with regard to the characteristics of the participants, application of orthodontic forces, devices employed, and methods used to collect GCF (Andrade et al., 2012), highlighting the lack of uniformity (Kapoor et al., 2014). Therefore, the role of cytokines in tissue remodeling triggered by orthodontic forces is still not fully elucidated with further research necessary,

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especially considering a potential diagnostic role for GCF cytokines for the monitoring of orthodontic tooth movement. The present longitudinal split-mouth controlled study was thus aimed to detect and quantify the GCF levels of IL-6, IL-8, IL-10, IL-13, IL −17, IFN- γ, GM −CSF, MCP-1, MIP −1β, and TNF-α during initial orthodontic treatment.

## 2. Materials and methods

### 2.1. Study population

The study sample consisted of 15 healthy patients (9 males and 6 females, 13.9 ±2.5 years) undergoing treatment at the Orthodontic Clinic of the Faculty of Dentistry, State University of Rio de Janeiro (FO-UERJ).

The inclusion criteria were as follows: a) age between 11 and 17 years; b) full permanent dentition; c) Little Irregularity index (Little, 1975) between 3 and 5 mm in the anterior segment of the lower arch, allowing use of continuous archwire for alignment and leveling; d) absence of deep overbite, which restrains bond brackets on the lower anterior teeth; e) absence of caries or restorations in the anterior teeth; f) full-mouth plaque score and full-mouth bleeding score ≤30% and ≤10%, respectively.

Patients with autoimmune diseases, pregnancy, and lactation were excluded from the sample. Additionally, participants exhibiting prolonged use of medication (e.g. antibiotics, antihistamines, cortisone, hormones, and any other medication that may interfere with the inflammatory process or adversely affect the periodontium) three months prior to the start of the study and during the study period were also excluded.

This study protocol was approved by the Research Ethics Committee of Pedro Ernesto University Hospital. A signed informed consent was obtained from the parents of the patients prior to entry into the study.

### 2.2. Periodontal monitoring

Patients received oral hygiene instructions, including demonstration of the correct use of toothbrush and floss, one week before the start of orthodontic treatment and throughout the collection period. A periodontal evaluation was performed at each time point. Specifically, the visible plaque and bleeding on probing were recorded on the buccal, lingual, mesial, and distal aspects of the upper and lower anterior teeth, from canine to canine, using a manual periodontal probe, type Goldman-Fox/Williams (Hu-Friedy, Chicago, USA). Presence of visible plaque and gingival bleeding within 15 s after probing were recorded before and after GCF collection (Perinetti et al., 2004), respectively, and were expressed as percentage of positive sites considering the patient as statistical unit.

### 2.3. Orthodontic appliance

Orthodontic brackets with a 0.022 × 0.028-inch slot (Mini Master Series, Roth prescription, American Orthodontics, Sheboygan, WI, USA) were bonded from the first molar to the molar on both the lower and upper arches.

After bonding, an 0.014-inch Nickel-Titanium archwire (Highland Metals Inc. San Jose, CA, USA) was inserted in the brackets of the lower teeth and tied with modular elastics (Morelli Orthodontics, Sorocaba, SP, Brazil). Only the modular elastics were placed in the upper arch. This condition was maintained for the whole experimental term.

### 2.4. Gingival crevicular fluid collection and cytokines quantification

The GCF samples were collected from the labial faces of two central incisors and one lateral incisor in the upper and lower arches (teeth 12, 11, 21, 31, 41 and 42) at different times, as follows: at the time of appliance installation (baseline), and after 1, 7 and 21 days of orthodontic activation.

Prior to GCF collection, the supra-gingival plaque was carefully removed and the sites were isolated with cotton rolls and dried with mild air jets. The samples were then collected by inserting absorbent strips of paper (Periopaper, Interstate Drug Exchange, Amityville, NY, USA) 1 to 2 mm into the gingival sulcus and holding it there for 30 s. Samples contaminated with blood were discarded, and a new sample was collected from the same point after a few minutes. The three paper strips from either the test or control teeth were pooled and stored dry in sealed plastic Eppendorf tubes at −80 °C until analysis.

Thereafter, the GCF samples were thawed and the total cytokines levels were detected using Luminex multi-analyte technology (Bio-Plex ProTM Human Cytokine Grp I Panel 17-Plex, Catalog no. M50-00031YV, BIO-RAD, Hercules, CA, USA) according to the manufacturer's instruction.

Prior to assay, the GCF contents were eluted from the periopaper strips by immersing each strip into 150 μl of the assay buffer provided at the kit and centrifuged for 5 min, in 8000 rotations per minutes. Subsequently, the supernatants were placed in a new tube and prepared for measurement of total cytokine levels.

The samples were incubated with antibodies immobilized on color-coded micro-particles to identify the molecules of interest, washed to remove unbound material, and then incubated with biotinylated antibodies to the molecules of interest. After further washing, a streptavidin-phycoerythrin conjugate that binds to the biotinylated antibodies was added, and this was followed by a final washing step. The Luminex analyzer determined the magnitude of phycoerythrin derived signal in a micro-particle specific manner. Cytokines were expressed as total levels in pg/sample.

### 2.5. Data analysis

The Statistical Package for Social Sciences Software 13.0 (SPSS Inc., Chicago, Illinois) was used for data analysis. The normality of the data was tested using a Shapiro-Wilk test and Q–Q normality plots and, based on the results, non-parametric methods were used for data analysis. The equality of variance among the datasets was also tested using a Levene test and Q–Q normality plots of the residuals.

Differences in the percentage of sites positive for plaque (%PL + ) or bleeding on probing (%BOP + ) over time between the test and control teeth were evaluated using a Friedman test. The significance of the differences between the groups at each time point was assessed using a Wilcoxon test. Moreover, a Spearman rank correlation test was used to assess the significance of the correlations between either the%PL+ or%BOP+ with the corresponding levels of any GCF cytokine, according to the experimental group and time point.

Differences in the levels of each GCF cytokine over time between the test and control teeth were evaluated using the Friedman test. When significant interactions were observed, the Bonferroni-corrected Wilcoxon paired sign rank test was used to evaluate the significance of the difference between baseline and the following time points. Moreover, the significance of the differences in each GCF cytokine between the groups at each time point was assessed using the Wilcoxon test.

A $p$-value less than 0.05 was considered as being statistically significant.