



## **A Study on the Effect of Orthodontic Tooth Movement on Activity of Aspartate Aminotransferase in Gingival Crevicular Fluid**

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### **Authors' contributions**

*This work was carried out in collaboration among all authors. Authors TS and CA designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors HP and SD managed the analyses of the study. Author CA managed the literature searches. All authors read and approved the final manuscript.*

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### **ABSTRACT**

**Background:** Gingival crevicular fluid (GCF) is an important source of biomarkers. These properties are useful in monitoring the effectiveness of orthodontic treatment. 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 are considered to be important in regulating alveolar bone resorption during orthodontic tooth movement.

**Aim:** The aim of the study is to evaluate the activity of AST in GCF in the tissue response during orthodontic tooth movement in order to assess whether this enzyme has potential as possible diagnostic aid of the periodontal metabolic changes during orthodontic tooth movement.

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**Materials and Methods:** Total 5 male and 5 female patients undergoing orthodontic treatment were included in study. The GCF was collected from mesial and distal gingival margins of canine at intervals 1<sup>st</sup>, 7<sup>th</sup>, 21<sup>th</sup> day after force application. Plaque index gingival index and probing depth scores for individual patients is obtained at different time interval. AST activity will be assessed for each subject during this interval.

**Results:** AST activity values in both mesial and distal sites increased significantly after 21 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.

**Conclusion:** Thus, it was concluded that within the limitations of the study, GCF AST activity can be considered as a biomarker of the periodontal metabolic changes during orthodontic tooth movement.

*Keywords: Orthodontic tooth movement; aspartate aminotransferase; gingival crevicular fluid.*

## 1. INTRODUCTION

Gingival crevicular fluid is transudate and an inflammatory exudate secreted by the gingiva that can be found in the crevices located at the point where the gum line meets the teeth. Concentrations of this fluid are usually low, but can spike when an inflammatory process occurs in the oral cavity [1,2].

Gingival crevicular fluid (GCF) is treated as a window for noninvasive analysis of inflammation, taking into account indicators and markers of connective tissue and bone destruction so it could be a useful indicator in determining the severity of gum related disease [3]. The volume of fluid coming out of the crevice increases together with raising vascular wall permeability caused by the action of inflammatory mediators. Its composition changes during the development of inflammation [2,4].

The forces that are exerted during orthodontic tooth movement cause distortion of the periodontal ligament extra-cellular matrix, resulting in some biological features that can lead to cellular activation by changing membrane polarity and ion channel activity. In addition, as the capillaries are stretched or compressed excessively, tissue damage may occur. Such events and interactions lead to the synthesis and secretion of extracellular matrix components, tissue-degrading enzymes, acids, and local factors; induce cellular proliferation and differentiation; and promote wound healing and tissue remodeling. *In vivo* studies suggest that as biologic reactions progress at varying rates and intensities during different periods of treatment, alternate combinations of biochemical molecules come into play. These combinations are dependent on alveolar remodeling dynamics, the

cycles of injury and healing, and the composition of the PDL cell population at each period [5,6,7].

GCF as an inflammatory exudate offers great potential as a source of factors associated with changes and destruction in the underlying periodontium due to orthodontic force application.

Host derived enzymes and their inhibitors, inflammatory and immune markers, tissue breakdown products and enzymes of bacterial origin have been determined from gingival crevicular fluid (GCF) samples in numerous biomarker studies. This has led to a shift of focus toward GCF as a potential diagnostic fluid.

Aspartate aminotransferase (AST) previously termed as glutamic oxaloacetate transferase (GOT) has proven to be a strong diagnostic indicator of periodontal inflammatory lesions [8,9]. It is a soluble cytosolic enzyme which is confined to the cell cytoplasm but is released by dead or dying cells. AST should be released during this process and should pass with the inflammatory exudate into GCF. GCF-AST levels help in determining the ongoing tissue destruction. AST level of 800  $\mu$ IU has been suggested as the most suitable cut-off point to distinguish the sites at risk and sites unlikely to progress and AST level  $\geq$ 1200  $\mu$ IU has the best positive predictive power.

Since the presence of Aspartate Aminotransferase (AST) enzyme in GCF has been demonstrated [10] several studies have observed that the levels of AST activity in GCF may reflect the magnitude of periodontal tissue destruction in periodontitis [11,12]. Therefore, it has been suggested that AST levels in GCF may

represent a potential marker for monitoring the periodontal metabolism [13,14]. However, there are only few studies which have investigated role of AST activity levels in tissue remodeling incidental to orthodontic forces.

## 2. MATERIALS AND METHODS

10 orthodontic patients (5 females, mean age of  $18.5 \pm 1.5$  years, and 5 males, mean age of  $20.6 \pm 2.5$  years) were randomly selected to participate in this study.

### Inclusion criteria includes:

- No history of any Systemic diseases;
- No history of any antibiotic therapy during the previous 6 months;
- No history of anti-inflammatory drug administration in the month preceding the study;
- Periodontally healthy with generalized probing depths  $\leq 3$  mm without radiographic evidence of periodontal bone loss.

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 thrice: At baseline (prior to orthodontic appliance placement) and on day 7 and day 21.

First of all the patient was asked to rinse his/her mouth with water. Prior to the collection of GCF, any supra-crevicular plaque and soft deposits from the sites were removed carefully at every visit without causing trauma to the gingival crevice. Canine tooth was selected as it is expected to have bone resorption. The area was then thoroughly irrigated with distilled water, isolated by cotton rolls and dried by a stream of air. Suction was also used to frequently aspirate the collected saliva to avoid contamination of the GCF.

Hirschmann micropipette was placed extracrevicularly parallel to tooth surface of the selected tooth and GCF was collected (mesial and distal site) up to first marking of micropipette corresponds to  $1\mu\text{l}$  of GCF volume (Figs. 1,2,3) GCF sample from the micropipette was transferred into test tube having  $100\mu\text{l}$  normal saline for dilution of the GCF sample and then the sample was sent for biochemical assay (Figs. 4,5,6,7).



Fig. 1. Baseline view



Fig. 2. Collection from distal side

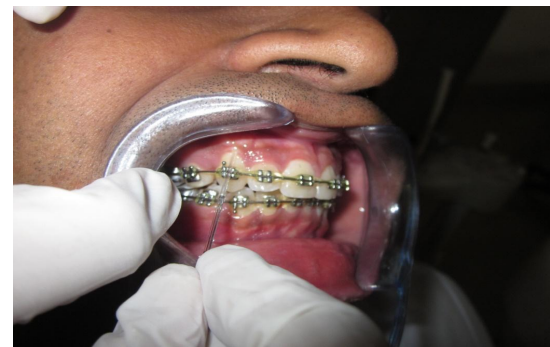
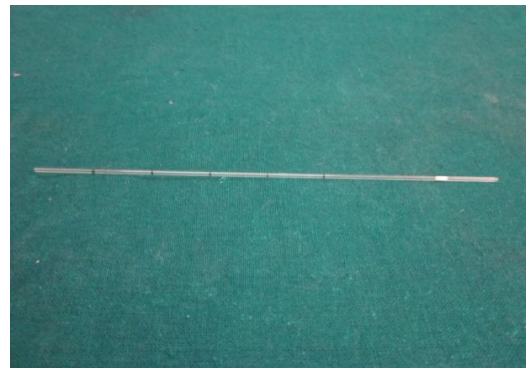


Fig. 3. Collection from mesial side







**Fig. 4. Micropipette and collection tube used for collecting GCF**



**Fig. 5. AST reagent kit**

### 2.1 Principle of Reaction

L-Aspartate + 2-Oxoglutarate => oxaloacetate + L-glutamate  
Aspartate Aminotransferase  
Oxaloacetate+NADH => D- malate +NAD + + H  
+Malate Dehydrogenase

### 2.2 Data Processing

The measurements AST activity were expressed. A probability of  $P < 0.05$  was considered 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®-2010).



**Fig. 6. Micropipette used for mixing reagent**



**Fig. 7. ERBA semi pro auto analyser for spectrometric analysis**

### 3. RESULTS

At the baseline, the clinical indices, expressed as the Mean ± SDs, were recorded as follows: 0.23±0.366, 1.67±0.513 mm, 0.23±0.253 for PI, PD and GI respectively, and on 7<sup>th</sup> days the indices were recorded as 0.12 ±0.234, 1.32 ±0.510 mm, 0.20±0.342 respectively and on 21<sup>st</sup> day the indices were recorded as 0.19±0.347, 1.50±0.453 mm, 0.30±0.360 respectively as shown in Table 1. No signs of periodontal destruction in any subject.

The AST activity at the tension sites increased gradually until it reached a maximum on day 21. This increase was statistically significant (P < 0.05). At the pressure sites, the AST activity values also increased gradually until they reached a maximum on day 21, and this increase was also statistically significant (P < 0.05) (Table 2).

### 4. DISCUSSION

The bone remodeling taking place during orthodontic tooth movement is a biologic process involving an acute inflammatory response in the periodontal tissues. The sequence characterized by periods of activation, resorption, reversal, and formation has been recently described as occurring in both tension and compression tooth sites during orthodontic tooth movement [15].

The early phase of orthodontic tooth movement involves an acute inflammatory response, distinguished by periodontal vasodilation and leukocyte migration from the periodontal ligament

capillaries [16]. The mechanism of bone resorption could also be related to the release of inflammatory mediators found in the gingival crevicular fluid [17].

GCF is a powerful source for clinical diagnosis since it contains different biochemical and cells in relation to different clinical situations indicating the state of periodontal health during orthodontic treatment [18].

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 have found an increase in certain GCF mediators (i.e., cytokines), that can act as markers of the clinical condition during orthodontic treatment. Aspartate Amino transferase 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. Since significant AST activities in GCF have been described [20,10]. 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 [12]. 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 [12].

**Table 1. Values of the clinical parameters used in the study**

Time indices	Baseline	7 <sup>th</sup> day	21 <sup>st</sup> day	ANOVA
PI	0.23±0.366	0.12 ±0.234	0.19±0.347	NS
PD	1.67±0.513	1.32 ±0.510	1.50±0.453	NS
GI	0.23±0.253	0.20±0.342	0.30±0.360	NS

*NS: no statistically significant difference of comparisons over the three time points within each group*

**Table 2. AST activity levels at mesial (tension) and distal (pressure) sites of the canines (µU/S)**

Time	AST activity levels at mesial sites mean (µU / S) ± SD	AST activity levels at distal sites mean (µU / S) ± SD
Baseline	1066.57±798.940	1140.85±765.680
Day 7	1011±402.550	1283.57±783.700
Day 21	1872±1294.163	1984.85±1092.983
ANOVA TEST	statistically significant difference P < 0.05 *	statistically significant difference P < 0.05 *

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 [21]. Histological animal research [22] 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. This process appears to occur on both pressure and tension sides of the alveolar wall.

## 5. CONCLUSION

According to the results obtained, and within the limitations of the study, it could be stated that AST activity values in both mesial and distal sites increased significantly after 21 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. This enzyme activity is further affected by the different stresses exerted on the periodontium by orthodontic forces and expresses the continuous processes which occur in the periodontal ligament. However, further studies are needed to establish procedures useful for clinical monitoring of biologic processes in the deeper periodontal tissues and the GCF during orthodontic tooth movement in human beings. The association between clinical parameters and tissue remodeling represented by AST GCF alterations can be clinically useful to biologically monitor and predict orthodontic treatment.

## DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

## CONSENT

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.

## ETHICAL APPROVAL

Ethical clearance was obtained from the institute (Narsinhbhai Patel Dental College and Hospital, Hemachandracharya North Gujarat University).

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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