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# The Activity of Aspartate Aminotransferase During Canine Retraction (Bodily Tooth Movement) in Orthodontic Treatment

[1]M.A.W. Rohaya, [2]Z.A. Shahrul Hisham and [1]K. Khazlina

The pattern of activity of aspartate aminotransferase enzyme (AST) following canine distalization stage in orthodontic treatment is being investigated. The enzyme is released to the gingival crevis following tooth movement resulted from focal necrosis in the adjacent periodontal ligament. The finding of enzymatic response could enhance our understanding of the enzyme's role during tooth movement. This study investigated the potential of AST as a biological marker to monitor tooth movement by determining its activity in Gingival Crevicular Fluid (GCF) during bodily tooth movement (canine distalization). About 13 patients age between 14-26 years participated in the study. All patients had orthodontic treatment using fixed appliances. For every subject, one upper canine being the test tooth while contralateral canine served as control. Distalization force (100 g) was applied only to the test tooth and GCF was collected from the mesial and distal sites of test and control teeth every week until week 12 (week 0, 1, 4, 8 and 12). The activity of AST in the GCF was determined spectrophotometrically (at 30°C, 340 nm). The AST activity in the GCF of test teeth in all patients significantly increased at week 1 ($p<0.05$) compared to control. The activities during the following weeks (4-12) were similar to one another and stabilized but significantly higher than week 1 ($p<0.05$). There was 100% increment of AST activity from week 1 to 4. AST activity in the distal site was also significantly higher ($p<0.05$) than the mesial site of test teeth. There is no difference in AST activities when age group (adult and adolescent) were compared ($p>0.05$). The AST activity appears to be enhanced with the applied force and showed no differences between adult and adolescent patient. Therefore, AST has the potential as a biological marker to monitor progress of orthodontic tooth movement.

**Key words:** Aspartate aminotransferase, gingival crevicular fluid, canine distalization, tooth movement, biological marker

[1]Department of Orthodontics, Faculty of Dentistry, Universiti Kebangsaan, Kuala Lumpur, Malaysia
[2]School of Biosciences and Biotechnology, Faculty of Science and Technology, Universiti Kebangsaan, Kuala Lumpur, Malaysia

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***For further information about this article or if you need reprints, please contact:***

M.A.W. Rohaya
Department of Orthodontic,
Faculty of Dentistry,
Jalan Raja Muda,
Abdul Aziz 50300,
Universiti Kebangsaan, Malaysia,
Kuala Lumpur, Malaysia

Tel: 603-92897756
Fax: 603-92897856

Z.A. Shahrul Hisham
School of Biosciences
and Biotechnology,
43600 Bangi,
Universiti Kebangsaan Malaysia,
Selangor, Malaysia

Tel: 603-89213245
Fax: 603-89252698

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## INTRODUCTION

Orthodontic tooth movement is a process that combines both pathologic and physiological responses following external applied forces (Wise and King, 2008). Orthodontic forces simulate the pathologic events which cause minor reversible injury to the tooth supporting tissues. Application of force to the teeth will initiate tissue damage that subsequently leads to production of inflammatory processes in the periodontal tissue and caused deformation of the alveolar bone. Applying the correct amount of force is vital in orthodontics especially during bodily tooth movement as to prevent iatrogenic damage to the supporting tissue, root resorption (Chan and Darendeliler, 2005), anchorage loss or delayed tooth movement from occurring since these would hamper treatment progress if inappropriate force is given. There were many studies that monitor progress of tooth movement using enzymes e.g., alkaline phosphatase (ALP) (Perinetti *et al*., 2002; Asma *et al*., 2008), where activity of osteoblastic cells was related to secretion of ALP during bone formation (Intan *et al*., 2008).

The current study which investigated the pattern of aspartate aminotransferase (AST) activity following application of force to distalize the canine (100 g) was a continuation of an earlier study by the same researcher which investigated the enzyme's activity after the application of force to align teeth (30-60 g). Distalization of canine involves bodily movement of the tooth. The force needed to induce this movement is higher than what is needed to align teeth which involve tipping with no translation displacement (Proffit, 1993). During bodily tooth movement, bone modeling process must occur that involved bone resorption and deposition. Bone resorption occurs in the site where the tooth moves toward (in this instance the distal site) and bone deposition occurs in the site where the tooth moves away from (the mesial site) (Sandy *et al*., 1993).

In this study, where higher level of force applied was done to assess the suitability of AST as a biological marker to monitor orthodontic tooth movement. If deemed suitable, it could lead to the production of a diagnostic kit that would help the orthodontist determine whether or not the correct amount of force has been applied by studying the level of activity with different force level.

Although AST was found to have the potential to serve as a biological marker for tooth movement in past studies, the number of studies is limited. The first study involved with different time of assessment that was 1 h, week 1, 2, 3 and 4 after applied force and also different type of teeth was used (molar) (Perinetti *et al*., 2003). On the other hand, more recent study in 2004 only involved the pulp tissue instead of AST activity in GCF (Perinetti *et al*., 2004). Present study involved a longer assessment of enzyme activity in GCF, which was over three months of observation with applied force.

There is a need to investigate the enzyme's responsiveness and pattern of activity when different forces are applied. The understanding and comparison between the patterns seen when higher forces are applied would enable better assessment of the enzyme's potential as a biological marker to monitor orthodontic tooth movement. Thus, the aim of this study was to further investigate the potential of AST as a biological marker by determining its activity in the gingival crevicular fluid (GCF) following application of force to bodily move teeth. The hypotheses were that the activities of AST in GCF would be elevated following force application and the activity in the distal site is higher than the mesial site.

## MATERIALS AND METHODS

This study was conducted from 2006 until 2007 at Department of Orthodontics, Dental Faculty, Universiti Kebangsaan Malaysia. Six adolescents and seven adults from a convenient list of patients from the Orthodontic Postgraduate Clinic, Dental Faculty, Universiti Kebangsaan Malaysia were invited to participate in the study. The mean ages of the adolescents and adults were 14.4±3.7 and 23.3±4.1 years, respectively. The inclusion criterias were: (1) need for orthodontic treatment, (2) extractions of upper and lower first premolars, (3) good general and periodontal health, (4) females are not pregnant, (5) no use of anti inflammatory drugs, antibiotics or chlorhexidine mouthwash in the month before and during the study, (6) written consent from patient/guardian and approval from ethical committee.

A transpalatal arch (0.9 mm stainless steel) was constructed and cemented to the first molars for anchorage purposes. Fixed appliances with orthodontic brackets (0.022×0.028; Roth, Minimaster, America) were placed on the remaining teeth. One upper canine was randomly chosen as the test tooth while the contralateral canine served as the control tooth. The test canine was distalized following extraction of the first upper premolar. A 0.016 round stainless steel archwire (A.J. Wilcock, Australia) was placed in the upper arch and a nickel titanium opening coil spring (American Orthodontics, USA) was placed between the upper lateral incisor and the upper canine. The compressed coil gave 100 g of force determined by the correx gauge (Dentaurum) to distalize (bodily movement) the canine and was changed every 4 weeks to maintain the amount of force given.

GCF was collected from the mesial and distal sites of test and control teeth before placement of the opening coil, i.e., week 0 and on weeks 1, 4, 8 and 12. Before GCF collection, any plaque present was removed and sampling sites isolated using cotton rolls and dried. A size 30 paper strip (Diadent, International Group, Korea) was inserted into the gingival sulcus at a depth of 1 mm for 30 sec. Each site was sampled 3 times at a minute interval. The samples were placed in a vial containing 100 μL Tris-HCl with pH 8.0 and stored at 4°C until analyzed.

GCF volume was calculated by weighing the paper points before and after collection and using the weight difference in expressing AST activity. AST activity determined spectrophotometrically at 30°C. Each sample was incubated for 5 min in a substrate containing 150 mmol of L-aspartate, 100 mmol of 2-oxoglutarate, 0.2 mmol NADH, 400 mU $mL^{-1}$ of malate dehydrogenase and 100 mmol of sodium phosphate buffer pH 7.4 in a total volume of 1.0 mL. In the presence of AST L-aspartate and 2-oxoglutarate exchange an amino group to produce oxaloacetate and L-glutamate. The rate of reaction was monitored by the reduction of oxaloacetate to L-malate by malate dehydrogenase with concurrent oxidation of NADH. As NADH was consumed, the change in absorbance at 340 nm was monitored. The mesial and distal values were averaged and the results converted to enzyme activity units (1 U = 1 μmol of NADH consumed per minute at 30°C).

Data analysis was carried out using The Statistical Package for Social Sciences Programme (SPSS, Version 12.0, SPSS Inc., Chicago, IL, USA). Wilcoxon paired signed rank test was used to assess any significant differences in AST activity in GCF between the test and control samples. The confidence interval was set at 95% and significant level was set, $p<0.05$. The study was registered for the clinical trials with identification number ISRCTN 47483728, validated by the criteria by World Health Organization (WHO) and also been approved by the Research and Ethics committee, Faculty of Dentistry, Universiti Kebangsaan Malaysia, Malaysia.

## RESULTS AND DISCUSSION

In the adult subjects, the test teeth showed increased activity at week 1 and increased further at week 4 (Table 1). At week 4, the AST activity was noted 100% increment and showed statistically significant different compared to week 0 ($p<0.05$). AST activity was also noted increased significantly at week 4 as compared to week 1 ($p<0.05$) (♦ at week 2; Table 1). However the activities at week 4, 8 and 12 seemed stabilized and were similar to each other where there were no statistical significant different found ($p>0.05$) (Table 1). Statistical analysis of AST activities in the mesial and distal sites of the test teeth were also showed significantly higher ($p<0.05$) than the mesial and distal sites of control teeth throughout the whole period of the study at week 1, 4, 8 and 12 (Table 1).

Similar findings were also noted for the adolescence subjects (Table 2). In the adolescent subjects, the test teeth also showed increased activity at week 1 and increased further at week 4. AST activity also showed 100% increment compared to week 0 and showed statistically significant different when compared between samples ($p<0.05$) (♦; Table 2). AST activity was noted increased significantly at week 4 when compared to week 1 ($p<0.05$) followed with a period of AST activities stabilization from week 4 to week 12, where there were no statistical significant different found ($p>0.05$) (♦; Table 2). Statistical analysis of both the mesial and distal sites of AST activities of the test teeth were also significantly higher than the mesial and distal sites of control teeth throughout the whole period of the study ($p<0.05$) at week 1, 4, 8 and 12 (*; Table 2).

Comparison of distal site and mesial site of AST activities for both adult and adolescent subjects showed statistical significant higher activities at distal compared to mesial site ($p<0.05$) (Table 3) at week 1, 4, 8 and 12. Statistical analysis comparing AST activities of both the adult and adolescent subjects of their respective distal and mesial sites of teeth showed no significant different in AST activities in week 1, 4, 8 and 12 (Table 4).

Meanwhile, the average tooth movement for adults was 0.88±0.5 mm $month^{-1}$ and for adolescents was 0.75±0.5 mm $month^{-1}$. There was also no significant different found on amount of tooth movement per month between different ages ranges ($p>0.05$).

Applying the wrong amount of force during orthodontic treatment can cause problems such as pain, gingival recession, delayed tooth movement, anchorage loss and prolonged treatment duration. Thus, orthodontists should always ensure that the amount of forces applied is suitable for the different types of tooth movement. One hundred gram of force to move canine distally (bodily tooth movement) was suggested by Proffit (1993). However due to patient's variation in their biological response to applied force, the study was design to determine the AST activities during canine distalization. It is hoped that by determining the enzyme activities in patient's GCF the treatment will be more of individual basis. A diagnostic kit to confirm that the correct amount of force has been applied can be useful to achieve this goal. Thus, the pattern of activity of a substance such as an enzyme that is produced during

Table 1: A comparison in AST activities in mU between the test and control teeth in adult subjects (n = 7) for mesial and distal sites

| | Week | | | | |
|---|---|---|---|---|---|
| Test | 0 | 1 | 4 | 8 | 12 |
| Test mesial | 60.4 (11.6) | 88.3 (4.3)♦ | 125.2 (24.9)♦ | 132.1 (25.0) | 131.2 (20.5) |
| Control mesial | 58.9(9.2) | 69.7 (9.9) | 72.3 (7.0) | 70.7 (6.3) | 71.4 (4.8) |
| p-value | 0.128 | 0.018* | 0.018* | 0.018* | 0.018* |
| Test distal | 60.8 (12.0) | 91.7 (5.6) ♦ | 145.1(43.6) ♦ | 147.0 (24.6) | 147.7 (25.9) |
| Control distal | 60.7 (10.6) | 72.8 (15.2) | 75.2 (8.0) | 74.4 (7.8) | 75.1 (6.2) |
| p-value | 0.398 | 0.018* | 0.018* | 0.018* | 0.018* |

The data represents median of AST activity with Interquartile [Median (IQR)] of tested weeks. Statistical analysis using Wilcoxon paired signed rank test to assess differential between the test and control samples. p<0.05 statistically significant of *comparing test tooth with control and ♦comparing test tooth with previous weeks of analysis

Table 2: A comparison in AST activities in mU between the mesial and distal sites of the distalized canines (test teeth) with the same sites in the control teeth in adolescent subjects (n = 6)

| | Week | | | | |
|---|---|---|---|---|---|
| Test | 0 | 1 | 4 | 8 | 12 |
| Test mesial | 58.3 (6.6) | 83.8 (4.1)♦ | 125.6 (11.1)♦ | 126.5 (9.7) | 127.6 (6.3) |
| Control mesial | 59.2 (4.6) | 70.7 (5.6) | 73.7 (8.1) | 72.4 (6.6) | 73.1 (6.6) |
| p-value | 0.248 | 0.028* | 0.028* | 0.028* | 0.028* |
| Test distal | 58.9 (6.0) | 91.0 (5.6)♦ | 146.6 (12.4)♦ | 146.5 (7.4) | 146.2 (9.3) |
| Control distal | 60.7 (7.4) | 76.3 (4.5) | 80.8 (6.7) | 79.6 (5.7) | 80.4 (4.4) |
| p-value | 0.833 | 0.028* | 0.028* | 0.028* | 0.028* |

The data represents median of AST activity with Interquartile [Median (IQR)] of tested weeks. Statistical analysis using Wilcoxon paired signed rank test to assess differences between the test and control samples. p<0.05 statistically significant of *comparing test tooth with control and ♦comparing test tooth with previous weeks of analysis

Table 3: A comparison in AST activities in mU between the mesial and distal sites of the distalized canines (test teeth) in adult (n = 7) and adolescent (n = 6) subjects

| | Week | | | | |
|---|---|---|---|---|---|
| Test | 0 | 1 | 4 | 8 | 12 |
| Adult mesial | 60.4 (11.6) | 88.3 (4.3) | 125.2 (24.9) | 132.1 (25.0) | 131.2 (20.5) |
| Adult distal | 60.8 (12.0) | 91.7 (5.6) | 145.1 (43.6) | 147.0 (24.6) | 147.7 (25.9) |
| p-value | 0.176 | 0.018* | 0.028* | 0.018* | 0.018* |
| Adolescent mesial | 58.3 (6.6.) | 83.8 (4.1) | 125.6 (11.1) | 126.5 (9.7) | 127.6 (6.3) |
| Adolescent distal | 58.9 (6.0) | 91.0 (5.8) | 146.6 (12.4) | 146.2 (9.3) | 146.2 (9.3) |
| p-value | 0.833 | 0.028* | 0.028* | 0.028* | 0.028* |

The data represents median of AST activity with Interquartile [Median (IQR)] of tested weeks. Statistical analysis using Wilcoxon paired signed rank test to assess differences between samples. *p<0.05 statistically significant

Table 4: A comparison in AST activities in mU between adult (n = 7) and adolescent (n = 6) subjects of the mesial and distal sites of the distalized canines (test teeth)

| | Week | | | | |
|---|---|---|---|---|---|
| Test | 0 | 1 | 4 | 8 | 12 |
| Adult mesial | 60.4 (11.6) | 88.3 (4.3) | 125.2 (24.9) | 132.1 (25.0) | 131.2 (20.5) |
| Adolescent mesial | 58.3 (6.5) | 83.8 (4.1) | 125.6 (11.1) | 126.2 (9.7) | 127.6 (6.3) |
| p-value | 0.600 | 0.249 | 0.463 | 0.753 | 0.753 |
| Adult distal | 60.8 (12.0) | 91.7 (5.6) | 145.1 (43.6) | 147.0 (24.6) | 147.7 (25.9) |
| Adolescent distal | 58.9 (6.0) | 91.0 (5.8) | 146.6 (12.4) | 146.5 (7.4) | 146.2 (9.3) |
| p-value | 0.463 | 0.917 | 0.917 | 0.917 | 0.917 |

The data represents median of AST activity with Interquartile [Median (IQR)] of tested weeks. Statistical analysis using Wilcoxon paired signed rank test to assess differences between samples. *p<0.05 statistically significant

tooth movement that represents the amount of force received by the teeth during that particular time could make it a suitable biological marker for a diagnostic kit. The enzyme of interest in this study is an intracellular enzyme, i.e., Aspartate aminotransferase (AST) which is normally found in most cells including periodontal and bone cells. Orthodontic forces typically cause cellular necrosis resulting in cell lysis and followed by the release of the enzyme into the gingival crevicular fluid (GCF).

In an earlier study done during tooth alignment treatment by the same researchers, AST's pattern of activity following the application of force to align teeth (30-60 g) was investigated. It was found that the enzyme's activity increased one week after the force was applied and gradually decreased in the next 3 weeks. From this study it was appeared that the AST had the potential to serve as a biological marker only after a week of applied force since the respective enzyme activity decrease at week 2 onwards although the applied force remain the

same. However, the applied forces level during earlier study was rather low due to tooth alignment treatment, i.e., tooth tipping movement. Current study showed that the level of AST activities is higher, i.e., (90-150 mU) compared to previous study (35 mU) due to higher level of force that was applied for bodily movement treatment, i.e., tipping movement (tooth alignment) need less force as compared to bodily movement (canine distalization). From this finding, it appeared again that the AST had the potential to serve as a biological marker. However in contrast with previous type of treatment that the respective enzyme can only be used as a biomarker during the first week of treatment, this study showed that during bodily movement treatment, AST can be used after a week and throughout the treatment since the pattern of enzymatic activity followed that of the force applied, i.e., activity level increased as force increased during treatment. However, due to the scarcity of studies that investigated AST's response to orthodontic force and thus its potential as a marker, more studies are warranted especially with difference in wire thickness or diameter. The results from current studies could further enhance our understanding and assessment of AST's response to orthodontic forces and suitability as a biological marker, respectively.

Following the application of force to distalize the canine (tooth bodily movement), it was found that the enzymatic activities in both the mesial and distal sites of the test teeth in all the subjects were significantly higher than the control teeth (tooth without applied force) showing once again AST's responsiveness to orthodontic force. The results also showed that in all the subjects, enzymatic activities in both sites increased at week 1 after force application and increased further at week 4. Enzymatic activities at weeks 4, 8 and 12 were similar but still higher compared to week 0 (Table 1, 2).

It was also found that in all of the subjects, AST's activity in the distal site was significantly higher than the mesial site at week 1, 4, 8 and 12 (Table 3). This indicates that there was more cell death in the compressed site (distal) than the tension site (mesial) which was not surprising since the distal site is where bone resorption occurs while the mesial site is where bone deposition occurs, that was also reported in by Perinetti *et al*., (2003). This particular finding also supports AST's suitability as an orthodontic biological marker since its activity reflected the biological processes that took place.

The current study found that there was no difference in the activity of AST between the adolescent and adult subjects during canine distalization. This is due to the fact that the test teeth in all subjects received a similar amount of force and had a similar inflammatory response leading to similar activity of enzyme been detected, thus AST activities are similar in both groups. When the results from this study and the previous study are considered, it appears that AST's potential as a biological marker is enhanced where firstly the enzyme responses to orthodontic force, secondly the tooth movement during bodily movement or canine distalization treatment reflect the concurrent biological process, i.e.,tissue inflammation that lead to bone resorption and formation (bone remodeling) and finally the third, its pattern of activity seems to correspond to the amount of force received by the teeth (showed by difference between test and control samples).

In terms of tooth movement, it was found that there was no bodily tooth movement in both groups (adult and adolescent) at one week of force application. This can be explained by the lag phase where more than seven days of bone remodeling is needed to induce bodily movement of teeth. Tooth movement only took place thereafter where it was found in the next three weeks (4, 8 and 12) and the test teeth moved at an average of 0.88 mm month$^{-1}$. About 1 mm of tooth movement per month is commonly seen after 4 weeks of force application when appliance is activated.

Some clinicians would attest that tooth movement is faster and treatment time is shorter in their adolescent patient compared to their adult patient. However the study found that there was no different in rate of tooth movement between adult and adolescent. The finding is also coincidence with Shimpo *et al*. (2003), who found the rate of bone formation in rats are similar even in an older or aged rat. This could be explained by the fact that once the teeth in the adult and adolescent have started to move, they will respond equally to orthodontic force and their rate of tooth movement will be similar. Martini (2001) also stated that when all subject were given the same level and duration of force and mechanics, their cells respond in a similar fashion, thus the pattern and level of AST activity were found to be similar.

## CONCLUSION

AST's potential as a biological marker to monitor orthodontic tooth movement is enhanced and the studies prove that during bodily tooth movement (canine distalization) it can be used as a better monitoring marker in treatment progress as a compared to during tipping movement (tooth alignment), where force level is important for different type of tooth movement. In contrast with common clinician perception, the result also showed that there was no different in AST activity and rate of tooth movement when adult and adolescent patients were compared.

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