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Effect of Low Level Laser Therapy on the Rate of Orthodontic Tooth Movement in Humans and the Levels of TNF-α in Gingival Crevicular Fluid.

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ABSTRACT

Aim: To appraise the effects of low level laser therapy (LLLT) on tumor necrosis factor-α (TNF-α) levels in gingival crevicular fluid (GCF) and its concordance with rate of orthodontic tooth movement (OTM) if any.

Materials and Methods: A split mouth investigation was done in 16 subjects in whom 150 g of force was used for individual retraction of each maxillary canine. A gallium-aluminum-arsenide diode laser (energy density, 8J/cm 2 ; wavelength, 940nm; power output, 100mW) irradiated only the experimental canine at 10 distinct points. The amount of canine retraction on the two sides was measured and compared. GCF levels of TNF-α was quantified using enzyme-linked immuno-sorbent assay (ELISA).

Results: A significant elevation in the levels of TNF-α was noted on the experimental side (P<0.001). The mean total retraction of the experimental canine (occlusograms and software, 4.1437 and 4.1563 mm, respectively) was higher than the control canine (occlusograms and software, 2.3438 and 2.3875 mm, respectively). The elevation of TNF-α was corroborated with accelerated retraction of the experimental canine (P<0.001).

Conclusion: The application of LLLT in conjunction with optimum orthodontic force accelerated the rate of OTM and elevated the GCF levels of TNF- α.

Keywords: Tumor Necrosis Factor; Gingival Crevicular Fluid; LLLT; ELISA.

INTRODUCTION

Orthodontic treatment is based on force application, a complex process inducing tooth movement in which a daunting array of well-coordinated synchronous biological reactions in the alveolar bone proper and the conterminous soft tissues restores the hosts' craniomandibular system to its state of physiologic equipoise.[1,2] The ensuing host response is characteristically a transitory, aseptic, acute inflammation marked by an alteration in the vascularity of the periodontal ligament (PDL) involving release of various immunoregulatory molecules produced by inflammatory cells that have migrated from the adjacent PDL capillaries.[3-5]

TNF-α, a vital pro-inflammatory monokine, executes a principal role in bone resorption by controlling osteoclastic activities in areas of compression.[6,7]The TNF family constituting two structurally related proteins namely TNF-α (or cachetin) and TNF-β (or lymphotoxin) is characterized by the pleiotropic nature of its actions.[8]The osteoclastogenic effect of TNF-α is exerted in the being of macrophage-colony stimulating factor (M-CSF) via its p55 (type1) receptor.[9]

Newer modalitiesacting independently or in tandem including mechanical, electrical and chemical to shorten treatment duration are nowadays being devised and lasers have been the centrioles of these investigations.[10] Altan et al reported that LLLT exacerbates the inflammatory response to orthodontic forces and triggers the release of receptor activator of nuclear factor kappa β – ligand (RANK-L) which regulates osteoclastic cell activity.[11]Fujita et al demonstrated that osteoblast precursors in vitro, expressed RANK at an earlier stage (days 2, 3) and to a greater amount following LLLT thus expediting tooth movement.[12]TNF-α stimulates RANK-L induced genesis of osteoclasts via the linking of RANK signaling pathways and TNF type 1 receptor.[9]

The gingival crevicular fluid, an osmotically derived inflammatory exudate, reflects the inflammatory state of the periodontium and thus provides a unique window for analysis of the activity of the periodontium and allied investing structures.[13]

Therefore, the current study intended to discern the effect of LLLT on the rate of OTM by evaluating levels of TNF-α in GCF in humans. Ample research published to date dwelled on the effect of LLLT on the rate of OTM but its role on GCF levels of TNF-α has not been investigated. Hence an assessment of TNF-α level following LLLT and its correlation with the rate of OTM

would further enhance the knowledge underlying the process.

MATERIALS AND METHODS

A prospective clinical study was carried out on patients reporting to the Department of Orthodontics and Dentofacial Orthopedicsafter obtaining ethical clearance from the institutional review board. Informed consent in writing was taken from all the participating patients.

16 subjects (9 female, 7 male) in the age ambit of 14-25 years were selected based on the aforementioned criteria. Patients with gingival index score below 1, without history of intake of anti-inflammatory drugs, steroids or antibiotics in the month preceding the study. Patients with poor oral hygiene, systemic diseases and those undergoing therapy that could potentially impact bone metabolism were excluded from the study.

All the patients has Angle's class I malocclusion requiring extraction of the maxillary first bicuspid. Labial fixed mechanotherapy using pre-adjusted-edgewise appliance with MBT prescription (0.022 x 0.028-in slot, 3M Gemini, 3M Unitek Corporation, Monrovia, California) was commenced following the extraction of the maxillary first bicuspids. After completion of leveling and alignment individual canine retraction was carried out on an 0.018-in stainless steel archwire (A.J. Wilcock, Birmingham, England) using nickel-titanium closed-coil springs (9,12mm; ORMCO, Sybron Dental SpecialitiesInc, Newport Beach, CA,USA), employing 150 g of distalizing force buccally. This force was gauged using a Dontrix gauge dynamometer (Leone® Dontrix Gauge, Leone, USA). The springs were recalibrated at the beginning of the fourth and eighth weeks. Anchorage was reinforced throughout the study using a transpalatal arch anchored on the maxillary first molars.

A gallium-aluminum-arsenide semiconductor diode laser (ezlaseTM, BIOLASE® Technology Inc. 4 Cromwell Irvine, CA) having energy density of 8J/cm2, power output of 100mW and wavelength of 940nm was used to irradiate the experimental canine immediately following the activation of the closed coil spring at 10 distinct points on the mucosa covering its root(Figure 1), for 10 seconds at each point on 3 consecutive days.1Laser irradiation was done at the commencement of distalization and after 4 and 8 weeks. Pseudo-irradiation of the control canine was done by enclosing the laser probe in a sleeve with a black opaque tip made of plastic i.e. the canine was distalized without laser application.

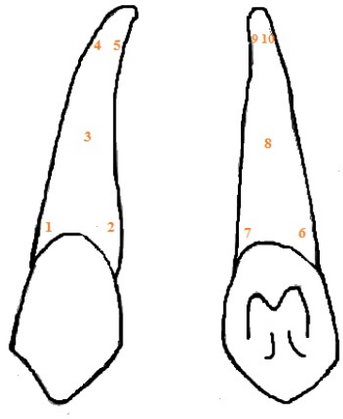

Figure 1. Points for laser irradiation (Labial surface): 1, mesiolabialgingival ridge; 2, distolabialgingival ridge; 3, midpoint of the root; 4, labial vestibule levelled at root apex analogous to point 1; 5: labial vestibule levelled at root apex analogous to point 2. Similar points on the palatal aspect giving a total of 10 such points.

Oral hygiene instructions were reinforced during placement of the appliance and the gingival and periodontal indiceswas recorded before collection of GCF. Gingival crevicular fluid collection was done using a calibrated volumetric microcapillary tube inserted at the entrance of the gingival sulcus till a volume of 5 µL was collected. Sample collection was always done between 3pm to 5pm, to account for the variability in volume of the GCF owing to the circadian rhythm.

Gingival crevicularfluid was sampled at 5 distinct time points from both the control and experimental sides, giving a total of 160 samples i.e. 16 subjects, 5 samples each from control and experimental sides at following time points:T_0, Baseline, before commencing retraction; T_{+3}, 3 days after LLLT application on experimental side and pseudo application on control side and commencing retraction; T_{+7}, 7 days after commencing retraction without LLLT; T_1 and T_2, i.e. 4 and 8 weeks after commencing retraction with LLLT application on experimental side and pseudo application on control side respectively. Human TNF-α ELISA kit (Krishgen Biosystems, Brea, California) was used to quantify the concentration of TNF-α (pg/µL).

To assess the rate of OTM, study models were obtained at T_0, T_1 and T_2 which were then compared to deduce the amount of tooth movement using the medial end of the ipsilateral 3rd palatal ruga as a reference landmark.The models was scanned using HP Scanjet G3010 Flatbed Scanner (Hewlett-Packard Co., Palo Alto, CA) and scanned images were printed andtraced. Landmarks for measuring the amount of canine retraction were marked on the traced images (Figure 2). The models were re-evaluated to ascertain the legitimacy of the occlusogram measurements by using software (AutoCAD 20.0, Autodesk, CA, USA) (Figure 3).

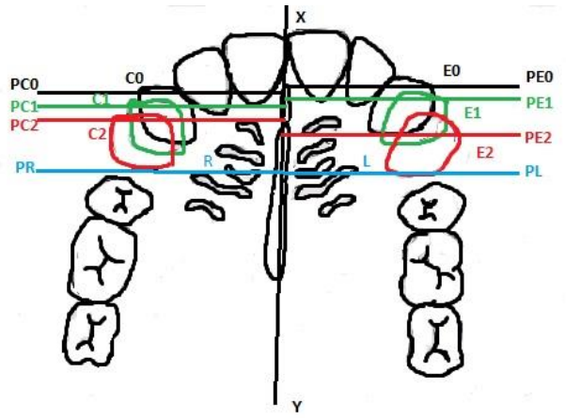

Figure 2. Schema of landmarks used to deduce canine distalization from occlusograms: XY, mid palatal suture plane; R and L, median most point of the right and left 3rd palatal ruga respectively; E0, pre-retraction experimental canine cusp tip; E1 and E2, experimental canine cusp tip 4 and 8 weeks post retraction commencement respectively; C0, C1,C2, points analogous to E0, E1, E2 on the control side respectively; PE0,PE1, PE2 perpendicular through E0, E1, E2 respectively to XY; PC0, PC1, PC2, Points analogous to PE0, PE1, PE2 respectively on the control side; PR and PL perpendiculars through point R and L respectively to XY.

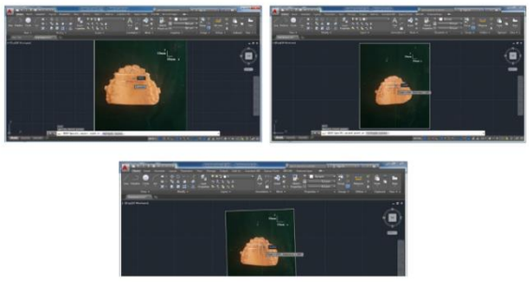

Figure 3. Measurement of the amount of individual canine retraction at T_0, T_1, T_2 by using the software (AutoCAD 20.0, Autodesk, CA, USA).

To measure the amount of canine retraction, the relative distances between the canine tip and the third palatal rugae were measured.The amount of canine distalizationwere measured as: R_1, amount of retraction at the end of 4th week; R_2, amount of retraction at the end of 8th week; R_T, total amount of retraction during the 8 week period, calculated as R_1+ R_2.

Statistical Analysis

Sample size was estimated using a power analysis. With a permissible error of upto 5% and power of the test of 90% a sample size of 10 was arrived at. A sample size of 16 was chosen to account for potential drop-outs, as a safety factor. The data was analyzed utilizing the SPSS software (SPSS for Windows, Version 20.0, Ill). Paired t-test and two-way repeated-measures of analysis of variance (ANOVA) were used to compare the levels of TNF-α and the amount of canine distalization as measured by occlusograms and the software between the groups at various time points (Table 1-3). A correlation between the two methods of measurement was assessed using Karl Pearsonscorrelation coefficient (Table 4).

RESULTS

As assessed by the gingival[14] and periodontal indices[15](both <1), the health of the periodontium was maintained throughout the course of the study.

The difference in the levels of TNF-α between the control and the experimental sides was insignificant at T_0, T_{+3}, T_{+7} though the experimental side constantly exhibited elevated levels of TNF-α at these time points (Figure 4). However, at T_1 and $T_2$the difference in the levels between the two sides was marked and significant with the experimental side exhibiting a two-fold and four-fold heightened increase respectively in comparison to the control side (Figure 4).

The amount of canine retraction (R_1, R_2, R_T) was higher on the experimental side compared to the control side (P<0.001) (Figures 5 and 6). The experimental canine distalization was 1.75 times greater than the control canineafter 8 weeks (P<0.001) (Tables 1-3; Figures 5 and 6).The Karl Pearson's correlation coefficient demonstrated a positive, statistically significant correlation between measures obtained by occlusograms and the software at all the time points (Table4).

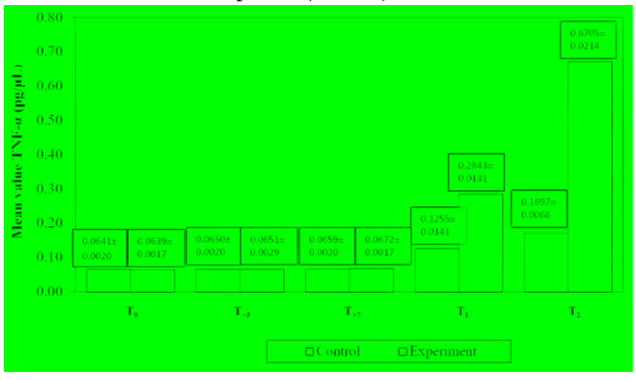

Figure 4. Comparison of TNF-α levels (mean± SD) between control and experimental sides at each time point.

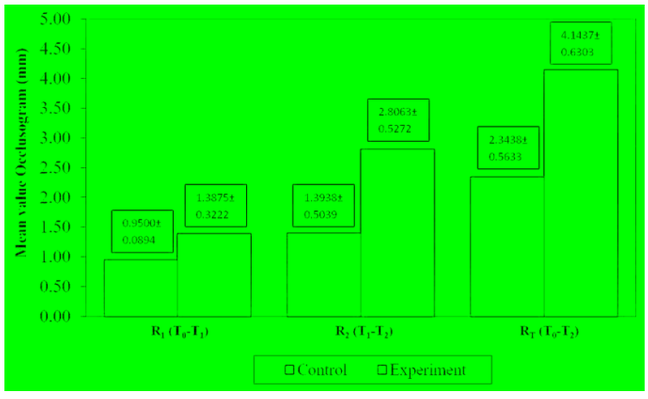

Figure 5. Comparison of amount of canine distalization(mean± SD) between the control and experimental sides at each time point by using occlusograms.

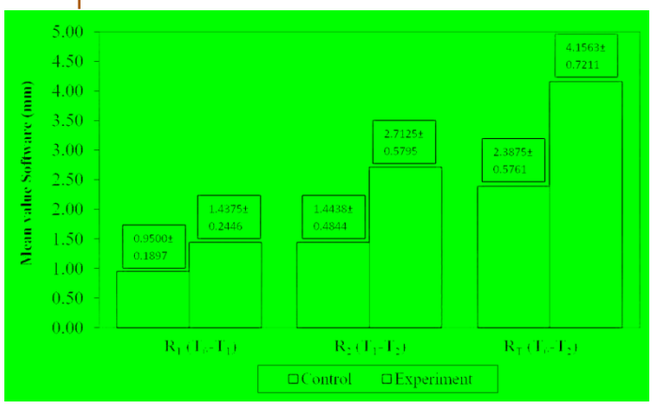

Figure 6. Comparison of amount of canine distalization(mean± SD) between the control and experimental sides at each time point by using the software.

commenced bone remodeling and triggered the release of a portion of the previously synthesized TNF-α into the gingival crevice. The experimental side, expressed higher amounts of TNF-α than the control side at T_{+3} compared to the baseline (an increase of 0.0012pg/µL, 0.0009 pg/µL respectively) which however, was not statistically significant (Fig 4). Laser irradiation for three consecutive days, has accounted for the marginally higher biological response on the experimental side. A progressive intensification in the TNF-α level was noted on day 7 which was greater on the experimental (0.0033pg/µL) than the control side (0.018pg/µL) which however was not statistically significant.

The heightened biological reaction on the experimental side signifies an amplification of local bone metabolism due to the laser irradiation. The experimental side exhibited a statistically significant two-fold heightened increase of the TNF-α levels in comparison to the control side at 4 weeks (T_1) and a four-fold higher increase in the level at 8 weeks (T_2) (P<0.05)attributable to the accumulative effect of the laser dose. Contrary to other approaches like corticotomies, whose effect deteriorates in about 4 weeks, the effect of laser irradiation was found to ameliorate over time.[18]

Table 1: Comparison of the TNF-α scores between the experimental and control sides at the various time points by two-way repeated-measures of ANOVA.

Source	*Type III Sum of Squares*	*Degrees of freedom*	*Mean Square*	F *value*	P *value*
Groups	0.6980	1	0.6980	6833.2500	0.0001*
Times	3.0890	4	0.7720	10526.9820	0.0001*
Groups * Times	1.5100	4	0.3770	3910.0400	0.0001*

DISCUSSION

On this present day, the specialty is juxtaposed between patient demands for shorter treatment duration against conventional treatment periods often lasting a few years. The current study was intended to discern the possible role of LLLT on osteoclastogenesis during OTM by evaluating the levels of TNF-α in the GCF in humans. An ample amount of research had been published on the role of LLLT on the rate of OTM but its role on the GCF levels of TNF-α had not been investigated to date.

Table 2: Comparison of the amount of canine distalization between the control and experimental side using the occlusogram method by two-way repeated-measures of ANOVA.

Sources	*Type III Sum of Squares*	*Degrees of freedom*	*Mean Square*	F *value*	P *value*
Groups	35.5270	1	35.5270	104.2860	0.0001*
Times	69.1310	2	34.5650	322.7900	0.0001*
Groups * Times	7.8860	2	3.9430	59.8170	0.0001*

TNF-α, a key intermediary of the acute phase inflammatory reaction is an early modulator of bone resorption. The PDL fibroblasts on the compression sites express higher quantities of TNF-α than in the tension zone, the imbalance between which activates the CD 4+ cells to express RANKL and facilitate OTM.[16]Its acts synergistically with IL-1β in bone remodeling following mechanical loading and also promotes the recruitment, activation and differentiation of osteoclasts.[17]

*p<0.05

A tepid increase in the levels of TNF-α was noted from T_0 - T_{+3} on both the sides. Transduction of the applied forces had

A statistically significant difference (P<0.001) was found between the experimental and control sides with respect to the accumulative movement of the canine which was higher on the experimental side by 1.75 times. (Tables 1-3; Figures 4 and 5). Alveolar bone stimulation enhances the recruitment, proliferation and differentiation of the osteoblasts and osteoclasts on the tension and compression sites respectively. The cells of bone remodeling are also recruited earlier on the laser irradiated side compared to the control side. Kawasaki and Shimizu have shown a 1.3 fold increase in the rate of OTM on the laser irradiated experimental side compared to the control side.[19]Varella et al investigated the effects of gallium-aluminium-arsenide semiconductor diode laser on orthodontic

tooth movement and noted a two-fold increase in the rate of OTM congruent with increase in the level of IL-1β at the end of 8 weeks.[20] This study was analogous to our study in terms of the laser parameters used and the dosage intervals.

We found that the temporal rise in the GCF levels of TNF-α on the experimental side was corroborated by an increase in the rate of the experimental canine movement at the same time intervals when measured using both, the occlusogram and the software (Tables 1-3). This is attributable to the potent osteoclastogenic effect of the cytokine. The persistent elevation in the levels of TNF-α is due to the bio-stimulatory effects of LLLT on the inflammatory cells (Table 1,Figure 4).

Table 3: Comparison of the amount of canine distalization between the control and experimental side using the software by two-way repeated measures of ANOVA.

Sources	*Type III Sum of Squares*	*Degrees of freedom*	*Mean Square*	F *value*	P *value*
Groups	33.1350	1	33.1350	90.9470	0.0001*
Times	69.6080	2	34.8040	276.8180	0.0001*
Groups * Times	6.6720	2	3.3360	48.8630	0.0001*

*p<0.05

Nuclear Factor-κβ, a transcription factor governs a variety of cell functions including inflammation and stress induced activation. Reactive oxygen species activate Nuclear Factor-κβ, either directly or in conjunction with TNF-α. LLLT generates reactive oxygen species which activate the redox sensitive Nuclear Factor-κβ signaling, which enhances the circulation of the pro-survival and the anti-apoptotic genes in the cells. This underlies enhanced PDL cell viability and many of the clinical effects seen after laser irradiation.[21]

general and dose dependent bio-stimulation or inhibition of cytokines in particular is obscure. Hence in our study, the effect of LLLT on OTM was evaluated with the concomitant changes in the expression of the cytokine TNF-α in the GCF.

A limitation of the current study was the use of scanned images of the casts rather than 3-dimensional models. The scope for future research is wide and further investigations with randomized control trials and long term evaluation for root resorption is the need of the hour to optimize laser treatment parameters to introduce this tool into daily clinical practice. Stereological studies on laser irradiated tissues are necessary to analyze the contradictory effects of laser used to alleviate pain following orthodontic force application and that used to accelerate OTM quantitatively.It is pertinent to consider follow up studies to explicate the effects of LLLT on a long term basis on the dental tissues.

CONCLUSION

By amalgamating the clinical results and findings of the biochemical quantification assay, we deduced the following:

1. Low level gallium-aluminium-arsenide semiconductor diode laser as an adjunct to a light orthodontic force accelerated OTM by 1.75 times accompanied by a two-fold elevation in the GCF levels of TNF-α at 4 weeks and four-fold higher increase at 8 weeks compared to the control side.
2. The bio-stimulatory effect of LLLT elevated levels of TNF-α in the GCF signifying the underlying mechanism associated with the acceleration in the rate of OTM following irradiation with lasers.

Table 4: Correlation between the amounts of canine distalization as measured by the occlusogram method and the software on the control and experimental side by Pearsons correlation coefficient.

Group	*Values*	*R_1 Occlusogram*		*R_2 Occlusogram*		*R_TOcclusogram*	
		r value	P *value*	*r value*	P *value*	*r value*	P *value*
Control	R_1 Software	0.6342	0.0083*				
	R_2 Software			0.9064	0.0001*		
	R_T Software					0.8994	0.0001*
Experimental	R_1 Software	0.8890	0.0001*				
	R_2 Software			0.9230	0.0001*		
	R_T Software					0.9120	0.0001*

*p<0.05

The knowledge on effects of laser on tissue remodeling in

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