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Diode Laser Irradiation Effects on Miniscrew Stability and IL-1β and TGF-β1 Levels: A Split-Mouth Randomized Controlled Clinical Trial

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Abstract

Introduction: This study aimed to assess the effect of low-level laser therapy (LLLT) on miniscrew stability and concentrations of interleukin-1β (IL-1β) and transforming growth factor-beta (TGF-β1) in peri-miniscrew crevicular fluid in the course of orthodontic treatment.

Methods: This randomized split-mouth double-blind clinical trial evaluated 18 patients requiring anterior retraction along with maximum anchorage. Miniscrews were placed between the maxillary second premolar and first molar. A diode laser was irradiated with a 980-nm wavelength and 100-mW output power in continuous-wave mode at four time points: T0 (1 hour after miniscrew placement), T1 (1 week later), T2 (at 1 month) and T3 (at 3 months) in one quadrant of the maxilla (laser group). The other quadrant of the maxilla underwent the pseudo-application of the laser (control group). The primary stability of miniscrews was measured by Periotest M and reported as Periotest value (PTV). Also, at each time point, samples were collected from the peri-miniscrew crevicular fluid one hour after laser irradiation to assess the concentration of IL-1β and TGF-β1.

Results: The mean PTV (inverse of the stability) was smaller in the laser group compared with the control group at all time points; this difference was significant at T2 and T3. The mean concentration of IL-1β in the laser group was lower than that in the control group at all time points, and this difference was significantly remarkable at T0 and T3. The mean concentration of TGF-β1 in the laser group was lower than that in the control group at T0, T1 and T3; however, the difference was not statistically significant.

Conclusion: The current results supported the efficacy of LLLT in increasing the miniscrew stability and decreasing the level of IL-1β pro-inflammatory cytokine.

Keywords: Miniscrew stability; Low-level laser therapy; Interleukin-1β; Transforming growth factor-β1.

Introduction

There has been a paradigm shift in the methods for providing anchorage during orthodontics treatment.[1] In this regard, the application of miniscrew implants (MSIs) has been of great clinical significance. MSIs opened a wide scope of dental movements and skeletal modifications, which were not possible before, by their absolute resistance to movement at orthodontic force levels. Therefore, the stability of MSIs is the cornerstone key to a successful treatment.

Two types of stability have been defined for MSIs: primary stability which is achieved through the mechanical contacts between the MSIs surface and bone, and secondary stability which is usually associated with bone remodeling around the MSIs. Several risk factors are associated with the failure of MSIs stability, including anatomical limitations, soft tissue thickness, cortical bone density/quality and insertion technique factors.[2]

Furthermore, inflammation in the surrounding mucosa of an MSI can lead to bone loss and irreversible peri-imlpantitis and ultimately MSI loss.[3] The reported success rate (no detectable movement or loss) for MSIs ranges from 71.4% to 100%.[4] Peri-implantitis accounts for 30% of MSI failures.[5] Cytokines, transforming growth factors (TGFs), and tumor necrosis factors regulate the inflammatory process of the oral cavity. Of these, Interleukin 1-β (IL-1β) is the most potent one which acts by attracting leucocytes and stimulating fibroblasts, endothelial cells, osteoclasts and osteoblasts to promote bone resorption and to inhibit bone formation.

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Recently, new approaches, including the application of such lasers as Er,Cr:YSGG and Er:YAG, for managing inflammation and accelerating healing have been introduced. These new methods have yielded better clinical outcomes.[6] Studies have revealed that the diode laser seems to be a valuable therapeutic tool for peri-implantitis.[7]

The use of low-level laser therapy (LLLT) to reduce inflammation has shown promising results.[7,8] That was attributed to the potential effects of the diode laser which could act as a bio-stimulator in tissue repair. Low-level diode irradiation can stimulate local microcirculation, decrease inflammatory mediators, and improve cell proliferation and collagen synthesis.[9] A study showed that LLLT seems to speed up the process of bone repair at implant sites.[10]

Considering the above reports that suggest LLLT can be used as a clinical adjuvant for improving clinical success with miniscrew treatment,[11] this study aimed to assess the effects of 980-nm diode laser irradiation on miniscrew stability and the concentration of IL-1β and TGF-β 1in peri-miniscrew cervical fluid (PMCF) at four time points following miniscrew placement.

Material and Methods

This is a controlled randomized double-blind clinical trial. The sample size was calculated to be 8 according to similar previous studies[12] assuming $\alpha = 0.05$ and study power of 80%. However, 11 patients were selected to lower the probability of dropouts during orthodontic treatment. Patients with class II division I malocclusion that met the following criteria were selected out of those presenting to the Orthodontics Department of the School of Dentistry, Shahid Sadoughi University of Medical Sciences, Yazd, Iran. The inclusion criteria were as follows: patients with class II malocclusion that requires extraction of maxillary first premolars and anterior retraction; patients whose age ranges between 12 and 15 years; patients without a history of systemic disease or consumption of medications that might affect the level of inflammatory factors; patients with no extraction of other permanent teeth; patients with suitable periodontal status. Patients with poor oral hygiene, not showing up for scheduled follow-ups or frequent bracket debonding were excluded from the study.

Diagnosis and treatment planning for each patient were performed based on their standard orthodontic records including photographs, study casts, lateral cephalograms and panoramic radiographs. All the patients were referred to the same surgeon for the extraction of the first maxillary premolars before orthodontic set-ups.

Orthodontic Treatment

After patient selection and prior to the onset of the study, all patients received oral hygiene instructions that were also repeated periodically during the study period.

Banding and bonding of teeth were performed by the first clinician for all patients using an MBT appliance with a 0.028×0.022-inch slot size (Discovery Smart, Dentaurum, Germany). Leveling and alignment of teeth were performed according to the standard protocols. After completion of this phase, MSI (1.6 x 8 mm, Bracketlike head, OSTEONIC, Korea) was placed under local anesthesia, consisting of 2% lidocaine with epinephrine (3M ESPE, St. Paul, Minnesota), between the roots of the second premolar and first molar in each maxillary quadrant according to the manufacturer's instructions by a hand insertion approach. One week later,[13] the maximum retraction of the anterior teeth was attempted using stainless steel rectangular wires with a NiTi close coil spring (Protect, China) with a 200 g load applied per side from the miniscrew to a hook crimped at the distal of the lateral incisor.

Laser Irradiation

This study had a split-mouth design in order to decrease the confounding factors. LLLT was performed by the second examiner in a double-blind fashion in such a way that the patient and the first examiner were blinded to the group allocation of each quadrant and the selected quadrant for laser therapy (Figure 1). The diode laser (GaAlAs; A.R.C. Laser, Nürnberg, Deutschland) with a 980-nm wavelength and 100-mW output power (detailed parameters described in Table 1) was used in this study. The laser was irradiated for 30 seconds to the occlusal and 30 s to the gingival part of the MSIs during four cycles in the laser group.[14] Selection of the maxillary quadrant for laser therapy was done randomly by the second examiner (laser group). The laser was also used for the control quadrant with the same timing but in off mode. By doing so, the patients were blinded to the group allocation of quadrants. It was irradiated at four time points: T0 (at the time of placement of mini-screw), T1 (at 1 week), T2 (at 1 month), and T3 (at 3 months).

Miniscrew Stability Measurement

The primary stability of MSIs was measured using Periotest M (Medizintechnik Gulden Modautal,

Figure 1. Low-Level Laser Application on the Experimental Side of the Patient Mouth

Germany). According to the manufacturer's instructions, the tip of the Periotest was held perpendicular to the MSI long axis at a 1-mm distance from the MSI head.[15,16] The Periotest with an electrically driven and electronically monitored tapping head percusses the MSI a total of 16 times. The tapping head has a pressure sensitive tip which records the duration of contact with the MSI and reports it as a number called Periotest value (PTV). The PTV ranges from -8 to + 50. The looser the MSI, the longer the contact time and the higher the PTV. PTV was measured three times for each sample, and the mean PTV for each miniscrew was calculated one hour after laser irradiation at the above-mentioned four time points.

Peri-Miniscrew Cervical Fluid Sampling

Peri-miniscrew cervical fluid (PMCF) samples were collected using high-purity filter papers used as paper strips (Munktell Filter AB, Falun, Sweden) to measure the concentration of IL-1β and TGF-β1. The samples were collected one hour after laser irradiation and prior to the measurement of PTV at the four time points (T0 to T3). A cotton roll was used for the isolation of the area, and the mini-screw site was dried with air spray for 15 seconds. The paper strip was inserted into the gingival sulcus around the mini-screw and remained there for 60 seconds. If the Periostrip was contaminated with blood or saliva, sampling was repeated. Immediately after PMCF collection, the paper strips were transferred into the sterile 1.5 mL microtubes containing 250 µL of phosphate buffered saline. Parafilm (Bemis; Neenah, Wisconsin, USA) was used to seal the tubes. The vials were stored at -20°C until the collection of all samples. The PMCF samples were used to measure the concentration of IL-1β by a kit designed for this purpose (Diaclone SAS, Besancon, France) and TGF-β1by another kit (IBL International GMBH, Germany) using the enzyme-linked immunosorbent assay according to the manufacturer's instructions.

Statistical Analysis

Statistical analysis was performed using PASW® version 18 (SPSS Inc., IL, USA). The normality of data was evaluated using the Kolmogorov-Smirnov test. Parameters that were not normally distributed were analyzed by the Wilcoxon test, and the remaining data with normal distribution were analyzed using the paired *t* test.

Table 1. Laser Parameters

Parameters	
Type of laser	Diode laser 980 nm (GaAlAs; A.R.C. Laser, Nürnberg, Deutschland)
Emission mode	continuous
Time on/Time off	-
Delivery system	Fiber 200µm Handpieces: HS11014
Energy distribution	-
Peak power	-
Average power	100 mW
Spot diameter at the focus	Round Spot 5mm spot at 15mm distance

Results

Assessment of PTV at the time of placement of miniscrews (T0) and at 1 week (T1) revealed no significant difference between the two groups ($P > 0.05$). However, PTV at 1 month (T2, P = 0.008) and 3 months (T3, $P = 0.003$) in the laser group was significantly smaller compared to the control group (Table 2).

The mean level of IL-1β in the laser group was significantly lower at T0 and T3 compared to the control group ($P < 0.05$). However, the difference in IL-1β concentration was not statistically significant between the study groups at T1 and T2 ($P > 0.05$, Table 3).

The mean difference in the level of TGF-β1 between the two groups was not significant at T0 to T3 ($P > 0.05$, Table 4).

One-way repeated measures ANOVA was used to assess the effect of time and laser irradiation and the interaction effect of the two on PTV. The effect of time ($P = 0.010$) and laser irradiation ($P = 0.004$) and the interaction effect of the two on PTV were all significant ($P = 0.002$, Figure 2).

Table 2. Mean PTV at Different Time Points in the Two Study Groups (n = 11)

Time	Laser Group		Control Group		Difference		*P* Value
	Mean	SD	Mean	SD	Mean	SD	
Initial	-7.01	1.10	-7.05	0.58	0.04	0.52	0.894**
1st week	-6.77	1.28	-6.74	0.87	0.03	0.41	0.877**
1st month	-6.33	1.25	-5.53	1.34	0.8	0.09	0.008**
3rd month	-6.67	0.87	-5.55	1.22	1.12	0.35	0.003**

Paired *t* test; ** Wilcoxon test.

Table 3. Mean IL-1β Concentration at Different Time Points in the Study Groups (n = 11)

Time	Laser Group		Control Group		Difference		*P* Value
	Mean	SD	Mean	SD	Mean	SD	
Initial	4.89	3.35	11.04	7.06	6.15	3.71	0.008**
1st week	6.73	4.67	7.90	4.87	1.17	0.2	0.333**
1st month	5.80	3.58	7.20	4.59	1.4	1.01	0.493*
3rd month	4.91	2.46	10.00	7.76	5.09	5.3	0.025*

* Paired *t* test; ** Wilcoxon test.

Table 4. Mean TGF-β1 Concentration at Different Time Points in the Two Study Groups (n = 11)

Time	Laser Group		Control Group		Difference		*P* Value
	Mean	SD	Mean	SD	Mean	SD	
Initial	51.62	4.83	52.49	7.03	0.87	2.2	0.609*
1st week	47.57	12.01	48.64	8.22	1.07	3.79	0.811*
1st month	49.85	9.61	49.71	10.94	0.14	1.33	0.964**
3rd month	49.18	11.23	56.16	7.79	6.98	3.44	0.135*

* Paired *t* test; ** Wilcoxon test.

The effect of time on IL-1β was not significant ($P=0.892$); however, the effect of laser irradiation on the level of IL-1β was significant ($P=0.001$). The interaction effect of time and laser irradiation on IL-1β level was not significant ($P=0.118$, Figure 3).

The effect of time ($P=0.393$) and laser irradiation ($P=0.372$) and the interaction effect of the two ($P=0.489$) on TGF-β1 concentration were not significant (Figure 4).

Discussion

This randomized controlled split-mouth double-blind clinical trial evaluated 18 patients with class II division I malocclusion requiring the extraction of maxillary first premolars and anterior retraction using MSIs.

A diode laser with a 980-nm wavelength in continuous-wave mode was irradiated in the laser quadrant while the control quadrant underwent pseudo-application of the laser. Availability, small size, reliability and affordability are among the advantages of this laser type compared with other types.[17] The diode laser with a 980-nm wavelength was chosen because studies showed that it is effective against bacteria and does not cause any changes in the surface texture of implants. This has been confirmed by electron microscopic assessments.[18,19] Some studies reported that LLLT with different wavelengths caused further stability of orthodontic miniscrews placed in the bone of animal models.[12,20-24]

The clinical failure of MSIs is related to different reasons, of which instability is considered the most important one. Primary stability is achieved by mechanical retention between the bone surface and miniscrew, and secondary retention is associated to bone remodeling. Inflammation around the MSIs, or so-called peri-implantitis, is an important negative factor resulting in MSIs loosening. The assessment of the factors involved in inflammation and investigating methods of controlling them could be a successful key for increasing MSIs stability. Previous studies showed the cellular dynamics and biologic events of peri- miniscrew crevicular fluid or gingival crevicular fluid controlled by a number of cytokines.[14] IL-1α, IL-1β and TNF- α are the major inflammatory mediators. IL-1β induces bone resorption by stimulating the synthesis of osteoclasts and affects neutrophil chemotaxis.[25] TNF-α upregulates the production of collagenases, prostaglandin E2, chemokines and cytokines, cell adhesion molecules, and bone resorption-related factors.[26] In this study, biochemical assessments were done on collected peri-miniscrew fluid samples to evaluate the IL-1β and TGF-β1 levels. Based on the results of the current study, the level of IL-1β decreased significantly between the two time extremes (T0 and T3). However, the difference between T0, T1 and T2 was not statistically significant.

Regarding TGF-β1, results showed that it was lower in the laser group compared to the control group at all the time points. However, this difference was not statistically significant. Interestingly, the level of TGF-β1 in the control group declined as well. This could state that the cytokine TGF-β1 decreased with time regardless of laser irradiation. Thus, it showed that the level of IL-1β and

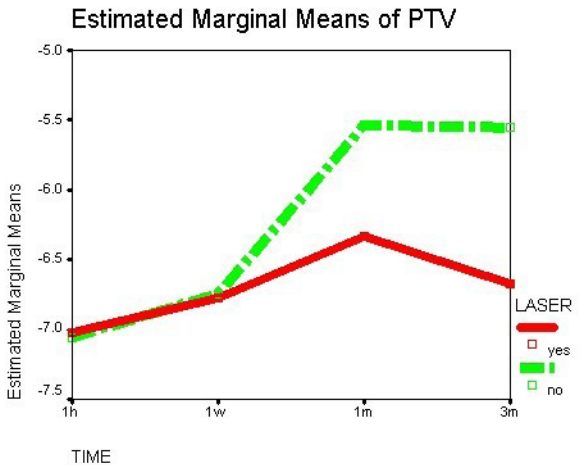

Figure 2. Mean PTV in the Two Study Groups. The red dotted line represents the laser group and the green line represents the control group

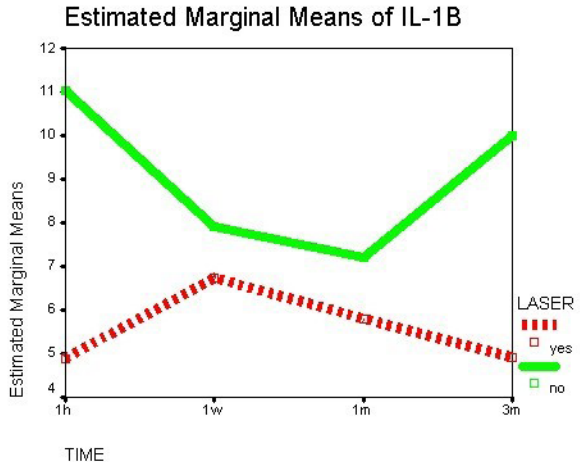

Figure 3. Mean Level of IL-1β in the Two Study Groups. The red dotted line represents the laser group and the green line represents the control group

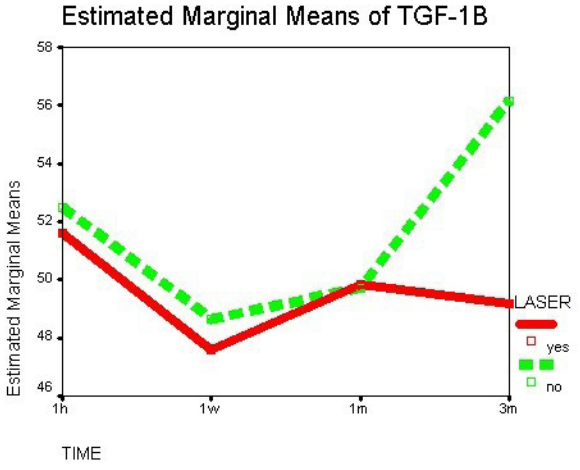

Figure 4. Mean Level of TGF-β1 in the Two Study Groups. The red dotted line represents the laser group and the green line represents the control group

TGF-β1 increased after miniscrew placement. However, laser irradiation only reduced the level of IL-1β.

Yanaguizawa et al assessed the effect of LLLT on inflammation due to mini-implant placement. They concluded that the level of IL-6 and IL-8 increased following mini-implant placement. However, in the laser group, the rate of inflammation and the level of IL-6 and IL-8 decreased following laser therapy.[5] Ekizer et al assessed the effect of a 618-nm diode laser on miniscrew stability and the level of IL-1βin GCF and PICF. They reported that despite the effect of the diode laser on miniscrew stability, it had no effect on the level of IL-1β, which was in contrast to our findings. This difference may be attributed to the intensity of the laser, which was 20 mW/cm^2 in their study and 100 mW in the present study.

In this study, the stability of MSIs was evaluated using the Periotest M device. Results showed that in the laser group the mean PTV significantly decreased with time which could be defined as the increase in MSIs stability. This finding was in line with the previous studies as several studies on animal models found that LLLT can increase the stability of mini-implants.[20,21,23,27] Ekizer et al evaluated the effect of 618-nm diode laser irradiation on miniscrew stability in a clinical trial design and reported that laser therapy had a positive effect on the stability of miniscrews,[15] which was in agreement with our findings. Abohabib et al reported that a 940-nm diode laser had a significant effect on miniscrew stability from weeks 3 to 10; however, it had no significant effects on its clinical success.[7] The finding of the present study showed the positive effect of LLLT, but it requires further evaluation with more study subjects, evaluation of molecular markers of inflammation, as well as gingival tissue changes with LLLT histologically.

Conclusion

According to the current results, LLLT could be recommended following miniscrew placement because:

- LLLT had a positive effect on MSIs stability during anterior reduction.
- LLLT showed promising effects on the reduction of IL-1β in PMCF.

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Competing Interests

None.

Ethical Approval

The study protocol was approved by the Ethics Committee of Shahid Sadoughi University of Medical Sciences (license number: IR.SSU.REC.1397.031) and registered at (identifier: IRCT20191008045028N1, https://irct.ir/trial/43051)

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