Original Research Article

Efficacy of low-level laser therapy enhances the rate of canine retraction by increasing β glucoronidase and pentraxin – 3 in gingival crevicular fluid: A clinical study

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A R T I C L E I N F O

A B S T R A C T

Aim: The aim of the study was to evaluate the efficacy of low-level laser therapy in enhancing the rate of orthodontic tooth movement using β glucoronidase and pentraxin-3 (TNF) biomarkers in gingival crevicular fluid.

Materials and Methods: A split-mouth design was used in 16 subjects (8 experimental and 8 controlled) aged 14 to 25 years, who's maxillary first premolars were extracted. A gallium-aluminium-arsenide semiconductor diode laser (wavelength, 810 nm; energy density, 10 J/cm2; power output, 100 mW) delivered low-level laser therapy to the experimental canine undergoing distalization at 10 points. The control canine was distalized without low-level laser therapy. The experimental and control canines were distalized using a force of 150 g provided by nickel-titanium closed coil springs. Gingival crevicular fluid was collected at 5 time points from the control and experimental sides, and the levels of β glucoronidase and pentraxin-3 were analysed by enzyme-linked immuno-sorbent assay (ELISA). The rate of canine retraction was calculated by using ortho analyser software.

Results: Increased levels of β glucoronidase and pentraxin-3 were observed in the experimental canines compared with the control canines (P <0.001). Cumulative tooth movements over an 8-week experimental period were greater for the experimental canines compared with the control canines. A positive correlation existed between the β glucoronidase and pentraxin-3 levels and the amounts of tooth movement across all time intervals.

Conclusion : Low level laser therapy in adjunct with continuous and light forces leads to increase in β glucoronidase and pentraxin-3 leading to accelerated orthodontic tooth movement.

Clinical Significance: Low-level laser therapy fastens the rate of canine retraction thus accelerating the orthodontic tooth movement. The levels of β glucoronidase and Pentraxin 5 are increased significantly after laser irradiation which aids in accelerating orthodontic tooth movement.

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1. Introduction

The widespread use of technology in modern years has revolutionised all the fields of medicine and dentistry. The era of Orthodontics has changed a lot from Angle's period to

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the present nanorobotic era in its concept, biomaterials, and technology. In the past few years new devices, techniques have made the Orthodontic treatment more systematic and effective but not reduced the orthodontic treatment time to great extent.

In present age of acceleration, humanity has grown to expect pace in all aspects of life. The diagnosis and treatment planning of adult Orthodontic treatment poses a greater challenge to the clinician because of the complex dentofacial structures and patients demanding speedy treatment.

Several strategies have been proposed to decrease the time in Orthodontic treatment. Various methods have been introduced to increase the rate of tooth movement which includes corticotomy, dento-alveolar distraction, periodontal distraction, peizocision, molecular therapy, mechanical/physical stimulation methods using direct electrical current, vibrators, magnets like samarium-cobalt etc and drugs. All these methods reduce treatment duration up to 70%. Yet these approaches cannot be applied clinically as they are not patient compliant.

Photo biomodulation (PBM), frequently referred to as low-level laser therapy. (LLLT) or cold laser therapy uses light energy to stimulate biological responses from the cell. Since last few years many histologic studies have endeavored to determine the effect of low-intensity laser therapy on the histochemical pathways directly associated with Orthodontic tooth movement. This LLLT Increases osteoblastic and osteoclastic activity.[1]

Orthodontic force induces a cellular response in the periodontal ligament, which brings about bone resorption on the pressure side and bone deposition on the tension side. This occurs via induction of osteoclasts via the RANK-RANKL pathway and presence of various inflammatory mediators such as IL-1, IL-8, and TNF-alpha etc. Macrophage colony stimulating factor (M-CSF), Receptor activator of nuclear factor kappa B ligand (RANKL), and osteoprotegerin (OPG) by osteoblasts play significant role in tooth movement.[2]

The gingival crevicular fluid (GCF) is a transudate of interstitial tissues which is produced by an osmotic gradient, that is released into the crevicular crevices. Orthodontic treatment is triggering an inflammatory process and it has been assumed that the quantification of specific biomarkers within the GCF can be determined using micropipette. However, there are contrasting results reported in the literature.[3]

Given that the orthodontic treatment is triggered by a set of inflammatory cytokines that are released into the crevicular fluid during the mechanical loading of forces, and its homeostasis is dependent on mechanical stimulation. An understanding of the biological response of crevicular fluid to mechanical loading of Orthodontic forces could advance the knowledge of orthodontic treatment.

The GCF biomarkers, β Glucoronidase (βG) and Pentraxin-3 (TNF) were selected for this study as: PTX-3 modulates aseptic inflammation as a response to the mechanical stress induced by orthodontic forces in first two weeks suggesting periodontal orthodontic remodelling. PTX-3, being an acute-phase protein, reflects with fidelity the inflammatory condition.[4] The lysosomal enzyme β-glucuronidase (βG) levels increases in the GCF of patients undergoing orthodontic treatment and a high enzyme activity suggests a greater cellular activity causing an apoptotic process to eliminate the hyalinized periodontal tissue formed during the early stages of orthodontic movement resulting in an accelerated tooth movement.[5]

Hence this study was undertaken to determine the efficacy of low-level laser therapy in enhancing the rate of Orthodontic tooth movement using β Glucoronidase (βG) and Pentraxin-3 (TNF) biomakers in Gingival Crevicular Fluid during the retraction phase of orthodontic treatment and to assess and compare the differences in rate of canine retraction in in experimental and controlled group after laser irradiation.

2. Materials and Methods

2.1. Sample source

This was an In-vivo. Patients reporting to the Department of Orthodontics and Dentofacial Orthopaedics at Bharati Vidyapeeth (Deemed to be) University Dental College and Hospital who fulfilled the following inclusion criteria were selected for the study. Eight patients were randomly selected and among these patients one side was experimental side while the other was controlled side. The study was approved by ethical committee of BV(DU)/MC&H/Sangli/IEC/Dissertation 2020-21/D-45.

2.2. Study sample size

The sample size was calculated using G- Power Software, from the data obtained (mean BPA values) from the previous study.

The sample size was determined by using following formula:

Tests - Means: Difference between two independent means (two groups)

Analysis: A priori: Compute required sample size

2.3. Criteria for selection of subjects

2.3.1. Inclusion criteria
1. Patients with Angle Class I malocclusion with bimaxillary protrusion and well aligned arches.
2. Patients indicated for maxillary first premolar extraction.
3. Patients indicated for Bilateral maxillary canine distalization.

Input:	Tail(s)	Two
	Effect size d	1.9611614
	α err prob	0.05
	Power (1-β err prob)	0.95
	Allocation ratio N2/N1	1
Output:	Noncentrality parameter δ	3.9223228
	Critical t	2.1447867
	Df	14
	Sample size group 1	8
	Sample size group 2	8
	Total sample size	16
	Actual power	0.9536757

4. Patients aged 14-25 years either male or female.
5. Patients with good periodontal condition and oral hygiene

2.3.2. Exclusion criteria
1. Patients with a history of orthodontic treatment
2. Patients with Angles Class I malocclusion with severe proclination and crowding of anterior teeth.
3. Patients with Systemic disease affecting bone and general growth.
4. Patients with poor periodontal condition and bone loss
5. Patients using anti-inflammatory analgesic drugs during the months preceding the study.
6. Patients taking any other medication that might hinder bone metabolism.
7. Patients with habit of smoking

3. Materials and Methods

Total of 16 patients who met the inclusion criteria were selected and divided into two groups of 8 patients using closed envelope method for control and study group. All the patients in both the groups were treated by same investigator with 0.022 slot MBT prescription brackets.

After diagnosis and treatment planning, the patients were referred for extraction of maxillary 1st premolars. The arches were levelled and aligned for two months since inception in all 16 patients. Then retraction was carried out using closed coil springs on 0.019×0.025 SS wire, with intermittent laser irradiation on $1^{st}, 3^{rd}, 7^{th}, 30^{th}, 60^{th}$ days for study group.

1. **Group 1**- experimental group and Group 2- control group.
2. **Group 1**: This group composed of 8 subjects who were treated with Laser therapy. Lasered group)
3. **Group 2**: This group composed of 8 subjects treated without laser (control group)

The patients were informed about the study and consent for performing the Laser therapy and for data collection was taken from the patients. Once levelling and alignment was achieved, 0.019" × 0.025" SS wire was left for 6 weeks for residual tip and torque to be expressed. Before retraction alginate impression of maxillary arch was taken, poured immediately and models were labelled. Prior to retraction all the maxillary anterior were consolidated with 0.010 SS ligature wire.(Diagram 1)

3.1. Laser irradiation

The procedure was carried in the experimental group. The device used in this study was a Simpler Diode Laser by Novo Laser, emitting infrared radiations at 810nm, by the output power of 100mW, dose of 10 J per sq.cm. and exposure time of 30 seconds. Laser irradiation was carried out on $1^{st}, 3^{rd}, 7^{th}, 30^{th}, 60^{th}$ days. The tip was held perpendicular and in contact with mucosa during laser procedure. A total of 10 irradiations each time, 5 by the buccal side and 5 by the palatal side were carried out and distributed in following order to cover the PDL fibres and alveolar process around the teeth. (Figure 1)

1. Two doses on cervical third (one mesial and one distal)
2. Two doses on apical third (one mesial and one distal)
3. One in the middle third (on the centre of the root)

The crimpable hooks were crimped on the main arch wire, 1mm distal to the lateral incisor in both the quadrants. Enmasse retraction of the anterior was performed in both the groups with light nickel – titanium closed coil spring (9x12mm) after having a basal 0.019" × 0.025" SS arch wire providing 150 grams of force measured with the help of a gauge dynamometer. The activation was done at an interval of 4 weeks. At each visit the force produced by the light nickel – titanium closed coil spring was checked.

3.2. Collection of GCF samples

After the isolation and drying of the site, capillary tubes of known internal diameter were inserted into the entrance of the gingival crevice and the fluid migrated up the tube by capillary action. The samples were collected, 1st, 3rd, 7th, 30th, 60th days and then taken to the laboratory for cytological examination. The GCF samples were collected form the area distal to the canine and mesial to the 2nd premolar. (Figure 2)

The specimens were clear and non-haemolysed. The samples were run at several dilutions to ensure accurate quantitation. They were centrifuged to remove debris prior to analysis. The samples were stored at temperature less than -80 °C.

3.3. Determination of rate of retraction

Impressions of the subjects were recorded during the 1st, 30th and 60th day. The plaster models were made. These plaster models were scanned at the lab and the NFT file was

made. And the rate of retraction was measured using the software Ortho Analyser. (Figure 3)
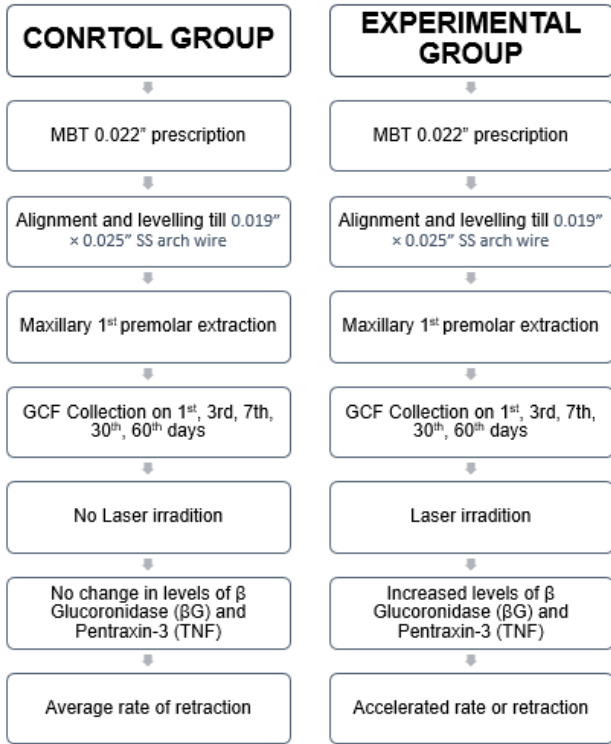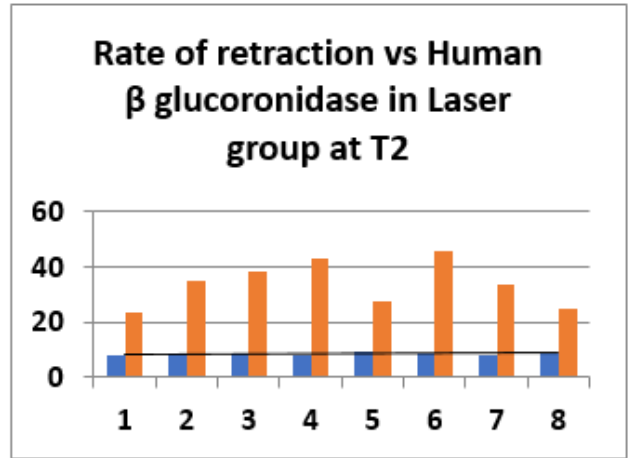

Diagram 1: Methodology

Graph 1: Rate of retraction vs Human β Glucoronidase in Laser group at T2

3.4. Statistical analysis
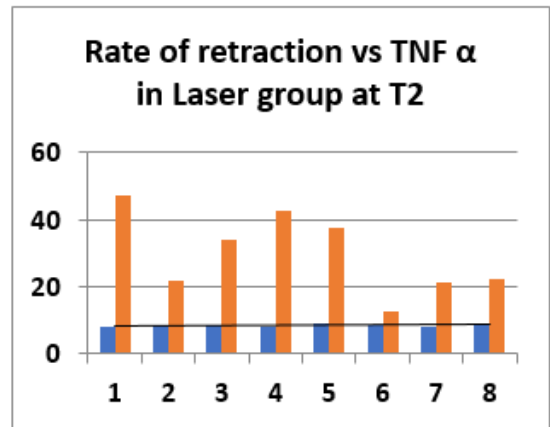
Two-way repeated measure ANOVA test was used to compare the level of β glucuronidase and TNF α with two groups (control and experimental). Bonferroni test was done for comparison of change in Human β glucuronidase and TNF α within each group. The software used for statistical analysis was SPSS software version 23.

Graph 2: Rate of retraction vs pentraxin 5 in laser group at t2

Figure 1: Laser irradiation

Figure 2: GCF collection

Table 1: Descriptive statistics of Human β glucoronidase among control and laser group

Groups	Control Group		Laser Group	
	Mean	Std. Deviation	Mean	Std. Deviation
1st day (T0)	43.43	9.54	41.81	15.82
3rd day (T1)	44.74	9.93	44.75	7.89
7th day (T2)	44.69	13.08	45.54	7.45
1st month (T3)	34.23	5.01	29.55	7.35
2nd month (T4)	34.70	4.82	33.73	8.26

**Table 2:** Descriptive statistics of TNF α among control and laser group

Groups	Control Group		Laser Group	
	Mean	Std. Deviation	Mean	Std. Deviation
1st day (T0)	31.71	14.98	39.15	4.50
3rd day (T1)	23.28	7.77	17.60	3.35
7th day (T2)	20.25	10.39	38.71	15.55
1st month (T3)	34.69	9.65	29.40	17.02
2nd month (T4)	18.06	4.54	29.86	12.10

Table 3: Descriptive statistics of rate of retraction among control and laser group

Groups	Control Group		Laser Group	
	Mean	Std. Deviation	Mean	Std. Deviation
T0	16.08	0.34	15.11	0.82
T1	15.21	0.22	11.43	0.57
T2	9.24	0.50	8.57	0.40

Table 4: Correlation of rate of retraction with human β glucuronidase and tnf-α in control group and study group

	Interval	Control group		Laser group	
		r value	p value	r value	p value
Rate vs β glucuronidase	T0	0.523	0.183	0.380	0.353
	T1	0.041	0.922	-0.287	0.491
	T2	0.355	0.388	-0.080	0.851
TNF-α	T0	0.428	0.290	-0.530	0.177
	T1	0.228	0.587	0.047	0.911
	T2	0.068	0.873	-0.432	0.285
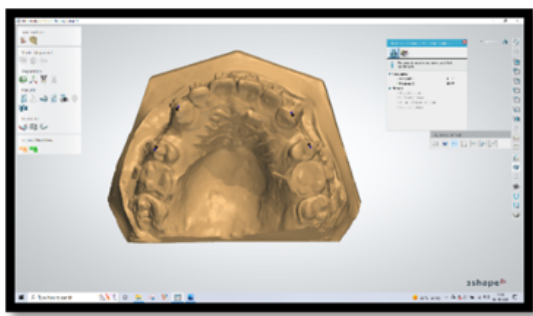
Figure 3: Ortho analyser

4. Result

For β glucuronidase the result was found to be significant with time with values of <0.001 and non-significant for treatment and time × treatment. The level of β glucuronidase increases with that of time. Bonferroni test showed significant result of 0.016 was observed at an interval of T1-T3. (Table 1) (Graph 1)

For TNF α ANOVA showed significant result for time, treatment, and time × treatment with values of 0.006, 0.044 and 0.002 respectively. On Comparison of change in TNF α within each group by Bonferroni test showed significant results at an interval of T0-T1. And Independent t test showed significant readings on T2 and T4.(Table 2) (Graph 2)

Rate of enmasse retraction showed significant results at all the intervals and more increased value for lased group. (Figure 4) (Table 3). Also, correlation of rate of enmasse retraction with Human β glucuronidase and Pentraxin (TNF) in control group and study group showed moderately strong correlation. (Table 4)

Orthodontic force in combination with low level laser therapy increases the levels of β glucuronidase and Pentraxin 3 (TNF) in GCF and accelerates OTM.
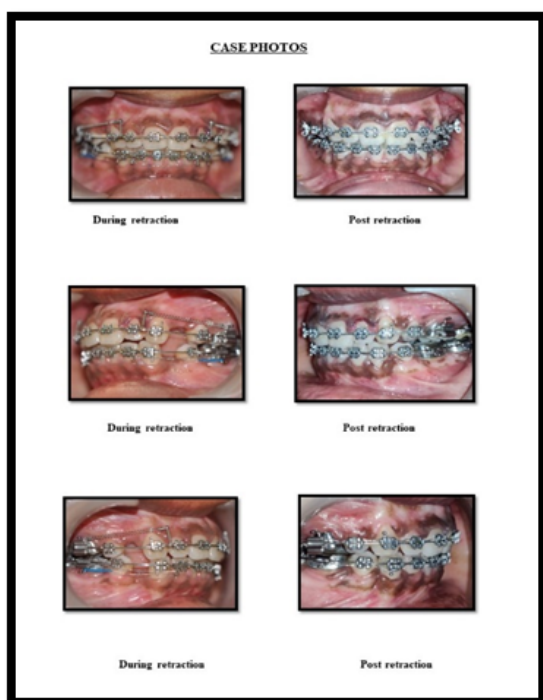
Figure 4: Case photo

5. Discussion

21st century is called to as the Century of the Biologist and the dentofacial dimension of the orthodontic specialty is a front stage player in the screenplay of scientific progress. The most relevant feature in this inevitable progress is the interdisciplinary collaboration of orthodontic specialty which has overtaken traditional orthodontic tooth movement (OTM) protocols and synthesized new method of low-level laser therapy for rapid orthodontic tooth movement. This new method has given young orthodontic clinician a protocol that helps to reduce side effects like root resorption, relapse, inadequate basal bone, and bacterial time/load factors like caries and infection.[6]

Orthodontic tooth movement have used GCF because of its non-invasive nature and ease of repetitive sampling from the same site with the help of micropipettes avoiding saliva contamination.

LLLT is found to be effective in alveolar bone remodelling processes by increasing osteoblast and osteoclast activity, leading to accelerated orthodontic tooth movement.[7] Many studies have been carried on LLLT in accelerating orthodontic tooth movement. Some studies found laser effective while others concluded the opposite.[8] These contradictory results can be evaluated by studying difference in laser parameters regarding its type, application methods, wavelength, dose of irradiation and exposure time.[9]

Mohamed Youssef et.al suggested low level laser therapy as a micro-invasive option able to accelerate orthodontics. So, acclimating low level laser therapy can accelerate tooth movement during orthodontic treatment and also effectively reduce pain level.[10] According to Burcu A.A et al in 2014, LLLT (Low Intensity Laser Therapy) is known as a stimulator of on-going biological process in tissue and is said to be effective in modulating cell activity and production of endogenous molecules which leads to Orthodontic Tooth Movement (OTM).[11]

According to Alissa M.V (2018), increased levels of βG, PTX-3 in combination with LLLT reflects one probable mechanism underlying increased orthodontic tooth movement. βG is involved in bone metabolism by triggering bone resorption and inhibiting bone formation.[12] Orthodontic forces evoked significant increases in levels of PTX-3 in periodontal tissue. Significant increases in βG, PTX-3 is observed from 4 hours to 42 days after application of forces. The concentrations of βG, PTX-3 were significantly increased in experimental groups than in controls at 24 hours after experiment were initiated.

We investigated the effects of LLLT on the rate of orthodontic tooth movement in enmasse retraction and comparison with the convention orthodontic technique. The results showed that, LLLT increased the rate of enmasse retraction by 2.1-foldin comparison to the control group. In another study done by Kau et al, the rates of tooth movement in the alignment face were 1.12mm per week for those in LLLT group compared to 0.49mm per week in controlled group.[13] LLLT decreases the treatment time; these results agree with results of studies by Sousa et al and Cruz et al.[14,15] 1.6-fold increases in canine retraction rate were found which agrees with results of Sousa et al, Youssef et al.[14–16]

6. Conclusion

Low level laser therapy (810nm GaAlAs diode laser) with irradiation parameter and protocol used in this study was found adjuvant tool in accelerating orthodontic tooth movement if properly used, with due consideration and good understanding of the principles and interaction between different treatment mechanics with biological tissue. This method helps to provide physical stimuli resulting in accelerated tooth movement, by varying the patient's biologic response and not by merely increasing forces or altering treatment mechanics.

The current study showed the significant amount of space closure obtained on day 90 suggesting the supporting role of LLLT in Orthodontic tooth movement, when compared to conventional method. Also, there is significant increase in the values of β Glucoronidase and Pentraxin-3 (TNF) in GCF which are important biomarker for Orthodontic tooth movement. And these results were significant.

Overall results of this study solicit to conclude that LLLT can be used as innovative treatment modality and can be effectively employed for accelerating OTM and improve the quality of treatment. In future perspective of this innovation, is decreased treatment cost and further research is needed to determine the effect of laser on space closure and to establish the parameter that results in the greater amount of tooth movement.

6.1. Limitations

GCF collection during orthodontic treatment there can be a major tissue damage require biochemical monitoring related to orthodontic treatment and the diurnal variations for collecting GCF which is a promising issue.

In the current GCF collection method it is mandatory to have periodontal health, oral hygiene, and optimal orthodontic forces.

6.2. Clinical significance

Low-level laser therapy fastens the rate of canine retraction thus accelerating the orthodontic tooth movement. The levels of β glucoronidase and Pentraxin 5 are increased significantly after laser irradiation which aids in accelerating orthodontic tooth movement. Thus, this therapy helps to decrease the orthodontic treatment time.

7. List of Abbreviations

1. TNF- Tumour necrosis factor
2. ELISA- Enzyme-linked immuno-sorbent assay
3. PBM- Photo biomodulation
4. LLLT- Low level laser therapy
5. βG-Beta glucoronidase
6. PTX- Pentraxin 5
7. OTM – Orthodontic tooth movement

8. Source of Funding

None.

9. Conflict of Interest

None.

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