Original Article

Interleukine-1β and Tumor Necrosis Factor–α Levels in the Human Gingival Sulcus during Orthodontic Treatment

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ABSTRACT

Objective: To test whether interleukine 1β (IL-1β) and tumor necrosis factor–α (TNF-α) levels differ from each other in different treatment levels.

Materials and Methods: Eighteen patients, nine female and nine male (aged 16–19 years; mean 17.4 ± 1.8 years), participated in this study. Each subject underwent a session on professional oral hygiene and received oral hygiene instructions. Two months later, a fixed orthodontic appliance was placed. The patients were seen at baseline, at days 7 and 21 and at the 3rd and 6th month as the leveling of the teeth occurred. Records of the baseline scores for the distalization forces were taken at the 6th month. Days 7 and 21 after 6 months of treatment were also recorded.

Results: There were increases in the volume of gingival crevicular fluid (GCF) and in the concentrations of IL-1β and TNF-α.

Conclusions: Leveling and distalization of the teeth evoke increases in both the IL-1 and TNF-α levels that can be detected in GCF.

KEY WORDS: Orthodontic tooth movement; Cytokines; Gingival cervicular fluid; Tumor necrosis factor; Interleukin-1

INTRODUCTION

When an orthodontic force is applied to a tooth for a prolonged period of time, an inflammatory response is initiated. As a result of this, a bone resorption process begins and this process accommodates tooth movement.[1]

The appearance of osteoclasts and bone resorption are critical factors that initiate tooth movement.[2] Mononuclear osteoprogenitor cells in local tissues require several developmental stages to turn into full functional multinucleated osteoclasts.[3] Various cytokines and hormones play an important role in this process.[3,4]

Interleukin–1 (IL-1) is a known cytokine that starts the bone resorption process by taking part in the survival, fusion, and activation of the ostoeclasts.[5,6] IL-1β, a major physiologic form of IL-1, is mainly secreted by monocytes and partially by macrophages, endothelial cells, fibroblasts, and epidermal cells. This secretion is activated by various stimuli.[5] All these studies demonstrate that mechanical stimuli activate inflammatory cytokines.[4,7] In a cat model, Davidovitch et al[8] have localized induced levels of IL-1β and tumor necrosis factor–α (TNF-α) in the periodontium of teeth undergoing movement.

IL-1β and TNF-α also affect bone metabolism directly. At extremely low concentrations, IL-1β and TNF-α have been implicated in the process of bone remodeling through specific receptors on the bone cell population.[9–11] Monocytes and macrophages do not constituently produce IL-1β or TNF-α, but on ''activation'' they synthesize and release these cytokines.[12,13]

Lynch et al[14] demonstrated that in the early stages of tooth movement (at 12 and 24 hours) cytokines are mostly seen in the periodontal ligament. All the studies that determined the level of cytokines in gingival crevicular fluid (GCF) evaluated subjects, both animals and humans, for short periods of time.[8,15–20] These studies applied distalization forces to the teeth and searched for early responses to the forces.

As a result of the great force that is applied to the

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tooth, a hyalinized zone occurs in the compressed periodontal ligament. This hyaline zone has also been described as an area of focal aseptic necrosis. From the tension side, an indirect resorption process initiates, which regulates bone resorption. But orthodontic treatment does not always use distalization forces that produce hyalinization as is considered in these studies. All the preceding studies applied heavy distalization forces on tooth. As a result of this pressure on the periodontium, hyaline zones occurred and the indirect resorption process initiated and induced levels of IL-1β and TNF-α. However, in the first stages of orthodontic treatment, low forces are used that do not result in hyalinized zones and indirect resorption. Therefore, there may be different levels of cytokines in the leveling and distalization stages of the orthodontic treatment. The null hypothesis of this study was whether IL-1β and TNF-α levels differ from each other in different treatment levels.

MATERIALS AND METHODS

Eighteen patients, nine female and nine male (age 16–19 years; mean 17.4 ± 1.8 years), who had presented at the Department of Orthodontics, Faculty of Dentistry, Dicle University, and who had been diagnosed for the extraction of their first premolars participated in this study. The inclusion criteria for the participants were: (1) a healthy systemic condition, (2) no use of anti-inflammatory drugs in the 6 months preceding the beginning of the study, (3) the need for extraction treatment with fixed appliances involving distalization of at least one maxillary/mandibular canine, (4) probing depth values (measured as the distance from the bottom of the sulcus to the most apical portion of the gingival margin) not exceeding 3 mm in the whole dentition, (5) no loss of periodontal attachment (measured as the distance from the bottom of the sulcus to the cemento-enamel junction) exceeding 2 mm in any interproximal site, and (6) no radiographic evidence of periodontal bone loss after a full-mouth radiographic periapical examination. Informed consent was obtained from the patients and the parents of patients under 18 years of age.

Clinical procedures

Maxillary or mandibular first premolars (or both) were extracted for each participant, and, during the following 2 months, all subjects received repeated oral hygiene instructions, which included the correct use of a toothbrush and an interdental brush. At the end of this period, before orthodontic treatment with full brackets (Omni Roth, GAC International Inc, Bohemia, NY) was initiated, GCF sampling was conducted (baseline). GCF was collected from the mesial and distal aspect of the upper canines in this study. At this appointment, orthodontic treatment was begun with 0.014-inch Nitinol arch wires (GAC International Inc). Seven days later, on the second appointment, patients were instructed to brush their teeth and not to eat anything 3 hours before the second sampling. The forces that are applied on the tooth were still active. Twenty-one days later, a third sampling was performed applying the same procedures. On the third month from the start of the treatment, the fourth GCF sample was collected. By this time, leveling was completed on most of the patients.

In the first 3 months into treatment, only leveling forces were applied on the canines. Any forces that would tip the tooth and produce hyaline zones were eliminated. It is very hard to design an appliance that delivers specific force types in vivo because of the irregularity of root surfaces. This is a limitation of in vivo studies, but we aimed to use light leveling forces, that will not produce hyaline zones initiating indirect resorption process, in the first 3 months of treatment. After the sixth month, heavier forces that produce hyaline zones that will initiate indirect resorption processes were used.

Leveling force was established by replacing the Nitinol archwires with larger ones when they were deactivated.

In the sixth month, before we proceeded with the distalization of the canines with rectangular stainless steel arch wires, the fifth GCF sampling was performed. This sampling served as a baseline score for distalization because it was performed before the application of retraction forces on the canines. A 7-mm Sentalloy closed coil spring (GAC International Inc) applied 150 g of force for the retraction of the canines. Seven days after the start of the retraction, the same sampling procedure was followed. The last sampling was performed on day 21 of the retraction.

The retraction forces were achieved using Sentalloy closed coil springs that were stretched between the canines and molars and applied 150 g of force. Sentalloy coil springs deliver constant force levels when they are activated. A full-bracketed 0.016 × 0.022″ stainless steel arch wire was used to retract the teeth.

GCF sampling

GCF sampling was obtained with paper strips (Periopaper, Pro Flow, Amityville, NY) using the method described by Rudin et al.[21] Sampling was performed only on the vestibular sides of the tooth to prevent salivary contamination.

Days 7 and 21 were chosen for GCF sampling because day 7 is the turnover time for enzymes and indirect resorption processes start on day 21.

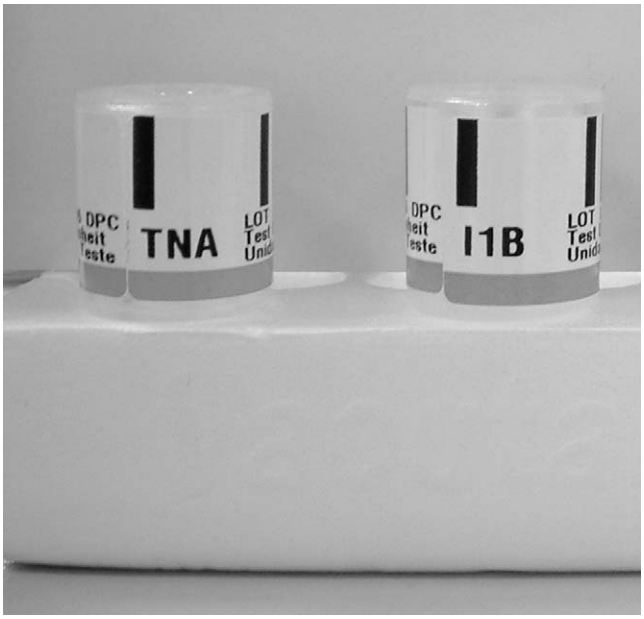

FIGURE 1. The kit used in the study.

Sample collections were done in the early hours of the day. Sample sites were isolated with cotton rolls, plaque was removed, and the tooth surfaces were air-dried. Paper strips were placed into the sulcus and, after waiting 30 seconds, an apparatus (periotron 8000, Ora Flow Inc, Plainview, NY) was used for determining the GCF volume. Paper strips were stored in sterile tubes at −20°C until the day of the experiment. Saliva and blood contamination was important; contaminated samples were excluded from the study. GCF sampling was done before all other clinical examinations were performed to prevent an increase in fluid volume. Before examination of the GCF, 1000 μL sterile NaCl (9 mg/mL) was added to paper strips, and the GCF was diluted at 3000 g at +5°C for 20 minutes.[22] The immunoassay system and the machine used for measuring IL-1β and TNF-α concentrations was Immulite (Diagnostic Products Corp, Los Angeles, Calif) (Figures 1 and 2). For the manual dilution of patient samples, IL-1β– and TNF-α–free non-human buffer matrix was used.

The amount of IL-1β and TNF-α detected in each sample was compared with an IL-1β and TNF-α standard curve that demonstrates a direct relationship between optical dentistry and cytokine concentration. The total amount of IL-1β and TNF-α was determined in picograms.

Statistical evaluation

A one-way paired *t*-test was used to determine differences between the amounts of IL-1β and TNF-α (Table 1). To understand the significant differences between the groups at a time interval, Mann-Whitney *U*-test was used by taking the differences between two adjacent points for IL-1β and TNF-α concentrations (Table 2). Descriptive measurements (mean, standard deviation, median, minimum, and maximum) of the volume of the GCF are given in Table 3. All the statistical evaluations were made using SPSS 10.0 software program.

RESULTS

The concentration of IL-1β and TNF-α are shown in Tables 1 and 2. The baseline levels for the concentration of IL-1β and TNF-α increased on days 7 and 21 of leveling and distalization. This increase was more prominent in the concentration of IL-1β but individual variations were very great. The only statistically significant difference was between the fourth and fifth examination in the concentration of TNF-α. There was a decrease between the last stage of the leveling and the starting stage of the distalization period ($P < .05$).

FIGURE 2. The machine used in the study.

Table 1. The concentrations of IL-1β and TNF-α (pg/ml)[a]

	Statistic	Baseline	Day 7	Day 21	Third mo	Sixth mo	Sixth mo + Day 7	Sixth mo + Day 21
	Mean	37.439	69.331	72.283	47.172	41.272	72.439	62.850
	SD	31.505	51.251	57.810	42.920	40.169	70.860	59.519
	SE	7.426	12.080	13.626	10.116	9.468	16.19	14.029
IL-1β	Maximum	115	214	247	192	187	314	271
	Minimum	7.0	14.5	21.3	10.9	12.4	19.7	10.7
	Significant	—	.102	.140	.673	.990	.109	.278
	F	—	2.833	2.228	0.181	0.000	2.710	1.214
	Mean	29.933	30.283	30.631	31.189	30.339	32.344	30.506
	SD	1.942	1.329	2.531	3.080	1.654	2.203	1.658
	SE	0.458	0.313	0.633	0.726	0.390	0.519	0.391
TNF-α	Maximum	33.6	32.8	37.2	39.4	32.9	35.9	33.3
	Minimum	24.4	28.1	24.4	26.5	26.6	27.5	26.1
	Significant	—	.421	.095	.098	.793	.224	.818
	F	—	0.663	2.956	3.105	0.070	1.534	0.054

[a] IL-1β indicates interleukin-1β; TNF-α, tumor necrosis factor–α.

Table 2. The Comparison of Two Adjacent Concentrations of IL-1β and TNF-α[a]

	Statistic	Day 7–21	Day 21–Third mo	Third mo–Sixth mo	Sixth mo (Sixth mo + 7 d)	(Sixth mo + 7 d) –(Sixth mo + 21 d)
	Mean	70.822	59.278	44.222	56.856	62.644
	SD	53.864	51.770	41.078	58.193	64.665
	SE	8.977	8.628	6.846	9.819	10.772
IL-1β	Maximum	247	247	192	314	314
	Minimum	14.5	10.9	10.9	12.4	10.7
	Significant	.920	.321	.720	.141	.592
	F	0.010	1.014	0.130	2.276	0.293
	Mean	30.550	31.003	30.764	31.342	31.425
	SD	2.285	2.765	2.745	2.173	2.136
	SE	0.586	0.461	0.412	0.362	0.356
TNF-α	Maximum	37.2	39.4	39.4	35.9	35.9
	Minimum	26.3	26.3	26.5	26.6	26.1
	Significant	.193	.306	.048*	.094	.100
	F	1.767	1.078	4.196	2.963	2.865

[a] Same as in Table 1.
* $P < .05$.

Table 3. The Volumes of Gingival Crevicular Fluid (mg)

Statistic	Baseline	Day 7	Day 21	Third mo	Sixth mo	Sixth mo + Day 7	Sixth mo + Day 21
Mean	0.0017	0.0023	0.0026	0.0024	0.0010	0.0016	0.0029
SD	0.0010	0.0007	0.0017	0.0012	0.0006	0.0009	0.0014
Median	0.0010	0.0008	0.0029	0.0023	0.0008	0.0014	0.0023
Minimum	0.0005	0.0011	0.0018	0.0009	0.0005	0.0002	0.0009
Maximum	0.0021	0.0027	0.0033	0.0037	0.0024	0.0036	0.0037

No statistically significant results were found between the other groups ($P > .05$).

GCF volumes are shown in Table 3. The GCF volume was greater on days 7 and 21 of leveling and distalization, and it returned to baseline level after leveling of the teeth. No statistically significant results were found between the groups ($P > .05$).

DISCUSSION

Orthodontic tooth movement occurs by the remodeling of the alveolar bone as a result of the force that is exerted on the periodontium. A cell-free hyalinized zone occurs on the pressure side of the periodontal ligament. Necrosis of this zone is established by os-

teoclasts that originate from the neighboring tissues. On the tension side, osteoblasts take place in the bone apposition process.[23] Proinflammatory cytokines play important roles in bone resorption as in any root resorption process.

The gingival sulcus was selected as the testing site because of its continuity with the PDL and its accessibility within the oral cavity. Tissue samples of the PDL or the bone undergoing resorption (or both) would provide a more direct site for measuring changes in cytokines but cannot be obtained from human subjects. Thus, cytokine values found in the sulcus provide an indirect measurement of changes in the PDL. Localization of cytokines in the PDL of animals can be accomplished by histologic techniques. In humans, a noninvasive method is needed. The prediction that compression of the PDL in humans could result in the migration of biochemical products into the gingival sulcus is the basis of our experimental design. Previous in situ techniques for biochemical analysis of the gingival crevice involved sampling crevicular fluid using paper strips.[8] The paper strip method was used in this study.

The upper canines of all patients were monitored in this study because these teeth are accessible and easily cleaned. It has also been reported that plaque accumulation depends on the site with more plaque accumulating in the anterior area than in the posterior area.[24]

It has been shown that levels of biochemical markers in the GCF might depend on different collection sites.[25] For this reason, the canines were used as both the test and control teeth in this study. The control data were collected at baseline, which were obtained before any force was applied. Serra et al[26] stated that age and sex does not increase enzymatic activity so age and sex differences were not considered in this study.

Gingival modifications incident to tooth movement have been reported in both histological and ultrastructural analyses, and clinically evident changes have also been detected.[27] Tuncer et al[17] and Samuels et al[28] have reported that GCF volumes significantly increase during orthodontic treatment. In this study, GCF volumes also increased in milligrams, especially when an orthodontic force was exerted on the tooth.

The increased mean concentrations of IL-1β and TNF-α were significantly higher compared with the baseline, but this was not statistically significant because of the large variation. This finding was the same as was shown in a previous study.[29] In the first 3 months of our experiment, four samplings were performed. The first one was the baseline scores and the others were carried out of the leveling stages. In general, the scores were elevated, but some individual variations were seen and no statistically significant results were obtained. In the first 3 months we tried to apply pure leveling forces. After 6 months, a Sentalloy coil spring was stretched between canine and molar to apply retraction forces. The evoked levels of IL-1β and TNF-α were nearly the same with both forces. In our study, our aim was simply to clarify the cytokine levels, not to normalize the cytokine or total protein concentrations. We aimed to measure the direct responses to different orthodontic forces in the periodontium.

The results of this study demonstrate that elevation of IL-1β and TNF-α levels do not significantly differ from each other. If IL-1β and TNF-α levels do not show significant differences from each other, it may be stated that lower forces may initiate bone resorption processes. So to achieve faster tooth movement, there is no need to apply heavy forces, which could be harmful to other tooth structures (ie, root resorption). This implies that the IL-1β and TNF-α levels might not return to baseline when a light leveling force or a heavy retraction force is applied.

The insignificant elevated mean level of the cytokines was sustained, but this might not be evidence that an orthodontic force induced IL-1β release from individual PDL cells. As Reitan[30] indicated, the irregularity of the root and alveolar bone surfaces might cause hyalinization in local areas even with minute forces because it is very difficult to design an appliance to apply even pressure on the entire PDL in vivo. Moreover, as the alveolar bone on the pressure side undergoes remodeling, the microscopic surface topography changes. Therefore, the resultant cytokine level measured in the crevicular fluid is the sum of the various cellular responses to the pressure experienced at various locations on the pressure side. This is considered a limitation of in vivo experiments.

Iwasaki et al[31] reported that IL-1β levels fluctuated with a 28-day cycle when a continuous orthodontic force was applied. The increased level at day 21 of our study seems to be related to this kind of periodicity.

In the early stages of orthodontic force application it has been shown that many PDL cells stain positively for IL-1β.[8] Also, Lynch et al[14] reported that in the early stages of tooth movement (12 and 24 hours) many PDL cell types stained positively for IL-1β. Lowney et al[20] demonstrated that TNF-α plays a pivotal part in the assessment of orthodontic tooth movement.

Tzannetou et al[18] used low and high forces to the maxillary molars to expand the palate. Low forces were produced by separator placement and higher forces by a palatal expansion device. They observed high levels of IL-1β levels with both the force levels. Also, Lee et al[29] demonstrated that the mean concen-

trations of IL-1β increase in the first 24 hours after continuous and interrupted forces. All these studies examined GCF in short time periods as compared with this study. They found that especially in the first 24 hours, cytokine levels increased and then equilibrium is reached, which is higher than the baseline levels.

As first described by Rygh,[32,33] bone remodeling determined by tooth movement is a continuous process characterized by bone resorption. However, recent histomorphometric findings[34] have shown that tooth movement is more complex than that described by Rygh.[32,33] King et al[34] described 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,[35,36] but in humans this timing seems to be longer than in rats. These studies might therefore support our finding of increased IL-1β and TNF-α levels in GCF.

CONCLUSIONS

- Leveling and distalization of the teeth evoked increases both in the IL-1β and TNF-α levels in the periodontal tissues that can be detected in GCF.

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