Original Article

Effects of Force Constancy on the Distribution of Interleukin-1 Beta and Tumor Necrosis Factor-Alpha Levels

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ABSTRACT

Objective: The purpose of this study was to evaluate levels of interleukin-1 beta (IL-1β) and tumor necrosis factor-alpha (TNF-α) in gingival crevicular fluid (GCF) with hybrid and PG retractors, which have differences in constancy of force.

Materials and Method: Forty canine teeth of 10 orthodontic patients were distalized with hybrid or PG retractors. The GCF was sampled from the distal sides of the canines at baseline, hour 1, day 1, month 1, and month 2. In the PG group, samples were re-collected 1 hour and 1 day after reactivation at month 1. Two-way ANOVA, paired *t* test, and Friedman and Wilcoxon tests were used for statistical analysis.

Results: The IL-1β level increased at hour 1 and month 2 in the upper and lower hybrid groups, whereas upper PG group increased at hour 1, month 1 + 1 hour, and month 2. The TNF-α level increased at hour 1 and declined afterward in the upper hybrid group. The only difference between the 2 retractors was found in TNF-α levels, which were higher at day 1 and month 2 in the upper PG group.

Conclusion: Continuous but diminishing forces produced by PG mechanics enhanced levels of TNF-α significantly at day 1 and month 2 compared with the constant and continuous forces applied by the hybrid retractor in the upper arch. Despite this difference, both retractors induced similar effects in IL-1β and TNF-α production and in the amount of tooth movement. (*Turkish J Orthod* 2013;26:7–18)

KEY WORDS: Cytokines, Hybrid Retractor, IL-1β, PG retractor, TNF-α

INTRODUCTION

An ideal retraction spring should apply continuous and light forces and be virtually constant throughout the entire range of its activation.[1] Hybrid and PG retractors are the best known retraction arches that have been claimed to provide ideal canine distalization. Although the initial force is the same, the magnitude of force decay and need for reactivation are the major differences between the retractors. The PG retractor was introduced by Poul Gjessing[2] in 1985. Horizontal force of PGs drops from 100 g to approximately 40 g over a 4-week period; therefore, PG retractors have to be reactivated every 4 to 6 weeks.[3] The hybrid retractor applies 100 g of constant force until 4 mm of deactivation. Sander[1] claimed that hybrid retractor require no more than one reactivation.

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Tooth movement induced by orthodontic forces is the end product of an inflammatory response of periodontal tissues.[4] Osteoblastic and osteoclastic activities occur as a result of inflammatory response of surrounding tissues.[5] Various cytokines play an important role in remodeling process.[6] Chemical analysis of gingival crevicular fluid (GCF) is a useful, noninvasive, and indirect method to investigate the cellular response of underlying periodontal ligament and levels of inflammatory cytokines during orthodontic treatment.[7] Interleukin-1 beta (IL-1β) is the first polypeptide mediator of immune-cell function to regulate bone remodeling by mechanical stress.[8] Tumor necrosis factor-alpha (TNF-α) is a typical mediator of inflammatory response that involves the bone resorption process and plays a prominent role in the control mechanism of osteoclast appearance at compression sites.[7,9]

Previous studies have evaluated the biological response of periodontal tissues when different magnitudes or types of orthodontic forces (continuous vs intermittent) were applied; most reported short-term changes.[10] Only 2 studies investigated force reactivation effects on the expression of TNF-α, IL-1β, and prostaglandin E2.[11,12] No studies have investigated the effects of constancy of continuous forces on cytokine levels in GCF and the difference between maxillary and mandibular arches. Thus, in the current study we intended to compare effects of hybrid (constant and continuous force) vs PG (continuous but diminishing force) retractors on IL-1β and TNF-α levels in GCF from baseline (pretreatment) to 2 months after orthodontic force application in maxillary and mandibular arches.

MATERIAL AND METHODS

Ten patients (6 girls and 4 boys; mean age, 14.3 ± 1.7 years) with an orthodontic treatment plan of extraction of 4 premolars and distal movement of all canines were randomly selected. The patients had no signs of periodontal destruction at clinical and radiographic examination and were excluded if they had received periodontal therapy, antibiotics, or an antimicrobial product in the previous 3 months, or if they had any systemic condition that might affect the periodontal treatment. Subjects were instructed to maintain good oral hygiene and avoid medications during the study. None of the participants had ever smoked. All of the participants were advised to maintain a soft diet and to alternate mastication on both sides during the study.

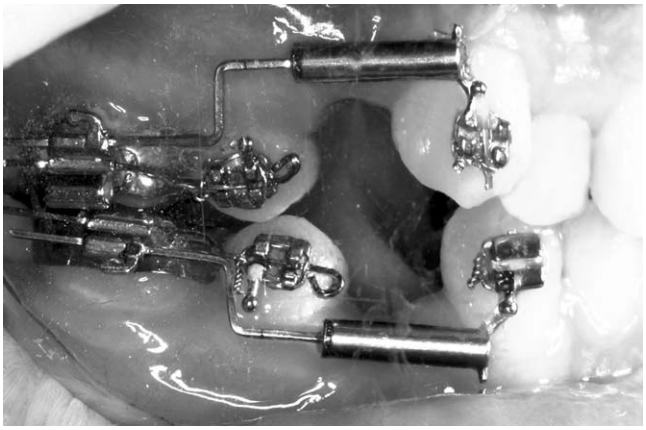

Figure 1. Hybrid retractor.

The study was conducted on both the maxilla and mandibula of these 10 patients. In total, 10 upper right canine teeth and 10 lower right canine teeth were retracted by hybrid retractors and 10 upper left canine teeth and 10 lower were retracted by PG retractors, one each in each patient. Consequently, 4 groups were constructed within each patient according to left and right side and maxillary and mandibular jaw. The participants' rights were protected, and informed consent and assent were obtained according to the Gazi University Ethical Committee Board.

Canine Distalization

Four weeks after the first premolar extractions, full fixed orthodontic treatment was begun. Canine brackets with vertical slot and molar bands were bonded. Maxillary and mandibular right canines were distalized by a hybrid retractor (Forestadent, Pforzheim, Germany) with 100 g (Fig. 1), whereas PG retractors applying 100 g were used for the left canines (Fig. 2). Hybrid retractors were activated 4.5 mm only once as recommended by Sander.[1] The PG spring was activated with a bend behind the molar tubes and reactivated at 1 month. The accuracy of the force was measured before canine retraction with a calibrated orthodontic force gauge. Hybrid and PG retractors both apply continuous forces; however, they have different activation methods and decay-force magnitudes. The horizontal force of PG retractors drops to approximately 40 g over a 4-week period, whereas the hybrid retractor applies 100 g constant force until 4 mm of deactivation.[1,3]

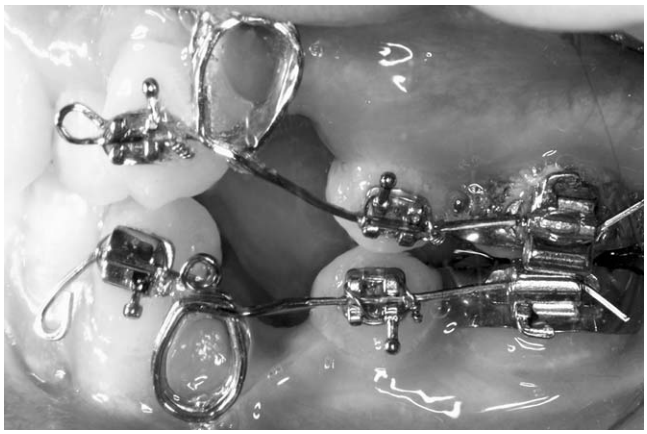

Figure 2. PG retractor.

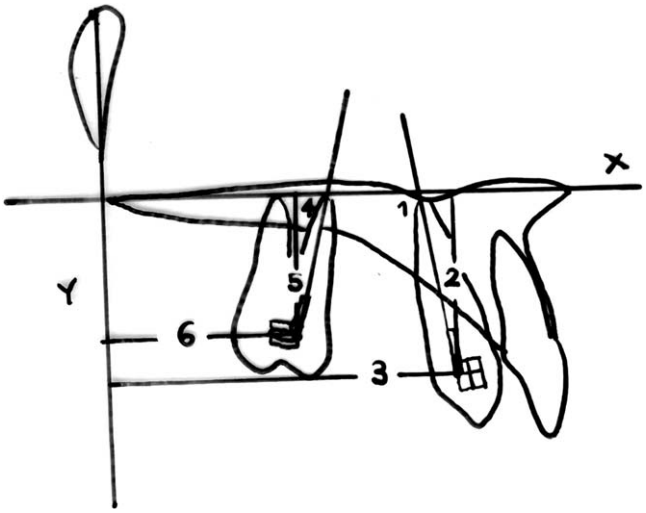

Figure 3. Measurements used to determine dental changes of upper canine and molar teeth on maxillary superimpositions. (1) x/U3 indicates angle between the long axis of the upper canine reference bar and x-axis (°); (2) x-U3, distance between the upper canine and x-axis (mm); (3) y-U3, distance between the upper canine and y-axis (mm); (4) x/U6, angle between the long axis of the upper first molar reference bar and x axis (°); (5) x-U6, distance between the upper first molar and x-axis (mm); (6) y-U6, distance between the upper first molar and y-axis (mm).

Clinical Parameters

Plaque index, gingival index, probing pocket depth, and bleeding on probing were recorded as clinical indices at baseline and at the end of 1 month and 2 months of initial orthodontic force application.

GCF Collection

The GCF was sampled twice from distal gingival crevices of each canine at baseline and repeated at hour 1, day 1, month 1, and month 2 of the initial orthodontic force application. The GCF samples were re-collected at only PG sites at 1 month + 1 hour and at 1 month + 1 day after the reactivation of the PG retractors at 1 month. Appliance activation (10 AM) and sampling (11 AM) were all done at the same hour at all appointments to reduce time-related variability.[13]

Before GCF collection, canines were washed with water, isolated with cotton rolls, and dried with air. The first paper strip (Periopaper-ProFlow Inc, Amityville, NY, USA) was inserted into the distal gingival crevice to a level of 1 mm below the gingival margin for 30 seconds. After a 1-minute interval, a second strip was inserted into the distal crevice for 30 seconds. Strips contaminated by saliva or blood were excluded from the sampled group. The paper strips were sealed in polypropylene containers separately. To determine the amount of GCF, an electronic scale was used to weigh the paper strips before and immediately after the collection, and differences were calculated. Each sample was stored at −30°C until analysis.

Enzyme-Linked Immunosorbent Assay Analysis

Each tube containing sample strips was eluted with 250 μL Hank's buffer containing 0.1 percent bovine serum albumin (Sigma, St Louis, MO, USA) by centrifugation (2000 × g; 4°C) for 5 minutes. After removal of the strips, supernatants were divided into 3 aliquots to determine each cytokine. The IL-1β and TNF-α amounts were determined by using commercially available enzyme-linked immunosorbent assays (AniBiotech Orgenium, Vantaa, Finland), according to manufacturer's instructions. After color development was stopped, optical density was measured at 450 nm. Total IL-1β and TNF-α were determined in picograms (pg) and concentration in each sample was calculated by dividing the amount of IL-1β and TNF-α by the volume of the sample (pg/μL). All the biochemical measurements were performed at the biochemistry laboratory of Medicine Faculty of Gazi University.

Cephalometric Evaluation

Cephalometric radiographs were taken before and at the end of 2 months of canine distalization. To minimize measurement errors, reference bars were inserted into canine brackets and molar tubes parallel to the long axis of the teeth. Cephalometric

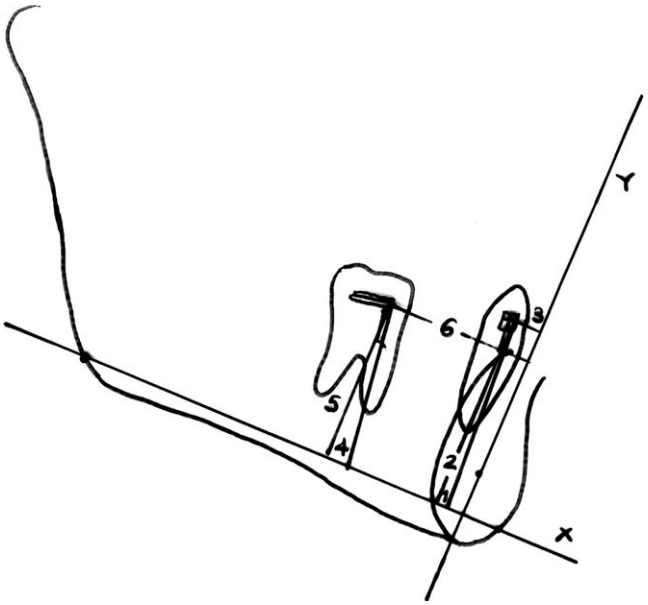

Figure 4. Measurements used to determine dental changes of lower canine and molar teeth on mandibular superimpositions. (1) x/L3 indicates angle between the long axis of the lower canine reference bar and x-axis (°); (2) x-L3, distance between the lower canine and x-axis (mm); (3) y-L3, distance between the lower canine and y-axis (mm); (4) x/L6, angle between the long axis of the lower first molar reference bar and x axis (°); (5) x-L6, distance between the lower first molar and x-axis (mm); (6) y-L6, distance between the lower first molar and y-axis (mm).

evaluations were carried out by only one researcher (B.I.A.) by linear and angular measurements. Local superimpositions of maxilla were carried out with reference to the palatal cortex of the maxilla. A coordinate system was set up on the pretreatment lateral cephalometric radiographs. The line through the pterygomaxillary point perpendicular to the ANS-PNS plane represented the y-axis, and the ANS-PNS plane represented the x-axis (Fig. 3). Similarly, a coordinate system for mandibular superimposition, the line through the midpoint of symphysis (D point) (y-axis) perpendicular to the GoGn plane (x-axis) was set up (Fig. 4).

Statistical Analysis

Data analysis was performed using the Statistical Package for Social Science (SPSS) version 11.5 software (SPSS Inc., Chicago, IL, USA) statistical program. Whether the distributions of continuous variables were normal or not was determined by the Shapiro Wilks test. Data were expressed as mean ± SD or median (minimum-maximum), where appropriate.

The mean differences in clinical and orthodontic measurements were evaluated by 2-way repeated measures ANOVA. When the *p* values were statistically significant, a multiple comparison test or paired *t* test was used to determine which measurement times differed from others.

Whether the differences among measurement times for cytokines were statistically significant or not was evaluated by Friedman test. When the *p* values were statistically significant, a Wilcoxon signed-rank test was used to determine which measurement times differed from others. A Wilcoxon signed-rank test was also used for intergroup comparisons for cytokines. Degrees of association between continuous variables were evaluated by the Spearman correlation test. A p value < 0.05 was considered statistically significant.

RESULTS

Clinical Parameters

There was a significant decrease only in the plaque index between baseline (T0) and 1 month (T1) ($p<0.05$) in total clinical indices (Table 1). No significant changes were determined in the clinical indices of canine teeth in all groups, which shows the constancy of oral hygiene status throughout the experiment (Table 2).

GCF Volume

Changes in the level of GCF volume at 1 day and 1 month were statistically significant compared with the baseline level in lower hybrid group ($p<0.05$)

Table 1. Total clinical indices

Parameter	Baseline (T0) (Mean ± SD)	1 Month (T1) (Mean ± SD)	2 Months (T2) (Mean ± SD)
Plaque index	1.35 ± 0.84[a]	0.94 ± 0.59[a]	1.03 ± 0.64
Gingival index	0.69 ± 0.50	0.66 ± 0.30	0.84 ± 0.27
Probing depth	1.73 ± 0.33	1.95 ± 0.31	1.91 ± 0.28
Bleeding on probing	0.23 ± 0.21	0.24 ± 0.20	0.21 ± 0.19

[a] Significant decrease in the plaque index between T0 and T1 ($p=0.047$).

Table 2. Clinical indices for canine teeth

Parameter	Baseline (T0)	1 Month (T1)	2 Months (T2)
Plaque index			
Upper hybrid group	1.24 ± 1.04	0.76 ± 0.63	1.04 ± 0.51
Upper PG group	1.28 ± 0.86	0.74 ± 0.40	1.08 ± 0.67
Lower hybrid group	1.23 ± 1.03	1.13 ± 0.88	1.15 ± 0.61
Lower PG group	1.08 ± 1.03	0.82 ± 0.35	1.14 ± 0.47
Gingival index			
Upper hybrid group	0.68 ± 0.54	0.55 ± 0.41	1.01 ± 0.37
Upper PG group	0.65 ± 0.61	0.58 ± 0.50	0.92 ± 0.38
Lower hybrid group	0.59 ± 0.53	0.65 ± 0.55	0.90 ± 0.30
Lower PG group	0.52 ± 0.50	0.70 ± 0.46	0.91 ± 0.42
Pocket depth			
Upper hybrid group	2.09 ± 0.53	2.08 ± 0.57	2.20 ± 0.43
Upper PG group	2.01 ± 0.48	2.29 ± 0.67	2.24 ± 0.41
Lower hybrid group	1.88 ± 0.46	1.98 ± 0.62	1.90 ± 0.40
Lower PG group	1.70 ± 0.41	2.04 ± 0.54	2.09 ± 0.56
Bleeding on probing			
Upper hybrid group	0.42 ± 0.33	0.23 ± 0.20	0.26 ± 0.32
Upper PG group	0.39 ± 0.34	0.39 ± 0.32	0.13 ± 0.17
Lower hybrid group	0.43 ± 0.47	0.23 ± 0.37	0.20 ± 0.28
Lower PG group	0.37 ± 0.38	0.27 ± 0.41	0.30 ± 0.32

(Fig. 5). The GCF volume increased significantly at 1 month, 1 month + 1 hour, and at 2 months compared with 1 hour in the upper PG group ($p<0.05$). In the lower PG group, GCF volume increased at 1 month and 2 months compared with baseline ($p<0.05$). No significant differences were determined in GCF volume between retractor types or between maxillary and mandibular arches ($p>0.05$) (Table 3).

Concentration of IL-1β

The IL-1β levels were significantly ($p<0.05$) upregulated at 1 hour and downregulated at 1 month, near to baseline levels ($p>0.05$); they again reached a significantly higher level at 2 months in both the upper and lower hybrid groups ($p<0.05$)

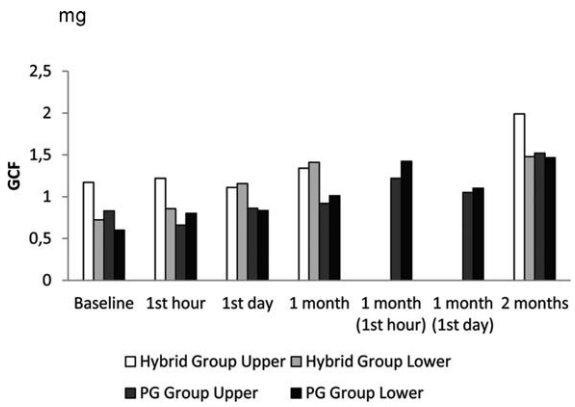

Figure 5. Distribution of the total amount of gingival crevicular fluid by time.

(Fig. 6). The IL-1β levels increased significantly ($p<0.05$) at 1 hour in the upper PG group and reached an even higher level at 1 month + 1 hour ($p<0.05$); these levels were significantly higher at 2 months compared with baseline levels ($p<0.05$). No significant difference was found in IL-1β levels between retractor types and between maxillary and mandibular arches at any time points ($p>0.05$) (Table 4).

Concentration of TNF-α

The TNF-α levels increased significantly ($p<0.05$) at 1 hour in the upper hybrid group and declined at 1 day compared with 1 hour ($p<0.01$); the TNF-α levels were significantly lower than 1 hour at both 1 month and 2 months ($p<0.05$) (Fig. 7). The levels of TNF-α at 1 day and 2 months in PG group were significantly higher than in the hybrid group in the upper arch ($p<0.05$). There were no significant changes in TNF-α levels between the maxillary and mandibular arches with both retractors ($p>0.05$) (Table 5).

There was no correlation between IL-1β and TNF-α levels at any time point. No correlation was found between any cytokines and clinical indexes ($p>0.05$).

Canine Distalization

The average amount of canine distalization at 2 months with hybrid retractors in the upper and lower

Table 3. Gingival crevicular fluid volume at the distal site of canine teeth (mg)

Groups	Statistics	Baseline	1 Hour	1 Day	1 Month	1 Month +1 Hour	1 Month +1 Day	2 Months
Hybrid (n=10) (upper)	Mean ± SD	1.17 ± 0.80	1.22 ± 1.00	1.11 ± 0.72	1.34 ± 0.81			1.99 ± 0.84
	Median	0.95	0.95	0.90	1.20			2.15
	Minimum	0.30	0.20	0.10	0.30			0.60
	Maximum	3.00	3.30	2.72	2.70			3.10
PG (n=10) (upper)	Mean ± SD	0.83 ± 0.38[a]	0.66 ± 0.30[b,c,d]	0.86 ± 0.70[e]	0.92 ± 0.38[b,f]	1.22 ± 0.47[c]	1.05 ± 1.41	1.52 ± 0.56[a,d,e,f]
	Median	0.95	0.65	0.55	0.90	1.20	0.85	1.45
	Minimum	0.20	0.20	0.30	0.30	0.20	0.20	0.80
	Maximum	1.40	1.10	2.50	1.40	1.80	3.40	2.30
Hyb rid (n=10) (lower)	Mean ± SD	0.72 ± 0.79[g,h]	0.86 ± 0.62	1.16 ± 0.68[g]	1.41 ± 0.99[h]			1.48 ± 0.66
	Median	0.30	0.70	1.00	1.10			1.20
	Minimum	0.20	0.20	0.30	0.40			0.90
	Maximum	2.20	2.20	2.50	3.10			2.70
PG (n=10) (lower)	Mean ± SD	0.60 ± 0.39[i,j]	0.80 ± 0.42[k]	0.83 ± 0.55[l]	1.01 ± 0.61[i]	1.42 ± 0.97	1.10 ± 0.71	1.47 ± 0.70[j,k,l]
	Median	0.50	0.90	0.80	0.90	1.10	0.90	1.20
	Minimum	0.20	0.10	0.20	0.20	0.30	0.40	0.80
	Maximum	1.50	1.40	2.10	2.20	3.40	2.60	3.00

[a] Significant difference within the upper PG group at baseline and 2 months (p=0.021).
[b] Significant difference within the upper PG group at 1 hour and 1 month (p=0.050).
[c] Significant difference within the upper PG group at 1 hour and 1 month + 1 hour (p=0.033).
[d] Significant difference within the upper PG group at 1 hour and 2 months (p=0.008).
[e] Significant difference within the upper PG group at 1 day and 2 months (p=0.043).
[f] Significant difference within the upper PG group at 1 month and 2 months (p=0.005).
[g] Significant difference within the lower hybrid group at baseline and 1 day (p=0.028).
[h] Significant difference within the lower hybrid group at baseline and 1 month (p=0.050);
[i] Significant difference within the lower PG group at baseline and 1 month (p=0.012).
[j] Significant difference within the lower PG group at baseline and 2 months (p=0.007).
[k] Significant difference within the lower PG group at 1 hour and 2 months (p=0.035).
[l] Significant difference within the lower PG group at 1 day and 2 months (p=0.027).

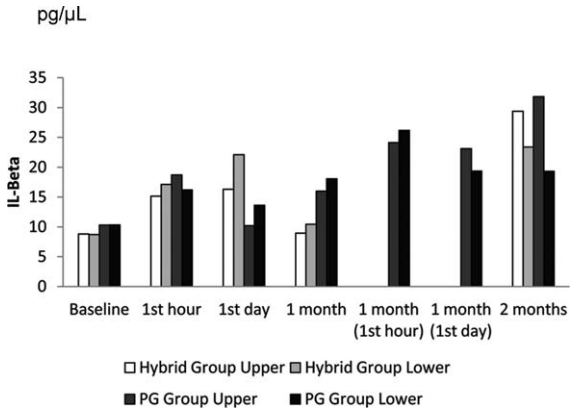

Figure 6. Concentration of interleukin-1 Beta.

arches was 2.65 ± 1.73 mm and 2.10 ± 1.41 mm; respectively, and with PG retractors was 2.25 ± 1.36 mm and 2.25 ± 0.92 mm, respectively ($p<0.01$). A significant amount of molar anchorage loss was observed with hybrid retractors in the maxilla ($p<0.01$) and mandible ($p<0.05$) but only in the mandible ($p<0.01$) with the PG retractor. Distal tipping of maxillary canines was significant with both retractors ($p<0.01$); however, distal tipping of the lower canines was significant only with the PG retractor ($p<0.05$). No significant differences were determined in any of the linear or angular measurements during 2 months of canine distalization between groups ($p>0.05$) (Table 6). There was no correlation between the amount of canine distalization and any of the cytokines ($p>0.05$).

DISCUSSION

Clinical studies on the up- and downregulation of cytokines in GCF have been carried out to provide a noninvasive way to show their involvement during orthodontic tooth movement.[9] Increased production of cytokine levels in the early stages of tooth movement has been shown in several studies.[10,11,14,15]

In this study, an attempt was made to evaluate the efficacy of hybrid vs PG canine retractors in terms of differences in force decay, magnitude, and activation procedures in conjunction with regulation of IL-1β and TNF-α levels. In the hybrid group (100 g), only one activation was applied for the 2-month experimental period, whereas in the PG group (100 g) reactivation was performed at 1 month. Age and sex differences were not considered as it has been found that age and sex do not increase enzymatic activity.[16] In the present study, no control teeth were used as all 4 quadrants required canine retraction as part of orthodontic treatment. Also, the anterior part could not serve as control teeth for possible movement because of interdental fiber, although these teeth were not engaged in the appliance. Consequently, baseline levels were used as control values. Some previous studies have also used baseline measurements as control.[12,15,17]

In the present study full fixed orthodontic treatment was begun 5 weeks after the first premolar extractions. During this 4-week period the focus was on instructing patients in how to maintain good oral hygiene. In similar studies orthodontic forces were applied 1 week,[12] 2 weeks,[18] 1 month,[19] or 2 months[17] after premolar extractions.

In the present study, no significant differences were found in clinical parameters, so we may assume that alterations of the plaque index, bleeding on probing, and probing depth did not have a major influence on the compositional changes of GCF.

The amount of GCF volume tends to increase with inflammation and capillary permeability. In current study, GCF volume generally increased over 2 months, regardless of retractor type or arch, because of the orthodontic forces. In accordance with our study, previous studies reported that GCF volumes significantly increase during orthodontic treatment, although no clinically gingival health changes occur.[15,17,20] Perinetti *et al*.[21] suggested that inflammation rather than orthodontic tooth movement affects GCF volume. In the study by Karacay *et al*.,[12] GCF volume decreased when heavy orthodontic forces were applied. Uematsu *et al*.[14] were unable to show differences in GCF volume between control teeth and those subjected to orthodontic movement.

Rygh[22] described bone remodeling by tooth movement as a continuous process characterized by bone resorption. King *et al*.[23] described 3 phases of tooth movement in rats: an early phase of bone resorption (3–5 days), its reversal (5–7 days), and a late phase (7–14 days) of bone deposition. A similar bone cycle has also been reported in humans, yet the timing in rats is longer than in humans.[24]

Cytokines play an important role in intercellular signaling and have been implicated in the pathology of periodontal diseases, bone destruction, and bone response to orthodontic tooth movement. One of the most important breakthroughs of bone biology has been the identification of the role of cytokines in bone remodeling, which is used by orthodontists

Table 4. Concentration of interleukin 1-beta (pg/μL)

Groups	Statistics	Baseline	1 Hour	1 Day	1 Month	1 Month + 1 Hour	1 Month + 1 Day	2 Months
Hybrid (n=10) (upper)	Mean ± SD	8.81 ± 4.61[a,b]	15.13 ± 4.83 [a]	16.28 ± 14.03	8.95 ± 5.75[c]			29.38 ± 25.55[b,c]
	Median	7.50	14.00	10.45	9.40			21.45
	Minimum	3.10	9.80	3.80	0.00			0.00
	Maximum	17.40	23.60	48.40	14.80			82.00
PG (n=10) (upper)	Mean ± SD	10.28 ± 5.11[d,e,f]	18.71 ± 10.55[d]	10.20 ± 9.09[g,h]	15.97 ± 16.21	24.11 ± 8.75[e,g]	23.12 ± 19.89	31.83 ± 26.20[f,h]
	Median	10.55	13.90	8.90	11.95	25.70	21.44	22.65
	Minimum	0.00	11.40	0.00	0.00	11.80	0.00	7.80
	Maximum	16.40	45.72	30.20	56.40	36.00	62.20	88.00
Hybrid (n=10) (lower)	Mean ± SD	8.70 ± 4.76[i,j]	17.09 ± 2.98[i]	22.08 ± 22.49	10.42 ± 10.24			23.36 ± 18.12[j]
	Median	8.45	17.60	10.55	9.70			19.20
	Minimum	2.40	12.50	4.00	0.00			0.00
	Maximum	15.60	22.80	58.00	33.90			63.00
PG (n=10) (lower)	Mean ± SD	10.32 ± 4.24	16.18 ± 7.14	13.61 ± 12.50	18.04 ± 21.48	26.14 ± 16.07	19.33 ± 19.85	19.32 ± 17.96
	Median	9.50	14.75	9.00	10.80	24.95	14.40	12.07
	Minimum	6.30	9.80	6.40	0.00	0.00	0.00	0.00
	Maximum	18.40	32.80	48.25	68.39	54.00	66.00	58.00

[a] Significant difference within the upper Hybrid group at baseline and 1 hour (p=0.005).
[b] Significant difference within the upper hybrid group at baseline and 1 month (p=0.022).
[c] Significant difference within the upper hybrid group at 1 month and 2 months (p=0.011).
[d] Significant difference within the upper PG group at baseline and 1 hour (p=0.021).
[e] Significant difference within the upper PG group at baseline and 1 month + 1 hour (p=0.005).
[f] Significant difference within the upper PG group at baseline and 2 months (p=0.013).
[g] Significant difference within the upper PG group at 1 day and 1 month + 1 hour (p=0.007).
[h] Significant difference within the upper PG group at 1 day and 2 months (p=0.013).
[i] Significant difference within the lower hybrid group at baseline and 1 hour (p=0.005).
[j] Significant difference within the lower hybrid group at baseline and 2 months (p=0.017).

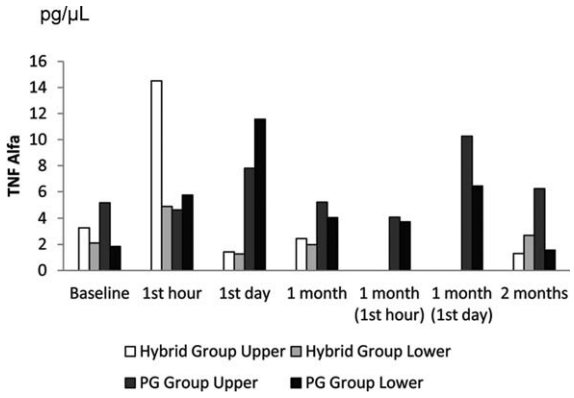

Figure 7. Concentration of tumor necrosis factor-alpha.

when forces are transmitted to the surrounding tissues of the periodontium.[25] Experiments prove that several neurotransmitters and cytokines are associated with the activation of bone resorption, by which the movement of teeth through bone is facilitated.[26,27]

In this study, IL-1β upregulated at 1 hour and downregulated at 1 month nearly to baseline levels; it upregulated again at 2 months in both upper and lower hybrid groups. The IL-1β levels increased at 1 hour in the upper PG group; the levels were high at 1 month and demonstrated an even higher level at 1 month + 1 hour until the end of 2 months. Similar up- and downregulations were seen in lower PG group; however, these changes were not significant. No significant changes were detected between retractors or jaws. The changes in IL-1β levels indicate early inflammatory response of cellular activities in periodontal ligament because of the orthodontic force. Previous studies investigating different force magnitudes showed peak levels in the early phase of tooth movement between 1 hour[5,28] and 48 hours.[29] Without force reactivation, IL-1β levels decreased significantly at 1 week to baseline level, but with force reactivation it reached higher levels similar to our findings.[13] Iwasaki *et al*.[30] reported that IL-1β levels fluctuated over a 28-day cycle when continuous orthodontic force was applied. In the present study, upregulation of IL-1β at 2 months in the hybrid group may be related to the mentioned cycle. The increment of IL-1β at 2 months might also be due to the breakdown of possible semi-hyalinized areas at the compression side and increment of osteoclastic activity. As Reitan[31] indicated, the irregularity of root and alveolar bone surfaces might cause hyalinization in local areas, even with minute forces. In accordance with current findings, Luppanapornlarp *et al*.[19] found that IL-1β levels increased at 1 day, declined to approximately normal levels at 1 week to 1 month, and increased again at 2 months with 50 g and 150 g of continuous forces.

In this study, initial orthodontic forces with the upper hybrid device caused TNF-α levels to increase at 1 hour as an early local host response toward orthodontic force; TNF-α levels declined afterward. In the PG group, TNF-α levels upregulated at 1 day and declined at 1 month and then upregulated again at 1 month + 1 day and downregulated again at 2 months. However, the changes in the PG group were not significant because of the large variation. The TNF-α levels were significantly higher at 1 day and 2 months in the PG group compared with the hybrid group in the upper arch. Different timing of cytokine elevations between retractors may be due to different biomechanical properties, force decay levels after activations, or differences between alloys of the wires. Optimizing biologic response is dependent not only on the initial force magnitude but undoubtedly also on the rate of decay force magnitude between activations. Therefore, the retraction spring should deliver forces within a physiologic force range.[3] Horizontal forces of PG retractors drop from 100 g to approximately 40 g over a 4-week period whereas the hybrid retractor applies 100 g constant force until 4 mm of deactivation.[1,3]

Karacay *et al*.[12] found that TNF-α levels increased at 1 day and declined at 1 week because of tissue adaptation to orthodontic force with the hybrid retractor. Significantly higher values were determined in the rapid canine distalization group at 1 hour and 1 week compared with the hybrid group, and tissue response continued for a longer time.

Ren *et al*.[32] reported that IL-1β and TNF-α reached significant levels 1 day after orthodontic force; however, no upregulation was observed during the long duration of tooth movement. It was stated that bioactivity of TNF-α and IL-1β was inhibited by a feedback mechanism within the periodontal ligament.[33]

In the present study no correlation was found between velocity of tooth movement and any cytokines. In contrast, Iwasaki *et al*.[30] found a positive correlation with IL-1β. Iwasaki *et al*.[18] also reported that many factors, such as cytokine levels, genotype, sex, growth status, and stress, affected speed of tooth movement.

As more is understood about biological activities of cytokines in bone remodeling, their involvement in

Table 5. Concentration of tumor necrosis factor-alpha (pg/μL) (n=10)

Groups	Statistics	Baseline	1 Hour	1 Day	1 Month	1 Month + 1 Hour	1 Month + 1 Day	2 Months
Hybrid (n=10) (upper)	Mean ± SD	3.26 ± 3.29[a]	14.51 ± 22.28[a,b,c,d]	1.42 ± 1.08[b,e]	2.44 ± 2.11[c]			1.30 ± 0.77[d,f]
	Median	2.45	5.10	1.45	2.00			1.30
	Minimum	0.00	1.40	0.00	0.00			0.00
	Maximum	9.60	68.00	3.50	6.60			2.80
PG (n=10) (upper)	Mean ± SD	5.15 ± 6.54	4.65 ± 4.44	7.81 ± 11.07[e]	5.20 ± 6.29	4.09 ± 3.70	10.26 ± 15.68	6.24 ± 9.26[f]
	Median	1.75	3.40	2.15	2.35	3.60	1.75	3.22
	Minimum	0.60	0.00	0.00	0.00	1.20	0.80	0.00
	Maximum	18.20	15.40	35.40	18.30	14.30	48.00	31.40
Hybrid (n=10) (lower)	Mean ± SD	2.11 ± 2.61	4.90 ± 4.79	1.27 ± 1.26	1.97 ± 1.99			2.69 ± 3.31
	Median	1.50	3.90	0.90	1.50			1.10
	Minimum	0.00	0.00	0.00	0.00			0.00
	Maximum	9.05	16.30	3.20	6.00			8.80
PG (n=10) (lower)	Mean ± SD	1.83 ± 1.19	5.74 ± 6.32	11.57 ± 22.68	4.06 ± 7.95	3.73 ± 2.04	6.44 ± 14.07	1.55 ± 1.15
	Median	1.50	3.35	1.45	1.60	3.95	1.55	1.70
	Minimum	0.10	0.00	0.00	0.00	0.00	0.00	0.00
	Maximum	3.80	22.00	66.00	26.30	6.30	46.00	3.70

[a] Significant difference within the upper hybrid group at baseline and 1 hour (p=0.037).
[b] Significant difference within the upper hybrid group at 1 hour and 1 day (p=0.007).
[c] Significant difference within the upper hybrid group at 1 hour and 1 month (p=0.013).
[d] Significant difference within the upper hybrid group at 1 hour and 2 months (p=0.005).
[e] Significant difference between the upper hybrid group and the upper PG group at 1 day (p=0.050).
[f] Significant difference between the upper hybrid group and the upper PG group at 2 months (p=0.050).

Table 6. Mean values and SDs before and after 2 months of canine distalization in each group and *p* values

Groups	Distance to x-Axis (mm)			Distance to y-Axis (mm)			Angular Measurement (°)		
	Baseline	2 Months	*p*	Baseline	2 Months	*p*	Baseline	2 Months	*p*
Upper canine									
Hybrid	26.50 ± 2.91	26.90 ± 2.75	0.087	46.25 ± 4.87	43.60 ± 4.81	.001**	106.35 ± 5.49	101.10 ± 4.50	0.009**
PG	27.25 ± 2.62	27.55 ± 2.30	0.111	48.70 ± 6.15	46.45 ± 6.50	.001**	107.55 ± 7.19	102.30 ± 5.37	0.006**
Lower canine									
Hybrid	37.55 ± 3.43	37.50 ± 3.24	0.678	7.05 ± 3.37	9.15 ± 2.46	.001**	86.20 ± 7.59	85.65 ± 6.68	0.287
PG	37.95 ± 3.39	37.90 ± 3.13	0.758	4.90 ± 3.78	7.15 ± 3.21	.000**	87.55 ± 9.33	85.45 ± 7.77	0.039*
Upper first molar									
Hybrid	17.00 ± 1.67	17.40 ± 1.70	0.087	24.75 ± 3.34	25.20 ± 3.41	.004**	85.65 ± 6.87	86.35 ± 6.67	0.066
PG	18.35 ± 1.16	18.35 ± 1.23	1.000	28.45 ± 5.02	28.75 ± 4.91	.111	86.85 ± 7.04	87.05 ± 6.26	0.733
Lower first molar									
Hybrid	27.85 ± 3.02	28.00 ± 2.88	0.279	25.85 ± 3.12	25.15 ± 3.12	.021*	86.05 ± 5.71	85.65 ± 4.72	0.589
PG	27.50 ± 3.11	27.80 ± 2.88	0.081	23.20 ± 2.55	22.55 ± 2.75	.009**	85.25 ± 5.28	84.80 ± 3.96	0.468

* $p < 0.05$; ** $p < 0.01$.

orthodontic tooth movement will become clearer. Further studies with larger sample sizes are still needed to understand the complexity of bone remodeling due to different magnitude and types of orthodontic forces.

CONCLUSIONS

Continuous but diminishing forces produced by PG mechanics enhanced levels of TNF-α significantly at 1 day and 2 months compared with constant and continuous forces applied by a hybrid retractor in the upper arch. Despite this difference, PG and hybrid appliances induce similar effects in IL-1β and TNF-α production and in the amount of tooth movement. Furthermore, no significant changes were found in any of the cytokine levels between jaws despite the compact bone structure in mandible.

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