Original Article

A Comparative Evaluation of Interleukin 1 Beta and Prostaglandin E2 with and without Low-level Laser Therapy during *En masse* Retraction

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Abstract

Background and Objectives: Orthodontic forces are known to produce mechanical damage and inflammatory mediators such as prostaglandins (PGs) and interleukin (IL)-1, in the periodontium and dental pulp. Low-level laser therapy (LLLT) is a stimulator of the on-going biological process in tissue and found to be effective in modulating cell activity, which is involved in orthodontic tooth movement. Here, a humble effort has been made to study two such cytokines, namely IL-1 β and PG E2 (PGE2) which are partially responsible for bone turnover. The purpose of this study was to compare the changes occurring in the values of IL-1 β and PGE2 in the gingival crevicular fluid (GCF) during *en masse* retraction with and without LLLT. **Methodology:** GCF was collected using micropipettes from the distal ends of upper canines. The experimental side was exposed to biostimulation using 810 nm gallium-aluminum-arsenide diode laser and the contralateral side taken as control. A total of 10 irradiations for 10 s per site were given, five on the buccal side and five on the palatal side, to cover the entire periodontal fibers and the alveolar process around the tooth. After 7 days and 21 days of retraction, GCF sample was collected. Quantitative analysis of IL-1 β and PGE2 in the GCF samples was assessed using a commercially available Raybiotech® IL-1 β and Human PGE2. **Results:** (1) IL-1 β and PGE2 levels showed significant results from baseline to 21 days after LLLT irradiation. (2) LLLT-assisted retraction was significantly faster than conventional retraction. **Interpretation and Conclusion:** It was concluded from the study that IL-1 β and PGE2 levels peaked after LLLT. The difference in the levels of both cytokines was statistically significant.

Keywords: *Cytokine, interleukin 1 beta, low-level laser therapy, prostaglandin E2*

Introduction

At the dawn of the 20th century, Edward H. Angle, the father of Modern Orthodontics, advocated including courses in the biological sciences in orthodontic training programs. At that time, these courses were mainly inside the domain of anatomy and physiology. However, at the onset of the 21st century, the scientific frontiers in biology have witnessed remarkable advances in molecular biology and molecular genetics. Angle's recommendation for biological sciences in the orthodontic curriculum most likely stemmed from his realization that orthodontic treatment was rendered to human patients. This fact had remained unaltered throughout the passing years as most orthodontic departments adopted the conviction that orthodontic excellence should be derived from a comprehensive knowledge of mechanics and biology. In reality, however, commanding knowledge of mechanics, material science, and metallurgy has prevailed, while biological sciences continue to play a minor role in clinical orthodontics.[1]

One of the major concerns of orthodontic patients is treatment time. Reducing the treatment time requires increasing the rate of physiologic tooth movement.[2] Many methods have been used in the past to accelerate the orthodontic tooth movement (OTM) such as electric and magnetic stimulation, drug injections of parathyroid hormone, misoprostol (prostaglandin [PG] E1 analog), and PG E2 (PGE2).[3] Although these substances stimulate the rate of tooth movement, they also have undesirable side effects such as local pain and discomfort during the injections. Recently, electric stimulation and resonance vibration have been tried in animals, but these methods require an apparatus that is not routinely used in dental practice. There have been several studies on the effects of lasers on soft and

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hard tissues in dentistry.[4] In orthodontics, there are *in vivo* studies on the biostimulatory effects of lasers during bone remodeling and dental movement.

The periodontal ligament (PDL), is the soft tissue matrix that joins the dental root to the alveolar bone, and also contains an intricate network of blood vessels and nerve endings. The fluid within the connective tissue of the PDL has three sources: cellular, vascular, and interstitial. This fluid being jelly-like when not in motion flows easily under pressure. The PDL ends cervically at the dentoenamel junction where the junctional epithelium acts as a weak barrier separating the PDL from the crevicular fluid.[5]

Orthodontic forces produce a distortion of the PDL matrix, resulting in an alteration in cellular shape and cytoskeletal configuration and neuropeptide release from afferent nerve endings. At the biomolecular level, these forces may induce the release of PGs, growth factors, and cytokines. Finally, orthodontic forces appear to generate cytokines that affect the formation and resorption of bone.[6]

The gingival crevicular fluid (GCF) is a transudate of interstitial tissues which is produced by an osmotic gradient, and it is released into the crevicular sulcus at a flow rate of about 3 μl/h. However, during periodontal inflammation, the main mechanism of GCF formation becomes exudative, with an increase in its flow rate, and thus volume increases up to 44 μl/h.[7]

Previous evidence has shown that the GCF volume might be a better indicator of gingival inflammation than standard clinical assessments. Considering that tissue remodeling is incident to OTM and is triggered by an inflammatory process, it has been hypothesized that the volume of GCF production will reflect these tissue changes. However, contrasting results have been reported in the literature, with studies showing both increased and unchanged GCF volumes incident to OTM. Orthodontic forces cause acute inflammatory reactions, vascular changes, and migration of leukocytes.[8] A number of previous studies have been focused on certain cytokines and enzymes in the GCF. In many previous studies, alkaline phosphatase, lactate dehydrogenase, 5 aspartate aminotransferase, PGE, interleukin (IL)-1, IL-6, IL-8, tumor necrosis factor (TNF), glucuronidase, and transforming growth factor have been evaluated. IL-1 is a known potent cytokine that facilitates the survival, fusion, and activation of osteoclast (OC), thus contributing to the initiation of bone resorption. IL-1 β, a major physiologic form of IL-1, is secreted mainly by monocytes and somewhat by macrophages, endothelial cells, fibroblasts, and epidermal cells, and its secretion is induced by various stimuli. Interestingly, IL-1 β is an inducer of PGE, and together with mechanical stress, it synergistically upregulates the formation of the PGs in the periodontal cells.[9]

Hence, the purpose of this study is to compare and evaluate the effects of IL-1 β and PGE2 with and without low-level laser therapy (LLLT) during *en masse* retraction of the teeth.

Aims and objectives of the study

1. To evaluate IL-1 β and PGE2 levels in GCF with LLLT during *en masse* retraction
2. To evaluate IL-1 β and PGE2 levels in GCF without LLLT during *en masse* retraction
3. To compare IL-1 β and PGE2 levels in GCF from the above groups
4. To draw clinical inferences from the same.

Methodology

The study subjects were patients seeking fixed orthodontic treatment from the Department of Orthodontics and Dentofacial Orthopaedics, Coorg Institute of Dental Sciences, Virajpet, Karnataka.

Inclusion criteria

- Patients undergoing first premolar extraction as a part of orthodontic treatment
- Patients with healthy periodontal conditions and good level of oral hygiene
- Patients with no history of antibiotic or anti-inflammatory drugs use in the month preceding the study.

Exclusion criteria

1. Patients with any systemic disorders
2. Patients with periodontal problems
3. Patients who have taken antibiotic medications during the past 3 months.

Armamentarium

Gingival crevicular fluid collection

- Graduated capillary pipettes
- Tin foil
- Cryovials
- Sample storage: −80°C

Enzyme-linked immunosorbent assay analysis

- Enzyme-linked immunosorbent assay (ELISA) reader (Sunrise, TESCAN)
- Microplate
- Micropipette
- Disposable micropipette tips
- ELISA kit (Raybiotech® Human IL-1 β and Human PGE2).

Sample collection

GCF samples of 5 μl were collected from the sites receiving treatment using a graduated microcapillary pipette by extracrevicular method. The samples from the treatment sites were collected at baseline, 7 days after laser irradiation, and 21 days after retraction. These pipettes were wrapped in tin foil, transferred to plastic vials, and stored at −70°C until the time of assay. Microcapillary pipette was procured from Sigma Aldrich, Bangalore, India.

The storage of GCF samples was done at Coorg Institute of Dental Sciences.

Laboratory analysis

Quantitative analysis of IL-1 β and PGE2 in the GCF samples was assessed using a commercially available ELISA test (Raybiotech® Human IL-1 β and Human PGE2).

Procedure summary for interleukin-1 beta

1. Prepare all reagents, samples, and standards as instructed
2. Add 100 µl standard or sample to each well. Incubate 2.5 h at room temperature
3. Add 100 µl prepared biotin antibody to each well. Incubate 1 h at room temperature
4. Add 100 µl prepared streptavidin solution. Incubate 45 min at room temperature
5. Add 100 µl TMB one-step substrate reagent to each well. Incubate 30 min at room temperature
6. Add 50 µl stop solution to each well. Read at 450 nm immediately.

Procedure summary for prostaglandin E2

1. Add 50 µL standard or sample to each well. Immediately add 50 µL biotinylated detection antibody to each well. Incubate for 45 min at 37°C
2. Aspirate and wash three times
3. Add 100 µL horseradish peroxidase conjugate to each well. Incubate for 30 min at 37°C
4. Aspirate and wash five times
5. Add 90 µL substrate reagent. Incubate for 15 min at 37°C
6. Add 50 µL stop solution. Read at 450 nm immediately
7. Calculation of results.

Procedure

The study participants were 12 in number. All cases were patients requiring first premolar extractions with good and normal periodontal condition.

Before commencement of the study, the patients were advised good oral hygiene methods and also were systematically checked for periodontal problems if any and were given oral prophylaxis 1 week before the study. GCF was collected using graduated microcapillary tubes from the distal ends of upper canine before sending for extraction of first premolars (T0) [Figure 1]. The patient was then sent for extraction of premolars. After leveling and aligning of the arches, laser irradiation on upper one side anterior segment will be done (experimental side). The experimental side was exposed to biostimulation using 810 nm diode laser and the contralateral side taken as control. All irradiations were done by the same operator using 810 nm gallium-aluminum-arsenide (GaAlAs) diode laser delivered with a power output of 100 mW in a continuous wave mode. A total of 10 irradiations was given, five on the buccal side and five on the palatal side, to cover the entire periodontal fibers and alveolar process around the tooth; the distribution and order were as follows – on the buccal and palatal side – (1) two irradiation doses on the cervical third of the root (1 mesial and 1 distal), (2) two on the apical third of the root (1 mesial and 1 distal), and (3) one on the middle third (center of the root) of canine lateral and central incisor of experimental side [Figures 2 and 3].

The experimental side was irradiated for 10 s per site. The total energy density (dose) at each application was 10 J (2 × 50 s × 100 mW). After 7 days of laser irradiation, one more sample of GCF was collected (T1) and the retraction process was started. After 21 days of retraction process had started, another GCF sample was collected (T2) before the laser irradiation [Figure 4]. Quantitative analysis of IL-1 β and PGE2 in the GCF samples was assessed using a commercially available Raybiotech® IL-1 β and Human PGE2.

A calibration curve was plotted by regression analysis, and the optical density of each sample was used to estimate the concentration of IL-1 β and PGE2 (pg/µL). This was corrected for the original volume of GCF, and the results were expressed as pgIL-1 β/µL and pgPGE2/µL.

These data were then compared with the data obtained from the GCF samples collected from controlled side where *en masse* retraction had been employed on both the segments and the data were then subjected to statistical analysis.

Results

In the present study, levels of IL-1 β and PGE2 in GCF were compared with and without LLLT during *en masse* retraction in patients where LLLT was used on the one side while on the other side was retracted using conventional retraction techniques.

In this study, split-mouth technique was used in all subjects; LLLT was performed distal to canine on the right quadrant of upper arch while left quadrant was left as control. GCF samples were collected from the distal ends of canines on the right and left side at baseline, 7 days after LLLT, and 21 days before LLLT. The collected GCF was then tested for IL-1 β and PGE2 using ELISA test (Raybiotech® Human IL-1 β and Raybiotech® Human PGE2).

Statistical methods applied for the study were

The data were collected, coded, and fed in the SPSS (IBM version 23 Statistical Analsis in Social Science). The descriptive statistics were calculated. The inferential statistics included parametric test, i.e. independent *t*-test, one-way ANOVA, and *post hoc* Tukey's test. The level of significance is set at 0.05 at 95% confidence interval.

Highly significant difference was seen while comparing IL-1 β values at control site at different time intervals [Table 1a].

At baseline, When compared to the baseline, IL-1 β showed a significant difference in 7 days and a highly significant

difference in 21 days. Also a highly significant difference was seen when IL-1 β was compared between 7 days and 21 days [Table 1b].

Highly significant difference was seen in the values of IL-1 β at LLLT site at all intervals [Table 2a].

At baseline, when compared, highly significant differences are seen in 7 days and 21 days. Between 7 and 21 days, when compared, a highly significant difference was seen [Table 2b].

Highly significant difference was seen while comparing PGE2 values at control site at different time intervals [Table 3a].

At baseline, when compared, highly significant differences are seen in 7 days and 21 days. Between 7 days and 21 days, when compared, a highly significant difference was seen [Table 3b].

Highly significant difference was seen in the values of PGE2 at LLLT site at all intervals [Table 4a].

At baseline, when compared, no significant difference was seen in 7 days and a highly significant difference was seen in 21 days. Between 7 days and 21 days, when compared, a highly significant difference was seen [Table 4b].

At baseline, no significant differences were seen between control and LLLT site. Highly significant differences were seen at 7 days and 21 days in IL-1 β levels at all time intervals in both control and LLLT sites [Table 5 and Graph 1].

At baseline, no significant differences were seen between control and LLLT site. Highly significant differences were seen at 7 days and 21 days in PGE2 levels at all time intervals in both control and LLLT sites [Table 6 and Graph 2].

Table 1a: Comparison of interleukins 1β value among study subjects at control site at different intervals (ANOVA)

Control	Mean	SD	*P*
Baseline	0.6307500	0.59009986	0.000 (HS)
7 days	4.4024167	2.51710322	
21 days	10.6041667	5.18800266	

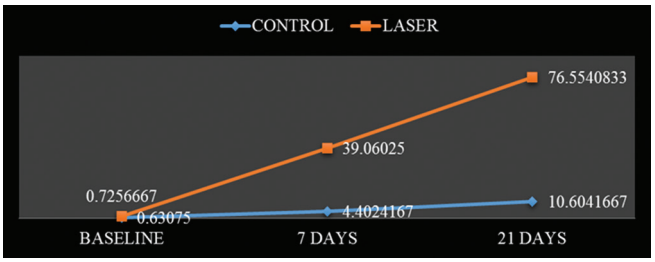
SD: Standard deviation; HS: Highly significant

Discussion

Orthodontic forces can result in capillary vasodilatation in the PDL, which results in migration of inflammatory cells as well as cytokine production. This in turn helps in the process of bone remodeling. Cytokines are proteins that act as signals between the cells of the immune system, which are produced during the activation of immune cells. Cytokines such as IL-1, IL-6, IL-8, and TNF-α have been proved to be associated with bone remodeling. The OTM acceleration was brought by raising the remodeling activity of bone. The velocity of OTM is related to cytokine release which can be detected in GCF.[10]

High concentration of cytokines such as ILs IL-1, IL-2, IL-3 IL-6, IL-8, and TNF-α was found to play a major role in bone remodeling; moreover, IL-1 stimulates OC function through its receptor on OCs. It was also found that mechanical stress due to orthodontic treatment increased the production of PGE and IL-1 β in the PDLs. IL-1 β has the most potent cytokine-stimulating OC activity and attracting white blood cells and other cellular mediators in the process of bone remodeling. It is the first polypeptide regulating the processes of resorption and neo-bone apposition in relation to mechanical stress. Moreover, IL-1 β is one of the mediators of inflammation which induces the secretion of substances causing pain. Besides, IL-1 β is produced by the PDL in quantity sufficient to diffuse into the GCF and has been identified as a biomarker of orthodontic movement.[11]

PGs, produced by deformed osteoblasts and gingival fibroblasts, are cytokines implicated in inflammation provoked by OTM. Among the subclasses of PGs, PGE2 is strongly related to bone resorption. PGE2 is induced by IL-1 β. IL-1 β synergically upregulates the formation



Graph 1: Comparison of IL 1β levels between control and LLLT site at different time intervals

Table 1b: Comparison of interleukins 1 β value among study subjects at control site at different intervals (*post hoc* Tukey's test)

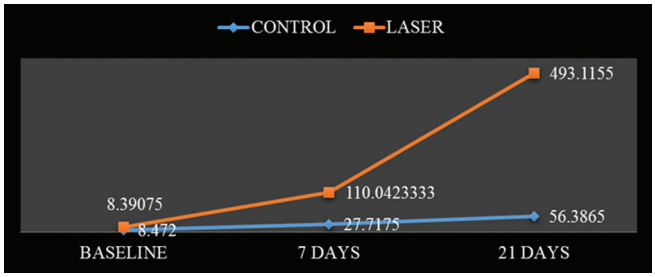
Control	Mean difference	SE	Significance value	95% CI	
				Lower bound	Upper bound
Baseline					
7 days	−3.77166667	1.366248	0.025 (S)	−7.1241582	−0.4191751
21 days	−9.97341667	1.366248	0.000 (HS)	−13.3259082	−6.6209251
7 days					
21 days	−6.20175000	1.366248	0.000 (HS)	−9.5542415	−2.8492585

SE: Standard error; CI: Confidence interval; HS: Highly significant; S: Significant

of PGs in the periodontal tissue subjected to stress orthodontic.

Photobiomodulation or LLLT is one of the most promising approaches today. Laser has a biostimulatory effect on bone regeneration. It has been found that laser light stimulates the proliferation of OC, osteoblast, and fibroblasts, thereby affecting bone remodeling and thus accelerating the tooth movement. The mechanism involved in the acceleration of tooth movement is by the production of ATP and activation of cytochrome C that low-energy laser irradiation enhanced the velocity of tooth movement via receptor activator of nuclear factor kappa-B (RANK)/RANK ligand (RANKL) and the macrophage colony-stimulating factor and its receptor expression.[12]

The major components of an LLLT system are the laser device itself, a delivery system, and a controller. All common commercially available LLLT systems use semiconductor diode lasers. These are generally variants of either GaAlAs, which emit in the near-infrared spectrum (wavelength 700–940 nm), or indium: gallium: arsenide: phosphorus devices, which emit in the red portion of the visible spectrum range (wavelength 600–680 nm).[13]



Graph 2: Comparison of PGE2 levels between control and LLLT site at different time intervals

Table 2a: Comparison of interleukins 1 β value among study subjects at low-level laser therapy site at different intervals (ANOVA)

Laser	Mean	SD	*P*
Baseline	0.7256667	0.58421436	0.000 (HS)
7 days	39.0602500	19.90972884	
21 days	76.5540833	29.91712001	

SD: Standard deviation; HS: Highly significant

The mechanisms of LLLT are complex but essentially rely upon the absorption of particular visible red and near-infrared wavelengths in photoreceptors within subcellular components, particularly the electron transport (respiratory) chain within the membranes of mitochondria. The electron transport chain is able to provide increased levels of promotive force to the cell, through increased supply of ATP, as well as an increased in the electrical potential of the mitochondria membrane, alkalization of the cytoplasm, and activation of nucleic acid synthesis. Because ATP is the "energy currency" for a cell, LLLT has a potent action that results in stimulation of the normal functions of the cell.[14]

In addition, it has been demonstrated that laser irradiation stimulates cellular proliferation and differentiation of osteoblast lineage nodule-forming cells, especially in committed precursors, resulting in an increase in the number of differentiated osteoblastic cells as well as in bone formation. A final aspect of the effect of LLLT on cells is related to the effects of laser light on the cytoskeleton. Several studies have suggested that LLLT can modulate cell behavior by causing re-arrangements of the cytoskeleton.[15]

Myofibroblasts are responsible for the contraction force during wound healing. These cells are observed in normal tissue, granulation one, and some pathological conditions. Because LLLT is an effective stimulator of differentiation to myofibroblasts, the process of wound healing should be accelerated.

LLLT has also been proven to reduce synthesis of inflammatory mediators in neural tissue, as well as more rapid maturation and regeneration, particularly axonal growth. It also reduces pain in patients suffering from postherpetic neuralgia, from cervical dentinal hypersensitivity, and from periodontal pain during OTM.

The present study was done to compare the changes in the values of IL-1 β and PGE2 in GCF on patients, in which *en masse* retraction was done by with and without LLLT.

Study procedure

In the present study, 12 subjects were selected according to the inclusion criteria. Split-mouth technique was used in all

Table 2b: Comparison of interleukins 1 β value among study subjects at low-level laser therapy site at different intervals (*post hoc* Tukey's test)

Laser	Mean difference	SE	Significance value	95% CI	
				Lower bound	Upper bound
Baseline					
7 days	−38.33458333	8.471431	0.000 (HS)	−59.1217351	−17.5474316
21 days	−75.82841667	8.471431	0.000 (HS)	−96.6155684	−55.0412649
7 days					
21 days	−37.49383333	8.471431	0.000 (HS)	−58.2809851	−16.7066816

SE: Standard error; CI: Confidence interval; HS: Highly significant

subjects wherein the upper right quadrant was designated as the reaction site and the upper left quadrant was designated as the control site. Before commencement of study, the patients were advised good oral hygiene methods and also were systematically checked for periodontal problems if any and were given oral prophylaxis 1 week before the study. LLLT was done on the upper right side, and retraction was started on the right and left side simultaneously. GCF samples were collected from distal aspect of canine on both control and reaction sites at baseline, 7 days after laser irradiation, and 21 days before laser irradiation. The GCF samples were put through Elisa test (Raybiotech® Human IL-1 β and Human PGE2) for determining the concentration of IL-1 β and PGE2. A calibration curve was plotted by regression analysis, and the optical density of each sample was used to estimate the concentration of IL-1 β and PGE2 (pg/μL). This was corrected for the original volume of GCF and the results were as pgIL-1 β/μL and pgPGE2/μL.

The statistical analysis in this study such as descriptive statistics, independent t-test, one-way ANOVA, and post hoc Tukey's test was done using SPSS (IBM version 23) for Windows were used. SPSS statistics software. Developer - IBM Corporation.

The mean value of IL-1 β and PGE2 was seen to peak after LLLT. When compared with independent *t*-test, it was found to be very highly significant in all 4 time intervals. This is attributed to the inflammatory reaction that is expected to happen when the LLLT is irradiated. IL-1 β and PGE2 have been found to directly stimulate human microvascular endothelial cells production of RANKL, enabling them to directly promote human OCs formation and bone resorption, as well as the release of other proresorptive factors such as TNF-α, IL-6, IL-8, fibroblast growth factor-2, and platelet-derived growth factor-AB. Such exposure may predispose precursors to develop into OCs once they enter bone tissue.

Hence, it is seen that higher levels of IL-1 β and PGE2 will increase the potential for much greater OCs formation and bone resorption in an inflammatory setting.

Comparison with other similar studies

Our study showed similarity with another study done by Grieve *et al.* (1994),[1] who compared the levels of two potent bone-resorbing mediators, PGE and IL-1 β during OTM. The study included ten patients, each having one treatment tooth undergoing orthodontic movement and a contralateral tooth taken as control. The GCF was sampled at control sites a treatment (compression) sites before activation and at 1, 24, 48, and 168 h. At 1 and 24 h, mean GCF IL-1 β levels were significantly elevated at treatment teeth compared with control teeth. The GCF levels of PGE

Table 3a: Comparison of prostaglandin E2 value among study subjects at control site at different intervals (ANOVA)

Control	Mean	SD	*P*
Baseline	8.4720000	0.91608832	0.000 (HS)
7 days	27.7175000	6.39997305	
21 days	56.3865000	18.40990998	

SD: Standard deviation; HS: Highly significant

Figure 1: Baseline sample collection from right and left side using capillary tubes from distal of canine

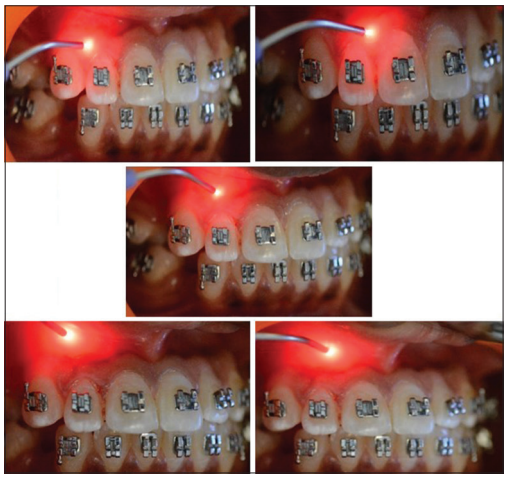


Figure 2: Laser irradiation on the buccal sites

Table 3b: Comparison of interleukins 1 β value among study subjects at control site at different intervals (*post hoc* Tukey's test)

Control	Mean difference	SE	Significance value	95% CI	
				Lower bound	Upper bound
Baseline					
7 days	−19.24550000	4.59905581	0.001 (HS)	−30.5306379	−7.9603621
21 days	−47.91450000	4.59905581	0.000 (HS)	−59.1996379	−36.6293621
7 days					
21 days	−28.66900000	4.59905581	0.000 (HS)	−39.9541379	−17.3838621

SE: Standard error; CI: Confidence interval; HS: Highly significant

for the treatment teeth were significantly higher at 24 and 48 h than the control teeth. The GCF levels of PGE and IL-1 β remained at baseline levels throughout the study for the control teeth, whereas significant elevations from baseline in GCF IL-1 β (24 h) and PGE levels (24 and 48 h) were observed over time in the treatment teeth. This was in similarity to our study as in our study there was a peak in the IL-1 β and PGE2 values after laser irradiation.

Our study also showed similarity with a study done by Lee *et al.* (2004),[16] which examined the effects of a light continuous force and an interrupted force with weekly reactivation on IL-1 (IL-1 β) and PGE2, and the possible interactions between these two potent mediators of the bone resorption process were assessed *in vivo*. Ten healthy young adults with four premolars extracted were assessed. In each subject, one maxillary canine (E1) received continuous force with a nickel-titanium coil spring. The opposite canine (E2) received an interrupted force with a screw-attached retractor; the force was reactivated weekly by two turns of the screw. An antagonistic canine was used as a control. GCF was collected from the distal side of each tooth, 10 times in 3 weeks, and IL-1 β and PGE2 levels were measured. Both experimental sites showed significant tooth movement compared with the control sites at 3 weeks. Well-controlled mechanical stresses with timely reactivation can effectively upregulate IL-1 β secretion, but there might be limitations in increasing the mediator levels, because of the feedback mechanisms *in vivo*. In addition, the analysis of crevicular fluid is a useful method for assessing cellular response to orthodontic force *in vivo*.

Our study was similar to another study done by Doshi-Mehta and Bhad-Patil (2012),[17] who assessed the efficacy of low-intensity laser therapy in reducing treatment time and orthodontic pain. In this study, twenty patients requiring extraction of first premolars were selected. The method used in this study was a randomly assigned incomplete block split-mouth design. Individual canine retraction by a nickel-titanium closed-coil spring was studied. The experimental side received infrared radiation from a semiconductor (GaAlAs) diode laser with a wavelength of 810 nm. The laser regimen was applied on days 0, 3, 7, and 14 in the 1st month, and thereafter on every 15th day until complete canine retraction was achieved on the experimental side. Tooth movement was measured on progress models. Each patient's pain response was ranked according to a visual analog scale. The result showed an

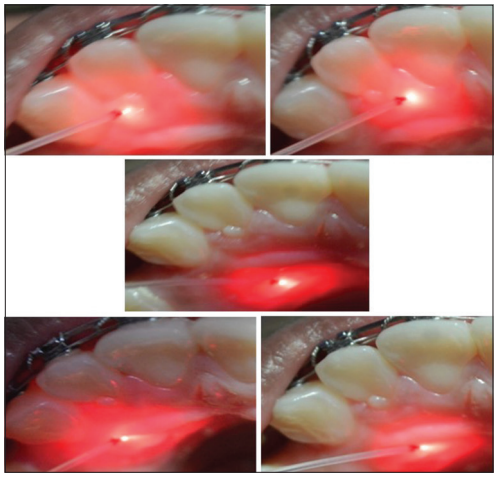


Figure 3: Laser irradiation on the palatal sites

Figure 4: Collection of samples 7 days after low-level laser therapy and 21 days before low-level laser therapy

Table 4a: Comparison of prostaglandin E2 value among study subjects at low-level laser therapy site at different intervals (ANOVA)

Laser	Mean	SD	*P*
Baseline	8.3907500	1.07671412	0.000 (HS)
7 days	110.0423333	32.43171381	
21 days	493.1155000	355.08317377	

SD: Standard deviation; HS: Highly significant

Table 4b: Comparison of interleukins 1 β value among study subjects at low-level laser therapy site at different intervals (*post hoc* Tukey's test)

Laser	Mean difference	SE	Significance value	95% CI	
				Lower bound	Upper bound
Baseline					
7 days	−101.65158333	84.04265969	0.456 (NS)	−307.8750011	104.5718344
21 days	−484.72475000	84.04265969	0.000 (HS)	−690.9481678	−278.5013322
7 days					
21 days	−383.07316667	84.04265969	0.000 (HS)	−589.2965844	−176.8497489

SE: Standard error; CI: Confidence interval; HS: Highly significant; NS: Not significant

Table 5: Comparison of interleukins 1 β levels between control and low-level laser therapy site at different time intervals (independent *t*-test)

Interleukins	Mean	SD	*t*	*P*
Baseline				
Control	0.630750	0.5900999	−0.375	0.711 (NS)
Laser	0.721167	0.5899873		
7 days				
Control	4.402417	2.5171032	−5.983	0.001 (HS)
Laser	39.060250	19.9097288		
21 days				
Control	10.604167	5.1880027	−7.524	0.000 (HS)
Laser	76.554083	29.9171200		

SD: Standard deviation; HS: Highly significant; NS: Not significant

Table 6: Comparison of prostaglandin E2 levels between control and low-level laser therapy site at different time intervals (independent *t*-test)

Prostaglandins	Mean	SD	*t*	*P*
Baseline				
Control	8.472000	0.9160883	0.199	0.710 (NS)
Laser	8.390750	1.0767141		
7 days				
Control	27.689083	6.4170623	−8.629	0.002 (HS)
Laser	110.042333	32.4317138		
21 days				
Control	56.386500	18.4099100	−4.255	0.000 (HS)
Laser	493.115500	355.0831738		

SD: Standard deviation; HS: Highly significant; NS: Not significant

average increase of 30% in the rate of tooth movement was observed with the low-intensity laser therapy. Pain scores on the experimental sides were significantly lower compared with the control sides. The study concluded that low-intensity laser therapy is a good option to reduce treatment duration and pain.

Conclusion

The present study was done to compare the changes in the values of IL-1 β and PGE2 in GCF on patients in which *en masse* retraction will be done with and without LLLT.

It was concluded from the present study that the levels of IL-1 β and PGE2 peaked at 7th day after LLLT and 21st day before LLLT from baseline.

This study showcased the molecular changes that are responsible for bone turnover in case of LLLT as compared to normal retraction procedures and also gave an insight into the reactions and responses that happen with OTM.

Summary

The present study was done to compare the changes in the values of IL-1 β and PGE2 in GCF on patients in which *en masse* retraction will be done with and without LLLT. Split-mouth technique was used in all subjects, wherein the upper right quadrant was designated as the reaction site and the upper left quadrant was designated as the control site. LLLT was done on the upper right side and retraction was started on the right and left side simultaneously. GCF samples were collected from distal aspect of canine on both control and reaction site at baseline, 7 days after LLLT, and 21 days before LLLT. The GCF samples were put through ELISA test (Raybiotech® Human IL-1 β and Human PGE2) for determining the concentrations of IL-1 β and PGE2.

Following parameters were compared:

1. IL-1 β levels variation in control site and reaction site at fixed time intervals
2. PGE2 levels variation in control site and reaction site at fixed time intervals.

The study concluded that:

1. The levels of IL-1 β and PGE2 gradually increased from baseline to 7th day after LLLT and 21st day before LLLT
2. The rate of retraction was significantly higher in LLLT when compared with conventional retraction.

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Conflicts of interest

There are no conflicts of interest.

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