

Comparison of Efficacy of Four Different Depigmentation Techniques Along With Melanocyte Analysis: A Clinical Histopathological Study.

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ABSTRACT:Introduction: The study of aesthetics, or the science of beauty, focuses on every specific element that appeals to the sight in both living and inanimate objects. On the other hand, improvements in cosmetic dentistry enable a dentist to create a setting that supports ideal dental aesthetics. The colour of gingiva plays very important role in overall appearance of the face. Despite being a benign condition, melanin hyperpigmented gingiva is aesthetically unappealing in people with high smile lines and prominent gingival display. Treatment for this melanin pigmentation is known as gingival depigmentation. Different depigmentation methods are used to remove or reduce this hyperpigmentation.

Aim: To clinically compare and evaluate the different techniques for gingival depigmentation along with melanocyte count.

Methodology: A total of 20 individuals, aged 18-40 years with hyperpigmentation on the facial aspect of the gingiva present in the esthetic zone included.Individuals melanin were with hyperpigmentation of gingiva were treated by different depigmentation techniques namely Scalpel technique (Conventional technique), Bur Abrasion, Diode Laser, Electrosurgery. For determining the melanocyte assay, a small amount of gingival tissue that was excised was studied under a special staining technique, i.e., Masson-Fontana method for melanin, a histochemistry procedure which determines the activity of melanin both quantitatively and qualitatively. Clinical and histological observations for the intensity of pigmentation were recorded at baseline and 6 months after surgery.

Results: The mean value of the Dummett Oral Pigmentation Index (DOPI) was 2.00 ± 0.71 at baseline; there was a statistically significant difference in the different depigmentation techniques of the mean DOPI when compared with that of the baseline (P = 0.001). The mean value of melanin histopathological count (MHC) was 91.40

 \pm 4.50 at baseline; the mean MHC value of different depigmentation techniques were not statistically significant when compared with that of baseline.

Conclusion:The observation from this study shows that Laser can be safe and effective alternative for depigmentation and shows low recurrence values compared to other techniques.

KEYWORDS:Melanin hyperpigmentation gingival depigmentation, Scalpel, Bur Abrasion, laser, electrosurgery, Melanocytes , Re pigmentation

I.INTRODUCTION:

AESTHETIC has become a significant aspect of dentistry. Clinicians are facing challenges in achieving acceptable gingival aesthetics as well as addressing biologic and functional problems. Among the various features depicting the appearance and health of the gingiva such as size, shape, consistency, contour and the colour of the gingiva plays an important role in overall aesthetics. Physiological pigmentation of the oral mucosa is clinically manifested as multifocal or diffuse melanin pigmentation with variable amount in different ethnic groups (**Cicek**, 2003)¹

On the other hand, advances in aesthetic/cosmetic dentistry allow a dental practitioner in achieving an environment that promotes optimal dental aesthetics.² Appearance of the face relies on a multitude of entities encompassing oral and extraoral factors.

Clinical melanin hyperpigmentation is not a medical problem and is a benign condition. However; it is an aesthetic concern in individuals with high smile line and excessive gingival display. The demand for cosmetic therapy is commonly made by individuals with gingival melanin hyperpigmentation.³

In some populations, regardless of age or gender, gingival hyperpigmentation is found which occurs due to an excessive production of melanin which is typically physiological and is a genetic



feature. The degree of pigmentation varies from person to person based on the activity of the melanoblasts.⁴

Procedure to remove this melanin hyperpigmentation is called gingival depigmentation, the gingival hyperpigmentation is eliminated or diminished using a variety of methods. The demand of a person for improved aesthetics is the primary indication for depigmentation therapy.⁵

Depending on the different depigmentation techniques used, this period of repigmentation varies. Re pigmentation may start as early as 6 to 24 months, primarily due to the activity of melanocytes.

Various methods have been used to try to achieve a gingiva that is free of pigmentation.⁶

METHODS AIMED AT REMOVING THE PIGMENTED GINGIVA:

A. Surgical methods:

a. Scalpel surgical technique

b. Bur abrasion method

c. Electrosurgery

d. Cryosurgery

e. Lasers : i. Neodymium: Yttrium-Aluminium Garnet (Nd:YAG) lasers.

ii. Erbium:YAG (Er :YAG) lasers iii. Carbon dioxide (CO2) lasers

f. Radiosurgery

B. Chemical methods:

a. 90% phenol and 95% ethanol b. Vitamin C

METHODS AIMED AT MASKING THE PIGMENTED GINGIVA:

a. Free gingival graft.

b. Sub-epithelial connective tissue graft.

c. Acellular dermal matrix allograft.

The present study was intended to evaluate the melanocyte response following depigmentation by different techniques at both clinical and histopathological levels.

II. SUBJECTS AND METHODS

Patient selection

A total of 20 systemically healthy patients, within the age group of 18-40 years with an aesthetic complaint of hyper pigmented gingiva were enrolled in this study from the outpatient department of periodontics of Goenka Research Institute Of Dental Science. The sample population had 10 males and 10 females. The patients were briefed about the surgical procedure. After obtaining the informed consent they were randomly divided in to 4 groups.

Group A - 5 patients - Depigmentation with Scalpel technique.

Group B - 5 patients - Depigmentation with Bur abrasion technique.

Group C - 5 patients - Depigmentation with Diode lasers. (Biolase®)

Group D - 5 patients - Depigmentation with Electrosurgery.

Inclusion criteria:

1. Patients of age group 18-40 years.

2. Both male and female patients.

3. Patients with physiological melanotic pigmented gingiva in relation to maxillary anterior region 13-23.

4. Patient with aesthetic concern.

5. Patient with thick gingival phenotype and healthy gingiva.

6. Patient with good oral hygiene.

Exclusion criteria:

1. Patient under medication.

2. Chronic Smokers.

3. Systemically compromised patients.

4. Pathological pigmentation other than physiological pigmentation.

- 5. Pregnant and lactating women.
- 6. Apprehensive patients

7. Patients with history of periodontal treatment for past 6 months.

8. Patient with history of post-surgical keloid.

Surgical procedure

Scalpel method: After administration of local anaesthetic, a Bard-Parker® (B-P) handle with a No. 15 blade was used to remove the pigmented layer. Pressure was applied with a sterile gauze soaked in local anaesthetic agent to control haemorrhage during the procedure. The entire pigmented epithelium along with a thin layer of connective tissue was removed with the scalpel. The exposed surface was irrigated with saline, and the surgical area was covered with a periodontal dressing. Patient was recalled after 1 week for removal of periodontal dressing and post-operative evaluation.





FIGURE: 1 Baseline intraoral photograph showing gingival pigmentation



FIGURE: 2 One-week re-evaluation after depigmentation with Scalpel method

Gingival Abrasion Technique:Depigmentation was performed with diamond round bur in a contraangled handpiece at low speed and saline irrigation. The surgical site was then covered with a periodontal dressing. Patient was recalled after 1 week for removal of periodontal dressing and post-operative evaluation.



FIGURE: 3Baseline intraoral photograph showing gingival pigmentation



FIGURE: 4 One-week re-evaluation after depigmentation with Bur Abrasion method

Diode laser technique: A local anaesthetic gel was applied topically before the depigmentation treatment. Through a 400-micron fibre optic tip, a diode laser (BIOLASE®) operating in pulsed mode with settings of 810 nm 60 J/sec was transmitted. In painting strokes, the pigmented gingival epithelium was ablated utilising the direct contact mode. Care was taken to include the epithelium at the tip of interdental papilla and the mucogingival junction on the other end without disturbing the marginal gingiva. postoperative instructions were given and patient is recalled after a week for follow-up and removal of periodontal dressing. The lasing process was conducted in accordance with laser safety regulations.





FIGURE: 5 Baseline intraoral photograph showing gingival pigmentation



FIGURE: 6 One-week re-evaluation after depigmentation with Laser method

Electrosurgery:Depigmentation was performed with single wire and loop electrode in contact mode with brush like strokes and saline irrigation. Care was taken to remove the remnants of pigmented epithelial layer if any. The surgical site was then covered with a periodontal dressing. Patient was recalled after 1 week for removal of periodontal dressing and post-operative evaluation.



FIGURE: 7 Baseline intraoral photograph showing gingival pigmentation



FIGURE: 8 One-week re-evaluation after depigmentation with Electro surgery method

Melanocyte assay:

For determining the melanocyte assay, a small amount of gingival tissue was excised using the B-P handle with a No. 11 blade, after determining the site for various procedure. The blade was positioned toward the interdental gingiva similar to that of an external bevel incision. Utmost care was ensured that a small triangular portion of the tissue was taken following the interdental gingival architecture. The shape of the interdental gingiva was maintained, and the contour of the gingiva was unaltered. The excised area was covered with a periodontal dressing for due comfort of the patient. After 6 months of postoperative period, a small amount of gingival tissue was excised using the B-P handle with a No. 11 blade from the same area where it was excised priorly for comparison of the melanocyte response. The excised gingival tissue was studied under a special staining technique, i.e., Masson-Fontana method for melanin, a histochemistry procedure which determines the activity of melanin both quantitatively and qualitatively.



POST OPERATIVE CARE:

Patients were instructed to continue with good oral hygiene and avoid trauma around the surgical site. Patients were prescribed with Paracetamol 500mg TD to be taken in case of pain only and 0.2% chlorhexidine Di gluconate rinse twice daily for 2 weeks. The patients were recalled after 1 week and were monitored monthly for 6 months. All the cases were examined by the same



operator to ensure that there was no examiner variability and also were put on gentle and soft brushing after pack removal for a week.

III. RESULTS

A total of Twenty patients, aged between 18 to 40 years, who reported to the Department of Periodontics, Goenka Dental College, with physiological gingival melanin pigmentation were recruited for the study.

The mean value of the Dummett Oral Pigmentation Index (DOPI) was 2.00 ± 0.71 at baseline.

✓ Following depigmentation with the scalpel technique, the mean DOPI was 0.80 ± 0.50 at 6 months.

- ✓ Following depigmentation with the Bur abrasion technique, the mean DOPI was 0.40 ± 0.55 at 6 months.
- ✓ Following depigmentation with the Laser technique, the mean DOPI was 0.20 ± 0.45 at 6 months.
- Following depigmentation with the Electrosurgery technique, the mean DOPI was 0.40 ± 0.55 at 6 months.

There was a statistically significant difference in the mean DOPI when compared with that of the baseline (P = 0.001)

When assessed after 6 months, the difference between the depigmentation values over the four techniques showed lower values of recurrence of pigmentation in Laser when compared to that of other techniques. [Table 1; Graph 1]

DOPI	$MEAN \pm SD$	
	PRE-OPERATIVE	POST-OPERATIVE
SCALPEL	2.00 ± 0.71	0.80 ± 0.75
BUR ABRASION	2.00 ± 0.71	0.40 ± 0.55
LASER	2.00 ± 0.71	0.20 ± 0.45
ELECTROSURGERY	2.00 ± 0.71	0.40 ± 0.55

⁽DOPI: Dummett Oral Pigmentation Index; SD: Standard deviation)

Table 1: Comparison of Mean Dummett Oral Pigmentation Index Pre- And Postoperatively



Graph 1: Comparison of Mean Dummett Oral Pigmentation Index Pre- And Postoperatively

The mean value of melanin histopathological count (MHC) was 91.40 ± 4.45 at baseline.

(Table:2; Graph 2)

- ✓ Following depigmentation with scalpel technique, the mean DOPI was 84.4 ± 5.39 at 6 months.
- ✓ Following depigmentation with the Bur abrasion technique, the mean MHC was 80.00 ± 4.86 at 6 months.
- ✓ Following depigmentation with the Laser technique, the mean MHC was 71.8 ± 4.26 at 6 months.
- ✓ Following depigmentation with the Electrosurgery technique, the mean MHC was 79.6 ± 2.42 at 6 months.
- ✓ However, the difference was not statistically significant in mean MHC when compared with baseline.



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MHC	$MEAN \pm SD$	
	PRE-OPERATIVE	POST-OPERATIVE
SCALPEL	91.40 ± 4.50	84.40 ± 5.39
BUR ABRASION	91.40 ± 4.50	80.00 ± 4.86
LASER	91.40 ± 4.50	71.80 ± 4.26
ELECTROSURGERY	91.40 ± 4.50	79.60 ± 2.42

(MHC: Melanin Histopathological count; SD: Standard deviation) Table 2: Comparison of mean melanin histopathological count pre- and postoperatively



Graph 2: comparison of mean melanin histopathological count pre- and postoperatively

MEAN \pm SD (POST- OPERATIVE)			
TECHNIQUE	DOPI	MHC	
SCALPEL	0.80 ± 0.75	84.40 ± 5.39	
BUR ABRASION	0.40 ± 0.55	80.00 ± 4.86	
LASER	0.20 ± 0.45	71.80 ± 4.26	
ELECTROSURGERY	0.40 ± 0.55	79.60 ± 2.42	

$MEAN \pm SD (POST- OPERATIVE)$			
TECHNIQUE	DOPI	MHC	
SCALPEL	0.80 ± 0.75	84.40 ± 5.39	
BUR ABRASION	0.40 ± 0.55	80.00 ± 4.86	
LASER	0.20 ± 0.45	71.80 ± 4.26	
ELECTROSURGERY	0.40 ± 0.55	79.60 ± 2.42	

(DOPI: Dummett Oral Pigmentation Index; MHC: Melanin histopathological count; SD: Standard deviation) Table 3: Comparison of mean postoperative Dummett Oral Pigmentation Index and melanin histopathological count between Four different groups



The mean value of melanin histopathological count (MHC) was 91.40 ± 4.45 at baseline.

- ✓ Following depigmentation with scalpel technique, the mean MHC was 84.40 ± 5.39 at 6 months.
- ✓ Following depigmentation with the Bur abrasion technique, the mean MHC was 80.00 ± 4.86 at 6 months.
- ✓ Following depigmentation with the Laser technique, the mean MHC was 71.8 ± 4.26 at 6 months.
- ✓ Following depigmentation with the Electrosurgery technique, the mean MHC was 79.6 ± 2.42 at 6 months.

However, the difference was not statistically significant in mean MHC when compared with baseline.

When compared with baseline, the DOPI showed the mean value of recurrence of pigmentation as 0.80 ± 0.50 forscalpel, 0.40 ± 0.55 for Burabrasion, 0.20 ± 0.45 for Laserand 0.40 ± 0.55 for Electrosurgery . This difference was statistically significant (P = 0.001)

The recurrence of pigmentation when assessed with DOPI showed a recurrence percentage of 40 % in the scalpel technique, 20% in Bur abrasion, 10 % in Laser and 20% in the Electrosurgery technique [Table 3].

The recurrence of pigmentation when assessed with regard to the mean histological count showed a recurrence of 92.34% in the scalpel technique, 87.53% in the Bur abrasion technique, 78.56% in the Laser technique and 87.09% in the Electro surgery technique.

This elicits a superiority of Laser over the other techniques With regard to the recurrence of pigmentation, clinically, Laser is far better than other techniques which has shown statistically significant values in our study, whereas for the mean histological count, though low recurrence rate was recorded in Laser , the effect of Laser over other techniques were not statistically significant.

IV. DISCUSSION:

Melanin pigmentation of the oral mucosa is a common finding seen in the oral cavity. It varies depending on the quantity, depth or location of the melanin pigment. Melanin pigmentation is occurred due to melanin deposition by melanocytes which are located at the basal and supra basal layers of the oral epithelium. The ascribable changes in the physiological pigmentation of the oral mucosa, which produce a colour range from light brown to almost black, are mainly caused by differences in the activity of melanocytes in the basal cell layer of the oral epithelium. Complaints of "black gums" are common which demands for depigmentation procedure is usually made for aesthetic reasons.⁷

This physiological pigmentation of the oral mucosa is common in Black persons, with the frequency being higher in dark-skinned Whites (Caucasians) than in light-skinned Whites, and the determination of the physiological oral pigmentation by genetic factors is strongly evident in the literature. Males and females are equally affected, with a greater incidence of bilaterally symmetrical involvement.

Elimination of these melanotic areas are done through surgical excision, lasers,^{8,9} cryosurgery through use of a gas expansion system,¹⁰ bur abrasion, scraping,electrocautery¹¹ and chemicals have been reported by many authors (Hirschfeld & Hirschfeld 1951,¹² Dummet & Bolden 1963,¹³Ginwalla et al 1966,¹⁴ Manchandia 1979, Tal et al 1987¹⁰ and Atsawasuwan et al 2000)¹⁵.

One of the most efficient, comfortable and reliable methods for gingival depigmentation has recently been acknowledged as laser ablation.¹⁶ Light amplification by stimulated emission of radiation, or laser is the abbreviation for this process. In 1960, Maiman ¹⁷created the first functional laser. Goldman et al. (1964)¹⁸ reported the first laser application to dental hard tissue, while Stern and Sognnaes (1972)¹⁹ discussed the effects of the ruby laser on enamel and dentin. In this investigation, an 810 nm 60 J/sec diode laser (Sirona Diode SystemTM, Germany) was chosen because of its nearly ideal absorption of haemoglobin and melanin.

Although various de-pigmentation methods have been advocated to attain a pleasing and esthetic smile, re-pigmentation is an alarming issue which is of concern to both the patient and the dentist. While the literature states that the mechanism of re-pigmentation is unclear, one hypothesis suggests that the melanocytes from the adjacent pigmented tissues migrate to the treated area and cause re-pigmentation. Studies by **Ginwalla et al.**¹⁴ showed repigmentation of 50% of the cases by using the abrasion technique after 24 -56 days. **Pal et al.**²⁰ showed that repigmentation occurred in 19% of the cases following gingival depigmentation by surgical bur. Hirschfeld and **Hirschfeld** (1951)¹² used phenol (90%) and alcohol (95%) to remove areas of oral pigmentation by destroying tissue down to and slightly below the basal layer of the mucous membranes. Repigmentation soon developed in three patients; the rest of the subjects met with the same results a



short while later. Dummett and Bolden (1963)¹³ excised pigmented gingiva by gingivectomy procedure in 9 cases. Repigmentation occurred in 67% of the areas, as early as 33 days after surgical removal. Ginwalla et al (1966)¹⁴attempted to remove gingival pigmentation in 6 cases using Slicing, three different techniques: Bone denudation and abrasion. Tal et al (1987)²¹ described depigmentation of the gingiva by cryosurgery, using gas expansion cryosurgical system based on the Joule-Thomson effect. Trelles et al (1993)²² were the first to treat patients with pigmented gingiva by argon laser. Atsawasuwan et al (2000)¹⁵ used Nd: YAG laser for the treatment of hyperpigmented gingiva and reported no recurrence in a period of 11 to 13 months of follow up. Chin JyhYeh (1998) described cryosurgical treatment of melanin-pigmented gingiva using direct application of liquid nitrogen with a cotton swab to the pigmented gingiva. Sameer $(2006)^{23}$ treated 3 cases of gingival hyperpigmentation by abrasion with a highspeed hand piece and diamond bur and no repigmentation in an 18 month follow up period but in a similar technique. **Farnoosh**(2008)²⁴ reported slight repigmentation in 2 cases. A free gingival auto graft can also be used to eliminate pigmented areas, but it is an extensive procedure which requires an additional surgical site and colour matching.

Taking this aspect into consideration, our study aimed at evaluating the melanocyte response following depigmentation by four different techniques. Our study included 20 patients who intended to undergo cosmetic therapy for hyperpigmented gums. After administering a routine oral prophylaxis and plaque control instructions and ensuring disease-free gingiva in the patients selected, different treatment modalities, namely scalpel, bur abrasion, laser and electrocautery techniques, were performed in the anterior region of the oral cavity.

On assessment after 6 months, the difference between the depigmentation values over the four different techniques showed Lower values of recurrence of pigmentation in laser when compared to that of other techniques.

V. CONCLUSION

Gingival melanin pigmentations can occur as a consequences of local, systemic environmental or genetic factors. Gingival depigmentation is one of the commonly executed perioesthetic procedures. To ensure treatment success, the potential causative or aggravating agent of the pigmentation should be identified and eliminated, if possible, before the surgical treatment. Various techniques are available with some advantages and some drawbacks. However, choice of the technique should be dependent on individual preference, clinical expertise and patient affordability.

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