Gingival crevicular fluid alkaline phosphate activity during the retention phase of maxillary expansion in prepubertal subjects: A split-mouth longitudinal study

Giuseppe Perinetti,[a] Fabrizia D'Apuzzo,[b] Luca Contardo,[c] Jasmina Primozic,[d] Katia Rupel,[a] and Letizia Perillo[e]
Trieste and Naples, Italy, and Ljubljana, Slovenia

Introduction: The aim of this study was to monitor the alveolar bone formation at the tension sites of teeth supporting the appliances for rapid maxillary expansion (RME) during the retention phase according to the local gingival crevicular fluid (GCF) alkaline phosphatase (ALP) activity. **Methods:** This split-mouth prospective study included 23 prepubertal subjects (15 girls, 8 boys; mean age, 9.0 ± 1.4 years) who had a constricted maxillary arch and were undergoing RME. Periodontal parameters, including probing depth, were recorded at 3 and 6 months after RME. Furthermore, the GCF ALP activity was measured at the tension sites of the supporting test teeth (TT) and at the antagonist control teeth (CT) sites. **Results:** Periodontal parameters were generally similar between the TT and CT sites during the study, with the exception that probing depth underwent a slight increase at the TT sites. At baseline, the GCF ALP activity was similar between the TT and CT sites; however, at both 3 and 6 months, significantly greater enzymatic activity was seen at the TT sites. The overall probing depth changes were not significantly correlated with the corresponding GCF ALP activity changes for either the TT or the CT sites. **Conclusions:** Alveolar bone formation at the tension sites would last up to 6 months of retention after RME. These results warrant more comprehensive studies to assess whether the GCF ALP activity has potential as a diagnostic tool for bone formation during the retention phase of RME.

When a skeletal constriction of the maxillary arch is diagnosed, orthopedic skeletal expansion involving separation of the midpalatal suture is the treatment of choice. The most common treatment is rapid maxillary expansion (RME).[1] It has been reported that mainly skeletal effects of RME are seen if this treatment is performed before puberty,[2] whereas at later development stages, more dental effects are to be expected, with possible tissue damage.[3] However, even when treatment is performed during the optimal skeletal maturation phase—ie, prepubertal—some dentoalveolar effects appear to occur.[1,2,4] Moreover, there is always some degree of skeletal or dentoalveolar relapse after RME, thus requiring hypercorrection followed by 4 to 5 months of retention.[1,5] Although most previous studies on RME treatment focused on the effects on the midpalatal suture or other sagittal and vertical changes[1] regarding dentoalveolar effects, a decrease in the buccal bone plate thickness of the supporting teeth has been reported at the end of active RME followed by a significant recovery of the original thickness after a retention period of 6 months.[4]

Gingival crevicular fluid (GCF) is a transudate with constituents from a variety of sources, including microbial dental plaque, host tissues, and serum, with a high site specificity.[6] A number of GCF constituents including host enzymes have been proposed as diagnostic indicators of periodontal status.[7] Among these enzymes, one of the first to be identified was alkaline phosphatase

[a]Research fellow, Department of Medical, Surgical and Health Sciences, University of Trieste, Trieste, Italy.
[b]Research fellow, Department of Orthodontics, Second University of Naples, Naples, Italy.
[c]Assistant professor, Department of Medical, Surgical and Health Sciences, University of Trieste, Trieste, Italy.
[d]Assistant professor, Department of Orthodontics and Jaw Orthopaedics, Medical Faculty, University of Ljubljana, Ljubljana, Slovenia.
[e]Professor, Department of Orthodontics, Second University of Naples, Naples, Italy.
All authors have completed and submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest, and none were reported.
Address correspondence to: Luca Contardo, Struttura Complessa di Clinica Odontoiatrica e Stomatologica, Ospedale Maggiore, Piazza Ospitale 1, 34129 Trieste, Italy; e-mail, l.contardo@fmc.units.it.
Revised and accepted, February 2015.

(ALP).[8] GCF ALP has a primary role in bone mineralization,[9] and it has been shown to be sensitive to alveolar bone formation during orthodontic tooth movement.[10-12] To date, only a few studies have investigated the levels of GCF constituents during maxillary expansion.[13-15] In particular, the levels of the inflammatory mediators interleukin-1β and β-glucuronidase[13] and prostaglandin E2[15] in GCF retrieved from the maxillary teeth of adolescents undergoing RME were increased. Interestingly, a few investigations on the GCF ALP activity changes during or after RME treatment have been reported,[14] in spite of the primary role of this enzyme in alveolar bone formation.[7] Moreover, none of these studies included prepubertal subjects.

Because of the small amount of data available on the metabolic changes at the palatal (tension) alveolar sites during the retention phase after RME treatment, the aim of this split-mouth prospective study in prepubertal subjects was to monitor alveolar bone formation at the tension sites of the first molars undergoing RME treatment. GCF ALP activity was used as a biomarker of tissue remodeling to determine the existence and duration of active alveolar bone formation during the retention phase.

MATERIAL AND METHODS

The study sample consisted of 23 healthy white children (15 girls, 8 boys; mean age, 9.0 ± 1.4 years; range, 6.7-11.9 years) who sought orthodontic treatment at the unit of orthodontics of the School of Dentistry at the Second University of Naples in Italy. The experimental protocol was approved by the institutional ethical committees, and voluntary informed consent was obtained from the patients after they received detailed information about the clinical trial.

Subjects were included according to the following criteria: (1) constricted maxillary arch with a unilateral or bilateral posterior crossbite; (2) prepubertal stage assessed by the cervical vertebral maturation method (stage 1 or 2),[16] (3) good general health, (4) variable degree of crowding, and (5) no use of anti-inflammatory drugs in the month preceding the beginning of the study.[11] Further periodontal inclusion criteria were (1) probing depth (PD) values not exceeding 3 mm in the whole dentition, (2) no radiographic evidence of periodontal bone loss evaluated by panoramic x-ray examination, and (3) full-mouth plaque and full-mouth bleeding scores of 20% or less. The full-mouth plaque and full-mouth bleeding scores were recorded as the percentages of tooth surfaces with supragingival plaque or bleeding within 15 seconds after probing with a 20-g controlled force probe (Vivacare TPS Probe; Vivadent, Schaan, Lichtenstein). Exclusion criteria were (1) absence of maxillary first molars; (2) previous or current periodontal disease, or attachment loss in the posterior teeth; and (3) previous orthodontic treatment.

The study design is shown in Figure 1. A preliminary visit took place 1 month before the baseline visit when the RME treatment began. At this preliminary visit, after the periodontal examination, full-mouth supragingival and subgingival scalings were carried out, and oral hygiene instructions were given to all patients. Moreover, in the month preceding the study and throughout it, all subjects received repeated oral hygiene instructions about the correct use of the toothbrush, dental floss, and interdental brush, and were not allowed to take any anti-inflammatory drugs that could have affected the results.[11] Moreover, they rinsed twice with 0.012% chlorhexidine mouthwash during the 2 weeks before the baseline visit and before the times when the GCF was sampled.[17]

At the baseline visit, the periodontal parameters were recorded, and the GCF was sampled immediately before the beginning of treatment, which consisted of mounting a hyrax rapid maxillary expander (Fig 2, *A*) cemented on the maxillary permanent first molars. The appliance was constructed with plain bands with a lowered vertical dimension such that they did not reach, or minimally entered, the crevicular sulcus. Laser welding was used, and the wires were modeled so that they were not in direct contact with the palatal gingival margins. An expansion screw with reduced dimension (model A0-0630-10; Leone, Sesto Fiorentino, Italy) was used and activated at 2 turns per day (0.20 mm per turn) for 16 to 23 days, thus reaching the total amount of expansion of 6.4 to 9.2 mm in all subjects (mean expansion, 7.5 mm). According to an in-vitro study, the force exerted by the screw used here, with arms orthogonal to the screw guide, is generally about 16 N per turn.[18] Then the screw was tied off with a ligature wire, and the expander was kept on the teeth as a passive retainer for 2 months. Immediately after the removal of the maxillary expander, a passive palatal bar (Fig 2, *B*), also constructed on plain bands with a reduced vertical dimension, was cemented on the maxillary permanent first molars. The palatal bar had short mesial arms on both sides and was kept for a further 4 months as a retainer.

The same clinical recordings and GCF samplings were repeated at 3 and 6 months after the RME active phase. The maxillary left and right permanent first molars were considered the test teeth (TT), and the mandibular left and right permanent first molars were considered the control teeth (CT).

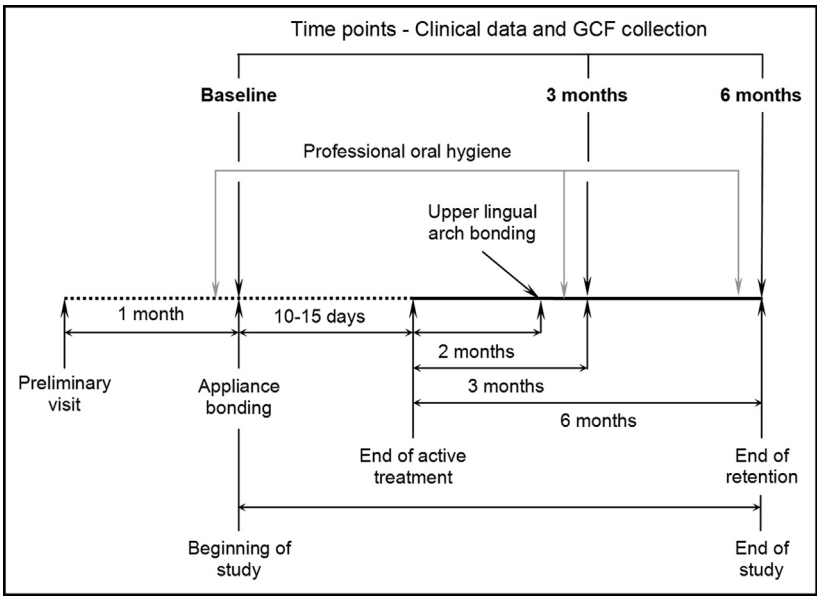

Fig 1. Diagram of the study design. A preliminary visit took place 1 month before the beginning of the study (baseline). At the baseline visit, periodontal parameters were collected, and the GCF was sampled immediately before bonding of the expander that was activated for 10 to 15 days. The appliance was kept as a passive retainer for 2 months and then replaced by a passive maxillary lingual arch kept for a further 4 months as a retainer. The same clinical recordings and GCF samplings were repeated at 3 and 6 months after the beginning of the study.

For all TT and CT, the periodontal parameters included the presence of supragingival plaque (PL+), bleeding on probing (BOP+) 15 seconds after probing with a 20-g controlled force probe, and PD. Clinical data were always collected at 6 sites per tooth (mesiobuccal, midbuccal, and distobuccal; and mesiopalatal or mesiolingual, midpalatal or midlingual, and distopalatal or distolingual) by the same operator (F.D'A.).

Contamination of the GCF samples was minimized by recording the plaque presence before carefully cleaning the tooth with cotton pellets, collecting the GCF from the isolated area, and then recording the PL+ and BOP+ as previously described.[17] GCF was collected using number 30 standardized sterile paper strips inserted 1 mm into the gingival crevice and left in situ for 30 seconds, after the sites had been isolated with cotton rolls and dried by a gentle airstream.[11] The GCF was collected at the mesiopalatal and distopalatal sites of the TT and at the mesiolinual and distolingual sites of the CT. The GCF samples were transferred to plastic vials and stored at −80°C until they were analyzed.

For the enzymatic activity determination, the biochemical assays were performed by blinded operators (J.P., K.R.) as previously described.[17] Briefly, the 4 samples from the 4 collection sites, for each resting and flow GCF sample, were resuspended in 250 μL of buffer containing 200 mmol/L of Tris and 20 mmol/L of magnesium chloride (pH, 9.8 ± 0.1) and 1 mg per milliliter of p-nitrophenol phosphate (N2770-5SET, Sigma Fast; Sigma-Aldrich, St Louis, Mo). The samples were then incubated at 37°C (± <0.1°C fluctuations) for 3 hours. During this incubation, the ALP in the samples hydrolyzed the p-nitrophenyl phosphate to p-nitrophenol and inorganic phosphate. The reactions were then stopped by the addition of 5 μL of 3-mol/L of sodium hydroxide, and the absorbance was read with a spectrophotometer at 405 nm. The relevant control for each analysis consisted of the reagent and the Tris buffer without the sample, and all samples were analyzed in 1 session. By using 18.45 as the p-nitrophenol mmol/L absorptivity, the absorbance was converted into enzyme activity units (1 unit = 1 mmol of p-nitrophenol released per minute at 37°C) and expressed as total activity in milliunits per sample.

A sample size of 24 subjects was necessary to detect an effect size coefficient of 0.8 for the GCF ALP activity in the comparison between sites (or time points), with an alpha set at 0.05 and a power of 0.80.[19] The effect size coefficient is the ratio of the difference between the recordings of the 2 sites, divided by the within-site standard deviation. Even though the effect size coefficient is not a measure of diagnostic accuracy, a greater intergroup difference compared with the corresponding

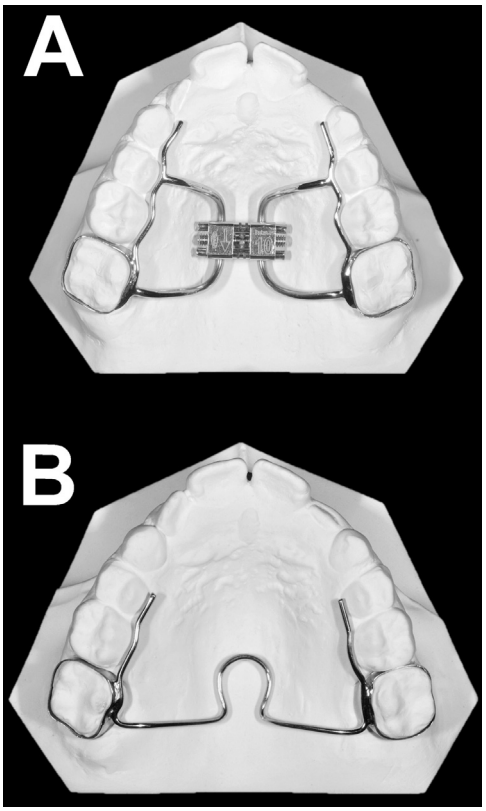

Fig 2. The appliances used in the study: **A,** hyrax maxillary expander; **B,** passive palatal bar.

variability means greater expected diagnostic accuracy of the GCF ALP activity in detecting bone formation. Therefore, an effect size of at least 0.8, regarded as a "large effect," was chosen.[19] During the study, 1 subject dropped out, leaving a sample size of 23 subjects.

On a separate group of 10 untreated matched subjects with the same features as the experimental group, GCF samples were collected twice 1 week apart with similar modalities on the maxillary and mandibular first molars. These data were used for the calculation of the method error in the GCF collection and ALP activity quantification through the method of moments variance estimator.[20] The results were expressed as percentages of mean error and 95% confidence intervals (CIs). The method of moments variance estimator has the advantage of not being affected by any unknown bias: eg, systematic errors between pairs of measurements.[20]

Statistical analysis

The Statistical Package for Social Sciences software (version 13.0; SPSS, Chicago, Ill) was used for data analysis, and the patient was the statistical unit. Within each subject and each group, PL+ and BOP+ were considered as the mean numbers of experimental sites positive for supragingival plaque and bleeding on probing; PD and GCF ALP activity were similarly considered as the mean values obtained by the TT or CT in the same palatal or lingual sites for each subject. These mean values were the statistical units of each data set considered in the analysis to reduce intersubject variability.

Each continuous data set was tested for normality of the data with the Shapiro-Wilk test and by Q-Q normality plots. Equality of variance was also tested with the Levene test and Q-Q normality plots of the residuals. Each data set was treated as ordinal data by nonparametric tests because of the failure to meet the required assumption for parametric analyses. Nevertheless, the means and standard deviations for the clinical parameters are reported for descriptive purposes.

For each periodontal parameter and GCF ALP activity, the Wilcoxon paired signed rank test was used to assess the significance of the differences between the TT and CT sites at each time point. The significance of the differences over time in the TT and CT sites was assessed using the Friedman test followed by a Bonferroni-corrected Wilcoxon paired signed rank test, where appropriate.

For either the TT or CT, the overall changes in PD and in GCF ALP activity (considering the subject as the statistical unit) were calculated as the difference between the scores recorded at 6 months and the corresponding baseline values. Finally, the Spearman rank correlation coefficient was used to evaluate the significance of the correlation between the overall changes in PD and GCF ALP activity in the TT and CT sites.

A P value less than 0.05 was used for rejection of the null hypothesis.

RESULTS

The method error for the GCF ALP activity expressed as the mean (95% CI) was 10.2 mU per sample (4.8-17.0), equivalent to 26.2% (12.3%-43.7%).

The clinical conditions were optimal throughout the study in all experimental teeth. For the merged TT and CT sites, the overall mean percentages of PL+ and BOP+ sites were 11.4% ± 8.3% and 1.8% ± 0.2%, respectively; the mean PD was 4.1 ± 3.5 mm. For PL+ and BOP+, no significant differences were seen between sites or within each site between time points (Table). Furthermore, no significant site differences of PD were observed at baseline between the TT and CT. However, at 3 and 6 months, significant increases in PD were observed in the TT site ($P = 0.028$), where significantly

Table. Clinical parameters and total GCF ALP activity in the TT and CT sites according to the time points (N = 23)

		Site		
Parameter	*Time*	*TT*	*CT*	*Diff*
PL+ (%)	Baseline	0 (0; 16.7)	0 (0; 33.3)	NS
	3 mo	0 (0; 33.3)	0 (0; 16.7)	NS
	6 mo	0 (0; 0)	0 (0; 16.7)	NS
	Diff	NS	NS	
BOP+ (%)	Baseline	0 (0; 16.7)	0 (0; 0)	NS
	3 mo	0 (0; 0)	0 (0; 0)	NS
	6 mo	0 (0; 0)	0 (0; 0)	NS
	Diff	NS	NS	
PD (mm)	Baseline	1.7 ± 0.5	1.7 ± 0.4	NS
	3 mo	1.9 ± 0.5	1.5 ± 0.4	$P = 0.003$
	6 mo	2.1 ± 0.5	1.5 ± 0.4	$P = 0.001$
	Diff	$P = 0.028$	NS	
GCF ALP (mU/sample)	Baseline	30.5 (11.5; 40.4)	25.0 (11.9; 59.7)	NS
	3 mo	46.7 (27.2; 58.1)*	27.8 (13.8; 37.9)	$P = 0.000$
	6 mo	52.3 (35.5; 70.9)*	32.0 (15.7; 38.9)	$P = 0.000$
	Diff	$P = 0.011$	NS	

Data are presented as median (25th; 75th percentiles) for PL+, BOP+, and GCF ALP activity, and as means and standard deviations for PD. *Diff*, Significance of the difference over time in each TT or CT site, or among the sites at each time point; *NS*, no statistically significant difference.
*Results of the pair-wise comparisons between the time points for each site are different from baseline.

higher values of PD were measured compared with the CT sites ($P < 0.01$, at least).

The GCF ALP activity was similar between the TT and CT sites at baseline, with no significant differences. A significant increase in the enzymatic activity was seen at the TT sites ($P = 0.011$); on the contrary, the GCF ALP activity was stable at the CT sites, with no significant differences among the time points. Pair-wise comparisons for the TT sites showed significantly greater GCF ALP activity at both 3 and 6 months compared with the corresponding baseline values. No significant difference was seen in the enzymatic activity between 3 and 6 months. At the comparisons between the TT and CT sites, the GCF ALP activity was significantly greater in the TT sites at both 3 and 6 months ($P = 0.000$).

The individual overall changes in PD and GCF ALP activity are shown in Figure 3. The changes in the PD ranged from −0.9 to 1.4 mm, and from −1.2 to 1.0 mm in the TT and CT sites, respectively. The changes in the GCF ALP activity ranged from −25.7 to 115.3 mU per sample, and from −48.5 to 80.4 mU per sample at the TT and CT sites, respectively. The Spearman rho correlation coefficients between the changes in PD and GCF ALP activity were 0.051 ($P = 0.816$) and 0.015 ($P = 0.994$) for the TT and CT sites, respectively.

DISCUSSION

In this study, we assessed GCF ALP activity up to 6 months after RME to evaluate alveolar bone formation at the tension sites of supporting teeth. The results show significant increases of GCF ALP activity at both 3 and 6 months without clinically relevant tissue inflammation.

The only human study evaluating GCF ALP activity related to RME treatment did not specifically evaluate the tension sites and was limited by a 28-day follow-up term, whereas bone turnover would be significantly longer.[14] Animal studies have shown that the bone remodeling cycle begins with an early wave of resorption, which requires 3 to 5 days, followed by its reversal (5-7 days) and a late wave of bone formation that continues for 7 to 14 days.[21] This process would occur at both the tension and compression stress sites.[21] Moreover, a similar process has been described for human bone,[9,22] where bone formation appears to begin 10 days[9] or 3 weeks[23] after the initial resorption.

The ALP is a host enzyme that allows bone mineralization by hydrolyzing inorganic pyrophosphate,[24] and it has been extensively correlated with the rate of bone formation.[9] In our study, the GCF ALP activity was up to 32.0 and 52.3 mU per sample at the CT and TT sites, respectively. However, because of the biochemical assay used, lacking a calibration curve but using the control sample with the reagent without the GCF instead, quantitative comparisons with previously reported data would not be fully reliable. In this study, the resting GCF was sampled because this has been shown to have greater repeatability compared with the flow GCF.[17] Whereas the method error was 26.2%, the intragroup variations were up to 71.5% for the tension sites at 6 months compared with the baseline values. Therefore, in spite

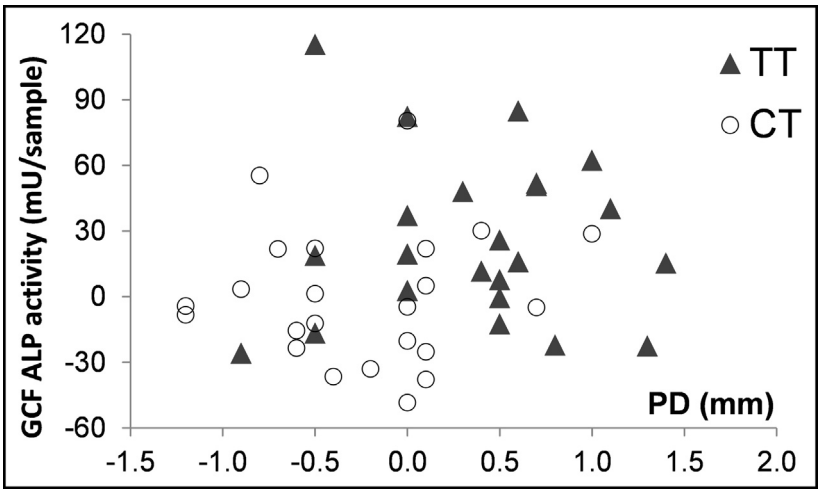

Fig 3. Scatter plot of total GCF ALP activity and PD overall changes in the TT and CT sites (N = 23). Nonsignificant correlations were seen between GCF ALP levels and pocket depths in the TT and CT, with Spearman rho correlation coefficients of 0.051 and 0.015, respectively.

of the method error, the variations seen were likely due to tissue reaction. Of interest, the GCF ALP activity recorded at 6 months was slightly, but not significantly, greater compared with that recorded at 3 months (Table). This would imply that alveolar bone formation is still active even after this period of retention. These results would be consistent with previous studies reporting that in addition to skeletal effects, RME has a relative amount of dentoalveolar effects, also in the prepubertal period.[1]

However, ALP is released also from polymorphonuclear cells during inflammation,[7] and several studies reported a significant elevation in GCF ALP activity around inflamed periodontal tissues.[8,25] Although other studies reported that during orthodontic treatment, increases in plaque accumulation and gingival inflammation are frequently seen,[26] adequate oral hygiene during orthodontic treatment can minimize the increase in plaque accumulation.[27] Moreover, a split-mouth protocol would prevent major biases caused by systemic and local metabolic conditions that might affect the GCF ALP activity.

In this study, no significant increase in either PL+ or BOP+ was detected (Table). Therefore, the increase of PD could be more properly related to a moderate gingival enlargement, without periodontal attachment changes, rather than attachment loss, because of the increased difficulty of effectively cleaning around the appliances.[28] It has been reported that GCF ALP activity is correlated with PD during inflammation.[29] On the contrary, in our study, the increase in the GCF ALP activity was not correlated with PD changes (Fig 3). This evidence would thus be consistent with the concept that an enzyme activity increase was a consequence of bone formation rather than periodontal damage.

The GCF ALP activity would thus be advantageous for clinicians because it provides the possibility of monitoring alveolar bone formation with an easy-to-use and noninvasive chairside kit that is already available.[30] It is well known that traditional diagnostic procedures—ie, probing depth or x-ray films—have inherent limitations because they are sensitive to periodontal disease history or remodeling but have no predictive capabilities. For this reason, the use of GCF biomarkers has been recommended for routine clinical practice.[31] This is particularly useful with the consideration that detectable enzymatic modifications at the GCF level occur earlier than clinically evident modifications.[29,30] However, in the treatment under investigation, antagonist, instead of contralateral, tooth sites were used as the controls, and monitoring was limited to the first 6 months of retention for the tension sites. Studies with a longer follow-up and evaluating the GCF ALP activity at compression sites during the retention phase are warranted. Moreover, monitoring of a combination of various GCF biomarkers, reflecting different biologic responses such as bone formation and resorption or inflammation, would also be useful to fully elucidate the tissue response to RME treatment.

The clinical implications of the results of this study show that alveolar bone formation at the tension sites of the supporting teeth is still active 6 months after RME. In light of this evidence, a period of retention not shorter than 6 months would be recommended to allow proper mineralization of these sites and prevent

relapse. Moreover, although not proved by our study, this evidence would be consistent with the indication of prolonged retention periods when RME is performed in older, pubertal patients, in whom greater dentoalveolar effects are expected to occur.

CONCLUSIONS

During the retention phase of RME, increases in GCF ALP activity may be detected at the tension sites at both 3 and 6 months without clinically relevant tissue inflammation. However, further comprehensive research including other GCF biomarkers and following long-term monitoring protocols are needed to propose that GCF ALP activity is a diagnostic tool for alveolar bone formation of the tension sites during the retention phase of RME.

ACKNOWLEDGMENTS

We thank Alex Bruno and Paolo Tonini (Ortotec, Gonars, Italy) for invaluable technical assistance.

REFERENCES

1. Lagravère MO, Major PW, Flores-Mir C. Long-term skeletal changes with rapid maxillary expansion: a systematic review. Angle Orthod 2005;75:1046-52.
2. Baccetti T, Franchi L, Cameron CG, McNamara JA Jr. Treatment timing for rapid maxillary expansion. Angle Orthod 2001;71: 343-50.
3. Starnbach H, Bayne D, Cleall J, Subtelny JD. Facioskeletal and dental changes resulting from rapid maxillary expansion. Angle Orthod 1966;36:152-64.
4. Ballanti F, Lione R, Fanucci E, Franchi L, Baccetti T, Cozza P. Immediate and post-retention effects of rapid maxillary expansion investigated by computed tomography in growing patients. Angle Orthod 2009;79:24-9.
5. Primozic J, Richmond S, Kau CH, Zhurov A, Ovsenik M. Three-dimensional evaluation of early crossbite correction: a longitudinal study. Eur J Orthod 2013;35:7-13.
6. Uitto VJ. Gingival crevice fluid—an introduction. Periodontol 2000 2003;31:9-11.
7. McCulloch CA. Host enzymes in gingival crevicular fluid as diagnostic indicators of periodontitis. J Clin Periodontol 1994;21: 497-506.
8. Ishikawa I, Cimasoni G. Alkaline phosphatase in human gingival fluid and its relation to periodontitis. Arch Oral Biol 1970;15: 1401-4.
9. Christenson RH. Biochemical markers of bone metabolism: an overview. Clin Biochem 1997;30:573-93.
10. Insoft M, King GJ, Keeling SD. The measurement of acid and alkaline phosphatase in gingival crevicular fluid during orthodontic tooth movement. Am J Orthod Dentofacial Orthop 1996;109:287-96.
11. Perinetti G, Paolantonio M, D'Attilio M, D'Archivio D, Tripodi D, Femminella B, et al. Alkaline phosphatase activity in gingival crevicular fluid during human orthodontic tooth movement. Am J Orthod Dentofacial Orthop 2002;122:548-56.
12. Perinetti G, Paolantonio M, Serra E, D'Archivio D, D'Ercole S, Festa F, et al. Longitudinal monitoring of subgingival colonization by Actinobacillus actinomycetemcomitans, and crevicular alkaline phosphatase and aspartate aminotransferase activities around orthodontically treated teeth. J Clin Periodontol 2004;31:60-7.
13. Tzannetou S, Efstratiadis S, Nicolay O, Grbic J, Lamster I. Comparison of levels of inflammatory mediators IL-1beta and betaG in gingival crevicular fluid from molars, premolars, and incisors during rapid palatal expansion. Am J Orthod Dentofacial Orthop 2008;133:699-707.
14. Wei FL, Wang CL, Liu DX, Guo J, Ke HF, Guo XX. Changes of aspartate aminotransferase and alkaline phosphatase activities in gingival crevicular fluid during rapid palatal expansion. Shanghai Kou Qiang Yi Xue 2007;16:168-71.
15. Sari E, Kadioglu O, Ucar C, Altug HA. Prostaglandin E2 levels in gingival crevicular fluid during tooth- and bone-borne expansion. Eur J Orthod 2010;32:336-41.
16. Baccetti T, Franchi L, McNamara JA Jr. The cervical vertebral maturation (CVM) method for the assessment of optimal treatment timing in dentofacial orthopedics. Semin Orthod 2005;11: 119-29.
17. Perinetti G, Di Leonardo B, Di Lenarda R, Contardo L. Repeatability of gingival crevicular fluid collection and quantification, as determined through its alkaline phosphatase activity: implications for diagnostic use. J Periodontal Res 2013;48:98-104.
18. Camporesi M, Franchi L, Doldo T, Defraia E. Evaluation of mechanical properties of three different screws for rapid maxillary expansion. Biomed Eng Online 2013;12:128.
19. Cohen J. A power primer. Psychol Bull 1992;112:155-9.
20. Springate SD. The effect of sample size and bias on the reliability of estimates of error: a comparative study of Dahlberg's formula. Eur J Orthod 2011;34:158-63.
21. King GJ, Keeling SD, Wronski TJ. Histomorphometric study of alveolar bone turnover in orthodontic tooth movement. Bone 1991;12:401-9.
22. Rodan GA. Introduction to bone biology. Bone 1992;13(Suppl 1): S3-6.
23. Frost HM. Some ABC's of skeletal pathophysiology. 6. The growth/modeling/remodeling distinction. Calcif Tissue Int 1991;49: 301-2.
24. Robison R. The possible significance of hexosephosphoric esters in ossification. Biochem J 1923;17:286-93.
25. Persson GR, DeRouen TA, Page RC. Relationship between gingival crevicular fluid levels of aspartate aminotransferase and active tissue destruction in treated chronic periodontitis patients. J Periodontal Res 1990;25:81-7.
26. Trossello VK, Gianelly AA. Orthodontic treatment and periodontal status. J Periodontol 1979;50:665-71.
27. Lundstrom F, Hamp SE, Nyman S. Systematic plaque control in children undergoing long-term orthodontic treatment. Eur J Orthod 1980;2:27-39.
28. Alexander SA. Effects of orthodontic attachments on the gingival health of permanent second molars. Am J Orthod Dentofacial Orthop 1991;100:337-40.
29. Perinetti G, Paolantonio M, Femminella B, Serra E, Spoto G. Gingival crevicular fluid alkaline phosphatase activity reflects periodontal healing/recurrent inflammation phases in chronic periodontitis patients. J Periodontol 2008;79:1200-7.
30. Chapple IL, Garner I, Saxby MS, Moscrop H, Matthews JB. Prediction and diagnosis of attachment loss by enhanced chemiluminescent assay of crevicular fluid alkaline phosphatase levels. J Clin Periodontol 1999;26:190-8.
31. Taba M Jr, Kinney J, Kim AS, Giannobile WV. Diagnostic biomarkers for oral and periodontal diseases. Dent Clin North Am 2005;49:551-71.