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"THE EFFECTS OF LOW LEVEL LASER THERAPY ON HEALING OF GINGIVA AFTER GINGIVECTOMY- AN IN VIVO STUDY"

Original Research Paper

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ABSTRACT: Aim: Healing involves interplay of cellular elements, cytokines and growth factors. It is a complex phenomenon which has been extensively researched. Low level laser therapy has been used as an adjunct to hasten healing in numerous clinical studies. It entails application of low level laser energy to increase the rate of epithelization, cell proliferation and migration. The present study aims at assessing the in-vivo healing after application of low level laser therapy.

Materials and Methods: The present study is a randomized controlled clinical trial with a split mouth study design in which a total of 15 subjects with bilateral gingival enlargement were selected. The selected sites (30 sites) were then randomly divided into two groups namely- the test group and the control group. The test group (15 sites) were irradiated with 940nm laser following gingivectomy on day 0, day3, day7,and day14 whereas the control group (15 sites) received placebo. The healing was assessed by applying Mira-2 tone dye on day 0, day3, day7, day14, day21 and day 30 and the dye uptake was observed by 3 observers.

Results: The laser irradiated site healed earlier than the contra lateral side, indicating that laser application accelerates healing in gingivectomy wounds. The sites treated with LLLT showed statistically significant results in terms of pain perception and staining of the tissues post-surgery.

Conclusion: Within the limits of this study and based of the clinical parameters assessed, it can be concluded that low level lasers can be successfully used as an adjunct to enhance the healing post gingivectomy.

Keywords:
Lasers, Gingivectomy, Healing

Source of support: Nil
Conflict of interest: None

INTRODUCTION: A major goal of periodontal therapy is to re-establish anatomical and physiological conditions conducive to long term health and function of the periodontium. The progression of the disease is mainly dependent on the immune and inflammatory responses of the individual.

Gingival hyperplasia is a rather common gingival condition with varied etiology and pathogenic process e.g. Bacterial plaque, mouth breathing, scurvy, hormonal disturbances, medications and neoplasms etc.[1] Gingivectomy or gingivoplasty is the procedure utilized for the elimination of suprabony periodontal pockets or pockets not extending beyond the mucogingival junction. It is also recommended for removal of diseased tissue, for prosthetic reasons, to improve aesthetics and/or establish normal gingival architecture, and to reduce probing depth of periodontal pockets[2]. Several techniques have been used to perform gingivectomy namely using scalpel, electrocautery, lasers, chemicals, burs etc.

The wound healing process after gingivectomy is by secondary intention which takes about 5 weeks to re-establish normal gingival epithelization thereby indicating that healing in this case is a slow process[3]. In order to accelerate healing several studies have shown improved healing of wounds by topical application of medicaments, antibiotics, or amino acids[4].

Lasers have now been used in modern dentistry for the past 30 years and their varied applications have made them an important adjunct in periodontal therapy. They have been reported to reduce bleeding, resulting in minimal post-operative discomfort, and reduce the need for sutures. A wide

range namely diode, CO2, Nd:YAG, Er:YAG lasers are used for soft and hard tissue ablation, detoxification of root surfaces, pocket debridement, bacterial elimination.

Despite the common use of these high power surgical lasers, when used in low intensity; are called low level lasers (LLL). These work in the mill watt range with wavelengths in the red or near infrared spectrum (400-900nm) [5]. The basic principle of Low Level Lasers is based on the biostimulation or the biomodulation effect[6], which consists of the fact that irradiation at a specific wavelength is able to alter cellular behavior[7]. which is achieved through mitotic activity induction of the epithelial cells, modification of capillary density, stimulation of the local microcirculation, and increase of the invitro and in vivo collagen synthesis[8].

Low level laser therapy (LLLT) has been used for promoting wound healing and reducing pain after gingivectomy[9], endodontic surgery[10] orthodontic treatment[11] and as an adjunct after non-surgical periodontal therapy.

The increasing application of laser technology and more specifically Low Level Laser Therapy in Periodontics will require a greater number of human clinical trials to completely establish its accelerated healing property. Thus, the present study was carried out to evaluate the effect of Low Level Laser Therapy on in vivo gingival wound healing in patients following gingivectomy/ gingivoplasty and to compare the healing obtained as an adjunctive therapeutic technique for healing against the natural healing of gingival tissues.

MATERIALS AND METHODS: A randomized controlled clinical trial with a split mouth design study was performed for the in vivo evaluation of the effect of low level laser therapy on healing of gingiva following gingivectomy and gingivoplasty.

A total of 15 patients of either sex, aged between 18-45 years were selected from Outpatient Department of Department of Periodontics.

The patients were selected with the inclusion criteria: 1) Patients with symmetrical gingival overgrowth on the maxillary or mandibular anterior region with at least 6 teeth affected, 2) Patients who were cooperative and committed to maintain oral hygiene and 3) Patients with no contraindication to periodontal surgery.

The exclusion criteria were: 1) Current smokers, tobacco and betel nut chewers, 2) Patients suffering from chronic systemic illness like diabetes; or compromised immunity or nutritional deficiencies, 3) Patients taking agents or medication that may alter gingival contour, 4) Patients who have undergone any periodontal treatment or antibiotics within the preceding 6 month and 5) Known allergy or hypersensitivity to any product used in the study.

A total of 30 sites from 15 patients with symmetrical gingival overgrowth were selected. The sites were then randomly divided into 2 groups namely: The test group and the control group.

CONTROL GROUP consisted of a total 15 sites that were subjected to placebo (the laser was applied on the site but the ignition point was not pressed) while the TEST GROUP comprised a total number of 15 sites that received Low Level

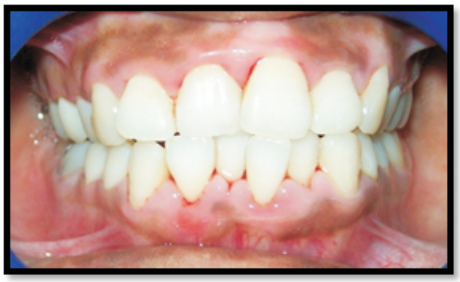
Laser Therapy:

The control group and the Test group were patient-blinded, placebo-controlled and split-mouth design. Three independent examiners were selected for the purpose of assessment of the parameters who were also blinded.

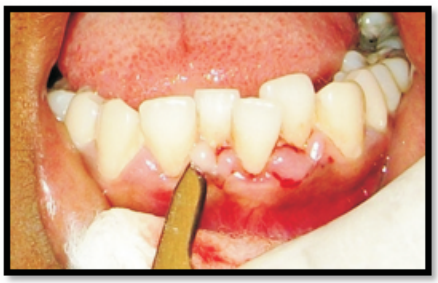
The study protocol was reviewed and approved by the Institutional Ethical Committee. Informed written consent was obtained from all the subjects. All patients received oral hygiene instructions and scaling and root planning if required. After 3 weeks, the physiological gingival contour was re-evaluated for gingivectomy or gingivoplasty.

Procedure:

During gingivectomy, a scalloped external bevel incision was made using Kirkland knife and a # 15 blade. Then, a sulcular incision was performed and interproximal tissue was released with the help of Orban's knife. Following excision of the enlarged tissue with curettes, gingivoplasty was performed using periodontal knives and Castroviezo Scissors.

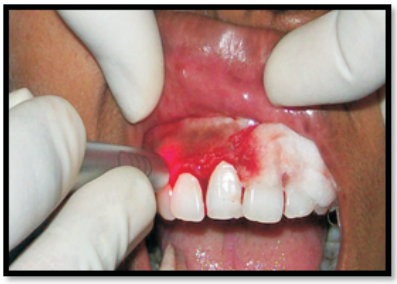


Pre -Operative View

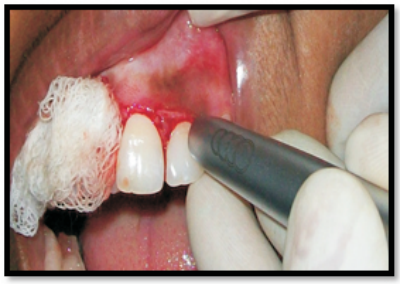


External Bevel Incision To Remove The Enlarged Gingiva

After this procedure, one of the symmetrical surgical sites was randomly assigned to receive LLLT. After haemostasis, LLLT was applied on one side of the surgical area (test site) in non- contact and continuous mode for 4 minutes whereas the adjacent symmetrical area (control site) did not receive laser therapy as it was placebo (the laser was applied on the site but the ignition point was not pressed.



Low Level Laser Being Applied On The Test Site On Day 0



Placebo Being Given On The Control Site On Day 0

Diode laser (Ezlaze, Biolaze) of 940nm was used for 4 minutes at 120mW delivering 4J/cm2, with pulse interval of 50 milliseconds and pulse length of 10 milliseconds energy.

Diode Laser Used In The Study

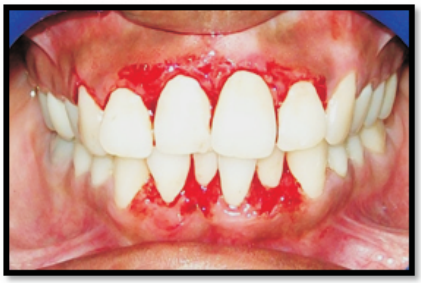
Once the LLLT was administered for the assessment of Visibility, the surgical area was disclosed with Mira-2-tone dye (HAGER & WERKEN GmbH & Co. KG, Germany) (disclosing solution) to better visualise the areas in which the gingival epithelium is absent, abraded or lacking sufficient keratinization and to distinguish areas from normal gingiva12

Mira-2-tone Dye Used In The Study

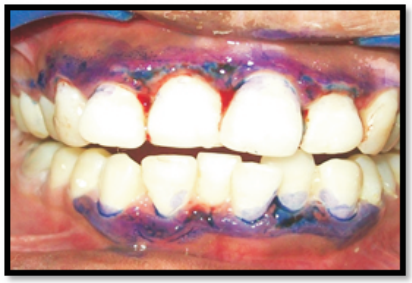
The darkly stained fields were considered as sites still undergoing wound healing with the lack of enough layers of epithelium.13 Then a Coe – pak dressing was applied in the surgically treated area.

This was followed by pain assessment procedure which was analysed only after the local anaesthesia effect had subsidized by using digital pressure over the lips on the surgically treated area. The pain experience on each of these test days was assessed by Visual Analogue Scale.[14]

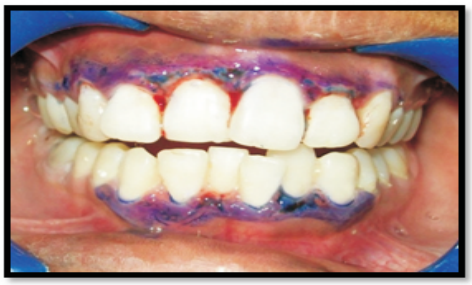
Surface area of stained fields on the LLLT applied sites and the controls were measured on the 3rd, 7th, 14th and 21stday and were then compared by statistical analysis. Laser application was repeated on the 3rd, 7th, and 14th day, and on these days Visibility as well as Pain assessment were observed. After each LLLT application, the surgical area was disclosed with Mira-2-tone dye.



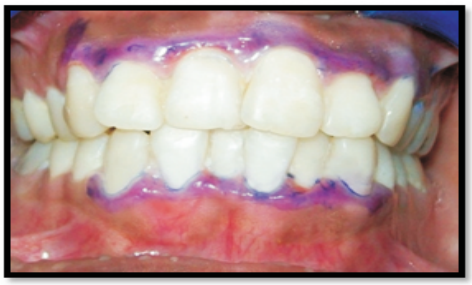
Surgical Sites After Low Level Laser Therapy



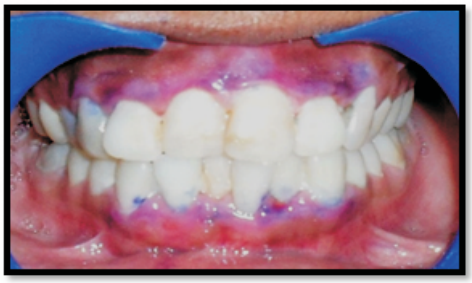
Dye Uptake At Day 0



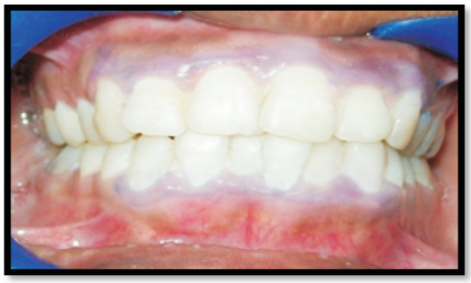
Dye Uptake On Day 3



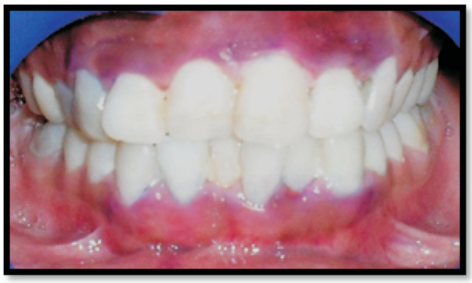
Dye Uptake On Day 7



Dye Uptake On Day 14



Dye Uptake On Day 21



Dye Uptake On Day 30

A follow up photograph was taken on day 30.

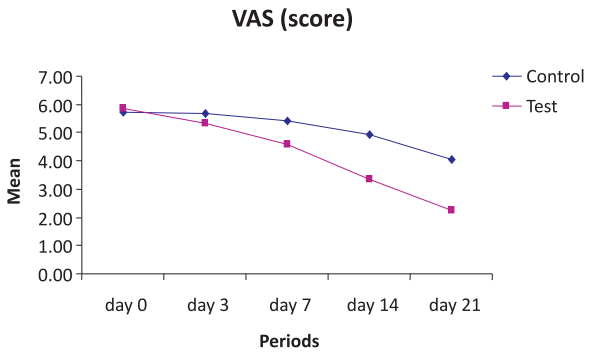
Statistical Analysis: Continuous data were summarized as Mean ± SD while discrete (categorical) in %. Continuous groups were compared by two factor (Groups X Periods) repeated measures analysis of variance (ANOVA) using general linear models (GLM) and the significance of mean difference within and between the groups was done by Tukey HSD (honestly significance difference) post hoc test after ascertaining the normality by Shapiro-Wilk test and the homogeneity of variance by Levene's test. Categorical groups were compared by chi-square (χ^2) test. A two-sided (α) $p < 0.05$ was considered statistically significant. All analyses were performed on SPSS (Windows version 15.0).

RESULTS: A. VISUAL ANALOGUE SCALE (VAS)

The pre day (0) and post treatments day (3, 7, 14 and 21) of two groups (Control and Test) are summarized in Table 1 and also shown graphically in Fig .1 . In both the groups, the mean VAS score (pain) decreases (improves) after the treatments and the decrease (improvement) was evident higher in Test group as compared to Control group .

Table 1 :Pre and post treatments VAS scores (Mean ± SD , n =15)of two groups

Groups	day 0	day 3	day 7	day 14	day 21	% change (day 30-day 0)
Control	5.73 ± 1.22 (3-7)	5.67 ± 1.18 (3-7)	5.40 ± 1.30 (2-7)	4.93 ± 1.39 (2-6)	4.07 ± 1.22 (1-5)	29.1%
Test	5.87± 1.25 (3-7)	5.33 ± 1.35 (3-7)	4.60 ± 1.18 (3-6)	3.33 ± 1.18 (2-5)	2.27 ± 1.03 (0-4)	61.4%



Comparing the mean VAS scores of two groups over the

periods, ANOVA revealed significantly different VAS between the Groups (F=4.84, p=0.036) and between the Periods (F=86.70, p<0.001). The interaction effect of both (Groups X Periods) on VAS was also found to be significant (F=12.68, p<0.001).

Table 2: ANOVA summary- VAS (score)

Source of variation (SV)	Sum of square (SS)	Degree of freedom (DF)	Mean square (MS)	Fvalue	Pvalue
Groups	29.04	1	29.04	4.84	0.036
Error	168.00	28	6.00	-	-
Periods	138.31	4	34.58	86.70	p<0.001
Groups x Periods	20.23	4	5.06	12.68	p<0.001
Error	44.67	112	0.40	-	-

Further, for each group, comparing the mean VAS within the groups (i.e. between periods) (Table 3 and Fig. 2), Tukey test revealed significantly (p<0.05 or p<0.001) different and lower VAS in Control group at day 14 and day 21 as compared to day 0. Further, in Control group, the mean VAS also lowered significantly (p<0.05 or p<0.001) at day 21 as compared to day 3 and day 7. In contrast, in Test group, the mean VAS lowered significantly (p<0.001) at day 7, day 14 and day 21 as compared to day 0 and day 3. Further, in Test group, the mean VAS also lowered significantly (p<0.001) at day 14 and day 21 as compared to day 7. Furthermore, in Test group, the mean VAS also lowered significantly (p<0.01) at day 21 as compared to day [14].

Table 3: For each group, significance (p value) of mean difference of VAS scores within the groups (i.e. between periods) by Tukey test

Comparisons	Control	Test
day 0 vs. day 3	1.000	0.389
day 0 vs. day 7	0.910	p<0.001
day 0 vs. day 14	0.025	p<0.001
day 0 vs. day 21	p<0.001	p<0.001
day 3 vs. day 7	0.977	0.057
day 3 vs. day 14	0.057	p<0.001
day 3 vs. day 21	p<0.001	p<0.001
day 7 vs. day 14	0.584	p<0.001
day 7 vs. day 21	p<0.001	p<0.001
day 14 vs. day 21	0.010	0.001

nsp>0.05 or *p<0.05 or ***p<0.001- as compared to day 0

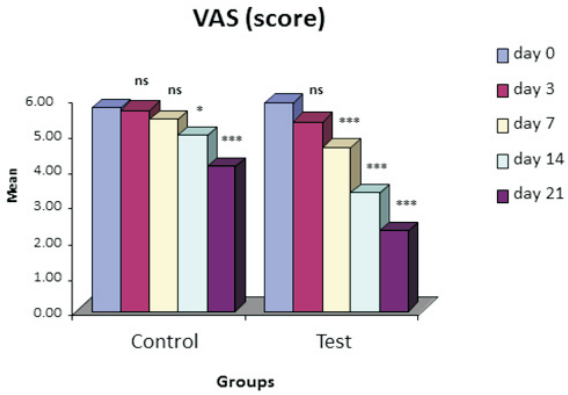


Fig. 2. For each group, mean VAS scores between the periods (i.e. within groups)

Similarly, for each period, comparing the mean VAS between the groups (Table 4 and Fig. 3), Tukey test revealed significantly (p<0.05 or p<0.01) different and lower VAS at day 14 and day 21 in Test group as compared to Control group. Further, at final evaluation (i.e. at the end of the treatments), the pain in Test group (61.4%) improved 2.1 (32.3%) fold more as compared to Control group (29.1%).

Table 4: For each period, significance (p value) of mean difference of VAS scores between the groups (i.e. between periods) by Tukey test

Comparisons	Control vs. Test
day 0	1.000
day 3	0.999
day 7	0.745
day 14	0.028
day 21	0.008

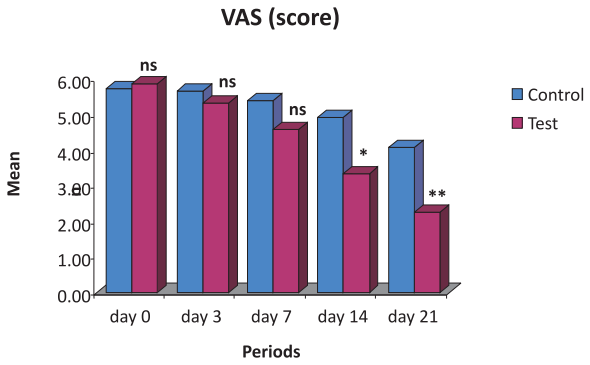


Fig. 3. For each period, mean VAS scores between the groups.

B. Visibility:

The pre and post treatments visibility scores (rated by three observers) of two groups are summarized in Table 5 and also shown graphically in Fig. 4. Like VAS, the visibility in both groups also improves after the treatments and improvement was evident further higher in Test group as compared to Control group.

Table 5: Pre and post treatments visibility scores of two groups

Periods	Control (n=45)	Test (n=45)	χ^2 value (DF=1)	p value
day 0	11 (24.4%)	9 (20.0%)	0.26	0.612
day 3	13 (28.9%)	15 (33.3%)	0.21	0.649
day 7	16 (35.6%)	21 (46.7%)	1.15	0.284
day 14	19 (42.2%)	29 (64.4%)	4.46	0.035
day 21	24 (53.3%)	37 (82.2%)	8.60	0.003
% change (day 21-day 0)	13 (28.9%)	28 (62.2%)	10.08	0.002

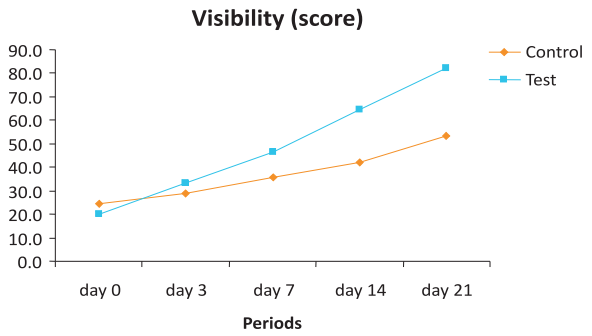


Fig. 4. Visibility scores of two groups over the periods.

For each group, comparing the Visibility scores within the groups (i.e. between periods) (Table 6 and Fig. 5), $\chi 2$ test revealed significantly ($p<0.05$ or $p<0.01$) different and higher Visibility in Control group at day 21 as compared to day 0 and day 3. In contrast, in Test group, the significant ($p<0.01$ or $p<0.001$) improvement in Visibility was evident at day 7 as compared to day 0.

Table 6: For each group, significance (p value) of visibility scores within the groups (i.e. between periods) by 2 tes

Comparisons	Control		Test	
	χ^2 value (DF=1)	p value	χ^2 value (DF=1)	p value
day 0 vs. day 3	0.23	0.634	2.05	0.153
day 0 vs. day 7	1.32	0.250	7.20	0.007
day 0 vs. day 14	3.20	0.074	18.22	p<0.001
day 0 vs. day 21	7.90	0.005	34.86	p<0.001
day 3 vs. day 7	0.46	0.499	1.67	0.197
day 3 vs. day 14	1.75	0.186	8.72	0.003
day 3 vs. day 21	5.55	0.018	22.04	p<0.001
day 7 vs. day 14	0.42	0.517	2.88	0.090
day 7 vs. day 21	2.88	0.090	12.41	p<0.001
day 14 vs. day 21	1.11	0.291	3.64	0.057

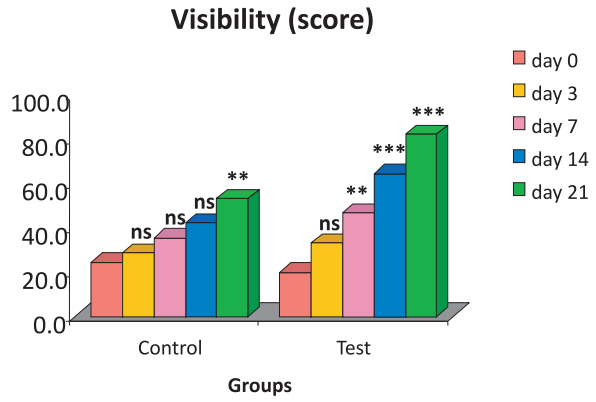


Fig. 5. For each group, visibility scoresbetween the periods (within groups).

Similarly, for each period, comparing the Visibility scores between the groups (Table 5 and Fig. 6), $\chi 2$ test revealed significantly ($p<0.05$ or $p<0.01$) different and higher Visibility in Test group at day 14 and day 21 as compared to Control group. At final evaluation (i.e. at the end of the treatments) the Visibility in Test group (82.2%) improved 2.2 (3.3%) fold more as compared to Control group (28.9%).

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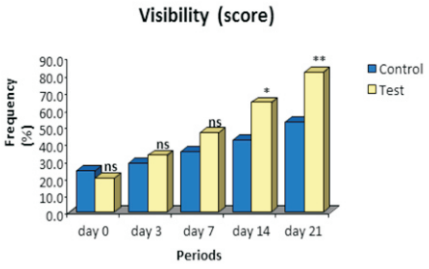

DISCU 1 in the elimination of suprabony periodontal pockets or pockets not extending beyond mucogingival junction. It may also be indicated to remove diseased tissue, for prosthetic reasons, to improve aesthetics and/or establish normal gingival architecture, and to reduce probing depth of periodontal pockets.2

The wound healing process after gingivectomy is by secondary intention and takes about 5 weeks to establish normal gingival epithelization3. Several studies have shown that the topical application of medicaments, antibiotics, or amino acids have resulted in improved wound healing of wounds by secondary intention.

Healing of periodontal tissue after surgical treatment has long been a subject of study. In this clinical trial, post-gingivectomy wounds were assessed over a number of days to clarify whether laser treatment could or could not improve the healing process and post-surgical patient comfort.

Soft tissue surgery using a variety of lasers has been reported8. The differences between the various types of laser beams produced are determined by their wavelengths: the shorter the wavelength, the greater its action and power of penetration. Additionally, lasers may be continuous or pulsed, and their potency is expressed in Watts (W),varying from deciwatts to megawatts. Energy is expressed in Joules per square centimetre (J/cm2), and therefore is equal to the potency multiplied by the duration of application[15]

Low level laser therapy (LLLT) is a light source treatment that generates light of a single wavelength. LLLT emits no heat, sound, or vibration. Instead of producing a thermal effect, LLLT may act via nonthermal or photochemical reactions in the cells, also referred to as photobiology or biostimulation[16].

The important factor in the effectiveness of low level laser therapy include dose, wavelength and the amount of energy applied17. Most experimental and clinical studies on LLLT were performed using semi-conductor diode lasers with wavelengths in the range 635-830nm. It has been suggested that the best LLLT-induced cellular mitochondrial respiratory chain activation, and subsequent fibroblastic activity, occurs between 562 and 600nm18. Many researchers using different parameters have investigated the effect of LLLT on wound healing. However, Almeida-Lopes et al have shown that lasers of equal power output present a similar biologic effect i.e., cell growth independently of their wavelengths.

There have been different concepts to explain the effect of laser radiation on the biological system. A number of LLLT mechanisms that could improve wound healing have been postulated, and they include ATP synthesis, fibroblast proliferation, collagen synthesis, phagocytosis of macrophages, and acceleration of the inflammatory phase of wound healing. All these mechanisms can result in cellular proliferation and acceleration of the wound healing process.[3]

Braun A (2010) did a study, to assess the subjective intensities of pain during supportive periodontal treatment using a sonic scaler or an Er:YAG laser using Visual Analogue Scale.[19]

The usage of the Mira-2-tone dye (HAGER & WERKEN GmbH & Co. KG, Germany) after each LLLT application to better visualise the areas in which the gingival epithelium is absent, abraded or lacking sufficient keratinization and to distinguish areas from normal gingiva has been suggested as an effective tool for the identification of areas lacking epithelium20 and was in accordance with the study performed by Demir T(2010). He also used the same dye for detecting areas of gingival abrasion.[21]

The pain experience on each of these test days was also assessed by the Visual Analogue Scale which is a numeric rating scale having a 10 cm line with “no” at one end, “worst imaginable” at the other end. This method makes it possible to quantify pain.[22]

The preoperatively (day 0) and postoperatively i.e. (day 3, day 7, day 14 and day 21) scoring was performed ,VAS scores (pain) of two groups (Control and Test) showed statistically significant result in Test group as compared to Control group. The % of improvement from day 0 to day 21 was 29.1% for the control and 61.4% for the test group.

The mean VAS score in between the appointments of laser application was also significantly lower in the control group ($p<0.05$ or $p<0.001$). In Test group, the mean VAS lowered significantly ($p<0.001$) at day 14 and day 21 as compared to day 7. Furthermore, In Test group, the mean VAS lowered significantly ($p<0.01$) at day 21 as compared to day [14].

Like VAS, visibility in both groups also improved after the treatments and improvement was evident further higher in Test group as compared to Control group. For each group, comparing the visibility scores within the groups (i.e. between periods), $\chi 2$ test revealed statistically significant ($p<0.05$ or $p<0.01$) difference in Control group at day 21 as compared to day 0 and day 3. In contrast, in Test group, the significant ($p<0.01$ or $p<0.001$) improvement in lightly stained was evident at day 7 as compared to day 0.

At final evaluation (i.e. at the end of the treatments), there was significantly lower staining of the tissues in the Test group (62.2%) as compared to Control group (28.9%).

Stahl et al (1968) & Ramfjord, S.P. (1991); The former stated that improved visibility accounts for the wound healing and during this period, cytokines and growth hormones expressed by immune cells such as neutrophils and macrophages orchestrate the wound healing process ,while the latter stated that after gingivectomy, collagen formation and a better gingival tissue organization occur gradually as the inflammation and the vascularity of the granulation tissue decrease. Collagen production of the granulation tissue occurs after fibroblast proliferation, which originates locally around the vascular, bone and lamina propria area2.

Acceleration of the wound healing process by laser can be explained by the higher collagen synthesis in the vascular and fibroblast proliferation on the connective tissue coupled with higher mitotic activity in the epithelial cells.[23]

LLLT improves the quality of histologic repair and is useful during wound healing. However, in this study the laser energy

did not minimize tissue inflammatory reactions [24].

A study conducted by Ozcelik et al 2008 had similar parameters as the present study patients with inflammatory gingival hyperplasia on their symmetrical teeth with bilateral involvement. A diode laser was randomly applied to one side of the operation area for 7 days daily while in our study LLLT was given on 3rd,7th,14thand 21stday. In both the studies surgical areas were disclosed by a solution (Mira-2-tones) to visualize the areas in which the epithelium is absent, and the results were similar and showed that LLLT enhances wound healing.[23]

LLLT may accelerate wound healing by increasing the motility of human keratinocytes and promoting early epithelization, increasing the fibroblast proliferation and matrix synthesis and by improving neovascularization. It has also been shown that the expression of fibroblast growth factor by macrophages and fibroblasts is increased by LLLT application. (Tuby et al 2006).This study was in accordance with study conducted by Donos et al 2005 who stated that LLLT application also increases the revascularization rate.

The healing period of laser therapy is slower than a scalpel wound but still laser wound is a sterile inflammatory reaction which do not require much of post-operative care.[17]

The results of this study are in accord with studies by Amorim et al where LLLT significantly promoted gingivectomy wound healing who utilized subjective criteria of improved healing. Similar results were also observed by Damante et al where laser was applied at 48hr intervals for four sessions. It has been shown that there is an acceleration of the wound healing process of experimental wounds in hard palate mucosa of mice at low level laser therapy with a He-Ne laser at energy densities of 3 and 7.5 J/cm2.[23]

After gingivectomy, collagen formation and a better gingival tissue organization occur gradually within 3–4 weeks as the inflammation and the vascularity of the granulation tissue decrease, even if the gingival surface appears to be completely healed clinically 2–3 weeks after the surgical procedure. Collagen production on the granulation tissue occurs after fibroblast proliferation, which originates locally around the vascular, bone, and lamina propria areas.2So there was no or very less uptake of dye on day [30].

This study had a few methodological limitations as the small sample size may affect the reproducibility of the result, thus, larger sample sizes are required to confirm the findings of the study and since the study was confined to a single wavelength use during low level laser therapy, so several wavelengths could be explored to deliver low level laser therapy.

CONCLUSION: Within the limits of this study and based of the clinical parameters assessed, it can be concluded that the encouraging results of the low level laser application on gingivectomy wounds emphasize its use as an adjuvant element for surgical periodontal treatment within the investigated parameters. Improved surface epithelization and pain compliance has been observed in the gingivectomy wounds with laser as an adjunct. However, a better understanding of this novel approach with larger sample sizes and variable irradiation regimen are needed to optimize the use of LLLT after periodontal surgery.

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