

## Pattern of Crevicular Alkaline Phosphatase During Orthodontic Tooth Movement: Leveling and Alignment Stage

(Corak Alkalin Fosfatase Krevikel Semasa Pergerakan Gigi Ortodontik: Peringkat Penyusunan Gigi)

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### ABSTRACT

*Bone formation has been associated with the presence of an enzyme called alkaline phosphatase (ALP). This longitudinal study was carried out to observe its activity in gingival crevicular fluid (GCF) during leveling and alignment stage of orthodontic fixed appliance treatment. Fourteen patients between the ages of 15 and 27 years old with moderate upper labial segment crowding were recruited from postgraduate orthodontic clinic. GCF from mesial and distal sites of upper canines were collected using endodontic paper point before the fixed appliance placement (week 0). The baseline level of ALP (week 0) acted as a control. Samplings of GCF were repeated at 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> week during orthodontic treatment. The activities of ALP were measured using spectrophotometer (405 nm). Paired sample t-test was used to assess the significance of difference over the 3 weeks. Although the results showed patterns of ALP activities on the test teeth throughout the 3 weeks of leveling and alignment stage, however the results were not significant ( $p > 0.05$ ) when compared to control. Therefore, it has been shown that there was no significant pattern of ALP activities in GCF in patients undergoing leveling and alignment stage of orthodontic treatment.*

*Keywords: Alkaline phosphatase; bone formation; orthodontic*

### ABSTRAK

*Pembentukan tulang telah dikaitkan dengan kewujudan sejenis enzim yang dikenali sebagai alkalin fosfatase (ALP). Kajian longitud ini dijalankan untuk memerhati aktiviti enzim tersebut di dalam cecair krevikel gingival (GCF) semasa peringkat penyusunan gigi rawatan aplian tetap ortodontik. Empat belas pesakit berumur di antara 15 dan 27 tahun yang mempunyai kesesakan sederhana di segmen labial atas telah diambil dari klinik pascasiswazah ortodontik. GCF dari kawasan mesial dan distal gigi kanin atas telah diambil menggunakan kertas poin endodontik sebelum peletakkan aplian tetap (minggu 0). Tahap aktiviti ALP pada minggu 0 ini bertindak sebagai kawalan. Pensampelan GCF telah diambil pada minggu pertama, kedua dan ketiga sepanjang rawatan ortodontik dijalankan. Aktiviti ALP diukur menggunakan spektrofotometer (405 nm). Ujian-t sampel berpasang digunakan untuk menilai perbezaan signifikan di antara 3 minggu tersebut. Walaupun hasil kajian mendapati bahawa terdapat corak pada aktiviti ALP sepanjang 3 minggu kajian dijalankan namun ia tidak signifikan ( $p > 0.05$ ) apabila dibandingkan dengan kawalan. Oleh itu, kajian ini telah membuktikan bahawa tiada corak yang signifikan berlaku pada aktiviti ALP di dalam GCF pada pesakit yang menjalankan rawatan ortodontik semasa peringkat penyusunan gigi.*

*Kata kunci: Alkalin fosfatase; ortodontik; pembentukan tulang*

### INTRODUCTION

Orthodontic tooth movement is based on the principle that an applied force to a tooth will cause it to move to a new intended position. Most contemporary fixed orthodontic appliance use light continuous force derived from archwire-bracket combination. This light continuous force will allow the teeth to be aligned in the dental arch without causing any iatrogenic damages such as root resorption. Application of continuous force produces concomitant bone resorption and formation at pressure area with increased activities of both tartrate-resistant acid phosphatase (TRAP)-positive osteoclast and osteoblast (Bonafe-Oliveira et al. 2003).

The increase in osteoblastic activity during bone formation will be accompanied by an increased expression of an enzyme called alkaline phosphatase (ALP) (Intan et al. 2008). To investigate the bone remodelling pattern based on ALP activity during an orthodontic treatment, body fluids such as saliva can be used (Shahrul Hisham et al. 2010). Apart from saliva, the source of ALP can be also obtained from a clear fluid excreted from the gingival crevice of a tooth. This clear transudate is known as gingival crevicular fluid (GCF). It reflects the biological body responses to periodontal healing in chronic periodontitis patients (Perinetti et al. 2008) or mechanical stimuli such as the orthodontic force (Batra et al. 2006; Perinetti et al.

2002; Perinetti et al. 2004). GCF can be easily collected using several methods such as micropipette or paper strips (Ozmeric 2004). These methods have been tested and shown to be reproducible, reliable and do not cause any iatrogenic damage to the patients (Insoft et al. 1996). Currently, there is no clinical study which uses GCF as source of ALP in order to investigate the bone remodelling pattern during the levelling and alignment stage of orthodontic treatment.

Few studies have looked into the ALP activity during active tooth movement using higher force of 100-150 gram in canine retraction (Asma et al. 2008; Insoft et al. 1996; Perinetti et al. 2004). It was found that the mesial and distal site of the tested tooth showed a significant increased in ALP activity when compared to the baseline (Perinetti et al. 2002). Furthermore, it was found that the tension sites showed more ALP activity when compared to the compression sites (Batra et al. 2006; Perinetti et al. 2002; Perinetti et al. 2004). By monitoring the enzymatic activity of ALP during an orthodontic treatment, the application of orthodontic force can be personalized to the patients' biological needs. Therefore, the aim of this longitudinal study is to look at the pattern of ALP during the first stage of orthodontic treatment i.e. the leveling and alignment stage.

## MATERIAL AND METHODS

### PATIENTS' SELECTION

Fourteen patients were recruited after an informed consent from the postgraduate orthodontic clinic, Dental Faculty, UKM. The inclusion criteria are listed below:

1. Patients presented with moderate crowding (4-8 mm)
2. Healthy with no systemic illness (as stated by patient)
3. Not pregnant (as stated by patient)
4. Periodontally healthy according to the following criteria:
  - a. Full mouth plaque score (FMPS) less than 20%
  - b. Full mouth bleeding score (FMBS) less than 20%
  - c. Periodontal pocket of less than 4 mm
  - d. No radiographic bone loss seen in OPG

Oral hygiene instruction and scaling and polishing were conducted prior to the study to ensure maintenance of good oral health. The oral hygiene and periodontal status of each subject were monitored throughout this study. Patients will not be included in this study if their oral hygiene and periodontal status deteriorated. During the period of this study, all patients were advised to restraint from using any anti-inflammatory drugs which has an effect on the orthodontic tooth movement (Kyrkanides et al. 2000) or chlorhexidine mouthwash which can alter the ALP-GCF level (Paolantonio et al. 2008). The study has been approved by the Faculty Ethical committee.

### ORTHODONTIC APPLIANCE AND TESTED TEETH

Orthodontic preadjusted appliance (0.056 cm × 0.071 cm) was bonded to the upper teeth. Bands were cemented to the upper 1<sup>st</sup> molars. No extraction was done prior to the bonding. A 0.030 cm round nickel titanium archwire was used for levelling stage. Oral hygiene instruction was given following bonding procedure. Patients were reviewed weekly up to 3<sup>rd</sup> week. The canines were chosen as the tested teeth and the level of ALP at week 0 (baseline) before the application of force acted as control.

### GINGIVAL CREVICULAR FLUID (GCF) SAMPLING

GCF samples were taken before bonding procedure (week 0), week 1, 2 and 3 from mesial and distal sites of the tested teeth (Figure 1). The 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 1 mm into the crevice for 30 s with a period of 90 s interval per sampling. Immediately, the 3 dipped paper points (per site) were placed in 1.5 mL eppendorf tube containing 700  $\mu$ L 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.

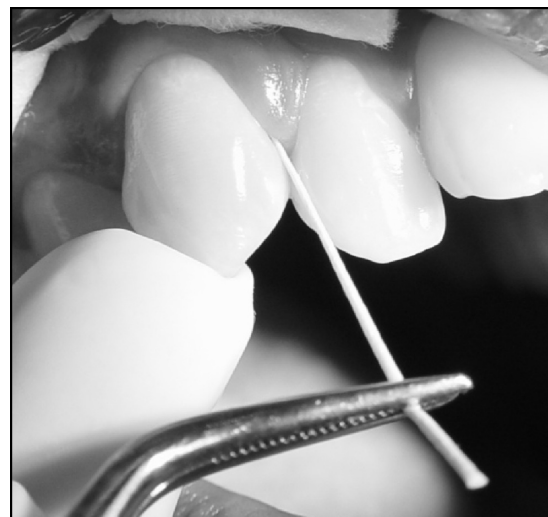


FIGURE 1. GCF sampling

### ALKALINE PHOSPHATASE (ALP) ASSAY

The ALP activity was determined by using spectrophotometer (Model 6330, Jenway UK) at 405 nm wavelength. Approximately 50  $\mu$ L of 40 mM carbonate buffer pH 9.8 with 3 mM MgCl<sub>2</sub> were pipette into a test tube using micropipette (Eppendorf). Subsequently, 50  $\mu$ L of GCF sample and 50  $\mu$ L of 3 mM p-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 *o*-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 *o*-nitrophenol released (µmol) per minute at 37°C.

#### STATISTICAL ANALYSIS

The Statistical Package for Social Sciences programme (SPSS® Inc., Chicago, IL USA) version 15.0 was used to analyse the data. Data was tested for normality using Shapiro-Wilk test. Paired *t*-test was used to assess the significance difference from baseline level (week 0) over 3 weeks as the data was normally distributed. A  $p < 0.05$  was considered as significant.

#### RESULTS

##### PATIENTS' CHARACTERISTICS

Ten females and four males were selected with age ranging from 13 to 27 years old. Seven patients had Class II division 1 malocclusion, six patients had Class I malocclusion and one patient had Class II division 2 malocclusion. (Table 1)

##### ALKALINE PHOSPHATASE ACTIVITY DURING LEVELLING AND ALIGNMENT STAGE

The ALP activities from week 0 to week 3 are shown in Table 2. At baseline (week 0), when there was no orthodontic force applied to the teeth, the mesial and distal sites of test teeth showed similar level of ALP activity. After 1 week of force application, the level of ALP in mesial and distal

TABLE 1. Patient's characteristics' characteristics

Patients' characteristics		Number of patients
Gender	Male	4
	Female	10
Age	min	13
	max	27
	mean	20.8
Malocclusion	CI I	6
	CI II/1	7
	CI II/2	1
	CI III	0

sites increased to 0.12U and 0.14U, respectively. At week 2, the level of ALP at the mesial site had increased further while the distal sites maintained from the previous week. At the last week of this study (week 3), a slight reduction in the ALP level can be observed at the distal sites while the mesial site level was maintained.

The pattern of ALP during the levelling and alignment stage at each weekly interval when compared to the baseline level (week 0) is shown in Figure 2. Percentages of ALP activity from each week were calculated by comparing the increment of each week to the baseline (week 0). At week 1, the mesial and distal sites had an increased level of ALP from the baseline of 12% and 21%, respectively. At week 2, the mesial sites had 18% increased of ALP level while the distal site had a slight increased of 4% from the previous week (week 1). At week 3, the level of ALP at mesial sites maintained to 31% from the baseline level. However, the distal site showed a reduction in the ALP level to 18% from the basal and 7% from the previous week (week 2). By using the paired *t*-test, all the changes in the ALP activities throughout this study were not statistically significant ( $p > 0.05$ ) as shown in Table 2 & 3.

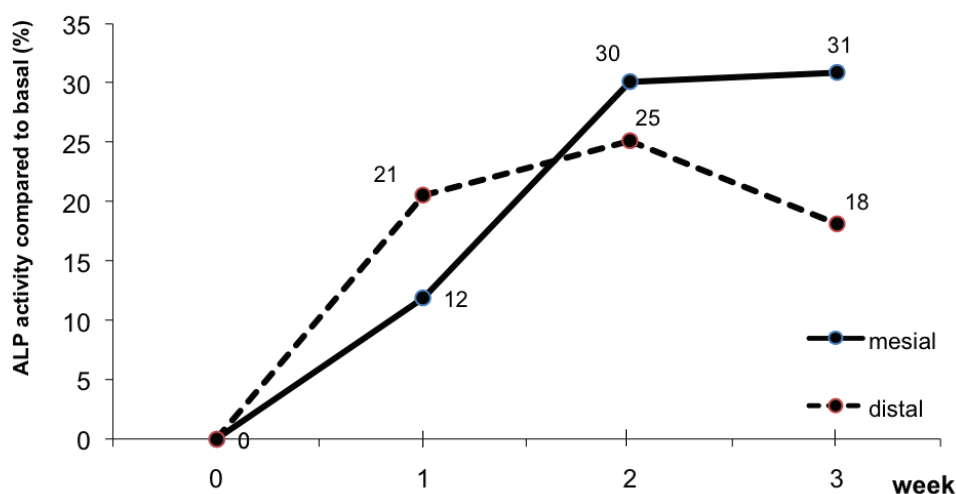


FIGURE 2. ALP activities when comparing to the baseline; week 0 as the baseline

TABLE 2. ALP activity from week 0 to week 3 as expressed in U

Week	Mesial (U)	Distal (U)	<i>p</i> value
0	0.11 ± 0.05	0.11 ± 0.04	0.828
1	0.12 ± 0.06	0.14 ± 0.07	0.234
2	0.15 ± 0.08	0.14 ± 0.06	0.850
3	0.15 ± 0.06	0.13 ± 0.03	0.448

TABLE 3. Paired t-test results comparing the same site at different week

Site	Week	<i>p</i> value	
Mesial	0	1	0.522
		2	0.260
		3	0.114
	1	2	0.409
		3	0.413
	2	3	0.973
Distal	0	1	0.365
		2	0.205
		3	0.117
	1	2	0.958
		3	0.677
	2	3	0.906

Overall, the level of ALP in the mesial and distal sites had increased gradually from week 0 until week 2. However, in week 3, the level of ALP in the mesial sites maintained but a further reduction of ALP level was observed in the distal sites.

## DISCUSSION

This is the first ever longitudinal study conducted to observe the activity of ALP expressed in the GCF during the first 3 weeks of leveling and alignment stage of orthodontic treatment. Leveling and alignment is the first stage of an orthodontic treatment sequence. During this stage, a flexible archwire usually a nickel titanium (NiTi) wire was tied into the bracket slot. The NiTi archwire exerts a light continuous force between 30 and 60 g onto the teeth in order to level and align the dentition. A tipping movement is produced through combination of bracket-archwire system in a fixed orthodontic appliance. Consequently, tooth will tend to rotate around its centre of resistance compressing the periodontal ligaments near the apex of the root on the same side of the force applied. On the other side, PDL around the crest of alveolar bone will be compressed.

During bone formation, osteoblast was found to express ALP (Intan et al. 2008). Therefore, based on the finding, we presumed that the ALP detected in GCF was from the action of osteoblast during bone formation surrounding

the test teeth. Consequently, by monitoring the pattern of ALP, bony turnover surrounding an orthodontically moved teeth can be observed.

After 1 week of force application, both level of ALP in distal and mesial site of test teeth increased by about 10-20%. Bonafe-Oliveira et al. (2003) in their study found that both bone formation as well as bone resorption does occur in respective of tension or compression sites. After orthodontic force is applied, bone will be formed at a higher rate to work concurrently with bone resorption in order to maintain the integrity of the alveolar bone that holds the dentition. Our finding also revealed that the level of ALP on the distal site was higher than the mesial site at week 1. This phenomenon could be due to the features of the bracket system. Bracket for canine in the preadjusted appliance has a built in system with mesial tip of 11°-13°. When an archwire is inserted into the bracket slot, the tooth is forced to tip mesially around its centroid. This will result in a compression area being created at the mesial site and tension area at the distal site around the cervical of the tooth. This phenomenon reflects in our finding whereby at week 1, ALP activity at the distal sites was more than the mesial sites. Other studies had also found a similar result where there was an increased of ALP activity at the tension sites (Perinetti et al. 2002; Perinetti et al. 2004).

The pattern of ALP at the mesial and distal sites was increased up to week 2. Batra et al. (2006) in his canine retraction study also found an increased of ALP activities in both the mesial and distal sites with the level peak at week 2. However, at subsequent week (week 3), the level of ALP was maintained in the mesial sites but decreased in the distal sites. The level of ALP at week 3 was more at the mesial sites than the distal counterpart. The bone formation sites seemed to be moving from distal to the mesial sites. This may be explained by the uprighting movement of teeth which transferred the area of tension from distal to mesial sites.

The overall enzyme activity produced in this study was insignificant and smaller than other similar studies (Asma et al. 2008; Batra et al. 2006; Perinetti et al. 2004). The small reading of the enzymatic activity may due to the lower force exerted on the teeth during the levelling and alignment stage as compared to the other studies which uses higher force to retract teeth (Asma et al. 2008; Batra et al. 2006; Perinetti et al. 2004). Patient recruitment that met all the inclusion criteria with time constraint were the limitation to this study. Given more time, biological variation of subjects can be eliminated by recruiting more subjects.

Expression of ALP reflects the biochemical changes which occurs in the supporting tissue after the application of an orthodontic force (Dhopatkar et al. 2005). Therefore, by monitoring the changes in ALP enzymatic activity, the application of force during orthodontic treatment can be customized according to the patient's needs.

## CONCLUSION

Therefore, this longitudinal study gives a new insight on the reaction of bone towards orthodontic forces which is reflected by the level of alkaline phosphatase. The low force and the small amount of tooth movement during the levelling and alignment stage may contribute to the low level of ALP. Consequently, ALP could be a promising bone formation biomarker to monitor the biological changes occurs during an orthodontic treatment. In the future, the response of bone to different types of orthodontic force could be observed.

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