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ORIGINAL ARTICLE

Comparative Evaluation of Clinical Efficacy of Diode LASER and Cryosurgery for Gingival Pigmentation: A Split-Mouth Randomized Clinical Study

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ABSTRACT

Objective: To compare and evaluate the clinical efficacy of diode laser and cryosurgery for treating melanin pigmentation of gingiva. **Material and Methods:** A total of twenty-five subjects with physiological gingival pigmentation on the facial aspect of both maxillary and mandibular anterior arches (50 sites), both male and female, with an average age ranging from 18-35 years, participated in the study. The sites were randomly divided into Group I: depigmentation by Laser and Group II: depigmentation by Cryosurgery. The following parameters were assessed for the evaluation of treatment results: Melanin Oral Pigmentation Index (PI), Visual Analogue Scale (VAS) for pain evaluation and Healing index (HI). The data collected was statistically evaluated. **Results:** On intergroup comparison, there was no statistical difference in the score from baseline ($p>0.05$); however, a statistically significant difference was seen at the end of 1 year ($p<0.05$). Moreover, 57-60% of arches showed recurrence of pigmentation in the laser group whereas; only 12.7-17% recurrence was seen in the cryosurgery group at the end of the first year. **Conclusion:** Treatment of gingival hyperpigmentation with laser and cryosurgery shows a marked improvement of gingival pigmentation in both groups, but the cryosurgery depigmentation sites showed more sustainability.

Keywords: Cryosurgery; Lasers; Melanins.

Introduction

A smile expresses a feeling of joy, success, sensuality, and affection and reveals self-confidence and kindness. It is more than a method of communication and is a means of socialization and attraction. The harmony of the smile is determined by both 'pink' and 'white' components i.e., shape, position and color of the teeth, and even by the adjacent gingival tissues [1]. Nowadays, in dentistry, gingival esthetics has become an important aspect of clinical practice. The color of the gingiva plays a key role and is an essential part of an attractive smile, but methods in the management of gingival pigmentation are not fully established [1].

Melanogenesis occurs in melanosomes of melanocytes, which produce two types of melanin, namely, phenomelanin and eumelanin, which differ in color and their synthesis. The pigmentation consists of three phases: activation of melanocytes, synthesis of melanin, and expression of melanin. The melanogenic enzymes are highly similar to metalloproteins: tyrosinase, TYRP1 or gp75, and TYRP2, and melanin is synthesized through a series of interactions catalyzed by these enzyme complexes [1-4].

Gingival pigmentation is multifactorial and may be caused by local and/ or systemic factors associated with endogenous and exogenous etiological factors [1]. The physiological pigmentation is multifocal/ diffuse pigmentation of melanin in the basal and supra-basal cell layer of the epithelium, and an increase in the deposition of melanin by melanocytes in the area leads to hyperpigmentation. The hyper-melanin pigmentation of the gingiva is esthetically unpleasant during speech and smile and is aggravated in individuals with gummy smiles [2].

Depigmentation of gingiva is a periodontal plastic surgery in which the hyperpigmented gingiva is removed/ reduced [2]. Many treatment modalities such as gingivectomy, electro surgery, cryosurgery, usage of chemical agents such as 90% phenol, abrasion with diamond bur, and lately lasers have been used for this purpose [1,3]. Among all the techniques, slicing by surgical blade is the most common and preferred method for depigmentation [3,4]. Recently, lasers (Nd:YAG, CO_2, Er:YAG, and Semiconductor Diode) have come up and laser ablation is recognized as the most effective, reliable and pleasant technique [4].

Another effective medical treatment modality is the cryosurgery technique, in which the destruction of undesired biological tissue occurs by freezing. It has many advantages, such as low bleeding, good esthetic results, minimal use of anesthetics, short period of recovery, and low cost of the procedure [5]. A low temperature is used to induce tissue necrosis with the intent of ablation by four processes, i.e., freezing, holding of freeze, thawing, and repetition of these cycles [5].

Various studies conducted so far involve the comparison of conventional depigmentation methods [3,4]. Limited literature is available comparing the recent types of equipment for the depigmentation of the gingiva and its effects on recurrence. Therefore, the present study was conducted to compare and evaluate the clinical efficacy of diode laser and cryosurgery for treating melanin pigmentation of gingiva.

Material and Methods

Study Design

A randomized split-mouth clinical study was conducted in the Department of Periodontology to compare and evaluate the clinical efficacy of diode laser and cryosurgery for treating melanin pigmentation of gingiva. The study was conducted in accordance with the Helsinki Declaration [6], approved by the Institutional Ethics Committee. An informed consent was obtained from the included subjects before the treatment.

Twenty-five subjects with physiological gingival pigmentation on the facial aspect of both maxillary and mandibular anterior arches (50 sites), male and female, with an average age ranging from 18-35 years, participated in the study.

Inclusion and Exclusion Criteria

The following inclusion criteria were adopted: Subjects with physiological pigmentation of gingiva involving the facial aspect of both maxillary and mandibular anterior teeth; Subjects with esthetic concern; Subjects with good oral hygiene, and Subjects with heavy and continuous melanin pigmentation [7,8].

As for the exclusion criteria, the following were adopted: Subjects with smoking habits; Subjects with compromised medical conditions; Subjects having thin gingival biotypes; Pregnant and/ or lactating women, and Systemic diseases associated with pathological gingival hyper-pigmentation.

Site Selection

The selected subjects having physiological gingival pigmentation on the facial aspect of both maxillary and mandibular anterior arches were divided randomly into two groups by the toss of a coin, with one arch among the mandibular/ maxillary arches per patient serving as group I and the other as group II.

- Group I: The sites treated with LASER (Diode LASER: Fona™, Sirona Dental Services GmbH, Bensheim, Germany).
- Group II: The sites treated with Cryosurgery (Cryo super AA4™, Appasamy Associates, Arumbakkam, Chennai, India).

Clinical Parameters

After oral hygiene instructions and the Phase I therapy, the subjects who fulfilled the inclusion criteria were recalled for surgery (Figure 1A), and the following parameters were assessed for the evaluation of treatment results:

1. Melanin Oral Pigmnetaion Index (PI)

Dummett-Gupta index [7] was used due to its simplicity and ease of use as it classified mild, moderate and heavy pigmentation, whereas Takashi et al. index [8] was used to measure gingival melanin pigmentation as solitary or continuous.

2. Visual Analogue Scale (VAS) [9] for pain evaluation.

3. Healing index (HI) [10].

The PI was recorded at baseline, 1st month, 3rd month, 6th month, 9th month and 1st year; VAS was recorded at 1st, 2nd, 3rd, 4th, 5th and 7th day, and; HI was recorded at 1st, 2nd and 3rd week.

Surgical Procedure

The extra-oral and intra-oral disinfection was performed using Savlon (3% w/v cetrimide; 1.5% v/v Chlorhexidine, ITC Limited, India) and betadine 2% and 5% povidone-iodine solutions, respectively. The surgical area was then anesthetized using local anesthesia (lignocaine hydrochloride 2% with 1:80,000 adrenaline).

In group I (Laser) (Figure 1B): After application of local anesthesia, melanin pigmentation was ablated by Diode Laser with a flexible fiber optic tip of 320µm diameter. The tip was applied at 1.5W power with 970nm wavelength in continuous mode using small brush-like strokes (back and forth) in a cervical-apical direction in

the pigmented area until the desired depth of pigmentation was removed. The remnants of the ablated tissue were removed with damp, sterile gauze.

In group II (Cryosurgery) (Figure 1C): After the application of local anesthesia, the site to be de-pigmented was isolated and then frozen using a Cryosurgery probe (tip no.- Derm p-0823, diameter- 08mm and length - 23mm) attached to the apparatus. The probe tip was placed with moderate pressure on the area to be treated for 30 seconds; as the tip reached -96°C, the area in contact froze. After 30 seconds, the probe tip attached to the gun of the apparatus is de-activated and allowed to thaw for 15-20 seconds. The tip is then removed, and the area is allowed to thaw further for 1 minute, after which mild erythema was seen in the treated area. The procedure is repeated with overlapping applications till the entire site is covered.

After completion of both procedures, the surgical area was covered by Coe-Pack (Coe-Pak™, GC America Inc., USA) for 7 days. The subjects were prescribed analgesics (Paracetamol, 500mg) and post-operative and oral hygiene instructions (Figures 1D and 1E). The subjects were recalled for clinical parameters readings as mentioned above.

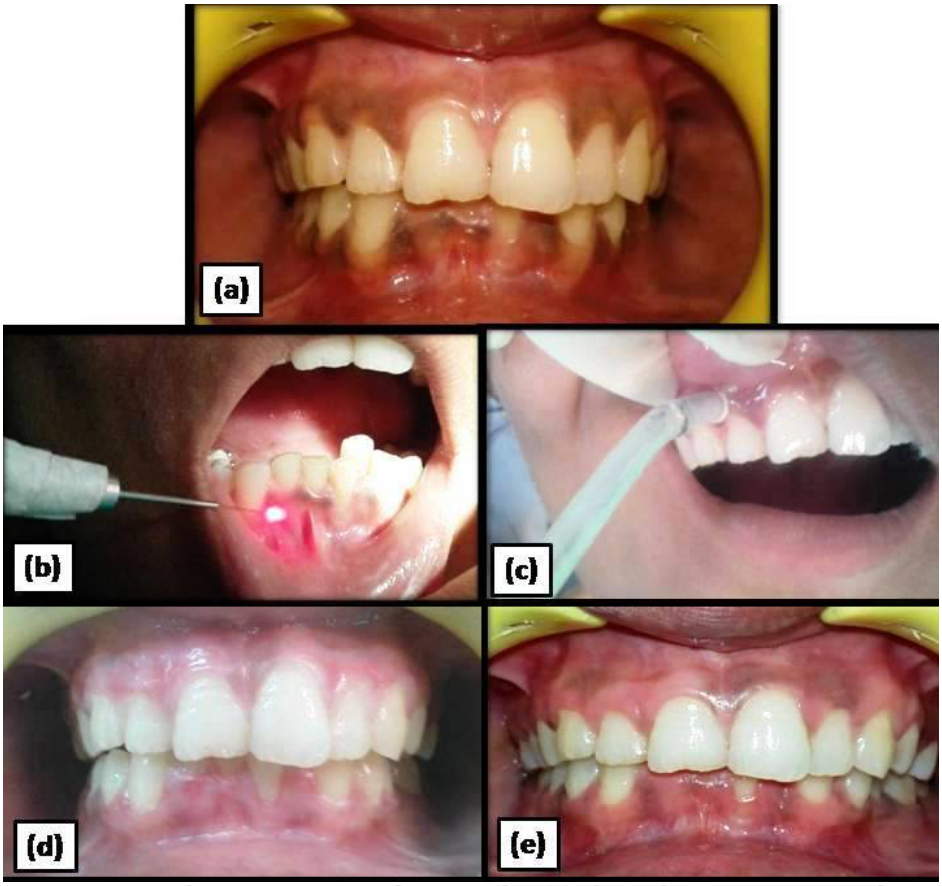

Figure 1. Pre-operative and post-operative photographs. (a) show the pre-operative photograph of the sites; (b) show the site treated for gingival pigmentation with LASER (group I); (c) show the site treated for gingival pigmentation with Cryosurgery (group II) and (d) & (e) shows post-operative photograph of the sites after 1 month and 1 year; respectively.

Statistical Evaluation

The data collected were analyzed by applying descriptive and inferential statistical analysis using SPSS version 22.0 (IBM Corp., Chicago, IL, USA). Fischer and paired t-test were used to compare the groups, and p-

values ≤0.05 were considered statistically significant, with alpha value calculated as 0.05 and confidence interval (CI) as 95%.

Results

Melanin Oral Pigmentation Index (PI)

Table 1 shows the comparison of PI between the two groups, laser and cryosurgery. At baseline, when PI was recorded, the pigmentation indexes were almost similar in both the groups (2.36 and 2.20) with no significant difference (p=0.26), reflecting that, the pigmentation levels were comparable in both the groups and bias was eliminated. At the 6th month, in a few subjects, slight pigmentation appeared in group I (mean=0.04), but no recurrence of pigmentation was observed in group II (mean=0), but the difference was not statistically significant (p=0.42). At 9th month, re-pigmentation was observed in both the groups in a few subjects (group I mean= 0.56 and group II mean=0.08), it was slightly more in the Laser group, but the differences were not statistically significant (p=0.076).

Table 1. Comparison of Melanin Pigmentation Index (PI) between laser and cryosurgery groups in different periods.

Period	Laser Group			Cryosurgery Group			t	p-value
	Mean	MD	Variance	Mean	MD	Variance		
Baseline	2.36	-	0.32	2.20	-	0.17	1.14	0.26
6 Months	0.004	2.32	0.39	-	2.20	0.17	0.80	0.42
9 Months	0.56	1.80	0.50	0.08	2.12	0.28	1.82	0.76
1 Year	1.36	1.00	0.33	0.28	1.92	0.41	5.34	0.00*

MD: Mean Difference; *Statistically Significant; t: paired t-test values.

Table 2 shows that at baseline, when PI was scored, the pigmentation was clinically almost similar in both groups (1.60 and 1.64), and it was substantively comparable as no statistical difference was found (p=0.78). In the Laser group, it was seen that at 6th month, in a few subjects, slight pigmentation reappeared (mean=0.04), but in the Cryosurgery group, no recurrence of pigmentation was observed (mean=0); statistically also, there was no significant difference (p=0.60). At 9th month, pigmentation appeared to some extent in both the groups, but it was more marked in the Laser group (mean=0.52) and was statistically significant (p=0.01). At 1 year, pigmentation appeared to some extent in a few subjects in both groups, but it was more marked in the Laser group (mean=0.96) than in the Cryosurgery group (mean=0.28) and was statistically significant (p=0.00). Thus, recurrence was more marked in the laser group and appeared earlier than in the cryosurgery group.

Table 2. Comparison of Melanin Pigmentation Index between the laser and cryosurgery groups at baseline, 6th month, 9th month and 1 year.

Period	Laser Group			Cryosurgery Group			t	p-value
	Mean	MD	Variance	Mean	MD	Variance		
Baseline	1.60	1.60	0.25	1.64	1.64	0.24	0.29	0.26
6 Months	0.04	1.56	0.34	-	1.64	0.53	0.53	0.60
9 Months	0.52	1.08	0.49	0.08	1.56	2.63	2.63	0.01*
1 Year	0.96	0.64	0.24	0.28	1.36	4.48	4.48	0.00*

MD: Mean Difference; *Statistically Significant; t: paired t-test values.

Healing Index (HI)

Table 3 represents post-operatively wound healing in groups I and II at the first, second, and third weeks. In the first week, when the healing index was compared between the two groups (mean 3.52 and 2.68, respectively), a significant difference was found (p=0.00), whereas clinically, group I showed better healing. In

the second week, the mean of the healing index in groups I and II was 4.64 and 3.72, respectively, a statistically significant difference (p=0.000). In 3rd week, when the healing index was compared in both groups, the difference was significant (p=0.003). The readings justify the clinical observation that early healing was observed in group I (Laser) at all periods.

Table 3. Comparison of Healing Index between laser and cryosurgery groups.

Week	Laser Group		Cryosurgery Group		t	p-value
	Mean	Variance	Mean	Variance		
Week 1	3.52	0.26	2.68	0.56	4.64	0.000*
Week 2	4.64	0.24	3.72	0.54	5.20	0.000*
Week 3	5.00	-	4.72	0.21	3.06	0.003*

*Statistically Significant; t: paired t-test values.

Visual Analogue Scale (VAS)

Table 4 shows the estimation of pain intensity. Measurements of pain intensity between the two groups, Laser and Cryosurgery at 1st day, 2nd day, 3rd day, 4th day, 5th day, 7th day, were estimated with the help of VAS. Patients experienced almost similar pain post-operatively in both groups, with a mean=3.36 and 4.28, respectively, on the 1st day, which was statistically not significant (p=0.08). On the 2nd day, pain was slightly reduced in the laser and cryosurgery groups, but the difference in the pain score was statistically insignificant (p=0.10). On 3rd, 4th, 5th day, post-operative day pain was markedly reduced in the laser group (mean=0.88, 0.24, and 0.08, respectively) as compared to the cryosurgery group (mean=2.20, 1.04, and 0.64, respectively), and statistically significant differences were seen (0.003, 0.008, and 0.009; respectively) in pain score at all time intervals. At the 7th day, there was no pain experienced in both laser and cryosurgery groups. Thus, the subjects in the laser group experienced less pain than those in the cryosurgery group at all periods. After a week, the lesion healed, and pain was not experienced in the groups.

Table 4. Comparison of Visual Analogue Scale (VAS) between laser and cryosurgery groups.

Days	Laser Group		Cryosurgery Group		t	p-value
	Mean	Variance	Mean	Variance		
Day 1	336	2.82	4.28	3.96	1.77	0.08
Day 2	2.28	2.71	3.08	2.99	1.68	0.10
Day 3	0.88	1.36	2.20	3.08	3.13	0.003*
Day 4	0.24	0.36	1.04	1.79	2.73	0.008*
Day 5	0.08	0.16	0.64	0.91	2.71	0.009*
Day 7	-	-	-	-	65,535.00	NA

*Statistically Significant; NA: Not applicable; t: paired t-test values.

Discussion

Oral pigmentation is seen in all the ethnic groups of the human race with no predilection of age and gender [11]. It occurs most frequently on gingiva among the intra-oral sites. Melanin pigmentation of the gingiva, also called "black gum", is benign in nature and is a common complaint of patients as it appears unaesthetic [2,11].

Several techniques have been used in the past to treat gingival depigmentation, such as chemical cauterization, gingivectomy, abrasion of gingiva, cryotherapy, free gingival graft, Acellular dermal matrix allograft (ADMA), Sub-epithelial connective tissue grafts (SECT), Electrocautery, and laser therapy [1,2].

The present study was conducted to determine the efficiency of semiconductor diode laser and cryosurgery for treating gingival pigmentation and compare the efficiency of the two techniques in terms of pain

felt by the patient, wound healing, and the rate of re-pigmentation. In this study, the semiconductor diode laser was preferred for depigmentation because of the poor absorption of light at 800-980nm in water but high absorption in hemoglobin and other pigments [12].

In the present study, the percentage of subjects with repigmentation from the 6th month to 1 year increases from 1.6% to 57% according to the Dummett-Gupta pigmentation index [7] and by Takashi et al. index [8] from the 6th to 1 year, increases from 2.5% to 60%. The results were in accordance with Kaur et al. [13] but were in contrast with Abraham et al. [14] and Gupta [15].

Cryosurgery is an established surgical procedure in medical practice with the main advantages of minimal post-operative infection, easy technique, no bleeding, and uneventful healing [5,16]. It is an effective technique in which tissue is destroyed by freezing, which in turn shows healing by 'complete regeneration' and 'sterile inflammatory reaction' [5,16].

In the present study, the percentage of subjects with repigmentation, which was recorded by the Dummett-Gupta pigmentation index [7] from the 6th month to 1 year, increased from 0% to 12% and by the Takashi et al. index [8] from 6th month to 1 year, increased from 0% to 17%. The results were in accordance with Ahmed et al. [17], Tal et al. [18] and Chin-Jyh Yeh [19].

The effects of laser and cryosurgery were compared on both indices. Both were found to be equally effective with no significant value ($p>0.05$) up to the 9th month. At one year, recurrence was more in the laser group (mean=1.36) than in the cryosurgery group (mean=0.28) with a statistically significant difference ($p<0.05$). The above findings are in accordance with Takashi et al. [8].

The healing was satisfactory in all the patients in the present study, with the laser group showing faster healing than the cryosurgery group. This could be due to the photobio-modular effect of laser, which helps stimulate fibroblasts, angiogenesis, and increased lymphatic flow, further fastening the healing process by repair and regeneration. The production of reactive oxygen species has a bactericidal effect, which enhances healing [12,14].

Our study showed a significant difference in the healing of depigmentation areas of the gingiva on comparison of the healing index between laser and cryosurgery groups at the first, second, and third weeks. The laser group showed a higher healing index than the cryosurgery group and significant differences at all three weeks were found ($p<0.05$). In 3rd week, the healing in both groups was uneventful as normal features of gingiva without scar formation were restored.

The possible explanation of high initial healing in the laser group is due to the production of a relatively thick coagulation layer on the treated surface, which behaves as a dressing. It secures the sensory nerve endings, causing less post-operative pain and homeostasis in the area, and provides a relatively dry and sterile wound site, causing minimal swelling [12,20].

The patient was under anesthesia, so evaluation of pain was not done immediately post-operatively. It was done at 1st, 2nd, 3rd, 4th, 5th, and 7th day post-operatively. At the laser-operated site, the score ranged between 1 and 2, and the score gradually decreased up to the 3rd day; after that, the score was equal up to the 7th day. Statistically significant differences were seen up to 3 days ($p<0.05$), and between the 4th, 5th and 7th days, there was no significant difference found ($p>0.05$). In the comparison of the visual analog scale in the cryosurgery group at 1, 2, 3, 4, 5, and 7 days, the score ranged between 1 and 8 and scored was statistically significant up to 3rd day ($p<0.05$), but in between 4, 5, 7 days, there was no significant difference seen ($p>0.05$).

In a comparison of VAS between laser and cryosurgery groups at 1, 2, 3, 4, 5, and 7 days, up to the 2nd day, no significant difference was found ($p>0.05$). Pain score gradually decreased from 3rd day onwards with a significant difference ($p<0.05$). This shows that the laser group experienced less pain than the cryosurgery group.

Various treatment approaches have been used to eliminate gingival pigmentation, including surgical ablation of the pigmented area free gingival grafts, and the recent approach includes lasers and cryosurgery. Of these various treatment modalities, lasers and cryosurgery have shown promising results in matters of patient comfort, safety, and aesthetically pleasing results. However, these procedures have been able to eliminate gingival pigmentation only for a short period, as there have been reports of recurrence of pigmentation after a few years.

Conclusion

In the present study, gingival depigmentation was attempted using two treatment modalities: laser and cryosurgery. In the initial months, both treatments responded very well. However, on follow-up, patients with laser started showing recurrence of pigmentation around the 6th month. However, the cryosurgery results were stable with slight recurrence in the 9th month onwards. Hence, it can be concluded that cryosurgical treatment offers consistent and superior results compared to laser. During the clinical observation of wound healing, it was observed that in the group treated with laser, healing occurred earlier than cryosurgery. Also, concerning patient pain perception, laser depigmentation had a significantly superior edge over cryosurgery.

Authors' Contributions

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All authors declare that they contributed to critical review of intellectual content and approval of the final version to be published.

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Conflict of Interest

The authors declare no conflicts of interest.

Data Availability

The data used to support the findings of this study can be made available upon request to the corresponding author.

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