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EVALUATION OF EFFECTS OF LOW-LEVEL LASER THERAPY ON ORTHODONTIC TOOTH MOVEMENT BY QUANTITATIVE ESTIMATION OF THE TARTRATE RESISTANCE ACID PHOSPHATASE LEVELS IN GINGIVAL CREVICULAR FLUID

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ABSTRACT

Aim: To test the hypothesis that mechanical forces combined with low-level laser therapy stimulates the rate of orthodontic tooth movement; by clinically measuring the canine retraction and histologically evaluating the Tartrate Resistant Acid Phosphatase level in the gingival crevicular fluid.

Method: A split-mouth study was conducted in 84 patients who required maxillary canine retraction following first premolar extraction using closed NiTi coil springs, delivering a constant force of 150 grams with Pre adjusted edgewise appliance. Low level laser therapy (LLLT) was done with GaAlAs laser irradiation at 8J/cm^2 and 100mW on the canine in the control side for 3 consecutive days. Tartrate Resistant Acid Phosphatase (TRAP) levels were estimated in gingival crevicular fluid (GCF) following irradiation. In the control side TRAP estimation was done in GCF without laser irradiation. Rate of tooth movement was assessed with serial study model analysis over an 8 week period.

Result: There was a significantly greater distal movement of the canine irradiated with LLLT at the end of both 4 and 8 weeks. There also was a positive correlation between the increased tooth movement on LLLT side and increased levels of TRAP after the 3$_{rd}$ day. (p value ≤ 0.05).

Conclusion: LLLT enhances the rate of orthodontic tooth movement which is also seen histologically by increased TRAP levels.

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Keywords: tartrate resistance acid phosphatase, gingival crevicular fluid, low level laser therapy, periodontal ligament

INTRODUCTION

Various research is being carried out in the field of orthodontics with the aim of enhancing the rate of tooth movement. Methods such as ultrasonic vibrations, electric stimulation, piezocisions, and parathyroid hormone injections have been proposed for accelerating orthodontic tooth movement.[1, 2], However, factors like patient discomfort, patient compliance, sophisticated apparatus and ethicality issues questions the practicality of these procedures.

Use of Low Level Laser Therapy (LLLT) for accelerating tooth movement has gained popularity since the past decade, due to its non-invasive nature and the ease of laser regimen to be followed [3]. Lasers have been used quite extensively, becoming an integral part of a surgeon's armamentarium. These lasers however have been used for their tissue ablation properties. Low level intensity lasers were then brought into picture with their use first in the medical field for tissue healing and laser assisted analgesia followed by its role in the field of dentistry and then orthodontics to promote bio-regeneration of the bone. Low Level Laser Therapy (LLLT) has been proposed for enhancing the rate of tooth movement by its bio stimulatory effect [4].

Efficiency with which bone is removed in the path of tooth movement is the rate limiting factor in orthodontics. Studies have shown a 1.3 fold increase in the rate of tooth movement on the laser irradiation side as compared to the control side owing to the increase in the number of osteoclasts on the compressed side of the tooth. Such an increase in the rate of tooth movement was attributed to the stimulation of the fusion of mononuclear macrophages to mature osteoclasts [5]. The considerations used while studying lasers mainly centre on the energy density and the application dose. Biologic tissues respond to laser by following the Arndt-Shultz law, which states that extremely small doses fail to stimulate, small doses stimulate living systems, medium doses impede, and large doses destroy them [6].

A bulk of published research outcomes indicate an increase in the rate of tooth movement after laser therapy compared to controls [3, 4, 7, 8], while other investigations concludes that lasers negatively affect tooth velocity [9]. These incongruous results, mainly due to the wide ranging dosage regimen combined with laser settings and the varying experimental designs make the elucidation of the data difficult [10, 11].

Out of various markers, Tartrate Resistant Acid Phosphatase (TRAP) has been found to be a major marker for osteoclastic activity at the sites of bone remodeling [12, 13]. Increased levels of TRAP have been found in saliva and gingival crevicular fluid (GCF) of patients undergoing orthodontic treatment [14]. A few studies that have been conducted on animals have shown increased levels of TRAP in the low level laser irradiated tissues, suggesting, enhanced rate of bone remodeling [8, 15].

Investigation of the crevicular fluid in humans after the tooth has been exposed to LLLT and moved at an accelerated rate, however is a mine still undug. The objectives of the study was to assess if LLLT using GaAlAs semiconductor diode laser can affect the rate of tooth movement and also to understand effects of LLLT at a histological level by quantifying the levels of Tartrate Resistant Acid Phosphatase (TRAP) in the gingival crevicular fluid (GCF).

METHODS

This was a prospective split mouth study design in which 84 patients within the age range of 14-25 years were evaluated for the effects of the low level laser therapy on the rate of tooth movement and its effect on the levels of Tartrate Resistant Acid Phosphatase (TRAP) in the gingival crevicular fluid (GCF) was estimated. This study was conducted after getting an approval from the Institutional Review Board (IRB) and Ethical Committee (IRB. No. 2013/P/OR/16). Informed consent was attained from the patient prior to the start of this study. The selected patients fulfilled the following criteria:

Inclusion criteria:

a. Patients requiring extraction of first premolars.
b. Patients with healthy periodontium with probing depth values not exceeding 3mm in the entire dentition.
c. No radiological evidence of periodontal bone loss.
d. No use of anti-inflammatory drugs during the month preceding the study.

Exclusion criteria:

a. Patients having any kind of systemic conditions.
b. Any medical treatment that may hinder bone metabolism.
c. Patients with poor periodontal status.
d. Patients on any other anti-inflammatory drugs or any medication during the month preceding the study.

Methodology

Each patient's maxillary arch was divided into experimental side and control side. Right side being the experimental side from central incisor to last erupted molar and left side being the control side from central incisor to last erupted molar. Leveling and aligning of the upper arch was carried out using progressing NiTi archwire, until an 0.018" stainless steel archwire could be placed passively for one visit. This was followed by making pre-experimental alginate impressions and obtaining a pre-experimental stone models (T1).

Gingival Crevicular Fluid (GCF) Collection

At this point of time a GCF sample was collected which served at baseline (G1). One week prior to the collection of GCF samples thorough oral prophylaxis was done. All patients followed the strict oral hygiene instructions to rinse twice daily with 0.5 ounces of 0.2% chlorhexidine gluconate throughout the duration of the study period. Supragingival plaque was removed from the canines and the region was flushed with water and gently dried with air. The isolation of the teeth was obtained using a suction, self-retaining retractor, and cotton rolls. All GCF samples were collected in the forenoon between 10 and 11 AM ((at same time of the day), to allow for the circadian variation seen in GCF volume [13]. A graduated

capillary of 5 μL capacity was introduced into the distal gingival crevice of the canine on both the control and experimental sides and a minimum of 3μL of GCF was collected. The collection of GCF was done by Brill technique [16]. The sample was collected for 20 min. It was then transferred to a sterilized plastic vial containing a phosphate buffer solution. The vial was frozen to −20°C until the sample was transported to the laboratory for analysis.

This was followed by individual canine retraction carried out with the help of NiTi closed coil springs of 9mm in length from the molar hook onto the canine hooks bilaterally after calibrating the force to 150gms using a CORREX gauge and recalibrated using a Dontrix gauge [Figure 1].

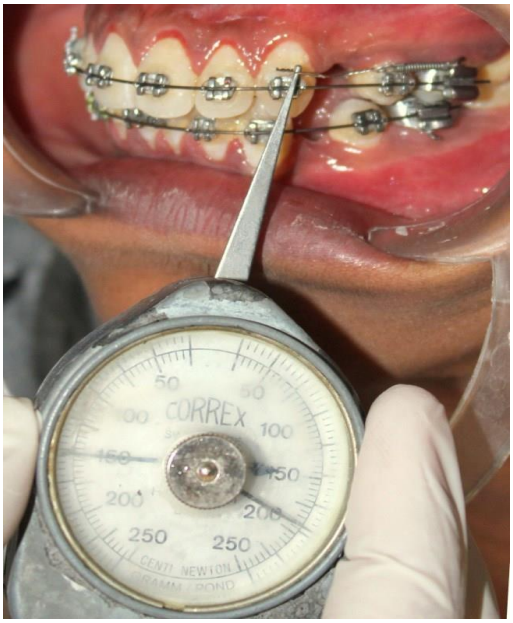


Figure 1. Correx gauge used to calibrate 150 gms of force to the closed coil Niti springs PAE (0.022" slot; MBT prescription) with 0.018" SS with closed coil NiTi springs between the canine and molar tube hook.

In this study Ezlase machine (Biolase: Irvine, CA), which is a Ga-Al-As semiconductor diode laser machine was used. A Ga-Al-As semiconductor diode laser with a wavelength of 940nm was used to deliver Low Level Laser Therapy onto the experimental canine side. The laser specifications were as follows: an energy density of 8J/cm^2 at 100mW power output. The power output in this study was kept at 100mW as the purpose was low level therapy without any thermal injury or cutting of tissues The application of laser was done for 10sec/point, onto 8 points at the apical, middle and cervical regions on the mucosa overlying the roots of the canine on the buccal and the palatal surfaces and on two points on the distal cervical margin of the canine to ensure an even distribution of the energy [Figure 2]. Since the tip was placed at a distance from the mucosa, the area being irradiated was approximately 1cm in diameter.

This laser regimen was followed for a period of three consecutive days, starting immediately after the application of the force. The GCF collection was done on the first (baseline), second, third, fifth and the fourteenth day.

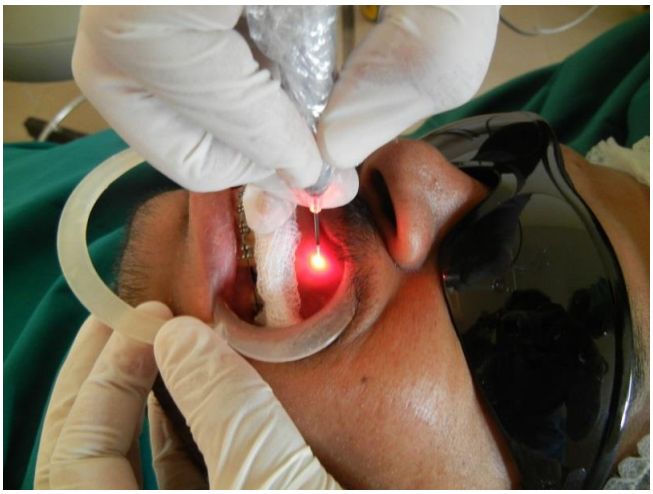


Figure 2. Low Level Laser irradiation delivery onto the experimental canine side onto the mucosa overlying the roots of the canine. (Energy density of 8J/cm^2 at 100mW power output).

At the end of 4 weeks, fresh impressions and casts were made (T2) and GCF samples were collected. The NiTi coil springs were reactivated to deliver 150gms of force and the three-day laser regimen was repeated once again. This procedure was done for a total duration of 8 weeks. At the end of 8th week, only impressions and models were made **(**T3) without the application of laser. The TRAP levels in GCF were estimated by using TRACP assay kit (Takara Bio Inc, Japan) by following manufacturer's instructions.

Method for Evaluating the Tooth Movement

The initial impression was poured in dental stone followed by pouring of the base in dental plaster. The model was then trimmed so that base of the model was parallel with the functional occlusal plane. It was checked using a spirit level. A silicone impression of the area covered by the mid-palatal raphe, second and third palatal rugae on either sides of the mid-palatal raphe was made on the baseline cast (T1). This is then poured in dental stone and an acrylic button was fabricated.

The acrylic palatal plug was prepared, which covered the medial portion of the palatal rugae with reference wires on the initial model. It was then re-checked for the fit on the successive models. A line was marked on the exposed surface of the palatal plug coinciding with the median raphe and incisive papilla point, which was useful to verify the superimposition of the plug on the progress models. The palatal plug was seated on the progress models so that the marked line coincided with the incisive papilla and the median raphe [Figure 3].

Photographs of the models with the acrylic plug were taken at each stage T1, T2 and T3. The base of the model was measured for its dimensions and it was found out that the dimension forming the distal most part of the model base (line joining the part distal to the maxillary tuberosity on either sides = X) had a uniform measurement of 75 mm in all the three models since the base former used in all the cases was the same [Figure 4].

Figure 3. Palatal Plug fabricated on the T1 cast for each patient.

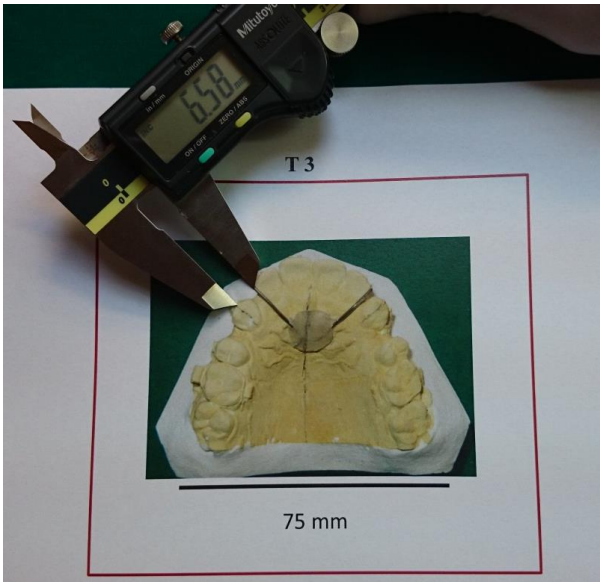


Figure 4. Measuring the distance between the canine cusp tip to the distal most part of the wire pointer on paper using a Mitotoyu digital caliper.

This measurement of 75mm digitally reproduced in a computer using a CorelDRAWX5 software. The photographs of the models were then resized and adjusted to meet the requirements so as to match the line "X." Coloured printouts were taken and the tooth movement was measured.

The palatal plug with reference wires was the reference device for all the study models of the same patient. The base value was set by measuring the distance from the tip of the wire to the respective canine on the first model (T1). The plug was then transferred to the progress casts (T2, T3) and the distance that the canine has moved is measured from the cusp tip to the reference wire end and then subtracted from the base value. The results thus obtained were then subjected to statistical analysis.

Statistical Analysis

The collected data was entered into the computer (MS-Office, Excel 2010) and subjected to statistical analysis using the statistical package- SPSS (version 20.0 Armonk, NY: IBM Corp). The mean difference and standard deviation values TRAP were calculated at different time interval. Paired t test was done to compare the difference in tooth movement between different stages in control group and in the experiment group. Statistical differences were determined at the 95% confidence level ($P < 0.05$).

Table 1. Comparison of tooth movement at T1 and T2 in the control group and experiment group

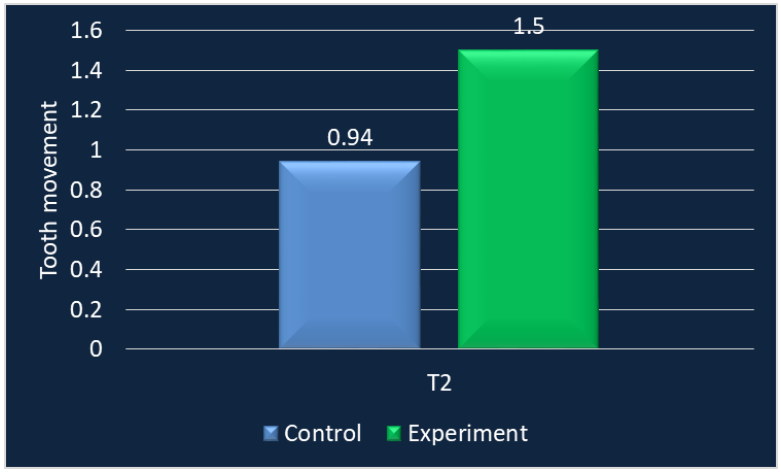
Group	T1 Mean ± S.D	T2 Mean ± S.D	p Value
Control	0 ± 0	0.94 ± 0.48	0.00*
Experiment	0 ± 0	0.15 ± 0.48	0.00*

T1- tooth movement recorded at the beginning of the study, T2 – tooth movement recorded at the end of 4th week; $p \leq 0.05$ is considered statistically significant.

Table 2. Comparison of tooth movement from T2 to T3 in the control group and experiment group

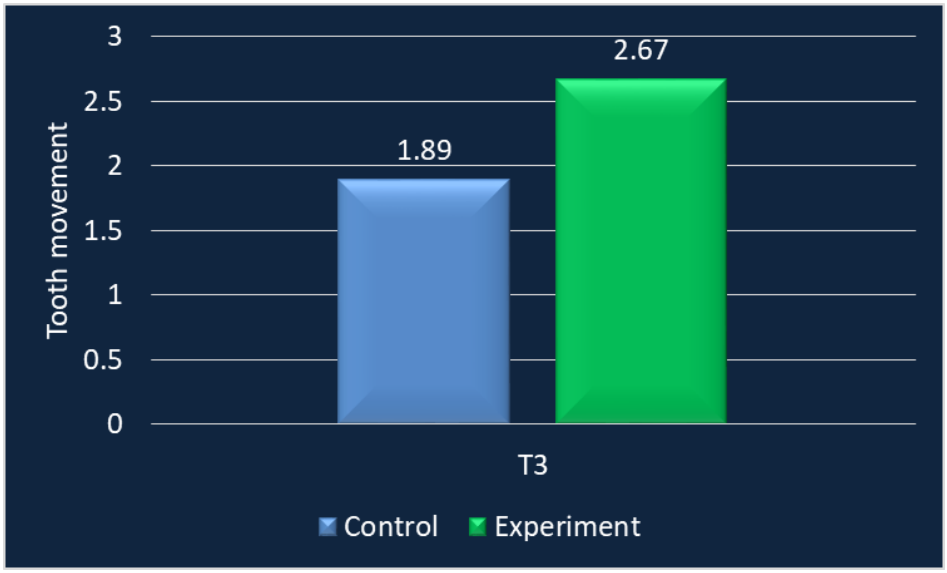
Group	T2 Mean ± S.D	T3 Mean ± S.D	p Value
Control	0.94 ± 0.48	1.89 ± 0.76	0.000*
Experiment	1.5 ± 0.48	2.67 ± 0.69	0.001*

T2 - tooth movement recorded at the end of 4th week, T3 – tooth movement recorded at the end of 8th week; $p \leq 0.05$ is considered statistically significant.



T2 - tooth movement recorded at the end of 4th between the control and the experiment group.

Graph 1. Comparison of tooth movement at T2 between the control and the experiment group.



T3 - tooth movement recorded between the 4th and the 8th week between the control and the experiment group.

Graph 2. Comparison of tooth movement recorded between the T2 and T3 between the control and the experiment group.

Table 3. Comparison of difference in tooth movement from T2 to T3 in control group to the difference in tooth movement from T2 to T3 in the experiment group

D2	Control (T2C2-T3C3)	Experiment (T2E2-T3E3)	p Value
	Mean ± S.D	Mean ± S.D	0.043*
	0.95 ± 0.36	1.16 ± 0.42	

$p \leq 0.05$ is considered statistically significant.

D2 - Comparison of difference in tooth movement from T2 to T3 in control group to the difference in tooth movement from T2 to T3 in the experiment group.

T2C2 – Control values at the end of 4 weeks. T2E2 – Experiment values at the end of 4 weeks. T3C3 – Control values at the end of 8th week. T3E3 - Experiment values at the end of 8th week.

Table 4. Comparison of difference in tooth movement from T1 to T3 in control group to the difference in tooth movement from T1 to T3 in the experiment group

D3	Control T1C1-T3C3	Experiment T1E1-T3E3	p Value
	Mean±S.D	Mean±S.D	0.001*
	1.89±0.76	2.67±0.69	

$p \leq 0.05$ is considered statistically significant.

D3 - Comparison of difference in tooth movement from T1 to T3 in control group to the difference in tooth movement from T1 to T3 in the experiment group.

T1C1 – Control values at the beginning of the study. T1E1- Experiment values at the beginning of the study.T3C3 – Control values at the end of 8th week. T3E3- Experiment values at the end of 8th week.

Table 5. Comparison of change in the levels of Tartrate Resistant Acid Phosphatase (TRAP) between various GCF collection days in the control side to the change in the levels of Tartrate Resistant Acid Phosphatase (TRAP) in the experiment side

	Control	Experiment	p value
	Mean±S.D	Mean±S.D	
D1 - D2	0.30±0.71	0.60±0.63	0.292
D2 - D3	0.67±0.65	0.72±0.67	0.882
D3 - D5	0.62±0.61	1.50±1.18	0.048
D5 - D14	1.29±1.34	1.40±0.85	0.782
D1 - D14	2.90±1.61	4.22±1.93	0.030

$p \leq 0.05$ is considered statistically significant. *D1 - D2*: Change in the levels of TRAP from Day 1 to Day 2, *D2 – D3*: Change in the levels of TRAP from Day 2 to Day 3, *D3 – D5*: Change in the levels of TRAP from Day 3 to Day 5, *D5 – D14*: Change in the levels of TRAP from Day 5 to Day 14, *D1 – D14*: Change in the levels of TRAP from Day 1 to Day 14.

RESULTS

Paired t –test was done for evaluation of tooth movement between the stages T1 (beginning of canine retraction) and T2 (at the end of 4 weeks) and also between the stages T2 and T3 (at the end of 8 weeks) in both the control group and the experiment group [Tables 1, 2].

Graph 1 compares tooth movement at the end of 4^{th} week between the control and the experiment group. Paired t test showed a significant difference in the amount of tooth movement at the end of 4th week between the control and the experimental group. The level of significance was recorded at the p value being 0.014 which corresponds to highly significant.

Graph 2 compares of tooth movement recorded between the 4^{th} and the 8^{th} week between the control and the experiment group. Paired t test showed a significant difference in the amount of tooth movement at the end of 8th week between the control and the experimental groups. The level of significance was recorded at the p value being 0.001 which corresponds to highly significant.

Paired t test was done to compare the difference in tooth movement from T2 to T3 between control group and experiment group. It was found that the difference in tooth movement was statistically significant ($p = 0.043$) [Table 3].

Paired t test was done to compare the difference in tooth movement from T1 to T3 in control group to the difference in tooth movement from T1 to T3 in the experiment group [Table 4]. It was found that the difference in tooth movement from T1 to T3 between control group and experimental group was statistically significant ($p = 0.001$).

Paired t test was done to compare the change in the levels of Tartrate Resistant Acid Phosphatase (TRAP) between various GCF collection days in the control side to the change in the levels of Tartrate Resistant Acid Phosphatase (TRAP) in the experiment side. (Table 5). Results showed that for the time intervals between Day 3 to Day 5 the increase in the level of TRAP was statistically significant ($p = 0.048$). Also, for the time intervals between Day 1

(baseline) and Day 14, the increase in the level of TRAP between the control and experimental sides was statistically significant (p = 0.03). There was no statistical significance observed in the TRAP levels of the control and experiment group between Day 1 and Day 2, between Day 2 and Day 3 and between Day 5 and Day 14.

DISCUSSION

Orthodontic tooth movement occurs when bone and other periodontal tissues undergoes remodeling on application of mechanical force. Bone remodeling is the sum of bone deposition by osteoblasts on tension areas and bone resorption by osteoclasts on pressure areas of the root. Tooth movement occurs when bone resorption and deposition occur at different places of the same tooth [17]. The rate of tooth movement is dependent on the rate of bone remodeling [18]. Low Level Laser Therapy (LLLT) has been proposed for enhancing the rate of tooth movement by its bio stimulatory effect [4].

Various histochemical studies have highlighted osteoclastic activity during bone remodeling as a result of orthodontic forces. Osteoclastogenesis is the rate limiting step in orthodontic tooth movement. Out of various markers, TRAP has been found to be a major marker for osteoclastic activity at the sites of bone remodeling [12, 13]. Increased levels of TRAP have been found in saliva and GCF of patients undergoing orthodontic treatment [14]. A few studies that have been conducted on animals have shown increased levels of TRAP in the low level laser irradiated tissues [8, 15].

In the present study, evaluation of tooth movement between the stages T1 and T2 and also between the stages T2 and T3 of both the control group and the experiment group using repeated measures of Paired t –test showed a highly significant amount of tooth movement was in each group at each time interval (p = 0.00). This is to show that irrespective of any additional means to enhance the rate of tooth movement, the force of 150gms delivered using a closed coil NiTi spring was efficient in moving the teeth even on the control side. This result indicates that the bone remodeling was active and hence the tooth movement was observed.

On comparing tooth movement at the end of 4th week between the control and the experimental group using Paired t test [Graph 1], a significant difference in the amount of tooth movement was observed at T2 between the control and the experimental group. The mean tooth movement recorded in the control side was 0.94 mm whereas in the experimental side it was 1.5 mm. The level of significance was recorded at the p value being 0.014 which corresponds to highly significant. Since all the other parameters were kept similar, it is believed that the increased tooth movement on the experimental side could be possible due to an increased bone metabolism due the irradiation with LLLT, a phenomenon that was studies extensively on rat sutures by Ozawa et al. [19].

On comparing the tooth movement taken place between T2 and T3 between the control and the experimental group. It was observed that a significant difference in the amount of tooth movement was noted at the end of 8th week between the control and the experimental groups. The mean tooth movement recorded in the control was 1.89 mm and on the experimental side were 2.67.

On comparing the difference in tooth movement from T2 to T3 in control group to the difference in tooth movement from T2 to T3 in the experiment group it was found that the

difference in tooth movement from T2 to T3 in control group to the difference in tooth movement from T2 to T3 in the experimental group was statistically significant ($p = 0.043$). This indicates that the canine in the experimental side had moved distally to a greater extent than the canine on the control side.

On comparing the difference in tooth movement from T1 to T3 in control group to the difference in tooth movement from T1 to T3 in the experiment group. it was found that the difference in tooth movement from T1 to T3 in control group to the difference in tooth movement from T1 to T3 in the experimental group was statistically significant ($p = 0.001$).

It must be noted that as the canine moves distally, an increase in the canine root prominence is noted onto the buccal cortical plates. This by itself can reduce the rate of tooth movement if adequate time for uprighting of roots is not provided in the mesiodistal plane. Also, the brackets used in this study included 0^0 torque canine brackets. Hence, the prominence of the root on the buccal cortex was unavoidable. In a study conducted by Shpack et al. it was reported that bodily tooth movement takes place faster than tipping movement due to shorter duration of root uprighting as reported by [20]. Risk of root resorption is also lesser in bodily moved canine due to the stress distribution along the roots as compared to the stress concentration at the apex resulting from tipping [18]. In our study, overall tooth movement, as noted by the T3 values of Table 4 and Graph 2, show that a significantly greater amount of tooth movement was noted at the end of the study in the experimental group in comparison to the control group.

A study conducted by Keeling SD et al. in rats found an increase in acid phosphatase activity on the tension sites of orthodontically treated teeth up to 7 days after appliance activation [21]. In our study, on comparing the change in the TRAP values during different time intervals between the control and experiment side, it was noted that for the time intervals between Day 3 to Day 5 the increase in the level of TRAP was statistically significant ($p = 0.048$). Also, for the time intervals between Day 1 (baseline) and Day 14, the increase in the level of TRAP between the control and experimental sides was statistically significant ($p = 0.03$). There was no statistical significance observed in the TRAP levels of the control and the experiment group between Day 1 and Day 2, between Day 2 and Day 3 and between Day 5 and Day 14. In the previously conducted studies, it was found that, there was an increased number of TRAP positive cells as early as 14 days from the start of orthodontic treatment [14, 22], which is comparable to our results.

This data suggests that the increase in the TRAP levels, i.e., the rate of osteoclastogenesis or bone resorption is higher between Days 3 and 5. This does indicate that the bone resorption at the experimental side had been taking place at a higher rate as compared to the control side.

CONCLUSION

The effect of low level laser therapy (LLLT) on the tooth movement is seen to be bio stimulatory, as it increases the rate of orthodontic tooth movement due to increased rate of osteoclastogenesis, which is observed by the increased level of Tartrate Resistant Acid Phosphatase (TRAP) level in the gingival crevicular fluid.

ETHICAL COMPLIANCE

The authors have stated all possible conflicts of interest within this work and all sources of funding for this work. If this work involved human participants, informed consent was received from each individual and it was conducted in accordance with the 1964 Declaration of Helsinki. If this work involved experiments with humans or animals, it was conducted in accordance with the related institutions' research ethics guidelines.

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