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Evaluation of Lactate Dehydrogenase Levels In Gingival Crevicular Fluid during Orthodontic Tooth Movement an *In-Vivo* Study.

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ABSTRACT

The purpose of the present in vivo study is to estimate the levels of lactate dehydrogenase enzyme in gingival crevicular fluid during orthodontic tooth movement. Twenty orthodontic subjects all requiring first premolar extractions were selected and treated with conventional straight wire mechanothreapy. Canine retraction was done using 125 g Nitinol closed coil spring. The maxillary canine on one side served as the experimental site while the contralateral canine served as the control. The gingival crevicular fluid samples were then collected from the experimental and control canines before the commencement of retraction, 1 hour after the initiation of canine retraction, followed by 1, 7 , 14 and 21 days. The LDH levels in the experimental site showed a steady increase from 0hour, 1hour, 1day and 7 days followed a steep increase from 7 to 14 days and a mild increase from 14 to 21 days. The difference between each of the group was statistically significant except between 0 hour and 1 hour. The levels of LDH in GCF on the control site revealed a mild increase from 0hour to 21 days, but the difference between the time groups were not statistically significant. It can be concuded that when constant, continuous and optimal orthodontic forces are applied activity of LDH in GCF showed a marked increase during orthodontic tooth movement with a statistically significant increase between 7th and 14th days compared with the control site, with the initiation of canine retraction.Hence, Lactate dehydrogenase, biomarker, gingival crevicular fluid, orthodontic tooth movement, canine retraction

Keywords: Lactate dehydrogenase; biomarker; gingival crevicular fluid; orthodontic tooth movement; canine retraction

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INTRODUCTION

Orthodontic force is an extrinsic mechanical stimulus that evokes a cellular response to restore equilibrium of the periodontal supporting tissues.Application of orthodontic force creates a pressure side and tension side within the periodontal ligament. Orthodontic tooth movement induced by mechanical stress is characterized by remodeling changes in the surrounding periodontium [1].

The strain induced by orthodontic force initiates inflammatory response such as alteration in blood flow and release of certain chemical mediators of inflammation such as cytokines, growth factors, neurotransmitters, arachidonic acid metabolites, colony stimulating factors, etc. The released mediators initiates the cellular response in various cell types in the tooth and surrounding periodontium and provides a favorable microenvironment for tissue deposition or resorption [2]. Subsequently various cell signaling pathways are activated that stimulates periodontal ligament turnover as well as alveolar bone resorption and deposition.

A biomarker is a substance that can be measured and evaluated objectively as an indicator of normal biological or pathogenic process [3]. Various biomarkers like alkaline phosphatase, aspartate aminotransferase, cathepsin B, myeloperoxidase, osteocalcin, interleukin 1β, interleukin 6, etc have been identified and proposed to be involved in orthodontic tooth movement.

Current studies support the use of gingival crevicular fluid for research purpose to study orthodontic tooth movement because of its non-invasive nature and repetitive sampling from the same site. Gingival crevicular fluid (GCF) is an inflammatory exudate that seeps into gingival crevices or periodontal pockets around teeth with inflamed gingiva [4]. The flow rate of gingival crevicular fluid is 0.05 to 20µl/min and the total fluid flow is between 0.5 and 2.4 ml/day.[5] Since 1960, when it was first suggested that analysis of GCF might be a way to quantitatively evaluate the inflammatory status of gingival and periodontal tissues, there has been intense interest in the diagnostic potential of GCF [5]. Recently, a number of GCF constituents have been shown to be diagnostic markers of active tissue destruction in periodontal diseases [6]. Therefore biochemical analysis of GCF provides a non- invasive model for investigating the cellular response of underlying PDL during orthodontic tooth movement [7]. The substances that are involved in bone remodeling are expressed in GCF by diffusion.

Lactate dehydrogenase (LDH) is an intracellular enzyme present in cell cytoplasm and is released extracellularly only after necrosis of the cell [8]. Lactate dehydrogenase exist in four distinct isoenzyme classes. Two of them are cytochrome - c dependent enzymes and the other two are nicotinamide adenine dinucleotide phosphatase - NAD(P)-dependent enzymes. Lactate dehydrogenase catalyzes the interconversion of pyruvate and lactate with concomitant interconversion of NADH and NAD^+. It converts pyruvate, the final product of glycolysis, to lactate when oxygen is absent or in short supply. Previous studies show lactate dehyrogenase activity increases in gingival crevicular fluid during gingival inflammation and periodontitis [9].

Recent studies have only evaluated lactate dehydrogenase as a biomarker for periodontal metabolism. But very few studies have been undertaken to evaluate the possible relation between lactate dehydrogenase and orthodontic tooth movement. Therefore the purpose of this study was to evaluate the lactate dehydrogenase levels in gingival crevicular fluid during orthodontic tooth movement as a biomarker for monitoring periodontal metabolism. The present study will help to improve our knowledge of molecular basis of orthodontic tooth movement and to monitor the progress of treatment.

MATERIALS AND METHODS

Study population

Twenty orthodontic subjects including 11 males and 9 females in the age group of 12- 25 years requiring fixed orthodontic appliance treatment constituted the sample. Comprehensive procedural information was given to all patients and written informed consent obtained. Ethical clearance was obtained from the Instuitional Ethical Committee

Inclusion Criteria

- Patients requiring fixed orthodontic treatment with extraction of upper first premolar
- Subjects with good health between 12-30 years with no predilection for sex
- Healthy periodontium with the gingival sulcus probing depth of ≤ 2mm
- No use of antimicrobial and anti-inflammatory drugs within 3 months before the baseline examination
- No radiographic evidence of bone loss

Exclusion Criteria

- Subjects with known history of systemic disorders
- Subjects who underwent periodontal treatment

Experimental design

All subjects underwent first premolar extraction and were treated with pre adjusted edge wise appliance 0.022" slot MBT prescription (Gemini, 3M Unitek) brackets bonded to incisors, canines and pre molars and bands on molars. After leveling and aligning, canine retraction was done on a base arch wire of 0.019 x 0.025"Stainless steel from molar hook to canine hook by a 9mm Nitinol closed coil spring(3M Unitek Monrovia)(Figure.1) exerting 125 g force measured by dontrix gauge (Figure.2). The maxillary canine on one side served as the experimental site while the contralateral canine served as the control.

Periodontal Screening

All the patients were subjected to oral prophylaxis two weeks prior to sample collection and strict oral hygiene instructions were given. Patients were instructed to avoid any medications during the course of the study period.

Gingival Crevicular Fluid Collection

Gingival crevicular fluid was collected using Hirschmann micropipette (BioVision Incorporated CA 95035 USA) (Figure.3) graduated from 1 µl to 5 µl. The teeth were gently dried with air spray and isolated with cotton rolls. Retraction of cheeks was done with cheek retractor. The gingival crevicular fluid samples were then collected by placing the micropipette at mesio labial line angle, mid labial surface, disto labial line angle, mesio palatal line angle, mid palatal surface and disto palatal line angle from the experimental and control canines, before the commencement of retraction, 1 hour after the initiation of canine retraction, followed by 1 day, 7 days, 14 days and 21 days (Figure 4&5; Table 1). Care was taken to avoid blood and saliva contamination.

Table 1: Time interval

T_0	Baseline
T_1	One hour after initiation of retraction
T_2	One day after initiation of retraction
T_3	7 days after initiation of retraction
T_4	14 days after initiation of retraction
T_5	21 days after initiation of retraction

Sample storage

The collected GCF samples were transferred to 1.5ml Eppendorf tubes (Figure.6&7) and sealed. Then it was labelled and stored at a temperature of -80°C (Figure.8 & 9) until the assay was performed.

GCF Lactate Dehydrogenase Assay

The activity of lactate dehydrogenase in gingival crevicular fluid samples were measured and analyzed spectrophotometrically and compared with the control site. The analysis was done using Lactate

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Dehydrogenase Activity Calorimetric Assay Kit (BioVision Incorporated CA 95035 USA) which contains LDH assay buffer, LDH substrate mix and LDH Positive Control (Figure.10 &11; Table 2). The reaction mix was prepared by mixing 48 µl Assay Buffer and 2 µl Substrate Mix Solution. Then the reaction mix was added to the GCF sample in the 96 well flat bottomed ELISA plates using micropipettes (Figure.12). In this colorimetric LDH quantification assay, LDH reduces NAD to NADH, which then interacts with a specific probe to produce a color (λmax = 450 nm) which was analyzed using spectrophotometer (Figure.13 &14). Results were first converted into enzyme activity units (1 unit = 1 µmol of NAD+ released per minute at 30°C) and finally expressed as total lactate dehydrogenase activity (µmol units/L) per sample.

Table 2: Lactate Dehydrogenase Activity Calorimetric Assay Kit contents

Components	K726-500	Cap Code	Part Number
LDH Assay Buffer	50 ml	NM	K726-500-1
LDH Substrate Mix (lyophilized)	1 vial	Amber	K726-500-2
NADH Standard (0.5µmol; lyophilized)	1 vial	Yellow	K726-500-3
LDH Positive Control	0.02 ml	Red	K726-500-4

Statistical Analysis

Descriptive statistics including mean, standard deviation and standard error were calculated for GCF lactate dehydrogenase levels of the test tooth and control tooth(Table 3). LDH levels at different time intervals were compared and analyzed using one way ANOVA and followed by Tukey HSD (Honestly Significant Difference) test. The data thus collected were assessed using SPSS (Statistical Package for Social Sciences IBM software).

Figure 1: NiTi Closed Coil Spring (9mm)

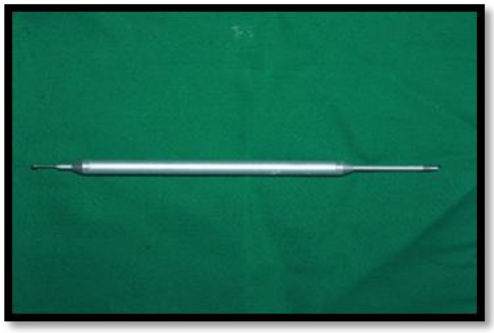

Figure 2: Dontrix Gauge

Figure 3: Hirschmann Micropipettes

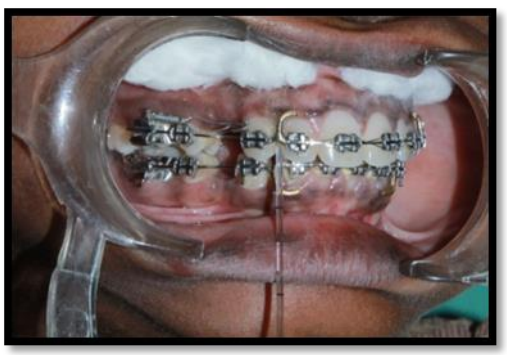

Figure 4: GCF sample collection –Experimental site

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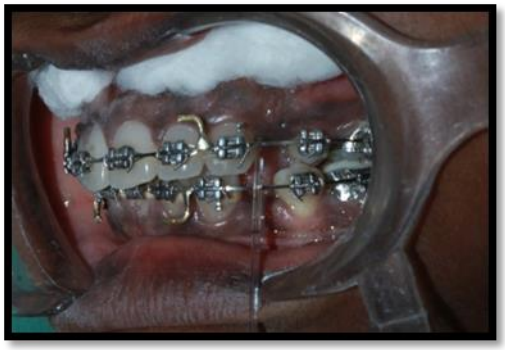

Figure 5: GCF sample collection – Control site

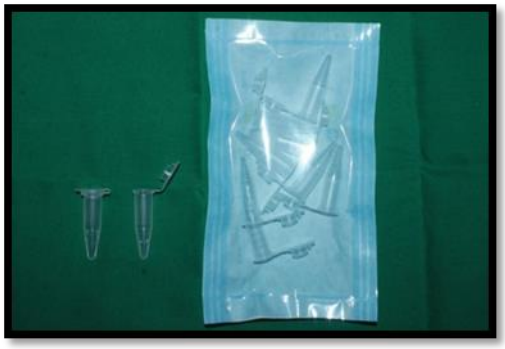

Figure 6: Ependorf Tubes

Figure 7: Collected GCF samples

Figure 8: Labeling of GCF samples

Figure 9: Storage of GCF samples

Figure 10: LDH Assay Buffer and Substrate Mix

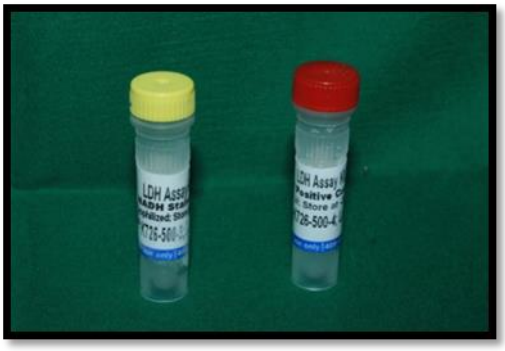

Figure 11: NADH standard and LDH positive control

Figure 12: Micropipettes

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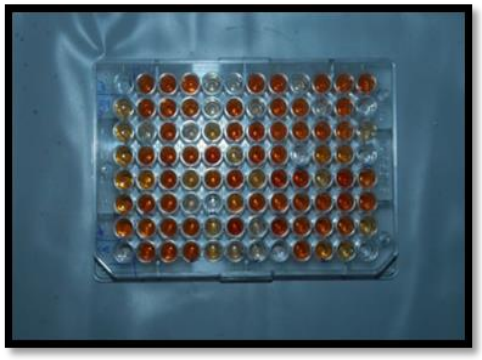

Figure 13: Colour formation during of GCF sample

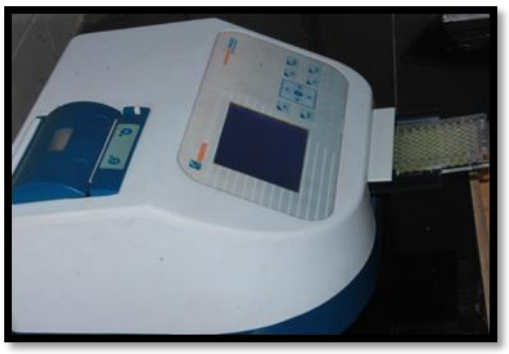

Figure 14: Spectrophotometric analysis Calorimetric assay

RESULTS

The gingival crevicular fliud samples were collected from experimental canine which was retracted using 9mm NiTi closed coil springs exerting 125g of force, and also from the contralateral canine that served as control without any retraction force. The sample collection was done at baseline, 1 hour, 1 day, 7 days, 14 days and 21 days after the initiation of canine retraction. The amount of NADH(Nicotinamide adenine dinucleotide reduced) formed by the reduction of NAD(Nicotinamide adenine dinucleotide) that reflected the activity of lactate dehydrogense in gingival crevicular fliud samples were analysed using spectrophotometer.

The mean, standard deviation and standard error were calculated for the obtained results. One way ANOVA and Tukey HSD test was used to test for any statistical significant difference in several related samples within either the control or experimental site at different time intervals

Table 3: Descriptive statistical data of LDH activity at different time intervals

		N	Mean	Std.Deviation	Std. Error	95%Confidence Interval for Mean
						Lower Bound
Time_0	control	20	194.8837	25.12742	5.61866	183.1237
	Experimental	20	195.8293	25.38198	5.67558	183.9501
	Total	40	195.3565	24.93373	3.94237	187.3823
Time_1	control	20	200.0692	24.08498	5.38557	188.7971
	Experimental	20	222.3967	16.47405	3.68371	214.6867
	Total	40	211.2330	23.29482	3.68324	203.7830
Time_2	control	20	209.6657	20.30059	4.53935	200.1648
	Experimental	20	244.7710	12.55749	2.80794	238.8939
	Total	40	227.2184	24.36373	3.85224	219.4265
Time_3	control	20	221.0605	15.10608	3.37782	213.9906
	Experimental	20	282.9288	26.18759	5.85572	270.6726
	Total	40	251.9946	37.77209	5.97229	239.9145
Time_4	control	20	227.6248	15.17094	3.39233	220.5245
	Experimental	20	463.4015	48.66842	10.88259	440.6240
	Total	40	345.5131	124.57965	19.69777	305.6706
Time_5	control	20	229.1533	15.22007	3.40331	222.0300
	Experimental	20	478.1565	47.96904	10.72620	455.7063
	Total	40	353.6549	130.88918	20.69540	311.7945

Post Hoc Tests
Dependant Variable: Control
Tukey HSD

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Table 4: Multiple comparisons between time intervals of control group by Tukey HSD test

(I)Count	(J) Count	Mean Difference (I-J)	Std Error	Sig.	95% Confidence Interval	
					Lowerbound	Upperbound
0	1	-5.18555	6.20966	.960	-23.1860	12.8149
	2	-14.78205	6.20966	.172	-32.7825	3.2184
	3	-26.17680*	6.20966	.001	-44.1772	-8.1764
	4	-32.74105*	6.20966	.000	-50.7415	-14.7406
	5	-34.26955*	6.20966	.000	-52.2700	-16.2691
1	1	5.18555	6.20966	.960	-12.8149	23.1860
	2	-9.59650	6.20966	.636	-27.5969	8.4039
	3	-20.99125*	6.20966	.012	-38.9917	-2.9908
	4	-27.55550*	6.20966	.000	-45.5559	-9.5551
	5	-29.08400*	6.20966	.000	-47.0844	-11.0836
2	1	14.78205	6.20966	.172	-3.2184	32.7825
	2	9.59650	6.20966	.636	-8.4039	27.5969
	3	-11.39475	6.20966	.448	-29.3952	6.6057
	4	-17.95900	6.20966	.051	-35.9594	0.0414
	5	-19.48750*	6.20966	.026	-37.4879	-1.4871
3	1	26.17680*	6.20966	.001	8.1764	44.1772
	2	20.99125*	6.20966	.012	2.9908	38.9917
	3	11.39475	6.20966	.448	-6.6057	29.3952
	4	-6.56425	6.20966	.897	-24.5647	11.4362
	5	-8.09275	6.20966	.783	-26.0932	9.9077
4	1	32.74105*	6.20966	.000	14.7406	50.7415
	2	27.55550*	6.20966	.000	9.5551	45.5559
	3	17.95900	6.20966	.051	-0.0414	35.9594
	4	6.56425	6.20966	.897	-11.4362	24.5647
	5	-1.52850	6.20966	1.000	-19.5289	16.4719
5	1	34.26955*	6.20966	.000	16.2691	52.2700
	2	29.08400*	6.20966	.000	11.0836	47.0844
	3	19.48750*	6.20966	.026	1.4871	37.4879
	4	8.09275	6.20966	.783	-9.9077	26.0932
	5	1.52850	6.20966	1.000	-16.4719	19.5289

*The mean difference is significant at the .05 level

Table inference

Multiple comparisons between time intervals of control group by Tukey HSD test is shown in table 4. Control group showed a gradual increase in LDH level from T_0 to T_5 though it was low compared to experimental site.

Post Hoc Tests
Dependant Variable: Exp
Tukey HSD

Table 5: Multiple comparisons between time intervals of experimental group by Tukey HSD test

(I)Count	(J) Count	Mean Difference (I-J)	Std Error	Sig.	95% Confidence Interval	
					Lowerbound	Upperbound
0	1	-26.567500	10.351139	.114	-56.57314	3.43814
	2	-48.941750*	10.351139	.000	-78.94739	-18.93611
	3	-87.099500*	10.351139	.000	-117.10514	-57.09386
	4	-267.57225*	10.351139	.000	-297.57789	-237.56661
	5	-282.32725*	10.351139	.000	-312.33289	-252.32161
1	1	26.567500	10.351139	.114	-3.43814	56.57314
	2	-22.374250	10.351139	.264	-52.37989	7.63139
	3	-60.532000*	10.351139	.000	-90.53764	-30.52636
	4	-241.00475*	10.351139	.000	-271.01039	-210.99911
	5	-255.75975*	10.351139	.000	-285.76539	-225.75411

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2	1	48.941750*	10.351139	.000	18.93611	78.94739
	2	22.374250	10.351139	.264	-7.63139	52.37989
	3	-38.157750*	10.351139	.005	-68.16339	-8.15211
	4	-218.63050*	10.351139	.000	-248.63614	-188.62486
	5	-233.38550*	10.351139	.000	-263.39114	-203.37986
3	1	87.099500*	10.351139	.000	57.09386	117.10514
	2	60.532000*	10.351139	.000	30.52636	90.53764
	3	38.157750*	10.351139	.005	8.15211	68.16339
	4	-180.47275*	10.351139	.000	-210.47839	-150.46711
	5	-195.22775*	10.351139	.000	-225.23339	-165.22211
4	1	267.572250*	10.351139	.000	237.56661	297.57789
	2	241.004750*	10.351139	.000	210.99911	271.01039
	3	218.630500*	10.351139	.000	188.62486	248.63614
	4	180.472750*	10.351139	.000	150.46711	210.47839
	5	-14.755000	10.351139	.712	-44.76064	15.25064
5	1	282.327250*	10.351139	.000	252.32161	312.33289
	2	255.759750*	10.351139	.000	225.75411	285.76539
	3	233.385500*	10.351139	.026	203.37986	263.39114
	4	197.227750*	10.351139	.000	165.22211	225.23339
	5	14.755000	10.351139	.712	-15.25064	44.76064

*The mean difference is significant at the .05 level

Table inference:

Multiple comparisons between time intervals of experimental groups by Tukey HSD method is shown in table 5. The experimental site showed a gradual increase in the LDH levels from baseline (To) - 21 days (T5). There was a statistically significant difference.in LDH levels at different time interval sexcept between T0&T1, T1&T2 and T4&T5.

Table 6: Multiple comparisons between experimental and control groups by ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
T0_Values	Between Groups	8.941	1	8.941	.014	.906
	Within Groups	24237.008	38	637.816		
	Total	24245.949	39			
T1_Values	Between Groups	4985.173	1	4985.173	11.709	.002
	Within Groups	16178.132	38	425.740		
	Total	21163.304	39			
T2_Values	Between Groups	12323.786	1	12323.786	43.256	.000
	Within Groups	10828.286	38	284.902		
	Total	23150.496	39			
T3_Values	Between Groups	38726.804	1	38726.804	83.758	.000
	Within Groups	17365.692	38	456.992		
	Total	55642.496	39			
T4_Values	Between Groups	555906.8	1	555906.758	427.822	.000
	Within Groups	49376.688	38	1299.387		
	Total	605283.688	39			
T5_Values	Between Groups	620026.2	1	620026.185	489.621	.000
	Within Groups	48120.914	38	1266.340		
	Total	668417.1	39			

Table Inference

Multiple comparisons between experimental and control groups is shown in table 6. There was a statistically significant difference in the levels of lactate dehydrogenase in gingival crevicular fluid between experimental and control groups at all the time intervals except at T_o

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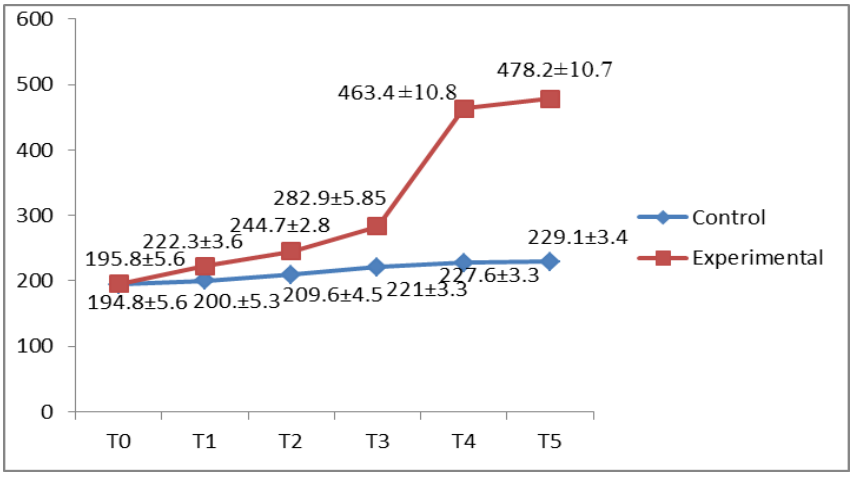

Graph 1: Gingival crevicular fluid lactate dehydrogenase level (µmol/L) in the control and experiment sites (values are mean ±SEM).

The levels of lactate dehydrogenase in gingival crevicular fluid varied from 194.88 ± 5.61 µmol/L at baseline to 229.15 ± 3.40 µmol/L on the 21st day in the control site.(Graph 1)

The LDH level in GCF steadily increased with time in the experiment site from 195.82 ±5.67 µmol/L at baseline to 282.9 ± 5. µmol/L at 7 days. This was followed by a statistically significant steep increase in LDH levels from 282.9 ± 5.85 µmol/L at 7 days to 4.63.4 ± 10.8 µmol/L at 14 days. There was a mild increase in LDH activity from 463.4 ± 10.8 µmol/L at 14 days to 478.15 ± 10.72. µmol/L at 21 days which was not statistically significant. Significantly higher LDH levels from the 7th day onwards until 21st day were observed at the experimental site where orthodontic force was applied.

DISCUSSION

Orthodontic treatment is based on the principle that if continuous pressure is applied to the tooth, movement occurs as a result of remodelling of the surrounding bone.[1] Tooth movement is a periodontal ligament phenomenon and the changes that produce the tooth movement are initiated in the periodontal ligament.[10] Due to sustained pressure, blood flow is decreased where the periodontal ligament is compressed and increased where the periodontal ligament is under tension.

The early changes that occur during orthodontic tooth movement are an active inflammatory response characterized by periodontal vasodilatation and migration of leucocytes out of capillary blood vessels. These cells are responsible for production of cytokines, the local biochemical signal molecules. After one or two days, the acute phase of inflammation subsides and is followed by a chronic phase. The chronic phase of inflammation involves proliferation of fibroblast, endothelial cells, osteoblasts, etc. During this phase there is continuous migration of leucocytes into the strained periodontal tissues which regulate the remodeling process [1].

Guyton reports bone resorption by formation of villous like projection of osteoclasts resulting in formation of ruffled border adjacent to bone [11]. These projections secrete proteolytic enzyme from lysosomes and several acids including lactic acid and citric acid from mitochondria and secretory vesicles. These acids are responsible for solution of the bone salts. The osteoclastic cells embibe by process of phagocytosis. Bony matrix particles and crystals eventually gets dissolved and released into the blood.

Lactate dehydrogenase is an oxido reductase enzyme that is present within the cytoplasm of the cell and is released from cells during necrosis. During anaerobic condition, cells depends on anaerobic glycolysis for respiration, during which the end product, lactic acid is formed. Lactate dehydrogenase helps in conversion of pyruvic acid into lactic acid. Cells that are able to adapt to the metabolic changes continue to thrive and cells that cannot adapt to the ischemic changes, undergo necrosis. The necrosed cells release its contents causing activation of local inflammatory response [8].

Several researchers found that cellular and tissue reaction starts after alteration in blood flow leading to extravasations of inflammatory cells and recruitment of osteoblasts and osteoclast. The released cells result in synthesis of various molecules and enzymes that interact with periodontal tissues for tissue remodeling [1].

Studies have demonstrated that the activity of lactate dehydrogenase in gingival crevicular fluid is significantly related to gingival inflammation and tissue destruction [9,12]. Therefore, lactate dehydrogenase activity in the gingival crevicular fluid has been recognized as a bio marker for monitoring periodontal metabolism.[13,14] Previous studies report that various biomarkers like myeloperoxidase, alkaline phosphatase, aspartate aminotransferrase, cathepsin B, interleukins (IL) - 2, IL - 6, IL - 8 that show alteration in their GCF levels after application of orthodontic force [15]. But very few studies have been carried out to evaluate the relationship between LDH and orthodontic tooth movement. Therefore this study was done to evaluate their levels in gingival crevicular fluid during orthodontic tooth movement by selecting twenty orthodontic subjects all requiring first premolar extractions. All the patients were treated with pre adjusted edge wise appliance 0.022 "slot MBT prescription using 3M brackets. After leveling and alignment, canine retraction was done on a base arch wire of 0.019 x 0.025"SS from molar hook to canine hook on one side by Nitinol closed coil spring (9mm) exerting 125g of force.[16] No force was given on the opposite side canine which acted as the control.

The gingival sulcus was chosen for experimenting LDH activity because of its continuity with periodontal ligament and compression of the periodontal ligament leads to migration of biochemical products into the gingival sulcus [17,18]. The gingival crevicular fluid samples were then collected by using Hirschmann micropipette graduated from 1 to 5 µl from the experimental and control canines before the commencement of retraction, 1 hour after the initiation of canine retraction, followed by 1 day, 7 days, 14 days and 21 days. The activity of lactate dehydrogenase in gingival crevicular fluid samples were measured and analyzed spectrophotometrically and compared with the control site.

The results of this study showed that there was increased LDH levels during orthodontic tooth movement compared to the control site and the difference was statistically significant at all the time intervals except the baseline levels at $T_{0.}$ The results correlates with the earlier study done by Emanuela Serra and Perinetti G who found that the levels of LDH increased in gingival crevicular fluid at the site where orthodontic force was applied compared to the contralateral control site [13,14].

The LDH levels in the experimental site after 125g of force application showed a steady increase from 0hour, 1hour, 1day and 7 days. There was then a steep increase from 7 to 14 days. This was followed by a mild increase from 14 to 21 days. The difference between each of the groups was statistically significant except between 0 hour and 1 hour. The steep increase between 7 annd 14 days can probably be explained by Burrstone's study who found that there were three phases of orthodontic tooth movement– initial, lag and post-lag phases.Initial phase is characterized by movement of teeth within the periodontal ligament space. Lag phase is characterized by hyalinized zone where no tooth movement takes place. Hyalinized tissues are focal aseptic necrosed areas which are acellular containing ground substance matrix. The post-lag phase is characterized by gradual or sudden increase in tooth movement followed by degeneration of hyalinized zones by cells of normal surrounding periodontium [19]. The steep increase found in this study coincides with the lag phase described by him.

The results of the study also correlated with Reitan whodescribed three distinct processes in periodontal tissue by orthodontic forces [20]. Tissue deformation occurs in the first phase followed by second phase during which the appropriate cells establish a microenvironment that allows for correct tissue modeling and remodeling. Tissue turnover occurs in the third phase to allow a reduction in the applied strain, which terminates in appliance deactivation. The tissue resorption or destruction attributes to the increase in the level of lactate dehydrogenase in GCF during orthodontic tooth movement [13,14,21] The high levels of LDH dehydrogenase reached after 14 days is probably due to this tissue turnover which occurred in the second phase.

The evaluation of LDH levels on the control site revealed a mild increase from T_0 to T_5, but the difference between the time groups were not statistically significant. The mild increase may have probably been due to residual tooth movement which could have occurred during the course of the study.

The effect of force applications on the tissues was explained by Brigit Thilander who divided the duration of tooth movement into two different periods, the initial and secondary periods. During the initial period, tooth movement occurs within the alveolar bone by narrowing of the periodontal membrane followed by differentiation of osteoclasts along the alveolar bone after 30 to 40 hours. Secondary period is characterized by undermining resorption after removal of hyalinized tissue [22]. Compression of blood vessels by application of orthodontic force causes damage to blood vessel wall and disintegration of blood elements. The damaged cells undergo sequential changes initially starting with swollen mitochondria and endoplasmic reticulum followed by rupture and disintegration of cytoplasmic membrane and the nucleus becomes pyknotic. After a certain period of time breakdown of nucleus takes place leaving behind the cells interspersed between collagen fibres. The results from this study establish a direct relationship between tissue breakdowns in the secondary period and an increase in the LDH levels.

Thus, from this study it can be concluded that lactate dehydrogenase enzyme can have a possible role as a biomarker of orthodontic tooth movement. A larger sample size and correlation of LDH with other established biomarkers can add more relevance to future studies. Studies can also be done using various force magnitudes over a longer period of time. Studies in the future can also include the use of LDH to facilitate orthodontic tooth movement in controlled clinical trials.

CONCLUSION

From the findings observed in this study it can be concluded that

- The activity of lactate dehydrogenase enzyme could be successfully evaluated in the gingival crevicular fluid.
- When constant, continuous and optimal orthodontic forces are applied activity of lactate dehydrogenase in gingival crevicular fluid showed a marked increase during orthodontic tooth movement with a highly significant increase statistically between 7th and 14th days compared with the control site, with the initiation of canine retraction.
- Hence, evaluation of activity of lactate dehydrogenase in gingival crevicular fluid can be used as a biomarker for periodontal metabolism during orthodontic tooth movement.

Further research is needed for evaluating the levels of lactate dehydrogenase in gingival crevicular fluid for different force magnitude and duration of application of force. Evaluation of lactate dehydrogenase enzyme in gingival crevicular fluid after 21 days can be done for improving the knowledge inmolecular basis for orthodontic tooth movement and determining whether active tooth movement is taking place. This is helpful in deriving an active appointment schedule and retention protocol. Further investigations are required to confirm whether this biomarker can be used to enhance the orthodontic tooth movement.

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