RADIOGRAPHICAL AND HISTOLOGICAL STUDY BY APPLYING LASER ON BONE REMODELING IN EXPERIMENTAL TOOTH MOVEMENT

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ABSTRACT

The aim of this study was to evaluate the effect of the Low-Level Laser Therapy (LLLT) on the rate of post-experimental tooth movement clinically, biologically and radiographically, and on the bone remodelling histologically. Sixty white rabbits were radiographic and histologically evaluated for the effect of local application of LLLT and VEGF (alone and in combination) on orthodontic movement.

Histological assessment for dental tissue response includes (periodontal ligament, cementum, and alveolar bone) to the application of LLLT and VEGF in orthodontic treatment. The data of the all experimental periods include the experimental tooth movement measurements (cumulative Measurements) and percentage; both of them were analyzed using both descriptive and inferential statistics shows Generally, combination group appear to be most effective and records a high value in all followings parameters in comparison to other groups.

INTRODUCTION

Low-Level Laser Therapy (LLLT) is a simple and inexpensive method that can be used easily in the dental practice for different purposes. The stimulatory effect of low-level laser therapy is well known and includes enhancement in tissue growth and tissue regeneration, resolvment of inflammation¹, pain reduction², enhancement of wound healing³. Some studies investigated the efficacy of low power lasers in reducing burning mouth pain⁴, promoting bone regeneration in the midpalatal suture during expansion and stimulating tooth movement⁵.

The vast majority of therapeutic lasers are semiconductor lasers today⁶. There are three diode types:

1. Indium, Gallium-Aluminum-Phosphide (InGaAlP) laser

2. Gallium-Aluminum Arsenide (GaAlAs) semiconductor laser
3. Gallium-Arsenide (GaAs) semiconductor laser

In recent research projects, the effect of laser therapy was tested regarding the stimulatory effect on bone remodelling with the potential to influence the tooth movement rate as related to photobiological responses of oral tissue after application of laser⁷.

Three effects commonly occur as a result of tissue exposure to laser photons⁸. They are:

Primary effects of photoreception are a result of the interaction of photons and cell mitochondria which capture, direct, and transduce photon energy to chemical energy used to regulate cellular activity.

Secondary effects occur in the same cell in which photons produced the primary effects and are induced by these primary effects. Secondary effects include cell proliferation, protein synthesis, degranulation, growth factor secretion, myofibroblast contraction and neurotransmitter modification—depending on the cell type and its sensitivity.

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Table 1: Descriptive statistics of Clinical Measurements of distance (mm) between MCIs among studied groups at different intervals with comparisons significant

Marker	Groups	Mean	SD	SE	ANOVA test	
					F-test	P- ^(*) Value
Week 1	Control	0.32	0.01	0.01	1290	0.000 HS
	Lazar	0.66	0.01	0.00		
	VEGF	0.34	0.02	0.01		
	Combination	0.79	0.01	0.01		
Week 2	Control	0.51	0.01	0.00	1111	0.000 HS
	Lazar	1.08	0.04	0.02		
	VEGF	0.78	0.01	0.01		
	Combination	1.22	0.01	0.00		
Week 3	Control	0.92	0.01	0.00	462	0.000 HS
	Lazar	1.78	0.03	0.01		
	VEGF	1.23	0.01	0.00		
	Combination	1.95	0.11	0.04		

(*) HS: Highly Sig. at P<0.01; S: Sig. at P<0.05; NS: Non Sig. at P>0.05

Table 2: Area under the curve (ROC) for Clinical Measurements of distance (mm) between MCIs in contrast of control group at different periods

Area Under the Curve							
Test Result :working side- intervals							
Periods	Area	Std. Error	Asymptotic Sig.	95% C.I.		Sen.	Spec.
				L.B.	L.B.		
1 week	0.056	0.047	0.001	-0.037	0.148	100	88.9
2 week	0.000	0.000	0.000	0.000	0.000	100	100
3 week	0.000	0.000	0.000	0.000	0.000	100	100

Cutoff Point (0.33) at 1 week; Cutoff Point (0.52) at 2 week; Cutoff Point (0.93) at 3 week

Tertiary effects are the indirect responses of distant cells to changes in other cells that have interacted directly with photons. They are the least predictable because they are dependent on both variable environmental factors and intercellular interactions. They are, however, the most clinically significant. Tertiary effects include all the systemic effects of phototherapy. Primary, secondary, and tertiary events summate to produce phototherapeutic activity.

Orthodontic tooth movement occurs in the presence of mechanical stimuli sequenced by remodelling of the alveolar bone and periodontal ligament (PDL). Bone remodelling is a process of both bone resorption on the pressure site and bone formation on the tension site⁹. Orthodontic tooth movement can be controlled by the size of the

applied force and the biological responses from the PDL. The force applied on the teeth will cause changes in the microenvironment around the PDL due to alterations of blood flow¹⁰, leading to the secretion of different inflammatory mediators such as cytokines, growth factors, neurotransmitters, colony-stimulating factors, and arachidonic acid metabolites¹¹. As a result of these secretions, remodelling of the bone occurs. The most important growth factor is vascular endothelial growth factor (VEGF), this protein is a member of the PDGF/VEGF growth factor family and encodes a protein that is often found as a disulfide linked homodimer. This protein is a glycosylated mitogen that specifically acts on endothelial cells and has various effects, including mediating increased vascular permeability, inducing angiogenesis, vasculogenesis and endothelial cell growth, promoting cell migration,

Table 3: Area under the curve (ROC) for Clinical Measurements of distance (mm) between MCIs in contrast of control group at different sources of variation

Area Under the Curve					
Test Result: working side-groups					
Control with others groups	Area	Std. Error	Asymptotic Sig.	95% Confidence Interval	
				Lower Bound	Upper Bound
	0.191	0.055	0.000	0.084	0.299

Table 4: Area under the curve (ROC) for Clinical Measurements of distance (mm) between MCIs in contrasts of all pair wise groups at different sources of variation

Area Under the Curve					
Test Result: working Side – study groups					
Contrasts	Area	Std. Error	Asymptotic Sig.	95% Confidence Interval	
				Lower Bound	Upper Bound
Control X Lazar	0.111	0.054	0.000	0.006	0.217
Control X VEGF	0.352	0.094	0.129	0.169	0.535
Control X Comb.	0.111	0.054	0.000	0.006	0.217
Lazar X VEGF	0.667	0.092	0.088	0.486	0.847
Lazar X Comb.	0.336	0.092	0.094	0.156	0.517
VEGF X Comb.	0.230	0.077	0.006	0.078	0.382

and inhibiting apoptosis. VEGF is involved in tissue neo-formation that is strictly correlated with the presence of blood vessels¹².

During orthodontic tooth movement, compressive forces induce angiogenesis of periodontal ligament together with the role of mediator of the VEGF. The localization of VEGF was analyzed in many in vivo researches and illustrated an increment in its expression in periodontal tissue during experimental tooth movement¹³. Therefore, VEGF exerts a fundamental role in remodelling

periodontal ligament and is also involved in bone resorption and formation.

MATERIAL AND METHOD

Orthodontic Appliance Design and LLL therapy

The orthodontic appliance consists of orthodontic bands, archwires, and NiTi open-coil spring. The bands were customized for each rabbit. Briefly, the animals were anaesthetized with general anaesthesia, induced by an intramuscular injection

Clinical Measurements of distance (mm) between MCIs

Figure 1: Cluster Bar Chart concerning Mean values for Clinical Measurements of distance (mm) between MCIs among studied groups at different intervals

Figure 2: Stem-Leaf Plots for Clinical Measurements of distance (mm) between MCIs among studied groups at 3rd week.

Table (5): Descriptive statistics for percentages of movement (P.M.) for each contrast's periods at different groups and Comparisons significant

S.O.V.		Mean of P.M.	Std. Dev.	ANOVA		ANOVA	
Periods	Groups			Periods	P ^(*) -value	Groups	P ^(*) -value
1w. & 2w.	Control	59.38	35.33	F=23.90	0.001 HS	F=2.715	0.138 NS
	Lazar	63.64					
	VEGF	129.41					
	Combination	54.43					
2w. & 3w.	Control	80.39	10.2484				
	Lazar	64.81					
	VEGF	57.69					
	Combination	59.84					
1w. & 3w.	Control	187.5	49.74				
	Lazar	169.7					
	VEGF	261.76					
	Combination	146.84					
R Squared = 0.903 (Adjusted R Squared = 0.822)							

(*) HS: Sig. at P<0.05; NS: Non Sig. at P>0.05

Figure 3: Receiver Operating Characteristic (ROC) curve for Clinical Measurements of distance (mm) between MCIs in contrast of control group at different intervals

of ketamine (50 mg/ml) at a dose of 50mg/kg body weight and muscle relaxant Orbarcaine 2% at a dose of 5mg/kg body weight. The two drugs were mixed

at the ratio of 2:1 (Ketamine: Orbarcaine), impression for mandibular central incisors (MCIs) of each rabbit was taken first with silicone material

Table (6): Multiple comparisons by (LSD) among all pairs of contrast's periods for (P.M.) parameter

Parameter	Statistical tests	periods	Periods	Mean Diff.	Sig.	C.S. ^(*)
Contrasts	LSD	1w. & 2w.	2w. & 3w.	11.03	0.604	NS
			1w. & 3w.	-114.74	0.001	HS
		2w. & 3w.	1w. & 3w.	-125.77	0.001	HS

(*) HS: Highly Sig. at $P < 0.01$; NS: Non Sig. at $P > 0.05$

Table 7: Descriptive statistics of Clinical Measurements of distance (mm) between MCIs among studied groups at different intervals with comparisons significant

Marker	Groups	Mean	SD	SE	Student test	
					t-value	P- ^(*) Value
Control	Radiographic	0.917	0.01	0.00	-0.319	0.756
	Clinical	0.918	0.01	0.00		NS
Lazar	Radiographic	1.768	0.03	0.01	-0.592	0.567
	Clinical	1.778	0.03	0.01		NS
VEGF	Radiographic	1.207	0.01	0.00	-4.568	0.551
	Clinical	1.225	0.01	0.00		NS
Combination	Radiographic	1.933	0.10	0.04	-0.221	0.830
	Clinical	1.947	0.11	0.04		NS

(*) HS: Highly Sig. at $P < 0.01$; S: Sig. at $P < 0.05$; NS: Non Sig. at $P > 0.05$

Table 8: Area under the curve (ROC) for Amount of width mandibular suture (mm) in contrast of control group

Area Under the Curve						
Test Result: Amount of width mandibular suture (mm)						
Control with others groups	Area	Std. Error	Asymptotic Sig.	95% C.I.		Sen.
				L.B.	L.B.	
	0.056	0.049	0.001	-0.040	0.151	100
						94.4

2Cutoff Point (0.23)

,study stone models of the MCIs and the surrounding region were made ,which used for preparing of individual resin trays for each rabbit; that then used to take precise final impressions with alginate material and the master stone models. Orthodontic bands were prepared to fit the teeth sizes, using band strips (Dentaurum-Germany) and then welded under pressure by using of Welder device. A round buccal tube with wings was then welded to the hand made bands in a horizontal direction and used as a labial tube. The bands were cemented to its co-related MCIs after the removing of the orthodontic elastic separator so that the superior border of the cemented bands was 3mm away from the incisal edge to allow for wear of the teeth and the lower border about 2mm away from the cervical area to avoid a trauma of the surrounding tissue.

Orthodontictooth movement was generated by the insertion of a stainless steel archwire with diameter of 0.016" and 15 mm in length through the labial tubes and the NiTi open-coil spring(ORTHO. TECHNOLOGY USA) with 3-4 mm in length (about 4-6 coils) was inserted along the archwire from the non-bend end and subjected to constrict pressure with tucker in order to be inserted between the labial tubes, so that it will apply a pushing force on both MCIs (in distal direction) with a total orthodontic force of (100 gm) divided into two teeth so that each incisor receives a light continuous force of (50gm) according to Proffit et al. This force was measured by pressure gauge (CORBLX, Dentarum –Germany). Two coils at both ends of the archwire were made in one plan, and it serves as a stopper for the arch wire and as non-traumatic end. Experimental tooth

movement was conducted for 21 days. The experimental group (A) was received the LLL therapy at 810 nm, with an output of 250 mW, and exposure of 20 s for each 7 days. While the experimental group (B) was received the 0.1µm of VEGF in pressure side and about 0.2 mm subgingivally, for each 7 day.

Experimental group (C) treated with a combination of LLL therapy and 0.1µm VEGF and for each 7 days.

Radiographical Study

An intraoral radiograph will be taken for the three groups before and after application of LLLT and VEGF separately and in combination. It a conventional Mono path size 2 ultra-speed type radiographic dental film.

RESULTS

Result of the Clinical Measurements

The data of the all experimental periods include the experimental tooth movement measurements (cumulative Measurements) and percentage; both of them were analyzed using both Descriptive and Inferential statistics as follows:

Measurements of distance (mm) between MCIs

A bodily tooth movement was observed in all groups. The means of the weekly measurements of the distance between the mandibular central incisors (MCIs) of each group and their comparisons among groups and within group are shown in Table

Figure 4: Receiver Operating Characteristic (ROC) curve Clinical Measurements of distance (mm) between MCIs in contrast of control group at different sources of variation

1 and Figure 1. It was found that there was a gradual increase in the amount of tooth movement from the 1st week. Till the 3rd week as follow:

After 1 week: the mean $\pm$ SD values of separation were increased, and the highest one was (0.79 ± 0.01), in the combination group, then (0.66 ± 0.01 mm) in laser, then (0.34 ± 0.02 mm) in VEGF and the least one was (0.32 ± 0.01 mm) in control.

After 2 weeks: the mean $\pm$ SD values of separation were increased, and the highest one was (1.22 ± 0.01), in the combination group, then (1.08 ± 0.04 mm) in laser, then (0.78 ± 0.01 mm) in VEGF and the least one was (0.51 ± 0.01 mm) in control.

After 3 weeks: the mean $\pm$ SD values of separation were increased, and the highest one was (1.95 ± 0.11), in the combination group, then (1.78 ± 0.03 mm) in laser, then (1.23 ± 0.01 mm) in VEGF and the least one was (0.92 ± 0.01 mm) in control.

Figure 2 illustrates Stem-Leaf Plots for clinical measurements of distance (mm) between MCIs among studied groups at the 3rd week.

By using Receiver Operating Characteristic Curve (ROC Curve) analysis was listed in Table 2 and Figure 3 that show area under the curve (ROC) for clinical measurements of distance (mm) between MCIs in contrast to control group at different periods, for 1 week the area (0.056) with sensitivity (100) and specificity (88.9), while for the 2 & 3 weeks they illustrate area (0.00) with sensitivity (100) and

Figure 6 Photo for Biopsies shows the difference in the distance that remained between MCIs after 3 weeks duration of experiment.

Figure 5: Receiver Operating Characteristic (ROC) curve for Clinical Measurements of distance (mm) between MCIs in contrasts of all pair wise groups at different sources of variation

specificity (100).

Table 3 & Figure 4 record (0.191) area under the curve (ROC) for distance (mm) between MCIs in contrast of control group with other groups.

The area under the curve (ROC) for clinical measurements of distance (mm) between MCIs in contrasts of all pair-wise groups was reported in Table 4 & Figure 5. Wide area (0.66) was recorded

for laser group pairwise VEGF group, with a high 95% Confidence Interval(LB 0.486-UB 0.847).

Percentages of movement (P.M.)

Descriptive statistics for percentages of movement (P.M.) for each contrast's periods at different groups and Comparisons significant was recorded in Table 5. High percentage recorded in **1w. & 3w.** and specifically to group VEGF (**261.76**).

Figure 7 Photo for Biopsies shows the difference in the distance that remained between MCIs after 3 weeks duration of experiment and after removing of orthodontic appliance.

The results shown in Table 6 illustrates that most pairs comparisons of contrasts of periods of (P.M.) factor are accounted highly significant at $P < 0.01$, except between (1w. & 2w. X 2w. & 3w.) at $P > 0.05$.

Figure 6 and 7 are the photographs for biopsies and demonstrate the difference in the distance that remained between MCIs after 3 weeks duration of the experiment.

Radiographic evaluation

At the end of the experiment period (at the end of 3rd week) all the study groups were subjected to x-ray to evaluate the distance that remained between MCIs as shown in Figure 8.

It was notice that, the radiographical measurement results of the distance that remained between MCIs at the end of experiment was coincide with the results of the clinical measurement at the same period, with statistically no significant difference ($P > 0.05$) between the two measurements of each group, as shown in Table 7 and Figure 9.

The Width of the Mandibular Suture (Mand.S)

The highest mean values of the Mand.S width at three points that measured through radiographs at the end of the experiment was in combination group, mean $\pm$ SD was (0.492 $\pm$ 0.04mm), and in laser (0.455 $\pm$ 0.01mm), while VEGF records a low value (0.242 $\pm$ 0.02mm) and control is the least one (0.215 $\pm$ 0.01mm), as shown in Figure 10.

Figure 8 Radiographical images for the study groups show the difference in the distance that remained between MCIs .

Table 8 & Figure 11 illustrate the area (0.056) under the curve (ROC) for the amount of width mandibular suture (mm) in contrast to control group with sensitivity (100) and specificity (94.4).

The Width of the Periodontal Ligament (PDL):

The mean $\pm$ SD values of the width of PDL (means of three points) were shown as follows

Figure 12 illustrates Stem-Leaf Plots for Width of PDL of Right and Left mesial side among studied groups. The highest values was recorded by combination group, M $\pm$ SD for the mesial right side was (0.285 $\pm$ 0.01mm) and for the left side (0.263 $\pm$ 0.02mm).

Figure 13 shows Stem-Leaf Plots for Width of PDL of Right and Left distal side among studied groups. The highest values were recorded by combination and laser groups, M $\pm$ SD for the mesial right side was (0.222 $\pm$ 0.02mm) that equal for both groups ,while for the left side, laser group records a high value

Figure 9: Cluster Bar Chart concerning Mean values for Clinical Measurements of distance (mm) between MCIs among studied groups at the end of third weeks

($0.232 \pm 0.01 \text{ mm}$), followed by combination ($0.227 \pm 0.01 \text{ mm}$).

Table 9 and Figure 14 show Area under the curve (ROC) for Width of PDL of Right and Left Mesial Side and Distal Side in contrast to control group at different intervals.

For right mesial side the area was (0.116) with sensitivity (100) and specificity (61.1), and for the left mesial side, the area was (0.199) with sensitivity (100) and specificity (50).

For right distal side the area was (0.287) with sensitivity (100) and specificity (44.4), and for the left distal side, the area was (0.199) with sensitivity (100) and specificity (55.6).

DISCUSSION

Low-Level Laser Therapy

The use of LLLT in present study depending on several in vitro studies, with adequate blinding of the observers, which have demonstrated that the effects of laser light are much greater than obtained with light from other sources, such as LEDs. (14) (15) treated three groups of patients with long-standing crural ulcers with a He-Ne laser, a combination of He-Ne and GaAs lasers, and non-coherent unpolarized red light. The two laser groups demonstrated excellent healing, while only a small percentage healing response was seen in the normal red light group.

LLLTh Equipment

Semiconductor diode lasers are compact and have high conversion efficiency from electrical energy to laser energy. Unlike He-Ne lasers, semiconductor laser diodes do not require a high voltage supply, and so can be used in portable,

Amount of width of mandibular suture (mm)

Figure 10: Stem-Leaf Plots for Amount of width mandibular suture (mm) among studied groups

battery-operated devices. It is also possible to pulse the light at various frequencies using simple external circuitry. Laser diodes have a typical life-expectancy of between 100,000 and 600,000 hours¹⁶.

The Voltage Regulator Device

The voltage regulator device was used in the present study to ensure of a stable electrical supply to the laser device since the intensity (power-P) is depend on voltage (**V**) and current (**I**) and directly proportional to them as $P = VI$

The Intensity and Duration of the LLLTherapy

In the present study, the conventional therapeutic laser device was adapted to exert effects on cells; interest has been concentrated on using lasers as a light source because of their greater therapeutic effect. Nowadays most LLLT clinical

Figure 11: Receiver Operating Characteristic (ROC) curve for Amount of width mandibular suture (mm) in contrast of control group

Width of PDL of Right and Left Mesial & Distal Side

Figure 12: Stem-Leaf Plots for Width of PDL of Right and Left Mesial Side among studied groups

procedures are undertaken using semiconductor diode lasers operating at 830 nm wavelengths. Since wavelength is the most important factor in any type of phototherapy, the clinician must consider which wavelengths are capable of producing the desired effects within living tissues. In our study LLLT with 800 nm wavelengths was used as the effects of different types of light on mast cells were well recognized. There is direct evidence that 660, 820, and 940 nm light can trigger mast cell degranulation. Mast cells are distributed preferentially about the microvascular endothelium in oral mucosa, dental pulp and in periodontium¹⁷. Mast cells in these locations contain the pro-inflammatory cytokine tumor necrosis factor in their granules. Release of this cytokine promotes leukocyte infiltration of tissues by enhancing expression of endothelial-leukocyte adhesion molecules. In addition, mast cell proteases, such as chymase, alter basement membranes and facilitate entry of leukocytes into tissues¹⁸. Because mast cells play a pivotal role in controlling leukocyte traffic, modulation of mast cell functions by LLLT can be of considerable importance

factor to cause modulation effects in many processes related to the oral cavity.

In present research the typical power output of a low-level laser device used for this therapy is in the order of 10-50 milliWatts, and total irradiances at any point are in the order of several Joules, in addition to estimate that thermal effects of LLLT on dental tissues will be not significant in order to have a biologic response with little or no tissue damage.

In the present study the laser probe was held perpendicular for 20 seconds in direct contact with the alveolar mucosa on each five points, in order to allow LLL penetrate soft and hard tissues from 3 mm to up to 15 mm. As the energy penetrates tissues, there is multiple scattering by both erythrocytes and microvessels. Because of this, both blood rheology and the distribution of microvessels in the tissue influence the final distribution pattern of laser energy¹⁹.

Vascular endothelial growth factor therapy

On the base of updated studies about the correlation between tooth movement and

Figure 13 :

inflammatory process. That, in concert with the mechanical responses of periodontal and oral tissues, which is essential for achieving tooth movement clinically²⁰.

Early effects of orthodontic forces are involved physical and biological response that includes extracellular matrix and cells of the periodontal ligament, blood vessels, neural and alveolar bone. As a consequence, many changes occur in these structures and various molecules are produced or induced, as well as cytokines, growth factors, colony-stimulating factors, and neurotransmitters²¹. The first stages before the orthodontic tooth movement are characterized by an acute inflammatory that appeared in the periodontium and involves the vasodilatation of capillaries which allows the migration of leucocytes in the periodontal tissue, where they are induced by biochemical signals to synthesize and to secrete several proinflammatory cytokines and chemokines, growth factors and enzymes²². Therefore, and up to above knowledge the present research was designed to use experimental group with orthodontic appliance that received 0.1µm of VEGF

in working side as VEGF is a biologically active substance that is expressed by cells within the periodontium in response to mechanical stimuli from orthodontic appliances with several possible of other biomarkers representing biological modifications during specific phenomena of inflammatory process²³.

The Clinical Measurement Results

Tooth Movement

In the present study by observing the results of the clinical measurements of the cumulative amount of tooth movement (teeth separation) in the four studied groups, it was obvious that combination group shows high records followed by laser group then VEGF and this may explained on the base of the followings:

1. Combination group received LLLT and VEGF, as each one has it's significant effects.
2. LLLT has been shown to cause vaso-dilation, with increased local blood flow, that brings in oxygen and allows for greater traffic of immune cells into tissue, followed by enhancement of remodeling processes that are need in tooth movement²⁴.

3. Vascular endothelial growth factor (VEGF) is the most important essential mediator of angiogenesis. Besides the stimulation of angiogenesis, VEGF has also been demonstrated to be involved in early hematopoietic development and chemotaxis of monocytes, which are essential cells in bone remodelling and then in tooth movement²⁴.

The present result also found that there was a gradual increase in the amount of tooth movement from the 1st week. Till the 3rd week and it could be explained according to²⁵ who reported that after 24h of orthodontic force loading, the periodontal space appeared considerably expanded. The periodontal fibres were stretched between the bone and the root. Three days after loading, the expanded periodontal space had slightly narrowed, the periodontal fibre arrangement was relaxed, and the blood vessels did not appear elongated. A considerable layer of osteoid was formed on the bone surface, and gradual tooth movement will record.

Radiographical Findings at the End of Experiment

Distance assessment between MCIs

In the present study the radiographic findings of teeth separation in the experimental and control groups, was used as support to the clinical findings of teeth separation that remained between MCIs at the last day of the experiment, as the radiographical and clinical measurements of the distance between MCIs for the same group was insignificant differed and it may be suggested for periodic records that are necessary for the description of tooth movement.

Width of the Mandibular Suture

In the present study the result of the radiographical measurement of the means of the three points (crestal, middle, and apical) for the width of the Mand.S revealed a high record for combination group followed by laser group then VEGF. These results could be attributed to the influence of LLLT on the biological function of a variety of cell types, that it is able to exert a range of several beneficial effects upon mandibular bone tissue²⁶, plus the angiogenesis effect of VEGF.

Width of the PDL

In this study, the highest value was recorded by combination group for the mesial right & left sides,

while laser group recorded the highest value for the distal right & left sides. Low-Level Laser (LLL) has been shown to have a positive effect on bone formation and the vasculature²⁷.

Assess of Tooth Movement (TM) and Bone Remodeling Histologically

The histological findings support and describe the clinical finding on the bases of science. The assessment of the effect of the LLLT and VEGF on the bone remodelling in the present study depends on analysis its effect on different histological parameters include osteoblasts, osteoclasts, and the blood vessels numbers.

Generally, combination group appear to be most effective and records a high value in all followings parameters in comparison to other groups.

Osteoblast cells number (Ob.)

By observing the result in the present study of the number of the Ob. Cells at different sides (pressure, and tension sides) it was noticed that its numbers were higher (active proliferation) in the experimental groups as compared to the control groups with a significant difference, this result may be related to the following explanations:

1. The effects of low-level laser therapy (LLLT) has been assumed responsible for promoting photostimulatory and photobiomodulatory effects, increasing cell metabolism, improving cell regeneration and invoking an anti-inflammatory response. A positive effect of LLLT on the bone proliferation of some cell types has been observed in many studies that coincide with our findings²⁸⁻³⁰.
2. The results indicated that exogenous VEGF acts as conditioned medium for inflammatory cytokine-activated stromal cells that can significantly promote osteoblast proliferation, migration, differentiation and ultimately enhance osteogenesis, depends and coincides with findings that were done by³¹⁻³³. Other current studies indicates a synergistic effect of VEGF and highlights the great potential of dual growth factors that delivery modality for regeneration of vascularized bone³⁴⁻³⁵.
3. Osteogenesis and angiogenesis are two closely correlated processes during bone growth, development, remodelling and repair. Vascular endothelial growth factor (VEGF) is an essential mediator of the process of angiogenesis³⁶.

Osteoclast cells number (OCL.)

By observing the results in the present study of the number of the OCL. A cell at different sides (pressure, and tension sides). It was noticed that its number was high in the experimental groups as compared to the control group with a significant difference, this result may be explained as follows:

1. The ability of the LLLT to effect directly or indirectly on the balance of OPG and RANKL, on which the osteoclastogenesis depends. Yamaguchi, M et al., 2009 mentioned that when the balance is inclined towards OPG, there will be fewer active osteoclasts; while when inclined towards RANKL, there will be more active osteoclasts¹⁸.
2. Brooks, P et al., (2009) results indicate that LLLT may reduce the relapse tendency, possibly due in part to bone formation in previous tension areas, and to redistribution of osteoclasts following removal of orthodontic force¹⁹.
3. The unique role of VEGF in osteoclastogenesis, Parker, S et al.,(2007) showed that Vascular endothelial growth factor (VEGF) has angiogenic, inflammatory, and bone-destructive roles in rheumatoid arthritis by activated of nuclear factor κ B ligand (RANKL) expression was determined in synovial fibroblasts³⁷.

Blood Vessels number (B.V)

Results analysis of this study for blood vessel count, show a high value in it's mean in combination group, followed by VEGF group, it was also noticed that, its number was higher in the experimental groups as compared to the control group and with a significant difference, the increase in the number of B.V in the experimental groups may be explained by the ability of the exogenous VEGF to effect on the stimulation of angiogenesis which is responsible for blood vessels formation . The use of laser radiation was associated with a statistically significant increase in proliferation of endothelial cells according to Tunér J, *et al.*,(2004)⁴⁰. In addition to LLLT that has been shown to influence directly on the number of cells associated with the repair process, and that is mediated by the increase in new blood vessel formation^{41, 42}.

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