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Effect of Low Intensity Laser Therapy (LILT) on MMP-9 expression in gingival crevicular fluid and rate of orthodontic tooth movement in patients undergoing canine retraction: A randomized controlled trial

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Keywords
LILT (Low Intensity Laser Therapy)
MMP-9 (Matrix Metalloproteinase 9)
GCF (Gingival Crevicular Fluid)

Summary

Introduction > Low Intensity Laser Therapy (LILT) has been shown to increase the rate of tooth movement. Since its use in orthodontics as a method of acceleration there has been a variety of views regarding its mode of action. MMP-9 is a known bone resorption factor studied in Bone remodelling. The aim of this study was to know the effect of LILT on rate of tooth movement and expression of MMP-9 in GCF.

Materials and methods > Ten patients (3 males and 7 females) who required maxillary first premolar extraction for routine orthodontic treatment were recruited. The individual canine retraction was studied, and the side of the experimental canine was randomly selected. The laser regimen was followed on the 1st, 3rd, 5th, 7th, 14th and then 15th days consecutively. GCF was collected at baseline, 14th day, 3 months and at the end of canine retraction on experimental side and MMP-9 was estimated quantitatively using a standard ELISA kit.

Results > The average increase in rate of tooth movement on experimental side at 3 months was 44% and MMP-9 concentration was also high. At the end of canine retraction (4.5 months) in the experimental group the average rate increase was 38% with MMP-9 concentrations similar in both the experimental and control group.

Conclusions > LILT increases the rate of tooth movement. LILT also has an effect of bio-stimulation as depicted by rise in MMP-9 concentrations in GCF. However, this bio-stimulatory effect is restricted to the initial part of the tooth movement.

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Introduction

The long duration of treatment is the major concern for orthodontic patients. Comprehensive orthodontic treatment usually lasts for a period of 24 months [1]. In recent times, there has been an increased tendency for research to focus on accelerating methods for tooth movement due to the huge demand by adults for a shorter orthodontic treatment time. Studies have shown that surgical procedures such as corticotomy and piezocision can accelerate the tooth movement up 1.5 to 2 times as compared to conventional orthodontics [2].

Although piezocision is considered as minimally invasive when compared to conventional corticotomy, both the procedures are invasive in nature [3,4].

Recently, studies have shown that acceleration of tooth movement can be produced by local injections of prostaglandins (PGs) [5,6], 1,25(OH)2D3 (1,25-Dihydroxyvitamin D: active form of vitamin D3) [7], and osteocalcin [8], around the alveolar socket. Even though these substances stimulate rate of tooth movement, they also have undesirable side effects as local pain and discomfort during the procedure of injection.

LILT (Low Intensity Laser Therapy) in its initial days found application only in medical sciences like orthopaedics, surgery and medicine. It is used to accelerate the callus formation at fracture sites, to accelerate wound healing.

Although most of the scientific literature states that LILT does accelerate the tooth movement [9,10], very few authors studied the effect of LILT at molecular level [11–13]. Most of the studies that evaluated the effects of LILT at molecular level in GCF (Gingival Crevicular Fluid) were on animals. There were few studies which used human subjects [14]. Hence, the Randomized Controlled Trial (RCT) was designed to study the effect of LILT (Intervention) on rate of orthodontic tooth movement and its effect on MMP-9 concentration (Outcome) in GCF of patients undergoing canine retraction (Patient population).

The objectives of this study were:

- to evaluate the effect of LILT on rate of orthodontic tooth movement and analyse the effect of LILT on expression of MMP-9 in GCF of teeth undergoing retraction (primary outcome);
- to evaluate whether there is any correlation between change in MMP-9 expression in GCF and rate of tooth movement (secondary outcome).

Materials and methods

Patient selection and eligibility criteria

Sample consisted of 10 patients (3 males, 7 females) who required maxillary first premolar extraction as a part of their orthodontic treatment. Patients with systemic illness, those under systemic medications, patients with impacted canines or canines having dilacerated roots were excluded from the study as these factors can affect the orthodontic tooth movement. Patients with sound periodontal health were included in the study and were motivated to maintain good oral hygiene throughout the treatment. Consort flow diagram for patient selection is shown in the flowchart (*figure 1*).

Informed consent was taken from each patient for laser irradiation and GCF collection before the procedures were carried out either from the patient or parent (in case the patient was a minor below the age of 18 years).

Study design

The study design was approved by Institutional ethics committee of Government Dental College and Hospital, Nagpur, Maharashtra, India, (Ref no. MUHS/PG-T/3882/2015).

It was a randomized controlled trial with split-mouth design where one side served as experimental group and other control group to eliminate any biological variables. Routine orthodontic records were taken for all the patients. Maxillary first premolars were extracted to meet the space requirements. Patient's right or left canines were randomly allocated to the experimental group. Patients were asked to pick up the chit from a bowl marked either left or right. Whichever side was picked by the patient was selected as experimental side. Experimental side received Low Intensity Laser Therapy (LILT) while control side did not receive LILT. Each group consisted of 10 quadrants.

Orthodontic protocol

On the 7th day after the extraction, separators were placed mesial and distal to maxillary first permanent molar. Two days after the separator placement, molar bands were customized (0.180 × 0.005 in). Triple molar tubes were welded on the buccal surface of the band. Pre- adjusted edgewise brackets with MBT 0.022'' prescription (Ortho Organizers, USA) were used. Alignment and levelling were initiated with 0.016 NiTi wire and followed by 16 × 22- NiTi, 17 × 25- nickel-titanium, 17 × 25- stainless steel, and 19 × 25- nickel-titanium. After alignment and levelling, a final working wire was placed: 19 × 25- stainless steel (Orthoforce; G&H Wire, Franklin, Ind). For anchorage a Temporary Anchorage Device (TAD) micro-implant (S.K. Surgicals, India) measuring 1.3 × 9 mm was inserted in between Maxillary second premolar and First molar. This was done to avoid the mesial movement of first molar so that distance calculated for assessment of rate of tooth movement only depended upon movement of canine.

After 21 days of 19 × 25'' in SS wire placement individual canine retraction was started. Incisors were consolidated with 0.009'' ligature wire. A constant force of 150 gm was used for canine retraction on both experimental and control side. Since the width of extraction space on both sides was different accurate spring selection was done using the manufacturer's guide (G&H, USA). The measuring gauge provided by the manufacturer has a hole to simulate the eyelet of the spring which is slid over the head of the micro implant and the closest landmark to the power arm of the canine bracket was then chosen as

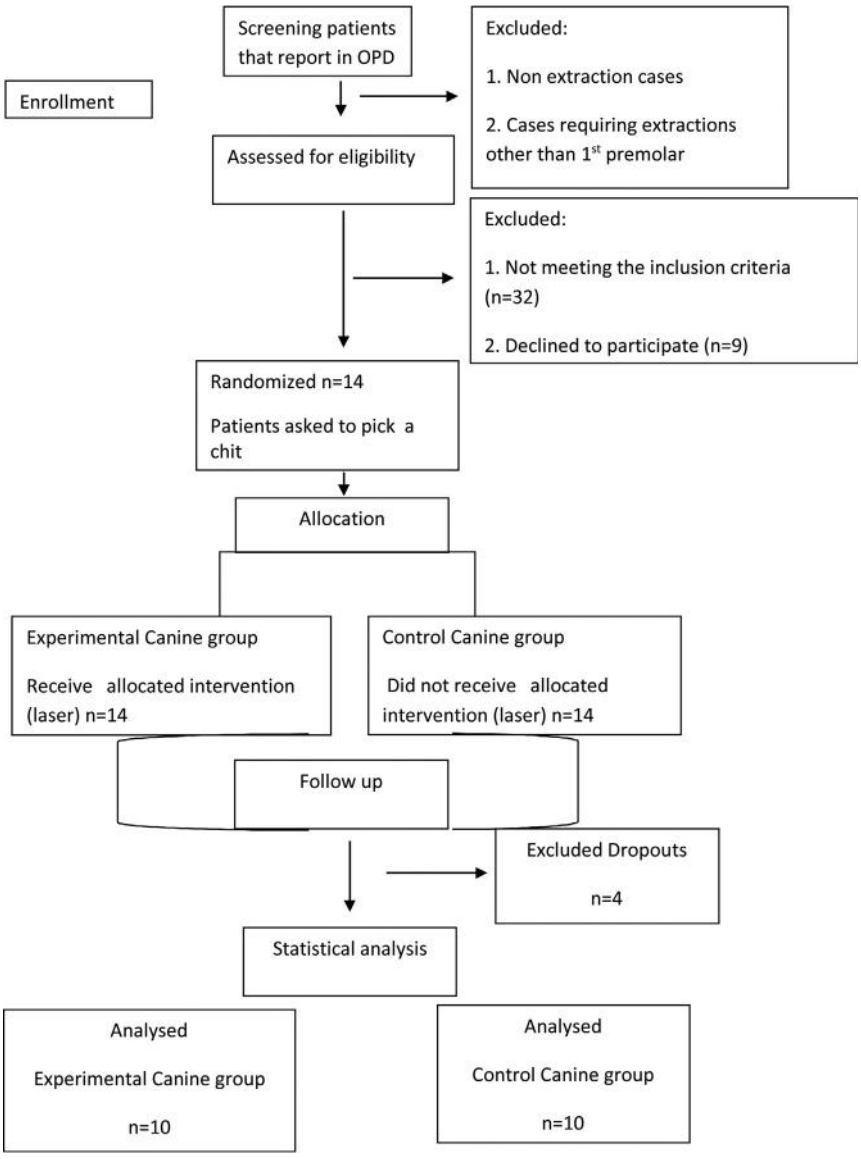

FIGURE 1

Flowchart showing the sample selection according to CONSORT guidelines

the distance which showed the desired length of the coil spring to be chosen. One end of the closed coil spring was secured with ligature wire to the head of the micro-implant and the other end of the power arm of the canine bracket. The exerted force values were confirmed with orthodontic dynamometer.

Laser protocol

The laser type used was a semiconductor (GaAlAs) diode laser emitting (Model: DenLase Version: DenLase-SY-A. 1c, China Daheng Group, Inc) infrared radiation with 980 ± 10 nm wavelength operated according to the manufacturer's recommendation as follows:

- wavelength - 980 nm;
- wave mode - continuous;
- output power - 0.3 W;
- exposure time - 30 sec (15 sec for labial and 15 sec for palatal side).

The handpiece houses the optical fibre core (Diameter 400 um) from where the laser beam is emitted. This handpiece is autoclavable and was disinfected after every use.

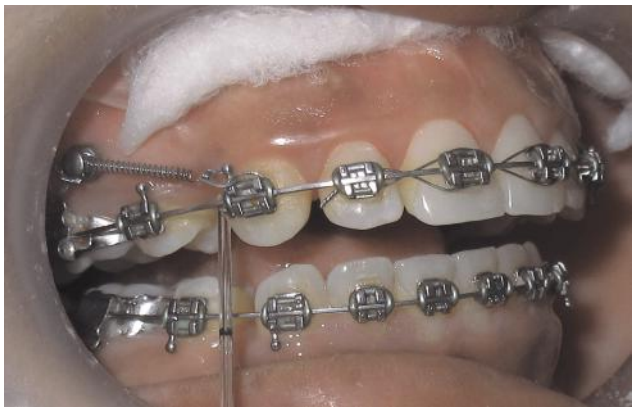

FIGURE 2
Laser Irradiation

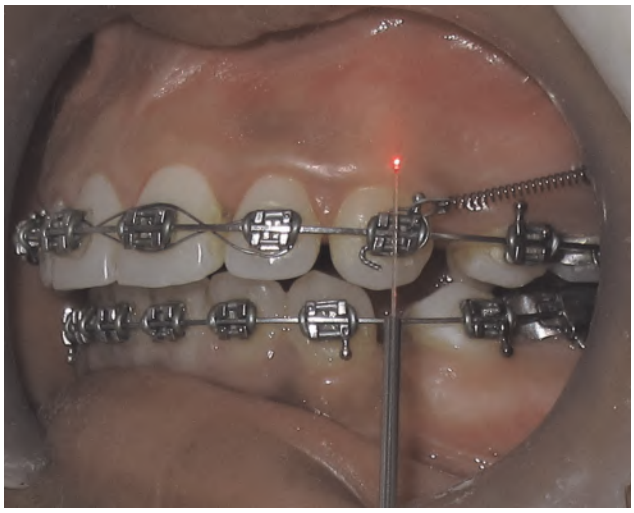

FIGURE 3
GCF collection

On day 1, closed coil springs were placed and a baseline GCF sample was collected from experimental/control side. After the baseline GCF sample was collected LILT irradiations were started on experimental side. A total of ten irradiations were done; five on the labial side and five on the palatal side. In order to cover the entire periodontal fibres and alveolar process around the canine teeth, the distribution and order was as follows:
On labial side:
(a) two irradiation doses on the cervical third of the canine root - one medial and one distal;
(b) two on the apical third of the canine root - one medial and one distal;
(c) one on the middle third, i.e., on the centre of the root.
On palatal side:
The irradiations were done similarly as above mentioned in (a), (b) and (c): the tip was held 2–3 mm away from the tissue (as per the manufacturer's guidelines) during application (*figure 2*). The total energy dose at each application was 9 J (2×15 s×0.3W). This procedure was followed for all subsequent appointments. The laser regimen was applied on 1, 3, 7, and 14-day intervals in the first month. Thereafter on every 15 days till the complete canine retraction on the experimental side (135.6 days; i.e., 4.5 months).
All the irradiations were done by a single operator. A red guiding light was irradiated without firing laser on control side too to blind the participants. After 6 months both experimental and control canines were examined by periapical radiograph which showed no undesirable changes and a vitality test was done on the canines which showed no damage to the pulp of retracted canines.

GCF collection protocol

The GCF was collected at following intervals as described below:
- at baseline i.e. before application of LILT: 1 sample, either from experimental or control canine before LILT was prescribed;
- at 14th day after the application of LILT: 2 samples (E1 - Experimental, C1 - Control);
- at 3 months after the application of LILT: 2 samples (E2 - Experimental, C2 - Control);
- at the end of retraction of canine. (E3 - Experimental, C3 - Control)

The total number of samples for each patient was 7 samples. The GCF sample collection was done by using a calibrated micro-capillary pipette (1-5ul) (*figure 3*). Prior to collection of GCF, any supragingival soft deposits were removed without causing trauma to the gingival crevice. The area was then thoroughly irrigated with distilled water, isolated by cotton rolls and dried by a stream of air. From both the maxillary canines (Experimental & Control), a standardized volume of 3 µL was collected. Collected GCF samples were immediately transferred to airtight plastic vials containing 97 µL of Phosphate Buffer Saline (pH: 7.4) to make 100 µL solution and were stored at −40 degree C (Cell Frost ltd.) until assayed.
Orthodontic treatment, Laser irradiation and GCF collection were carried out by single operator.

MMP-9 estimation

ELISA assay procedure was carried out with ELISA kit for Human MMP 9 (RayBio®, RayBiotech, Inc., USA, Cat#: ELH-MMP9). The standard curve was plotted as the relative O.D. 450 (Observed Optical Density at 450 nm) of each standard solution (Y-axis) vs. the respective concentration of the standard solution (X-axis). The MMP-9 concentration of the samples was then interpolated from the standard curve by using Sigmaplot software.

Measurement of tooth movement

The measurement of tooth movement was done on progress models. Three models were made for each patient:

- after completion of alignment and levelling, i.e., on day 1 of canine retraction;
- at the end of 3 months of canine retraction;
- at completion of canine retraction on experimental side.

On the models the mesial cusp tip of first molar (R1) and the canine cusp tip (R2) were taken as reference points. The distance between R1 and R2 was measured on all three models for each patient by a digital caliper (AEROSPACE, China) accurate to ± 0.02 mm. This distance was recorded as described below:

- T0 = after completion of alignment and levelling i.e. on Day 1 of canine retraction;
- T1 = at the end of 3 months of canine retraction;
- T2 = on completion of canine retraction on experimental side. It should be noted here that at the time point T2, control side canine is yet to be retracted completely.

The difference between T0 and T1 (T0 –T1) was taken as amount of tooth movement over the period of 3 months. The rate of Orthodontic Tooth Movement (OTM) was calculated as follows:

- rate of OTM = amount of tooth movement/time period;
- rate of OTM at the end of 3 months, recorded as M1 = (T0 – T1)/3;
- rate of OTM on completion of canine retraction on experimental side recorded as–M2. M2 = (T1 – T2)/time required in months.

M1 and M2 readings were calculated for both the experimental and control side and compared.

All the measurements were carried out by a data collector who was blinded and did not know which was the experimental side.

Statistical analysis

The data on distance between cusp tip of canine to cusp tip of mesiobuccal cusp of 1st molar for control and experimental canines were obtained at baseline and post-treatment time points. Also, the expression levels of MMP9 were obtained at different study time points. The displacement of canine on control and experimental side was compared using paired t-test. The levels of MMP9 on control and experimental sides, at different time points were compared using paired t-test. Further, the change in the marker expression from baseline to time points T1 and T2 were compared using paired t-test. The rate of displacement from baseline to T1 and T1 to T2 were compared between control and experimental canines using paired t-test. Also, the correlation of change in the MMP levels and rate of displacement was obtained for control and experimental canines using Pearson's correlation coefficient.

All the analyses were performed using SPSS version 20.0 (IBM Corp.) and the statistical significance was tested at 5% level.

Results

table I shows the rate of displacement on control and experimental side from T0 to T1 and from T0 to T2. The mean rate of displacement between T0 to T1 on control side was 0.94 mm with SD of 0.29 mm, while on experimental side was 1.36 mm with SD of 0.35 mm. The difference in the rate of displacements was statistically significant ($P \leq 0.05$) with *P*-value of 0.0026, using paired t-test. Further, the mean rate of displacement between T0 to T2 was 0.96 mm with SD of 0.33 mm on control side, while it was 1.33 mm and 0.48 mm on experimental side. The difference in the rate of displacements was statistically significant ($P \leq 0.05$) with *P*-value of 0.0267. The difference between rate of displacement during T1-T2 between groups was statistically insignificant as revealed by *P*-value of 0.3589 ($P > 0.05$).

table II shows the MMP-9 concentration (in ng/mL) in GCF measured at different time points and obtained from ELISA test. *table III* gives the descriptive statistics for change in the marker expression on control and experimental side. The mean change in levels up to 3 months on control side was 9.62 ng/mL with SD of 2.24 ng/mL, while on experimental side, it was 11.09 ng/ml with SD of 1.69 ng/mL. The difference of changed levels between the two groups was statistically significant ($P = 0.0296$). The mean change in the levels up to time of retraction on control side was 10.13 ng/mL with SD of 3.64 ng/mL, while on experimental side was 11.09 ng/mL with SD of 3.09 ng/mL. The difference was statistically insignificant ($P = 0.2022$). The difference of change in levels between T1 and T2 between two groups was also statistically insignificant ($P = 0.5681$).

Table I

Rate of displacement for control and experimental canine (mm/month).

Time difference	Control (*n* = 10)			Experimental (*n* = 10)			*P*-value
	Mean	SD	Median	Mean	SD	Median	
T0 to T1 (M1)	0.94	0.29	0.92	1.36	0.35	1.32	0.0026**
T0 to T2 (M2)	0.96	0.33	0.97	1.33	0.48	1.20	0.0267*
T1 to T2	0.34	0.16	0.35	0.29	0.11	0.28	0.3589 (NS)

Obtained using paired t-test; NS: Not significant. Significant, $^{*}P \leq 0.05$, $^{**}P \leq 0.01$.

Table II

MMP-9 concentration (in ng/mL) in GCF measured at different time points: baseline, 14 days (E1 & C1), 3 months(E2 & C2), end(E3 & C3) of canine retraction) obtained from ELISA test.

Patient	Baseline	E1	C1	E2	C2	E3	C3
1.	5.488	0.685	0.587	13.658	11.488	10.918	11.946
2.	3.165	0.587	0.293	14.605	14.108	13.79	7.331
3.	1.314	0.247	0.121	14.649	13.973	14.462	13.84
4.	3.642	0.37	0.176	15.645	13.257	10.517	8.591
5.	4.115	0.356	0.192	15.584	14.583	15.59	14.83
6.	1.057	0.345	0.109	11.452	11.777	15.534	15.755
7.	1.881	0.945	0.463	13.799	8.492	16.134	14.913
8.	6.161	1.554	1.14	14.379	14.16	15.16	16.745
9.	2.096	0.164	0.147	14.291	10.995	14.788	14
10.	1.544	0.587	0.219	13.399	13.831	14.437	13.829

Table III

Change in marker expression for control canine and experimental canine (ng/mL).

Duration	Control (n = 10)			Experimental (n = 10)			P-value
	Mean	SD	Median	Mean	SD	Median	Duration
Baseline to T1	9.62	2.24	10.04	11.09	1.69	11.66	0.0296*
Baseline to T2	10.13	3.64	11.31	11.09	3.09	12.08	0.2022 (NS)
T1 to T2	0.51	3.92	0.35	0.013	2.55	0.25	0.5681 (NS)

Obtained using paired t-test; NS: Not significant. Significant, $^{*}P \leq 0.05$.

table IV shows the mean MMP9 levels at baseline and at subsequent time points till retraction for Experimental and Control groups. Comparison of means across times was performed using repeated measure analysis of variance. The analysis revealed that the difference of means across times in both groups was statistically highly significant with P-value < 0.0001. *table V* provides the comparison of MMP9 levels between different times obtained for Experimental and Control groups. The mean difference of MMP9 levels between all the time intervals was statistically significant except for 3 month- end of canine retraction, both in the Experimental group (P = 0.9865) and the control one (P = 0.6892).

table VI provides the correlation between change in the levels of MMP9 (ΔMMP9) and rate of displacement in two different

Table IV

Comparison of mean MMP-9 levels with time in Control and Experimental groups.

Groups	Baseline	14 days	After 3 months	At canine retraction	P-value
Experimental	3.05 ± 1.78	0.58 ± 0.41	14.15 ± 1.20	14.13 ± 1.92	< 0.0001***
Control	3.05 ± 1.78	0.34 ± 0.32	12.66 ± 1.94	13.18 ± 3.04	< 0.0001***

Obtained using repeated measures ANOVA; $^{***}P \leq 0.001$.

Table V
Paired comparison of MMP9 levels between times 1: at 14 days; 2: at 3 months; 3: at the end of canine retraction.

	P-value	
Comparison	Experimental	Control
Baseline–1	0.0008 (S)	0.0003***
Baseline–2	< 0.0001 (HS)	< 0.0001***
Baseline–3	< 0.0001 (HS)	< 0.0001***
1–2	< 0.0001 (HS)	< 0.0001***
1–3	< 0.0001 (HS)	< 0.0001***
2–3	0.9865 (NS)	0.6892 NS

*Obtained using paired t-test followed by Bonferroni correction. NS: Not significant. Significant, $^{*}P \leq 0.05$, $^{**}P \leq 0.01$, $^{***}P \leq 0.001$.

Table VI
Correlation between change in levels of MMP9 and rate of displacement for control and experimental canines.

Duration	Control		Experimental	
	*r**	*P*-value	*r**	*P*-value
Baseline to T1	0.35	0.3211	0.17	0.634 NS
Baseline to T2	0.51	0.1354	0.28	0.4371 NS

r*: Pearson's correlation coefficient. NS $P > 0.05$.

durations for control and experimental canines. For baseline to T1, on the control side, the correlation between ΔMMP9 and rate of displacement was 0.35. In other words, with the increase of ΔMMP9, the rate of displacement increased; however, the relationship was statistically non-significant ($P = 0.3211$). For baseline to T2, the correlation was of 0.51, thus positive but also statistically non-significant ($P = 0.1354$). And, on the experimental side, for the baseline to T1 duration, the correlation was of 0.17 ($P = 0.634$). With the increase of ΔMMP9, the rate of displacement also increased on this side; but here again, the relationship was statistically insignificant ($P > 0.05$). It is the same for baseline to T2.

Since T1 is taken at 3 months from the start of the canine retraction, for all the patients T1-T0 interval duration is 3 months. T2 is taken at the end of canine retraction on the experimental side. Thus, T2-T1 interval duration is different for all the patients. At T2, on the control side, the canine was not yet finished.

table VII shows that it took 135.6 days in total (4.5 months) for completion of canine retraction on experimental side. With this the average rate of tooth movement on the experimental side was 1.36 mm/month but on control side was 0.96 mm/month. Considering this the treatment time was calculated to be around 6.25 months (187.5 days) on the control side.

Table VII
Time duration for canine retraction.

Patient	T2-T1 (days)	T2-T1 (months)	T0-T2 (months)
1.	60	2.00	5
2.	45	1.50	4.5
3.	45	1.50	4.5
4.	60	2.00	5
5.	30	1.00	4
6.	40	1.33	4.33
7.	30	1.00	4
8.	60	2.00	5
9.	40	1.33	4.33
10.	45	1.50	4.5

Discussion

Study protocol

In this study the laser (Model: DenLase Version: DenLase-SY-A. 1. c, China Daheng Group, Inc) used was a semiconductor type (AlGaAs) with wavelength of 980 nm in continuous mode with power output 0.3 W according to the manufacturer's guidelines for Biostimulation. In previous studies these parameters were different as compared to ours because of different reasons: animal models, different laser systems and guidelines. Therefore, the direct comparison between ours and previous studies is limited.

TAD (Temporary Anchorage Devices) were placed so that the question for anchorage loss in form of mesial movement of maxillary first molar does not arise. Placement of TAD would have caused Regional Acceleration Phenomenon (RAP) but since the placement site was away from retraction site, it is unlikely to affect the rate of tooth movement. Furthermore, since TADs were placed on both the sides, the rate of retraction on both sides were comparable.

GCF is a predominantly inflammatory exudate and is often demonstrative of underlying remodelling activity of bone. Collection of GCF is a non-invasive procedure in contradiction to collection of blood serum. Moreover, GCF is better representative of molecular changes happening during tooth movement as compared to saliva. It was collected on day 1 before LILT irradiation (Baseline) from either the experimental or control side, 24 hours after the start of irradiation (E1 & C1), 3 months

(E2 & C2) and finally at the completion of canine retraction on experimental side (E3 & C3).

The GCF collection was timed at such intervals because earlier studies by Ozawa [15] and Kim [16] reported early response to LILT. Furthermore, a previous study by Doshi-Mehta and Bhad-Patil [17] reported more acceleration during the first 3 months of the individual canine retraction as compared to the following phase of decreased acceleration. Moreover, Saito and Shimizu [18] reported better osteoblastic response in midpalatal sutures of rats that were irradiated earlier than those which were irradiated later.

Bone remodelling is a combination of bone deposition and bone resorption. Bone resorption is considered to be rate limiting step in orthodontic tooth movement. So, in order to study tooth movement, it is best to choose bone resorption marker rather than bone deposition marker.

Matrix metalloproteinase 9 (MMP-9, 92-kD gelatinase/type IV collagenase = gelatinase B) is a member of the MMP gene family and implicated in tissue destruction in the various pathophysiologic conditions. MMP-9 can cleave the cross-link-containing NH (2)-terminal telopeptides of the alpha 2 chain of type I collagen and collagen types III, IV, and V as well as gelatines. MMP-9 is produced by osteoclasts in the human bone tissues and suggest that it can degrade bone collagens in concert with MMP-1 and cysteine proteinases in the subosteoclastic microenvironment. Hence MMP-9 is a predominantly bone resorption marker which can be studied on pressure side during tooth movement [19]. Yamaguchi et al. [20] also studied MMP-9 on resorption side in rats. Therefore, the expression of MMP-9 was studied in GCF on pressure side.

Rate of orthodontic tooth movement

The mean rate of Orthodontic Tooth Movement (OTM) on control side at the end of 3 months was 0.94 mm/month while on experimental side it was 1.36 mm/month and the difference amongst them was statistically significant (*table I*). In other words, the mean rate of tooth movement on experimental side was 1.44 times more as compared to that of control side. This shows that there was 44% increase in rate of orthodontic tooth movement on experimental side as compared to the control side. Similar results were obtained by Ge. et al. [10] who showed 20-40% increase in the rate of tooth movement. Also, AlSayed Hasan [21], also reported 30% decrease in treatment time throughout the treatment.

But, Kim et al. [22] reported a higher rate of tooth movement than the present study over a period of 2 months in dogs. 150 gm of force was used to retract canine. They found 2.08 fold increase in tooth movement for their experimental LILT sample as compared to 1.44-fold increase over a period of 3 months in the present study. Also, they have used pulsed mode as opposed to continuous mode used in the present study (as per the manufacturer's guidelines). Yoshida et al. [23] stated that laser units show more bio-stimulatory response when functioning in pulsed mode, but Bradley et al. [24] and Takeda [25] used the continuous mode effectively. The mean rate of Orthodontic Tooth Movement (OTM) on experimental side at the end of canine retraction was 1.33 mm/month while on control side it was 0.96 mm/month and the difference amongst them was statistically significant (*table I*). This implies that the overall mean rate of tooth movement on experimental side was 1.38 times more as compared to that of control side, showing that there was a 38% increase in the rate of orthodontic tooth movement on experimental side as compared to the control side.

So, there was decrease in acceleration from 44% in the first three months as compared to 38% during the overall canine retraction. However, Youssef et al. [26] reported the rate of canine retraction as almost twice as fast as control canines over a six-month period in human subjects using split mouth design. He used 8 J application dose. The reason could be increased irradiation frequency of 4 times/month till the end of canine retraction as compared to 5 times/month in the first month and 2 times/month in subsequent months in our study.

MMP-9 levels in GCF

However, after three months of canine retraction and until the completion of treatment (T1-T2) the change in marker expression on experimental side was 0.013 ng/ml while on control side it was 0.51 ng/ml. The difference was statistically insignificant. The mean change in MMP-9 throughout the canine retraction on experimental side was 11.09 ng/mL while on control side it was 10.13 ng/ml and the difference was statistically insignificant (*table III*).

Thus, LILT increased the expression of MMP-9 in GCF in the first three months of canine retraction. However, this effect decreased after the first 3 months as change in MMP-9 concentration were similar in both experimental and control groups. LILT being effective in the earlier part of orthodontic tooth movement is evident from previous studies by Kim et al. [16], Doshi-Mehta and Bhad-Patil [17] and Saito and Shimizu [18].

When mean MMP-9 concentrations were compared within the group at different time intervals (Baseline, 24 hours, 3 months, at the end of canine retraction on experimental side) using ANOVA, the difference between them was highly significant. There was statistically significant change in MMP-9 levels throughout the canine retraction within both groups (*table IV*). When MMP-9 levels were compared between different time intervals within the groups using Paired t-test followed by Bonferroni correction the difference was statistically significant from baseline to 3 months, but it was insignificant thereon in both the groups. This indicates that MMP-9 levels are influenced most during initial part of the tooth movement and then tends to remain constant till the end of retraction (*table V*).

Yamaguchi et al. [20] studied effect of LELI (Low Energy Laser Irradiation) on orthodontic tooth movements in rats and expression of matrix metalloproteinase-9, cathepsin K, and alpha(v) beta (3) integrin. On the pressure side they found out higher expression of MMP-9, cathepsin K, and alpha (v) beta (3) integrin as well as significant increase in velocity of orthodontic tooth movement on laser side. However, this study used animal model and Immunohistochemical staining (IHC) to determine the expression of biomarkers, the results were quite similar to that of our study. Since the animals were sacrificed for tissue sectioning after 7 days, long term effect of Laser in this study was not determined.

Correlation of tooth movement and MMP-9 levels in GCF

When mean changes in MMP-9 levels were correlated with rate of orthodontic tooth movement for both experimental and control side, at any point of time during the canine retraction the correlation was positive. This means that with increase in the change in MMP-9 marker expression in GCF the rate of orthodontic tooth movement also increased. This correlation was positive throughout the canine retraction, but it was statistically insignificant (*table VI*).

Lee et al. [27], in their study on rats consistently reported positive correlation between relapse rates and MMP levels throughout the experiment. Since relapse is nothing but PDL remodelling after removal of orthodontic force, such a correlation is also evident during orthodontic tooth movement. Findings of our study were consistent with this but the statistical significance of such a correlation could have been assessed better if the sample size was larger.

Acceleration by LILT

It took 135.6 days in total (4.5 months) for completion of canine retraction on experimental side. Considering average rate of canine retraction on control side as 0.96 mm/month the treatment time comes to around 6.25 months (187.5 days). Thus, there was 38% reduction in total treatment time on experimental side as compared to control side at the end of canine retraction (*table VII*).

When compared to other methods such as Piezocision (1.5 times) and Corticotomy (1.5 to 2 times)[2], LILT produced lesser acceleration (1.38 to 1.44 times) as compared to conventional orthodontic tooth movement.

Limitations of present study

The limitation of this study was a smaller sample size with which we obtained statistically insignificant correlation between rate of tooth movement and change in marker expression.

Moreover, the age range of the selected participants was too broad (14–24 years). Within a patient, rate of retraction can be compared but while calculating acceleration, age can contribute as a confounding factor. Bigger and age matched samples would have been more appropriate for the study design.

CONSORT checklist [28] and flow diagram for patient selection has been shown.

There was no external funding received for the trial.

Conclusions

In the condition of present RCT, the following conclusions were drawn:

- LILT increases the rate of orthodontic tooth movement by 38%;
- application of LILT increases the concentration of MMP-9 in GCF, in the initial period of orthodontic tooth movement;
- changes in the application schedule for LILT are desirable as it is more effective in the earlier part of tooth movement as compared to the later part;
- long term studies with larger sample size with age matched samples and more biomarkers are required to fully understand the role of LILT in accelerating orthodontic tooth movement at molecular level.

Disclosure of interest: the authors declare that they have no competing interest.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.ortho.2020.01.008.

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