

Aspartate Aminotransferase Enzyme Activity in Gingival Crevicular Fluid is affected by Human Orthodontic Tooth Movement

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Abstract

Aim: Aspartate Aminotransferase (AST) is a soluble enzyme that is normally confined to the cytoplasm of cells, but is released to the extra cellular environment upon cell death. The activity levels of AST in the gingival crevicular fluid (GCF) are considered to be important in regulating alveolar bone resorption during orthodontic tooth movement. The aim of this study was to evaluate the activity of AST in the gingival crevicular fluid in order to assess whether this enzyme has potential as possible diagnostic aid to express the tissue response during orthodontic tooth movement.

Materials and Methods: Fourteen upper and lower canines of patients having different Angle classification were selected for the study. After the extraction of the first premolars, the maxillary / mandibular canines were subjected to orthodontic retraction in a distal direction. Gingival crevicular fluid was sampled from mesial and distal gingival crevices of each canine separately at baseline, 1 hour, 7 days, 14 days, 21 days and 28 days after the application of the orthodontic distal retraction. AST activity was determined spectrophotometrically, and results were expressed as total AST activity ($\mu\text{U} / \text{sample}$).

Results and Discussion: AST activity values in both mesial and distal sites increased significantly after 14 days compared to baseline. The increase of AST activity was greater at the distal sites (compression sites) than at the mesial sites (tension sites). Local host response toward the orthodontic forces might lead to an increase in AST activity levels.

Conclusions: The increase in the AST levels in the GCF may reflect the biological activity and bone remodeling processes which occur in the periodontum during controlled orthodontic tooth movement.

Key words

Aspartate Aminotransferase, Bone remodeling, Orthodontic tooth movement.

Introduction

Over the past 40 years, several studies have tried to explain the biomechanical and the biological phenomena that allow tooth movement caused by an orthodontic appliance [1-3].

Bone remodeling during orthodontic tooth movement has been classically described as a continual and balanced process, characterized by bone deposition at sites of tension and bone resorption on the pressure side [4-7].

With moderate to severe forces, compression of the connective tissue fibers on the pressure side occurs, oxygen supplies and nutrition are restricted, and ischemia of the periodontal ligament begins. After several days of trauma, osteoclastic activity is seen, and the relatively healthy ligament cells observed near the necrotic tissue are available for repair activities.

In order to monitor the expression of biologically active substances non-invasively in humans, changes in the composition of gingival crevicular fluid (GCF) during orthodontic tooth movement have been studied [8, 9]. These substances are involved in bone remodeling and produced by the periodontal ligament (PDL) cells in sufficient quantities to diffuse into the GCF. Grieve et al. [10] reported that prostaglandin-E and interleukin-1 beta (IL - 1 β) in GCF were elevated during orthodontic tooth movement. Uematsu et al. [11] also demonstrated that (IL - 1 β), interleukin-6 (IL-6), tumor necrotizing factor-alpha (TNF - α) and epidermal growth factor (EGF) were elevated in the GCF during such movement. Thus, the amount of these substances in the fluid apparently increases during tooth movement.

Since the presence of Aspartate Aminotransferase (AST) enzyme in GCF has been demonstrated [12], several studies have observed that the levels of AST activity in GCF may reflect the magnitude of periodontal tissue destruction in periodontitis [13, 14]. Therefore, it has been suggested that AST levels in GCF may represent a potential marker for monitoring the periodontal metabolism [15, 16]. However, there are only few studies which have investigated a possible role of AST activity levels in tissue remodeling incidental to orthodontic forces.

The aim of this study was to determine whether AST activity in GCF reflects the changes occurring in periodontal tissues during human orthodontic tooth movement.

Materials and Methods

Study Population

Seven orthodontic patients (four females, mean age of 16.5 ± 1.5 years, and three males, mean age of 17.6 ± 2.5 years) from the Department of Orthodontics, College of Dental Medicine - Damascus University were selected to participate in this study.

To be eligible for the study, those patients had to meet the following criteria: (1) good general health; (2) lack of antibiotic therapy during the previous 6 months; [10] absence of anti-inflammatory drug administration in the month preceding the study; (4) periodontally healthy with generalized probing depths ≤ 3 mm without radiographic evidence of periodontal bone loss; and (5) requirement of upper and/or lower first premolars extraction and canine distal tooth movement as part of their orthodontic treatment plan.

Signed informed consents, from the patients to be subjected to the study, or from the parents of patients less than 18 years of age, were obtained prior to the commencement of the study.

Experimental Design

Through a complete orthodontic treatment plan, 6 upper first premolars and 8 lower first premolars had to be extracted for all of the patients as a first step.

Three weeks after the extraction of the first premolars, the canines next to the extraction areas [**6 upper canines** (4 canines in 2 female patients and 2 canines in 1 male patient)] and [**8 lower canines** (4 canines in 2 female patients and 4 canines in 2 male patients)] were moved in a distal direction using 90g forces for the mandibular canines and 115g forces for the maxillary canines. The forces were generated through an archwire using a retraction spring and verified with a calibrated orthodontic force gauge which was modified according to every case separately (Figure.1).

Gingival crevicular fluid samples from each tooth was collected at baseline (before the application of the orthodontic force) and one hour, seven days, 14 days, 21 days and 28 days after the application of the orthodontic force. During the observation period no orthodontic activation was performed.

Clinical monitoring, GCF samples collection and Aspartate Aminotransferase analysis

The status of the periodontal tissues was determined by clinical periodontal assessments including plaque index (PI), gingival index (GI) and probing depth (PD). These clinical parameters were assessed twice: at baseline (prior to orthodontic appliance placement) and on day 28. One week before the baseline examination, all patients underwent a session of supra – and subgingival ultrasonic scaling.

GCF was sampled separately from the mesial and distal gingival crevices of each canine where the orthodontic forces were applied using the method of Offenbacher et al. (1986) [17].

Supragingival plaque was removed in conjunction with a record of the PI. GCF samples were collected firstly by isolating the area with cotton rolls as well as by drying the teeth and adjacent marginal gingival with air (to minimize saliva contamination), and then using paper strips (Roeko Inc®, Germany) inserted for 60 seconds into the crevice to a level of one millimeter below the gingival margin. After removing the first strip and waiting for one minute, a second strip was placed at the same site for another 60 seconds. Strips contaminated by saliva or blood were excluded from the sampled group (Figure. 1).



Fig. 1) The orthodontic spring used for canine retraction, and the collection of GCF samples from the gingival sulcus

GCF volume was measured with an electronic instrument (Periotron 8000, Ora Flow) that had been calibrated by an established method. We then used a software program (Mlconvert.exe, Ora Flow) to convert the measurements to microliters [18]. Directly after collection of GCF samples, the paper strips were incubated in 200 μ L of saline serum for 15 minutes. Then, all the strips were thrown out

and the tubes with the fluids remained were taken to the laboratory of biochemistry at *Al – Assad* hospital (Damascus – Syria) for the procedures of chemical analysis.

All the tubes were put in the spectrophotometric automatic apparatus (Roche Hitachi 912® - Diamond Diagnostics - USA) to be automatically analyzed without any manual procedures. The Roche/Hitachi 912 is a flexible system for both classical and special clinical chemistries, homogeneous immunoassays. It has an on-board capacity of 35 tests and throughput of up to 360 photometric tests per hour for analysis of serum, plasma, urine, cerebrospinal fluid and hemolysate (Figure. 2).

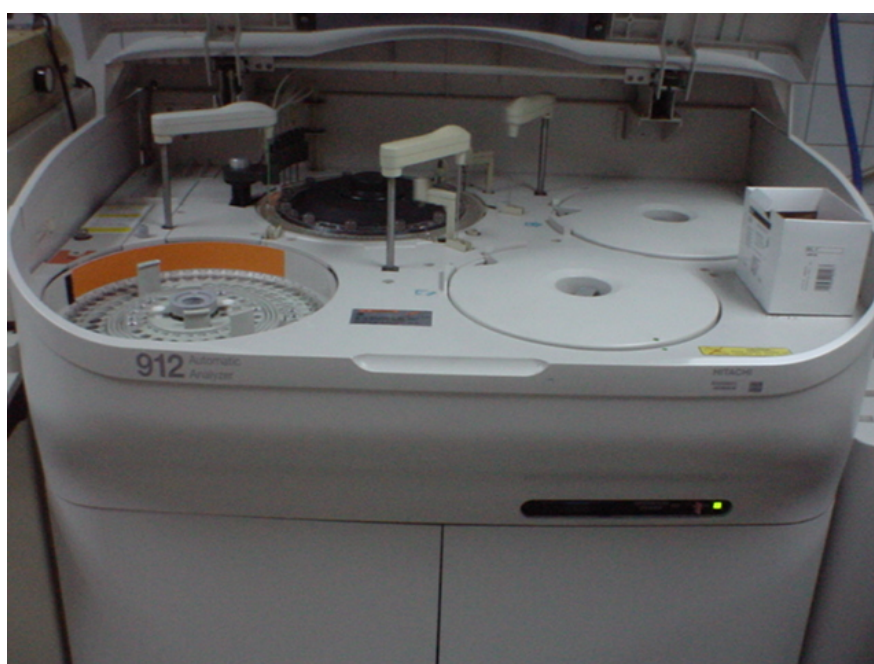


Fig. 2 Spectrophotometric automatic apparatus (Roche Hitachi 912®) used to analyze AST activity levels

By means of this apparatus, the total volume of GCF was expressed in (microliter) (μL), and the AST activity was expressed as total AST activity (micro unit per sample) ($\mu\text{U} / \text{Sample}$).

Data Processing

The values were calculated as the mean \pm standard deviation (SD) and Analysis Of Variance (ANOVA), a calculation procedure to allocate the amount of variation in a process and determine if it

is significant or is caused by random noise, was used to evaluate the statistical significance of the differences of the clinical measurements among the experimental categories in each group/column.

The measurements of GCF volume and AST activity were expressed as the overall volume for each experimental group considering the tooth itself as the statistical unit.

A probability of $P < 0.05$ was accepted for rejection of the null hypothesis and to state that with a 95% level of confidence that the two parameters are not the same.

All the statistical analyses were done by means of a computer software program (SPSS®-2006).

Results

At the baseline, the clinical indices, expressed as the Mean \pm SDs, were recorded as follows: 0.24 ± 0.367 , 1.68 ± 0.514 mm and 0.23 ± 0.253 for PI, PD and GI respectively, and after 28 days the indices were recorded as 0.19 ± 0.347 , 1.50 ± 0.453 mm and 0.30 ± 0.360 respectively as shown in Table 1.

Time Indices	Baseline	Day 28	ANOVA Test
<i>Plaque Index PI</i>	0.24 \pm 0.367349	0.19 \pm 0.34744	<i>NS</i>
<i>Probing Depth PD</i> Mean (mm) \pm SD	1.68 \pm 0.514194	1.50 \pm 0.453133	<i>NS</i>
<i>Gingival Index GI</i>	0.23 \pm 0.253004	0.30 \pm 0.360479	<i>NS</i>

Table (1): The values of the clinical parameters used in the study

NS: no statistically significant difference of pair wise comparisons over the two time points within each group

No signs of periodontal destruction were observed in any subject.

The GCF volume was slightly greater at tension sites after 21 and 28 days than at the other observation periods (Table 2). However, No statistically significant results were found between the groups during

the whole period of observation ($P > 0.05$). At pressure sites, the GCF volume was slightly greater after 28 days than at the other observation period, and similar to the results at tension sites, no statistically significant results were found between the groups at the pressure sites ($P > 0.05$). More details are demonstrated in Table 2.

Time	GCF Volume at mesial sites Mean (μ L) \pmSD	GCF Volume at distal sites Mean (μ L) \pmSD
Baseline	0.46 \pm 0.1533	0.50 \pm 0.1534
After 1 hour	0.50 \pm 0.1532	0.50 \pm 0.0882
Day 7	0.47 \pm 0.1411	0.44 \pm 0.0944
Day 14	0.46 \pm 0.1051	0.47 \pm 0.1093
Day 21	0.53 \pm 0.1203	0.50 \pm 0.1196
Day 28	0.55 \pm 0.1585	0.56 \pm 0.1756
ANOVA Test	<i>NS</i>	<i>NS</i>

Table (2): (GCF) volumes at mesial (tension) and distal (pressure) sites of the canines (μ L)

NS: no statistically significant difference of pair wise comparisons over the time points within each group

The AST activity at the tension sites increased gradually until it reached a maximum on day 14. This increase was statistically significant ($P < 0.05$). The values of AST activity showed a decrease after 21 and 28 days (Table 3). At the pressure sites, the AST activity values also increased gradually until they reached a maximum on day 14, and this increase was also statistically significant ($P < 0.05$). Then,

after 21 days all the values decreased non – significantly, and after 28 days a slight non – significant increase was observed.

Time	AST Activity Levels at mesial sites Mean ($\mu\text{U} / \text{S}$) \pm SD	AST Activity Levels at distal sites Mean ($\mu\text{U} / \text{S}$) \pm SD
Baseline	1068.57 \pm 799.950	1142.85 \pm 769.689
After 1 hour	914.28 \pm 732.726	747.14 \pm 665.252
Day 7	1010 \pm 403.555	1288.57 \pm 785.707
Day 14	1870 \pm 1295.169 [*]	1982.85 \pm 1093.984 [*]
Day 21	1468.57 \pm 658.376	1235.71 \pm 521.251
Day 28	1394.28 \pm 993.956	1538.57 \pm 752.376
ANOVA Test	statistically significant difference P < 0.05 *	statistically significant difference P < 0.05 *

Table (3): AST activity levels at mesial (tension) and distal (pressure) sites of the canines ($\mu\text{U} / \text{S}$)

Results of pair wise comparisons over the time points within each group:

* Baseline versus day 14

After 14 days, the AST activity values were significantly greater at pressure sites than those at the tension sites.

Discussion

This study was designed to evaluate the AST activity occurring during human orthodontic tooth movement, with the aim of investigating the relationship between the GCF levels of this enzyme and periodontal tissue remodeling incidental to controlled occlusal trauma (in this case; orthodontic force).

Tissue remodeling incidental to controlled occlusal trauma may be detectable by changes in GCF, as previously observed in cross-sectional and longitudinal human studies [19]. In particular, some studies [20] have found an increase in certain GCF mediators (i.e., cytokines), that can act as markers of the clinical condition during orthodontic treatment . Aspartate Aminotransferase enzyme is widely distributed in tissues, with the highest levels in heart and liver. Since this enzyme is normally confined to the cytoplasm, the increase in its extra cellular levels is considered to be a sign of increased cell necrosis [12, 14]. Indeed, its serum levels are considered to be a biochemical marker of myocardial infarction or hepatitis [20]. Since significant AST activities in GCF have been described [21] [12], different investigations have documented a positive relationship between the enzyme activities and the severity of tissue destruction, using both the experimental gingivitis model and periodontitis patients [14]. AST activity in GCF has been correlated with clinical parameters of periodontal health, including attachment loss, alveolar bone levels, and gingival index. Moreover, it has been demonstrated that an increase in the AST activity in GCF is related to periodontitis activity [14].

In periodontal tissues, orthodontic tooth movement produces a biological process previously described as a continuous phenomenon, leading to bone resorption in pressure sites and bone deposition in tension sites [4-7]. Histological animal research [20] has demonstrated that both bone deposition and resorption take place in both tension and compression sites in the alveolar bone undergoing mechanical stress by tooth movement. According to these data [20], an early wave of resorption, which requires 3 to 5 days, is followed by its reversal (5 to 7 days), and by a late wave of bone formation that continues for 7 to 14 days. This process appears to occur on both pressure and tension sides of the alveolar wall. This model is delineated by an initial asynchronous phase in which bone resorption is greater than bone deposition, while, at later times, resorption and deposition may become synchronous.

In the present study, a slight increase in the volume of GCF occurred at the mesial sites one hour after the force had been started. After one week, all the values of GCF volume returned to decrease and then to increase gradually after 21 and 28 days. However, all the recent changes were not statistically significant.

Fixed orthodontic appliances, when applied in the mouth, play an important role to attract the dental plaque layer which results in gingival inflammation, and the latter usually causes an increase in GCF volume. Although the slight increase of GCF volumes in this study was not statistically significant but it might have occurred due to a slight gingival inflammation which existed after 28 days. This gingival inflammation did not reach – at any time – destructive values (0.23 ± 0.253 , 0.30 ± 0.360 at baseline and after 28 days respectively).

Thus, the slight increase in GCF volume could be explained by the mechanical forces under which the tooth had been subjected.

It should be noted that a GCF AST level of $800 \mu\text{U} / \text{Sample}$ is approximately 20 times greater than levels found in serum of normal periodontally healthy subjects; and the finding that both healthy and diseased periodontal sites exhibit $\text{AST} \geq 800 \mu\text{U} / \text{Sample}$ in GCF, indicates that significant local tissue destruction does occur at healthy and diseased sites [22]. However, this destruction does not necessarily result in clinically significant attachment level changes.

According to these data, it seems to be a little bit difficult to address a reference value of AST in GCF since it is normal to detect higher levels of $800 \mu\text{U} / \text{Sample}$. In our study, five time points (including the Baseline point) were adjusted to monitor the trend of AST, and for statistical purposes in order to detect where the apex of the AST levels was, we considered only the differences of values between each time point and the baseline point. Therefore, after 14 days, the values of AST activity in GCF have been shown to increase significantly at both sites (pressure and tension sites). This increase of AST activity may be explained as a consequence of tissue remodeling. Indeed, the compression of the periodontal ligament induces a hyalinization of the most compressed area. This hyaline zone is described as an area of focal aseptic necrosis that is resistant to degradation and persists in the pressure zone, depending on the magnitude of the force.

The AST activity levels on day 14 at compression sites, when compared to those at tension sites, were greater, although the significance of this comparison was not statistically processed.

Thus, within the limitations of the current study and the restricted sample volume, it could be stated that AST activity in GCF is indeed affected by controlled occlusal trauma, which causes tissue

remodeling, and that its value may also be partially influenced by factors other than mechanical stress, such as gingival inflammation. However, when gingival inflammation is kept under control, AST activity in GCF may be considered to be a suitable indicator of the biologic effects produced by orthodontic treatment.

Conclusion

This study demonstrated a slight increase in the gingival fluid content of AST in teeth subjected to an orthodontic force system. This enzyme activity is further affected by the different stresses exerted on the periodontum by orthodontic forces and expresses the continuous processes which occur in the periodontal ligament.

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