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Evaluation of efficacy of low-intensity laser therapy on acceleration of orthodontic tooth movement by measuring IL-6 and TNF- α levels in GCF- A randomized clinical controlled trial

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Abstract: *Aims and Objectives:* There is a profuse tendency for researches to focus on accelerating methods for tooth movement due to the huge demand from adults for a shorter orthodontic treatment time. Photobiomodulation is an emerging area of science that has clinical applications in number of human biological processes. Low-Intensity laser therapy (LILT) has been discussed in many fields of dentistry and hence this study was undertaken to evaluate the efficacy of Low Intensity Diode Laser to accelerate orthodontic tooth movement and correlating the IL6 and TNF α - levels in gingival crevicular fluid. *Materials and Methods:* Twenty patients aged from 15 to 22 years were randomly selected and among these patients, ten patients were allotted for experimental and ten patients served as controlled groups by flip coin method. Treatment was initiated by bonding fixed appliances in both the arches. The experimental group was treated with LILT using Diode Laser. GCF was collected by intrasulcular technique at different period of intervals and subjected to laboratory assessment. *Results:* There was statistical significance in the rate of tooth movement in the experimental group (LILT). Increased levels of TNF-α and IL-6 were also observed in GCF samples of experimental group as compared with the control group. *Conclusion:* Application of low-level laser therapy in adjunctive to orthodontic forces accelerated orthodontic tooth movement along with increased the levels of TNF-α and IL-6 in gingival crevicular fluid.

Keywords: Enmasse Retraction, IL-6 and TNF- α, Low Intensity Laser Therapy (LILT).

Introduction

Orthodontic treatment is based on the principle that when force is delivered to a tooth and transmitted to the adjacent tissues, certain mechanical, chemical and cellular events take place within these tissues, which allows structural alteration and contribute to the movement of the tooth [1]. Barlow reported that teeth moves 0.8 to 1.2mm per month when continuous forces are applied [2].

There is a huge expectations from adults for a shorter duration of orthodontic treatment and hence exist an enormous tendency for researchers to focus on accelerating the methods for tooth movement. The conventional orthodontic treatment time poses several disadvantages like higher predisposition to caries, gingival recession and tooth resorption. Thus there is always a thrust for researches to find new techniques with least possible disadvantages [3].

According to literature available many methods and techniques have been deployed to accelerate Orthodontic tooth movement which include Corticotomy, Dento-alveolar distraction, periodontal distraction, peizoincision, molecular therapy, magnets like samarium-cobalt , drug injections [1], electric stimulation [2], and ultrasound application [4].

Although these methods reduce treatment duration up to 70%, but do have their limitations that could be associated to discomfort and pain with drug injections, invasive procedures or a sophisticated apparatus that demands applications for a long term to achieve its therapeutic effect.

The periodontal tissues express extensive macroscopic and microscopic changes leading to alterations in extracellular matrix, cell membrane, cytoskeleton, nuclear protein matrix, and genome when an orthodontic force is applied. The changes in Periodontal ligament vascularity as well as mechanical alterations in the cytoskeleton of Periodontal ligament and bone cells will result in local synthesis and release of various key mediators such as chemokine's, cytokines, and growth factors.

These are the main molecules involved in bone cell recruitment, activation, proliferation, differentiation, and survival. These molecules stimulate periodontal ligament and bone cells to orchestrate an inflammatory response followed by osteoclastogenesis and bone resorption in compression sites, and new bone formation at Periodontal ligament tension sites. These molecules can modulate the outcome of the application of orthodontic force, accelerating orthodontic tooth movement [5]. There seemed to be increased volume of Interleukins in GCF (gingival crevicular fluid) during orthodontic treatment [6].

Laser therapy has vast applications in different fields of dentistry. Low level laser therapy is used for reduction of pain and increases bone absorption in the length of mid palatal sutures during expansion. But to the best of our knowledge, hardly published literature is available on effects of biomodulation promoted by laser to accelerate human teeth movement. Hence an innovative study was carried to evaluate the efficacy Low-Intensity Laser Therapy during an orthodontic movement by measuring the levels of cytokines TNF-α and IL-6 in GCF.

Aims and Objectives: The aim of this study was to evaluate the efficacy Low-Intensity Laser Therapy during an orthodontic movement by measuring the levels of cytokines IL-6 and TNF-α in GCF pre and post retraction.

The Objectives includes:

1. To determine the rate and compare of enmasse retraction in the experimental and control group with Low intensity laser therapy.
2. To determine anchorage loss in the experimental and control group with Low intensity laser therapy.
3. To Evaluate and compare levels of TNF-α and IL-6 in GCF samples in experimental and control groups.

Material and Methods

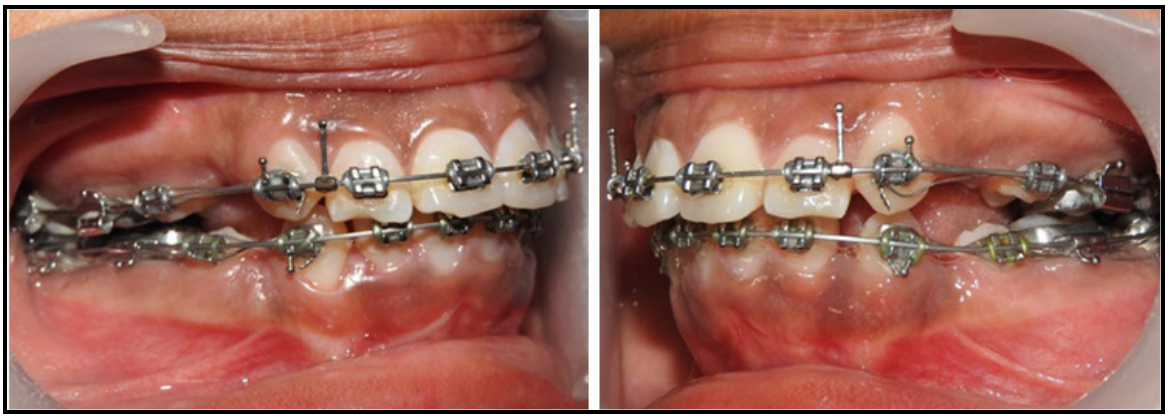
Patients reporting to the Department of Orthodontics and Dentofacial Orthopedics at Al-Ameen Dental College and Hospital, Vijayapur, who met the following inclusion criterias were selected for the study. Twenty patients were randomly selected and among these patients, ten patients were allotted for experimental and ten patients served as controlled group by flip coin method.

The Inclusion criteria included patients with Angle's class-2 div-1 or bimaxillary protrusion, required maxillary 1st premolar extraction as part of orthodontic treatment and age ranging from15 to 22 years. The patients with poor compliance, smoking, drug abuse, systemic diseases, Periodontitis and with thin gingival biotype were excluded. The sample size 10 per group (i.e. 20 nos) with 90% power and 5% level of significance with anticipated mean difference of mean SBS (MPa) between the two study groups as 0.31 and anticipated SD 0.19 were selected.

Methodology: The selected patients were labelled as Group I (Experimental) and Group II (Control) with ten participants in each by randomization. The patients were informed about the study and consent was obtained. The patients were referred for extraction of maxillary first premolars after recording the detailed case history. Treatment was initiated by bonding fixed appliances in both the arches. The arches were levelled and aligned.

After levelling and aligning, retraction was carried out using 0.019x0.025 stainless steel wire in 0.022 slot MBT prescription brackets [Fig no.1]. The duration of study was set up for 60 days. Once levelling and aligning was achieved, 0.019''x0.025'SS wire was left for 6 weeks for residual tip and torque to be expressed. Prior to the retraction all the maxillary anterior were consolidated with 0.010 SS ligature wire.

Fig-1: Retraction with 0.019 x 0.025 stainless steel wire



Laser irradiation: This procedure was done only in the experimental group. The equipment used in this study was a Simple Diode Laser by doctor smile, emitting infrared radiations at 980nm, by the output power of 12mW, dose of 5J per sq.cm. And exposure time of 10 seconds. Laser irradiation was carried out on 1st, 8th, 21st, 28th, 45th, and 59th day in contact mode. A total of ten irradiations, five each on buccal and palatal side were distributed as two doses on cervical third (one mesial and one distal), two doses on apical third (one mesial and one distal) and one in the middle third (on the center of the root) in following order to cover the periodontal ligament fibers and alveolar process around the teeth [7] (Fig no. 2).

Fig-2: Laser Irradiation

Enmasse retraction of the anteriors was performed in both the groups with active tie-backs after having a basal 0.019*0.025 SS arch wire providing 150 grams of force measured with the help of a Dontrix gauge. All the patients were recalled for routine activation at an interval of 4 weeks. At each visit the force produced by the active tie-backs was checked.

Collection of GCF Samples: GCF was collected after isolation and drying of the site with capillary tubes by intrasulcular technique from distal to the canine and mesial to the second premolar sites in both the groups at 2nd, 9th, 29th, 46th and 60th day [Fig no.3]. The samples were sent to laboratory at Almeen Medical college research center, Vijaypur to evaluate quantitative detection of TNF-α and IL-6 interleukins. The ELISA kit procured was Human IL-6 ELISA kits from Krishgen Bio Systems [Fig no.4].

Fig-3: Collection of GCF samples using capillary tube

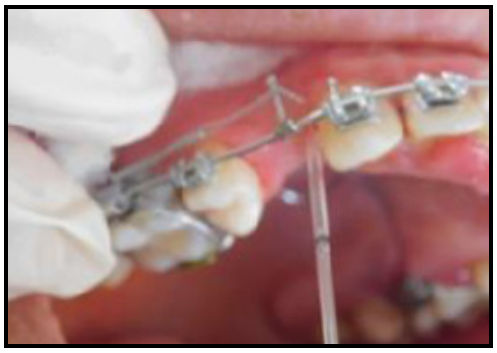


Fig-4: Human ELISA Kit

Determination of Rate of Retraction: The rate of retraction was defined as the distance travelled, divided by the time required to complete space closure. This was recorded in millimeters per interval where an interval was defined as a four week period. The width of the extraction space, closure and duration of retraction were recorded with Vernier caliper.

Determination of Anchor Loss: Pre and post space closure lateral cephalometric radiographs were taken for all patients and the change in the position were superimposed and anchorage loss was measured.

Statistical Analysis: Data was analyzed using SPSS software v.23.0 and Microsoft office 2007. The rate of retraction was assessed by one way anova followed by post hoc tukey's HSD test to evaluate and compare the rate of space closure between the time intervals.

Results

On Inter Group Comparison, Independent unpaired t test showed statistical significant difference in the rate of encase retraction at the end of 4^{th} week D1 ($p< 0.024$), 8^{th} week D2 ($p<0.029$), 12^{th} week D3 ($p<0.001$), 16^{th} week D4 (0.001) [Table 1].

Table-1: Inter group comparison between experimental and control group

			Mean	Standard deviation	T	P value
D1	Right	Experimental	1.7420	0.04492	20.861	0.024 (S)
		Control	1.0860	0.08872		
	Left	Experimental	1.7420	0.03795	20.674	0.005 (H.S)
		Control	1.0720	0.09520		
D2	Right	Experimental	1.6390	0.03381	25.077	0.029 (S)
		Control	1.0410	0.06740		
	Left	Experimental	1.6390	0.02846	26.619	0.004 (H.S)
		Control	1.0260	0.06703		
D3	Right	Experimental	1.3660	0.01350	17.699	0.000 (H.S)
		Control	1.0220	0.05996		
	Left	Experimental	1.3670	0.01767	18.852	0.001 (H.S)
		Control	1.0070	0.05774		
D4	Right	Experimental	1.3260	0.01350	18.250	0.001 (H.S)
		Control	0.9970	0.05539		
	Left	Experimental	1.3190	0.01853	17.574	0.000 (H.S)
		Control	0.9830	0.05755		
D5	Right	Experimental	0.9820	0.41507	0.053	0.003 (H.S)
		Control	0.9750	0.05421		
	Left	Experimental	0.9810	0.42120	.194	0.002 (H.S)
		Control	0.9550	0.05442		
D6	Right	Experimental	0.2150	0.31585	-7.177	0.014 (S)
		Control	0.9440	0.05835		
	Left	Experimental	0.2240	0.33060	-6.542	0.011 (S)
		Control	0.9320	0.35210		

Anchor Loss was determined by measuring the difference in values of pre and post retraction recorded by cephalograms. Pre and post retraction values were compared by paired t test and the difference was statistically non-significant (p value = 0.4) [Table 2].

Table-2: Comparison of anchor loss between Experimental group and Control group					
Anchor loss		**Mean**	**Standard deviation**	**T**	**p value**
Experimental	Pre	20.80	1.549	-0.997	0.487 (N.S)
	Post	21.60	2.011		
Control	Pre	20.6000	1.50555	-1.259	0.432 (N.S)
	Post	21.6000	2.01108		
Difference b/w pre and Post	Experimental	0.8000	0.78881	-0.514	0.400 (N.S)
	Control	1.0000	0.94281		

Table-3: Comparison of Mean TNF-α between Experimental and Control group by Mann-Whitney u test									
Day	**Cases**			**Controls**			**Mann-Whitney U**	**Z**	**P value**
	Mean	**SD**	**Mean Rank**	**Mean**	**SD**	**Mean Rank**			
Day 02	1.207	0.542	13	0.968	0.234	8	25	-1.89	0.059
Day 09	1.201	0.388	12.9	0.894	0.218	8.1	26	-1.815	0.07
Day 29	1.174	0.373	13.5	0.854	0.221	7.5	20	-2.268	0.023*
Day 46	1.134	0.363	12.1	0.921	0.202	8.9	34	-1.209	0.226
Day 60	1.199	0.360	13.5	0.877	0.193	7.5	20	-2.268	0.023*
Note: * significant at 5% level of significance (p<0.05)									

Table-4: Comparison of Mean IL-6 between experimental and Control group by Mann-Whitney U test									
Day	**Cases**			**Controls**			**Mann-Whitney U**	**Z**	**p value**
	Mean	**SD**	**Mean Rank**	**Mean**	**SD**	**Mean Rank**			
Day 02	0.959	0.316	13.3	0.651	0.242	7.7	22	-2.117	0.034*
Day 09	0.894	0.292	13.1	0.610	0.253	7.9	24	-1.967	0.049*
Day 29	0.909	0.264	13.7	0.586	0.254	7.3	18	-2.419	0.016*
Day 46	0.783	0.259	12.8	0.549	0.240	8.2	27	-1.739	0.082
Day 60	0.847	0.141	14.6	0.477	0.224	6.4	9	-3.099	0.002*
Note: * significant at 5% level of significance (p<0.05)									

Table-5: Comparison between mean TNF-α & IL-6 among Experimental group by Mann-Whitney u test									
Day	**TNF-α**			**IL-6**			**Mann-Whitney U**	**Z**	**p value**
	Mean	**SD**	**Mean Rank**	**Mean**	**SD**	**Mean Rank**			
Day 02	1.207	0.542	12.6	0.959	0.316	8.4	29	-1.587	0.112
Day 09	1.201	0.388	13.1	0.894	0.292	7.9	24	-1.965	0.049*
Day 29	1.174	0.373	12.6	0.909	0.264	8.4	29	-1.587	0.112
Day 46	1.134	0.363	13.3	0.783	0.259	7.7	22	-2.117	0.034*
Day 60	1.199	0.360	13.3	0.847	0.141	7.7	22	-2.117	0.034*
Note: * significant at 5% level of significance (p<0.05)									

The difference of the means of analysis variables between two independent groups for TNF-α and IL-6 was tested by Mann-Whitney U test. Comparison of mean TNF-α and IL-6 in experimental and control group observed an increase in TNF-α and IL-6 values in experimental group form day 2nd to 60th day which was statistically significant [Table 3 & 4]. On comparison between TNF-α and IL-6 in experimental group, mean value of TNF-α from day 2nd to 60th day was higher than IL-6 [Table 5].

Discussion

The prolong treatment duration is one of the main deterrents in orthodontic therapy as it prompts many patients, especially adults to either avoid treatment or to seek shorter alternative solutions with compromised results [8]. Clinicians are constantly striving towards developing potential strategies and techniques to enhance the rate of orthodontic tooth movement [9], where Low Intensity Laser Therapy (LILT) has found to be beneficial in inducing remodeling of alveolar bone by increasing osteoblast and osteoclast numbers, which leads to acceleration of orthodontic tooth movement [10].

According to Burcu A.A et al, LILT is known as a stimulator of on-going biological process in tissue and has been found to be effective in modulating cell activity and production of endogenous molecules which are involved in Orthodontic Tooth Movement (OTM) [11]. The results of our present study observed that LILT increased the rate of enmasse retraction by 2.1 fold when compared to the controlled group. These findings were in accordance to study done by Kau where the rates of tooth movement in the alignment face were 0.49mm per week in LLLT and 1.12mm per week for controlled group [12].

Our study also observed that duration of treatment was reduced and these results were similar to studies conducted by Sousa et al [13], Cruz et al [14], Kocher et al [15] and Youssef et al [16]. Our findings suggest that LILT does accelerate human teeth movement and could therefore considerably shorten the whole treatment duration in accordance with Delma R. Cruzet al [7] but these findings were inconsistent with reported study by Mohamed Abd El-Ghafour et al [17].

The rate of tooth movement is influenced by bone density, rate of osteoclast recruitment and activation which in turn are dependent on age. In our study age group between 15 to 22 years were included to avoid bias. The anchor loss in the present study was minimal i.e. 0.8mm in experimental group and 1.0mm in control group which was statistically non-significant (p value=0.4) and these similar findings were observed by Mohamed Abd El-Ghafour et al [17].

It has been reported that chemical analysis of Gingival Crevicular Fluid (GCF) is a promising technique to investigate the response of dental and periodontal tissues to orthodontic force load in a biochemical manner. Cytokines are extracellular signaling proteins directly involved in the bone remodeling and inflammatory process during OTM, which act directly or indirectly to facilitate bone and PDL cells differentiation, activation, and apoptosis [6].

In our study the comparison of mean TNF-α and IL-6 in experimental and control group observed an increase in TNF-α and IL-6 values in experimental group form day 2 to day 60 which was statistically significant. These results were in accordance with a study where significant increases in IL-1β, IL-8, TNF-α from 4 hours to 42 days after application of forces where shown. The concentrations of IL-1β, IL-6, TNF-α were significantly higher in experimental groups than in controls at 24 hours after experiment was initiated [18]. On comparison between TNF-α and IL-6 in experimental group, mean value of TNF-α from day 2nd to 60^{th} day was higher than IL-6.

Previous studies have independently assessed the levels of inflammatory cytokines and LILT (Low Intensity Laser Therapy) on rate of Orthodontic Tooth Movement (OTM), but combination of LILT and cytokines levels during OTM has been unspecified. The assessment of TNF-α and IL-6 during laser irradiation could provide insight into the basis of accelerated tooth movement observed with this modality as this first of its kind study.

Sample size arrived was small and hence effect size was not able to validate which was limitation of this study and also correlation between the levels of IL-6 , TNF-α and the amount of tooth movement across all time intervals was not assessed. The other limitation in this study was the disability to blind both the participants and the operator, but blinding to the assessor was done.

Conclusion

An increase in the rate of orthodontic tooth movement in the experimental group with

minimal anchorage loss in both experimental and controlled group was observed with significant increase levels of cytokines. Lasers were well accepted by patients as it was noninvasive, painless with short duration. Longitudinal studies with different wave length of lasers are needed to be carried out. Hence histological studies on animal models must be performed to better understand the biological effects of the device.

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Conflicts of interest: There are no conflicts of interest.

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