Assessment of Pain Sensation and Release of RANKL In Gingival Crevicular Fluid Accompanying Laser Application In Orthodontic Treatment: A Randomized Controlled Clinical Trial

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ABSTRACT

Purpose: This study was made to evaluate the level of receptor activator of nuclear factor- kappa B ligand (RANKL) in gingival crevicular fluid (GCF) and pain perception during orthodontic tooth treatment to evaluate the efficacy of low-level laser therapy (LLLT).

Subjects and methods: A number of 10 patients (age range: 15-20) requiring extraction of maxillary first premolars as a part of orthodontic therapy were selected randomly. A split-mouth technique was used. The test side received (LLLT) from a semiconductor (aluminium galliumarsenide) diode laser. The laser was irradiated on days 0, 2, 7, and 14. The canine distalization was achieved with a force of 150 g per side using nickel titanium closed coil spring. GCF samples were collected from canines on days 0, 7, 14 and 30 using perio paper point #35. Enzyme-linked immunosorbent assay (ELISA) was used to assess levels of RANKL. Pain was assessed for one week from the intervention using a visual analogue scale (VAS). Gingival index and pocket depth were assessed at baseline and after 30 days.

Results: There was no significant difference between RANKL concentrations in the two groups at baseline, 7 and 14 days. After 30 day; Laser side showed statistically significant lower mean RANKL (p=0.011) than control side. For pain: non significant difference was found between the two groups during the tested periods.

Conclusion: LLLT showed no additional benefits over conventional canine retraction regarding pain and RANKL release. However, the laser group showed the least RANKL level at the end of the study which denotes biostimulatory effect of laser on bone cells.

KEYWORDS

Laser,
Orthodontics and RANKL.
INTRODUCTION

Tooth movement in orthodontics is caused by bone resorption and deposition and factors that influence the rate of these processes might affect orthodontic treatment. The role of osteoclasts in tooth movement is very important, factors that promote this activity while decreasing bone density can lead to faster tooth movement. A great number of researchers have used different biochemical ways involving medications to develop the speed and quality of orthodontic treatment, but the systemic effect on the metabolism of body makes this difficult to be appropriate in orthodontics. Low intensity laser therapy can be used to solve this problem as a non-invasive way of accelerating movement of teeth in a physiological manner. However the existing evidence is still inconclusive.

The precise balance between resorption of bone by osteoclasts and bone deposition by osteoblasts is required for bone remodeling and repair. Fastening alveolar socket repair might be a good way to improve preservation of alveolar ridge. Many researches have studied the influence of low intensity laser therapy on modulation of bone resorption and deposition, inflammation control and pain relief during orthodontic treatment since rebuilding bone necessitates a controlled inflammatory response. Really, the use of LLLT to speed up bone repair could be studied. This procedure is relatively painless and non-invasive, with low discomfort and no risk of drug interactions or adverse effects. In this respect, the use of LLLT after oral procedures may be useful, and its benefits on bone healing following tooth extraction have been reported in various studies.

Osteoblasts and stromal cells of the bone marrow express RANKL, while preosteoclasts and other cells in this family express its receptor RANK. By activating multiple transcription factors that drive osteoclastogenesis, the contact between RANKL and its receptor RANK enhances the creation, fusion, activation, differentiation and survival of osteoclast cells, resulting in bone resorption. OPG is a decoy receptor formed by osteoblasts and other cells that competes with RANKL for binding to its receptor RANK. This interaction reduces bone resorption by inhibiting osteoclast cell proliferation and differentiation.

According to surveys of orthodontic patients, pain is one of the most commonly mentioned negative effects of treatment, and even when compared to the pain of invasive operations like extractions, patients reported orthodontic pain to be more common and severe. The VAS (visual analogue scale) is often regarded as the most accurate and trustworthy method for assessing subjective feelings such as pain. The VAS is a set of descriptors that define various levels of pain intensity. Patients are required to read a list of adjectives and choose the one that best represents their level of discomfort. Adjectives indicating two extremes, such as “no pain” and “excruciating/extremely acute pain” are included in an acceptable VAS scale.

According to a recent study that looked at the effect of low-intensity laser therapy on orthodontic pain caused by the force of canine retraction, a single dosage of diode laser therapy (660nm) can be an effective method for decreasing the orthodontic discomfort caused by canine retraction force.

Another recent study showed that LLLT has promising benefit. LLLT and self-ligating bracket system results the best and LLLT and conventional bracket system as the 2nd best in reducing pain sensation during the first week of OTM.

According to a recent study, using 100 mW LLLT for 6 minutes each day for 6 days could increase orthodontic tooth movement, up-regulate tissue gene expressions, and actively promote bone remodeling in rats undergoing orthodontic tooth movement. Intensity pulsed ultrasound promoted orthodontic treatment, resulting in higher release of RANKL and a shorter orthodontic treatment duration. The number of bone cells also increased by the use of this technique.
A limited literature had been carried for evaluation of the effect of low intensity laser on canine retraction and pain sensation during retraction, so this study was conducted to estimate the effect of LLLT on the level of RANKL and pain during OTM.

SUBJECT AND METHODS

This study was randomized prospective split-mouth controlled clinical trial. The participants were 10 female orthodontic patients with an age ranged from 15 to 20 years. Inclusion criteria were: patients requiring maxillary first premolars extraction and maxillary canines retraction as a part of the orthodontic treatment plan. Medically compromised patients or patients under medical treatment that affect orthodontic tooth movement rate were excluded.

The Research ethics committee of Al-Azhar University, Faculty of Dental Medicine for Girls, Cairo, Egypt. had approved this study with a final code (RES-OR-18-030). The patients and/or guardians were fully informed about the procedure and signed informed written consents.

Sample size:

The accepted sample size according to statistical sample size equation is 10. The sample size for this study depends on: 1-Acceptable level of significance p<0.05 (Type I or α error=5%). 2-Power of the study =0.8. The “power” of the study then is equal to (1 -β).

The following records had been taken: Orthodontic study model, Standardized Panoramic Radiograph, Lateral Cephalometric Radiograph, Extraoral photographs, Intra-oral photographs.

Study design and randomization method:

The sample of 10 patients was randomly selected for the study side (laser side) and control side using Microsoft Office Excel 2007, with considering that the left side was the experimental side for the first 5 patients and the right side was the experimental side for the last 5 patients.

Methods:

After the separation phase, molar bands with buccal tubes of “0.022”x 0.028” (Washbon first molars, Ormco, Clifornia, USA) were selected for the right and left maxillary first molars. Trans palatal arch (TPA) with NANCE appliance was banded and cemented to the upper first permanent molar to achieve anchorage for canine retraction.

An orthodontic appliance constructed with brackets with “0.022 x 0.028” slot (Atlas Mini, Dinaflex, Missouri USA) were bonded on upper arch from the right second premolar to the left second premolar except the maxillary right and left first premolars which will be extracted. Successive arch wires were progressively placed until “0.019 x 0.0025” inch stainless-steel wires (Acti-4S Stainless Steel Archwire, Modern Orthodontics LLC, California, USA).

Then extraction of upper first premolars was performed, prior to the canine retraction phase, both right and left maxillary first molars and second premolars were ligated together using 0.009 -inch wire in the form of figure of 8. This aided in increasing the anchorage. Similarly, ligation of the upper incisors was performed for anterior segment stabilization.

The retraction of the maxillary canines was performed using a prefabricated 9mm super elastic Nickel-Titanium closed coil spring (Vector Tas Niti coil spring, Ormco Corp, California, USA), applying force of 150 g measured by a force gauge (VST Corp., China) and was activated every two weeks. The distal wing of the canine bracket was fixed using 0.009-inch ligature wire to the arch wire to avoid rotation of canine during retraction. At every appointment, the appliance in every subject was assessed for damage as a quality-control measure, if a bracket, arch wire or a spring involved in canine retraction was damaged the subject was excluded from the study.

The equipment used in this study was a Gallium Aluminum Arsenide (GaAlAs) semiconductor diode laser (EpicX, BioLase, USA) using whitening handpiece emitting continuous infrared radiation of
wavelength 940 nm in a continuous contact wave mode. The following parameters were used\(^{(3)}\): power (100 mW) irradiation time (25 s), energy (2.5 J), energy density (3,937 J/cm\(^2\)). All irradiations were performed by the same operator. The hand piece was held perpendicular and in contact gently labially with the mucosa at the middle third of the root of the canine as in (Fig. 1). The canine was irradiated directly after the application of retraction force, this considered day 0, then irradiations were repeated in days 2, 7 and 14 for a total treatment dose of 10 J after four sessions of laser irradiations.

Precautions were taken before LLL application procedure where both the patient and the operator used appropriate protective glasses specific for the wavelength used according to the safety rules. The patient wore a self-retaining retractor and the surface exposed to the low level laser was air dried.

![Buccal application of laser at the middle third of canine](image)

**Gingival crevicular fluid samples (GCF)** were collected using PERIOPAPER POINTS size\(^{(35,30)}\), the site to be sampled was isolated with cotton rolls and plaque was gently removed with cotton pellets then washed with water and air dried. The filter paper point was inserted 1-2 mm into the gingival sulcus until mild resistance is felt, it was left in position for 60 sec while GCF is absorbed into it, then transferred to the plastic eppendorf. The samples were collected on days 0, 7, 14 and 30, then stored at -80°C until analysis. Probing depth (PD) and gingival index were assessed at baseline and after one month\(^{(29)}\).

**Detection of RANKL:**

Fine Test kit cat number (E-3-021-1) of ELISSA was used for detection of RANKL. The test samples were imbedded in phosphate buffer saline (PBS) with pH 7.5 shortly after collection, aliquoted, and refrigerated at 80°C for long term storage to avoid numerous freeze-thaw cycles. Warming the reagents at room temperature (37°C) for at least 30 minutes was required; The samples were diluted and blended before being used. The standard was settled, and the positions of the test sample and control (zero) wells on the pre-coated plate were recorded.

The measured parameter’s concentration was determined by the use of this equation: (the relative O.D.450) = (the O.D.450 of each well) – (the O.D.450 of Zero well); the standard curve was displayed as the relative O.D.450 of each standard solution (Y) vs. the relevant concentration of the standard solution (X). The standard curve was used to obtain the level of the measured parameters in the samples; the curve was displayed using a particular professional software; and finally, the obtained results from the samples were multiplied by the dilution factor to produce the concentration before dilution.

**Statistical analysis:**

Tests of normality (Kolmogorov-Smirnov and Shapiro-Wilk tests) were used to investigate numerical data for normality. RANKL level and PD data showed normal (parametric) distribution while pain scores and GI data showed non-normal (non-parametric) distribution. The test used for parametric data was two-way repeated measures ANOVA test. For pair-wise comparisons, Bonferroni’s post-hoc test was used when ANOVA test is significant. For GI scores, Wilcoxon signed-rank test was used for non-parametric data. Friedman’s test was used to study the changes by time in pain scores. When Friedman’s test is significant, Dunn’s test was used for pair-wise comparisons. IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY: IBM Corp, was used to perform the statistical analysis.
RESULTS

A-RANKL level:

1- Comparison between Laser and control sides:

Non significant difference was found between RANKL levels in the two groups at base line, after 7 as well as 14 days. After 30 days; Laser side showed statistically significant lower mean RANKL level than control side ($P$-value = 0.011, Effect size = 0.529).

Table (1) Descriptive statistics and results of two-way repeated measures ANOVA test for comparison between RANKL levels (nmol/ml) in Laser and control sides:

<table>
<thead>
<tr>
<th>Time</th>
<th>Laser (n = 10)</th>
<th>Control (n = 10)</th>
<th>$P$-value</th>
<th>Effect size (Partial Eta Squared)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean  SD</td>
<td>Mean  SD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Base line</td>
<td>194.9 57.2</td>
<td>163.7 25.9</td>
<td>0.091</td>
<td>0.284</td>
</tr>
<tr>
<td>7 days</td>
<td>149.7 15.1</td>
<td>140 27.5</td>
<td>0.286</td>
<td>0.125</td>
</tr>
<tr>
<td>14 days</td>
<td>142.4 55.6</td>
<td>127.4 25.9</td>
<td>0.279</td>
<td>0.129</td>
</tr>
<tr>
<td>30 days</td>
<td>129.5 10.3</td>
<td>161.8 30.2</td>
<td>0.011*</td>
<td>0.529</td>
</tr>
</tbody>
</table>

*: Significant at $P \leq 0.05$

2- Changes by time within each side(Fig 2):

As regards Laser side; a statistically significant change was found in RANKL levels at different time periods ($P$-value = 0.012, Effect size = 0.772). Pair-wise comparisons between time periods showed that there was a statistically significant decrease in RANKL level from base line to seven days followed by non-statistically significant change from seven to 14 days. From 14 to 30 days; there was a statistically significant decrease in RANKL level. The mean RANKL level after 30 days showed statistically significant lower mean value compared to base line level.

B- Pain (VAS) scores:

As regards control side; there was a statistically significant change in RANKL concentrations at different time periods ($P$-value = 0.003, Effect size = 0.851). Pair-wise comparisons between time periods showed statistically significant decrease in RANKL level from base line to seven days as well as from seven to 14 days. From 14 to 30 days; there was a statistically significant increase in RANKL level. The mean RANKL level after 30 days showed non-statistically significant difference from base line level.

Figure (1) Line chart representing mean and standard deviation values for RANKL levels at different time periods within each group.
C. Pocket Depth (PD):

At base line; irradiated side showed statistically significant higher mean PD than control side ($P$-value = 0.037, Effect size = 0.4). After 30 days; there was no statistically significant difference between the two sides.

As regards Laser side; there was a statistically significant decrease in PD after 30 days ($P$-value = 0.001, Effect size = 0.736). As regards control side; PD remained constant.

D. Gingival Index (GI):

At base line; there was no statistically significant difference between median GI scores in the two sides. After 30 days; Laser side showed statistically significant lower median GI than control side ($P$-value = 0.007, Effect size = 3.344).

At both sides; there was no statistically significant change in GI scores by time.
DISCUSSION

The effect of laser therapy on orthodontic treatment has been evaluated in several studies for better and faster tooth movement and for pain reduction during separator placement, archwire insertion, and canine retraction\(^4\,31,32\).

Many procedures are being employed to speed up orthodontic treatment\(^3\). The effect of LLLT on pain level and RANKL release during orthodontic treatment was investigated in this study.

The present study was performed on canine retraction to localize the pain at this area and thus reducing the measuring error in reporting pain. Moreover, the previous studies were few and yielded controversial findings\(^4,5\).

Previous studies have showed that LLLT reduces duration of tooth movement by improving remodeling of bone through increasing mineralized bone formation, osteoclast’s number and periodontal cellular proliferation \(^3,23,6\).

The same wavelength of this study (940 nm) was used in another study\(^7\) that studied the influence of LLLT on OTM rate throughout canine distalization in patients with class II division 1 malocclusion. According to another study\(^8\), the most critical elements determining tissue response are the wavelength of laser and energy density. A study stated that\(^9\), the most effective energy density range for initiating a photobiological tissue reaction is 0.5–4 J/cm\(^2\). The energy density employed in the current study, according to these findings, was 3.9 J/cm\(^2\), which was determined using the following parameters: Energy Density = Energy (J)/Area (cm\(^2\)).

GCF samples were taken in this investigation to determine the amount of RANKL. In one investigation\(^10\) GCF samples were taken from the mesiobuccal and distobuccal sides by periopaper strips. Biomarker’s concentration was measured in picograms (pg). Another study \(^11\) used the same method of sampling in investigating the difference between adults and adolescents in GCF composition during orthodontic treatment.

In this study there was no statistically significant difference between irradiated group and conventional group on RANKL level at day 0, 7, 14 and also in Pain level at different follow up periods. This result was supported by a study \(^12\) that looked at the influence of LLLT on the rate of OTM, pain, and concentration of RANKL in GCF. Although RANKL concentration levels improved and increased, the rate of OTM and pain perception did not change substantially from the control group. The findings of the current study were consistent with earlier research that found LLLT did not statistically increase orthodontic tooth movement\(^13,14,15\).

The current conclusion contradicted a study\(^19\) that examined the influence of two different wavelengths of LLLT on movable molars during orthodontic treatment; its finding revealed that the irradiated groups had a greater and obvious increase in RANKL levels as compared to the control groups. A study\(^18\) looked into the influence of LLLT on OTM rate and RANKL concentration levels and found the same results. Another study\(^16\) found that LLLT has a favorable effect on RANKL levels. This study used pre-osteoclast-like cells to determine the level of RANK after radiation in vitro. RT-PCR and Immunohistochemical staining demonstrated increased amount of RANK and RANKL in the irradiated group than in the conventional group. A study\(^17\) used two immunohistochemical analysis to determine the quantity of RANK/RANKL. From the beginning through the completion of the trial, they discovered that RANKL levels in the laser group were much higher.

Pain perception relies on pain threshold, age and sex. Therefore, to avoid individual variations, the present study utilized a split-mouth design similar to many previous studies rather than the parallel design. The major advantage of this method is the elimination of most interfering factors, thus making the results more reliable. A low-powered diode laser...
is usually used in various dental fields. This laser has shown positive effects on soft tissues and bone, including faster and better osseous remodeling, better tissue repair, disinfection of the dental canal, better and faster osseointegration in dental implants and pain reduction\(^5,33,3\).

To evaluate pain level, visual analogue scale was used over the first 7 days of the study. The VAS questionnaire is thought to be reliable, sensible, repeatable, and understandable by patients. Moreover, it is both practical and easy to use and was utilized for this study\(^5,20\). Therefore, almost all studies in this field use this specific questionnaire\(^20\). The findings from articles on canine retraction can be divided into two distinct categories: some reported that the laser was effective in pain reduction while others found it to be ineffective\(^4\).

The current study showed that there was no statistically significant difference between median pain scores in the two sides at all follow up periods. This result comply with one study that showed that the laser had no effect on pain relief\(^5\).

On the contrary, the results of this study do not comply with other studies that conclude that LLLT can be effective for pain relief in canine retraction. This difference may be due to differences in the wavelengths and types of lasers used in these studies\(^7,21\). Although a few studies have concluded that lasers are not effective in pain reduction, no technical mistakes were observed in these studies\(^22\).

The main purpose of all canine retraction studies except for the current study and another two studies\(^5,21\) was to evaluate the effect of LLLT on orthodontic movement and assessment of pain was a secondary objective. As a result, pain evaluation may have been somewhat deprioritized in these studies; therefore, further studies are needed to obtain definite findings, and these studies should consider the following points: 1- Use of a wave length between 600 and 800nm. According to the previous studies\(^5,21\): Laser at this wavelength may be effective, but more studies are needed to confirm this result. 2- Performing data analysis without the data for patients who did not experience pain. 3- Applying the laser on the canine and the first molar on the experimental side.

The results of this study concluded that low level laser had no stimulatory influence on acceleration of orthodontic treatment agreed with the findings which were reported by many human studies and disagreed with other studies which showed effect of LLLT on tooth movement\(^23\). This controversial results in the previous studies may be due to the different energy densities they used; which mentioned in Arndt-Schulz law; who stated that low doses have stimulatory and high dosages have inhibitory effects\(^25\) or may be due to the longer wavelengths they used\(^24\).

**CONCLUSION**

The effect of low intensity laser therapy (LLLT) application (within the parameters used in this study) during canine retraction showed no additional benefits over conventional canine retraction regarding pain level and release of RANKL at base line, 7 and 14 days. However, the laser group showed the least RANKL level at the end of the study which denotes biostimulatory effect of laser on bone cells.

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**RECOMMENDATION**

Further studies with longer period and different biomarkers are needed.

**CONFLICT OF INTEREST:** There was no conflict of interest.

**FUND:** No funding had been received for this study.
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