Comparative evaluation of diode laser ablation and surgical stripping technique for gingival depigmentation: A clinical and immunohistochemical study

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ABSTRACT

Introduction: There are various treatment modalities to remove the black patches of melanin pigmentation. The aim of the study is to clinically compare the diode laser ablation and surgical stripping technique for gingival depigmentation and to evaluate their effect on the histological changes in melanocyte activity.

Materials and Methods: A total of 40 sites of 20 patients with bilateral melanin hyperpigmentation were treated with the surgical stripping and diode laser ablation technique. Change in Hedin index score, change in area of pigmentation using image analyzing software, pain perception, patient preference of treatment were recorded. All 40 sites were selected for immunohistochemical analysis using HMB-45 immunohistochemical marker.

Results: At 12 months post-operative visit, in all sites, repigmentation was observed with different grades of Hedin index. Paired t-test, analysis of variance, and Chi-square tests were used for statistical analysis. Repigmentation in surgical stripping is significantly lesser compared to laser ablation. Lesser numbers of melanocytes were found on immunohistological examination at 12 months postoperatively. Comparison for patient preference and pain indices give statistically significant values for diode laser techniques.

Conclusion: Gingival hyperpigmentation is effectively managed by diode laser ablation technique and surgical stripping method. In this study, surgical stripping technique found to be better compared to diode laser ablation.

Key words: Depigmentation, diode laser, esthetics, immunohistochemistry, repigmentation

Introduction

The color of the gingiva is determined by several factors, including the number and size of blood vessels, thickness of epithelium, degree of keratinization of epithelium, and pigments within the epithelium. Melanin, carotene, reduced hemoglobin, and oxyhemoglobin are the main pigments contributing to the color of the oral mucosa.¹

Melanin, a brown pigment, is the most common natural pigment contributing to endogenous pigmentation of gingiva. It is produced by the melanocytes, which are mainly present in the basal and suprabasal layers of the gingival epithelium.²,³ Active melanocytes synthesize tyrosinase enzyme, which accumulates in vesicles in the golgi system, leading to the formation of pre-melanosomes.⁴ Tyrosinase enzyme leads to oxidation of tyrosin via a number of intermediate products, including dihydroxyphenylalanine, resulting in the formation of dense pigment melanin, which forms homogeneous, opaque melanosomes.⁵,⁶ Premelanosomes are transferred by melanocytes to the adjacent keratinocytes by cytocrine ability. This process is known as “inoculation,” wherein keratinocytes play an active phagocytic role.⁷,⁸ This structural and functional relationship give rise to the concept of epithelial-melanin unit.⁹ A ratio of 1:15 (melanocyte:basal keratinocyte) has been found in the human gingival epithelium.⁹ Melanophores are situated at epithelium-connective tissue junction, and their long thin dendritic processes ramify among neighboring keratinocytes. This arrangement allows the transfer of melanosomes into keratinocytes which determines the pigmentation. All melanophores produce melanin, which are of two types: The eumelanins, which are brownish black, and the pheomelanins.

Physiologic pigmentation of gingiva is clinically manifested as multifocal or diffuse melanin pigmentation with variable
amounts in different ethnic groups and in different races.\textsuperscript{9-11} It is a common esthetic concern, which is aggravated in patients with excessive gingival display. Studies done in the past have shown that dark patches of pigmentation on the facial aspects of the gingiva may have psychological effects on individuals, and that attitude toward pink gingival coloration are found to be more favorable compared to brown, blue-black or mixed colorations.\textsuperscript{10,12}

Gingival depigmentation is a treatment modality used to remove the melanin hyperpigmentation. Several techniques have been employed for this purpose, which include mechanical, surgical, electrosurgical, cryosurgical, free gingival grafts, and lasers.\textsuperscript{13-15} Scalpel technique is a time tested technique and remains the gold standard.\textsuperscript{16} The commonly used lasers for gingival depigmentation are the diode (810 nm), CO\textsubscript{2} lasers (10,600 nm), neodymium-doped yttrium aluminum garnet (YAG) lasers (1,064 nm).\textsuperscript{15,17} Gingival depigmentation using lasers requires melanocytes to lie within its range of penetration and to contain melanin to absorb and convert light energy into heat by photothermolysis.\textsuperscript{18}

Gingival repigmentation refers to the reappearance of melanin pigmentation following a period during which clinically pigmented tissues were depigmented. It is a common concern in the treatment of gingival hyperpigmentation and starts with the migration of melanocytes from the adjacent gingiva. The extent and time interval of recurrence varies with regard to the treatment modalities used and the duration of follow-up. Repigmentation can be assessed clinically as well as histologically.\textsuperscript{19}

Based on this background, the aim of this study was to evaluate and compare the effects of the surgical stripping and diode laser techniques for gingival depigmentation.

**Materials and Methods**

All patients were selected from the outpatient Department of Periodontics, Pacific Dental College and Hospital, Udaipur, India. Patients who were concerned about their esthetics and with uniformly dense bands of bilateral physiologic gingival hyperpigmentation on facial aspects were selected. Inclusion and exclusion criteria for the patient selection are as follows:

**Inclusion criteria**

- Diffuse continuous physiologic pigmentation of the gingiva, involving facial aspect of maxillary second right premolar to second left premolar region
- Patient with Hedin index score Grade 4
- Patients with esthetic concerns
- Patients with high and very high smile line
- Periodontally healthy subjects
- Patients with age between 18 and 30 years.

**Exclusion criteria**

- Patients with any systemic diseases
- Subjects who are current or former smokers
- Pregnant and lactating women
- Patients taking any medication
- Patients with any metallic restoration or undergoing orthodontic treatment
- Patients with thin gingival biotype
- Patients with any occlusal disharmony
- Patients with missing anterior teeth
- Gingival pigmentation associated with occupational hazards
- Patients of Caucasian and Negroid race.

A total of 20 patients (12 males, eight females) with age range of 18-30 years were enrolled in the study. Patients were made aware of the procedures and the purpose of the study, and prior written informed consents were obtained. Each patient underwent professional scaling, one week before therapy. Allocation of sites was done by coin toss by a single examiner. Accordingly, sites divided into two groups. Group I includes site treated with surgical blade, Group II includes site treated with diode laser. All 40 sites were selected for the immunohistological examination.

**Clinical assessments**

**Hedin melanin index for extent of pigmented area**

The index was scored as:

- 0 = No pigmentation;
- 1 = One or two solitary units pigmentation in the papillary gingiva;
- 2 = >3 units of gingival pigmentation without formation of a continuous band;
- 3 = >1 short continuous ribbons of pigmentation;
- 4 = One continuous band including the entire area.\textsuperscript{20}

All sites selected were of score 4 preoperatively. The index was recorded at 3, 6, 9, and 12 months post-operative period.

**Change in area of pigmentation using image analyzing software**

Changes in the gingival color and area of pigmentation were assessed using standardized photographs.\textsuperscript{21} Pre-operative and 3, 6, 9, 12 months post-operative images were obtained using a digital camera (Canon SLR EOS D1100 Camera [14.2 megapixel camera]) (Figure 1). Camera used at the following settings: F stop (focal stop) = f/4.5; exposure time = 1/60 s; focal length = 35 mm; ISO = ISO-250; flash mode = No flash compulsory mode.

A customized registration tray was placed in the patient’s mouth, and the connecting rods which are attached to the camera were fixed to the customized tray (Figure 2e). This setup helped in maintaining a standardized distance and...
angulations of the camera in relation to the patient, thus providing photographs of the same area taken at different intervals of time. The photographs were obtained in JPEG format. Surface area of the pigmented surface was measured using the image analyzing software (Image J Software, Version 1.43, National Institute of Health, USA). Pigmented area on the photograph was traced in the software and surface area was measured in mm$^2$ (Figure 2f).

Visual analog scale (VAS)

The VAS was used at 1 day and 1 week post-operative visits to measure the intensity of pain experienced during and after the treatment. Pain was assessed on 10 cm horizontal, continuous interval scale with the left end point was marked as “no pain” and the right end point marked “severe pain.” The patient placed a mark to coincide with the level of pain. Scores were calculated as follows:

$0 = $No pain
$0.1-3.0$ cm = Slight pain
$3.1-6.0$ cm = Moderate pain
$6.1-10.0$ cm = Severe pain.

Clinical evaluation of bleeding and healing

Following depigmentation, the patient was recalled at 1 day, 1 week, 1 and 3 months post-operative period for evaluation of bleeding and wound healing using criteria given by Ishii et al. and Kawashima et al.

Bleeding scores: A = None; B = Slight; C = Moderate; D = Severe.

Wound healing scores: A = Complete epithelialization; B = Partial epithelialization; C = Ulcer; D = Necrosis.

Histological assessment

The activity of melanocytes was measured at baseline and 12 months postoperatively, based on the density of the pigmentation, by microscopically viewing the density of melanin granules. The grading was done using the scale as follows:

$0 = $Absence of melanin granules (no pigmentation)
$1 = $Rare or scattered melanin granules (mild pigmentation)
$2 = $Dense but not aggregated melanin granules (moderate pigmentation)
$3 = $Dense and aggregated melanin granules (heavy pigmentation).

The melanocytes communicate with at least 30-40 keratinocytes in the epithelial–melanin unit. The activity was thus assessed by counting the percentage of keratinocytes of basal and suprabasal layers which were laden with the melanin pigment, using an eyepiece with a millimeter grid.

Patient’s preference of treatment procedure

Patients were asked about comfort during treatment and to identify their preferred modality at 1 day post-operative period as follows:

$1 = $Surgical stripping;
$2 = $Diode laser ablation.

Procedures performed in the study

Surgical stripping technique (Figure 2b)

After local anesthetic infiltration, the procedure was performed with the surgical blade no. 11 or 15. A partial dissection technique was used, and the epithelium and a portion of lamina propria were gently dissected out in the form of strip. Tissue tags which were left behind were removed using tissue nippers.

Laser ablation technique (Figure 2a).
Topical anesthetic spray was applied to the operating area. Energy output, pulse duration, and focal distance were determined based on manufacturers instruction, with some variations from published reports and a series of previously done cases. Depigmentation was carried out using semiconductor diode laser (Piccaso 810 nm Diode Unit, AMD Lasers, Indianapolis, USA). With proper isolation, depigmentation procedure was done using 300 µm tip in contact mode (Power setting 3W, continuous mode). Laser ablation was started from the mucogingival junction toward the free gingival margin, including interdental papilla. The laser beam was applied using the “brush technique,” as described by Tal et al.1 and the tip was kept in motion all the time. Remnants of the ablated tissue were removed using sterile gauze dampened with saline solution.

All above-mentioned procedures were completed in each patient in a single session.

**Histological procedure**

Pre-operative punch biopsies were obtained from the distal aspect of the canine using a disposable punch (Figure 2c and 2d). The samples were immersed in 10% neutral buffered formalin and sent to the laboratory for further processing. At the end of 12 months, tissue samples were obtained again for histological analysis to assess the change in degree of pigmentation.

Samples were stained using HMB-45 marker for immunohistological examination. It was performed using a sterptavidin-biotylatedimmunoperoxidase reaction as described by Barrete et al.27 Positive cells with marker were identified by the presence of a stained cell body that had at least one associated dendrite (Figure 3).

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**Summary of various clinical and histological examinations at different time interval is as follows:**

| Time Period       | Examination                                                                 |
|-------------------|------------------------------------------------------------------------------|
| Pre-operative     | Pre-operative biopsy and immunohistological examination                      |
|                   | Hedin index score                                                           |
|                   | Area of pigmentation                                                        |
| 1 day post-operative | VAS                                                                          |
|                   | Bleeding                                                                    |
|                   | Wound healing                                                               |
| 1 week post-operative | VAS                                                                          |
|                   | Bleeding                                                                    |
|                   | Wound healing                                                               |
| 1 month post-operative | Wound healing                                                                |
| 3 months post-operative | Hedin index score                                                          |
|                   | Area of repigmentation                                                      |
| 6 months post-operative | Hedin index score                                                          |
|                   | Area of repigmentation                                                      |
| 9 months post-operative | Hedin index score                                                          |
|                   | Area of repigmentation                                                      |
| 12 months post-operative | Hedin index score                                                          |
|                   | Area of repigmentation                                                      |
|                   | Biopsy and immunohistochemical examination                                  |

VAS: Visual analog scale

Data analyses were performed using statistical software (SPSS version 11.5 for windows, IBM, Armonk, NY).

To test the statistical significance of the difference of categorical variables across two treatment groups, $\chi^2$ test of independence of attributes was used if the cell frequency was >5. Otherwise, Fisher exact test was used. To test the statistical significance of the difference of quantitative variables, one-way analysis of variance (ANOVA) test was performed. Paired t-test was used to test the statistical significance of difference between the baseline and 12 months parameters. $P<0.05$ was considered statistically significant.

**Results**

**Changes in Hedin index (Table 1)**

In both groups, pre-operative Hedin index grade was 4. None of the patients showed repigmentation at 3 months post-operative period in both treatment groups. In Group I, at 6 months post-operative period repigmentation was not observed in 16 (80%) sites and 4 (20%) sites were observed with Grade 1. At 12 months post-operative period, 6 (30%) sites with Grade 1, 10 (50%) sites with Grade 2 and 4 (20%) sites with score 3 were observed. In group II, at 6 months post-operative period repigmentation was not observed in 10 (50%) sites, 8 (40%) sites observed with Grade 1 and 2 (10%) site observed with Grade 2. At 12 months post-operative period, 4 (20%) sites with Grade 1, 4 (20%) sites with Grade 2 and 12 (60%) sites were observed with Grade 3.
The intragroup comparison of repigmentation among sites of surgical stripping and diode laser was done at 6 and 12 months post-operative period. The intergroup comparison at 6 and 12 months post-operative period was done using $\chi^2$ test. Repigmentation was significantly lesser on sites treated with a surgical blade.

Comparison of changes in area of pigmentation (Table 1)
The comparison between groups for changes in area of pigmentation at baseline, 6 and 12 months post-operative period was done by ANOVA. Median values for intergroup comparison were calculated. Intergroup comparison shows that significantly lesser area of repigmentation was found in surgical blade site at 12 months post-operative period. Intragroup comparison shows a significant reduction in area of pigmentation at 12 months post-operative period in both the treatment groups ($P < 0.05$).

VAS scores (Table 1)
At day 1 postoperatively, the median scores for VAS, obtained by ANOVA were 3.8 (0-8.2) for surgical stripping and 1.8 (0-7.0) for diode laser group. The median scores for VAS at 1 week post-operative period were 0 (0-1.0) for both the groups. $P$ values as obtained by ANOVA with Bonferroni’s correction for multiple corrections at the day 1 post-operative period were 0.01 (Group I vs. II) which is statistically significant. At 1 week post-operative period $P$ values were non-significant ($>0.05$). The diode laser treated sites resulted in least pain intraoperative and postoperatively.

Preference of treatment procedure (Table 1)
A significantly higher number of patients preferred diode laser first 16 (80%) followed by surgical stripping 8 (40%). $P$ values were obtained using $\chi^2$ tests were statistically significant ($P < 0.05$).

Post-operative bleeding and wound healing (Table 2)
Bleeding was significantly higher in sites treated with surgical blade compared to diode laser treated sites. Healing was significantly delayed in sites treated with diode laser.

Changes in histological parameters (Table 3 and Figure 3)
12 months postoperatively, the pigment density was mild to moderate in all cases. Histologic recurrence was seen in the form of pigment deposit along the basement membrane, in 8 (40%) of surgical treated sites and 8 (40%) laser treated sites. Intergroup comparison by $\chi^2$ test showed $P > 0.05$, which are not statistically significant. Pigment-laden keratinocytes at baseline and 12 months postoperatively were counted. Intergroup comparison was carried out using ANOVA test. $P$ values were obtained and showed that it was not statistically significant ($P > 0.05$).

Discussion
The present study is a 1 year split-mouth clinical and immunohistochemical longitudinal study comparing diode laser ablation and surgical stripping technique for gingival depigmentation. The semiconductor diode laser is emitted in continuous wave or gates pulsed modes and is usually operated in a contact method using a flexible fiber optic delivery system. Laser light at 800-980 nm is poorly absorbed in water but highly absorbed in hemoglobin and other pigments. Since the diode laser basically does not interact with dermal hard tissues, the diode laser is an excellent soft tissue surgical laser, indicated for cutting and coagulating gingival and oral mucosa. The diode laser exhibits “hot-tip” effect caused by heat accumulation at the end of the fiber, and produces a relatively thick coagulation layer on the treated surface. Thermal ablation means that energy delivered by laser is coupled into irradiated material by an absorption process, yielding a temperature rise in that tissue. As the temperature increases at the treated sites, the soft tissues are subjected to warming (37-60°C), protein denaturation, coagulation (>60°C), welding (70-90°C), vaporization (100-150°C) and carbonization (>200°C). In surgical stripping technique, gingival epithelium is completely removed along with some part of connective tissue. Later on, new epithelium will be formed which is devoid of pigmented cells.

The treated sites were observed with the signs of repigmentation. The recurrence rate varies with regards to the treatment modalities used and duration of follow-up. Several authors have quoted different rates of recurrence with different techniques. Singh et al. compared diode laser and cryosurgery for gingival depigmentation and concluded that the depigmentation achieved using both techniques were found equivalent and satisfactory. At 18 month follow-up, repigmentation was found in one case in each group.

Lagdive et al. presented a case series which describe scalpel blade surgery and diode laser for gingival depigmentation. Better results were achieved with diode laser compared to conventional surgical stripping technique.

Hedge et al. compared surgical stripping; erbium:YAG laser and CO₂ laser. They concluded that surgical stripping for gingival depigmentation remains the gold standard. Lesser repigmentation was observed with surgical stripping technique after 6 months of follow-up.

In the present study, clinical repigmentation appeared in the form of multiple units in the papillary areas and gingival margin, possibly due to the relative difficulty in treating...
these areas with both the techniques. Both the techniques are relatively easy to perform in attached gingiva because of the broader area and relatively firm base.

The visual analog scale was used to assess the pain perception during and after the treatment. Less of pain was experienced following treatment with diode laser due to the formation of protein coagulum on the wound surface, which acts as biologic dressing. In addition, it may be due to the sealing of sensory nerve endings. This is in contrast to the conventional surgical stripping technique, which leaves behind a raw, bleeding surface with exposed nerve endings.

Intensity of clinical pigmentation depends upon the branching of melanocytes, number and degree of dispersion of melanosomes, degree of melanization of melanosomes, capacity of melanocytes to transfer melanin, capacity of keratinocytes to receive melanin and functional activity of melanocytes.\(^6\) In this

### Table 1: Various clinical parameters at baseline, intraoperative and 12 months post-operatively

| Parameters                                         | Treatments                                      | Group I (surgical [n=20]) (%) | Group II (laser [n=20]) (%) | Group I versus II |
|----------------------------------------------------|-------------------------------------------------|-------------------------------|-------------------------------|------------------|
| Pigmentation at baseline (Hedin index)             |                                                 | 20 (100)                      | 20 (100)                      |                  |
| Repigmentation at 6 months (Hedin index)           |                                                 |                               |                               | <0.05            |
| Grade 0                                            |                                                 | 16 (80)                       | 10 (50)                       |                  |
| Grade 1                                            |                                                 | 4 (20)                        | 8 (40)                        | <0.05            |
| Grade 2                                            |                                                 | 2 (10)                        |                               |                  |
| Repigmentation at 12 months (Hedin index)          |                                                 |                               |                               | >0.05            |
| Grade 1                                            |                                                 | 6 (30)                        | 4 (20)                        |                  |
| Grade 2                                            |                                                 | 10 (50)                       | 4 (20)                        | <0.05            |
| Grade 3                                            |                                                 | 4 (20)                        | 12 (60)                       | <0.05            |
| Area (mm\(^2\)) median (minimum to maximum)       |                                                 |                               |                               | >0.05            |
| Baseline*                                          |                                                 | 58.5 (25-130)                 | 60.0 (30-155)                 |                  |
| 6 months postoperatively*                          |                                                 | 2.8 (0-8)                     | 3.4 (0-8)                     | >0.05            |
| 12 months postoperatively*                         |                                                 | 16.6 (10-40)                  | 32.8 (20-60)                  | <0.05            |
| Intra group comparison                             |                                                 |                               |                               | <0.05            |
| VAS                                                |                                                 |                               |                               |                  |
| 1 day postoperatively*                             |                                                 | 3.8 (0-8.2)                   | 1.8 (0-7.0)                   | <0.05            |
| 1 week postoperatively*                            |                                                 | 0 (0-3.0)                     | 0 (0-3.0)                     | -                |
| Patient preference (1 day post-operative)         |                                                 |                               |                               | <0.05            |
| First                                              |                                                 | 16 (80)                       | 8 (40)                        |                  |
| Second                                             |                                                 | 4 (20)                        | 12 (60)                       | <0.05            |
| Number of sites with repigmentation                |                                                 |                               |                               | >0.05            |
| 6 months postoperatively                           |                                                 | 4                             | 10                            | <0.05            |
| 12 months postoperatively                          |                                                 | 20                            | 20                            | -                |

*Values are median (minimum to maximum). P values are obtained by ANOVA with Bonferroni correction for multiple corrections. The rest of values are n (%), whose P values were obtained by \(\chi^2\) test. Otherwise, paired t-test was used. VAS: Visual analog scale, ANOVA: Analysis of variance.

### Table 2: Various clinical parameters at immediate, 1 day, 1 week and 1 month post-operative period

| Parameters                                      | Group I (n=20) | Group II (n=20) | Group I versus II |
|-------------------------------------------------|---------------|-----------------|------------------|
| Bleeding                                        | A  | B  | C  | D  | A  | B  | C  | D  | P value |
| Immediate post-operative                        | 0  | 4 (20) | 12 (60) | 4 (20) | 0  | 18 (90) | 2 (10) | 0  | <0.05 |
| 1 day post-operative                            | 18 (90) | 4 (10) | 0  | 0  | 20 (100) | 0  | 0  | 0  | >0.05 |
| 1 week post-operative                           | 20 (100) | 0  | 0  | 0  | 200 (100) | 0  | 0  | 0  | -     |
| Wound healing                                   | A  | B  | C  | D  | A  | B  | C  | D  | P value |
| 1 day post-operative                            | 0  | 8 (40) | 12 (60) | 0  | 0  | 4 (20) | 16 (80) | 0  | <0.05 |
| 1 week post-operative                           | 16 (80) | 4 (20) | 0  | 0  | 4 (20) | 16 (80) | 0  | 0  | <0.05 |
| 1 month post-operative                          | 20 (100) | 0  | 0  | 0  | 20 (100) | 0  | 0  | 0  | -     |

Values are n (%). P values were obtained by \(\chi^2\) test.
study, pre- and post-operative histologic scores correlated with the clinical picture. Lesser rate of repigmentation may suggest that either migration of melanocytes did not occur during this period or the melanocytes which has migrated are not active, or the treatment modalities have some specific effect on the cells, which reduced their activity. The presence of melanin pigments was variable but markedly reduced in different regions of the basement membrane of basal layer compared to pre-operative levels of pigments. These findings are consistent with those achieved by Bergamaschi et al. who observed behavior of melanocytes following gingivectomy procedure using electron microscope.

One of the major disadvantages of the surgical blade is unpleasant bleeding during and after the procedure due to the damage to the underlying connective tissue including blood vessels, which also hampers the visualization of the surgical area. The reason of less bleeding is the coagulation of the blood vessels in lased area which prevents extravasation of blood. This finding is consistent with the findings of Lagdive et al. In this study, significantly delayed healing was observed on diode laser treated site compared to surgical blade. This finding is consistent with the finding of Lagdive et al. who had observed delayed wound healing in diode laser treated sites. The finding is also in consistent with the results of Rossmann et al. who had reported slower wound healing following use of laser compared to scalpel technique. In this study, diode laser was used in contact mode in sweeping motion, and sufficient care was taken not to overexpose the surgical area with laser radiation to avoid excessive necrosis of the tissue. After laser application, a carbonized layer of tissue was found at the impact site. This carbonized gingiva was removed from the field by wiping with moist sterile gauze sponge. If the char is allowed to accumulate and lasing is attempted through it, there will be a rapid jump in temperature to 150-200°C and the lasing point will begin to incandesce to an orange glow, causing extensive thermal damage which may cause delayed healing.

In an experimental study, Luomanen explained the reasons of delayed healing with use of lasers. The results from the study suggested that retarded proliferation of capillaries during healing and slower infiltration of inflammatory cells caused by thermal coagulation and denaturation of some vasculogenic polypeptides by laser is the cause of delayed wound healing on laser treated sites. D’Arcangelo et al. concluded that thermal damage around the lased site is responsible for delayed wound healing. He also postulated that wavelength of the laser, power setting (watts), continuous/pulsed mode, pulse duration, pulse frequency, and exposure time are important parameters governing extent of thermal injury to the tissues.

Patient preference of the treatment modalities was given importance in this study. Patients who preferred diode laser most felt that there was less pain during and after the procedure and they did not have to go for an additional injection of local anesthetic. They also appreciated the lesser time taken and minimal bleeding which was experienced. Those who preferred laser less complained of burning smell, and many complained of slight discomfort and a dry, itchy feeling, at some point of time during the treatment. Patients who preferred surgical stripping more, felt that other than the unpleasant experience of administration of anesthesia, the rest of the procedure was silent and uneventful.

Conclusions

Following conclusions can be drawn out from the study:
- Repigmentation was significantly less in surgical stripping side after 12 months follow up.
- Comparison of activity of melanocytes showed a definite decline following treatment with both the modalities.
- Bleeding and pain parameters were found to be significantly lesser in diode laser side.
- Healing was found to be delayed on diode laser-treated areas.

Table 3: Immunohistological parameters at baseline and 12 months post-operative period

| Parameters                                      | Group I (n=20) | Group II (n=20) | Group I versus II P value |
|-------------------------------------------------|----------------|-----------------|--------------------------|
| Number of cells laden with melanin pigments     |                |                 |                          |
| Pre-operative*                                  | 192 (70-280)   | 187 (60-270)    | >0.05                    |
| 12 months post-operative*                       | 32 (10-90)     | 39 (10-80)      | >0.05                    |
| Intra group comparison*                         | P<0.05         | P<0.05          |                          |
| Density of pigments at baseline                 |                |                 |                          |
| Grade 2 (%)                                     | 2 (10)         | 4 (20)          | >0.05                    |
| Grade 3 (%)                                     | 18 (90)        | 16 (80)         | >0.05                    |
| Density of pigments at 12 months post-operative period |            |                 |                          |
| Grade 0                                         | 6 (30)         | 2 (10)          | >0.05                    |
| Grade 1                                         | 8 (40)         | 10 (50)         | >0.05                    |
| Grade 2                                         | 6 (30)         | 8 (40)          | >0.05                    |

*Values are median (minimum to maximum). P values are obtained by ANOVA with Bonferroni correction for multiple comparisons. The rest of the values are n (%), whose P values were obtained by χ² test. ANOVA: Analysis of variance
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