Efficacy of liquid nitrogen and electrocautery assisted gingival depigmentation in term of patient’s perception, histological wound healing - A randomized triple blind clinical trial

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Abstract:
Background: Hyper-melanin pigmentation of the gingiva (GMP) is one of the imperative contributory factors for smile-sensitive individuals. Numerous gingival depigmentation (GD) procedures have been attempted in the literature to evaluate the clinical outcome mostly. Hence, a randomized clinical-histopathological triple-blinded trial was planned to evaluate the pain experienced by the patient, gingival wound healing, and density of melanocytes following liquid nitrogen-assisted GD (LNAGD) and electrocautery-assisted GD (ECAGD) procedures. Materials and Methods: Thirty-two arches with bilateral physiologic labial/buccal GMP extending from distal aspect tooth #14–24 and #34–44 in 16 healthy individuals were selected and were equally treated with LNAGD and ECAGD techniques. Dummett oral pigmentation index and Hedin melanin index were evaluated at baseline and 3 months’ postoperatively (PO). The visual analog scale was utilized for the intensity of pain assessment at baseline (immediately after treatment) and 1st day and 7th day PO. Histological wound healing and density of melanocytes were evaluated using Gal et al.'s wound-healing assessment index and Patsakas et al.'s criterion, at baseline (0), 8, 24, 72, and 96 h; 1st, 2nd, 3rd, and 4th week; and 3 months and at 0 and 3 months' PO, respectively. Statistical analysis was done using one-way ANOVA, post hoc Tukey, unpaired, and paired "t" test. Results: Both groups showed a statistically significant influence on the parameters evaluated. Conclusion: The LNAGD had a substantial superior result in terms of early wound healing, reduction in density of melanocytes, reduction in pain experienced by the patient, with reduction and delay in the recurrence of GMP.

Key words: Density of melanocytes, electrocautery, gingival melanin pigmentation, liquid nitrogen, pain experienced, wound healing

INTRODUCTION

Different pathological diseases, conditions and factors like tissue vascularity, epithelial thickness, the magnitude of keratinization, physiological pigment/stains such as melanin, carotene, reduced haemoglobin and oxyhemoglobin contribute colour to the gingiva,[1,2] but hypermelanin pigmentation of the gingiva (GMP) is one of highly acknowledged and crucial factor other than face, facial symmetry, lip, teeth etc., which play a substantial role in the harmony of a smile. Advancement in cosmetic dentistry raised awareness and esthetic expectations among dental patients including considerable interest regarding gingival depigmentation (GD) procedures. GMP in the anterior esthetic region associated with unpleasant appearance is a prime factor that restricts the conscious patient to smile in a social circle/public gathering. Different invasive, minimal invasive, masking GD procedures are published in the web of literature, but histological evaluation of the outcome and patient’s perceptions have not been explored. Hence, a triple-blinded randomized clinico-histopathological trial was performed with the aim to evaluate the effect of liquid

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nitrogen-assisted GD (LNAGD) and electrocautery-assisted GD (ECAGD) on pain experienced by the patient, gingival wound healing, and density of melanocytes.

**MATERIALS AND METHODS**

The present randomized clinical trial was conducted in the Institutional Department of Periodontology and Oral Implantology, India. The clinical trial had performed as per the ethical standard outlines in the 1964 Declaration of Helsinki as revised in 2013. The institutional ethical committee provided their approval for the trial and was duly registered with ClinicalTrials.gov No. ID CTRI/2017/11/010718.

A total of 16 patients (3 females + 13 males) with 32 arches of GMP with an average age of 21.75 and 23 years, respectively, were selected out of 20 patients after listening in detail to the procedural techniques, objectives, benefits, and risks associated with the study protocol, after submission of informed written consent to participate in the trial as per the study design [Table 1].

15-30 years of systemically and periodontally healthy, otherwise aesthetically cognizant patients with bilateral physiologic GMP pigmentation on the labial gingiva extending from the distal aspect of tooth no. (#)14-24 and #34-44 of permanent dentition [Figures 1a and 2a], with minimal Grade 4 HMI score and Grade 3 DOPI including 3-4 months of availability of all the selected patients for study tenure were the inclusion criteria. Smokers, pregnant and lactating mothers, patients with syndromes, patients on drugs or metals exposure related to hyperpigmentation are the exclusion criteria. For the present trial, considering \( \alpha = 0.05 \), power: 90\%, confidence interval: 85\%, coefficient of variation (CV\%): 17.5, and \( n = 14 \), considering the unknown error, the sample size for the trial was increased to 16.

Trial patients underwent scaling 1 week before the GD procedure. In each patient, the maxillary and mandibular arch were randomly allocated to LNAGD (Group I) and ECAGD (Group II) by coin-toss method, respectively, by another trained clinician who was not the part of the trial. All the universal precautions were taken by the trained principal investigator during both the procedures at a different time interval.

In Group I, the labial gingival surface of the anterior jaw was thoroughly dried utilizing cotton gauze and air spray. The cotton ball was soaked with 15% lidocaine solution local anesthetic solution and kept over the labial gingiva for 10 min. Just before the surgery, baseline gingival partial-thickness biopsy was taken [Figure 1b] from first/second premolar of the first quadrant in all the cases without deep epithelization, and hemorrhage was controlled by direct pressure with gauze soaked in sterile saline after evaluating gingival thickness with a sterile endodontic k file with stopper. Rolled cotton of an earbud size and shape was dipped in liquid nitrogen for 5 s and applied for 20 s from canine site adjacent to the baseline biopsy to the first premolar of cross arch in a rolling motion each till the thawing occurred [Figure 1c]. Approximately, average of 18 ml of liquid nitrogen was utilized for each case and freezing and thawing time taken was approximately 45 s.

In Group II, after achieving local anesthesia as described in Group I, baseline gingival biopsy was taken [Figure 2a] as in case LNAGD. Bonart electrosurgical unit’s fine wire and loop electrode was utilized at mode 1 with 3RF/2MHz intensity in continuous feather brushing stroke [Figure 2b] method for 5-second interval application each under high power suction and intermittent cooling for 10 seconds till through gingival depigmentation were achieved [Figure 2c].

**Table 1: Dummett oral pigmentation index, Hedin index, and visual analog scale analysis at different time interval in Group I (liquid nitrogen-assisted gingival depigmentation) and Group II (electrocautery assisted gingival depigmentation) utilizing one-way ANOVA and intragroup comparison at different time intervals separately evaluated utilizing post hoc Tukey test**

| Time Interval | Mean±SD | Minimum | Maximum | F | P | Significant Pairs |
|---------------|---------|---------|---------|----|---|------------------|
| At 0 h        | Mean±SD | Minimum | Maximum | F  | P  | Significant Pairs |
| Mean±SD       | 3±0     | 0.25±0.462 | 2.875±0.353 | 141.67 | <0.001 | At 0 h versus 1 month |
| Minimum       | 0       | 0       | 3       | 1  | 1  | At 0 h versus 1 month |
| Maximum       | 3       | 1       | 2       | 2  | 2  | At 0 h versus 1 month |
| Pain at 0 h (immediately after GD) | Mean±SD | Minimum | Maximum | F | P | Significant Pairs |
| Pain at 0 h (immediately after GD) | Mean±SD | Minimum | Maximum | F | P | Significant Pairs |
| Mean±SD       | 0.125±0.353 | 0       | 3       | 73.405 | <0.001 | At 0 h versus 1 month |
| Minimum       | 0       | 0       | 3       | 3  | 3  | At 0 h versus 1 month |
| Maximum       | 3       | 1       | 4       | 4  | 4  | At 0 h versus 1 month |
| Pain at 1 day | Mean±SD | Minimum | Maximum | F  | P  | Significant Pairs |
| Pain at 1 day | Mean±SD | Minimum | Maximum | F  | P  | Significant Pairs |
| Mean±SD       | 0.875±0.64 | 0       | 3.5±0.534 | 77.237 | <0.001 | At 0 h versus 1 month |
| Minimum       | 0       | 0       | 3       | 3  | 3  | At 0 h versus 1 month |
| Maximum       | 3.5     | 1.2     | 4       | 4  | 4  | At 0 h versus 1 month |
| Pain at 1 week| Mean±SD | Minimum | Maximum | F | P | Significant Pairs |
| Pain at 1 week| Mean±SD | Minimum | Maximum | F | P | Significant Pairs |
| Mean±SD       | 3.5±0.462 | 0       | 0.25±0.462 | 77.237 | <0.001 | At 0 h versus 1 month |
| Minimum       | 0       | 0       | 0.5     | 0.5 | 0.1 | At 0 h versus 1 month |
| Maximum       | 0.25±0.462 | 0       | 0.375±0.744 | 0.0371 | <0.05 | At 0 h versus 1 month |

*P<0.05 significant. GD – Gingival depigmentation; SD – Standard deviation; F – test statistics; P – Probability Value*
Patients were instructed with postoperative (PO) oral hygiene measures such as 0.2% chlorhexidine mouth use two times a day with 10 ml of solution, quit brushing for 7 days on the site of treatment, and SOS analgesic (acetaminophen 500 mg) only if patient experience severe pain in both the groups. Patients have asked to define the intensity of pain experienced using visual analog scale (VAS) consisting of a horizontal line of 10 cm long, anchored by two ends, where the left end at “0” denotes no pain and right end at “10” represents unbearable pain; score pain as: 0 - no pain, 1–3 slight, 3–6 - moderate but well tolerable, 6–10 - severe pain at 0 (immediately after treatment) h, 1 day, and 1 week after procedures and number of analgesics consumed if any. Every second arch treatment in both the groups was carried out only after the completion of healing and follow-up of the first site.
DOPI was utilized for the intensity of pigmentation and HMI for the extent of pigmentation (clinically) was evaluated at baseline (0 - before procedure) and 3 months’ PO. The intensity of pain experienced by the patient was evaluated during the surgery, at 1 day, and 1 week PO using VAS. To validate the influence of both GD techniques on density of GMP and periodontal wound healing evaluated histologically through eight sets of minimally invasive three partial-thickness gingival biopsies for each operated site without deepithelization from the first/second premolar at baseline (0h), first premolar of cross arch and canine (0.8 h and 3 month; 0.24h and 3 month; 0.72h and 3 month; 0.96h and 3 month; 0h,1week and 3 month; 0h,2 weeks and 3 month; 0h,3 weeks and 3 month and 0h, 4week and 3 months) respectively in both groups after obtaining written signed consent of the patients to prevent ethical bias.

The blinded gingival biopsies were sent to the institutional department of oral pathology for routine tissue processing and histological assessment utilizing indices of Gal et al.[3] and Patsakas et al.[4]

The oral pathologist, principal investigator, and statistician were blinded by coding the specimens, which was carried out by a trained clinician who was not part of the trial. The preoperative and PO values obtained were statistically analyzed using one-way ANOVA, post hoc Tukey, unpaired “t” test, paired “t” test by the principle investigator utilizing SPSS Inc., PASW Statistics for Windows, Version 18.0., Chicago, IL, USA. After the statistical analysis, the decoding was done and the outcome obtained.

RESULTS

DOPI and HMI were statistically significant (P < 0.05) at different intervals for both groups. DOPI, HMI, and VAS for pain assessment between the different time intervals (0–1, 0–3, and 1–3-month PO) in Group I showed significant pair difference (P < 0.05) between 0–1 and 0–3 months whereas in Group II at all intervals [Table 1]. Intergroup comparison of the DOPI as well as HMI score was reported to be significant (P < 0.05) only at 3-month comparison [Table 2].

Intragroup comparison of the VAS reported at a different interval for both groups showed statistically significant results (P < 0.05). Intragroup comparison of the VAS between 0–1 day, 0–1 week, and 1–7 days PO showed significant pair difference (P < 0.05) between 0–1 day and 0–1 week in Group I whereas in Group II between all intervals [Table 1]. Intergroup comparison of the VAS score reported a statistically significant difference (P < 0.05) between the 0 and 1 day PO comparison only [Table 2].

Intragroup comparison of the density of GMP at baseline and 3 months’ PO for Group I and Group II showed highly significant results (P < 0.001) only [Table 3].

Wound-healing status of Group I

At baseline: Para-keratinized stratified squamous epithelium (PKSSE) showed hyperplastic long rete ridges with focal melanin pigmentation in the basal and parabasal layer. The underlying fibrous connective tissue (CT) showed foci of chronic inflammation and few endothelium-lined channels [Figure 1d]. At 8h: PKSSE showed with foci of degeneration

**Figure 3:** (a) Long rete ridges, (b) focal keratinization, (c) hyperplastic rete ridges (blue arrow), and (d) complete epithelization (yellow arrow) with focal melanin pigmentation (brown arrow) of the gingival biopsies taken at 2, 3, and 4 weeks and 3 months’ postoperatively of Group I (H and E, ×10). (e) and (f) Clinical observation at baseline and 3 months’ postoperative of Group I

**Figure 4:** (a-d) Photomicrographs of gingival biopsies taken at 2, 3, and 4 weeks and 3 months’ postoperatively of Group II (description details in the image, H and E, ×10). (e) and (f) Clinical observation at baseline and 3 months’ postoperative of Group II
## Table 2: Intergroup comparison of Dummett oral pigmentation index, Hedin index, and visual analog scale of Group I (liquid nitrogen-assisted gingival depigmentation) and Group II (electrocautery-assisted gingival depigmentation) utilizing independent $t$-test

| Parameters | Group I versus II | Group II versus II | VAS Group I versus II |
|------------|-------------------|--------------------|----------------------|
| **DOPI** | At 0 h | At 1 month | At 3 months | At 0 h | At 1 month | At 3 months | Pain at 0 h | Pain at 1 day | Pain at 1 week | Pain at 0 h | Pain at 1 day | Pain at 1 week | Pain at 0 h | Pain at 1 day | Pain at 1 week |
| Mean | 2.875 | 3 | 0.125 | 0.25 | 0.875 | 0.25 | 3.375 | 3.5 | 0.375 | 0.25 | 1.125 | 0.375 | 3.625 | 0.625 | 1.75 | 0.125 | 0.0 |
| Variance | 0.125 | 0 | 0.125 | 0.2142 | 0.4107 | 0.2142 | 0.2678 | 0.2857 | 0.5535 | 0.2142 | 0.6964 | 0.553571 | 0.553571 | 0.5 | 0.125 | 0 | 0 |
| $t$ statistic | -1 | 0.6069 | 2.2360 | 0.4751 | 0.4034 | 1.8973 | 8.064258 | 5.813777 | 65535 | 0.125 | 0.0 |
| $P$ ($T \leq t$) two-tail | 0.3506 | 0.5543 | 0.0435 | 0.6419 | 0.6963 | 0.0785 | <0.001 | <0.001 | NA |

$P$<0.05 significant. DOPI – Dummett oral pigmentation index; VAS – Visual analog scale; LNAGD – Liquid nitrogen-assisted gingival depigmentation; ECAGD – Electrocautery-assisted gingival depigmentation; NA – Not available; $P$ – Probability value; $t$ – Calculative value

## Table 3: Intragroup and Intergroup evaluation of the density of melanocytes in Group I (liquid nitrogen-assisted gingival depigmentation) and Group II (electrocautery-assisted gingival depigmentation) at baseline and 3 months’ postoperative utilizing paired $t$-test and unpaired $t$-test, respectively

| Parameters | Intragroup comparison of melanocyte density in Group I and II | Test Parameters | Intergroup comparison of melanocyte density in Group I and II |
|------------|-------------------------------------------------------------|-----------------|-------------------------------------------------------------|
| **Intragroup** | Mean density at 0 h | Mean density at 3 months | Mean density at 0 h | Mean density at 3 months | Mean density at 0 h | Mean density at 3 months | Mean density at 0 h | Mean density at 3 months |
| Group I | 2.5 | 1.125 | 2.875 | 0.625 | 0.744024 | Mean | 2.500 | 2.875 | 1.125 | 0.625 |
| Group II | 0.534522 | 0.834523 | 0.353553 | 0.740247 | 0.125 | 0.696 | 0.554 |
| $t$ statistic | 3.666667 | 7.19516 | 0.007999 | 0.000181 | -1.655 | 1.265 | 0.124 | 0.227 |
| $P$ ($T \leq t$) two-tail | <0.001 | <0.001 | NA |

$P$<0.05 significant. $P$ – Probability value; $t$ – Calculative value
and loss of rete ridges with focal necrosis [Figure 1e]. The underlying CT showed a moderate amount of chronic inflammatory cells with lymphocytes predominance. At 24 h: The PKSSE showed degeneration in some areas with desquamation and complete loss of rete ridges. The underlying CT showed diffuse inflammation [Figure 1f]. At 72 h: The underlying cellular CT showed areas of degeneration and foci of chronic inflammation along few endothelium-lined vascular channels. The PKSSE showed hyperplasia in few rete ridges [Figure 1g]. At 96 h: The PKSSE showed hyperplasia with foci of degeneration. The underlying CT showed degeneration with foci of extravasated erythrocytes [Figure 1h].

At 1st week, PKSSE showed the incomplete formation of rete ridges. The underlying CT stroma was loose in nature with marked chronic inflammatory cell infiltrates predominately lymphocytes and few small budding capillaries [Figure 1i]. At 2nd, 3rd, and 4th week: PKSSE showed long rete ridges in the 2nd week [Figure 3a], foci of hyperkeratosis in the 3rd week [Figure 3b], and hyperplastic rete ridges by the 4th week [Figure 3c]. The underlying CT stroma was dense, fibrous with small and medium-sized capillaries and extravasated erythrocytes. At 3 months: PKSSE showed long rete ridges and foci of hyperkeratosis with well-defined architecture. Focal chronic inflammatory cell infiltrate is evident within the Fibrous CT [Figure 3d]. The gingiva showed rare and scattered melanin granules corresponding to mild pigmentation 3 months post operatively [Figure 3f].

**Wound healing status of Group II**

At baseline: PKSSE with hyperplasia and hyperkeratosis with foci of melanin pigmentation in the basal and parabasal region. The underlying fibrous CT showed mild inflammation and few endothelium-lined channels [Figure 2d]. At 8 h: Histopathological examination showed complete deep epithelization; the underlying CT showed degeneration, focal inflammation predominantly of lymphocytes, and extravasated erythrocytes [Figure 2e]. At 24 h: there is no evidence of epithelium; the underlying CT was loose fibrous in nature with foci of granulation tissue [Figure 2f]. At 72 h: It showed a moderate degree of inflammation within the degenerated CT [Figure 2g]. At 96 h: Epithelial formation is seen in focal areas. The underlying CT is fibrous and cellular with foci of chronic inflammation [Figure 2h].

At 1st week: PKSSE is seen increasing in thickness with a cellular CT [Figure 2i]. At 2nd week: PKSSE increases in thickness with decreased inflammation in the CT [Figure 4a]. At 3rd week: PKSSE showed broad elongated rete ridges with focal hyperplasia along with dense, fibrous CT with mild inflammation [Figure 4b]. At 4th week: PKSSE has increased in thickness, and the underlying fibrous CT showed mild inflammation [Figure 4c]. At 3 months: PKSSE is of variable thickness showing hyperplasia in few areas and underlying fibrous CT showed mild inflammation and few endothelium-lined channels [Figure 4d]. The gingiva showed dense but not aggregates of melanin granules corresponding to moderate pigmentation at 3 month post operatively [Figure 4f].

All biopsy sites healed uneventfully without any complications. No other complication observed in both groups except mild–moderately tolerable pain and recurrence of melanin histologically in four cases of ECAGD.

**DISCUSSION**

Endogenous melanin is the most common inherent brownish/black stain that imparts color to the gingiva. GMP may be physiological, may be pathological, or may be the source of local and systemic influences, such as tobacco use and prolonged anti-malarial.\(^9\) Therefore, except physiological GMP, rest of the pathological, local, and systemic factors that might influence/precipitate the GMP were excluded from the trial.

GD is a plastic operating procedure for the elimination or reduction of GMP by surgical scalpel techniques,\(^6-9\) electrosurgery,\(^9,10\) lasers,\(^11-13\) free gingival graft,\(^14\) cryosurgery-assisted GD,\(^15-17\) etc., Diode laser, ECAGD, and LNAGD techniques are minimally invasive techniques that can be performed under topical anesthesia. Looking after the cost of diode laser equipment, technique sensitization and cost of diode laser-assisted GD, it was dropped. Controlled tissue destruction by freezing can be achieved by utilizing different materials such as liquid CO\(_2\), liquid nitrogen, a mixture of ice and salt, and liquid NO\(_2\) in day-to-day medical practice. Liquid nitrogen is an easily available, economical, safe, and popular cryogen. Its popularity is because of its low temperature achievable (−197°C) which makes it suitable option for the management of orofacial lesions, such as vascular malformations, hyperkeratosis and leukoplakia, granulomatous and hyperplastic conditions, mucus cysts and polyps, erosive conditions, and recurrent nasopharyngeal carcinoma, as cited in the report of Bansal et al.\(^18\) It is also an effective treatment modality against a broad range of benign problems.\(^19\) Benign skin lesions include actinic keratosis, viral wart, molluscum contagiosum, and dermatofibroma,\(^20\) condyloma acumina (CA) is a common viral sexually transmitted disease.\(^21\) As liquid nitrogen freezes the pigmented tissue quickly and destroys the pigmented cells that may be the reason, its use is recommended in the management hyper-melanin depigmentation techniques. Electrosurgery has a strong influence in the retarding migration of melanin cells from locally situated cells\(^22\) and has advantages such as the absence of bleeding and patient discomfort.\(^23\) Therefore, liquid nitrogen and electrocautery techniques were considered for the present trial. To the best of our information, our study is the first triple-blinded randomized clinical trial evaluated the effect of LNAGD and ECAGD on pain experienced by the patient, gingival wound healing, and density of melanocytes. Hence, direct comparisons with former studies were not possible.

LNAGD was highly effective in reducing the intensity and extent of GMP (DOPI and Hedin) up to 3 months [Figure 3e]. The outcome achieved was in agreement with the clinical trials of Narayankar et al.,\(^24\) Esfandiary et al.\(^25\) concluded that cryosurgery in GD offered effective clinical outcome; it is as effective as diode laser-assisted GD till 6 months without recurrence, respectively. Reports of Patil et al.\(^26\) and Kumar et al.\(^27\) reported that cryosurgery is a better and effective GD technique as it destroys gingival epithelium without causing the damage to the CT, which is also in consistency with the
trail. LNAGD treatment was extremely effective in decreasing the density of melanocytes too [Figure 3d]. The reason for the same might be because (i) gingival thawing occur due to liquid nitrogen application induce rapid freezing of water within the gingival tissue and slow melting responsible for tissue destruction and (ii) liquid nitrogen also participated in cell dehydration, enzyme inhibition, protein denaturation and thermal shock-induced cell death, alteration of tissue vasculature, and immune reaction, which leads to cell expiry.\textsuperscript{[30]} However, the relevance of immunological injury is still controversial.\textsuperscript{[29,30]} Finally, it has been theorized that freezing involves vascular injury.\textsuperscript{[31]} The hypothesis is that capillary blood flow stasis with in the tissue, resulting in ischemia leads to tissue necrosis.

In LNAGD group of patients, the gingival deepithelization completed by 96 h PO, but reepithelization healing process started from approximately 1 week and completed in 2 weeks. The outcome achieved is consistency with the report of Kumar et al.\textsuperscript{[27]}

Pain induction reported till 1-day PO but is well tolerable utilizing VAS.\textsuperscript{[32]} VAS method was selected because it is well recognized, reviewed extensively, and found to be a trustworthy method.\textsuperscript{[33]} The outcome achieved was in accordance with the reports of Narayankar et al., Rehmati et al., Parvez M which disclosed mild pain in their patients\textsuperscript{[24,34,35]} respectively but in contrary to Shirazi et al observed no pain following deepigmentation.\textsuperscript{[36]} The exact reason for the early reepithelization is not known till date, but the release of blood constituents from damaged vascular channel begins the process of tissue healing.\textsuperscript{[37]}

ECAGD effectively reduced the intensity and extent of GMP (DOPI and Hedin) up to 3 months’ follow-up. The outcome achieved was in consistency with the reports of Gupta et al.\textsuperscript{[3]} being reported electrocautery is better than a surgical technique for the management of GD. Gufran\textsuperscript{[38]} observed only the intensity of pigmentation utilizing DOPI index at 0, 1, and 6 months’ interval, which is inconsistence with our report on intragroup comparison only but in contrary on intergroup comparison; the reason for the same may be because they have compared electrocautery versus surgical technique. In the present trial, repigmentation was observed in four cases out of 16 sites treated which is in accordance to the report of Elavarasu et al.\textsuperscript{[22]} who concluded that diode laser GD has less gingival repigmentation than electrocautery at 3-month PO.

ECAGD treatment was highly effective in reducing the density PO too. The reason for outcome achieved may be because (i) melanin cells present in the basal and suprabasal cell layers of operated and surrounding sites experienced molecular disintegration following electrical energy application-assisted stimulation by electrocautery and delaying the relocation of melanin cells from the locally situated cells.\textsuperscript{[39]}

Pain induction is mild–moderate up to 1st day PO but well tolerated by Group II patients. The outcome achieved was in accordance with the reports of Gupta et al.\textsuperscript{[30]} and Chandana and Kedige.\textsuperscript{[31]} Although the pain is reported in both the groups, pain score is moderately higher in ECAGD; the reason for the same may be because of the utilization of topical anesthesia but none of the patients consumed even single analgesic tablet.

In ECAGD, gingival deepithelization completed at 0 h PO. Reepithelization started from approximately at 96 h and almost completed at 3–4 weeks PO which was the first report to evaluate the periodontal wound healing histologically from ECAGD, so direct comparison is not available, but clinically, it is consistency with the report of Ksagani et al. who evaluated clinical epithelization only.\textsuperscript{[40]}

Different authors utilized different histological assessment of wound-healing criterion in their proposed indices. Gal et al.\textsuperscript{[41]} used both semi-quantitative assessment (wound re-epithelialization: migration of keratinocytes, bridging of cells, keratinization; inflammatory cells: Absence/presence; fibroblasts: Absence/presence; new vessels: absence/presence mild/moderate/marked; collagen: Absence/presence mild/moderate/marked; and quantitative method assessment (polymorph nuclear leukocytes/tissue macrophages ratio; percentage of reepithelialization and area of the granulation tissue). Patsakas et al. determined the distribution of melanin granules in different anatomical areas of the gingiva and to relate the density of melanin granules to the degree of gingival inflammation. Abramov et al.\textsuperscript{[42]} evaluated that the surgical wound-healing process in both the vagina and abdomen includes transient acute and chronic inflammation, fibroblast proliferation, and neovascularization, as well as progressive maturation of granulation tissue, reepithelialization, and collagen deposition and scored as 0–3, but none of them disclosed the grade of wound healing (good/fair/poor). Therefore, on the basis of the histological findings of our study samples, Sanjeev Kumar Salaria, Karthikeyan Ramalingam and Sanjeev Kamboj proposed a modified, simple, economical, semi-quantitative wound-healing assessment index after GD [Table 4] adapted from proposed healing indices by Gal et al.,\textsuperscript{[41]} Patsakas et al.,\textsuperscript{[42]} Abramov et al.,\textsuperscript{[42]} and Gupta and Kumar.\textsuperscript{[43]} If we apply this index to our study sample, we attained an average score of 13 for LNAGD (excellent healing) and 22 for ECAGD (good healing).

**CONCLUSION**

Both the treatment modalities were effective in the management of GMP, but LNAGD had a significantly superior edge over ECAGD technique in terms of wound healing and reduction in density of melanocytes and pain experienced by the patient. Looking after the limitations such as small sample size and short-term trial, further, long-term randomized clinical controlled trial on large sample size is recommended to validate the conclusion.

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Table 4: Salaria and Karthikeyan Ramalingam proposed modification of semi-quantitative wound-healing assessment index after gingival depigmentation

| Score | Nature of epithelialization | Degeneration of epithelium | Melanin pigmentation preoperative* | Melanin pigmentation postoperative* | Distribution of inflammation | Neutrophils | Lymphocytes | Plasma cells | Fibroblasts | Nature of collagen | New blood vessels | Extravasation of erythrocytes | Granulation tissue |
|-------|----------------------------|-----------------------------|-----------------------------------|-----------------------------------|-----------------------------|-------------|-------------|--------------|-------------|-----------------|----------------|------------------------|------------------|
| 0     | Absence of epithelium      | Absent                      | Absent                            | Absent                            | Mild, focal                | Few, focal | Few, focal | Few, focal   | Few, focal   | Immature fibrils  | Absent         | Absent                 | Absent           |
| 1     | Partial epithelial closure | Restricted to basal third   | Rare and scattered melanin granules | Rare and scattered melanin granules | Mild diffuse               | Few, diffuse| Few, diffuse| Few, diffuse | Few, diffuse | Reticular pattern | Mild (<2 per HPF)| Absent                  | Minimal, subepithelial/superficial lamina propria |
| 2     | Complete epithelial closure| Involving up to middle third | Dense but not aggregated melanin granules | Dense but not aggregated melanin granules | Moderate and focal | Moderate, focal | Moderate, focal | Moderate, focal | Vertical fibers | Moderate, focal | Moderate, focal | Moderate, extension into deep lamina propria |
| 3     | Epithelial closure with spinous cell/granulocyte differentiation | Involving more than middle third | Dense and aggregated melanin granules | Dense and aggregated melanin granules | Moderate and diffuse | Moderate, focal | Moderate, focal | Moderate, focal | Moderate and diffuse | Mixed | Moderate, diffuse, filling the deep lamina propria |
| 4     | Epithelial hyperplasia/epithelium with complete maturation and keratinization | Involving the entire epithelium | Dense aggregations of melanin granules with melanin incontinence into subepithelial connective tissue | Dense aggregations of melanin granules with melanin incontinence into subepithelial connective tissue | Intense inflammation (focal/diffuse) | Intense (focal/ diffuse) | Intense (focal/ diffuse) | Plenty | Horizontal fibers or fascicles | Marked (>10 per HPF) | Plenty/abundant granulation tissue |

Adapted from wound-healing model proposed by Abramov et al., Gal et al., Gupta et al., and Patsakas et al. The proposed criteria use Hematoxylin and Eosin stain for assessment. *Melanin location could be enhanced using Masson-Fontana stain, #Early and mature collagen could be differentiated with Masson trichrome stain. Healing status: Excellent – 9–13, Good – 14–26, Fair – 27–39, Poor – 40–52. RBCs – Red blood cell, HPF – Histopathological findings.
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Conflicts of interest
There are no conflicts of interest.

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