Levels of Cytokines in Gingival Crevicular Fluid during Rapid Maxillary Expansion and the Subsequent Retention Period

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Objective: *To monitor the effects of rapid maxillary expansion (RME) on bone metabolic activities during and after 3 months of retention.* ***Study Design:*** *Fifteen patients with a mean age of 12.9 ± 0.6 years were treated with a bonded expansion device, activated 2 turns per day. The retention period was 3 months. Clinical periodontal parameters were recorded at baseline and after retention. Gingival crevicular fluid (GCF) samples were collected from maxillary first molars from the compression sides at baseline, then at 1 and 10 days and after retention. Tension side samples were obtained at baseline and after retention. Interleukin-1beta (IL-1β), transforming growth factor beta1 (TGF-β1), prostaglandin E_2 (PGE_2) and nitric oxide (NO) levels were specifically measured.* ***Results:*** *Periodontal parameters increased significantly after retention relative to baseline values. Levels of IL-1β, TGF-β1 and PGE_2 increased on day 10, and decreased after retention on the compression side. NO levels were elevated on day 10, and remained higher after retention on the compression side. Tension side cytokine levels remained higher relative to baseline values after retention.* ***Conclusions:*** *The results of this study indicate the importance of ongoing adaptive bone activities after 3 months of retention with RME, which should be considered questionable as an effective retention period.*

Keywords: *rapid maxillary expansion, gingival crevicular fluid, bone remodeling, retention*

INTRODUCTION

Rapid maxillary expansion (RME), the most common treatment for patients with transverse maxillary discrepancies, is orthopedic expansion through the separation of the midpalatal suture achieved with strong forces.[1] If these strong forces are not tolerated by maxillary structures, severe relapse and tipping of the anchorage teeth might be encountered, thereby requiring retention appliances to maintain the results until completion of bone remodeling.[2] The results of a systematic review demonstrated that approximately 25% of the initially achieved expansion remains stable in the long-term.[3] At this point, clinicians are faced with making decisions about the retention period in order to stabilise the result with an adequate duration of retention.[4] There is currently no consensus on the minimal retention period, which usually varies between 3 and 6 months in literature,[5-8] and therefore, relapse continues to be unavoidable.[9]

Orthodontic forces change the biological system within the cells in the periodontal ligament (PDL) and alveolar bone, providing release of several biological mediators, such as cytokines, chemokines, neurotransmitters, prostaglandins and growth factors.[10,11] In response to mechanical force, compression of the PDL occurs on one side, and tension occurs on the opposite side. Therefore, catabolic changes are predominantly mediated on the compression sides, and anabolic changes occur on the tension sides.[12,13] Studies have focused on the cellular activities during orthodontic treatment in order to provide knowledge about the changes in bone and tissue metabolism within gingival crevicular fluid (GCF).[14,15] The analysis

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of GCF is a useful and non-invasive method for examining cellular dynamics.[16] Several biomarkers can be detected in GCF to evaluate the role of cytokines in bone remodeling. Interleukin 1β (IL-1β), and prostaglandin E_2 (PGE_2) are increased during the early stages of orthodontic tooth movement, and induce bone resorption.[15] Another biological agent, transforming growth factor-β1 (TGF-β1), which is released from the organic matrix of the resorbed bone into the local microenvironment, mediates proliferation and differentiation of osteoblast precursors, and thereby promotes bone formation.[12]

Nitric oxide (NO) also plays an important role in regulating bone response to mechanical stress.[11]

There have been few studies in literature which have evaluated the biochemical response of periodontal tissues during RME treatment.[17,18] A previous study assessed levels of inflammatory mediators during active and retention periods of RME.[19] Reports in literature have also shown buccal and palatal bone thickness changes during RME, when evaluated with computed tomography (CT).[19,20] A recent study also assessed alveolar bone formation during the retention phase.[21] However, evaluations of the cellular activities of the periodontal tissues during and after maxillary expansion are still needed for clinicans to obtain more data on the appropriate retention protocol. Therefore, the aim of this study was to evaluate the changes in IL-1β, TGF-β1, PGE_2, and NO levels during and after a 3-month retention period of RME by means of GCF analysis, in order to assess if 3 months of retention would be sufficient for bone remodeling.

MATERIALS AND METHOD

The research was approved by the Ethics Committee of Gazi University (25901600-5565), and informed consent was obtained from the parents or legal guardian of each participant. Sample size was calculated by considering the detection limits of the cytokines according to the manufacturers instructions with 80% theoretical power, and the number of patients was required to be a minimum of 12. Therefore, the study sample consisted of 15 adolescent patients (8 female, 7 male; mean age 12.9±0.6 years) who had applied for orthodontic treatment at the Department of Orthodontics, Faculty of Dentistry, Gazi University. All patients were selected based on the following criteria: (1) presence of posterior crossbite with transverse maxillary arch deficiency, (2) maxillary first molars present and fully erupted, (3) no history of orthodontic treatment, (4) no restorative/prosthetic restorations on maxillary first molars, (5) good periodontal health with no clinical and radiographic evidence of bone loss, (6) no signs of gingival inflammation or history of periodontal treatment, (7) no systemic diseases, or use of anti-inflammatory agents, antibiotics, or immunosuppressants in the past 6 months, and (8) still in the skeletal growth period.

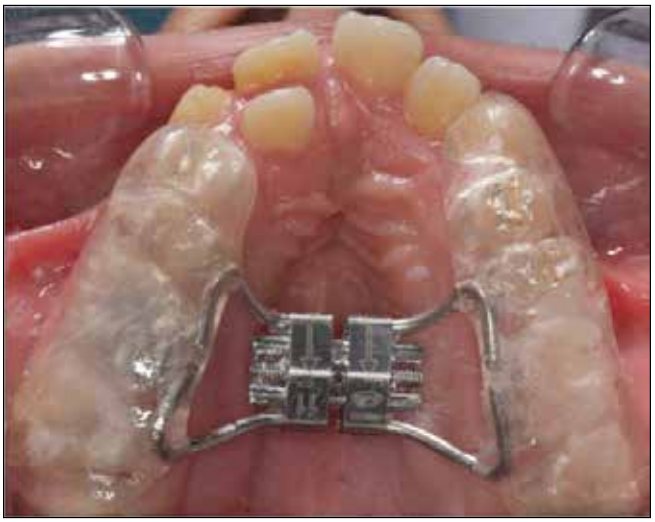
Periodontal evaluation

All patients initially received periodontal prophylaxis including scaling and polishing. They were instructed to brush their teeth twice a day for a minimum of 3 minutes in order to maintain oral hygiene throughout the treatment. None of the patients used any oral antiseptic solutions or mouthwash during the study period. After two weeks, the status of the periodontal tissues was determined by clinical periodontal assessments. Levels of plaque index (PI),[22] and gingival index (GI)[23] were recorded. Probing depth (PD) was measured with a periodontal probe (Hu Friedy, Chicago, Illinois, USA) by positioning the device in the sulcus parallel to the long axis of the tooth, without any pressure. Bleeding on probing (BOP) was recorded according to the presence of bleeding and measured as the percentage of sites with bleeding.[24] A blunt periodontal probe was passed along the gingival crevice and if bleeding occurred within 10 to 15 seconds, a positive score was given. The number of positive units was divided by the number of sites examined and the result was multiplied by 100 to express the index as a percentage. All periodontal assessments were performed at baseline and at the end of the retention period. In addition, the patients were encouraged to implement oral hygiene throughout the observation period.

RME appliance

All patients were treated with an acrylic bonded expansion device. The appliance consisted of a Hyrax-type expansion screw (Leone orthodontic products, Sesto Fiorentino, Firenze, Italy), two 0.045-inch stainless steel wires extending to the palatal surfaces of the premolars and molars, and acrylic covering the occlusal surfaces of the posterior teeth, extending 2-3 mm away from the gingiva. Care was taken to ensure that the arms of the screw were parallel to the palatal mucosa (Figure 1). The Hyrax screw was activated twice per day with a one-quarter turn in the morning and in the evening, until sufficient expansion and overcorrection was obtained. After the active phase, the screw was fixed and the appliance was left in place as a retainer for 3 months.

Figure 1. Intra-oral view of the rapid maxillary expansion device.



GCF sample collection

GCF samples were collected from the compression and tension sides of the right and left maxillary molars. The compression side samples were obtained from the mesio-buccal gingival crevices of the first molars at baseline (prior to the start of expansion), on the 1st and 10th days after initial activation of the screw, and at 3 months after retention. Tension side samples were collected from the mesio-palatal gingival crevices at baseline and at the end of the 3-month retention period. GCF sampling took place in a temperature-controlled area between 09.00-11.00 a.m.

Prior to sample collection, the area was isolated with cotton rolls, and the teeth and the adjacent marginal gingival area were dried with air. Then, cotton paper strips (Periopaper, OraFlow Inc,

Smithtown, NY) were inserted gently into the gingival crevices below the gingival margin (level of approximately 1mm) for 30 seconds. After removing the first strip and waiting for one minute, a second strip was placed on the same side for another 30 seconds. Strips which were contaminated with saliva or blood were excluded. The paper strips from the buccal and palatal sides of each tooth were sealed separately in polypropylene containers.

Periotron 8000 (Oraflow Inc, Plainview, New York, USA) was used to save the samples as Periotron units and GCF volume was calculated after conversion to microliters with software (MLCONVERT.exe.Software Version 2.52, Oraflow Inc, Amityville, NY). Each sample was then stored at −80°C until assay.

Cytokine analysis

Each strip was eluted twice with 200 µL of Hank's balanced salt solution containing 0.5% bovine serum albumin by centrifugation (3000 × *g*, 4°C, 15 minutes). All of the absorbance measurements were performed using ChemWell 2900 (Awareness Technology, Inc., USA). The total amounts of biomarkers were determined in picograms, and concentrations were calculated by dividing the amounts by the GCF volume of each sample (pg/µL).

IL-1β-EASIA (KAP1211): The immunoenzymetric assay for the quantitative measurement of human IL-1β was performed using a commercial kit (DIAsorce IL-1β-EASIA kit, katalog no: KAP1211, DIAsorceImmunoAssays S.A., Belgium). DIAsorce IL-1β-EASIA is a solid phase Enzyme Amplified Sensitivity Immunoassay performed on a microtiterplate. The assay uses monoclonal antibodies directed against distinct epitopes of IL-1β. Bound enzyme-labelled antibody is measured through a chromogenic reaction. The amount of substrate turnover was determined colorimetrically by measuring the absorbance at 450 nm., which was proportional to the IL-1β concentration. The results were expressed as pg/mL.

TGF-β1 Platinum ELISA (BMS249/4/BMS249/4TEN): The human transforming growth factor-β1 (TGF-β1) ELISA is an enzyme-linked immunosorbent assay for the quantitative detection of human TGF-β1 (eBioscience, Affymetrix Co., Bender MedSystems GmbH, Austria). An anti-human TGF-β1 coating antibody is adsorbed onto microwells. Human TGF-β1 present in the sample/standard binds to the antibody. A biotin-conjugated anti-human TGF-β1 antibody is added and binds to human TGF-β1 captured by the first antibody. Following incubation, Streptavidin-HRP and subsequently substrate solution is added to the wells. A coloured product is formed in proportion to the amount of human TGF-β1 present in the sample and absorbances are measured at 450 nm. The results were expressed as pg/mL.

Prostaglandin E_2 Express EIA Kit (Item no. 500141): This assay is based on the competition between PGE_2 and $PGE_{2\text{-acetylcholinesterase}}$ conjugate for a limited amount of PGE2 monoclonal antibody (Cayman Chemical Company, USA; Item no. 500141). The product of the enzymatic reaction coloured by Ellman's Reagent, absorbs strongly at 412 nm. The intensity of this colour is proportional to the amount of PGE2 tracer bound to the well, which is inversely proportional to the amount of free PGE2 present in the well during incubation. The results were expressed as pg/mL.

Nitrite/Nitrate Colorimetric Assay Kit (Item no:780001): This kit provides a method for the measurement of total nitrate (nitrite concentration in a sample (Cayman Chemical Co., USA; Item no.780001). The first step is the conversion of nitrate to nitrite utilizing nitrate reductase. Following the addition of the Griess reagent, which converts nitrite into a deep purple azo compound, photometric measurement of the absorbance at 540 nm determines the nitrite concentration. The results were expressed as µM.

Statistical analysis

Data analysis was performed using the Statistical Package for Social Sciences software (SPSS, Version 20.0, USA). The Shapiro Wilks test was used to determine the normal distribution of the data. If the distribution was normal, two-way ANOVA, and if the distribution was not normal, Friedman's test were performed at all the time points. The significant parameters were evaluated with a multiple comparison test. The differences between time points were analyzed with the Mann-Whitney-U test, when parametric test assumptions were not satisfied. When the distribution was normal, the paired-t test was used. Descriptive statistics were presented as median and minimum-maximum ranges. Correlations between periodontal clinical indices and levels of cytokines and biochemical mediators were evaluated with Spearman's correlation or Pearson correlation tests. A value of $p<0.05$ was considered statistically significant.

RESULTS

A total of 15 patients were enrolled in the study, and 1 patient was subsequently excluded following breakage of the appliance. The sample revealed an average transverse discrepancy of 7 mm. The mean activation period of the screw was 20 days, retention period was 3 months and the mean expansion was 8 mm.

Clinical periodontal parameters: The descriptive measurements (mean, median, standard deviation (SD), minimum and maximum values) of the clinical periodontal assessments in the sampling area are shown in Table 1. The results demonstrated that PI and GI were significantly increased at the end of retention relative to the baseline values ($p<0.01$, respectively).

Levels of biomarkers on the compression side: The compression side GCF volume, total amounts and concentrations of IL-1β, TGF-β1, PGE_2, and NO and comparison between the periods are shown in Table 2. GCF volume was significantly elevated on the 10th day relative to baseline, followed by a significant decrease in the 3rd month compared to the 10th day ($p<0.01$, respectively). The total amount of IL-1β was increased on the 1st and 10th days compared to the baseline level at a significance level of $p<0.01$. There was a significant decrease in the 3rd month compared to the 1st and 10th days ($p<0.01$).

The total amounts of TGF-β1 were increased on the 1st and 10th days compared to the baseline level ($p<0.01$, respectively), and a significant decrease was determined in the 3rd month relative to the 10th day ($p<0.01$). The PGE_2 total amount was increased on the 10th day compared to baseline, revealing a significant decrease in the 3rd month relative to the 1st day ($p<0.01$, respectively). Increases were found on the 1st and 10th days, and at the 3rd month in the total amount of NO, compared to baseline at the significance level of $p<0.01$. No statistically significant differences were determined between the measurement time points in respect of the concentration of biomarkers ($p>0.05$).

Levels of biomarkers on the tension side: The tension side GCF volume, total amounts and concentrations of IL-1β, TGF-β1, PGE_2, NO and comparison between the periods are shown in Table 3. A

Table 1. Descriptive measurements of clinical periodontal assessments.

	Baseline (n=14)					Post-retention (n=14)					
	x	SD	Median	Min	Max	x	SD	Median	Min	Max	p
PI	1.20	0.59	1.00	0.25	2.50	1.50	0.50	1.50	0.75	2.75	0.001
GI	0.80	0.51	1.00	0.00	1.50	1.20	0.58	1.40	0.25	2.00	0.002
PD (mm)	2.40	0.68	2.40	1.00	3.50	2.40	0.41	2.40	1.75	3.00	0.489
BOP (%)	33.36	14.12	28.12	16.20	52.00	33.40	15.07	29.18	16.09	53.00	0.445

x, mean; SD, standard deviation; *p<0.05; **p<0.01; ***p<0.001; ns, p>0.05.

Table 2.Medians and ranges for parameters at baseline(0), 1st day (1), 10th day (2), and 3rd month (3), and comparison between periods at compression side.

	Baseline (0)	1st day (1)	10th day (2)	3rd month (3)	p-value					
					0-1	0-2	0-3	1-2	1-3	2-3
GCF volume (µl)	0.04 (0,03-0,06)	0.05 (0,03-0,10)	0,07 (0,05-0,13)	0,05 (0,03-0,10)	ns	0.001	ns	ns	ns	0.001
IL-1β total amount (pg)	557.71 (278,86-797,14)	697.36 (560,00-973,14)	784,49 (624,86-1034,12)	624,26 (342,77-896,57)	0.001	0.001	ns	ns	0.001	0.001
IL-1β concentration (pg/µl)	13142.86 (6971,43-19385,73)	14867.39 (5600-21819,27)	12815.48 (6318,89-18469,58)	12635.76 (5356,78-29885,71)	ns	ns	ns	ns	ns	ns
TGF-β1 total amount (pg)	22.67 (12-81,33)	28,33 (14,33-86,89)	38,67 (18,24-102,67)	19,80 (13,33-84,33)	0.001	0.001	ns	ns	ns	0.001
TGF-β1 concentration (pg/µl)	495.00 (240,00-1716,70)	566.67 (286,66-2133,33)	538.89 (186,67-1746,66)	483.33 (138,00-2108,33)	ns	ns	ns	ns	ns	ns
PGE2 total amount (pg)	182.01 (125,00-531,25)	223.79 (136,70-536,70)	255,58 (181,55-770,31)	200,01 (115,60-450,14)	ns	0.001	ns	ns	0.001	ns
PGE2 concentration (pg/µl)	3986.28 (2529,83-10904,25)	4861.13 (1770,80-8945,00)	4024.23 (1610,62-9505,17)	4101.88 (1770,80-14716,33)	ns	ns	ns	ns	ns	ns
NO total amount (pg)	0.67 (0,05-3,45)	1,15 (0,10-3,84)	1,56 (0,35-3,95)	1,36 (0,24-3,58)	0.001	0.001	0.001	ns	ns	ns
NO concentration (µM)	15.96 (0,79-86,27)	20.14 (1,36-76,82)	22.84 (3,80-65,75)	32.43 (4,76-82,15)	ns	ns	ns	ns	ns	ns

*p<0.05; **p<0.01; ***p<0.001; ns, p>0.05.

Table 3. Medians and ranges for parameters at tension side, and comparison between periods.

	Baseline	3rd month	p
GCF volume (µl)	0,04 (0,01-0,06)	0,05 (0,04-0,08)	0.011
IL-1β total amount (pg)	560,91 (435,32-728,00)	614,51 (482,56-924,00)	0.005
IL-1β concentration (pg/µl)	14535,40(9790,48-45356,70)	12411,00 (8066,89-17844,91)	ns
TGF-β1 total amount (pg)	15,67 (12,00-77,33)	17,33 (14,00-78,00)	0.001
TGF-β1 concentration (pg/µl)	449,92 (211,12-3866,67)	366,79 (233,33-1866,68)	ns
PGE2 total amount (pg)	146,48 (107,46-462,77)	198,22 (114,17-485,12)	0.008
PGE2 concentration (pg/µl)	4732,17 (2487,00-13130,00)	3738,88 (2127,25-12128,00)	ns
NO total amount (pg)	0,37 (0,05-2,31)	0,96 (0,10-2,98)	ns
NO concentration (µM)	20,82 (0,79-76,95)	18,39 (1,90-74,50)	ns

*p<0.05; **p<0.01; ***p<0.001; ns, p>0.05.

significant increase was found in GCF volume in the 3rd month relative to baseline (p <0.05). The total amounts of IL-1β, TGF-β1, and PGE_2 were increased in the 3rd month relative to baseline at a significance level of p <0.01.

There were no significant correlations between periodontal clinical indices and mediator levels p >0.05.

DISCUSSION

As an orthodontic or an orthopedic force is applied on the dental structures, consecutive events occur leading to bone resorption on the compression side and osteogenesis on the tension side. These reactions are essential for tooth movement in orthodontics, but another important issue is the adaptive responses to forces through re-organization of the supporting tissues, providing stability of the achieved results. There is individual variability in dental and skeletal responses in respect of RME treatment.[19] Clinicians must be able to achieve adequate mineralization of periodontal structures and palatal sutures in order to minimize the relapse tendency before initiating the subsequent orthodontic treatment. Determining cellular activities after RME treatment has some clinical relevance in this regard. Previously, CT scans have been used in several studies to identify changes in basal bone after RME.[20,25] The purpose of this study was to evaluate the cellular responses of periodontal structures at the initial stages and after 3 months of retention induced by RME in growing patients. For this purpose, GCF analysis, which is a useful and non-invasive method, was performed.[14,16,26] GCF samples were collected from maxillary first molars, which are vulnerable to strong forces during this treatment, and both compression and tension sides were taken into consideration. Although strict oral hygiene instructions were given throughout the study, there was an increase in clinical periodontal indices, which might have been related to poor compliance of the patients by the end of the retention period. This result showed that the potential irritation effect of the appliance cannot be ignored.

One of the major findings of this study was that the total amounts of IL-1β, TGF-β1and PGE_2 reached the highest levels on the 10th day, followed by decreases at the end of retention approaching baseline levels on the compression side. Similarly, NO levels were elevated on the 10th day, but remained at high levels at the end of retention when compared to baseline levels on the compression side. The increase in the total amount of IL-1β on the 1st and 10th days may represent the early inflammatory response of the tissues on the compression side evoked by RME. Increased levels of this biomarker might show phagocytosis of necrotic tissue, degradation of hyalinized bone and repair of the bone.27 Recently, the reduced bone thickness of the supporting teeth on the buccal aspect has been shown with the use of CT, during RME treatment.20 Another study reported the effects of continuous and interrupted orthodontic forces on bone resorption processes, and significant elevations in IL-1β and PGE2 levels were found at 24 hours after force application.15 These findings have been associated with the direct effect of the mechanical force, and the enhanced local bone remodeling. Similarly, the current results showed up-regulation of these mediators in the early active periods of RME.

Studies which have evaluated the retention periods have reported elevated levels of IL-1β from the first day of RME until 28 days of retention, and the results have been attributed to the tendency for relapse.18 Similarly increased levels of PGE2 have been reported after 7 and 14 days of retention, indicating the possible relapse tendency of the maxillary segments.28 Conversely, the current study results showed decreased levels of these markers on the compression side after 3 months of retention. These conflicting results may be due to differences between treatment techniques, retention periods, and/or patient selection. According to the interpretation of a previous report,18 the current study findings may be associated with the recovery of alveolar remodeling around the supporting teeth after 3 months of retention on the compression side. In another aspect, reduction of the force during the retention phase of RME might be related to the lower biomarker levels, indicating the low risks for root resorption and dental tipping.

It has been stated that orthodontic loading and overloading may cause micro damage to bone, and the consequent release of mediators, cytokines, PGE2, TGF-β1, and NO to stimulate bone remodeling.12 As the expanded maxillary arch has a strong tendency to relapse to its previous form, researchers have focused on accelerating bone formation to prevent relapse and shorten the retention period.29 In this context, TGF-β1 has been reported to promote bone formation in the expanding suture, but the role of TGF-β1 during bone remodeling is complex. While it presents anabolic properties on bone tissues through bone matrix protein production, its involvement in bone resorption has also been emphasized.30 During orthodontic tooth movement its presence has been reported to be increased on both the compression and tension sides.10 It has been explained by the fact that TGF-β1 favors bone formation during the early stages of osteoblastogenesis.31 In contrast, during the late stages of osteoblastogenesis, TGF-β1 inhibits the differentiation of mesenchymal stem cells into osteoblasts and the mineralization of mature osteoblasts in culture.32 In respect of the hematopoietic osteoclastic lineage, TGF-β1 affects bone resorption in a dose-dependent manner.33 In the current study, the levels of TGF-β1 on the compression side demonstrated a significant elevation on the 1st day, reaching a maximum level on the 10th day, and a significant reduction in the 3rd month. It has been stated that TGF-beta possesses separate facilitative and suppressive effects on osteoclast differentiation and bone resorption.34 It is released from the bone matrix after the initiation of resorption, first allowing monocytes to develop into osteoclasts, and then limiting the extent and duration of resorption after its release from the bone matrix. Accordingly, it can be concluded that, at the compression side, elevation of TGF-β1 levels in the early stages of force application might present as bone resorption, while the reduction of signaling in the later stages might reveal increases in bone mass, elastic modulus and hardness, mineral concentration, and resistance to fracture of the bone.[35]

NO plays a role through decreasing osteoclastic formation and activity, leading to positive bone formation due to a decreased recruitment of osteoclasts. In the current study, the NO levels on the compression side increased gradually showing the highest level on the 10th day. This seems to be an adaptive response of the compressed structures for remodeling. The levels remained high after 3 months of retention, which might depend on the influence of bone activity,[36] and may also be related to the different isoforms of NO synthase (NOS), which are neuronal nitric oxide synthase (nNOS), endothelial nitric oxide synthase (eNOS), inducible nitric

oxide synthase (iNOS), and bacterial nitric oxide synthase (bNOS). The important regulatory effect of iNOS and eNOS in bone remodeling has been shown during orthodontic force application.[37] In addition, while iNOS mediates inflammation-induced bone resorption on the compression side, eNOS interferes with bone formation on the tension side.[37,38] The results of another study showed that tooth pulp tissues under orthodontic force reacted differently, revealing high levels of iNOS and low levels of eNOS in the first 15 days, and a decrease of iNOS and increase of eNOS levels after 30 days. The authors attributed this difference to the reduction of inflammation in the later stages of orthodontic treatment.[39]

There is still no clear consensus on the minimal retention time after RME. Many practitioners have retention concepts varying between three and six months. In a previous study, researchers fixed the expansion screws for 2 weeks after expansion, and used a removable retention appliance passively for approximately 3 months.[40] However, a recent study showed that a retention time of 6 months was not sufficient for reorganization of the suture in post-pubertal patients, and advised a retention period longer than 6 months.[41] In the current study, it was aimed to compare baseline and the 3-month time point results on the tension side, to evaluate if the cellular activities were completed after 3 months of retention. As there was sustained cytokine presence, given that IL-1β, TGF-β1, and PGE_2 expression is associated with ongoing inflammatory processes and bone remodeling, this association may be clinically relevant in the decision of the clinician to terminate the treatment. In this regard and through the hypothesis of the study it does not seem logical to observe the intermediary phases of orthodontic force effects on cytokine level on the tension side. Current findings have shown that cellular activities were still ongoing after 3 months of retention, indicating a possible risk of relapse. In a previous report, the periodontal changes at the end of the active phase of RME and 6 months of retention were examined with CT evaluations.[19] Although a decrease was determined in the buccal bone plate thickness of the supporting teeth at the end of the active phase, there was significant bone apposition on the palatal side after 6 months of retention. The authors related this finding to the adequate duration of retention after RME. A recent study also monitored alveolar bone formation on the tension sides of supporting teeth during the retention phase of RME and found that alveolar bone formation could last for up to 6 months.[21] The NO levels in this study did not change significantly on the tension side, which could be explained by the absence of the orthopedic force after the retention period. As stated in a previous study, new results are needed to clarify whether NO levels vary depending on the force magnitude.[42] In these regards, the current findings may represent ongoing metabolic bone activities on the tension side, thus indicating the absence of compensatory bone apposition after 3 months of retention. Clinically, these outcomes may highlight the importance of an adequate duration of retention of more than 3 months.

Some of the limitations of the current study were the small sample size and the lack of a control group. It would also have been beneficial to record the periodontal indices at intermediate intervals. Furthermore, the findings are limited to a 3-month retention period. Follow-up findings at 6 months could not be performed due to the different continuing treatment procedures. However, the analysis for the 3-month follow-up, which is a common period of retention for RME, allowed focus on the importance of the detection of periodontal tissue remodeling. Future studies should be designed with larger sample sizes to more appropriately generalize the results to the general population and should include longer retention periods to evaluate the risks of relapse.

CONCLUSION

This study represents a first step in monitoring periodontal tissue responses on a side-specific basis during and after 3 months of retention with RME therapy. It can be recommended that further studies with varying retention periods will be both useful and of interest.

The mechanical stress induced by RME revealed increased levels of biomarkers in the initial stages, but there was a decrease at the end of 3 months of retention on the compression side. This was evident despite the increased periodontal indices, which might show that the mechanical force could have more effect on the periodontal responses.

Findings on the tension side demonstrated that cellular activities were still ongoing after 3 months of retention, indicating a possible risk of relapse.

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