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The Effect of Low Level Laser Therapy on Cytokines Release during Orthodontic Tooth Movement

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ABSTRACT

Objective: Bone resorption is the cornerstone in bone remodeling affecting the rate of orthodontic tooth movement. Different cytokines have a direct effect on osteoclastogenesis. The aim of this study is to evaluate the effect of Low Level Laser Therapy (LLLT) RANKL release during orthodontic tooth movement.

Materials and methods: 20 patients requiring orthodontic therapeutic extraction of the maxillary first premolars were randomly selected. A randomized controlled trial was preformed; Laser group (LG) was assigned using 940 nm diode laser irradiations (100 mW, 2.5 J, 3.9 J/cm^2) at days 1, 3, 8 and 15. Canine retraction was done using nickel-titanium closed-coil spring applying a force of 150 g/side. Gingival crevicular fluid (GCF) samples were collected from distal surface of the canines on both sides, 1 day before the intervention treatment (T0), day 3 after the intervention (T1), day 15 (T2). RANKL concentration levels were assessed using Enzyme linked immunosorbent assay.

Results: There was statistically significant increase in RANKL concentration levels in the Laser Group from T0 to T1, there was no statistically significant difference in concentration level from T1 to T2, but there was a statistically significant difference in RANKL concentration levels between T0 and T2 in both groups.

Conclusion: Low Level laser therapy can increase the RANKL release during orthodontic tooth movement.

Keywords: Laser therapy, Cytokines, Orthodontic tooth, Antiretroviral therapy

INTRODUCTION

Orthodontic tooth movement (OTM) is a result of bone resorption and apposition; factors influencing the rate at which these processes take place may affect OTM. It has been shown that osteoclast activity is important in tooth movement. Factors that can increase this activity and decrease bone density can be expected to result in faster tooth movement [1,2].

The receptor activators of nuclear factor kappa B ligand (RANKL) molecule exerts counterbalancing regulatory effects on osteoclastogenesis, including osteoclast differentiation, activation and survival and are as a result critical for initiation and maintenance of orthodontic tooth movement. Osteoclast differentiation and function appear to be regulated by a counterbalancing system, which has been referred to as the RANKL/RANK/OPG regulatory axis. An increased RANKL/OPG ratio will favor osteoclast formation and activation, so bone resorption will occur [3-6].

Low level laser therapy (LLLT) has yielded important outcomes in orthodontics, with positive effects on bone remodeling and acceleration of new vascularization and acceleration of tissue healing and repair. Youssef et al. [7] evaluated the effect of the low-level (GaAlAs) diode laser (809 nm, 100 mW) on the canine retraction their findings suggested that low-level laser therapy can highly accelerate tooth movement during orthodontic treatment and can also effectively reduce pain level. Altan et al. [8] studied the effects of 820 nm diode laser on osteoclastic and osteoblastic cell proliferation-activity and RANKL/OPG release during orthodontic tooth movement they concluded that low-level laser irradiation accelerates the bone remodeling process by stimulating osteoblastic and osteoclastic cell proliferation and function during orthodontic tooth movement. Fujita et al. [9] study was designed to examine the effects of low-

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energy laser irradiation on expressions of RANK, RANKL and OPG during experimental tooth movement; results suggested that low-energy laser irradiation stimulates the velocity of tooth movement via induction of RANK and RANKL. Suzuki et al. [10] study evaluated the biological effects of low-level laser therapy (LLLT) on bone remodeling, tooth displacement during the orthodontic tooth movement; results indicated that LLLT influenced bone resorption by increasing the number of TRAP-positive osteoclasts and the RANKL expression at the compression side.

The aim of this study is to evaluate the effect of LLLT applications during orthodontic tooth movement on the release of RANKL.

MATERIALS AND METHODS

This study was reviewed and approved for scientific validity and methodology by the committee of postgraduate studies and research, Faculty of Dentistry, Suez Canal University. Subjects were selected from the orthodontic outpatient clinic, faculty of dentistry, Suez Canal University and Misr International University. Adult patients with full permanent dentition seeking orthodontic treatment were free from any medical condition that may interfere with the orthodontic treatment. Patients having good oral hygiene and free from periodontal problems. Patients who did not have previous orthodontic treatment. Patients diagnosed for Class II division 1 malocclusion with increased over jet or patients with bimaxillary dentoalveolar protrusion malocclusion; that will require therapeutic extraction of upper first and second premolars and retraction of the anterior segment. Potential participants were informed of the study rationale and design. In addition, they were provided with a written consent for approval to participate in this study. The full sample following the approval of the informed consent comprised of 20 patients (8 males, 12 females) age ranging from 18 years to 29 years.

Sample size calculation was based upon the results of Nishijima et al. [11] using RANKL level as the primary outcome; the effect size was large (1.02). Using alpha (α) level of (5%) and Beta (β) level of (20%), i.e., power=80%; the minimum estimated sample size was 10 subjects. Sample size calculation was performed using IBM® SPSS® SamplePower® Release 3.0.1; accordingly, the sample size was determined to be 20 subjects.

For every patient a fixed upper and lower orthodontic appliance (OrmcoTM mini 2000 Roth slot 0.22, USA) were used. After leveling and alignment of the upper arch, mini-screws (MCT BIOTM, Qmedical®, South Korea) were placed bilaterally between the upper first molar and second premolar below the mucogingival junction in the attached mucosa.

After leveling and alignment and reaching a heavy arch wire (0.016 × 0.022 stainless steel wire) and for each patient the intervention technique was assigned to a quadrant: Low Level Laser Therapy (LLLT) was assigned to the other side of the maxillary arch also at the canine region.

On the side Low level laser therapy was applied; in this study near infrared 940 nm InGaAsP Diode laser (EPIC XTM by BIOLASE®, USA) was used. For delivering laser irradiation the Deep tissue hand-piece by BIOLASE® was used with a laser beam diameter of 9 mm with irradiation area of 0.635 cm^2 **(Figure 1)**. For Low level laser Therapy, the following parameters and settings were used: Power (100 mW), Irradiation Time (25 s), Energy (2.5 J), Energy density (3.937 J/cm^2). All irradiations were done by the same operator, the hand piece was held perpendicular and in contact gently labially with the mucosa at the middle point of the root of the canine **(Figure 1)**. The canine was irradiated directly after the application of retraction force with the nickel-titanium closed-coil spring, this was considered Day 1, then the irradiation was repeated in days: 3, 8 and 15; for a total treatment dose of 10 J after 4 sessions of laser irradiations **(Figure 2)**.

Figure 1. EPIC X™ by BIOLASE®, USA set on the required parameters.

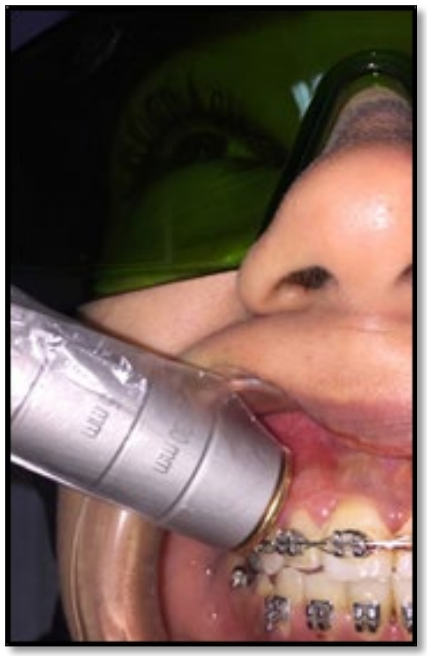

Figure 2. Laser irradiation delivered by deep tissue hand piece directed perpendicular to the root of the canine and in contact with the mucosa.

Gingival crevicular fluid (GCF) samples were collected using PERIOPAPER® strips (Harco, Tustin, Calif). The site to be sampled was isolated with cotton rolls and plaque was gently removed with cotton pellets. Sites were then washed with water and air dried prior to sampling. The filter paper strip was inserted 1-2 mm into the gingival sulcus until mild resistance is felt. It was left in position for 30 s whilst GCF is absorbed into it. Care was taken to avoid damage to the soft gingival tissues **(Figure 2)** [12].

Samples were collected from the distobuccal sites of the examined canines from both groups as follows **(Figure 3 and Table 1)**:

Table 1. Groups samples collection follow-up times.

Time	Group 2 (LLLT)
T0	1 day before Laser application
T1	Day 3 of laser Application
T2	Day 15 of laser application

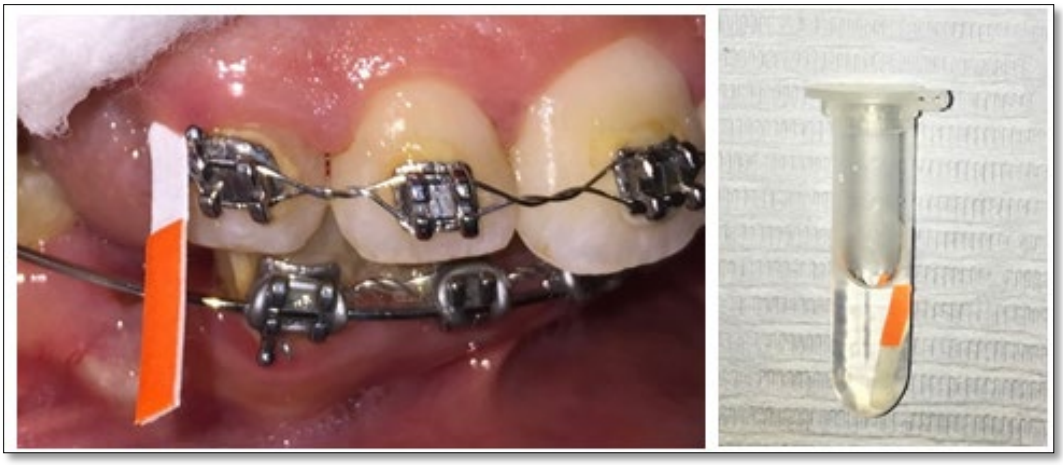

Figure 3. GCF sample collection.

The detection of RANKL was done by ELISA technique using Fine Test kit cat number (EH0313). This technique was based on sandwich enzyme-linked immune-sorbent assay technology. The test samples; gingival percravicular miniprep were embedded in phosphate buffer saline (PBS) with pH 7.5 soon after collecting and aliquot and stored at -80°C for long term with avoidance of multiple freeze-thaw cycles. The reagents allowed warming for at least 30 min at room temperature (37°C); the samples were diluted and mixed completely and evenly. The standard was settled, test sample and control (zero) wells on the pre-coated plate respectively and then their positions were recorded. The standard was used in different gradient concentrations according to the manufacture instructions. The prepared standards were added into the appropriate wells at a volume of 0.1 ml; similarly, samples were added also into test sample wells. The plate was sealed with a cover and incubated at 37°C for 90 min. The cover was removed and the plate content was discarded, the plate was clapped on the absorbent filter papers or other absorbent material. Do NOT let the wells completely dry at any time. The 0.1 ml of Biotin-detection antibody working solution was added into the above wells (standard, test sample and zero wells). The solution was added at the bottom of each well without touching the side wall. The plate was sealed with a cover and incubated at 37°C for 60 min. The cover was removed and the plate washed 3 times with Wash Buffer A 0.1 ml of SABC working solution was added into each well, the plate covered and incubated at 37°C for 30 min. The plate washed 5 times with Wash buffer and each time we let the wash buffer stay in the wells for 1-2 min. A 90 µl of TMB substrate was added into each well; the plate was coved and incubated at 37°C in dark within 15-30 min. A 50 µl of Stop solution was added into each well and mixed thoroughly. The color changed into yellow immediately. The O.D. absorbance was read at 450 nm in a microplate reader immediately after the stop solution has been added. The concentration of the measured parameter was calculated using the following equation:

The relative O.D.450 = The O.D.450 of each well – The O.D.450 of Zero well

The standard curve was plotted as the relative O.D.450 of each standard solution (Y) vs. the respective concentration of the standard solution (X). The concentration of the measured parameter in the samples was interpolated from the standard curve; the Curve was plotted using specific professional software; finally, the samples calculated results were multiplied by the dilution factor to the concentrations from interpolation to obtain the concentration before dilution.

Numerical data were explored for normality by checking the distribution of data and using tests of normality (Kolmogorov-Smirnov and Shapiro-Wilk tests). Data showed normal (parametric) distribution. Data were presented as mean, standard deviation (SD) and 95% Confidence Interval (95% CI) for the mean values.

Two-way repeated measures Analysis of Variance (ANOVA) was used to study the effect of treatment, time and their interaction on mean sRANKL concentration. Bonferroni's post-hoc test was used for pair-wise comparisons when ANOVA test is significant. The significance level was set at $P \leq 0.05$. Statistical analysis was performed with IBM® [1] SPSS® Statistics Version 20 for Windows.

RESULTS

Demographic data

The present study was conducted on 20 subjects; 8 males (40%) and 12 females (60%). The mean and (standard deviation) values for age were 23.4 (3.5) years with a minimum of 18 and a maximum of 29 years old.

sRANKL concentration

With LLLT treatment; there was a statistically significant change in mean sRANKL concentration by time (P-value <0.001, Effect size=0.880). Pair-wise comparisons between the follow up times revealed that there was a statistically significant increase in mean sRANKL concentration from T0 to T1 followed by non-statistically significant decrease in sRANKL concentration from T1 to T2. The mean sRANKL level at T2 showed statistically significantly higher value compared to T0 concentration **(Figure 4 and Table 2)**.

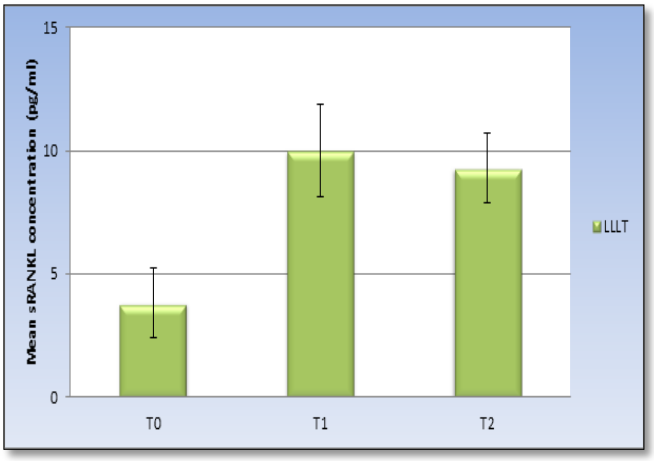

Figure 4. Bar chart representing mean and standard deviation values for sRANKL at different time periods within LLLT.

[1] ® IBM Corporation, NY, USA
® SPSS, Inc., an IBM Company

Table 2. The mean, standard deviation (SD), 95% Confidence Interval (95% CI) values and results of two-way ANOVA test for comparison between sRANKL at different time periods within LLLT group.

Time	LLLT	
	Mean (SD)	95% CI
T0	3.8 (1.4) ^{B}	3.1-4.5
T1	10.0 (1.9) ^{A}	9.2-10.9
T2	9.3 (1.4) ^{A}	8.6-9.9
Partial Eta Squared *(Effect size)*	0.880	
P-value	<0.001*	

*: *Significant at P ≤ 0.05, Different superscripts are statistically significantly different*

DISCUSSION

Several techniques are currently used to accelerate orthodontic tooth movement. In this study we investigated the effect of the release of RANKL during orthodontic tooth movement. We distinguished noninvasive approach: Low Level Laser Therapy (LLLT).

The sample size used in this present study was larger than the sample size in Nishijima et al. [11] study investigating the levels of RANKL and OPG in GCF for 10 subjects undergoing orthodontic tooth movement. Again it was larger than the sample size used in Barbieri et al. [13] study where Levels of RANK, OPG, OPN and TGF-ß1 were also analyzed in 10 volunteers undergoing orthodontic treatment. However, our sample size in the present study matched the same size as in Grant et al. [14] study investigated changes in cytokines and biomarkers of bone and tissue metabolism within gingival crevicular fluid (GCF) from patients undergoing orthodontic treatment.

The effect of LLLT on the rate of OTM was previously investigated in previous studies. Seifi et al. [17], Gama et al. [18] and Marquezan et al. [19] performed animal studies and they all shared similar results and stated that there was no statistically significant difference between the irradiated group and non-irradiated groups on the rate of OTM. In contrast to their results Altan et al. [8], Suzuki et al. [10], Yoshida et al. [20], Yamaguchi et al. [21] and Cossetin et al. [22] performed animal studies and stated that LLLT can improve the rate of OTM through high expression of RANKL release stimulating alveolar bone remodeling. Youssef et al. [7], Dosh-Mehta et al. [16], Genc et al. [23], Caccianiga et al. [24], Qamruddin et al. [25] and Arumughan et al. [26] in their clinical studies on patients stated that LLLT had a significant effect on accelerating the rate OTM.

In the current study near infrared 940 nm InGaAsP Diode laser was used, Qamruddin et al. [25] used the same wavelength 940 nm in their clinical study investigating the effect of LLLT on rate of OTM during canine retraction in Class II div 1 patients.

Baxter and Diamantopoulos [27] stated that laser wave length and energy density are the most important factors determining the tissue response. Mester et al. [28] stated that energy density in the 0.5-4 J/cm^2 is the most effective range in start of a photobiological tissue reaction. According to these findings the energy density used in the study was 3.9 J/cm^2; that was calculated from the given parameters:

$$\text{Energy Density} = \text{Energy (J)}/\text{Area (cm}^2\text{)}$$

In the current study GCF samples were collected from the distobuucal sites using periopaper strips and the markers for RANKL were evaluated using ELISA test and the concentration of RANKL was expressed in pictogram/millimeter (pg/ml), in the study done by Bariebri et al. [13] RANKL was measured through GCF samples collected using periopaper strips at the mesiobuccal (tension side) and distobuccal (compression side) sites. The amount of each biomarker was determined in picograms (pg). Another study using a similar technique for sampling was Rody et al. [29] investigating differences in the GCF composition between adults and adolescents undergoing orthodontic treatment.

The results of the current study stated that after applying LLLT there was a significant increase of sRANKL concentration levels, there was a significant increase of from T0 (before laser application) to T1 (day 3 of laser application), the mean sRANKL level at T2 (day 15 of LASER application) showed statistically significantly higher value compared to T0 concentration; P-value<0.001, Effect size=0.880. These results comply with results stated by Fujita et al. [9] where they investigated the concentration levels of RANKL at days 2, and 3 after low laser laser irradiation during orthodontic treatment; they stated that the positive immunoreactions to the primary antibodies of RANKL and RANK were significantly increased in the irradiation group on day 2 and 3, compared with the non-irradiation group, they concluded that These findings suggest that low-energy laser irradiation stimulates the velocity of tooth movement via induction of RANK and RANKL.

Altan et al. [8] investigated the effect of different laser irradiations parameters in comparison to control group on the rate of OTM, they found that the group irradiated with lower energy density findings showed that RANKL immune-reactivity was stronger than in the other groups. Milligan et al. [30] designed a study to evaluate the effect of two different wattage parameters of LLLT on orthodontically moved molars, exhibited differences in the amount of tooth movement and molecular and histological changes in the adjacent periodontal areas. Their findings suggested that regardless of wattage used the laser irradiated groups exhibited higher and significant increase level of RANKL concentration levels when compared to control non-irradiated groups. Similar results were also stated by Suzuki et al. [10]; in their study they investigated the effect of LLLT on rate of OTM and concentration levels of RANKL at days 3, 6, 9 and 21. The immunohistochemistry analysis showed increasing number of TRAP-positive osteoclasts and the RANKL expression at the compression side, during all examination times. Which complies with this current study results that showed significant increase of RANKL concentration at day 3 (T1) and day 15 (T2) when compared to T0; also there was no significant change of RANKL concentration between day 3 (T1) and day 15 (T2), implying that the used frequency of irradiations (day 1, day 3, day 5, day 8, day 15) maintained high level of RANKL and we can postulate that with repeating the fore-mentioned protocol we can maintain RANKL concentration throughout the canine retraction procedure. Another study stating the positive effect of LLLT on RANKL concentration was the study of Aihara et al. [31] that evaluated preosteoclast-like cells to measure the amount of RANK after radiation in vitro. Immunohistological staining and RT-PCR expressed higher levels of RANK and RANKL in the laser therapy group as compared to the control group. Kim et al. [32] also evaluated the amount of RANK/RANKL using 2 immunohistochemistry analyses. They realized that RANKL levels were significantly in higher concentration levels in the laser group from the beginning to the end of the study.

In contrast to the results of Dominguez et al. [33], where they investigated the effect of LLLT on accelerating rate of OTM, pain and RANKL concentration in GCF. Their results showed that although there was improvement and increase in RANKL concentration levels, in the rate OTM and pain perception, yet the change was not significantly different from the control group. Furthermore, three studies showed that LLLT did not statistically accelerate orthodontic tooth movement; these are studies done by Marquezan et al. [19], Limpanichkul et al. [34] and Kansal et al. [35]. The literature reports that energy density is the most important laser parameter to be considered as it determines the amount of energy received by the tissues per area. An acceptable energy density is between 0.5 and 12 J/cm^2; however, the ideal energy density seems concentrated between 1 and 6 J/cm^2. Marquezan et al. [19] used in his study energy density of 6000 J/cm^2, in Limpanichkul et al. [34] study the energy density used was 25 J/cm^2 while in the current study the energy density used was 3.9 J/cm^2. The higher energy density may be the reason they showed no difference between the experimental low level-intensity laser therapy subjects and the controls in both studies. In the study of Kansal et al. [35] used outcome power was 12 mW that would unlikely produce a direct photochemical or biostimulatory effect. In the present study the output power used was 100 mW.

Aligning with the results found in this study, the review article published by Yassaei et al. [36] stated that based on different researches, it may be concluded that low level laser therapy may increase the rate of tooth movement during orthodontic treatment through the mechanism of increasing levels of RANKL in PDL which leads to increased osteoclastogenesis.

CONCLUSION

Low level laser therapy facilitated orthodontics applications will increase the RANKL release during orthodontic tooth movement. As RANKL is a key molecular factor in osteoclastogenesis, we can assume that LLLT and CFO can accelerate the rate OTM.

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