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Low-level laser therapy increases interleukin-1β in gingival crevicular fluid and enhances the rate of orthodontic tooth movement

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Introduction: The aim of this investigation was to evaluate the effects of low-level laser therapy on interleukin-1β (IL-1β) levels in gingival crevicular fluid and its correlation with orthodontic tooth movement. **Methods:** A split-mouth design was used in 10 subjects (6 female, 4 male) aged 14 to 25 years, whose maxillary first premolars were extracted. A gallium-aluminum-arsenide semiconductor diode laser (wavelength, 940 nm; energy density, 8 J/cm2; power output, 100 mW) delivered low-level laser therapy to the experimental canine undergoing distalization at 10 points. The control canine was distalized without low-level laser therapy. The experimental and control canines were distalized using a force of 150 g provided by nickel-titanium closed-coil springs. Gingival crevicular fluid was collected at 5 time points from the control and experimental sides, and the levels of IL-1β were analyzed by enzyme-linked immuno-sorbent assay (ELISA). The distal movements of the maxillary canines were measured and compared. **Results:** Increased levels of IL-1β were observed in the experimental canines compared with the control canines ($P < 0.001$). Cumulative tooth movements over an 8-week experimental period were greater for the experimental canines (occlusogram and software, 4.450 and 4.4903 mm, respectively) compared with the control canines (occlusogram and software, 2.025 and 2.0501 mm, respectively). A positive correlation existed between the IL-1β levels and the amounts of tooth movement across all time intervals. **Conclusions:** In combination with light orthodontic force, application of low-level laser therapy increased the levels of IL-1β in gingival crevicular fluid and accelerated orthodontic tooth movement. (Am J Orthod Dentofacial Orthop 2018;154:535-44)

Orthodontic tooth movement is a highly complex process defined as an adaptive biologic response to interference in the physiologic equilibrium of the dentofacial structures by an externally applied force.[1] As a result of the organized periodontal tissue remodeling after the application of mechanical forces, bone remodeling during tooth kineticism is a biologic mechanism that involves an acute inflammatory response.[2] At the cellular level, remodeling of the periodontium encompasses bone resorption contiguous to the periodontal ligament in the zone of compression, bone deposition in the zone of tension, and degeneration and reorganization of the periodontal ligament.[3] The force-induced tissue strain generates local alterations in vascularity, in conjunction with cellular and extracellular matrix reorganization evoking a synthesis and release of various neurotransmitters, cytokines, colony-stimulating factors, growth factors, and metabolites of arachidonic acid.[4]

Cytokines are diminutive protein molecules that regulate cell communication and function by inducing cellular proliferation and differentiation; they are actively secreted by diverse cell types in response to external stimuli.[5,6] Interleukin 1 (IL-1) is a cytokine that exists in 2 forms—alpha (α) and beta (β)—of which IL-1β is pertinent in bone metabolism.[4] One of the most potent cytokines in the periodontal milieu during the initial stage of orthodontic tooth movement, IL-1β

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is secreted by fibroblasts, macrophages, cementoblasts, osteoblasts, and osteoclasts.[7] Since the survival, fusion, and activation of osteoclasts correlate with IL-1β, it can be relevant as a biomarker in determining the magnitude of orthodontic tooth movement dependent on alveolar bone-remodeling efficiency.[8]

Iwasaki et al[9] observed a rapid velocity of orthodontic tooth movement and elevated IL-1β/IL-1 receptor antagonist levels in the gingival crevicular fluid of experimental teeth. IL-1β levels determined by radioimmunoassay[10] and enzyme-linked immuno-sorbent assay (ELISA)[11] showed significantly greater elevations in the experimental teeth compared with the corresponding controls, attributed to inflammation in response to mechanical stress. Also, the *P2X7R* gene plays a crucial role in bone biology and mechanotransduction, and primarily promotes necrotic tissue metabolism.[12] Adenosine triphosphate-activated *P2X7R* is involved in IL-1β processing and release as a result of mechanical stress.[13]

The classic 2- to 3-year span for orthodontic treatment is burdensome for patients; thus, it is of paramount importance to expedite alveolar bone remodeling during orthodontic treatment to abbreviate the time required.[14] Presently, there is heightened inclination for researchers to concenter on accelerating methods for tooth movement due to the immense need to curtail orthodontic treatment time.[15]

Nonsurgical and device-assisted therapies, because of their minimal invasiveness, have been used to biologically accelerate tooth movement.[15] Substantial orthodontic tooth movements of 2 to 3 mm per month have been attained using vibratory stimuli with significant patient acceptance and compliance.[16]

Expedited tooth movement with laser irradiation, however, has been the cynosure of recent studies. Kawasaki and Shimizu[14] reported that low-level laser therapy stimulated tooth movement and osteoclast formation on the compression side during experimental tooth movement in vivo in rats. Fujita et al[17] and Yamaguchi et al[18] demonstrated that low-level laser therapy accelerated tooth movement via receptor activator of nuclear factor kappa B (RANK), RANK ligand (RANKL), and macrophage-colony stimulating factor along with its receptor (c-fms) expression in vitro, respectively. Other studies have also shown that low-level laser therapy enhances the rate of orthodontic tooth movement.[19-21] Therefore, low-level laser therapy appears to be a good alternative, due to its stimulatory effects on orthodontic tooth movement with few detriments.

Previous studies have independently assessed the levels of inflammatory cytokines[5-8] and low-level laser therapy[17-21] on the rate of orthodontic tooth movement, but to date the amalgamation of the response of mediators of bone remodeling combined with low-level laser therapy and orthodontic force has been unspecified. The assessment of IL-1β during laser irradiation could provide insight into the basis of accelerated tooth movement observed with this modality.

The value of gingival crevicular fluid in the appraisal of the biologic state of the innate tissues of the bone, periodontium, and allied tooth-investing structures extends itself as a source of biomarkers of specific clinical conditions.[22] This has merit in monitoring the outcome and efficiency of orthodontic treatment, primarily the response of the alveolar bone to forces during tooth movement.

In this study, we aimed to identify and assess the gingival crevicular fluid levels of IL-1β during orthodontic tooth movement and the correlation with low-level laser therapy using a gallium-aluminum-arsenide semiconductor diode laser, and to determine whether low-level laser therapy can accelerate orthodontic tooth movement.

MATERIAL AND METHODS

Patients reporting to the Department of Orthodontics and Dentofacial Orthopedics, SDM College of Dental Sciences and Hospital, in Dharwad, Karnataka, India, for treatment were included in this prospective study, after approval from the institutional review board and ethical committee. Written and informed consents were obtained from all patients in the study.

Ten patients (6 female, 4 male), aged 14 to 25 years (mean age, 17.7 years), were evaluated for the effects of low-level laser therapy on the rate of orthodontic tooth movement and the levels of IL-1β in gingival crevicular fluid.

All patients had Angle Class I malocclusion with bimaxillary protrusion and well-aligned arches, and were indicated for maxillary first premolar extraction and bilateral maxillary canine distalization. All subjects had good oral hygiene, probing depth values less than 3 mm, gingival index scores less than 1, no radiologic evidence of periodontal bone loss, and no use of anti-inflammatory drugs during the month preceding the study. Professional oral hygiene instructions were rendered before the study. Patients with systemic diseases, medical treatment, or medication that might hinder bone metabolism were excluded from the study.

A split-mouth study was designed in which the experimental side was assigned by a lottery method with a sealed envelope. To prevent bias, this was done before subject recruitment. Each patient was fitted with preadjusted edgewise brackets having an MBT

prescription (3M Gemini brackets; 3M Unitek, Monrovia, Calif) with 0.022 × 0.028-in slots. The maxillary canines were distalized on an 0.018-in stainless steel archwire (AJ Wilcock, Birmingham, United Kingdom) after complete leveling and aligning on a round 0.016-in nickel-titanium archwire (Ortho Organisers, San Marcos, Calif). A light nickel-titanium closed-coil spring (9 × 12 mm) (Ormco; Sybron Dental Specialities, Orange, Calif) was used to retract each canine, secured with a ligature wire, to deliver an initial force of 150 g between the maxillary canine and first molar. The amount of force was calibrated using a gauge dynamometer (Dontrix gauge; Leone, Florence, Italy). Retraction force was applied to the buccal side of the experimental and control canines. The same force was reapplied at the start of weeks 4 and 8.

At the beginning of retraction, the experimental and control canines were allocated by the lottery method with a sealed envelope. A gallium-aluminum-arsenide semiconductor diode laser (Ezlase; BIOLASE Technology, Irvine, Calif) with a wavelength of 940 nm, energy density of 8 J/cm^2, and 100 mW power output was used to deliver low-level laser therapy to the experimental canine undergoing distalization.

Application of low-level laser therapy was done immediately after spring activation at 10 points (Fig 1), 10 seconds per point, for 3 consecutive days at the following intervals: start of canine retraction, 4 weeks later, and 8 weeks later.

The control canine was subjected to distalization without low-level laser therapy. The laser probe was enclosed in a sheath. On the irradiation side, the probe sheath had a clear plastic end that was substituted for pseudo-irradiation on the control side with a sheath having a black plastic end. Dark green laser protective glasses were used by the operator (A.M.V.) to prevent identification of the laser sheath used. A third person (A.V.R.) controlled the use of the laser and the placebo sheath tubes.

Gingival index[23] and periodontal disease index[24] scores were recorded before the collection of gingival crevicular fluid. Gingival crevicular fluid was collected from the control and experimental canines of each maxillary quadrant using a calibrated volumetric microcapillary tube with graduated markings at every 1 μL until a standardized volume of 5 μL was collected after adequate isolation. Gingival crevicular fluid was collected at 5 time points from each subject on both the control and experimental canines, amounting to 100 samples, at the following times: T0, before starting canine retraction; T+3, after canine retraction for 3 days with and without low-level laser therapy on the experimental and control sides, respectively; T+7, after canine retraction for 7 days without low-level laser therapy; T1, after canine retraction for 4 weeks with and without low-level laser therapy on the experimental and control sides, respectively; and T2, after canine retraction for 8 weeks with and without low-level laser therapy on the experimental and control sides, respectively.

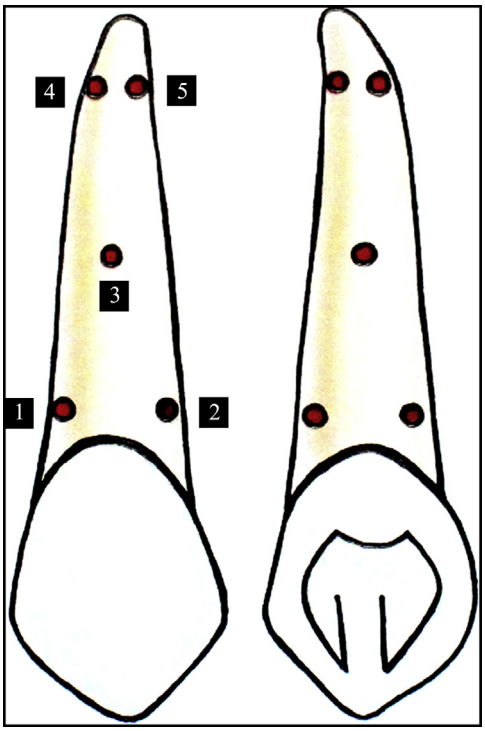

Fig 1. Points for laser application (buccal surface): *1*, distobuccal gingival ridge; *2*, mesiobuccal gingival ridge; *3*, buccal central point, central in relation to the other points; *4*, bottom of the oral vestibule at the root apex parallel to point 1; *5*, bottom of the oral vestibule, at the root apex parallel to point 2. The same points were selected for application on the lingual aspect, totaling 10 points.

The samples were then diluted with 250 μL of sterile phosphate-buffered saline solution (pH 7.4) and stored at −80°C until analysis.

The samples were assayed to determine the concentration of IL-1β (pg/μL) using a commercially available human IL-1β ELISA kit (Krishgen BioSystems, Brea, Calif).

The amount of canine retraction was measured. At the beginning of each set of irradiation, before placing the archwire and retracting the springs, an alginate impression was made and poured in dental stone to prepare a cast. A sequel of models (Supplemental Figs 1-4) was used to compute the amount of canine retraction relative to the stable landmark of the ipsilateral medial end of the third palatal rugae.

These landmarks were used to measure canine retraction from the occlusogram (Fig 2): XY, midpalatine raphe

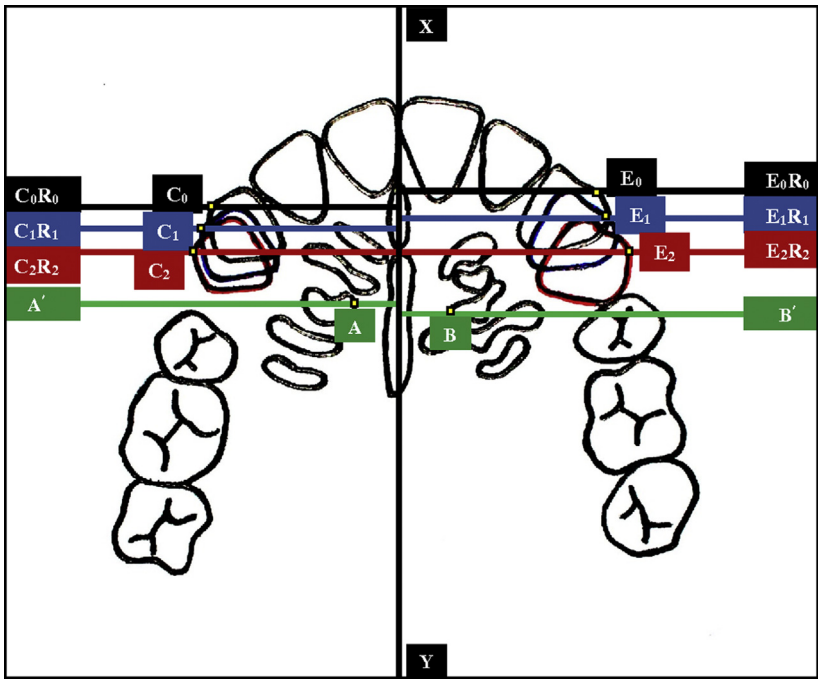

Fig 2. Schematic representation of the landmarks used to measure canine retraction from the occlusogram: *XY*, midpalatine raphe plane; *A*, median end of the right third palatal rugae; *B*, median end of the left third palatal rugae; *C0*, preretraction canine cusp tip (control); *C1*, 4 weeks postretraction canine cusp tip (control); *C2*, 8 weeks postretraction canine cusp tip control); *E0*, *E1*, and *E2*, same as C0, C1, and C2, respectively, on the experimental side; *C0R0*, line drawn perpendicular to XY passing through point C0; *C1R1*, line drawn perpendicular to XY passing through point C1; *C2R2*, line drawn perpendicular to XY passing through point C2; *E0R0*, *E1R1*, and *E2R2*, same as C0R0, C1R1, and C2R2, respectively, on the experimental side; *A′*, line drawn perpendicular to XY passing through point A; *B′*, line drawn perpendicular to XY passing through point B. Differences between the perpendiculars determined the amount of retraction.

plane; A, median end of the right third palatal rugae; B, median end of the left third palatal rugae; C0, preretraction canine cusp tip (control); C1, 4 weeks postretraction canine cusp tip (control); C2, 8 weeks postretraction canine cusp tip control); E0, E1, and E2: identical to C0, C1, and C2, respectively, on the experimental side; C0R0, line drawn perpendicular to XY passing through point C0; C1R1, line drawn perpendicular to XY passing through point C1; C2R2, line drawn perpendicular to XY passing through point C2; E0R0, E1R1, and E2R2, identical to C0R0, C1R1, and C2R2, respectively, on the experimental side; A′, line drawn perpendicular to XY passing through point A; and B′, line drawn perpendicular to XY passing through point B. The difference between the perpendiculars determined the amount of retraction.

Impressions were recorded at T0, T1, and T2.

The amount of canine retraction was measured as follows: R1, amount of canine retraction at the end of week 4; R2, amount of canine retraction from the end of week 4 to the end of week 8; and RT (R1 + R2), total amount of canine retraction.

The relative distances between the tips of the canines and the third rugae were measured using occlusograms (Fig 2), and the reevaluation of the amount of canine retraction was confirmed with software (CATIA V5R20; Dassault Systèmes, Vélizy-Villacoublay, France) (Fig 3).

Statistical analysis

A power analysis was performed to determine the sample size needed to detect significant differences in the levels of IL-1β and the amounts of canine retraction. The study was designed to have power of 90%, with a permissible α error of 5%. Normality of the variables was ascertained by the Kolmogorov Smirnov test in the control and experimental groups. Data analyses were done using software (SPSS for Windows, version 15.0; SPSS. Chicago, Ill). Two-way repeated measures of analysis of variance (ANOVA) was used to compare the levels

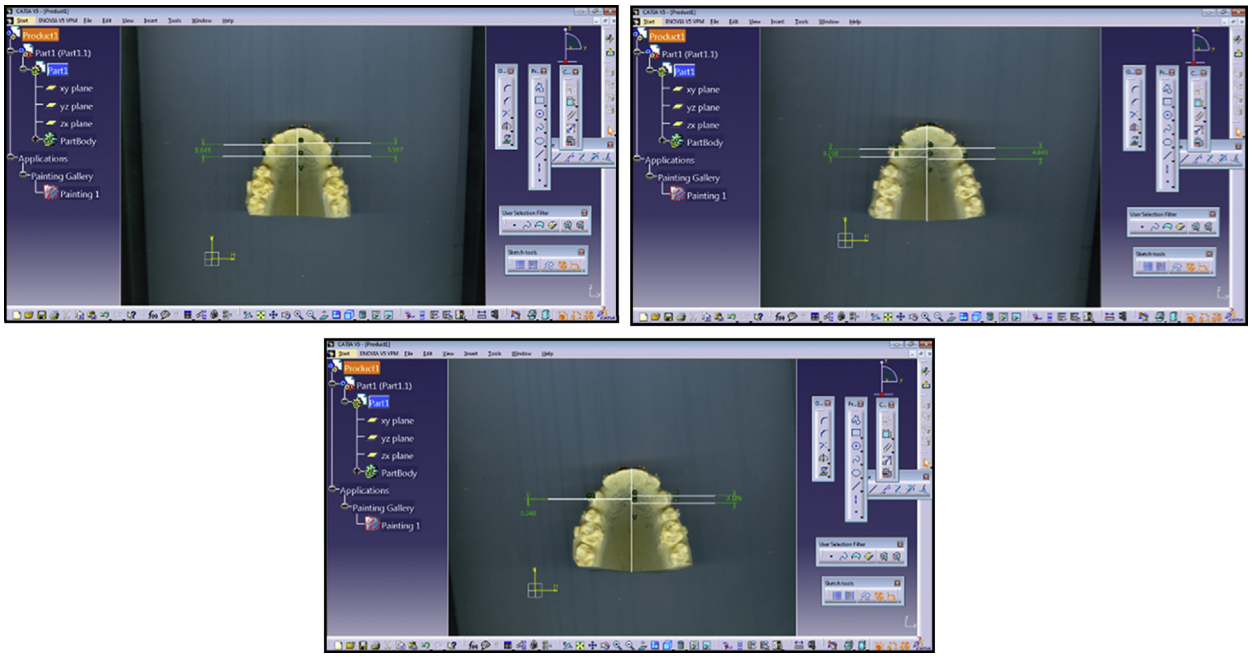

Fig 3. Amounts of canine retraction measured at T0, T1, and T2 on the control and experimental sides by the software.

Table I. Comparison between the 2 study groups (control and experimental) with respect to IL-1β values at T0 (baseline), T+3 (3 days), T+7 (7 days), T1 (1 month), and T2 (2 months) by 2-way repeated-measures of ANOVA

Source	*Type III sum of squares*	*Degrees of freedom*	*Mean square*	*F value*	P *value*
Groups	0.5990	1	0.5990	10053.7820	<0.001*
Times	2.5520	4	0.6380	7950.7290	<0.001*
Groups * times	1.2680	4	0.3170	5312.5540	<0.001*

*$P < 0.05$.

Table II. Comparison between the 2 study groups (control and experimental) with respect to the amount of canine retraction (occlusogram) by 2-way repeated-measures of ANOVA

Source	*Type III sum of squares*	*Degrees of freedom*	*Mean square*	*F value*	P *value*
Groups	39.2040	1	39.2040	383.1720	<0.001*
Times	43.7270	2	21.8640	377.0480	<0.001*
Groups * times	6.6020	2	3.3010	129.0540	<0.001*

*$P < 0.05$.

Table III. Comparison between the 2 study groups (control and experimental) with respect to the amount of canine retraction (software) by 2-way repeated-measures of ANOVA

Source	*Type III sum of squares*	*Degrees of freedom*	*Mean square*	*F value*	P *value*
Groups	39.6970	1	39.6970	360.9260	<0.001*
Times	43.6770	2	21.8390	331.7790	<0.001*
Groups * times	6.3390	2	3.1690	117.9000	<0.001*

*$P < 0.05$.

of IL-1β at the various times between the groups (Table I). The amounts of canine retraction measured by the occlusogram (Table II) and the software method (Table III) in both groups were compared using 2-way repeated-measures ANOVA. The Pearson correlation coefficient test was used to correlate the IL-1β values with the amounts of canine retraction by the occlusogram and software methods (Table IV). Correlations between the 2 methods were also assessed by the Pearson correlation coefficient (Table V).

RESULTS

Plaque accumulation was nominal (<1) as determind by the gingival index.[23] Periodontal destruction was not

Table IV. Correlation between IL-1β values and amounts of canine retraction measured by the occlusogram and software methods in the control and experimental groups by Pearson correlation coefficient

		(T0-T1) IL-1β		*(T1-T2) IL-1β*		*(T0-T2)-IL-1β*	
Group	*Value*	r *value*	P *value*	r *value*	P *value*	r *value*	P *value*
Control	R1 occlusogram	0.7837	0.0073*				
	R1 software	0.7678	0.0095*				
	R2 occlusogram			−0.0104	0.9772		
	R2 software			0.0643	0.8600		
	RT occlusogram					0.7552	0.0115*
	RT software					0.7867	0.0069*
Experimental	R1 occlusogram	0.8463	0.0020*				
	R1 software	0.8102	0.0045*				
	R2 occlusogram			0.4510	0.1908		
	R2 software			0.4486	0.1935		
	RT occlusogram					0.7286	0.0169*
	RT software					0.6874	0.0280*

*$P < 0.05$.

Table V. Correlation between amounts of canine retraction measured by the occlusogram and software methods in the control and experimental groups by Pearson correlation coefficient

		R1 occlusogram		*R2 occlusogram*		*RT occlusogram*	
Group	*Value*	r *value*	P *value*	r *value*	P *value*	r *value*	P *value*
Control	R1 software	0.9925	0.0001*				
	R2 software			0.9867	0.0001*		
	RT software					0.9917	0.0001*
Experimental	R1 software	0.9900	0.0001*				
	R2 software			0.9784	0.0001*		
	RT software					0.9946	0.0001*

*$P < 0.05$.

detected in any subject during the study as appraised by the periodontal disease index.[24]

Significantly higher levels of IL-1β were observed in the experimental canines compared with the control canines at all time intervals (Fig 4). Both the control and experimental groups demonstrated significant increases in the levels of IL-1β from T0 to T2; however, the percentage change in the experimental group was greater compared with the control group (Fig 4). In the experimental group, the levels of IL-1β compared with T0 were markedly elevated at T1 by approximately 4 times, and to approximately 10 times the initial value at T2 (Fig 4).

At all time points (T0-T1, T1-T2, and T0-T2), the amounts of canine retraction (R1, R2, and RT) were greater for the experimental canines compared with the control canines ($P < 0.001$) (Figs 5 and 6). At the end of week 8 of retraction combined with low-level laser therpay, the amount of canine retraction on the experimental side doubled compared with the end of week 4 and was higher than that on the control side by both the occlusogram (Fig 5) and the software (Fig 6) methods. The Pearson correlation coefficient demonstrated a positive correlation between the amounts of canine retraction calculated by the occlusogram and the software (Table V).

DISCUSSION

In orthodontics, no consensus exists on the most proficient method to maneuver teeth. An ideal approach ought to produce the most conceivable rate of orthodontic tooth movement without irreversible damage to the root, periodontal ligament, or alveolar bone.[25] Our study aimed at evaluating the stimulatory effects of low-level gallium-aluminum-arsenide semiconductor diode laser irradiation on experimental tooth movement, and comparing the IL-1β levels in gingival crevicular fluid on the irradiated and nonirradiated sides. Application of lasers intermittently for 8 weeks markedly elevated the levels of IL-1β on the laser-irradiated side compared with orthodontic force alone (Fig 4) and was

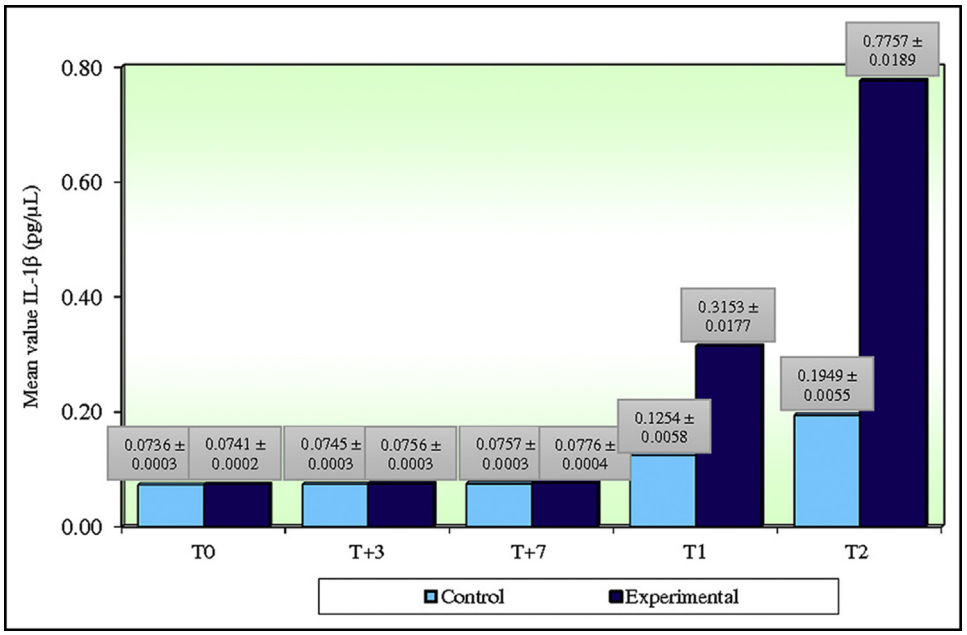

Fig 4. Comparison between the control and experimental groups with respect to IL-1β values (mean ± SD) at T0, T+3, T+7, T1, and T2.

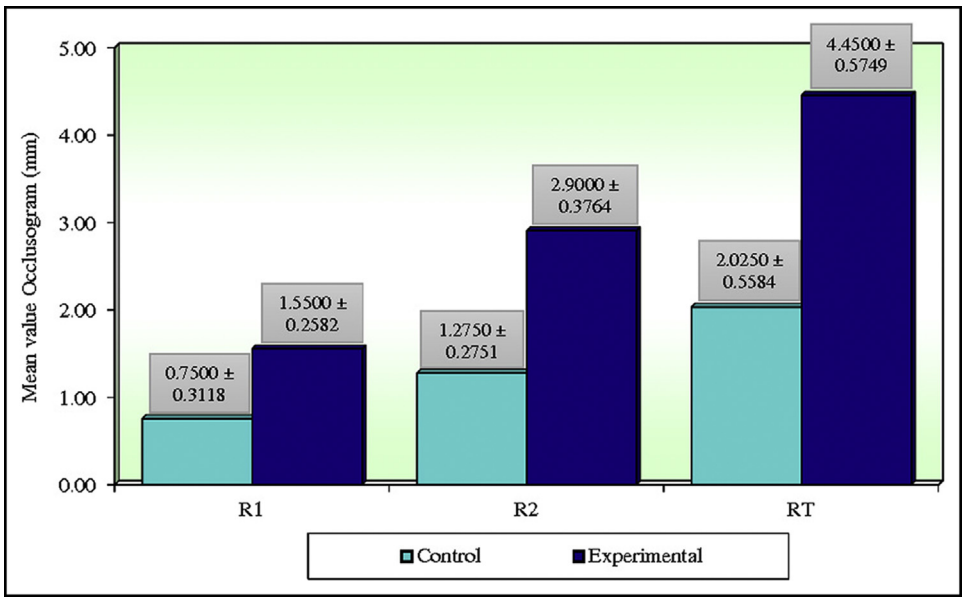

Fig 5. Comparison between the 2 study groups (control and experimental) with respect to the amount of canine retraction (mean ± SD) by the occlusogram method.

concurrent with increased rates of orthodontic tooth movement (Table IV).

Contemporary literature advocates that downstream from the germinal mechanotransduction event at focal adhesions, which link the extracellular framework to the cytoskeleton, mechanically actuated remodeling is mediated by an intricate feedback mechanism that involves the synthesis and release of cytokines such as IL-1, interleukin-6, and RANKL by cells of the osteoblast or fibroblast lineage.[26] These successively act either in an

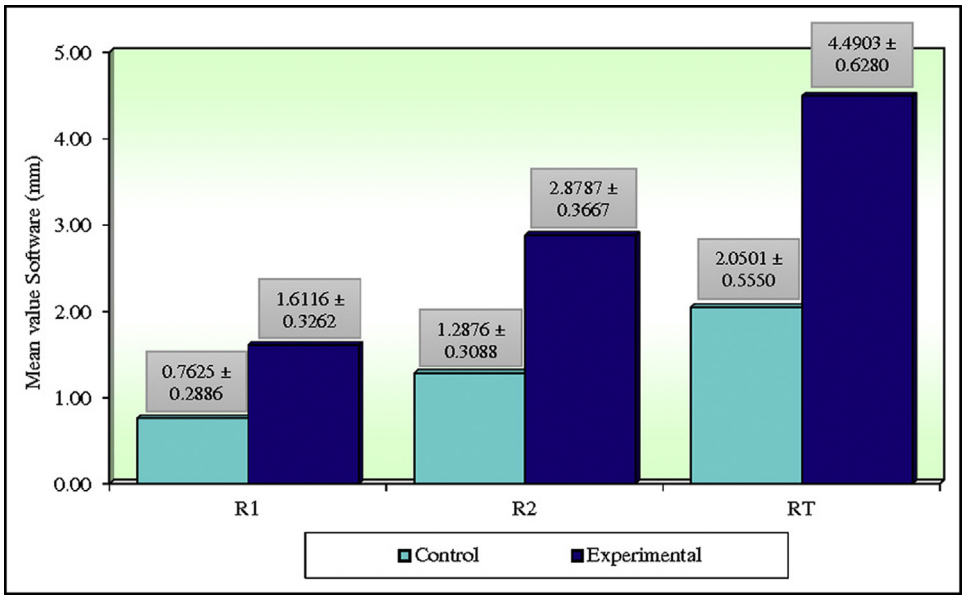

Fig 6. Comparison between the 2 study groups (control and experimental) with respect to the amount of canine retraction (mean ± SD) by the software method.

autocrine or a paracrine manner to regulate the expression of transcription factors, cytokines, structural molecules, and growth factors implicated in the proliferation, differentiation, and function of mesenchymal and other distinct cell types.

Secreted by osteoclasts as an immediate reaction to mechanical stress during the elementary stage of orthodontic tooth movement and by macrophages at later stages, IL-1β aggregation has been observed in compressed areas of the periodontal ligament. This cytokine determines the amount of tooth movement depending on alveolar bone remodeling efficiency, since the fusion, survival, and activation of osteoclasts corresponds with it.[8] Also, IL-1β is directly associated with bone resorption because it induces the expression of RANKL in osteoblasts and periodontal ligament cells and stimulates the differentiation of osteoclast precursors.[8,27] Therefore, in this study, we evaluated the IL-1β levels during orthodontic tooth movement since it had potential to be a valuable biologic marker in monitoring the bone remodeling process by initiating bone resorption and also playing a cardinal role in the inflammatory process associated with orthodontic tooth movement.

A slight increase in the IL-1β concentration was observed from T0 to T+3 in both groups (Fig 4; Supplemental Tables I and II); this suggests that bone remodeling was not progressive enough to effect a conspicuous increase in the IL-1β level and was caused primarily by a distortion of the periodontal ligament before bone remodeling. However, the experimental group demonstrated a greater increase in the levels of IL-1β compared with the control group (0.0015 and 0.0009 pg/μL, respectively) (Supplemental Table I). This can be attributed to the low-level laser irradiation on the experimental canine for 3 consecutive days that may have elicited an enhanced biologic response in the paradental tissues on the experimental side. Bone remodeling induces a release of IL-1 and interleukin-6 from the periodontal ligament that upregulates RANKL and matrix metalloproteinases by osteoblasts during orthodontic tooth movement. RANKL further stimulates the formation and function of osteoclasts from the mononuclear precursor cells that ingress the bone surface and degrade the mineralized matrix.

Low-level laser irradiation triggered a self-propagating cascade of events as evident by the increase in the IL-1β levels from T+3 to T+7 on the irradiated side (Fig 4). Low-level laser therapy targets the mitochondria, primarily cytochrome-c oxidase in the electron transport chain and porphyrins on the cell membrane. Light photons, when absorbed, stimulate adenosine triphosphate synthesis by activating the electron transport chain, transiently stimulating the reactive oxygen species (which successively increases the conversion of adenosine diphosphate to adenosine triphosphate) and also temporarily releases nitric oxide from its binding site on cytochrome-c oxidase.[28] These key factors play

significant roles in the clinical efficacy of low-level laser therapy.

In the experimental group, the mean concentration of IL-1β increased 4 fold at T1 and 10 fold at T2 compared with T0 (Fig 4). This agreed with the findings of Leethanakul et al,[29] who demonstrated increases in the IL-1β levels at 1 month and 2 months of canine retraction along with vibratory stimulation using a powered toothbrush. Analogous to a study by Uematsu et al,[30] the levels of inflammatory mediators in gingival crevicular fluid underwent significant elevations during orthodontic tooth movement. Increased IL-1β concentrations detected in our study indicated a potential biologic reaction to external stimuli adjunct to the application of conventional orthodontic forces.

Incremented IL-1β levels quantified in this study are within the restraints of an acceptable physiologic response. Furthermore, after the continuous application of laser irradiation, increased accumulation of IL-1β may occur over more than 8 weeks of canine retraction, and this prospect should be cautiously considered before low-level laser therapy is adopted as a routine intervention.

Safe, minimally invasive and cost-efficacious treatments are being sought to abbreviate treatment time; hence, it is consequential to expedite alveolar bone remodeling during orthodontic treatment. Histologic studies have attempted to actuate the effect of low-level laser therapy on the histochemical pathways directly associated with orthodontic tooth movement.[17,18,31,32]

The rate of canine retraction was significantly greater in the irradiated group compared with the nonirradiated group (Figs 5 and 6; Supplemental Tables III-VI); this agrees with other studies.[14,17,18] The irradiated group exhibited a 2-fold increase in the amount of canine retraction at the end of week 8 that correlated with an increase in the IL-1β secretion observed to be maximum at the end of the week-8 experimental period (Table IV). The greater amount of canine retraction at the experimental site can be accredited to the effect of elevated IL-1β secretion as a result of low-level laser therapy.

Low-level laser therapy has additional advantages of being minimally invasive, less traumatic, and precise. It also has a dose-dependent effect on alveolar bone remodeling and proliferation in vivo. In this study, a dose of 8 J/cm^2 was used at which significant biostimulatory effects were observed as accelerated tooth movement. This agreed with other studies and also demonstrated an increase in the rate of orthodontic tooth movement.[14,17-21]

To our knowledge, the effects of low-level laser therapy on orthodontic tooth movement with the evaluation of inflammatory cytokines involved during bone remodeling have not been investigated until now. Assessment of biomarkers of bone remodeling during laser irradiation could provide an understanding of the mechanism of accelerated tooth movement with this novel approach.

We exclusively elucidated the combined effects of low-level laser therapy on orthodontic tooth movement and its response on the inflammatory mediators of bone remodeling in conjunction with orthodontic force. This study is the first of its kind.

Future studies with a larger sample are warranted. Since IL-1β is closely related to root resorption, it should be analyzed to discern the extent and severity of the phenomenon. Also, studies evaluating the effects of different irradiation dosages, prolonged use of lasers on orthodontic tooth movement, and cell-to-cell interactions in the periodontium in response to low-level laser therapy are necessary. However, low-level laser therapy at an early stage in orthodontic treatment is practicable and may have a substantial therapeutic benefit to abbreviate treatment time.

CONCLUSIONS

In combination with light orthodontic forces, low-energy gallium-aluminum-arsenide semiconductor diode laser irradiation accelerated tooth movement with an increase in the level of IL-1β in gingival crevicular fluid.

Inflammatory cytokines expressed during orthodontic tooth movement can be measured by gingival crevicular fluid analysis, and increased levels of IL-1β reflects one probable mechanism underlying increased orthodontic tooth movement.

Low-level laser therapy-facilitated orthodontics is approximately 2 times faster than conventional orthodontics and can be used to provide physical stimuli resulting in accelerated tooth movement, by varying the patient's biologic response and not by merely increasing forces or altering treatment mechanics.

SUPPLEMENTARY DATA

Supplementary data related to this article can be found online at https://doi.org/10.1016/j.ajodo.2018.01.012.

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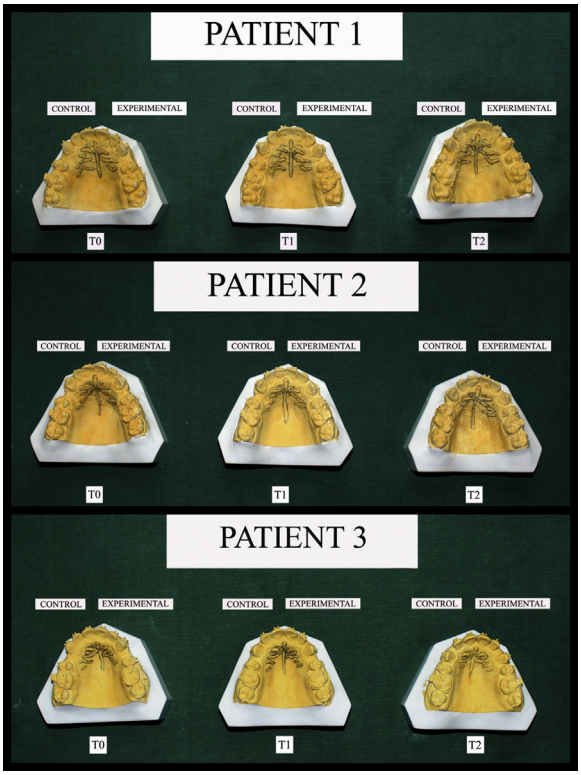
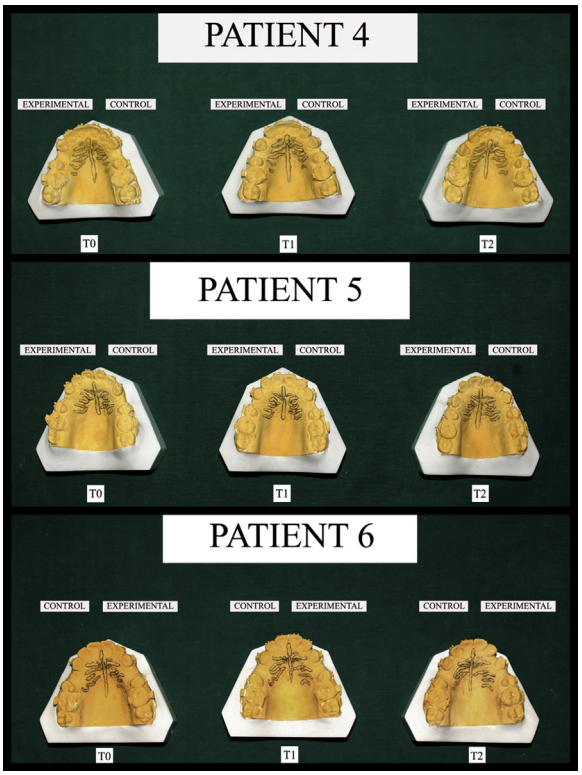
APPENDIX

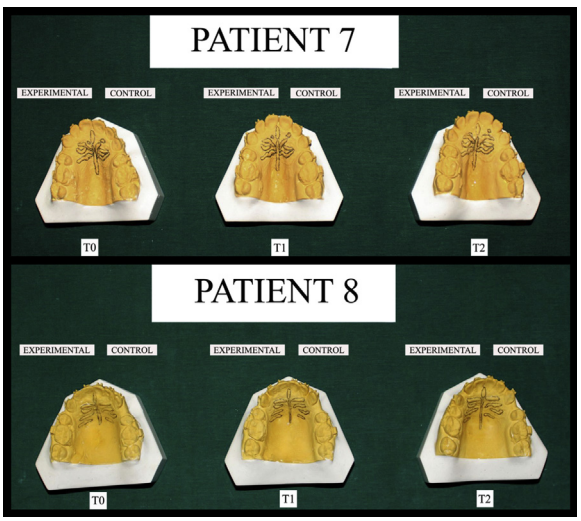


Supplemental Figs 1-4. Model pictures showing the amounts of canine retraction at T0, T1, and T2.

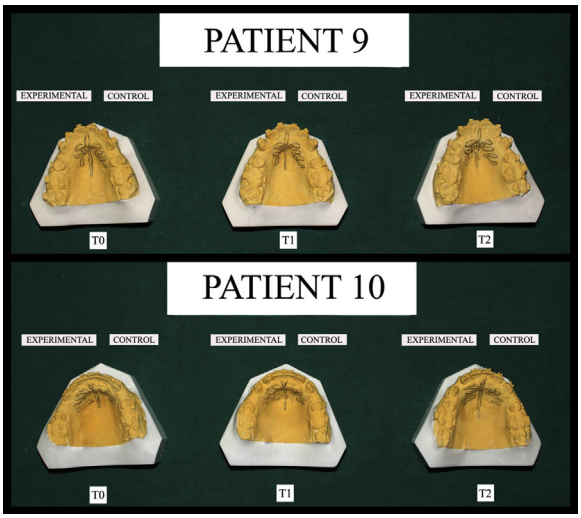
Video



Supplemental Figs 1-4. (*continued*).



Supplemental Figs 1-4. (*continued*).



Supplemental Figs 1-4. (*continued*).

Supplemental Table I. Bonferroni adjustment for multiple comparisons: IL-1β scores between control and experimental groups

				95% CI for difference	
Group	*Mean difference*	*SE*	P *value*	*Lower bound*	*Upper bound*
Control vs experimental	−0.1550	0.0020	<0.001*	−0.1580	−0.1510

*$P < 0.05$.

Supplemental Table II. Bonferroni adjustment for multiple comparisons: IL-1β scores at T0 (baseline), T+3 (3 days), T+7 (7 days), T1 (1 month), and T2 (2 months)

					95% CI for difference	
Time	*Time*	*Mean difference*	*SE*	P *value*	*Lower bound*	*Lower bound*
T0	T+3	−0.001	0.0001	<0.001*	−0.0010	−0.0010
	T+7	−0.003	0.0001	<0.001*	−0.0030	−0.0030
	T1	−0.146	0.0030	<0.001*	−0.1590	−0.1340
	T2	−0.411	0.0030	<0.001*	−0.4230	−0.4000
T+3	T+7	−0.002	0.0000	<0.001*	−0.0020	−0.0020
	T1	−0.145	0.0030	<0.001*	−0.1580	−0.1330
	T2	−0.410	0.0030	<0.001*	−0.4220	−0.3980
T+7	T1	−0.144	0.0030	<0.001*	−0.1560	−0.1310
	T2	−0.409	0.0030	<0.001*	−0.4200	−0.3970
T1	T2	−0.265	0.0040	<0.001*	−0.2800	−0.2500

*$P < 0.05$.

Supplemental Table III. Bonferroni adjustment for multiple comparisons: amounts of canine retraction (occlusogram) between control and experimental groups

				95% CI for difference	
Group	*Mean difference*	*SE*	P *value*	*Lower bound*	*Upper bound*
Control vs experimental	−1.6170	0.0830	<0.001*	−1.8030	−1.4300

*$P < 0.05$.

Supplemental Table IV. Bonferroni adjustment for multiple comparisons: amounts of canine retraction (occlusogram) at various time intervals

					95% CI for difference	
Time	*Time*	*Mean difference*	*SE*	P *value*	*Lower bound*	*Upper bound*
R1 (T0-T1)	R2 (T1-T2)	−0.9370	0.0470	<0.001*	−1.0740	−0.8010
	RT (T0-T2)	−2.0880	0.0890	<0.001*	−2.3500	−1.8250
R2 (T1-T2)	RT (T0-T2)	−1.1500	0.0850	<0.001*	−1.3990	−0.9010

*P <0.05.

Supplemental Table V. Bonferroni adjustment for multiple comparisons: amounts of canine retraction (software) between control and experimental groups

				95% CI for difference	
Group	*Mean difference*	*SE*	P *value*	*Lower bound*	*Upper bound*
Control vs experiment	−1.6270	0.0860	<0.001*	−1.8210	−1.4330

*P <0.05.

Supplemental Table VI. Bonferroni adjustment for multiple comparisons: amounts of canine retraction (software) at various time intervals

					95% CI for difference	
Time	*Time*	*Mean difference*	*SE*	P *value*	*Lower bound*	*Upper bound*
R1 (T0-T1)	R2 (T1-T2)	−0.8960	0.0530	<0.001*	−1.0520	−0.7400
	RT (T0-T2)	−2.0830	0.0920	<0.001*	−2.3530	−1.8130
R2 (T1-T2)	RT (T0-T2)	−1.1870	0.0920	<0.001*	−1.4570	−0.9170

*$P < 0.05$.