

## Effect of local injection of platelet-rich plasma on level of IL-1 $\beta$ and TNF- $\alpha$ in gingival crevicular fluid during orthodontic tooth movement in dogs

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### ABSTRACT

**Objectives:** This study sought to assess the effect of local injection of platelet-rich plasma (PRP) on level of interleukin 1 $\beta$  (IL-1 $\beta$ ) and tumor necrosis factor-alpha (TNF- $\alpha$ ) in gingival crevicular fluid (GCF) during orthodontic tooth movement in dogs.

**Materials and Methods:** Six dogs, aged 10 to 12 months, were used in this study. one quadrant of the maxilla in six male mixed breed dogs was randomly selected as the test group and the contralateral quadrant served as the control group. After bilateral extraction of maxillary first premolars, a nickel-titanium closed coil spring was placed between the second premolar and canine teeth to apply 200 g force for space closure. A mixture of PRP and thrombin-calcium chloride was injected into the periodontal ligament of the second premolar in the test quadrant at 0, 21 and 42 days. A mixture of thrombin-calcium chloride was injected into the periodontal ligament of the second premolar in the control quadrant as placebo at the same time points. The levels of IL-1 $\beta$  and TNF- $\alpha$  in GCF was measured using ELISA. Data were analyzed using repeated measures ANOVA, Bonferroni test, and independent t-test.

**Results:** The levels of IL-1 $\beta$  and TNF- $\alpha$  in the GCF of the test group were significantly higher than the corresponding values in the control group at all time points ( $P < 0.001$ ). A significant increase in GCF levels of IL-1 $\beta$  and TNF- $\alpha$  was also noted most all the time ( $P < 0.05$ ).

**Conclusion:** Local injection of PRP can significantly increase the level of IL-1 $\beta$  and TNF- $\alpha$  in GCF of teeth under orthodontic force application.

### Keywords

Platelet-Rich Plasma; Orthodontic Tooth Movement; Interleukin 1 $\beta$ ; Tumor Necrosis Factor-Alpha

### Introduction

Attempts are ongoing to accelerate orthodontic tooth movement (OTM) due to high demand of patients to shorten the course of their orthodontic treatment. Prolonged orthodontic treatment course can increase the risk of dental caries, gingival recession and root resorption.<sup>1</sup> Thus, researchers are searching for strategies to enhance OTM with minimal complications.

In presence of mechanical stimuli, OTM can cause alveolar bone and periodontal ligament remodeling.<sup>1</sup> Bone remodeling includes bone resorption at the pressure site and bone formation at the tension site.<sup>2</sup> Orthodontic forces applied to the teeth cause some changes in the periodontal ligament, alter the blood flow, and lead to release of many pro-inflammatory mediators such as cytokines, growth factors, neurotransmitters, and arachidonic acid metabolites. Bone remodeling is the result of interactions of these mediators.<sup>3,4</sup> A number of cytokines and hormones are involved in the biological mechanism of OTM. Tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), interleukin-1 $\beta$  (IL-1 $\beta$ ), interleukin-6, prostaglandin E2 and some other pro-inflammatory cytokines can enhance the process of osteoclastic bone resorption by activating RANKB and RANKL.<sup>5</sup> OTM increases the level of cytokines such as TNF- $\alpha$  in the gingival crevicular fluid (GCF).<sup>6,7</sup> The role of TNF- $\alpha$  in OTM has been previously documented.<sup>8,9</sup> Thus, increasing the level of TNF- $\alpha$  may be able to enhance OTM.<sup>10</sup>

Evidence shows that invasive techniques such as the conventional corticotomy are significantly more effective than the non-surgical and non-invasive techniques.<sup>11</sup> Mechanical stimulation increases osteoclastic activity and decreases

alveolar bone density, which does not occur in use of non-invasive methods.<sup>12-15</sup> Thus, biochemical supplements may be used to achieve a more effective biological response. These supplements include cytokines, prostaglandins and hormones such as relaxin. However, use of these products may have unfavorable systemic effects. Platelet-rich plasma (PRP) is extensively used for tissue regeneration with minimal invasiveness.<sup>16-23</sup> However, controversy still exists regarding its efficacy for bone regeneration.<sup>24</sup> PRP has a high concentration of autologous platelets in a small volume of autologous plasma. The platelets present in PRP release their alpha granules at the wound site following the coagulation process. The alpha granules contain a group of growth factors that cause cell proliferation and differentiation, and are imperative for osteogenesis. Thus, in addition to the pro-coagulative effects of PRP, it serves as a source of growth factors to enhance wound healing, bone regeneration and fibroblast proliferation. PRP gel is formed by mixing the PRP (obtained by centrifugation of autologous whole blood) with thrombin and calcium chloride (CaCl<sub>2</sub>). Addition of thrombin and calcium chloride to PRP automatically releases the alpha granules for subsequent secretion of biological growth factors such as platelet derived growth factor, transforming growth factor-beta, vascular endothelial growth factor, and epidermal growth factor.<sup>25,26</sup> Considering the increasing demand for orthodontic treatment and recent claims regarding acceleration of OTM by PRP and the controversial results on this topic, this study sought to assess the effect of PRP on GCF level of IL-1 $\beta$  and TNF- $\alpha$ , as two key biomarkers involved in OTM.

## 2. MATERIAL AND METHODS

A power analysis was designed to have adequate power to apply a two-sided statistical test of the research hypothesis (null hypothesis) that there was no difference between the two sides. Based upon the work of Mostafa et al.<sup>25</sup>

The study was conducted in accordance with the Declaration of Helsinki, and followed the guidelines for the care and use of laboratory animals. The inclusion criteria were mixed breed male dogs between 10 to 12 months, and weighing 15 to 20 kg. The dogs were selected using convenience sampling. Sample size was calculated to be 6 in each group considering  $\alpha=0.05$ , power of 80%, and  $\beta=0.2$ .

The maxillary right and left first premolars of the dogs were extracted under general anesthesia by a veterinarian, and a 9-mm nickel-titanium closed coil spring (G & H Ortho) was placed in the empty space to apply 200 g load from the canine tooth (as the anchorage unit) to the second premolar (as the movement unit) for space closure. In order to fix the coil spring, 0.014-inch ligature wire (Orthotechnology, USA) was tightened around the second premolar and canine teeth as two rings. Next, the surfaces of the canine and second premolar teeth were etched with 37% phosphoric acid (Denfil, Vericom Co., Korea) for 30 s. Bonding agent (Adper<sup>TM</sup> Single Bond 2, 3M ESPE, St Paul, MN, USA) was then applied on the tooth surface and light-cured (Dentamerica Litex 680A Curing Light, Taiwan) for 20 s. Composite resin (Denfil, Vericom Co., Korea) was applied around the teeth, covered the ligature wires, and cured for 40 s (Figure 1). In each dog, a mixture of PRP and thrombin-CaCl<sub>2</sub> was injected into the periodontal ligament of the second premolar in one randomly selected maxillary quadrant. This quadrant served as the test group. The contralateral quadrant served as the control group and received an intraligamentary injection of thrombin-CaCl<sub>2</sub> (as placebo) in the second premolar. The study period was 63 days, and intraligamentary injections were performed around the second premolar tooth at 8 points of midbuccal, midlingual, distobuccal, distolingual, midpoint of distal surface, mesiolingual, mesiobuccal and midpoint of mesial surface at 0, 21 and 42 days. After isolation by #15 paper points (Diadent, Korea), samples were collected from the GCF at 0, 1, 2, 7, 21, 42 and 63 days from the pressure and tension sides in both the test and control groups using paper points. After each sampling, The samples were coded during data analysis to ensure blinding. the paper points were immediately placed in a medium and sent to a laboratory in 2-5°C. In the laboratory, they were frozen at -70°C.

Next, the level of IL-1 $\beta$  and TNF- $\alpha$  mediators was measured by ELISA using specific kits for this purpose (BioTek, USA).

### 2.1 Preparation of PRP

Blood samples were obtained from the cephalic vein of dogs, transferred into 5-cc test tubes containing sodium citrate, and centrifuged in plasma rich in growth factors (PRGF) centrifuge system (BTI, Spain) operating at 3600 rpm for 10 min. Next, 0.5 cc of PRP was mixed with 0.5 cc of thrombin and CaCl<sub>2</sub> and injected into the periodontal ligament at 8 points (midbuccal, midlingual, distobuccal, distolingual, midpoint of distal surface, mesiolingual, mesiobuccal and midpoint of mesial surface) around the second premolar. Next, 0.5 cc of CaCl<sub>2</sub> was mixed with 0.5 cc of thrombin and injected at the same sites in the control quadrants.

### 2.2 Measuring the GCF level of IL-1 $\beta$ and TNF- $\alpha$

According to the manufacturer's instructions provided in the IL-1 $\beta$  measurement kit, 150  $\mu$ L of Assay Buffer redcap was added to each well followed by 20  $\mu$ L of STD/Sample/Ctrl and was gently shaken. Next, 50  $\mu$ L of AB was added to each well and gently mixed. The plate was covered and stored at 18-24°C for 4 h. All wells were then aspirated and 300  $\mu$ L of wash buffer was added to each well and gently shaken. Washing was repeated for three times. Next, 200  $\mu$ L of CONJ solution was added to each well, the plate was covered and incubated at 18-24°C for 1 h. Next, the wells

were rinsed with 300  $\mu$ L of wash buffer 5 times and then 200  $\mu$ L of SUB solution was added to each well and the wells were incubated at 18-24°C in the dark for 30 min. Next, 50  $\mu$ L of STOP solution was added to each well. The samples were transferred to ELISA Reader (BioTek, USA) at 450 nm wavelength. The same was performed to measure the level of TNF- $\alpha$ ; the only difference was that the TNF- $\alpha$  kit was used for this purpose. The values were reported in micromole per liter ( $\mu$ mol/L).

### 2.3 Statistical analysis

Data were analyzed using SPSS version 21 (SPSS Inc., IL, USA). The mean and standard deviation of the GCF level of IL-1 $\beta$  and TNF- $\alpha$  were reported. The Shapiro-Wilk test was applied to assess the normality of data distribution, which showed that the data regarding the GCF level of IL-1 $\beta$  and TNF- $\alpha$  had normal distribution in both groups. Thus, data were compared using independent samples t-test, repeated measures ANOVA and Bonferroni test at 0.05 level of significance.

### 3. RESULTS

Table 1 compares the mean level of IL-1 $\beta$  in the GCF at different time points following the application of orthodontic force in the test and control groups using repeated measures ANOVA. The results showed an increase in GCF level of IL-1 $\beta$  from day 0 to day 63 in both groups. Table 2 shows pairwise comparisons of different time points regarding the mean GCF level of IL-1 $\beta$  in the test and control groups. No significant change was noted in the mean level of IL-1 $\beta$  in the GCF in the control group from day 0 to day 63 ( $P>0.05$ ). However, in the test group, significant differences were noted in most all pairwise comparisons of different time points ( $P<0.05$ ) except for day 0 and day 1 ( $P=0.051$ ) such that the mean GCF level of IL-1 $\beta$  significantly increased at each time point compared with the previous time point. The GCF level of IL-1 $\beta$  in the test group was significantly higher than the control group at all time points ( $P<0.001$ ) (Figure 1).

**Table 1.** Mean level of IL-1 $\beta$  ( $\mu$ mol/L) in GCF at different time points following the application of orthodontic force in the test and control groups using repeated measures ANOVA

| Group<br>Time point | Test |                | Control |                | P value |       |                    |
|---------------------|------|----------------|---------|----------------|---------|-------|--------------------|
|                     | Mean | Std. deviation | Mean    | Std. deviation | Group   | Time  | Interaction effect |
| Day 0               | 1.35 | .28            | .65     | .21            | <.001   | <.001 | .002               |
| Day 1               | 1.55 | .25            | .77     | .14            |         |       |                    |
| Day 2               | 2.02 | .31            | .95     | .12            |         |       |                    |
| Day 7               | 2.15 | .29            | 1.00    | .13            |         |       |                    |
| Day 21              | 2.62 | .61            | 1.05    | .19            |         |       |                    |
| Day 42              | 2.80 | .67            | 1.08    | .22            |         |       |                    |
| Day 63              | 3.13 | .72            | 1.15    | .19            |         |       |                    |

**Table 2.** Pairwise comparisons of different time points regarding the mean GCF level of IL-1 $\beta$  ( $\mu$ mol/L) in the test and control groups using the Bonferroni post-hoc test

| Day (I) | Day (J) | Test            |         | Control         |         |
|---------|---------|-----------------|---------|-----------------|---------|
|         |         | Mean difference | P value | Mean difference | P value |
| Day 0   | Day 1   | -.200           | .051    | -.117           | .859    |
|         | Day 2   | -.667*          | .003    | -.300           | .458    |
|         | Day 7   | -.800*          | .000    | -.350           | .168    |
|         | Day 21  | -1.267*         | .001    | -.400           | 1.000   |
|         | Day 42  | -1.450*         | .000    | -.433           | 1.000   |
|         | Day 63  | -1.783*         | .000    | -.500           | .886    |
| Day 1   | Day 2   | -.467*          | .008    | -.183           | 1.000   |
|         | Day 7   | -.600*          | .001    | -.233           | .635    |
|         | Day 21  | -1.067*         | .005    | -.283           | 1.000   |
|         | Day 42  | -1.250*         | .002    | -.317           | 1.000   |
|         | Day 63  | -1.583*         | .001    | -.383           | 1.000   |
| Day 2   | Day 7   | -.133*          | .018    | -.050           | 1.000   |
|         | Day 21  | -.600*          | .036    | -.100           | 1.000   |

|        |        |         |      |       |       |
|--------|--------|---------|------|-------|-------|
|        | Day 42 | -.783*  | .010 | -.133 | 1.000 |
|        | Day 63 | -1.117* | .001 | -.200 | 1.000 |
| Day 7  | Day 21 | -.467   | .004 | -.050 | 1.000 |
|        | Day 42 | -.650*  | .016 | -.083 | 1.000 |
|        | Day 63 | -.983*  | .001 | -.150 | 1.000 |
| Day 21 | Day 42 | -.183*  | .047 | -.033 | 1.000 |
|        | Day 63 | -.517*  | .000 | -.100 | 1.000 |
| Day 42 | Day 63 | -.333*  | .001 | -.067 | 1.000 |



**Figure 1.** Fixing the ligature wire using composite resin

Independent t-test revealed no significant difference between the test and control groups in the mean GCF level of IL-1 $\beta$  in day 1 compared with baseline (day 0). However, this difference was significant at all other time points such that the increase in the mean GCF level of IL-1 $\beta$  in the test group was significantly greater than that in the control group ( $P < 0.05$ ).

Table 3 compares the mean level of TNF- $\alpha$  in the GCF at different time points following the application of orthodontic force in the test and control groups using repeated measures ANOVA. The results showed an increase in the GCF level of TNF- $\alpha$  from day 0 to day 63 in both groups. Table 4 shows pairwise comparisons of different time points regarding the mean GCF level of TNF- $\alpha$  in the test and control groups. No significant change was noted in the mean level of TNF- $\alpha$  in the GCF in the control group from day 0 to day 63 ( $P > 0.05$ ). However, in the test group, significant differences were noted in most all pairwise comparisons of different time points ( $P < 0.05$ ) except for day 1 and day 2 ( $P = 0.304$ ) such that the mean GCF level of TNF- $\alpha$  significantly increased at each time point compared with the previous time point.

According to the Bonferroni post-hoc test, the mean GCF level of TNF- $\alpha$  in the test group was significantly greater than that in the control group at all time points ( $P < 0.001$ ) (Figure 2).

**Table 3.** Mean level of TNF- $\alpha$  ( $\mu\text{mol/L}$ ) in GCF at different time points following the application of orthodontic force in the test and control groups using repeated measures ANOVA

| Group<br>Time point | Test  |                | Control |                | P value |       |                    |
|---------------------|-------|----------------|---------|----------------|---------|-------|--------------------|
|                     | Mean  | Std. deviation | Mean    | Std. deviation | Group   | Time  | Interaction effect |
| Day 0               | 14.72 | 2.44           | 2.98    | .72            | <.001   | <.001 | .001               |

|        |       |       |      |     |
|--------|-------|-------|------|-----|
| Day 1  | 17.68 | 4.28  | 3.37 | .59 |
| Day 2  | 20.57 | 6.98  | 3.70 | .67 |
| Day 7  | 24.42 | 8.22  | 3.83 | .74 |
| Day 21 | 28.20 | 9.39  | 3.97 | .73 |
| Day 42 | 30.65 | 11.01 | 4.17 | .67 |
| Day 63 | 33.45 | 12.58 | 4.35 | .49 |

**Table 4.** Pairwise comparisons of different time points regarding the mean GCF level of TNF-a in the test and control groups using the Bonferroni post-hoc test

| Day (I) | Day (J) | Test            |         | Control         |         |
|---------|---------|-----------------|---------|-----------------|---------|
|         |         | Mean difference | P value | Mean difference | P value |
| Day 0   | Day 1   | -2.967*         | .032    | -.383           | 1.000   |
|         | Day 2   | -5.850*         | .036    | -.717           | 1.000   |
|         | Day 7   | -9.700*         | .004    | -.850           | 1.000   |
|         | Day 21  | -13.483*        | .001    | -.983           | 1.000   |
|         | Day 42  | -15.933*        | .002    | -1.183          | 1.000   |
|         | Day 63  | -18.733*        | .002    | -1.367          | 1.000   |
| Day 1   | Day 2   | -2.883          | .304    | -.333           | 1.000   |
|         | Day 7   | -6.733*         | .006    | -.467           | 1.000   |
|         | Day 21  | -10.517*        | .002    | -.600           | 1.000   |
|         | Day 42  | -12.967*        | .002    | -.800           | 1.000   |
|         | Day 63  | -15.767*        | .002    | -.983           | 1.000   |
| Day 2   | Day 7   | -3.850*         | .001    | -.133           | 1.000   |
|         | Day 21  | -7.633*         | <.001   | -.267           | 1.000   |
|         | Day 42  | -10.083*        | <.001   | -.467           | 1.000   |
|         | Day 63  | -12.883*        | <.001   | -.650           | 1.000   |
| Day 7   | Day 21  | -3.783*         | .001    | -.133           | 1.000   |
|         | Day 42  | -6.233*         | .001    | -.333           | 1.000   |
|         | Day 63  | -9.033*         | .001    | -.517           | 1.000   |
| Day 21  | Day 42  | -2.450*         | .021    | -.200           | 1.000   |
|         | Day 63  | -5.250*         | .014    | -.383           | 1.000   |
| Day 42  | Day 63  | -2.800*         | .013    | -.183           | 1.000   |



**Figure 2.** Pairwise comparisons of different time points regarding the mean GCF level of IL-1B ( $\mu\text{mol/L}$ ) in the test and control groups

The difference in the mean GCF level of TNF- $\alpha$  at each time point compared with baseline (day 0) was also compared between the test and control groups, which revealed that this increase was greater in the test group than the control group at all the time points ( $P < 0.05$ ).

#### 4. DISCUSSION

This animal study sought to assess the effect of PRP on GCF levels of IL-1 $\beta$  and TNF- $\alpha$ , as two key biomarkers involved in OTM. The results showed significantly higher levels of both mediators in the test group compared with the control group at most all time points. In the test group, the mean GCF level of IL-1 $\beta$  increased over time and this increase was significant between each two consecutive time points except between day 0 and day 1. The same was true for GCF level of TNF- $\alpha$  except between day 1 and day 2. Lack of a significant change between the aforementioned two time points can be due to short interval between the time of injection and GCF sampling (which was too short to allow the production of mediators) or the increase in the cumulative effect of PRP in the next injections compared with the first injection. In total, it may be concluded that local injection of PRP in dogs during OTM can increase the GCF level of IL-1 $\beta$  and TNF- $\alpha$ , which have a confirmed role in OTM.<sup>26,27</sup> Akbulut et al.<sup>27</sup> assessed the effect of PRP on OTM in 48 rats and showed that it was not efficacious since it did not accelerate OTM in rats. Their results were different from our findings, which may be due to the fact that they evaluated rats in their study while our study was conducted on dogs. Also, the assessment time points and the method of preparation of PRP were different in the two studies. We used the one-step protocol for preparation of PRP while they used a two-step protocol for this purpose. Padişar et al.<sup>28</sup> evaluated the GCF level of IL-6 and TNF- $\alpha$  during OTM in 10 patients and showed that the level of both biomarkers in the test group was higher than the control group at all time points. Also, the level of both biomarkers in the tension side was higher than the pressure side, but not significantly. They did not inject PRP for the control patients and similar to our study, they observed insignificant increase in GCF level of biomarkers in the control group. Thus, it may be concluded that PRP can be effective for significant increase in GCF level of biomarkers during OTM. Güleç et al.<sup>29</sup> assessed the effect of PRP on OTM in 76 rats and reported that PRP injection decreased the density of alveolar bone, increased osteoclastic activity, and accelerated OTM. Their results were in agreement with ours. Rashid et al.<sup>30</sup> assessed the effect of PRP on OTM in dogs and reported that local PRP injection accelerated OTM with no clinical or histological side effects. Their results were in line with ours. Sufarnap et al.<sup>31</sup> evaluated the efficacy of PRP for OTM acceleration in 19 pigs. They found no significant difference between the two groups. However, within group changes were significant over time. Controversy in the results of studies can be due to different animals tested, different magnitudes of load applied for OTM, and difference in frequency of injections of PRP such that Sufarnap et al.,<sup>31</sup> only injected PRP once, which may be responsible for insignificant difference between the two groups. Yoshimatsu et al.<sup>9</sup> assessed OTM in normal rats and those with a defect in TNF- $\alpha$  receptor. Measurements showed increased OTM and higher number of osteoclasts in normal rats. Their study highlighted the role of TNF- $\alpha$  as a key biomarker in OTM. Thus, we assessed the GCF level of TNF- $\alpha$  and found a significant increase in its level after injection of PRP. Nakornnoi et al.<sup>32</sup> evaluated the efficacy of L-PRP for acceleration of OTM in 21 New Zealand rabbits. They showed that injection of L-PRP accelerated OTM and increased the number of osteoclasts. They also showed increased remodeling during OTM due to injection of L-PRP, and their results and methodology were similar to ours. Alomari et al.<sup>33</sup> evaluated the efficacy of PRP injection for prevention of alveolar bone resorption following rapid maxillary expansion (RME) in 18 patients using cone-beam computed tomography. They concluded that PRP did not decrease alveolar defects following RME and could not prevent them. Our results showed that PRP increases the GCF level of two pro-inflammatory mediators and probably enhances bone remodeling following OTM as such. However, it may not be able to regenerate periodontal defects caused due to the application of heavy loads. Our results showed that PRP injection increased the GCF level of IL-1 $\beta$  and TNF- $\alpha$ , and this increase continued by repeated injections. Considering the confirmed role of cytokines such as IL-1 $\beta$ , IL-6, IL-8 and TNF- $\alpha$  in bone remodeling during OTM<sup>28</sup> and the association of these cytokines with higher osteoclastic activity and increased OTM,<sup>15</sup> it may be concluded that PRP can accelerate orthodontic treatment by increasing the GCF level of IL-1 $\beta$  and TNF- $\alpha$  as two major cytokines related to OTM.

Future clinical trials are required on the efficacy of PRP injection for acceleration of OTM. Also, the efficacy of different concentrations of PRP should be evaluated in future studies.

#### 5. CONCLUSION

Injection of PRP can significantly increase the level of IL-1 $\beta$  and TNF- $\alpha$  in GCF of teeth under orthodontic force application.

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