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Crevicular Alkaline Phosphatase Activity During Orthodontic Tooth Movement: Canine Retraction Stage

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To observe pattern of ALP activity in Gingival Crevicular Fluid (GCF) during canine retraction stage of fixed orthodontic treatment. Ten patients age between 15 to 27 years old with moderate upper labial segment crowding were recruited from postgraduate orthodontic clinic. The tested canines were distalized using nickel titanium push coil spring. The level of ALP at week 0 acted as the baseline. Samples were taken at day 0, at week 1, 4, 8 and 12. The activities of ALP were measured using spectrophotometer (405 nm). Paired sample t-test was used to assess the differences at weekly interval and between test and control groups over the study period. The ALP level was decreased in both the mesial and distal of the test teeth for the first month and stabilizes months after. Significant difference were detected from week 4 upward when compared to the baseline ($p<0.05$). The bony turnover specifically the bone formation can be monitored through the expression of ALP in the gingival crevicular fluid during orthodontic treatment.

Key words: Alkaline phosphatase, gingival crevicular fluid, orthodontic

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INTRODUCTION

Orthodontic tooth movement is based on the biological principle that prolonged pressure on teeth results in remodeling of the alveolar bone. There will be a balance between bone formation and resorption where formation predominantly on the tension site and resorption on the pressure site (King and Keeling, 1994). The tissue surrounding an orthodontically moved tooth will be influenced by the magnitude of force applied. Heavy forces will cause substantial bone destruction and root resorption. An optimal force will reduce the risk of damaging the hard tissue. However, the biological response of bone turnover of each individual may varies.

By monitoring the bony turnover of a patient, an optimal force can be applied which caters to the patients' needs. Thus, in the quest of searching a suitable bone turnover biomarker for orthodontic treatment, many potential bone biomarkers have been studied such as acid phosphatase and prostaglandins. In this study, an enzyme called alkaline phosphatase (ALP) is chosen. Alkaline phosphatase has been found to be synthesised and secreted by the osteoblast cells during bone formation process. Alkaline phosphatase catalyses the hydrolysis of phosphatase ester, which is a potent inhibitor of mineralization process in alkaline pH that associated with the formation of calcified tissue. Alkaline phosphatase has been found to be synthesised and secreted during bone regeneration processed as demonstrated by Stucki *et al.* (2001) in their histochemical observations.

Few investigations have been conducted to explore the activity of ALP in orthodontic patients. A study by Insoft *et al.* (1996) showed that the subjects had a peak of ALP activity between 1 to 3 weeks when there was little tooth movement. However, during the period of peak tooth movement, the level of ALP is reduced (Insoft *et al.*, 1996). ALP was also found to be elevated on the tension site compared to the pressure site of an orthodontically moved tooth (Perinetti *et al.*, 2002, 2004). However, another study found that the pattern of ALP was similar in both mesial and distal site where there was a gradual increased of ALP level with a peak at day 14 (Batra *et al.*, 2006). Currently it is not easy to evaluate the level of ALP during tooth movement as laboratory procedure is needed to perform the enzymatic assay. Thus, there is an intention of our study to design a diagnostic kit for easier evaluation of ALP in the future.

The source of ALP in these studies were taken from Gingival Crevicular Fluid (GCF) found in the sulcus of an orthodontically moved tooth (Perinetti *et al.*, 2002, 2004; Batra *et al.*, 2006). GCF is a transudate which reflects the body immune and inflammatory responses to chemical or mechanical stimuli (Griffiths, 2003). Currently, GCF represents a potential source of biomarker for diagnostic purposes since it contains biochemical and cellular factors which represent the biological changes occurring in the oral environment (Griffiths, 2003). GCF components can be utilized as a diagnostic test for clinical biomarker as multiple samples can be collected from a patient without causing iatrogenic damage (Lamster, 1997).

By selecting an appropriate bone turnover biomarker, the treatment progress of an individual can be monitored and the amount of force applied can be modified accordingly to avoid iatrogenic damages (Krishnan and Davidovitch, 2006). In addition, the duration of retention for each patient can be customized according to the expression of a suitable bone formation biomarker.

Thus, the aim of this longitudinal study is to explore the potential of ALP as a suitable bone formation biomarker during the first 3 months of canine distalization stage of an orthodontic treatment.

MATERIALS AND METHODS

Selection of patients: Ten patients were recruited after an informed consent from the Postgraduate Orthodontic Clinic, Dental Faculty, UKM. Oral hygiene instruction, scaling and polishing were conducted prior to the study to ensure maintenance of good oral health. Patients were requested not to use any anti-inflammatory drugs (Kyrkanides *et al.*, 2000) or mouthwash containing chlorhexidine during the period of this study. This study has been approved by the Faculty Ethical Committee. The inclusion criteria are listed below:

- Patients presented with moderate crowding who need extraction of at least upper first premolars.
- Patients that need an upper fixed appliance treatment to distalize maxillary canine.
- Healthy with no systemic illness (as stated by patient).
- Not pregnant (as stated by patient).
- Periodontally healthy according to the following criteria:
  - Full month plaque score (FMPS) less than 20%.
  - Full month bleeding score (FMBS) less than 20%.
  - Periodontal pocket of less than 4 mm.
  - No radiographic bone loss seen in OPG.

Orthodontic appliance and teeth to be tested: Orthodontic preadjusted appliances (0.022"×0.028") were bonded to the upper and lower teeth. All patients must complete the

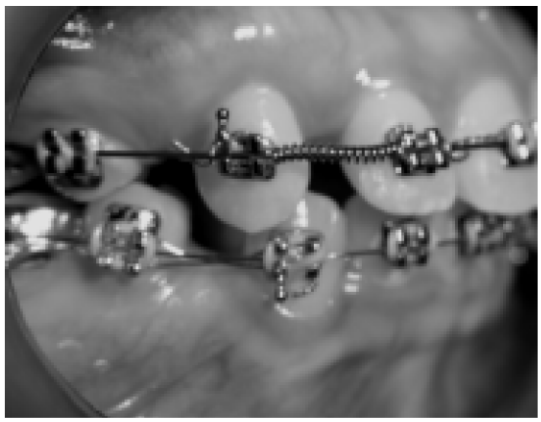

Fig. 1: Canine distalization technique

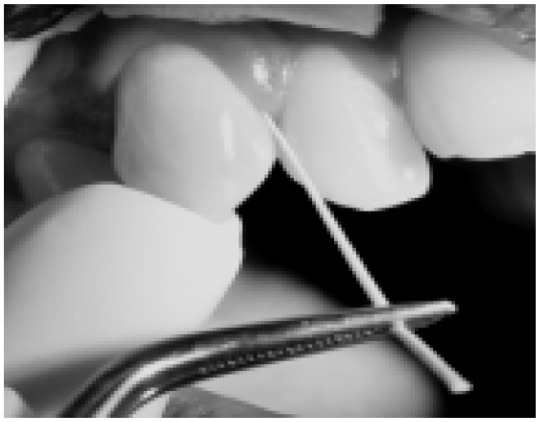

Fig. 2: GCF sampling

leveling and alignment stage and be on an upper 0.016' stainless steel archwire (special plus AJ Wilcock, Whittlesea, Australia) for at least 1 month prior to the canine distalization. The incisors were gathered together with stainless steel ligature for extra anchorage. Nickel Titanium (NiTi) push coil spring with force of 150 g was applied between lateral incisor and canine to distalized canine. The force was measured using correx gauge (Dial-type stress and tension gauge; Dentaurum, Germany). Lateral incisor and canine brackets were ligated with 0.09' stainless steel ligature while other teeth were secured with elastomeric modules as shown in Fig. 1. Oral hygiene instruction was given post-operatively. Patients were reviewed after one week, a month and two months and after three months. The level of ALP at week 0, i.e., before the application of force acted as the baseline.

Gingival Crevicular Fluid (GCF) sampling: GCF sampling were taken at day 0 (prior to the force application) followed by at week 1, 4, 8 and 12. The test teeth were cleaned with cotton pellet to remove any supragingival plaque, isolated using cotton roll and dried using gentle air stream. Three standard endodontic paper points (size 30) were inserted approximately 1 mm into the crevice for 30 sec with a period of 90 sec interval per sampling to increase the volume of the GCF collected per side (Fig. 2). Immediately, the 3 dipped paper points (per site) were placed in 1.5 mL eppendorf tube containing 350 μL of physiological saline.

The eppendorf tube containing 3 dipped paper points per site was centrifuged using the centrifuge machine (MicroCentaur, UK) for 5 min at 2000 g in order to elute completely GCF components. The paper points were removed and the supernatant stored at -40°C until analysed for a maximum of 1 week.

Alkaline phosphatase assay: The ALP activity was determined by using spectrophotometer (Model 6330, Jenway UK) at 405 nm wavelength. Approximately 50 μL of 40 mM carbonate buffer pH 9.8 with 3 mM $MgCl_2$ were pipette into a test tube using micropipette (Eppendorf). Subsequently, 50 μL of GCF sample and 50 μL of 3 mM ρ-nitrophenyl phosphate were added into the same tube. The samples were then incubated at 37°C for 30 min. The enzymatic reaction was terminated by adding 50 μL of 0.6 M sodium hydroxide and the absorbance was measured immediately at 405 nm. The amount of ρ-nitrophenol formed was measured using a standard curve prepared from phosphatase substrate (Sigma 104®, Sigma-Aldrich, St Louis, USA). The ALP activity is presented in enzyme unit (U). U is defined as the amount of ρ-nitrophenol released (μmol) per minute at 37°C.

Statistical analysis: The Statistical Package for Social Sciences programme (SPSS® Inc., Chicago, IL USA) version 12.0 was used to analyze data. Data was tested for normality using Shapiro-Wilk test. As data were normally distributed, paired t-test was used to assess the difference between different sites at specified time and at one site at different time with $p<0.05$ was considered as significant.

RESULTS

Demographic data: Patients studied were eight females ranging in age between 15 to 27 years old. Majority had Class I and Class II division 1 malocclusion with only one Class III malocclusion case (Table 1).

ALP activity during canine distalization stage: At baseline (week 0), before the insertion of the NiTi coil spring, level of ALP were similar in the mesial and distal

Table 1: Demographic data

Gender	Male	2
	Female	8
Age	Min	15
	Max	27
	Mean	22
Malocclusion	Cl I	4
	Cl II/1	5
	Cl II/2	0
	Cl III	1

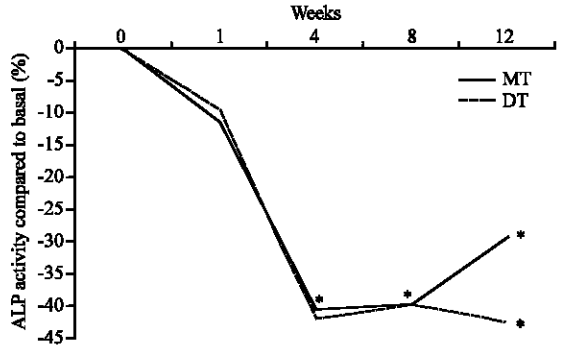

Fig. 3: ALP activities when comparing to the baseline; MT-mesial test, DT-distal test; week 0 as the baseline; *p<0.05 when compared to baseline level (week 0)

sites. At week 1, level of ALP activity was decreased in mesial and distal sites. On week 4, both the tested sites showed a reduction of ALP level to 0.06 U when compared to week 1. At week 8, the level of ALP in the mesial sites was maintained but the distal sites showed a slight increased from the previous week (week 4). On the last week of this study (week 12), the mesial site slightly increased but the distal site had reduced (Fig. 3).

Statistical analyses of paired t-test were also used in our studies. The changes in the level of ALP at each weekly interval when compared to the baseline level (week 0) is shown in Fig. 1. At week 1, ALP activity at the mesial and distal sites decreased by an average of 10% from the baseline level (week 0). The reductions were not statistically significant when using paired t-test ($p>0.05$). A marked reduction of ALP activity was noted after 1 month (week 4) at both of the tested sites. The level of ALP on the mesial and distal sites was 40.3 and 41.6%, respectively. These reductions in the ALP activity were statistically significant when paired t-test was used ($p<0.05$). At week 8, both the tested sites showed similar level of ALP. The reduction of 39% from the baseline was not significantly difference from the previous week (week 4) when paired t-test was used ($p>0.05$). At week 12, the pattern of ALP changed in the mesial and the distal sites. The mesial sites level increased by 10% but the distal sites showed further reduction from the previous

Table 2: Amount of canine movement measured at each weekly interval

Week	Mean±SD (mm)
0-1	0.38±0.31
0-4	0.72±0.44*
0-8	1.71±0.74*
0-12	2.24±0.76*

*p<0.05

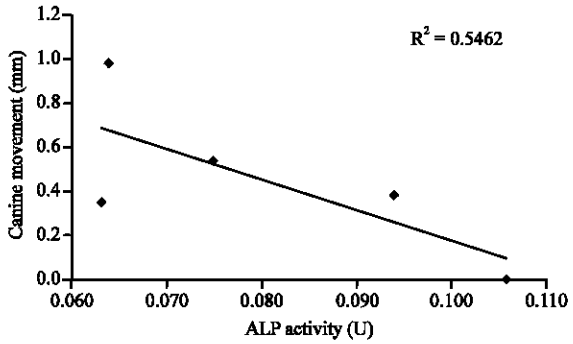

Fig. 4: Correlation between the amount of canine movement and the level of ALP in the mesial test canine

week (week 8). However, these changes were not significant when compared to the previous week (week 8) ($p>0.05$) but significant difference were detected when compared to the baseline (week 0) ($p<0.05$) when using paired t-test.

Overall, pattern of ALP in the mesial and distal sites were similar where there was a marked decreased of ALP level starting from week 4. The level of ALP in the mesial and distal sites stabilized afterwards (week 4, week 8 and week 12). There were no significant differences in the ALP activity between the mesial and distal sites throughout the study period ($p>0.05$) when statistical analysis of paired t-test were used. The levels of ALP in the mesial and distal sites were significantly decreased from week 4 onwards when compared to its baseline level ($p<0.05$) when using paired t-test statistical analysis.

Alkaline phosphatase and canine movement: After 1 week of force application, the test canine moved on average of 0.38±0.311 mm. After a month, the movement was 0.72±0.44 mm. The utmost movement of the test canine was detected after 2 month (week 8) where it moved in an average of 1.71±0.74 mm. In the last month (week 12), the canine had moved 2.24±0.76 mm from the initial position at week 0. In average, the test canine has moved of an average of 0.75±0.2 mm per month (Table 2).

The amount of canine movement was negatively correlated with the level of ALP expressed in the mesial and distal sites of the test groups. Both the mesial and distal site of the tested canine had r-value of -0.7 which were moderately correlated with the canine movement (Fig. 4).

DISCUSSION

This longitudinal study gives a new insight on the reaction of bone towards orthodontic forces which is reflected by the level of alkaline phosphatase (ALP). Secretion of ALP during bone formation is due to the activity of osteoblastic cells (Intan *et al*., 2008). Based on this study we presumed that the osteoblast's ALP was secreted to GCF, thus by monitoring the respective enzyme activity would represented as monitoring the bone formation. Currently, no study has been conducted to observe the level of ALP in GCF and correlated it with the amount of bone formation from the same sample. During this study, patients were asked to come at week 1, 1st month, 2nd month and 3rd month of treatment. This recall time were formulated to explore pattern of bone formation presented by the level of ALP during the time before the next activation of the orthodontic appliance. Furthermore, this will make the recall visit interval more convenient to patients. Traditionally, patients were called every 4-6 weekly.

Canines are forced to move distally by the continuous action of the NiTi push coil spring. The movement is a combination of bodily and tipping movement. Therefore, there would be no pure tension and compression area around the tooth. There would be a combination of both compression and tension area at mesial and distal site of the tooth (King and Keeling, 1994). Thus, it would be expected to observe concurrent bone formation and resorption around an orthodontically moved tooth.

The pattern of ALP during canine retraction stage reflects the biochemical changes which occurs in the bone surrounding canine. The result from this study which showed similar pattern of ALP during canine movement in the mesial and distal sites is in agreement with other previous studies (Batra *et al*., 2006). This indicates that bone formation occurs at the tension sites as well as at the pressure site. Bonafe-Oliveira *et al*. (2003) has also observed the similar pattern of concomitant bone resorption and formation at the same site after force application to teeth. It is believed that a reduction in bone formation would be expected and bone resorption prevails during orthodontic tooth movement. This was observed in the finding as the canines are distalized, less ALP activity was expressed in GCF when compared to baseline (week 0). The balance between the normal bone remodeling process is now interrupted by the force application. An orthodontically moved tooth will experienced less bone formation and more bone resorption around it as shown in the ALP activity level.

In few other studies, an increased in the ALP level was detected during orthodontic tooth movement with a peak in between 1-3 week (Perinetti *et al*., 2002, 2004). This may be due of the different in the time interval where ALP was sampled. The longest observational period was one month (Perinetti *et al*., 2002; Batra *et al*., 2006) whereby in this study, the enzyme activities were obtained for 3 consecutive months. However, in a preliminary study done by Isik *et al*. (2005), the pattern of ALP were similar to our study where there were decreased in ALP level from basal (day 0) up to the 4th week.

As the canines were pushed to move further away from the initial position, gap will be created in the bony socket at the mesial sites of the tooth. Thus, the rate of bone formation will be increased to maintain the original bony socket morphology. This phenomenon is called frontal or direct resorption where the tooth is said to move with the bone (Melsen, 1999). This will explain the result seen on week 12 where a different pattern of ALP at the mesial and distal sites. At that point in time, bone formation will be more on the mesial sites than the distal sites in order to preserve the original tooth's socket morphology. The distal sites will continue to experience more bone resorption and less bone formation for the canine to move into the intended position.

During this study, canine movement was measured at each weekly interval. After one week, canine was distalized at about 0.4 mm which is which is similar to Batra *et al*. (2006) study. The amount of ALP activity expressed in the GCF represents the amount of bone formation at a particular time. Thus during canine movement, less bone formation is expected. The explanation confirmed by the finding in this study when the canine movement was correlated with the amount of ALP activity, a negative correlation was revealed. This phenomenon has also been reported by Insoft *et al*. (1996) whereby level of ALP activity was found to decrease during active tooth movement.

Thus, it can be concluded that the bony turnover specifically the bone formation can be monitored through the expression of ALP in the gingival crevicular fluid during orthodontic treatment. In the future, the potential of ALP as a bone formation biomarker can be further investigate during different stages of orthodontic treatment such as retention stage.

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