African Journal of Pharmacy and Pharmacology Vol. 5(20), pp. 2213-2219, 29 November, 2011
Available online at http://www.academicjournals.org/AJPP
DOI: 10.5897/AJPP11.304
ISSN 1996-0816 ©2011 Academic Journals

Full Length Research Paper

Crevicular tartrate resistant acid phosphatase activity and rate of tooth movement under different continuous force applications

Rohaya Megat Abdul Wahab[1]*, Maryati Md Dasor[1], Sahidan Senafi[2], Asma Alhusna Abang Abdullah[1], Abdul Aziz Jemain[3], Nurfathiha Abu Kasim[2], Zulham Yamamoto[2] and Shahrul Hisham Zainal Ariffin[2]

[1]Department of Orthodontics, Faculty of Dentistry, Universiti Kebangsaan Malaysia, Kuala Lumpur, Malaysia.
[2]School of Bioscience and Biotechnology, Faculty of Science and Technology, Universiti Kebangsaan Malaysia, Bangi, Malaysia.
[3]DELTA, School of Mathematics, Faculty of Science and Technology, Universiti Kebangsaan Malaysia, Bangi, Malaysia.

Accepted 9 November, 2011

This study investigated specific activities of tartrate resistant acid phosphatase (TRAP) in gingival crevicular fluid (GCF) and cumulative canine movement when two orthodontic forces were applied during orthodontic treatment. Each selected subjects (n = 12) randomly received a 100 and 150 g force for canine retraction either on the right or left site of maxillary arch. GCF was collected at the mesial and distal sites of the canines for 5 consecutive weeks where baseline activity served as control. Canine distance was measured before retraction and weekly (5 weeks) after force application. TRAP activity was determined using spectrophotometer. At the distal site of test canine (150 g force), TRAP activity peaked significantly ($P < 0.05$) at week 4 while tested canine (100 g force) at week 5. At the mesial site of test canine, no significant ($P > 0.05$) TRAP activities were found between 150 and 100 g of forces. There was significantly ($P < 0.05$) more cumulative canine movement by 5 weeks in 150 g as compared to 100 g force. TRAP enzyme can be a useful biomarker for monitoring orthodontic tooth movement. A force of 150 g was found to be more optimal in canine retraction than 100 g force.

Key words: Biomarker, bone remodeling, orthodontic forces, tartrate resistant acid phosphatase, tooth movement.

INTRODUCTION

Optimal orthodontic force of certain magnitude and temporal characteristic whether it is continuous or intermittent, constant or declining is capable of producing maximum rate of tooth movement, without tissue damage and with maximum patient comfort (Proffit et al., 2007). Force application as an extrinsic mechanical stimulus, triggers biological cellular response in the periodontium that initiates orthodontic bone remodeling to obtain optimal tooth movement.

The orthodontic bone remodeling is a balanced and continuously process of bone deposition by osteoblast and bone resorption by osteoclast (Keeling et al., 1993). Osteoclasts are multinucleated cells derived from granulocyte-monocytes located in hematopoietic bone marrow. Their activity was found to be predominant at compression site (Rody et al., 2001) and these cells functioned by forming a highly acidic extracellular space, thereby solubilizing bone minerals. Osteoclast expressed high amount of tartrate resistant acid phosphatase (TRAP) which localized within the ruffled border area, the lysosomes, the Golgi cisternae and vesicles (Halleen et al., 2000).

Several studies have investigated TRAP enzyme as a useful osteoclast biomarker (Keeling et al., 1993; Rody et

*Corresponding author. E-mail: shahroy7@gmail.com. Tel: +603-92897761. Fax: +603-92897794.

al., 2001; Insoft et al., 1996; Noxon et al., 2001) to determine osteoclast activity during tooth movement. The sample was collected mainly from gingival crevicular fluid (GCF) found in the gingival sulcus. GCF is an inflammatory exudate derived from number of sources, including serum, the primary source of aqueous component of GCF, the connective tissue and the epithelium, in addition to inflammatory cells and bacteria present in the tissue and crevice (Uitto, 2000). GCF was chosen because it is readily available for diagnostic purposes and the sampling is non-invasive, relatively simple and easy in repetitive procedures. Thus, its collection poses minimal risk such as local irritation of the patient's gingival. Changes in composition of GCF highly correlated to any changes that occurred deep in the periodontium during orthodontic tooth movement. For this reason, GCF can represent the biologic condition of the alveolar bone. Hence, analysis of GCF sample provides better understanding of the dynamic and metabolic status associated with orthodontic tooth movement (Kavadia-Tsatala et al., 2002).

Several TRAP studies in orthodontic were performed mainly on animal model using force range from 20 to 60 g (Keeling et al., 1993; King and Keeling, 1995). Only few studies had investigated TRAP activity in a human model, such as, Insoft et al. (1996) who investigated only three female subjects with premolar tooth moved buccally using 100 g force and a study by Shahrul Hisham et al. (2010) that had monitored pattern of TRAP in saliva during orthodontic tooth movement. At present, there is no study that compares the effect of different continuous force to the crevicular TRAP activity during canine retraction. Therefore, the objectives of this study are to compare the effect of different continuous orthodontic force (100 or 150 g) to the specific tartrate resistant acid phosphatase activities in gingival crevicular fluids and to compare the rate of canine movement during 5 weeks of retraction period between two forces. The hypotheses of this study are the specific tartrate resistant acid phosphatase activities and the rate of canine movement is different between forces.

MATERIALS AND METHODS

Subject selection

A total of twelve healthy orthodontic subjects (age range: 14 to 24 years, mean age: 19.7 ± 5.0 years) participated in the study. These subjects were selected based on the following inclusion criteria; (1) healthy with no known systemic diseases; (2) good general and periodontal health and not pregnant; (3) mild to moderate crowding of the maxillary and mandibular arch; (4) canine relationship of class II ½ unit or more; (5) class II/1 incisal relationship with overjet more than 6 mm; (6) overbite not more than 50%; (7) no use of any anti-inflammatory drugs during the study; (8) no previous orthodontic or orthopedic treatment and (9) no craniofacial anomalies. All subjects were prohibited from taking any anti-inflammatory drugs and mouthwash containing chlorhexidine throughout the study period. These subjects also received periodontal prophylaxis; full mouth scaling and polishing. Optimal oral health was ensured prior to the study. An informed consent was obtained from the subjects or the parents or guardians (for subjects under 16 years of age) to participate in this study, after written and verbal explanation. The protocol was reviewed and approved by the Research Ethical Committee of the Universiti Kebangsaan Malaysia (No:1.5.3.5/244/DD/034 (1)/2009).

Orthodontic appliances and experimental teeth

A Nance appliance was fitted to the maxillary first molars prior to the maxillary first premolars extractions. The buccal surface of the maxillary teeth (e.g. incisors, canine and premolar) were bonded with a 0.022 × 0.028 inch pre-adjusted edgewise appliance (American Orthodontics, Mini Master; MBT prescription). The initial alignment was obtained with a 0.014 inch NiTi archwire and the leveling and alignment stage was completed in three to four consecutive reviews until 0.018 × 0.025 inch NiTi was reached. The working archwire of 0.019 × 0.025 inch stainless steel archwire inserted and left *in situ* for four week to allow passivity of the archwire before canine retraction stage. Canine retraction was performed on a 0.019 × 0.025 inch stainless using light nickel titanium (NiTi) push coil spring (sds Ormco). The NiTi coil spring was placed between maxillary lateral incisor and maxillary canine. These teeth were ligated with a 0.009 inch stainless steel ligature wire to prevent rotation. In a split-mouth design, subjects received a 100 or 150 g force either on the right or left side of maxillary arch, determined through "toss of coin". The force was measured using Correx gauge (dial-type stress and tension gauge; Dentaurum Germany). All four maxillary incisors were also ligated together using 0.009 inch stainless steel ligature wire to increase anterior anchorage. Oral hygiene instruction was given post-operatively. Subjects were reviewed and gingival crevicular fluid (GCF) was collected on a weekly basis for six consecutive weeks (week 0 to week 5) of canine retraction where baseline (week 0) activity acted as control.

Canine movement and radiographs evaluation

Alginate impressions (Kromopan 100 h LASCOD S.P.A., Italy) of maxilla were taken for fabrication of study models at each visit after GCF collection. Canine distance was measured from the distal margin of the maxillary lateral incisor bracket to the mesial margin of the maxillary canine bracket on the study model using a digital caliper (KERN, Germany; resolution: ± 0.01 mm). Weekly canine movement as well as cumulative canine distances from baseline to week 5 of canine retraction was obtained at the end of experimental term. Periapical (pa) radiographs were taken to monitor the maxillary canines (test teeth; 13 and 23) root changes. The pa radiographs were taken pre-operatively prior to the placement of the NiTi Coil springs and post-operatively after GCF sample collection at week 5 and 6 months post retraction. These pa radiographs were projected on a screen and magnified tenfold. They were assessed for apical root and lateral surface root resorptions according to Liou and Huang (1998). The apical root resorption was assessed by the following scores: 0 = no apical root resorption, 1 = slight blunting of the canine root apex, 2 = moderate resorption of the root apex beyond blunting and up to one fourth of the root length and 3 = excessive resorption of the root apex beyond one fourth of the root length. The lateral root resorption on the distal side of the canine root was assessed according to the following scores: 0 = smooth lateral root surface and periodontal ligament, 1 = slightly irregular lateral root surface not beyond one third of the dentine width between the distal side periodontal ligament and pulp chamber, 2 = moderate irregular lateral root surface beyond one third and up to two thirds of the dentine width

Table 1. Crevicular tartrate resistant acid phosphatase activity on the tension (mesial) and compression (distal) sites of distalised maxillary canines under 150 g force and 100 g force.

Time	150 g	Kruskal-Wallis	100 g	Kruskal-Wallis	100 g vs. 150 g
	Specific enzyme activity (U/mg)	(baseline vs. week)	Specific enzyme activity (U/mg)	(baseline vs. week)	t-Test
(A) Mesial					
Baseline	1.74 ± 0.88	-	1.65 ± 0.64	-	NS
Week 1	2.15 ± 0.97	NS	2.24 ± 0.79	NS	NS
Week 2	1.85 ± 1.07	NS	2.82 ± 1.87	NS	NS
Week 3	3.23 ± 1.48	Sig	2.20 ± 0.98	NS	NS
Week 4	2.44 ± 1.16	NS	2.50 ± 1.04	NS	NS
Week 5	2.10 ± 0.97	NS	1.87 ± 1.31	NS	NS
(B) Distal					
Baseline	1.53 ± 0.59	-	1.57 ± 0.48	-	NS
Week 1	1.85 ± 1.18	NS	3.04 ± 1.93	NS	NS
Week 2	1.67 ± 0.94	NS	1.96 ± 0.87	NS	Sig
Week 3	2.34 ± 1.61	NS	3.36 ± 1.92	NS	NS
Week 4	4.70 ± 2.65	Sig	3.07 ± 0.75	NS	NS
Week 5	2.48 ± 1.60	NS	4.36 ± 2.14	NS	Sig

Data presented as median ± minimum median absolute deviation of specific enzyme activity (n = 12) with unit of U/mg. NS, No statistically significant difference. Sig, Significance (P < 0.05).

between the distal side periodontal ligament and pulp chamber and 3 = excessive irregularity of the lateral root surface beyond two thirds of the dentine width between the distal side periodontal ligament and pulp chamber. Root resorptions of the experimental canines were investigated, as one of the detrimental effects of the different continuous orthodontic forces exerted to the test teeth as compared to the control.

TRAP in GCF samples collection and assay

Gingival crevicular fluid was collected using methylcellulose filter paper strips (Periopaper, Proflow, Amityville, N.Y). The maxillary canine was isolated prior samples collection with cotton rolls and tooth surface was gently dried for 5 s. The native GCF was collected at the mesial and distal sites of the test and control teeth. Each periopaper was inserted 1 mm in the gingival crevice and left *in situ* for 60 s while maintaining isolation. A total of three periopapers were used at intervals of 60 s to maximize the volume of GCF collected per site. The three dipped periopapers were inserted into 1.5 ml microcentrifuge tubes containing 80 µl of physiologic saline. Later, the tube was centrifuged for 5 min at 4000 ×g using a microcentrifuge machine (Hettich Zentrifugen Mikro 22R) to completely elute the GCF components. The supernatant was immediately analysed.

Enzyme activity was determined using a spectrophotometer (Varian Cary 50UV-Vis) at 405 nm. The GCF samples of 50 µl were incubated for 60 min at 37°C in a substrate containing 50 µl of p-nitrophenyl phosphate (100 mM), 50 µl of acetate buffer at pH 5.8 (0.1 M), 50 µl of 1.0% v/v triton X-100, 50 µl of potassium chloride (0.15 M), 50 µl of ascorbic acid (1 mM), 50 µl of ferum chloride (0.1 mM), 50 µl of sodium tartrate (10 mM) and lastly 0.1 ml of sterile distill water was added to increase the total volume to 0.5 ml. Enzyme activity was then terminated by the addition 0.7 ml of NaOH (0.9 M) to the components (sample and substrate). Immediately, the absorbance was measured in a spectro-photometer. Standard curve employing 1 mM of ρ-nitrophenol solution to convert absorbance into enzymatic activity unit (1 U = 1 µmol of ρ-nitrophenol released per minute at 37°C) was used. The TRAP specific activities were determined based on units (U) of activity per total protein content (mg) and were stated as U/mg. A standard curve of bovine serum albumin (Sigma, USA) protein was prepared earlier to determine the total protein content (mg) for each assay. Non-parametric statistical analyses were used in analyzing data after non normality distribution of the data were determined using the Kolmogorov-Smirnov test. The Kruskal-Wallis test was utilised to compare the specific TRAP activities at different times (week) to the baseline (week 0). The comparison of cumulative canine movements (mm) with time (week) between 150 and 100 g groups were analysed using the paired t-test and P < 0.05 was considered as significant difference.

RESULTS

A total of twelve healthy orthodontic patients (11 female and 1 male) with age ranging from 14 to 25 years completed this study. Mean age of the participants was 19.7 ± 5.0 years. The pattern of TRAP activities during canines' retraction under the 150 and 100 g were observed from week 0 to week 5 at both mesial and distal sites. In the 150 g group, TRAP activity peaked at week 3 (1.8 times higher) than baseline at mesial sites, statistically significant difference (P < 0.05) when the Kruskal-Wallis test was used (Table 1). Later in week 4 and 5, the TRAP activities reduced towards the baseline value. However, at the distal sites TRAP activity was

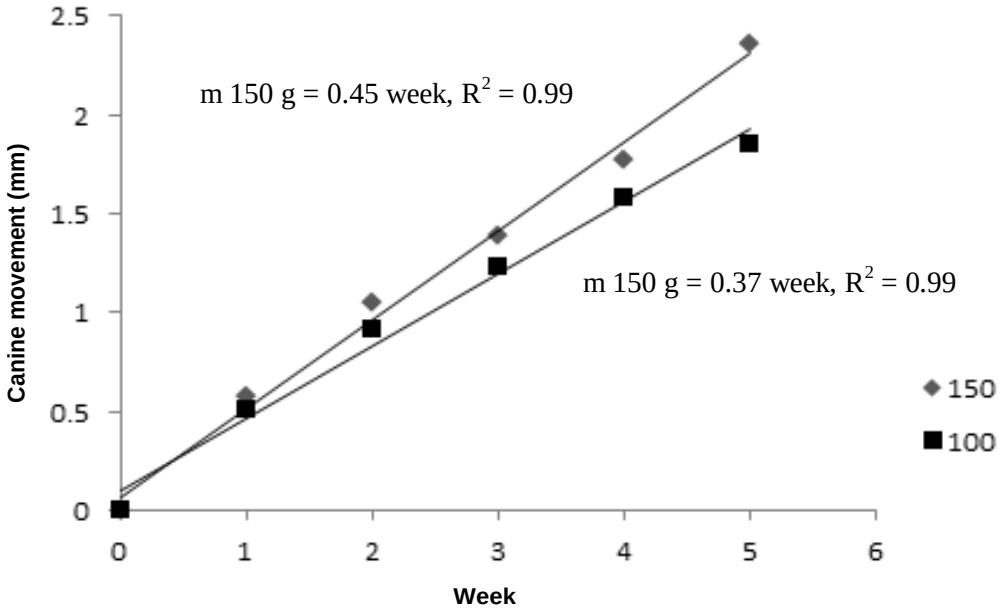

Figure 1. Comparison between mean cumulative movement (in mm) of distalised maxillary canines with 150 and 100 g orthodontic force over 5 consecutive weeks. (n = 12). R^2 = coefficient of determination.

Table 2. Comparison between the measurement of canine movement at 150 and 100 g orthodontic forces.

Time	Canine movement		P-value
	150 g (mm)	100 g (mm)	
Week 1	0.58 ± 0.43	0.51 ± 0.44	NS
Week 2	1.05 ± 0.69	0.91 ± 0.87	NS
Week 3	1.39 ± 0.72	1.23 ± 0.94	NS
Week 4	1.77 ± 0.76	1.58 ± 0.97	NS
Week 5	2.36 ± 0.96	1.85 ± 0.94	Sig

Data presented as mean ± standard deviation of canine movement (n = 12) with unit of mm. NS, No statistically significant difference. Sig, Significance (P < 0.05).

peaked at week 4 (3 times higher) than baseline activity with statistically significant (P < 0.05) when Kruskal-Wallis test was used. In the 100 g group, TRAP activity was peaked at week 2 (1.7 times higher) than baseline at mesial sites, but not statistically significant (P > 0.05) when Kruskal-Wallis test was used (Table 1A). TRAP activities later showed a fall from week 3 to week 5 towards the baseline value. At the distal sites, TRAP activities peaked at week 5 (2.7 times higher) than baseline activity but not statistically significant (P > 0.05) when the Kruskal-Wallis test was used (Table 1).

TRAP activities were also compared between the 150 and 100 g of orthodontic forces (Table 1). At the distal sites, TRAP activity peaked in week 4 with 150 g force but showed no significant difference (P > 0.05) than 100 g while with the 100 g force, TRAP activity peaked significantly (P > 0.05) in week 5 than 150 g. However, the pattern of TRAP activity observed at the mesial sites showed with increased orthodontic force (150 g) produced later peaked TRAP activity (week 3) as compared to 100 g (week 2), but no statistically significant difference (P > 0.05).

There was a linear relationship of cumulative canine movements (mm) with time (week 0 - week 5) between the 150 and 100 g groups (Figure 1). The mean of cumulative canine movement was 2.36 ± 0.96 mm with the 150 g of force with a rate of 0.45 mm per week and 1.85 ± 0.94 mm with the 100 g of force with a rate of 0.37 mm per week. Therefore, maxillary canine with the 150 g of force moved 22% faster, statistically significant (P < 0.05) than those with the 100 g force using paired t-test (Table 2).

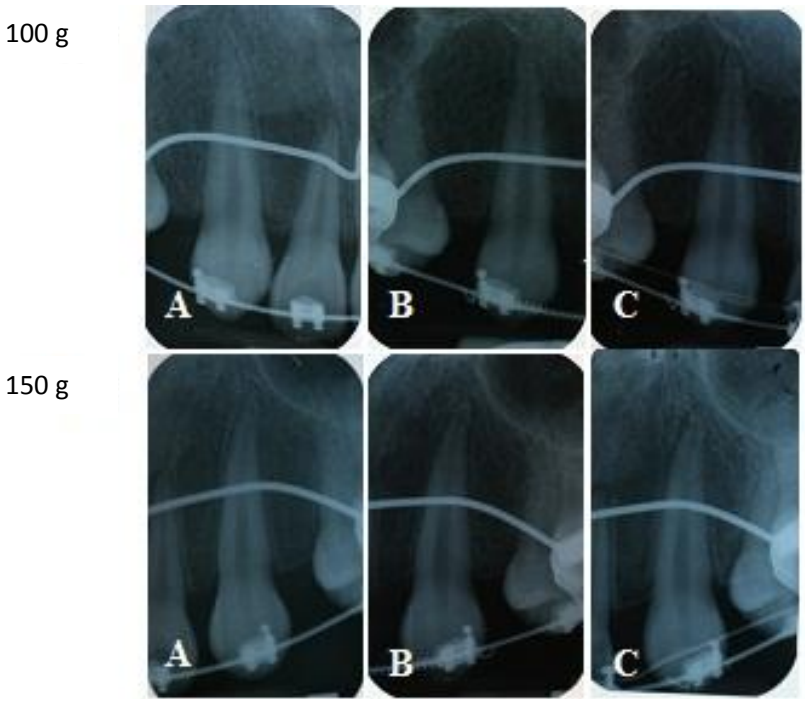

Figure 2. Periapical radiographs of canine at 100 and 150 g groups. Apical and lateral root resorptions were assessed using periapical radiographs taken at baseline (A), week 5 (B) and 6 months post retraction (C).

Signs of canine root resorption was monitored preoperatively and postoperatively from 5 weeks until six months post retraction using periapical radiographs. There were no lateral or apical root resorptions (score 0) in the canines at 5 weeks of retraction and six months post retraction in 100 and 150 g orthodontic force groups (Figure 2).

DISCUSSION

The composition of GCF increased with orthodontic tooth movement (OTM), showing that GCF can be used as a sample for further observation during OTM (Samuels et al., 1993). Careful monitoring of the periodontal condition of the subjects helped to minimize the influence of the gingival inflammation to the composition of GCF. In this study, subjects with gingivitis were excluded from the study. GCF was utilised as a sample in this prospective study to investigate and to compare the pattern of activity between orthodontic force on TRAP under different continuous orthodontic forces (150 and 100 g) and rate of OTM.

Maxillary teeth was levelled and aligned prior to canine retraction for 3 to 4 months. The archwire 0.019 × 0.025 inch stainless steel (SS) was placed 1 month prior to canine retraction. It was done to allow passive sliding of the teeth along the archwire with minimal friction. The rectangular wire (0.019 × 0.025 inch SS) was used to promote more of bodily movement. By placing the rectangular wire into the slot, a couple is created within the bracket slot. Thus, it will control root position and allow the tooth to move bodily in the direction of applied force. Bodily canine movement can also occurred faster (38 days) than tipping movement due to a shorter duration of root uprighting (Shpack et al., 2008). Furthermore, risk of root resorption was lesser in bodily moved canine due to stress distribution along the roots as compared to the stress concentration at the apex resulting from tipping (Reitan, 1964).

Phosphatase changes have been described in orthodontically treated tissues. However, only few studies have reported about TRAP. Casa et al. (2006) used TRAP histochemical method to detect the distribution of clastic cells during the application of torque over 4 weeks. They found that the mononuclear TRAP positive cells appeared at week 2 and while multinucleated TRAP positive cells were numerous at 3 and 4 weeks. Other studies looking at the specific enzyme activity, such as Rody et al. (2001) who found an increased of acid phosphatase at compression site. In addition, King et al. (1993) found decreased alkaline activity on compression/distal site and increased acid and alkaline phosphatase activities on the tension/mesial sites of orthodontically treated teeth up to 7 days after appliance activation in rats.

Insoft et al. (1996) also found that acid phosphatase to increase between the third and sixth weeks with more activity of acid phosphatase enzyme at mesial than distal sites, while the current study observed slight increment of TRAP activity at week 2 at the mesial and peaked at week 4 at the distal sites in 150 g. Similarly, for the 100 g, slight increment TRAP activity was found at week 3 at the mesial but peaked at week 5 in the 100 g at the distal site. We found that the level of TRAP activity was significantly higher at the distal site in the 150 and 100 g force as compared to the mesial sites. This result indicated that bone resorption was more predominant at the distal/compression sites compared to the mesial/tension sites. On the other hand, pretreat-ment/baseline showed a variation of readings specific TRAP activities (Table 1) due to residual bone activity as a result of prolong bone remodeling (King and Keeling, 1995) activity in response to the masticatory force.

Rate of canine movement was investigated with different forces magnitudes. This study found 22 percent faster cumulative canine movement in the 150 g group and was statistically significant as compared to the 100 g force in 5 weeks of retraction. This is in agreement with Samuels et al. (1998) who recommended that the optimal force for space closure at the 150 g was more effective than the 100 g spring when using a nickel-titanium coil springs, but increasing the force to 200 g produced no further increase in rate of closure. Boester and Johnston (1974) also found increased canine movement with increasing force from 60 to 150 g (0.8 to 1.3 mm/month), but force beyond 150 g (240 and 330 g) results in 0.8 to 1.0 mm canine movement per month. Similar study by Asma et al. (2008), found the canine to move only 0.72 ± 0.44 mm per month with 100 g force as compared to our study which was 1.77 ± 0.76 and 1.58 ± 0.97 mm in the 150 and 100 g forces, respectively.

The periapical radiographs were taken before and after the application of force to observe any signs of apical and lateral root resorptions around the maxillary canines for six months post retraction. According to Artun et al. (2005), six months observation period was enough to detect early formation of the root resorption. Methodology by Liou and Huang (1998) was adopted in assessing root resoprtion for this study. Smale et al. (2005) also found that root resorption can be detected as early as the early stage of leveling and alignment. Another study by Artun et al. (2005) also monitor root resorption using periapical radiographs over six to twelve months and found that apical root resorption identified in the first six months of active orthodontic treatment will progress in the following six months period. This study found no apical or lateral root resorptions of the canines after six months post retraction (Figure 2).

A study done by Shahrul Hisham et al. (2010) who have investigated the pattern of TRAP specific activity in saliva during orthodontic tooth movement. The study finding was concordance with the current findings where the TRAP specific activity increased during the treatment. The earlier peak in the TRAP activity with 150 g of force showed more canine movement. This finding was important to clinical practice in orthodontic treatment where earlier peak of TRAP specific activity could indicates faster tooth movement when 150 g of orthodontic force was applied.

Conclusions

Our study suggested that TRAP enzyme can be a useful biomarker of bone remodeling in orthodontic tooth movement. There was significant increased of specific TRAP activity at weeks 3 and 4 at tension and compression sites, respectively as compared to baseline of the 150 g force and faster canine tooth movements of 22%. Furthermore, no lateral and apical of root resorption were noted in periapical radiograph during 5 week of retraction period and after six months post retraction.

ACKNOWLEDGEMENTS

The authors thank Univerisiti Kebangsaan Malaysia for the research fund; (UKM-OUP-FGG-2011 and UKM-OUP-KPB-33-170-2011), Ministry of Higher Education (UKM-DD-03-FRGS0030-2010) and post-graduate orthodontic students who have contributed patients to this research. We also thank Nuraliza Ab Wahab for her assistance with laboratory work of the enzymes assay.

REFERENCES

Artun J, Smale I, Behbehani F, Dopel D, Hof MV, Kuijpers-Jagtman AM (2005). Apical root resorption six and 12 months after initiation of fixed orthodontic appliance therapy. Angle Orthod., 75: 919-926.

Asma AAA, Rohaya MAW, Shahrul Hisham ZA (2008). Crevicular alkaline phosphatase activity during orthodontic tooth movement: canine retraction stage. J. Med. Sci., 8(3): 228-233.

Boester C, Johnston LE (1974). A clinical investigation of the concepts of differential and optimal force in canine retraction. Angle Orthod., 44: 113-119.

Casa MA, Faltin RM, Faltin K, Arana-Chavez VE (2006). Root reosption on torqued human premolars shown by tartrate resistant acis phosphatase histochemistry and transmission electron microscopy. Angle Orthod., 76: 1015-1020.

Halleen JM, Alatalo SL, Suominen H, Cheng S, Janckila AJ, Vaananen K (2000). Tartrate resistant acid phosphatase 5b: a novel serum marker of bone resorption. J. Bone Miner. Res., 15(7): 1337-1345.

Insoft M, King GJ, Keeling SD (1996). The measurement of acid and alkaline phosphatase in gingival crevicular fluid during orthodontic tooth movement. Am. J. Orthod. Dentofacial Orthop., 109: 287-296.

Kavadia-Tsatala, S, Kaklamanos EG, Tsalikis L (2002). Effects of orthodontic treatment on gingival crevicular fluid flow rate and composition: Clinical implications and applications. Int. J. Adult Orthod. Orthognath. Surg., 17: 191-205.

Keeling SD, King GJ, Mc Coy EA, Valdez M (1993). Serum and alveolar bone phosphate changes reflect bone turnover during orthodontic tooth movement. Am. J. Orthod. Dentofacial Orthop., 103: 320-326.

King GJ, Keeling SD (1995). Orthodontic bone remodeling in relation to appliance decay. Angle Orthod., 65: 129-140.

King GJ, Keeling SD, Wronki TJ (1993). Histomorphometric study of alveolar bone turnover in orthodontic tooth movement. Bone, 12(6): 401-409.

Liou EJW, Huang CS (1998). Rapid canine retraction through distraction of the periodontal ligament. Am. J. Orthod. Dentofacial Orthop., 114: 372-82.
Noxon SJ, King GJ, Gu G, Huang G (2001). Osteoclast clearance from periodontal tissues during orthodontic tooth movement. Am. J. Orthod. Dentofacial Orthop., 120: 466-476.
Proffit WR, Fields HW, Sarver DM (2007). Contemporary Orthodontics. 4th edn. St. Louis: Mosby Year Book.
Reitan K (1964). Effects of force magnitude and direction of tooth movement on different alveolar bone types. Angle Orthod., 34(4): 244-255.
Rody WJ, King GJ, Gu G (2001). Osteoclasts recruitment to sites of compression in orthodontic tooth movement. Am. J. Orthod. Dentofacial Orthop., 120: 477-489.
Samuels RH, Pender N, Last KS (1993). The effects of orthodontic tooth movement on the glycosaminoglycan components of gingival crevicular fluid. J. Clin. Periodontol., 20: 371-377.
Samuels RH, Rudge SJ, Mair LH (1998). A clinical study of space closure with the nickel-titanium closed coil spring and an elastic module. Am. J. Orthod. Dentofacial Orthop., 114: 73-79.
Shahrul Hisham ZA, Mohd Faiz E, Rohaya MAW, Yosni B, Sahidan S (2010). Profile of lactate dehydrogenase, tartrate resistant acid phosphatase and alkaline phosphatase in saliva during orthodontic tooth movement. Sains Malaysiana, 39(3): 405-412.
Shpack N, Davidovitch M, Sarne O, Panayi N, Vardimon AD (2008). Duration and anchorage management of canine retraction with bodily versus tipping mechanics. Angle Orthod., 78: 95-100.
Smale I, Artun J, Behbehani F, Doppel D, Van't Hof M, Kuijpers-Jagtman AM (2005). Apical root resorption 6 months after initiation of fixed orthodontic appliance therapy. Am. J. Orthod. Dentofacial Orthop., 128: 57-67.
Uitto VJ (2000). Gingival crevice fluid- an introduction. Periodontology, 31: 9-11.