Effectiveness of Er:YAG and CO_2 Lasers in the Management of Gingival Melanin Hyperpigmentation

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Abstract

Background: Although clinical melanin pigmentation does not present itself as a medical problem or a disease entity, "black gums" is a major esthetic complaint for many people, who often requests cosmetic corrections. Gingival depigmentation can be carried out using many procedures; lasers of various types being a new addition. This study was undertaken to evaluate and compare the effectiveness of CO_2 and Er:YAG lasers for the treatment of gingival melanin hyper pigmentation.
Materials & Methods: Twenty young age and gender matched subjects were selected for a randomized split mouth depigmentation procedure using Er:YAG (Group A) and CO_2 laser (Group B). Parameters evaluated were: Dummet index, Hedin melanin Index, Gingival and Plaque Index, time taken for the procedure, bleeding during the procedure, VAS scale for pain perception and wound healing and patient preference for the procedure. Wilcoxon signed rank test, Chi-square test, paired t test were used to analyze statistical significance between different variables.
Results: CO_2 laser treatment caused increased pain and delayed wound healing when compared to Er:YAG laser treatment.
Conclusion: Although both treatment modalities are highly effective depigmentation procedures, giving excellent esthetics results; when pain, wound healing and patient preferences were considered Er:YAG outscored CO_2 Laser.
Summary: The effectiveness of the Er:YAG and CO_2 laser for the treatment of gingival melanin depigmentation was evaluated clinically and histologically, although both treatment modalities are highly effective, giving excellent esthetics results, however, when pain and wound healing were considered Er:YAG was better than CO_2 laser.

Key words: Er-YAG, Lasers, Laser therapy, CO_2 laser, Melanins, Hyperpigmentation

Introduction

The gingival complex plays a vital role in the overall esthetics of a smile. The color of the gingiva is an integral part of many epidemiological evaluations of gingival health, ranging from pale pink to deep red or violet [1]. Its color is determined by several factors, including the number and size of blood vessels, epithelial thickness, quantity and quality of keratinization, and pigments within the epithelium. Melanin, carotene, reduced hemoglobin and oxyhemoglobin are the main pigments contributing to the normal color of the oral mucosa [2]. Melanin, a brown pigment, is the most common natural pigment contributing to endogenous pigmentation of the gingiva. Physiological pigmentation of the oral mucosa (mostly gingiva), is clinically manifested as multifocal or diffuse melanin pigmentation with variable amounts in different ethnic groups worldwide[3] and it occurs in all races [3]. The gingiva is the most frequently pigmented intraoral site [2]. Physiological gingival hyper pigmentation is caused by excessive melanin deposition by the melanocytes mainly located in the basal and suprabasal layers of the epithelium.

Gingival melanin hyper pigmentation is neither a medical problem nor a disease entity, but "black gums" is a common complaint, and fair-skinned people and they frequently request cosmetic correction. Gingival depigmentation is the treatment modality used to remove the melanin hyper pigmentation for esthetic reasons [4]. Several techniques have been employed for this purpose, [5,6] employing mechanical, [7] surgical, [5,8,9] chemical, [9] electrosurgical, [10] and cryosurgical [4,11] techniques. Recently, lasers have been used to ablate cells containing and producing the melanin pigment [12,13]. The advantages and disadvantages for each techniques was recently discussed in one of our reviews [14].

Different lasers have been used for gingival depigmentation, including carbon dioxide (CO_2,wavelength 10,600 nm), [13] semiconductor diode (wavelength 820 nm), [15] neodymium-doped: yttrium, aluminum, and garnet (Nd: YAG, wavelength1,064 nm), [6] erbium-doped:yttrium, aluminum garnet (Er: YAG, 2940 nm), [2] and erbium and chromium doped: yttrium, scandium, gallium garnet (Er,Cr: YSGG wavelength 2,780 nm) lasers. However the use of Er: YAG laser for depigmentation has gained increasing importance in recent years. The aim of the present study was to evaluate and compare the effectiveness of CO_2 and Er: YAG lasers both intra-operatively and post-operatively for the treatment of gingival melanin hyper pigmentation.

Materials and Methods

The study was carried out from November 2010 to August 2011. The study population consisted of 20 age and gender balanced (10 males, 10 females, age range: 18-30 years) systemically healthy subjects who complained of black gums and requested any cosmetic correction. Subjects were either recruited from the outpatient section, Department of Periodontology and Oral Implantology, Dr. DY Patil Dental College and Hospital, Pune, India, or were referred. Written informed consent was obtained from those who agreed to participate voluntarily. Ethical clearances were obtained from the institution's ethical committee and review boards. Subjects with moderate to severe melanin pigmentation in the maxillary anterior gingiva from canine to canine (esthetic zone) were included. Subjects with any systemic diseases, especially those

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associated with healing disturbances (uncontrolled diabetes, auto-immune disease, etc.) and tobacco users were excluded from the study. Subjects were randomly divided based on a computer generated list into two split mouth groups: group A (n=20) treated by Er:YAG (wavelength 2940 nm) laser, group B treated by CO_2 (wavelength 10600 nm) laser. Histological section was also undertaken to evaluate the density of melanin pigmentation and the activity of melanocytes pre-operatively and 6 months postoperatively. Incisional biopsies were done to obtain a tissue from the distal aspect of the canines. Percentage and intensity of staining for Masson Fontana was evaluated for basal, spinous and superficial layers of epithelium.

Pre-Operative assessment included Dummet index [16] for intensity of pigmentation; Hedin melanin index [17] for extent of pigmentation. Intra-operatively evaluation included severity of bleeding during the procedures (1: None, 2: Slight, 3: Moderate, 4: Severe) as estimated by Simoes DS, [18] time taken [13] by each procedure (minutes) and intensity of pain experienced using Visual Analogue Scale (VAS, 0: No pain, 0.1-3.0: slight pain, 3.1- 6.0: moderate pain, 6.1- 10: severe pain) [19]. Safety glasses were worn by the operator, patient and assistant. Highly reflective instruments or instruments with mirrored surfaces were avoided. For Er:YAG laser (2940nm 180mJ, 10 Hz total power of 1.8W, long pulse, no water, no air and RO_2 hand piece was used in a non-contact, defocused mode) standard settings were used. The laser beam was applied using the 'brush technique' (*Figure 1*) as described by Tal [2] Continuous and slow movements with overlap of the laser spots till the entire area was free of pigmentation. The CO_2 laser (10,600nm) was set at 2-4 watts. The ablation was performed in a non-contact, continuous wave, defocused mode with focal distance almost 1 inch away from the pigmented area (*Figure 2*). The "Epithelial peel" technique was used. High attention was given to avoid passing the beam on teeth structures and over the mucosa. In areas close to the tooth surface and near the margin, a focused mode was used to prevent the beam targeting a larger surface area. After completion of each procedure, operated area was finally cleaned with gauze soaked with normal saline and no dressing was given in any of the treated sites. The patient was instructed to avoid spicy, hard, sour and hot food, avoid smoking and brushing on the treated area and was instructed to maintain oral hygiene by regular rinsing after meals and advised warm saline rinses from the next day. Post-operative evaluation was carried out on the 1st, 7th day, and subsequently after 30, 90 and 180 days following depigmentation. Pain assessment was done using VAS and gingival and plaque indices [20] to evaluate the efficacy of oral hygiene after the treatment and clinical evaluation of gingival wound healing [21,22] (0: Tissue defect or necrosis, 1: Ulcer, 2: Incomplete or partial epithelization, 3: Complete epithelization). Patient preference for each procedure was recorded.

Figure 1. *Application of Er:YAG laser.*

Figure 2. *Application of CO_2 laser.*

Statistical Analysis

Wilcoxon signed rank test, Chi-square test, paired *t* test were used to analyze statistical significance between different variables. P value of <0.05 was considered statistical significant.

Results

Wilcoxon Signed Ranks Test and Chi-square tests ware used to analyze Dummet index at baseline and at 180 days respectively, and it was found that either values at baseline and at 180) were not significant (p<0.05) (*Table 1*). Wilcoxon Signed Ranks Test was used to analyze Hedin Melanin index. The mean value of both groups A and B at 30, 90 and 180 days were highly significant (p<0.001) (*Table 2*). Hedin melanin index between groups A and B at baseline and at 180 were not significant (p=0.564) & (p=0.157) respectively (*Table 2*).

Paired t test was used to analyze plaque and gingival indices. For both groups A and B the values for plaque and gingival indices at 30, 90 and 180 days were highly significant (p<0.001) (*Table 3*). Paired t test was used to analyze time taken for procedure (minutes). The mean ± SD time for group A was 22.45 ± 2.72 minutes compared to 17.70 ± 2.97 minutes for group B which was highly significant (p<0.001) (Table not shown).

Bleeding during the procedure was analyzed according to the grade of the bleeding, graded as none (1), Slight (2), Moderate (3), and Severe (4). Wilcoxon Signed Ranks Test was used to analyze the severity of bleeding during the procedure. The mean ± SD value for Group A was 2.20 ± 0.41 compared to 1.35+0.49 for group B which was highly significant (p<0.001) (*Table 4*). Paired t test was used to analyze VAS intra-operatively at days 1 and 7. Intra-operatively and at day 1, VAS was highly significant between groups A and B (p<0.001). However at day 7 the values were not significant (*Table 5*). Gingival wound healing on Day 1 the mean ± SD value for Group A was 1.75 ± 0.44 compared to 1.35 ± 0.59 for group B which is significant (p=0.005) (*Table 6*). Sixteen out of 20 patient preferred Er: YAG which was statistically significant with p=0.0139* (*Table 7*).

Table 1. *Dummet index to check for intensity of melanin pigmentation.*

Dummet Index	**N=20 (M: F = 10:10) Age range 18-30 years**	**Mean ± SD**	**Wilcoxon Signed Ranks Test (Z)**	**P**	**Recurrence (N=20)**	**χ^2 (df=1)**	**P**
Baseline	**Group A**	2.70 ± 0.47	1.414	0.157	-	-	-
	Group B	2.60 ± 0.60			-	-	-
180 days	**Group A**	2.70 ± 0.47	-	-	8	0.45	0.05*
	Group B	2.60 ± 0.60			5		

*Statistically significant P< 0.05

Table 2. *Hedin Melanin Index for extent of pigmented area.*

Hedin Melanin Index	**Group A**			**Group B**			**Groups A & B**	
	Mean ± SD	**Wilcoxon Signed Rank Test (Z)**	**P**	**Mean ± SD**	**Wilcoxon Signed Rank Test (Z)**	**P**	**Wilcoxon Signed Rank Test (Z)**	**P**
Baseline	3.70 ± 0.470	-	-	3.60 ± 0.598	-	-	0.577	0.564
Day 30	0.1000 ± 0.30779	4.053	<0.001*	0.050 ± 0.22361	4.042	<0.001*		
Day 90	0.3000 ± 0.47016	4.008	<0.001*	0.150 ± 0.36635	4.030	<0.001*		
Day 180	0.50 ± 0.688	3.992	<0.001*	0.30 ± 0.571	3.994	<0.001*	1.414	0.157

*Statistically significant P< 0.05

Table 3. *Plaque Index and Gingival Indices.*

Time	**Group A**			**Group B**		
	Mean ± SD	**Paired t**	**P**	**Mean ± SD**	**Paired t**	**P**
Plaque Index						
Baseline	1.18 ± 0.427	-	-	1.25 ± 0.323	-	-
Day 30	0.98 ± 0.289	4.59	<0.001*	1.03 ± 0.200	5.67	<0.001*
Day90	0.785 ± 0.245	4.54	<0.001*	0.90 ± 0.228	4.67	<0.001*
Day180	0.775 ± 0.275	4.85	<0.001*	0.77 ± 0.172	6.47	<0.001*
Gingival Index						
Baseline	1.316 ± 0.363	-	-	1.395 ± 0.491	-	-
Day 30	1.03 ± 0.152	4.03	0.001*	1.06 ± 0.283	4.44	<0.001*
Day 90	0.93 ± 0.236	4.70	<0.001*	0.98 ± 0.295	4.54	<0.001*
Day 180	0.82 ± 0.173	5.14	<0.001*	0.86 ± 0.254	5.34	<0.001*

Table 4. *Bleeding during procedure.*

Bleeding during the Procedure	**A**	**B**	**Wilcoxon Signed Ranks Test (Z)**	**P**
None-1	-	-	1.35+0.49	<0.001*
Slight-2	-	13		
Moderate-3	16	7		
Severe-4	4	-		

Table 5. *VAS scale to check for the intensity of pain intra and post operatively.*

VAS	**Intra-Op**		**Day 1**		**Day 7**	
	Group A	**Group B**	**Group A**	**Group B**	**Group A**	**Group B**
Mean ± SD	2.15 ± 1.04	4.85 ± 1.26	1.25 ± 1.12	2.65 ± 1.31	0.1 ± 0.31	0.2 ± 0.41
Paired T	3.85		3.08		1.41	
P	<0.001*		0.002*		0.157	

*Statistically significant P< 0.05

Wound healing in all patients was uneventful and showed excellent esthetics at 1, 3 and 6 months postoperatively (*Figures 3, 4 and 5*). The Hedin index evaluated the extent of pigmented area and compared with the baseline values, in which most of the cases, extended from canine to canine at 6 months post-operatively, Hedin index showed presence of

Table 6. *Gingival wound healing.*

Gingival Wound Healing (Day 1)	A	B	Wilcoxon Signed Ranks Test (Z)	P
Score 0	-	1		
Score 1	5	11		
Score 2	15	8	2.828	0.005*
Score 3	-	-		
Mean ± SD	1.75 ± 0.44	1.35 ± 0.59		

*Statistically significant P< 0.05
Score: 0- Tissue defect or necrosis, 1- Ulcer, 2- Incomplete or partial epithelization, 3- Complete epithelization

Table 7. *Patient preference for procedure.*

Patient's Preference for- A	16
Patient's Preference for- B	4
χ^2 (df=1)	6.05, P=0.0139*

Figure 3. *1 month post-operative wound healing.*

Figure 4. *3 months post-operative wound healing.*

Figure 5. *6 months post-operative wound healing with isolated areas of repigmentation.*

pigments in isolated marginal and papillary areas. Only 5 of the CO_2 and 7 of the Er:YAG laser treated sites showed more than one or two isolated areas of pigmentation. The scores for gingival index and plaque index at baseline were similar for all the allocated sites, across the two treatment groups.

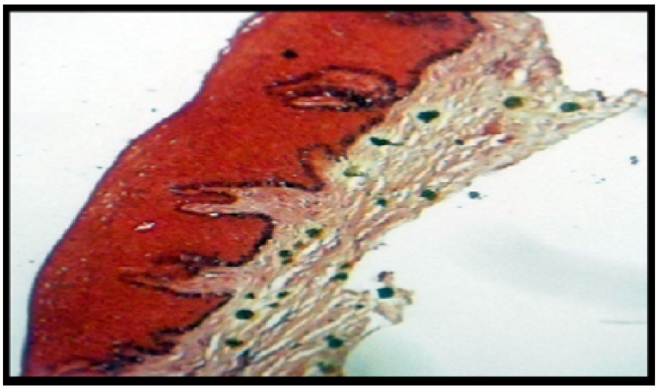

Figure 6. Preoperative biopsy stained with Masson Fontana.

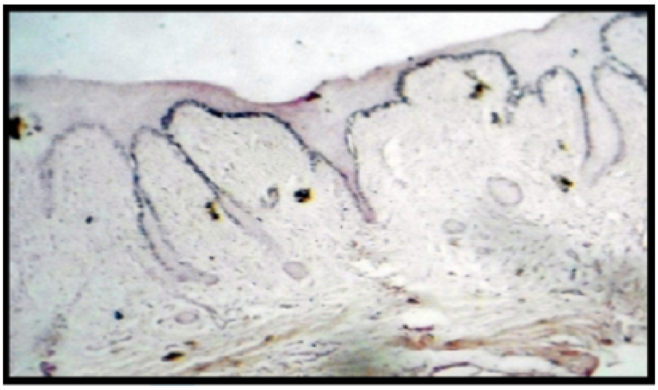

Figure 7. *Biopsy form Er:YAG treated site.*

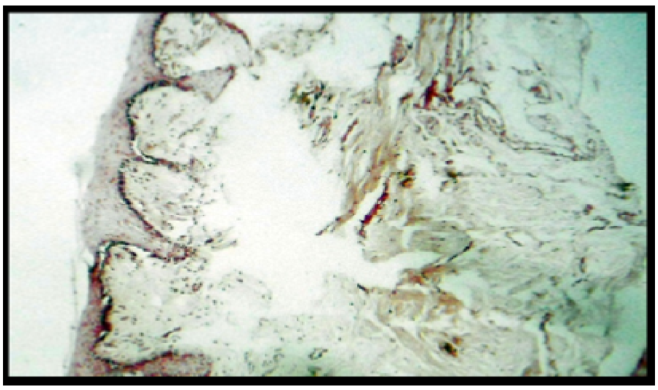

Figure 8. *Biopsy from CO_2 treated site.*

There was a steady increase in the gingival index scores in the first 3 weeks of the treatment, significantly more for both the treated sites. Even though the patients were regularly being reinforced about the oral hygiene maintenance, during the initial healing phase, there might have been less compliance from the patients. At the end of 6 months, the values were comparable to baseline across all the treated sites.

Repigmentation was assessed in terms of change in Hedin Index and DOPI (change in density) from baseline to 6 months post-operatively. There was a steady fall in the area of pigmentation from baseline to 6 months in all the three treatment groups ($p<0.001$).

Preoperative biopsy stained with Masson Fontana of basal layer showed strong staining positivity (>77%), spinous layer showed moderate positivity (50-75%) and superficial layer showed mild patchy positivity (<25%) (*Figure 6*). Postoperative biopsy specimen form Er:YAG treated site showed basal cells with moderate staining positivity (50-75%) (*Figure 7*), whereas biopsy from CO_2 treated sites showed mild to moderate staining (<50%) positivity (*Figure 8*).

Discussion

Within group A and group B, there was a statistically significant change in DOPI (change in density, Dummet index) on the 7th (not shown) and on 180th day compared to the baseline ($p<0.000$) in all the treated sites with CO_2 laser and Er: YAG laser. This was in agreement with the observations made by Azzeh MM [13] TMS Ginwala [23] TK Pal [24] Ozbayrak S [21] who found low levels of pigmentation scores throughout the study.

Only 4 out of 20 patients preferred CO_2 to Er: YAG laser as they complained of the irritating "bullet type noise" of Er:YAG laser. They also appreciated the lesser time and minimal bleeding of the CO_2 laser treatment. 16 out of 20 patients preferred Er:YAG laser since felt that there was minimal pain, no burning smell during the procedure and they did not have to be anesthetized as often required intermittently during the CO_2 type of treatment. Treatment with Er:YAG laser took slightly more time than CO_2 laser, approximately 22.45 minutes, possibly because considerable bleeding was encountered in those areas. This is in agreement with the studies done by Azzeh MM [13] who took approximately 20-25min. Bleeding encountered was in the form of spots. In one treated site, there was severe bleeding at one point, may be owing to the laser beam penetrating deeper than required. It was observed that the bleeding was directly correlated with the depth of the ablation. The CO_2 laser treated surfaces showed a dry ablated surface, with a similar whitish hue; however some areas of carbonization and charring were visible, especially in the papillary region. Essen [29] in his report concluded that CO_2 laser is an effective and safe method for gingival depigmentation with minimal carbonization and almost no bleeding and post-operative pain [25].

Hedge [27]. in their recent reported compared surgical stripping, Er: YAG and CO_2 lasers fir gingival melanin depigmentation, patient preference, perception of pain, change in Dummett oral pigmentation index, Hedin index, and change in area of pigmentation from baseline to 6 months postoperatively were evaluated. The authors concluded that all the three techniques showed statistically significant change in DOPI, Hedin index, compared to baseline. Repigmentation areas were almost similar with all the techniques at 6 post-operative months. However, patient preference and pain indices gave statistically significant values for Er:YAG lasers similar to our results [26].

In the present study, VAS was highly significant intra-operatively and at Day 1 (*Table 7*). Results of the VAS showed that most of the Er:YAG treated sites had slight pain (0.1-3.0) whereas CO_2 laser treated patients reported slight to moderate pain (0.1-3.0, 3.0-6.0) and 2 patients complained of severe pain (6.1-10). Decreased pain with Er:YAG laser and to some extent in CO_2 laser could be due to the protein coagulum that is formed on the wound surface, thereby acting as a biological dressing. In addition, it may be due to the sealing of the ends of sensory nerves. The Er:YAG laser has the least thermal damage and least tissue penetration [13] (1μm), resulting in low tissue necrosis, thus reducing pain. This is in agreement with the studies done by Azzeh MM [13], Ozbayrak [28] Essen [29]. In addition to the low tissue necrosis and reduced pain and discomfort Er: YAG laser treatment does not require any local anesthesia and the procedure can be completed in lesser time as compared to other lasers [29]. Tal reported that Er: YAG laser is a suitable treatment modality for gingival depigmentation and satisfies most of the requirement [31].

In our study, Er:YAG laser showed faster wound healing at day 1, day 7 and 1 month post-operatively. Re-epithelization was complete in 1 week; however, healing area appeared slightly translucent and immature. At 10-15 days, epithelization was complete. At 1 month, the gingiva was similar to the normal untreated gingiva and the healing was mature, completely devoid of pigment. Repigmentation was assessed in terms of change in Hedin Index and DOPI from baseline to 6 months post-operatively. There was a steady fall in the area of pigmentation from baseline to 6 months in both the treatment groups ($p<0.001$). The repigmentation that appeared was in the form of small dots in the interdental areas, or streaks on the attached gingiva. Only one of Er:YAG laser repigmentation was in the form of distinct patches on the attached gingiva and the rate of recurrence was also fast. This could be due to the high activity of the melanocytes. Therefore high attention needs to be paid to avoid leaving behind any melanocytes in the periphery of the lesion so as to prevent the so called "Migration effect", which is a primary cause for repigmentation as explained by Tal and Perlmutter in 1986 [32].

Conclusion

The present randomized split mouth study showed that Er:YAG and CO_2 lasers are highly efficient techniques for the treatment of gingival hyperpigmentation. They have shown numerous advantages, such as painless, bloodless, sterile field, minimal topical anesthesia and better esthetic outcome. The average scores for pain assessment by VAS intra-operatively and post-operative at day 1 was significantly higher in CO_2 laser group compared to Er:YAG Laser treatment group. Although CO_2 laser treatment was quicker than Er:YAG the lasing energy and time differed according to the degree of pigmentation, the epithelial thickness, and the pigmented surface treated. Significantly higher proportion of patients in Er:YAG Laser group showed better healing appearance at 7th post-operative day compared to CO_2 Laser. When pain, wound healing and patient preferences are considered Er:YAG outscored CO_2 laser. The patients were satisfied with the esthetic outcome, which was the ultimate goal for us.

References

1. Jones J. A Photometric study of the color of healthy gingiva. *Journal of Periodontol*ogy. 1977; **48**: 21-26.

2. Tal H, Oegiesser D, Tal M. Gingival Depigmentation by Erbium:YAG Laser: Clinical Observations and Patient Responses. *Journal of Periodontol*ogy. 2003; **74**: 1660-1667.

3. Dummett CO. Oral pigmentation. *Journal of Periodontology*. 1960; **31**: 356-360.

4. Chin- Jyh Yeh. Cryosurgical treatment of melanin pigmented gingiva; *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology.* 1988; **86**: 660-663.

5. Tamizi M, Taheri M. Treatment of severe physiologic gingival pigmentation with free gingival autograft. *Quintessence International.* 1996; **27**: 555-558.

6. Atsawasuwan P, Greethong K, Nimmanon V. Treatment of gingival hyper pigmentation for esthetic purposes by Nd:YAG laser: Report of 4 cases. *Journal of Periodontology.* 2000; **71**: 315-321.

7. Farnoosh AA. Treatment of gingival pigmentation and discoloration for esthetic purposes. *International Journal of Periodontics & Restorative Dentistry.* 1990; **10**: 312-319.

8. Almas K, Sadig W. Surgical treatment of melanin pigmented gingiva; An esthetic approach. *Indian Journal of Dental Research.* 2002; **13**: 70-73.

9. Hirschfeld I, Hirschfeld L. Oral pigmentation and a method of removing it. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology.* 1951; **4**: 1012-1016**.**

10. Gnanasekhar JD, Al-Duwairi YS. Electrosurgery in dentistry. *Quintessence International.* 1998; **29**: 649-54.

11. Tal H, Landsberg J and Kozlovsky A. Cryosurgical depigmentation of the gingiva – A case report. *Journal of Clinical Periodontology.* 1987; **14**: 614-617.

12. Sharon E, Azaz B, Ulmansky M. Vaporization of melanin in oral tissues and skin a carbon dioxide laser: A canine study. *Journal of Oral and Maxillofacial Surgery.* 2000; **58**: 1387-1393.

13. Azzeh MM. Treatment of gingival hyper pigmentation by erbium-doped:yttrium, aluminum, and garnet laser for esthetic purposes. *Journal of Periodontology*. 2007; **78**: 177-184.

14. Kathariya R, Pradeep AR. Split mouth de-epithelization techniques for gingival depigmentation: A case series and review of literature. *Journal of Indian Society of Periodontology.* 2011; **15**: 33-40.

15. Nakamura Y, Funato A, Wakabayashi H, Matsumoto K. A study on the removal of the melanin pigmentation of dog gingiva by CO_2 laser irradiation. *Journal of Clinical Laser Medicine & Surgery.* 1992; **10**: 41-46.

16. Dummett CO, Gupta OP. Estimating the Epidemiology of Oral Pigmentation. *Journal of the National Medical Association*. 1964; **56**: 419-20.

17. Hedin CA. Smoker's melanosis. Occurrence and localization on the attached gingiva. *Archives of Dermatology.* 1977; **113**: 1533-1538.

18. Ozbayrak S, Dumlu A, Ercalik-Yalcinkaya S. The treatment of melanin pigmented gingiva and oral mucosa with CO2 laser. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology*. 2000; **90**-14-15.

19. Simoes DS, Aranha AC, Eduardo C, Aoki A. Esthetic Treatment of Gingival Melanin Hyperpigmentation with Er: YAG Laser: short –Term Clinical Observations and Patient Follow-Up. *Journal of Periodontology.* 2007; **78**: 2018-2025.

20. Hoffman A, Marshall RI, Bartold PM. Use of the Vector™ scaling unit in supportive periodontal therapy: a subjective patient evaluation. *Journal of Clinical Periodontology.* 2005; **32**: 1089.

21. Loe H. The gingival index, the plaque index and the retention index systems. *Journal of Periodontology.* 1967; **38**: 6(supplement).

22. Ishii S, Aoki A, Kawashima Y, Watanabe H, Ishikawa I. Application of an Er: YAG laser to remove gingiva melanin hyperpigmentation-Treatment procedure and clinical evaluation. *Japanese Society for Laser Dentistry.* 2002; **13**: 89-96.

23. Kawashima Y, Aoki A, Ishii S, Watanabe H, Ishikawa I. Er:YAG laser treatment of gingival melanin pigmentation. In: Ishikawa I, Frame JW, Aoki A (Editors) The 8th International Congress on Lasers in Dentistry. Yokohama, Japan: Elsevier; **2003**, pp. 245-248.

24. Ginwalla TMS, Gomes BC and Varma BRR. Surgical removal of gingival pigmentation (A preliminary study). *Journal of Indian Dental Association*.1966; **38**: 147.

25. Pal TK, Kapoor KK, Parel CC and Mukherjee K. Gingival Melanin Pigmentation – a study on its removal for esthetics. *Journal of Indian Society of Periodontology.* 1994; **3**: 52- 54**.**

26. Esen E, Haytac MC, Oz IA, Erdoğan O, Karsli ED. Gingival melanin pigmentation and its treatment with the CO2 laser. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology.* 2004; **98**: 522-527.

27. Hegde R, Padhye A, Sumanth S, Jain AS, Thukral N. Comparison of surgical stripping; erbium-doped:yttrium, aluminum, and garnet laser; and carbon dioxide laser techniques for gingival depigmentation: a clinical and histologic study. *Journal of Periodontology.* 2013; **84**: 738-748.

28. Ozbayrak S, Dumlu A, Ercalik-Yalcinkaya S. Treatment of melanin-pigmented gingiva and oral mucosa by CO_2 laser. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology*. 2000; **90**: 14-15.

29. Essen E, Haytac MC, Oz IA, Erdogan O, Karsli ED. Gingival melanin pigmentation and its treatment with the CO_2 laser. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology.* 2004; **98**: 522-527.

30. Simşek Kaya G, Yapici Yavuz G, Sümbüllü MA, Dayi E. A comparison of diode laser and Er:YAG lasers in the treatment of gingival melanin pigmentation. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology.* 2012 Mar; **113**: 293-299.

31. Tal H, Oelgiessr D, Tal M. Gingival depigmentation for aesthetic purposes using erbium:YAG laser: rationale and technique. *Refuat Hapeh Vehashinayim.* 2002; **19**: 25-32.

32. Perlmutter S, Tal H. Repigmentation of the gingival following surgical injury. *Journal of Periodontology.* 1986; **57**: 48-50.