

## Original Article

# Analysis of salivary biomarkers during orthodontic tooth movement with conventional bracket and self-ligating brackets: An *in vivo* study

### ABSTRACT

**Objective:** The aim of this study is to evaluate and compare salivary enzyme levels during orthodontic tooth movement with conventional brackets and self-ligating brackets.

**Materials and Methods:** Twenty patients (15–25 years of age) where 10 patients treated with mechanical biological treatment prescription and 10 patients were treated with Damon prescription requiring after first premolar extraction participated in the study. The canine retraction was started with nickel-titanium (NiTi) coil spring with 0.019 0.025" stainless steel wire. Saliva sampling was done after initial alignment before retraction and at 1, 2, 3, 4, and 5 weeks after the application of orthodontic force. A volume of 5 ml of unstimulated whole saliva will be collected from the subject for each prescription. Aspartate aminotransferase (AST), alkaline phosphatase (ALP), and lactate dehydrogenase (LDH) enzyme samples will be analyzed with fully automated clinical chemistry analyzer model TOSHIBA 120R from Agappe Diagnostics. The salivary sample for tartrate-resistant acid phosphatase (TRAP) will be analyzed with the enzyme-linked immune sorbent assay (ELISA) technique ELISA.

**Results and Discussion:** During canine retraction with NiTi coil spring the salivary enzyme levels for LDH and TRAP showed a significant difference from baseline to week 5 with Group A (conventional bracket) after the initiation of compressive orthodontic. The salivary enzyme levels for LDH, AST, TRAP, and ALP showed no significant difference from baseline to week 5 with Group B (self-ligating bracket) after the initiation of compressive orthodontic force. When compared between Group A and Group B at different time intervals for LDH, AST, TRAP, and ALP salivary enzyme levels, Group B showed a significant difference. The significant difference was seen with LDH at week 0 to week 2, AST at week 5, and TRAP at week 4, whereas ALP showed no significant difference. A significant difference with Group A was only seen with TRAP enzyme at week 1.

**Conclusion:** The LDH, AST, TRAP, and ALP level in Group A showed a significant increase whereas Group B showed no significant difference after the initiation of orthodontic.

**Keywords:** Alkaline phosphatase, aspartate aminotransferase, conventional bracket, lactate dehydrogenase, nickel-titanium coil spring, self-ligating bracket, tartrate-resistant acid phosphatase

## INTRODUCTION

Tooth movement can be classified into physiological tooth movement and orthodontic tooth movement (OTM).<sup>[1]</sup> The application of orthodontic force can change the dental and paradental tissues. Previous studies have shown that several enzymes are expressed during these phases. These enzymes have been described as biomarkers during bone remodeling.<sup>[2-5]</sup> Biomarkers are biologically active substances which are classified as biomarkers of inflammation,

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**How to cite this article:** Thomas S, Raghunath N. Analysis of salivary biomarkers during orthodontic tooth movement with conventional bracket and self-ligating brackets: An *in vivo* study. Int J Orthod Rehabil 2018;9:49-54.

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<b>Website:</b> <a href="http://www.orthodrehab.org">www.orthodrehab.org</a>	<b>Quick Response Code</b> 
<b>DOI:</b> 10.4103/ijor.ijor_46_17	

bone resorption, cell necrosis, bone deposition, and mineralization. The enzymatic biomarkers include lactate dehydrogenase (LDH), aspartate aminotransferase (AST), Tartrate-resistant Acid Phosphatase (TRAP), and alkaline phosphatase (ALP).<sup>[6,7]</sup> There is a need to evaluate the levels of biomarkers during the phase of tooth movement with force delivering systems to understand the biomechanics of tooth movement. The present study is aimed to measure the level of LDH, AST, ALP, and TRAP of young patients undergoing orthodontic canine retraction with nickel-titanium (NiTi) coil spring. The study is also aimed to compare the levels of LDH, AST, ALP, and TRAP between conventional brackets and self-ligating brackets.

### Aims and objectives

1. To evaluate salivary enzyme levels during OTM with conventional ligating bracket
2. To evaluate salivary enzyme levels during OTM with self-ligating bracket
3. To compare the salivary enzyme levels during OTM between conventional and self-ligating bracket at different time intervals.

### MATERIALS AND METHODS

In this study, a total of 20 Orthodontic patients aged between 15 and 25 years, irrespective of gender were selected from the outpatients in the Department of Orthodontics and Dentofacial Orthopedics, JSS Dental College and Hospital, JSS University, Mysuru. A total of 10 Orthodontic patients were treated with mechanical, biological treatment prescription [Figure 1], and 10 orthodontic patients were treated with Damon prescription [Figure 2]. The selected patients underwent orthodontic treatment with sequential wire changes until 0.019 × 0.025" [Figure 3] stainless steel wire were placed. Saliva sampling was done after initial alignment before retraction and at 1 week, 2 weeks, 3 weeks, 4 weeks, and 5 weeks after the application of orthodontic force. The retraction was done with NiTi closed coil spring,



Figure 1: 3M Unitek

size 0.010 and length 9 mm [Figure 4]. A total of 5 ml of unstimulated whole saliva was collected from the subject for each prescription. The subject will be asked to salivate directly into sterile containers [Figure 5]. AST, ALP, and LDH enzyme samples will be analyzed with fully automated clinical chemistry analyzer model TOSHIBA 120R from Agappe Diagnostics [Figure 6]. The salivary sample for TRAP will be analyzed with the enzyme-linked immune sorbent assay (ELISA) technique ELISA [Figure 7].

### RESULTS

#### Statistical methods applied

##### Descriptive statistics

The descriptives procedure displays univariate summary statistics for several variables in a single table and calculates standardized values (z scores). Variables can be ordered by the size of their means (in ascending or descending order), alphabetically, or by the order in which the researcher specifies.

Following descriptive statistics were employed in the present study – mean, standard deviation, frequency, and percent.

##### Paired-samples t-test

The Paired-Samples *t*-test procedure compares the means of two variables for a single group. It computes the differences between values of the two variables for each case and tests whether the average differs from 0.

##### Repeated measure ANOVA

General linear model (GLM) repeated measures analyze groups of related dependent variables that represent different measurements of the same attribute. This dialog box lets you define one or more within-subjects factors for use in GLM repeated measures. Note that, the order in which you specify



Figure 2: Damon Q (Damon prescription)



Figure 3: Arch wire



Figure 4: NiTi closed coil spring



Figure 5: Sterile containers



Figure 6: Chemical Analyser - Toshiba 120FR

within-subjects factors is important. Each factor constitutes a level within the previous factor.

### Independent-samples *t*-test

The independent-samples *t*-test procedure compares means for two groups of cases. Ideally, for this test, the patients should be randomly assigned into two groups and hence that any difference in response is due to the treatment (or lack of treatment) and not to other factors.

When groups were compared across different time intervals for changes in LDH values, nonsignificant differences were observed, indicating the similarity in the pattern of changes across 2 groups ( $F = 1.041, P = 0.399$ ).

When groups were compared across different time intervals for changes in AST values, nonsignificant differences were observed, indicating the similarity in the pattern of changes across 2 groups ( $F = 1.604, P = 0.167$ ).

When groups were compared across different time intervals for changes in TRAP values, nonsignificant differences were observed, indicating the similarity in the pattern of changes across 2 groups ( $F = 4.372, P = 0.001$ ).

When groups were compared across different time intervals for changes in ALP values, nonsignificant differences were

observed, indicating the similarity in the pattern of changes across 2 groups ( $F = 1.817, P = 0.117$ ).

### DISCUSSION

OTM is a process characterized by bone remodeling with bone deposition on tension side and bone resorption on the compression side.<sup>[8]</sup> An optimal force is important for adequate biological response in the periodontal ligament. A force level of 150–250 g is considered as the optimum force range for retraction of the canine tooth.<sup>[2,3,9]</sup>

A biomarker is defined as an indicator of normal biological, pathogenic processes, pharmacological responses to a therapeutic and other healthcare intervention.<sup>[4]</sup>

LDH, AST, TRAP and ALP in human saliva and gingival crevicular fluid (GCF) have the potential to serve as a biological marker for OTM monitoring.<sup>[5,7,10]</sup>

Enzyme levels of LDH in Group A (Conventional Bracket) found a significant increase ( $P \leq 0.05$ ) from baseline to week 5 [Graph 1]. This phenomenon shows the inflammatory process takes place 7–42 days after the start of treatment.



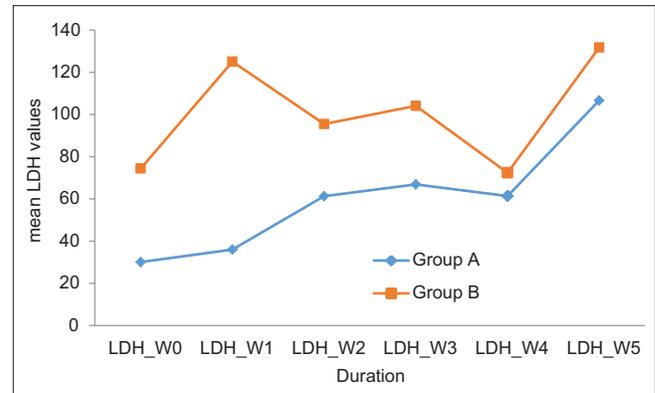
Figure 7: Elisa kit

During orthodontic treatment, inflammatory processes occur as a result of mechanical pressure imposed on the teeth. LDH will be released when lysis cells during inflammatory processes occur.<sup>[11]</sup> The inflammatory process that occurs during this orthodontic treatment will cause the death of part of a cell resulting from mechanical stress which, in turn, causes LDH enzymes to be released. The same increased LDH level is observed in GCF studies when inflammation occurs when orthodontic treatment is administered.<sup>[11]</sup>

LDH enzyme levels of Group B (self-ligating bracket) showed significant increase ( $P \leq 0.05$ ) at week 0 and week 1 [Graph 1] resulting inflammation taking place at 7 and 14 days, this minimal response of LDH in the saliva might have been caused by southern leaf blight, which has the potential to reduce the frictional resistance produced by the archwire and the bracket. Moreover, saliva itself is one of the factors that can affect frictional resistance. This finding is in agreement with the previous studies by Rohaya *et al.* and Shahrul Hisham *et al.*<sup>[5-7,10]</sup>

AST is an intracellular enzyme that is normally confined to the cell cytoplasm but is released into the extracellular environment on cell death.<sup>[12]</sup> We found a significant difference ( $P \leq 0.05$ ) of enzyme levels of AST with Group A (conventional bracket) between week 1 and week 5 and week 2 to week 5 [Graph 2], this shows that there is cell necrosis taking place during 14–42 days, after inflammation.

According to von Böhl *et al.*,<sup>[13]</sup> necrotic tissue was formed during the second phase of tooth movement (after approximately 2 days of force application). However, the level gradually decreased over the next 3 weeks of treatment. We observed no significant difference ( $P > 0.05$ ) with enzyme levels of AST of Group B (self-ligating bracket) between baseline to week 5 in saliva during this study.



Graph 1: Mean lactate dehydrogenase values

Meanwhile, TRAP enzyme levels for Group A (conventional bracket) shows a significant increase ( $P \leq 0.05$ ) on 28, 35, and 42 days and is followed by bone formation from week 1 to week 3 and week 1 to week 4 [Graph 3].

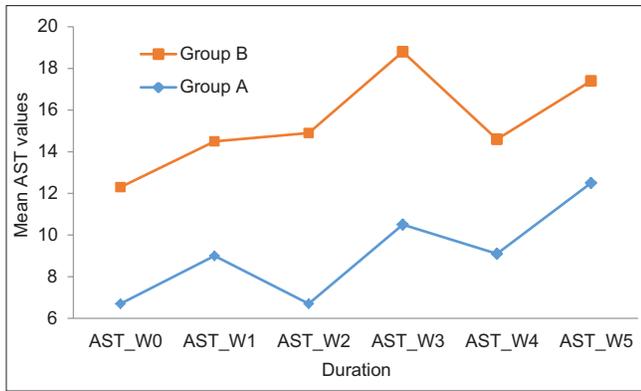
Therefore, the profile of TRAP enzyme levels obtained for Group A (Conventional Bracket) indicates that the resorption process by osteoclastic cells is active on days 28, 35, and 42 days. In this phase, the resorption process by osteoclasts involves the degradation of organic and mineral elements in the bone matrix (Hill 1998). Next, the bone remodeling process will take over the role of completing the bone modeling cycle during orthodontic treatment. This phenomenon can be seen when ALP activity used as an indicator of the presence of active osteoblast cells where a significant increase ( $P \leq 0.05$ ) on 28 and 35 days after the increase of TRAP or osteoclast activity. Similar studies by Perinetti *et al.* (2002) showed ALP activity increased in GCF after 7 days of orthodontic treatment was imposed on patients.

There was a significant increase ( $P \leq 0.05$ ) of TRAP enzyme levels of Group B (Self-ligating bracket) at week 4, meaning bone resorption taking place. ALP enzyme levels showed no significant difference. These enzyme biomarker profiles showed that the remodeling cycle in Group B (self-ligating bracket) might be completed earlier, thus shortening the treatment time. However, the enzyme levels in saliva were very low and thus did not present a clear picture of the remodeling. Therefore, saliva samples would require more sensitive detection methods to obtain a clearer picture of the remodeling cycle. The use of self-ligating bracket tends to induce less bone resorption and bone formation, with desired tooth movement.<sup>[14]</sup>

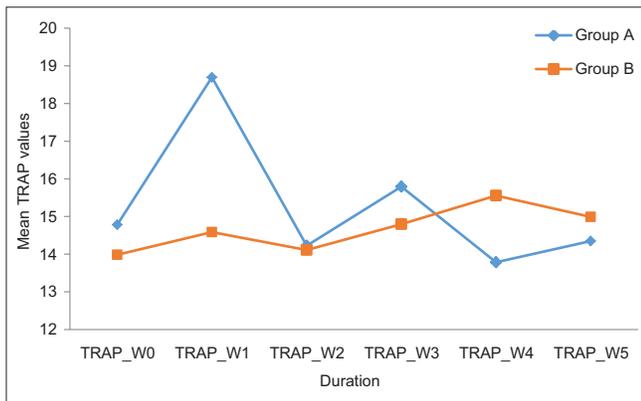
## CONCLUSION

### The conclusions drawn from the present study were

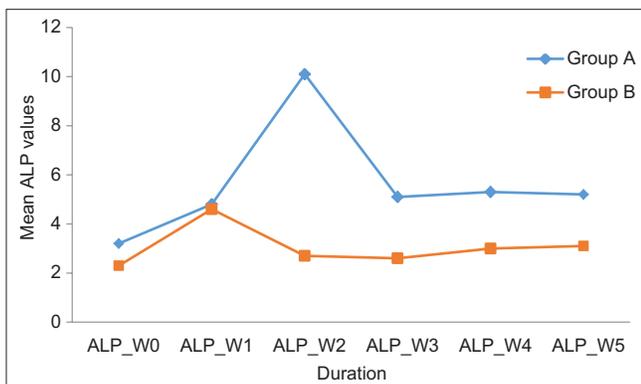
- During canine retraction with NiTi coil spring, the LDH salivary enzyme levels in Group A (conventional bracket)



Graph 2: Mean aspartate aminotransferase values



Graph 3: Mean tartrate-resistant acid phosphatase values



Graph 4: Mean alkaline phosphatase values

were increased at baseline to week 5 after the initiation of compressive orthodontic force

- During canine retraction with NiTi coil spring, the LDH salivary enzyme levels in Group B (self-ligating bracket) showed no significant difference from baseline to week 5 after the initiation of compressive orthodontic force
- During canine retraction with NiTi coil spring, the AST salivary enzyme levels in Group A (conventional bracket) showed no significant difference from baseline to week 5 after the initiation of compressive orthodontic force
- During canine retraction with NiTi coil spring, the AST

salivary enzyme levels in Group B (self-ligating bracket) showed no significant difference from baseline to week 5 after the initiation of compressive orthodontic force

- During canine retraction with NiTi coil spring, the TRAP salivary enzyme levels in Group A (conventional bracket) showed a significant difference from baseline to week 5 after the initiation of compressive orthodontic force
- During canine retraction with NiTi coil spring, the TRAP salivary enzyme levels in Group B (self-ligating bracket) showed no significant difference from baseline to week 5 after the initiation of compressive orthodontic force
- During canine retraction with NiTi coil spring, the ALP salivary enzyme levels in Group A (conventional bracket) showed no significant difference from baseline to week 5 after the initiation of compressive orthodontic force
- During canine retraction with NiTi coil spring, the ALP salivary enzyme levels in Group B (self-ligating bracket) showed no significant difference from baseline to week 5 after the initiation of compressive orthodontic force
- Comparison of LDH salivary enzyme levels between Group A and Group B at different time intervals where Group B (self-ligating bracket) shows a significant difference at week 0, week 1, and week 2 after the initiation of compressive orthodontic force
- Comparison of AST salivary enzyme levels between Group A (conventional bracket) and Group B (self-ligating bracket) at different time intervals where Group A shows a significant difference at week 5 after the initiation of compressive orthodontic force
- Comparison of TRAP salivary enzyme levels between Group A (conventional bracket) and Group B (self-ligating bracket) at different time intervals where Group A shows a significant difference at week 1 and Group B at week 4 after the initiation of compressive orthodontic force
- Comparison of ALP salivary enzyme levels between Group A (conventional bracket) and Group B (self-ligating bracket) at different time intervals where Group A and Group B showed no significant difference after the initiation of compressive orthodontic force [Graph 4].

**Generalized conclusion from the present study is**

- The LDH, AST, TRAP, and ALP level in Group A (Conventional Bracket) showed a significant increase after the initiation of orthodontic force
- Where the salivary enzyme levels of LDH, AST, TRAP and ALP in Group B (Self-ligating Bracket) showed no significant difference after the initiation of orthodontic force.

**Financial support and sponsorship**

Nil.

### Conflicts of interest

There are no conflicts of interest.

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