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Lactate Dehydrogenase Activity During Tooth Movement under 1.0 N and 1.5 N Continuous Force Applications

(Aktiviti Laktat Dehidrogenase Semasa Pergerakan Gigi dengan Aplikasi Tekanan 1.0 N dan 1.5 N Secara Berterusan)

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ABSTRACT

The aim of this study was to observe the pattern of lactate dehydrogenase (LDH) activity in GCF and the rate of tooth movement at two different orthodontic forces (1.0 N and 1.5 N). Twelve subjects participated in this study and was chosen based on the inclusion criteria. Each subject received forces of 1.0 N and 1.5 N for tooth movement either on the left or right side of the maxillary canine. GCF sample was collected at mesial and distal sites of the canines before applying the appliance (week 0) and every week for 5 weeks after tooth movement (week 1 to week 5) where baseline activity served as control. LDH activity was assayed spectrophotometically at 340 nm. The tooth movements were measured from casted study models. LDH specific activity at mesial sites in 1.0 N and 1.5 N force groups, respectively increased significantly ($p<0.05$*) only on week four and throughout the treatment when compared with baseline. At distal sites, LDH specific activity with 1.5 N was higher than 1.0 N throughout the five weeks of tooth movement. LDH specific activity with 1.5 N force increased at both mesial (week 2) and distal sites (week 3) with significant different (*$p<0.05$*) when compared with 1.0 N force. The tooth movement with 1.5 N showed significantly faster (*$p<0.05$*) at the end of week 5 when compared with 1.0 N. LDH has the potential as a biological marker of inflammation during tooth movement. A force of 1 N was more suitable to be used although less tooth movement was produced because less inflammation caused by the force can be useful in orthodontic treatment for patients with stabilised periodontal diseases compared with 1.5 N force.*

Keywords: Biological marker; inflammation; lactate dehydrogenase; orthodontic force; tooth movement

ABSTRAK

Kajian ini bertujuan untuk melihat corak aktiviti laktat dehidrogenase (LDH) di dalam GCF dan kadar pergerakan gigi pada dua daya tekanan ortodontik yang berbeza (1.0 N dan 1.5 N). Dua belas orang subjek telah mengambil bahagian dalam kajian ini dan mereka dipilih berdasarkan beberapa kriteria yang telah ditetapkan. Setiap subjek menerima 1.0 N dan 1.5 N daya tekanan untuk pergerakan gigi sama ada pada bahagian kanan atau kiri gigi taring maksila. Sampel GCF dikumpul dari bahagian mesial dan distal gigi taring sebelum dipakaikan pendakap gigi (minggu 0) dan setiap minggu untuk lima minggu selepas gigi digerakkan (minggu 1 hingga minggu 5) dengan aktiviti basal dijadikan sebagai kawalan. Aktiviti LDH diasai menggunakan pendekatan spektrofotometri pada 340 nm. Pergerakan gigi diukur daripada model-model kajian yang telah dibentuk. Aktiviti spesifik LDH pada bahagian mesial dalam kumpulan tekanan 1.0 N dan 1.5 N masing-masing meningkat secara signifikan ($p<0.05$*) hanya pada minggu 4 dan sepanjang rawatan berbanding kawalan. Pada bahagian distal, aktiviti spesifik LDH dengan 1.5 N adalah lebih tinggi berbanding 1.0 N sepanjang lima minggu pergerakan gigi. Aktiviti spesifik LDH dengan tekanan 1.5 N meningkat (*$p<0.05$*) pada kedua-dua bahagian mesial (minggu 2) dan distal (minggu 3) berbanding tekanan 1.0 N. Pergerakan gigi dengan 1.5 N lebih pantas (*$p<0.05$*) pada akhir minggu 5 berbanding dengan 1.0 N. LDH berpotensi sebagai penanda biologi untuk inflamasi semasa pergerakan gigi. Daya tekanan 1.0 N berbanding 1.5 N lebih sesuai digunakan walaupun ia menghasilkan kurang pergerakan gigi kerana penghasilan inflamasi yang rendah adalah penting dalam rawatan ortodontik kepada pesakit periodontal yang telah stabil.*

Kata kunci: Inflamasi; laktat dehidrogenase; penanda biologi; pergerakan gigi; tekanan ortodontik

INTRODUCTION

Application of an appropriate orthodontic force to a tooth during orthodontic treatment results in tooth movement. Orthodontic force is an extrinsic mechanical stimulus that elicits a biologic cellular response in order to restore equilibrium of the periodontal supporting tissues (Ren et al. 2003). Theoretically, orthodontic force application will generate a pressure side and tension side within the periodontal ligament (Roberts-Harry & Sandy 2004). Based on this, orthodontic treatment depends upon remodelling

of the periodontal ligament (PDL), gingival soft tissue and alveolar bone (periodontium) in order to allow tooth movement, which is when tissue is removed ahead and deposited behind the tooth (Serra et al. 2003).

Orthodontic force application of tooth movement is characterized by remodelling changes in dental and periodontal tissues, including dental pulp, PDL, alveolar bone and gingiva. When these tissues are exposed to varying degrees of magnitude, frequency and duration of mechanical loading, they will express extensive macroscopic and microscopic changes (Krishnan & Davidovitch 2006). The changes produced a number of substances such as interleukin-1 and alkaline phosphatase that are involved in the bone remodelling process which later can diffuse into the gingival crevicular fluid (GCF) (Kavadia-Tsatala et al. 2002). Therefore, GCF sample analysis could help in understanding the on-going biochemical processes associated with the bone turnover during orthodontic tooth movement.

Gingival crevicular fluid is an exudate or transudate that arises at the gingival margin. The transudate normally will become an exudate under tissue inflammation (Lamster & Ahlo 2007). Many recent studies have performed non-invasive analyses of various cell mediators or enzymes in the GCF to better describe biological responses to orthodontic force in human. In the GCF, studies have reported significant elevations in alkaline phosphatase (Perinetti et al. 2002) and aspartate aminotransferase (Perinetti et al. 2003; Rohaya et al. 2008) activities during the first month of orthodontic treatment. In another study, tartrate resistant acid phosphatase activity in GCF peaked significantly during the first month of tooth movement (Rohaya et al. 2011). These findings demonstrated that several processes, such as inflammation and cell death, can occur in the periodontal tissues surrounding the mechanically stressed teeth (Roberts-Harry & Sandy 2004).

Orthodontic treatment for patients with periodontal disease differs considerably from that performed in subjects with a healthy periodontium. Periodontal disease such as periodontitis occurs when inflammation takes place at the gingiva and spreads to the ligament and bone that support the teeth. However, in tooth movement, inflammation is the important event which developed after the application of the orthodontic force and followed with tooth movement. Therefore, one should be aware of the treatment sequence for periodontal patients, including the use of lighter force with greater moment and force ratios (Jin 2007). Moreover, absolute magnitude of orthodontic force can be reduced and a countervailing moment must be applied accordingly.

Lactate dehydrogenase (LDH) is an essential cytoplasmic enzyme present in all major organ systems and the appearance of LDH at extracellular occur after cell damage or cell death (Drent et al. 1996). Therefore, LDH activity in GCF has been suggested as a potential marker for observing periodontal metabolism. There are few studies on LDH as a biological marker for inflammation, which is considered as a phenomenon in tissue destruction that leads to tooth movement. A recent study of LDH activity during orthodontic treatment has shown significant increasing levels of LDH activity but in this study, only one type of orthodontic force (1.25 N) was use to the test teeth (Sarah & Sukumaran 2011). Therefore, the objective of this study was to observe the pattern of LDH activity in GCF and rate of tooth movement when two different orthodontic forces, (1.0 N and 1.5 N) were applied. The hypothesis of this study was that LDH specific activity in GCF show a different activity profile and rate of tooth movement when applied with different forces and 1.0 N force produced less inflammation than 1.5 N forces.

Material and Methods

The study was performed from March until August 2011. All experiments involving the orthodontic procedures/ samplings were conducted at the Orthodontic Postgraduate Clinic, Department of Orthodontics, Dental Faculty, Universiti Kebangsaan Malaysia while experiments involving laboratory analysis were conducted at the School of Bioscience and Biotechnology, Universiti Kebangsaan Malaysia.

Subject Selection

Twelve healthy orthodontic subjects (14-24 years old) were selected for the study. The subjects were given periodontal prophylaxis treatment, full mouth scaling and polishing prior to the study to ensure maintenance of good oral hygiene. An informed consent was obtained from the subjects or guardian or parents (patients below 16 years old) in order to participate in this study. The study has been approved by the Research Ethical Committee of Universiti Kebangsaan Malaysia (No: 1.5.3.5/244/DD/030(1)/2010). The inclusion criteria of the subjects selected are as follow: healthy with no known systemic disease and good general and periodontal health; do not taking any anti-inflammatory drugs and mouthwash containing chlorhexidine; not pregnant (as stated by the patient); mild to moderate crowding of the maxillary and mandibular arch; canine relationship of class II ½ unit or more; class II/I incisal relationship with over jet more than 6 mm; overbite not more than 50%; no previous orthodontic and orthopaedics treatment and no craniofacial anomalies.

Orthodontic Appliances and Experimental Teeth

A Nance appliance was fitted to the maxillary first molars prior to the maxillary first premolars extractions. The buccal surface of the maxillary teeth; incisors, canine and second premolars were bonded with a 0.056 cm × 0.071 cm pre-adjusted edgewise appliance (American Orthodontics, Mini Master; McLaughlin Bennet Trevisi (MBT) prescription). The initial alignment was acquired with a 0.036 cm Nickel Titanium (NiTi) archwire and the levelling and alignment stage was completed when a 0.046 cm × 0.064 cm NiTi was attained (around three to four

consecutive visits). The working archwire of 0.048 cm × 0.064 cm stainless steel archwire was inserted and left *in situ* for four weeks to allow passivity of the archwire before tooth movement stage. Tooth movement was performed on a 0.048 cm × 0.064 cm stainless steel using a NiTi push coil spring (sds Ormco). The NiTi coil spring was placed between the maxillary lateral incisor and maxillary canine. These teeth were ligated with a 0.023 cm stainless steel ligature wire to prevent rotation. In a split-mouth design, subjects received a 1.0 N or 1.5 N orthodontic force either on the right or left side of maxillary arch. The side of the force was determined through 'toss of coin'. The force applied was measured using a Correx gauge (dial-type stress and tension gauge; Dentaurum Germany). All four maxillary incisors were also ligated together using a 0.023 cm stainless steel ligature wire to increase anterior anchorage. Subjects were reviewed on weekly basis for six consecutive weeks of tooth movement and GCF were collected during the reviews (week 0 to week 5) where week 0 activity served as the control. GCF baseline was collected a week before the placement of appliance.

GCF SAMPLING

GCF was collected from mesial and distal sites of test teeth before placement of appliance (baseline), at week 0 (before tooth movement), 1, 2, 3, 4 and 5 using methylcellulose filter paper strips (Periopaper, Proflow, Amityville, N.Y.). Before GCF collection, any supragingival plaque was removed from sampling sites and isolated using cotton rolls together with gently dried of tooth surface using air stream for 5 s. The periopaper strip was inserted 1 mm depth into the gingival sulcus of each site and left *in situ* for 60 s (Perinetti et al. 2011) before placed into 1.5 mL microcentrifuge tube containing 80 µL of normal saline (0.9% w/v sodium chloride). After that, the tube was centrifuged for 10 min at 4°C and 4,000 × g using centrifuge machine (Hettich Zentrifugen Mikro 22R) to elute the GCF component completely. The sample was analysed immediately.

LACTATE DEHYDROGENASE ASSAY

LDH activity was determined spectrophotometrically at 340 nm wavelength using a Varian Cary 50 UV-Vis spectrophotometer. Sample was added into a test tube containing 16.2 M of natrium pyruvate, 54 M of phosphate buffer, pH 7.4 and 0.2 M of NADH after 5 min incubation at 30°C in a total volume of 1.2 mL. The change in the absorbance was recorded every 30 s for 3 min. Then, the result was converted to enzyme activity units (1 U = 1 mol NADH consumed per minute at 30°C). The final results were reported as LDH specific activities, which were determined based on units of activity (U) per total protein content in a milligram (mg) and were stated as U/mg. The protein content of the sample was determined using Bradford's method (1976) with some modification and standard curve of bovine serum albumin.

CANINE MOVEMENT MEASUREMENT

Canine movement was measured using a digital caliper (KERN, Germany) with a sensitivity of ± 0.01 mm from the distal margin of the lateral incisor bracket to the mesial margin of the canine bracket from the dental cast fabricated at every visit. Then, cumulative canine distances were obtained at the end of the experimental term.

STATISTICAL ANALYSIS

Normality distribution of the data was determined using the Kolmogorov-Smirnov test. As the data were normally distributed, t-test was used to compare the LDH specific activity at a different time (week) with baseline between different forces application and sites and also cumulative canine movements (mm) with times (week) between the 1.0 N and 1.5 N groups with $p<0.05$ was considered as significant using the statistical R-2.7.1 program.

RESULTS

Twelve healthy orthodontic subjects with age ranging from 14 to 24 years completed this study successfully. The mean age of the subjects was 19.7 ± 5.0 years. The changes in LDH activities during tooth movement under the 1.5 N and 1.0 N force were observed from week 0 to week 5 at both mesial and distal sites of maxillary canines with baseline (before the placement of appliance) acted as a control.

In the 1.0 N group (Table 1), LDH specific activity at mesial sites of test teeth was higher than the baseline at week 0 to week 4. However, the increment at week 1 to week 3 showed no significant differences ($p>0.05$) while at week 4, the increment was statistically significant ($p<0.05$) than the baseline when analysed using paired t-test. In contrast, at week 5, LDH specific activity decreased towards the baseline value with no significant differences ($p>0.05$). At the distal sites, LDH specific activity decreased at week 1 to week 3 than the baseline but showed no significant differences ($p>0.05$). At week 4, the activity was slightly higher than the baseline but showed no significant differences ($p>0.05$). Later, at week 5 the activity decreased when compared with baseline with no significant differences ($p>0.05$).

In the 1.5 N group (Table 2), LDH specific activity at mesial sites of test teeth increased from week 1 to week 5 and the increment of LDH specific activity at week 1 to week 5 showed significant increases ($p<0.05$) when compared with baseline. At the distal sites, LDH specific activity significantly decreased ($p<0.05$) at week 1 when compared with baseline. Later, at week 2 to week 4, the activity of LDH increased when compared with baseline but showed no significant differences ($p>0.05$) as compared with baseline. Lastly at week 5, the LDH activity decreased when compared with baseline but showed no significant differences ($p>0.05$).

LDH activities were also compared between the 1.5 N and 1.0 N of orthodontic forces (Figure 1) at both mesial

TABLE 1. Mean differences of LDH specific activity between test teeth and baseline at different week on mesial and distal sites of maxillary canine with 1.0 N orthodontic force

Baseline (B)	Week (W)	Mesial	t-test	Distal	t-test
		Mean Difference ± SE ($\times 10^{-5}$) [W-B]		Mean Difference ± SE ($\times 10^{-5}$) [W-B]	
Before appliance placement	1	0.38 ± 0.4	NS	-0.06 ± 0.45	NS
	2	0.28 ± 0.20	NS	-0.40 ± 0.24	NS
	3	0.03 ± 0.59	NS	-0.45 ± 0.49	NS
	4	0.83 ± 0.31	S	0.29 ± 0.72	NS
	5	-0.61 ± 0.45	NS	-0.18 ± 0.38	NS

Data presented as mean differences ± standard error of specific enzyme activity (n=12) with unit of U/mg. NS, no significant difference and S, significant difference (p<0.05)

TABLE 2. Mean differences of LDH specific activity between test teeth and baseline at different week on mesial and distal sites of maxillary canine with 1.5 N orthodontic force

Baseline (B)	Week (W)	Mesial	t-test	Distal	t-test
		Mean Difference ± SE ($\times 10^{-5}$) [W-B]		Mean Difference ± SE ($\times 10^{-5}$) [W-B]	
Before appliance placement	1	0.67 ± 0.23	S	-1.14 ± 0.52	S
	2	0.77 ± 0.47	S	0.27 ± 0.37	NS
	3	0.60 ± 0.25	S	0.37 ± 0.48	NS
	4	0.95 ± 0.33	S	0.56 ± 0.38	NS
	5	0.80 ± 0.34	S	-0.42 ± 0.38	NS

Data presented as mean differences ± standard error of specific enzyme activity (n=12) with unit of U/mg. NS, no significant difference and S, significant difference (p<0.05)

and distal sites. At the mesial sites, LDH activity for 1.0 N force group peaked at week 4 but showed no significant differences (p>0.05) when compared with 1.5 N force group. On the other hand, the 1.5 N force group produced highest LDH activity at week 2 and showed significant differences (p<0.05) when compared with 1.0 N force. Furthermore, at the distal sites, LDH activity was highest at week 4 for 1.0 N force group but showed no significant differences (p>0.05). LDH specific activity of 1.5 N force was higher than 1.0 N force at the distal sites throughout five weeks of treatment with only week 3 produced significant differences (p<0.05) when compared with 1.0 N force. The higher LDH activity with 1.5 N force indicated that more inflammation and more tooth movement occur at the test teeth.

The cumulative canine movement of the test teeth is shown in Figure 2. There was a linear relationship of cumulative canine movement (mm) over time (week 0 to week 5) for both 1.5 N and 1.0 N groups. The mean of cumulative canine movement over five weeks of treatment was 2.36 ± 0.28 mm with 1.5 N force and at the rate of 0.47 mm per week as compared with 1.85 ± 0.27 mm with 1.0 N force and at the rate of 0.37 mm per week. Therefore, maxillary canine with 1.5 N moved faster than those with 1.0 N force, however only showed significant differences (p<0.05) at week 5 using t-test when compared between 1.0 N and 1.5 N force groups.

DISCUSSION

There are four phases involved in the tissue remodelling cycle, i.e. activation, resorption, reversal and formation (Wise & King 2008). Bone remodelling process will occur when orthodontic force is applied during orthodontic treatment. An acute inflammatory response is known to be the early phase during orthodontic tooth movement (activation). It is distinguished by periodontal vasodilatation and leukocyte migration from the periodontal ligament capillaries (Apajalahti et al. 2003). When a force is applied to a tooth, the periodontal tissues undergo either tension or compression stress, depending on the tooth movement (Roberts-Harry & Sandy 2004). Therefore, in this study, we investigated the enzyme specific activities at mesial (tension) and distal (compression) sites. Early studies in rat showed that bone resorption occur in the compression sites (Rygh 1972) whereas, bone deposition in the tension sites (Rygh 1976).

The presence of LDH at extracellular space is known related to cell necrosis and tissue breakdown (Victor et al. 2007). For this reason, LDH was studied as a biomarker for

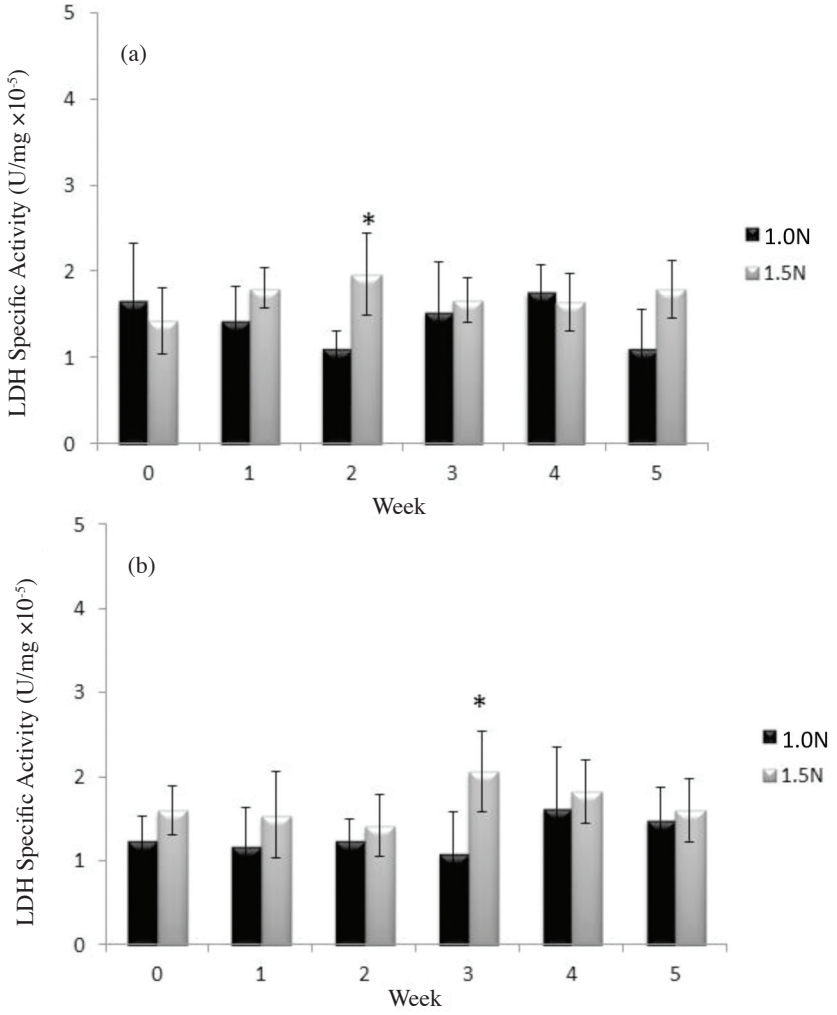

FIGURE 1. Specific activities of LDH with standard error at mesial and distal canine of 1.5 N and 1.0 N orthodontic forces. (a) LDH activity of mesial sites with 1.0 N and 1.5 N and (b) LDH activity of distal sites with 1.0 N and 1.5 N, * : statistically significant (p<0.05).

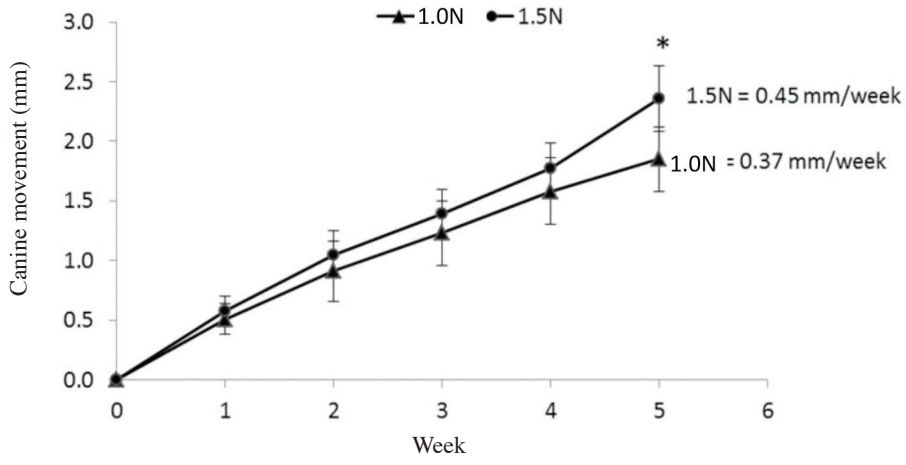

FIGURE 2. Comparison between mean cumulative of maxillary canine movement (in mm) with standard error of 1.5 N and 1.0 N orthodontic forces over 5 consecutive weeks (n=12), *: statistically significant (p<0.05)

inflammation during tooth movement (Perinetti et al. 2005; Sarah & Sukumaran 2011; Shahrul Hisham et al. 2010) as the inflammation process is a part of the tissue remodelling cycle. Most of the studies performed have used GCF to test various cell mediators or presence of enzymes during tooth movement (Asma et al. 2011; Rohaya et al. 2011). Until now, only a few studies have investigated LDH levels in GCF during orthodontic tooth movement (Roberts-Harry & Sandy 2004). These studies have indicated that the LDH level in GCF could reflect the biologic activity in the periodontium during orthodontic tooth movement.

Orthodontic force applied during orthodontic treatment is optimal when it can move teeth efficiently from original position into their desired position, without causing discomfort or tissue damage to the patient (Toms et al. 2002). In periodontal diseased patients, a lighter force should be used. In this study, 1.0 N force group was compared with 1.5 N force group. The results showed slightly higher of LDH specific activity with 1.5 N force than 1.0 N force both at mesial and distal sites. Moreover, LDH specific activity peaked earlier with 1.5 N force at mesial and distal sites compared with 1.0 N force. These findings suggested that 1.5 N force may be a better force than 1.0 N force as it produced more inflammation hence faster tooth movement. However, the 1.0 N force being the lighter force showed delayed peak activity at week 4 as compared with week 2 and 3 for 1.5 N force. Therefore, 1.0 N force is more suitable to be used in stabilised periodontal diseased patients. This is because an increase in the magnitude of the force may cause greater pressure on the periodontal ligament due to the apical shift in the centre of resistance. This may finally increase the damage to periodontal tissues and the root (Jin 2007).

The maxillary canine with 1.5 N force moved significantly faster at week 5 than those with 1.0 N force (Figure 2). These are similar to past studies which reported the distal movement of canines in orthodontic patients with an optimum range of pressure (1.5 N-2.0 N) on the tooth-bone interface produced a faster rate of tooth movement compared with lower force such as 1.0 N force (Wise & Keeling 2008). In another study, the magnitude of the mean horizontal tooth movement significantly increased 50% when a force of 2.0 N was applied as compared with 0.5 N force (3.4-5.1 mm on average) (Py et al. 1996).

Specific activity of LDH during orthodontic treatment was observed for 5 weeks. LDH specific activity with 1.5 N force at the mesial sites in this study was significantly increased throughout five weeks of treatment as compared with the baseline. This is supported by a previous study which LDH specific activity increased significantly at test teeth than control teeth although the study does not distinguish between compression and tension sites (Sarah & Sukumaran 2011). Furthermore, application of force to the teeth has initiated two changes: tissue damage with the subsequent production of inflammatory processes in the periodontal ligament and bone resorption process (Serra et al. 2003). The LDH activity in the test teeth has increased from week 1 to week 5 and week 4 of treatment using 1.5 N and 1.0 N forces, respectively. The increase of enzyme activity might be as a result of tissue resorption in both the compressed and tensional sites (King et al. 1991) and cell necrosis in the periodontal ligament during the orthodontic treatment (Serra et al. 2003).

Moreover, our results were also in agreement with an earlier study which investigated LDH activity during orthodontic tooth movement when 1.25 N force was applied to test teeth (Sarah & Sukumaran 2011). The results revealed significantly higher LDH activity on the 7th, 14th and 21st day at the sites where orthodontic force had been applied. This finding was similar to our result although we used different force.

Conclusion

In this study, LDH in GCF was shown to be a potential biological marker for monitoring inflammation during orthodontic tooth movement. The LDH specific activity at tension and compression sites peaked earlier at week 2 during the tooth movement with 1.5 N than 1.0 N force (week 4). The specific activity of the enzyme and the rate of tooth movement showed variation according to the type of orthodontic force used. The 1.5 N force was shown to produce higher LDH activity and rate of tooth movement as compared with 1.0 N force. However, it is suggested that 1.0 N force is more suitable to be used in patients with stabilised periodontal conditions.

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