MEANDROS MEDICAL AND DENTAL JOURNAL
June 2024 25(2):154-167

Meandros Medical and Dental Journal
doi:10.69601/meandrosmdj.1496245

Research Article

INVESTIGATION OF BONE REMODELING IN GINGIVAL CREVICULAR FLUID IN PATIENTS TREATED WITH RAPID MAXILLARY EXPANSION DURING PUBERTAL PERIOD

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ABSTRACT

Objective: We aimed to evaluate bone remodeling following 3-month and 6-month retention periods of rapid maxillary expansion (RME) in gingival crevicular fluid (GCF).

Materials and Methods: 23 pubertal participants (15 girls- 8 boys, 12-15 years) with maxillary transversal deficiency were enrolled in the study. Following banded-type RME appliance was introduced into the mouth, RME protocol was initiated by turning the Hyrax screw twice daily (morning/evening) with ¼ activation. GCF samples were taken from right maxillary first molars at four different time points (T0: before the appliance was deployed, T1: following active phase, T2: at the end of the 3-month retention period, T3: at the end of the 6-month retention period) using paper strips. Probing depth, gingival index, plaque index, and bleeding percentage at probing were recorded. BALP, OPG, RANKL, and TNF-α levels were measured in GCF samples using ELISA kits.

Results: Our study revealed that GCF's BALP and OPG levels remained unchanged over time in tension and pressure regions, and there were no statistically significant differences among regions at each time point ($p>0.05$). RANKL level was statistically significantly increased in the buccal region (the pressure region) at time T1 ($p=0.042$). The palatinal region's TNF-α level was statistically significantly higher than the buccal region at T0, T2, and T3 time points ($p=0.005$, $p=0.042$, and $p=0.006$, respectively). Our study showed no correlation between 3-month and 6-month retention periods and biomarkers indicating RME's bone metabolic activities.

Conclusion: Further studies with different retention periods and larger sample sizes are suggested.

Keywords: Rapid maxillary expansion, Gingival crevicular fluid, Bone remodelling

Received: 05 June 2024
Revised: 30 July 2024
Accepted: 08 August 2024
Published: 31 August 2024

MEANDROS MEDICAL AND DENTAL JOURNAL
June 2024 25(2):154-168
Meandros Medical and Dental Journal
doi:10.69601/meandrosmdj.1496245

INTRODUCTION

The main goal of orthodontics is to provide patients with ideal function and aesthetics by treating anomalies in the stomatognathic system caused by genetic or environmental factors or a combination of these and to achieve a permanent result. These irregularities can occur in all three spatial dimensions: sagittal, vertical, and transversal (1).

In transversal anomalies, skeletal and/or dental, unilateral or bilateral posterior cross-bite is frequently observed (2). The posterior cross-bite incidence was between 2.7% and 18.2% in different populations, whereas this rate was reported as 2.7% in the Turkish population (3). Posterior cross-bite is a common malocclusion with prevalence rates of 7.5% to 22.2% in deciduous and mixed dentition and 10.2% to 14.4% in permanent dentition (4). Many researchers have suggested that anomalies in the transversal direction should be treated in the early period regardless of their clinical presentation and etiology (3,5).

Rapid maxillary expansion (RME), the most common treatment modality for patients with maxillary transversal misalignments, is an expansion type that involves opening the midpalatal suture with the application of significant forces. Many appliance designs have been developed to treat maxillary transversal deficiency with RME. Today, the most commonly used appliance designs are the tooth- and tissue-supported Haas appliance and the tooth-supported Hyrax appliance (6).

Expansion appliances usually generate orthopedic forces ranging from 3 to 10 pounds. If the maxillary structures do not tolerate these heavy forces, severe relapse and tipping of the anchored teeth might be encountered. Therefore, using retention appliances is necessary until the bone remodeling is completed to retain the results achieved (7).

Ekström et al. determined satisfactory mineralization of the expanded suture after the third month (8). The midpalatal suture reorganization and a stable relationship between the maxilla and facial skeleton's joints with other bones will determine the RME appliance retention time. Besides, the time needed to reorganize all structures during retention seems to depend on the residual load remaining after the termination of appliance activation.

If RME treatment is performed in the prepubertal period, mainly skeletal effects of RME have been reported, whereas in later developmental stages, more dental effects can be expected, along with potential tissue damage (9). Dentoalveolar effects might occur even when RME treatment is performed in the prepubertal period (9-11).

Even though most previous studies on RME treatment have focused on the effects on the midpalatal suture or other sagittal and vertical changes, a decrease in the buccal bone thickness of the anchored teeth at the end of the active expansion period of RME and a significant improvement in the original bone thickness after 6 months of retention have been reported regarding dentoalveolar effects (11).

Lagravère et al. reported that approximately 25% of the initial amount of expansion remained stable in the long term (10). Clinicians must decide on an adequate retention time to stabilize the result. The literature has no consensus on the minimum retention time, ranging from 3 to 6 months (12).

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June 2024 25(2):154-168

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Gingival crevicular fluid (GCF) analysis is a valuable and non-invasive method to investigate cellular dynamics. Several biomarkers, such as bone alkaline phosphatase (BALP), osteoprotegerin (OPG), Receptor Activator of Nuclear Factor Kappa-B Ligand (RANKL), and tumor necrosis factor (TNF)- α, which are biomarkers of bone formation, resorption, and inflammation, can be assayed in GCF to assess the cytokines' role in bone remodeling. The literature contains many studies evaluating the periodontal tissues' biochemical responses during RME treatment (13-15).

This prospective clinical study aimed to evaluate bone remodeling in GCF after 3-month and 6-month retention periods in RME. This study also aimed to determine the optimal retention period to prevent the maxillary expansion relapse and contribute to the literature.

Our study's null hypothesis was 'There is no correlation between the bone metabolic activities of RME and 3-month and 6-month retention periods'. Our study's alternative hypothesis was 'A correlation is observable between 3-month and 6-month retention periods and bone metabolic activities of RME'.

MATERIALS AND METHODS

This observational descriptive prospective clinical study investigated the bone formation and destruction phenomena occurring during RME treatment of pubertal patients in the gingival crevicular fluid.

Constructing the study sample

This study involved pubertal individuals who presented to Aydın Adnan Menderes University, Faculty of Dentistry, Department of Orthodontics between March 2021 and March 2022 for orthodontic treatment and were diagnosed with maxillary transversal deficiency by clinical examination. A total of 23 individuals, including 15 girls and 8 boys, whose ages ranged between 12 and 15 years, participated in the study.

Since the partial eta-squared value required to calculate the study's sample size was unavailable in the relevant literature, (0.06) was used to represent the medium effect size. Based on this, the number of patients required was calculated as 23 with a type 1 error of 0.05, a type 2 error of 0.20, and an effect size of 0.2526 for four repeated measurements.

Study inclusion criteria were as follows: individuals with transversal maxillary deficiency (≥4mm) and unilateral or bilateral posterior cross-bite, individuals in the pubertal period (S-MP3cap stages) determined based on the wrist bone maturation stages, absence of orthodontic treatment history, presence and complete eruption of maxillary first molars, no history of antibiotic treatment for the last three months and no use of anti-inflammatory drugs within one month prior to the commencement of the study, no systemic disease, no history of gingivitis symptoms or periodontal treatment, and optimal periodontal health.

Exclusion criteria were as follows: a history of orthodontic treatment, absence or incomplete eruption of maxillary first molars, a history of antibiotic treatment for the last three months and use of

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anti-inflammatory drugs within one month prior to the commencement of the study, presence of a systemic disease, gingivitis symptoms or a history of periodontal treatment, and poor periodontal health.

The decision of Aydın Adnan Menderes University Faculty of Dentistry Clinical Research Ethics Committee# ADUDHF2021/07, dated February 24, 2021, was obtained. In addition, the approval of the Turkish Ministry of Health, the Turkish Pharmaceuticals and Medical Devices Agency has been obtained. Then, the consent forms, written straightforwardly and understandably, were read and signed by the patients and their parents to enable the study to be conducted.

Application method

In order to plan patients' treatment, cephalometric, panoramic, wrist, and AP radiographs were obtained, intraoral and extraoral photographs were acquired, and digital models were obtained by performing intraoral scans. A banded-type RME appliance consisting of a modified Hyrax screw was applied to treat the patients (Figure 1). For the construction of the appliance, the right and left maxillary first molars were banded, and impressions were taken with alginate from the maxilla while the bands remained in the mouth. A plaster model was obtained from the impression. The dimensions of the Hyrax screw (model AO-0620-13, model AO-0620-11, model AO-0620-9; Leone, Sesto Fiorentino, Italy) were selected according to the extent of maxillary narrowness. The appliance was then leveled and polished to make it suitable for placement in the mouth.

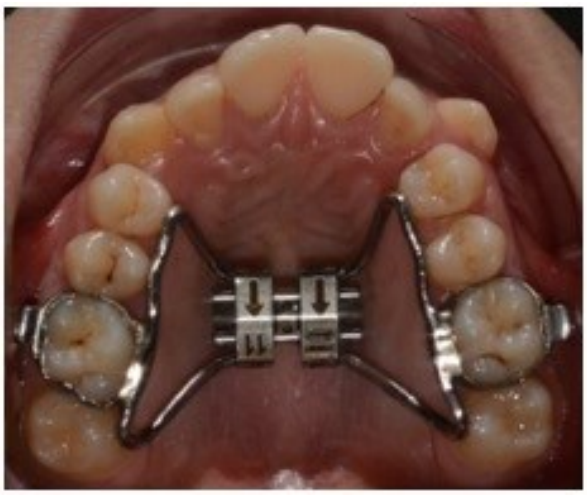

Figure 1. A banded-type RME appliance used in the study

Glass ionomer cement (3M Unitek, 3M UNITEK Dental Products, Monrovia, USA) was mixed with a 3/1 powder-to-liquid ratio. After the prepared glass ionomer cement was coated on the bands' inner surfaces, the appliance was applied to the mouth by placing the bands on the first molar teeth.

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Immediately after placing the appliance in the mouth, the Hyrax screw was turned twice daily with ¼ rotation in the morning and evening by the patient's guardian; thus, the RME protocol was initiated.[1] Activation of the screw was continued twice daily, once in the morning (0.2 mm) and once in the evening (full activation 0.4 mm) until the palatinal tubercles of the maxillary teeth were at the same level as the buccal tubercles of the mandibular teeth. Thus, sufficient expansion and overcorrection were achieved after 3 to 6 weeks of RME treatment. The screw was then ligated with a ligature wire, and the same appliance was kept on the teeth as a passive retainer throughout the 6-month retention period.

Sampling procedure

Samples were taken from all patients' right maxillary first molars at four different times to achieve standardization as follows: T0 (Baseline) (Samples from this time were taken as the control group): Immediately before appliance placement; T1: After the active phase (after finishing turning the screw); T2: End of the 3-month retention period after the active phase; T3: End of the 6-month retention period after the active phase.

GCF samples were taken prior to recording the periodontal parameters. At each time point, GCF samples were taken from the buccal and palatinal surfaces of the right maxillary first molar. Before GCF sampling, the field was isolated with cotton rolls and a salivary absorbent, and the adjacent teeth and marginal gingival zone were gently air-dried. Then, paper strips (Periopaper, Oraflow, NY, USA) were gently placed in the gingival crevice of the right maxillary first molar below the mesiobuccal gingival margin (at approximately 1 mm) with the help of a tweezer until mild resistance was encountered. Blood and saliva-contaminated paper strips were excluded from the study. The paper strips were kept in the gingival pocket for 30 seconds to ensure standardization (13-15).

Each periopaper's GCF volume was measured in µl using the Periotron 8010 device (Periotron 8010, Oraflow, Amityville, NY, USA). Similar procedures were conducted in the right maxillary first molar's distobuccal, mesiopalatinal, and distopalatinal gingival regions. The arithmetic mean of the periotron values of two samples from the same area was calculated, and a single value was recorded. All GCF samples were obtained in a temperature-controlled area between 09.00-11.00 A.M and by the same researcher (C.G.). Paper strips from the same buccal and palatinal sites were transferred to empty Eppendorf tubes (Lamtek, Istanbul Teknik Kimya, Istanbul, Turkey) and coded for each patient. The samples were stored at -80°C until the time of analysis.

The probing depth (PD), gingival index (GI), plaque index (PI), and bleeding percentage at probing (%BOP) were recorded using a Williams periodontal probe (Hu Friedy, Chicago, Illinois, USA)(16-18). All periodontal parameters were recorded by the same investigator (Ç.G.).

Biochemical analysis

Analyses were performed at the Department of Biochemistry, Faculty of Medicine, Aydın Adnan Menderes University. According to the manufacturer's procedure, OPG, RANKL, BALP, and TNF-α levels in the samples were measured using commercial Enzyme Linked-Immuno-Sorbent Assay (ELISA) kits (BT Lab, Shanghai, China).

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Statistical analysis

Royston's test analyzed the multivariate normal distribution of biochemical and pocket depth measurements, and Shapiro-Wilk's test analyzed the suitability of age for univariate normal distribution. Age was expressed as mean ± standard deviation (mean±sd), and range of variation (minimum-maximum: min-max), and all other measurements were summarized as mean±sd and median (interquartile range, IQR: first quartile - third quartile).

Because biochemical and pocket depth measurements had contradictory observations with Mahalanobis distance and did not show a multivariate normal distribution, the differences in the measurements' time variability between regions were analyzed with a nonparametric LD-F2 design. The ANOVA-type test statistic (ATS) and p-value derived from the LD-F2 design for the time and region interaction effect were given. As the effect of time and site interaction was not statistically significant, the Friedman test analyzed the difference of the measurements by time in each site. The Friedman test also compared plaque index (PI), gingival index (GI), and bleeding on probing (BOP) measurements by time and pocket depth measurements by region at each measurement time. When necessary, the method proposed by Campbell and Skillings was applied as a post-hoc test after the Friedman test. At each measurement time, biochemical measurements were compared by region using the Wilcoxon test. Statistical significance level $p \leq 0.05$ was considered.

The Royston test and LD-F2 design were implemented in the R programming language (v.4.3.1) and RStudio software (v.2022.12.0.353) (R Core Team, 2022; Team, 2022). The mvn function of the MVN package (v.5.9) was used for the Royston test, and the nparLD function of the nparLD package (v.2.2) was used for the LD-F2 design. The IBM SPSS Statistics 22.0 (IBM Corp. Released 2013. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.) package software was used for all other statistical analyses.

Table 1. Distribution of PI, GI, and BOP by time

Measurement time	PI Mean±SD Median (IQR)	GI Mean±SD Median (IQR)	BOP Mean±SD Median (IQR)
T0	0.15±0.30	0.23±0.41	3.26±8.61
	0.00 (0.00-0.25)[a]	0.00 (0.00-0.25)[a]	0.00 (0.00-0.00)[a]
T1	0.59±0.37	0.88±0.61	27.17±29.11
	0.50 (0.25-1.00)[b]	0.75 (0.25-1.50)[b]	25.00 (0.00-50.00)[b]
T2	0.75±0.35	1.13±0.52	33.70±26.77
	1.00 (0.25-1.00)[b,c]	1.25 (0.75-1.50)[b,c]	25.00 (25.00-50.00)[b,c]
T3	0.87±0.27	1.34±0.38	44.57±19.88
	1.00 (0.75-1.00)[c]	1.50 (1.25-1.50)[c]	50.00 (25.00-50.00)[c]
$\chi 2$, p-value	38.787, <0.001	35.420, <0.001	35.952, <0.001

SD: Standard deviation, IQR: Interquartile range.

[a,b, and c] indicate homogeneous times ($p>0.05$) regarding the respective column measurement.

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doi:10.69601/meandrosmdj.1496245

RESULTS

The mean age of the study participants was 13.57±1.13 (min-max: 11.50-15.67) years. PI, GI, and BOP measurements increased significantly with time ($p<0.001$ for all three) (Table 1). Despite such an increase, no statistically significant differences existed between the T1 and T2 and between T2 and T3 time points ($p>0.05$). However, the measurements were significantly lower at T0 compared to the other time points and at T1 compared to T3.

Regarding pocket depth distribution over time and regions, the results showed statistically significant differences in the pocket depth measurements of the buccal and distal regions over time ($p=0.019$ and $p=0.010$, respectively; Table 2). However, there were no significant differences among the measurement times in the palatinal and mesial regions ($p=0.209$ and $p=0.082$, respectively). No statistically significant difference was detected when pocket depth changes over time were compared among the regions [Anova-type test statistic (ATI)=0.721, $p=0.621$]. Pocket depth in the buccal and distal regions increased at T1 and T2, even though it was insignificant compared to T0, and at time T3, it was similar to T1 and T2 but increased in a way that constituted a statistically significant difference compared to T0 ($p<0.05$). A statistically significant difference was obtained when the regions' pocket depths were compared for each measurement time point ($p<0.001$ for all times). At time T0, although the pocket depth in the buccal region was the lowest, the buccal and palatinal regions were similar; pocket depths in the mesial and distal regions were significantly higher than in the buccal region but similar to the palatinal region ($p<0.05$).

Table 2. Distribution of pocket depth by time and regions

PD (mm)	Region				Comparison result	
Measurement time	Buccal Mean±SD Median (IQR)	Palatinal Mean±SD Median (IQR)	Mesial Mean±SD Median (IQR)	Distal Mean±SD Median (IQR)	χ^2	p-value
T0	1.52±0.51	1.74±0.54	2.17±0.39	2.04±0.21	28.761	<0.001
	2 (1-2)^{a,1}	2 (1-2)^{1,2}	2 (2-2)^{2}	2 (2-2)^{a,2}		
T1	1.70±0.47	1.83±0.39	2.17±0.39	2.22±0.42	24.571	<0.001
	2 (1-2)^{a,b,1}	2 (2-2)^{1,2}	2 (2-2)^{1,2}	2 (2-2)^{a,b,2}		
T2	1.83±0.39	1.91±0.29	2.39±0.50	2.17±0.39	25.517	<0.001
	2 (2-2)^{a,b,1}	2 (2-2)^{1}	2 (2-3)^{2}	2 (2-2)^{a,b,1,2}		
T3	1.83±0.39	1.96±0.21	2.43±0.51	2.43±0.51	28.191	<0.001
	2 (2-2)^{b,1}	2 (2-2)^{1}	2 (2-3)^{2}	2 (2-3)^{b,2}		
χ^2, p-value	9.900, 0.019	4.538, 0.209	6.709, 0.082	11.455, 0.010		

SD: Standard deviation, IQR: Interquartile range.

^{a,b} Indicates homogeneous times regarding pocket depth for the region in the respective column($p>0.05$).

^{1,2} Indicates homogeneous regions regarding pocket depth for the region in the respective line ($p>0.05$).

The ANOVA-type test statistic for the Time x Region interaction effect=0.721, $p=0.621$

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Regarding the GCF volume distribution by time and regions, the GCF volume in the buccal and palatinal regions changed significantly over time ($p<0.001$ for both regions), yet the change was not significantly different between the two regions (ATI=1.100, p=0.342). The volume of GCF obtained from the buccal region increased significantly at T1 and T2 time points but remained similar to T2 at time point T3. The volume of GCF collected from the palatinal region was significantly higher at T1 than at T0 and increased at T2 and T3 compared to T1, although not significantly. The GCF volume was significantly higher at T2 and T3 than at T0. Moreover, the two regions were comparable regarding GCF volume at each measurement time ($p>0.05$).

When BALP measurements were considered, it was observed that the measurements were similar over time for both regions (p=0.673 and p=0.837, respectively), and for each time point, there was no significant difference between the regions ($p>0.05$; Table 3).

Table 3. Distribution of BALP levels by time and regions

t-BALP (IU/L)	Region		Comparison result	
Measurement time	Buccal Mean±SD Median (IQR)	Palatinal Mean±SD Median (IQR)	Z	p-value
T0	159.00±41.16	141.43±47.75	1.582	0.114
	170.84 (128.92-193.55)	152.28 (98.24-176.88)		
T1	153.31±44.15	142.75±32.21	0.882	0.378
	142.69 (117.45-183.55)	151.87 (112.85-169.80)		
T2	153.11±34.67	153.80±24.14	0.061	0.951
	147.49 (129.13-185.01)	153.65 (133.92-172.09)		
T3	150.45±29.60	154.88±44.21	0.213	0.831
	146.34 (124.54-172.68)	151.66 (126.13-163.13)		
χ^2, p-value	1.539, 0.673	0.852, 0.837		

SD: Standard deviation, IQR: Interquartile range.

The ANOVA-type test statistic for the Time x Region interaction effect=0.818, p=0.460

The distribution of RANKL over time and regions is tabulated in Table 4. For buccal and palatinal regions, RANKL measurements remained unchanged over time (p=0.509 and p=0.860, respectively). While there was no statistically significant difference in RANKL measurements between buccal and palatinal regions at T0 (p=0.316), the buccal region had a significantly higher RANKL level than the palatinal region at time point T1 (p=0.042). At T2 and T3, there were no significant differences between the two regions ($p>0.05$).

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doi:10.69601/meandrosmdj.1496245

Table 4. Distribution of RANKL levels by time and region

t-RANKL (pg/mL)	Region		Comparison result	
Measurement time	Buccal Mean±SD Median (IQR)	Palatinal Mean±SD Median (IQR)	Z	p-value
T0	6.68±1.38	6.34±1.57	1.004	0.316
	6.61 (5.56-7.65)	6.7 (5.14-7.41)		
T1	6.88±1.03	6.13±1.37	2.038	0.042
	6.69 (6.04-7.70)	6.24 (4.80-7.23)		
T2	6.44±1.67	6.21±1.58	0.578	0.563
	6.55 (5.58-7.63)	6.54 (5.29-7.23)		
T3	6.15±2.14	6.26±1.22	0.274	0.784
	6.63 (4.07-7.76)	6.47 (5.18-7.25)		
χ^2, p-value	2.319, 0.509	0.757, 0.860		

SD: Standard deviation, IQR: Interquartile range.

The ANOVA-type test statistic for the Time x Region interaction effect=0.636, p=0.584

Regarding OPG measurements, it was determined that the measurements remained unchanged over time for both regions (p=0.311 and p=0.622, respectively), and no statistically significant difference was present between the regions at each time point (p>0.05) (Table 5).

Table 5. Distribution of OPG level by time and regions

t-OPG (ng/mL)	Region		Comparison result	
Measurement time	Buccal Mean±SD Median (IQR)	Palatinal Mean±SD Median (IQR)	Z	p-value
T0	220.63±53.19	222.60±52.70	0.639	0.523
	224.48 (174.12-268.25)	227.47 (182.95-272.26)		
T1	224.45±63.68	226.22±51.03	0.243	0.808
	255.81 (178.70-273.72)	209.09 (186.88-271.63)		
T2	224.69±45.94	227.68±44.17	1.247	0.212
	243.41 (183.80-268.04)	232.15 (193.52-268.47)		
T3	227.64±41.54	227.87±49.96	0.213	0.831
	236.76 (186.32-270.79)	243.18 (185.39-279.04)		
χ^2, p-value	3.574, 0.311	1.769, 0.622		

SD: Standard deviation, IQR: Interquartile range.

The ANOVA-type test statistic for the Time x Region interaction effect=0.219, p=0.848

For buccal and palatinal regions, TNF-α measurements remained unchanged over time (p=0.216 and p=0.931, respectively; Table 6). At T0, T2, and T3 time points, the palatinal region had significantly higher t-TNF-α levels than the buccal region (p=0.005, p=0.042, and p=0.006, respectively). On the other hand, TNF-α levels were similar between the buccal and palatinal regions at T1 (p=0.248).

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doi:10.69601/meandrosmdj.1496245

Table 6. TNF-α level distribution by time and regions

t-TNF-α (ng/L)	Region		Comparison result	
Measurement time	Buccal Mean±SD Median (IQR)	Palatinal Mean±SD Median (IQR)	Z	p-value
T0	282.52±104.42	343.98±78.2	2.829	0.005
	296.45 (173.77-366.80)	339.25 (288.76-373.82)		
T1	331.99±78.33	349.10±70.37	1.156	0.248
	318.50 (281.31-376.71)	365.61 (312.83-391.62)		
T2	305.74±70.92	352.92±71.47	2.038	0.042
	294.36 (248.23-356.61)	335.62 (308.45-397.62)		
T3	308.67±48.07	353.46±46.32	2.737	0.006
	313.17 (257.52-342.63)	355.09 (306.41-390.52)		
χ^2, p-value	4.461, 0.216	0.443, 0.931		

SD: Standard deviation, IQR: Interquartile range.

The ANOVA-type test statistic for the Time x Region interaction effect=0.360, p=0.763

DISCUSSION

Our study investigating bone remodeling in gingival crevicular fluid in patients with an indication for maxillary expansion in the pubertal period and treated with RME revealed that plaque index, gingival index, and bleeding at probing measurements increased with time but remained within healthy limits so as not to affect the health of periodontal tissues. Pocket depth measurements of the buccal and distal regions differed over time. The GCF volume in the buccal and palatinal regions increased over time. However, the GCF's RANKL and TNF-α levels remained primarily unchanged or differed marginally in buccal and palatinal tension and pressure zones and in time. On the other hand, although not statistically significant, an increase was detected in the palatinal region at T2 and T3 times compared to T1. Regarding OPG level, an increase was observed in both regions over time, although not statistically significant.

Gingival index

With the onset of puberty and increasing sex hormones, gingivitis prevalence rises around the age of 11 years. Gingival inflammation in the absence of plaque increase has been reported to be enhanced during this period (19). In our study, despite repeated encouragement for oral hygiene, increases in the gingival index might have occurred due to age and the appliance's plaque retentiveness.

Pocket depth

It has been recognized that the reduction in buccal bone thickness and buccal marginal bone levels of posterior maxillary teeth after performing RME is one of the first expected effects of RME (20). Thus, our study's buccal pocket depth increase in the buccal region might have been associated with the attachment loss in the buccal region of the tooth to which the support is applied with the effect of the

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doi:10.69601/meandrosmdj.1496245

compressive force exerted by the RME appliance. On the other hand, our study's finding of increased pocket depth in the distal region can be explained by moderate gingival overgrowth with no attachment loss due to the increasing challenge of cleaning the appliance effectively. Perinetti et al. also observed significant increases in pocket depth measurements during the third and sixth-month retention periods (14).

Gingival crevicular fluid volume

In Topal et al.'s study, the buccal GCF volume was higher at day 10 compared to baseline, followed by a significant decrease at 3 months compared to day 10. On the palatal side, GCF volume was significantly increased at 3 months compared to baseline (13). On the other hand, GCF volume increase may not only be caused by orthodontic forces alone but may also be related to regional periodontal health. The GCF volume tends to increase in individuals undergoing orthodontic treatment (21, 22). In our study, the increase in GCF volume starting at T1 might have been associated with aseptic inflammation caused by the palatal suture's opening upon the force applied during appliance activation. Increased GCF volumes at T2 and T3 compared to T1 for both regions may be related to mild plaque retention and mild gingival inflammation onset due to prolonged appliance stay in the mouth. The higher GCF volumes on the buccal and palatal surfaces at T2 and T3 compared to T0 are attributable to orthopedic relapse and continuing exertion of residual forces on the dental arch.

Gingival crevicular fluid biomarkers

Perinetti et al. studied a split-mouth cohort to evaluate alveolar bone formation in the tension zones of the first molars of patients undergoing RME treatment in the prepubertal period and noted increased BALP activity in the GCF at the tension zones during the retention phase of RME, both at 3 and 6 months (14). Animal studies have shown that the bone remodeling cycle begins with an early resorption phase requiring 3 to 5 days, followed by its reversal (5-7 days) and a late bone formation phase lasting 7 to 14 days in both tension and compression zones (23). The lack of a significant increase in BALP level in our study can be attributed to our study's relatively small sample size.

The RANKL/OPG ratio was reported to be significantly higher during orthodontic tooth movement (24). It has been shown in vivo that the RANKL/OPG ratio in GCF is significantly higher in patients with periodontal disease than in healthy individuals (25). Moreover, Grimaud et al. stated that increasing RANKL in the OPG and RANKL systems may play a role in bone resorption mechanisms (26). In our study, the expression of RANKL increased in the buccal region, the pressure zone, at the T1 time point (the active phase), which might have stimulated osteoclastogenesis and osteoclastic activity, supporting bone remodeling and subsequently reducing bone mass and altering the microstructure of bone tissues in early days of RME. Increases in RANKL might reflect an acute inflammatory phase and bone resorption process caused by RME and could be considered potential markers of bone remodeling. Even though statistically insignificant increases in OPG levels at T2 and T3 time points can be explained by increased bone formation. The lack of significant increases in RANKL and OPG levels in our study can also be attributed to our study's relatively small sample size.

TNF-α enhances the phagocytic capability of neutrophils and may induce an inflammatory response. In addition, it increases osteoclastic activity, resulting in connective tissue damage and impairing oral tissue repair (27, 28). It has been reported that TNF-α levels increased in the gingival crevicular fluid during orthodontic tooth movement and induced bone resorption in the pressure zone (29). Regarding the TNF-α – RME treatment relationship, Tang et al. determined a gradual increase in TNF-α levels in adolescent patients treated with RME (30). TNF-α levels started increasing gradually at the 24th hour and reached a maximum level at the end of the first week. After the 1-week active RME treatment, the TNF-α gradually decreased during the first, fourth, and seventh-week retention periods. Nevertheless, the level was still significantly higher than the baseline level. Our study revealed no change in TNF-α measurements for buccal and palatinal regions over time. However, TNF-α levels of the palatinal region were significantly higher than those of the buccal region except for the T1 time point.

Meanwhile, regarding bone metabolic activities, our study's alternative hypothesis, "There is a correlation between 3-month and 6-month retention periods and bone metabolic activities of RME ", was rejected. The null hypothesis, "There are no correlations observable between 3-month and 6-month retention periods and bone metabolic activities of RME," was accepted.

CONCLUSION

In conclusion, we suggest that inflammatory substances cause damage and then participate in the periodontal tissue repair process, and thus, the inflammatory reaction causes an increase in TNF-α in the gingival crevicular fluid during RME. Further studies with different retention periods and larger sample sizes are strongly recommended.

Acknowledgments

We thank our patients and their families for their kind cooperation.

Authorship contributions

CG: Conceptualization; Data Curation; Investigation; Methodology; Validation; Writing-Original Draft. MGC: Supervision; Project administration; Methodology; Conceptualization; Writing-Review and Editing. AT: Biochemical analysis; Methodology; Writing-Review and Editing.

Data availibity statement

Data can be requested from the authors.

Declaration of competing interest

The authors have no conflicts of interest to declare.

Ethics

The decision of Aydın Adnan Menderes University Faculty of Dentistry Clinical Research Ethics Committee ADUDHF2021/07, dated February 24, 2021, was obtained. In addition, the approval of the Turkish Ministry of Health, the Turkish Pharmaceuticals and Medical Devices Agency has been obtained.

Funding

This study was supported by Aydin Adnan Menderes University Scientific Research Projects Unit by project number of DHF-21005.

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