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Research Article

Salivary Lactate Dehydrogenase Activity in Correlation with Orthodontic Force Application

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Abstract

Orthodontic tooth movement generates mechanical forces to periodontal ligament and alveolar bone. The forces correlate with initial responses of periodontal tissues and involving many metabolic changes. One of the metabolic changes detected in saliva is lactate dehydrogenase (LDH) activity.

Objectives: To evaluate the correlation between orthodontic interrupted force applications, lactate dehydrogenase activity and the distance of tooth movement.

Methods: 140 Samples were collected from 20 Subjects , consisted of saliva at Pre-extraction of First Upper Premolar, Pre-retraction of Upper Canine and 1, 7, 14, 21 and 28 days Post-retraction of upper canine with100g interrupted orthodontic force.

Results: ANOVA showed significant differences between (LDH) activites and duration of force (F=11.926; *p=0.000*). Significant differences between Pre-retraction of Upper Canine group and 14 and 28 days Post-retraction of canine. The region of retraction correlated with the distance of tooth movement (F=7.377; *p=0.007*). The duration of force correlated with the distance of tooth movement (F=66.554; *p=0.000*). The differences were significant between Pre-retraction of Upper Canine group and 1, 7, 14, 21 and 28 days Post-retraction of canine.

Conclusion: This study concluded that orthodontic interrupted force application on canine could increase the distance of tooth movement and LDH activity in saliva.

Keywords: *Lactate Dehydrogenase; Crevicular Fluid; Orthodontic Force; Enzymes; Saliva*

Introduction

Biomechanics is the major principle in the forces application during orthodontic tooth movement [1]. Biomechanical response correlates with the changes of applied orthodontic force. Previous studies in biomechanics focused on bone metabolism [2]. Tooth movement by orthodontic force application is characterized by remodeling changes in dental and paradental tissues including dental pulp, periodontal ligament, alveolar bone and gingiva. When these tissues exposed to varying degrees of magnitude, frequency and duration of mechanical loading , they express extensive macroscopic and microscopic changes [3].

Orthodontic forces induce periodontal ligament and alveolar bone remodeling. It also stimulates inflammation reaction in the periodontal tissue. This reaction triggers alveolar bone resorption and apposition by inflammatory mediators [3]. The earlier responses of periodontal tissues to mechanical stresses involve several metabolic changes resulted in tooth movement. One of the metabolic changes is an increase activity of lactate dehydrogenase (LDH) which can be detected in saliva [4-7]. Lactate dehydrogenase is an enzyme normally present in the cytoplasm and release extracellularly when cells undergoing cell death. An increase of lactate dehydrogenase level has been

reported during bone remodeling due to orthodontic treatment [8]. It is believed that lactate dehydrogenase has the potential to be used as a marker for inflammation process during orthodontic treatment [9].

Previous studies have demonstrated the use of gingival crevicular fluid and saliva for diagnosis of Periodontitis [10]. Research showed that activity of LDH in gingival crevicular fluid (GCF) increased significantly after orthodontic force application [11]. Although a number of evidences have accumulated on the use of (GCF) for the diagnosis of periodontitis, this approach requires special technique for sampling. In addition, it is difficult to obtain (GCF) from all the sides of the dentition. GCF may be acceptable for clinical use but not for epidemiological purposes, and especially not for mass screening [10]. Recently, saliva has been used for diagnostic purpose because it has wide range of elements for diagnosis.

The use of saliva for diagnostic purposes is considered to be less invasive and relatively easy compared to other method. Furthermore, taking saliva as a sample does not cause stress for the patient [10,12]. Currently, there are three orthodontic force types based on their duration: Continuous, Interrupted and Intermittent Forces. Continuous force could be maintained at some appreciable friction of the original from one patient visit to the next. Interrupted force could decline to zero between activation. Intermittent force occur when a force level decline abruptly to zero intermittently once the orthodontic appliance is removed by the patient or when a fixed appliance is temporarily deactivated and return to the original level some time later. The intermittent force could become interrupted between adjustments of the appliance is performed [1]. The objective of this study was to evaluate the correlation of orthodontic interrupted force application on canine with lactate dehydrogenase activity and the distance of tooth movement. Interrupted force with module chain was used in this study for upper canine retraction.

Methods

Twenty orthodontic patients were included in this study. The inclusion criteria were as follows: men or women, age 12-40 years old, required orthodontic treatment with upper first premolar extraction and upper canine retraction, did not have ectopic tooth, good general health of tooth and mouth, good oral hygiene, did not have vertical or horizontal bone defects, and did not have consuming all medications or drugs including non-steroidal anti-inflammatory drugs and antibiotic drugs 1 month before the study period, did not have periodontal diseases and systemic diseases and willing to follow the research procedures by signing the informed consent. The study was initiated after receiving the signed informed consents from the patients. For each patient, 100g force measured with push-pull gauge (ORMCO*ETM–Lot No.05G46G, USA) was applied to retract upper canine distally. Previous study has reported the optimal force for orthodontic tooth movement was 70-120g [1]. Close module chain was used and attached from molar band hook to canine bracket hook. The distance of tooth movement was measured from distal side of bracket at lateral incisive to mesial side of bracket at canine using caliper 0.01 mm precision (Mitutoyo, No. NTD12-15PMX, Japan) at the Pre-retraction upper canine 1, 7, 14, 21 and 28 days post retraction upper canine. The saliva was collected simultaneously for LDH activity comparison. Pre-extraction and Pre-retraction LDH activity was measured and used as control groups.

One mL unstimulated saliva was collected using 1.5mL tubes. All subjects were asked to rinse the mouth with sterile water for approximately 10 seconds before saliva collection. The collected saliva samples were directly put on ice, transported to Immuno-endocrinology Division, College of Medicine, King Faisal University, Al-Ahsa, Kingdom of Saudi Arabia, and stored at -80°C freezer. The LDH activity was measured with LDH FS*IFCC reagent (DiaSys Daignostic System GmbH, D-65558 Holzheim, Germany). The absorbance value were detected with spectrophotometer at 340nm wavelength (BIO-RAD Smart Spec V3.00.13.e, Italy).One thousand µL reagent 1 containing phosphate buffer (50m mol/L) and pyruvate (0.60m mol/L) was incubated at 26°C for 1 minute. Then reagent 2 (Good's buffer and NADH) was added and the sample was incubated at 26°C for another 1 minute. One hundred microL of saliva was taken and put in the spectrophotometer to measure LDH activity. Statistical analysis of Kolmogorov Smirnov, ANOVA, HSD post hoc Tukey and Pearson Correlation were used for the test.

Results

Tooth movement and LDH activity reached their peaks at day 21 (Figure 1 and 2). The duration of force affected the distance of tooth movement (F=66.554, *p*=0.000), while the region of retraction correlated with the distance of tooth movement (F=7.377, *p*=0.007). It showed that the duration of force affected LDH activity (F=11.926, *p*=0.000).

The region of retraction was not correlated with duration of force (*p*=1.000), distance of tooth movement (*p*=0.079) nor LDH activity (*p*=1.000). This study demonstrated the correlation between duration of force with distance of tooth movement (*p*=0.000) and LDH activity (*p*=0.000), also the correlation exist between distance of tooth movement with LDH activity (*p*=0.000).

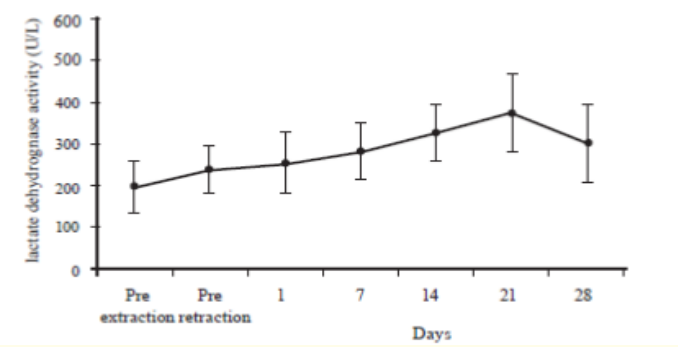

Figure 1: *Means and standard deviations of the distance of tooth movement (mm).*

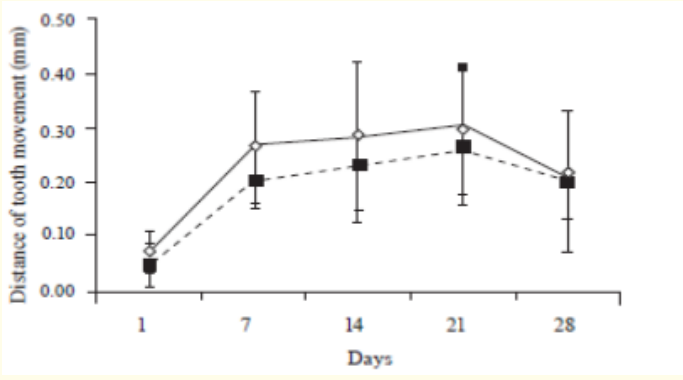

Figure 2: *Results of Measurement of LDH activity (U/L) at different time after orthodontic force application.*

Discussion

Orthodontic tooth movement occurs by the remodeling of the alveolar bone as a result of the force exerted on the periodontal tissue. When a force greater than capillary blood pressure applies to a tooth; hyaline zone occurs in the direction of force. This hyaline zone, free of cells, is necrotic area caused by osteoclast activity that originates from the tension site. On the tension site, osteoblasts occur in the bone apposition process [8]. The bone remodeling that occurs 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 [13]. Orthodontic tooth movement induces a biological process leading to bone resorption in the pressure sites and bone apposition in tension site. Histological studies showed that first wave of resorption occur in 3 to 5 days followed by its reversal in 5 to 7 days. This is followed by a late wave of bone formation between 7 and 14 days [11].

Lactate dehydrogenase, an enzyme normally limited to the cytoplasm of cell, is only released extracellularly after cell death. It is a functionally related intracellular, cytoplasmic enzyme that is release into the extracellular environment upon cell death. Its extracellular presence is related to cell necrosis and tissue breakdown. LDH activity measured from GCF have also been positively correlate with tissue inflammation during gingivitis and tissue destruction cause by periodontitis in humans [4].

LDH significantly increase in saliva on patients with periodontal diseases compared to healthy patients [5]. LDH is a ubiquitous enzyme that plays a significant role in the clinical diagnosis of pathologic processes. Screening periodontal disease by measuring salivary levels of LDH may be feasible, simple and convenient approach that does not require expert examiners [10]. In this study, the statistical tests showed that the LDH activity is affected by the duration of force. Moreover statistical test results also showed significant differences in LDH activity before and after bracket bonding. The reason might be due to stimulation of plaque retention at the bracket which cause gingival inflammation.

The present study was in line with research stated that increment of lactate dehydrogenase activity is often related to tissue inflammation commonly caused by gingivitis and periodontitis respectively [6]. This research showed that the activity of LDH increased significantly after 7days and reached their peak in 21 days by orthodontic force application. These features might occur because the initial wave of absorption is taken place from 3-5 days after the application of orthodontic force. This condition is similar with previous studies that showed the increased LDH activity at 7 to 21 days post-retraction of canine [11].

Increasing LDH activity was possibly caused by pressure on alveolar bone when canine retraction occurred during orthodontic treatment. Therfore, this LDH might be released since there were cells death during inflammation process [9]. This study also showed that the duration of orthodontic force affected the distance of tooth movement. Moreover, this study showed that tooth movements can be seen after 7 days applicaton of force. Furthermore, the peak movement of tooth was at 21 days after application of force and after that time the tooth movement is decreased. The initial wave of resorption occurred at 3-5 days after application of orthodontic force and followed by the opposite at 5-7 and the final wave of resorption occurred at 7 to 14 days which is similar to the results of this research and was in concordance with previous studies [11].

The average activity of LDH and tooth movement decreased after 21 days application of orthodontic force. The possibility in decreasing of LDH might be related to the presence of tissue repair and force decays from the module chain. Based on these findings it might be appropriate to do adjustment of orthodontic appliance after 21 days application of force.

Conclusion

Duration of orthodontic force application influence lactate dehydrogenase activity. Lactate dehydrogenase activity in saliva also correlates with the distance of tooth movement. As a summery, this study confirmed that , the level of lactate dehydrogenase in whole saliva could also add information about tooth movement activity related to orthodontic treatment.

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