Original Article

DOI: 10.7860/JCDR/2023/64252.18598

Dentistry Section

Gingival Depigmentation by Application of Diode Laser at 810 nm (Denlase) and 470 nm (Bluelase) Wavelengths: A Split-mouth Randomised Clinical Trial

GAUTAMI S PENMETSA[1], LAHARI KARUTURI[2], GNVS SRUTHIMA[3], AV RAMARAJU[4], RAJYALAKSHMI MIKKILI[5], MOHANA KONDAPALLI[6], SRIVIDYA INTI[7], SRAVANTHI VUNDAVALLI[8]

ABSTRACT

Introduction: Gingival hyperpigmentation is a common aesthetic concern that often requires treatment to achieve a pleasing smile. While various methods for depigmentation have been described in the literature, the scalpel method is widely advocated. Laser technology, particularly diode lasers, has emerged as a preferred treatment option due to their unique properties.

Aim: To compare the efficacy of two different diode wavelengths, namely Denlase and Bluelase lasers, in terms of pain intensity, aesthetic appraisal, and the rate of repigmentation.

Materials and Methods: This split-mouth randomised clinical trial was conducted in the Department of Periodontics at Vishnu Dental College, Bhimavarm, Andhra Pradesh, India. over a period of one year with follow-up. A total of 16 patients were included, and the right and left quadrants (both maxilla and mandible) were randomly assigned to either the Denlase or Bluelase group using the coin toss method. Depigmentation using diode lasers at 810 nm and 470 nm wavelengths was carried out in the respective groups. Pain perception, aesthetic appraisal, and recurrence of pigmentation were evaluated at 1, 3, 6, 9 months, and one year. The data were analysed using Friedman's analysis and the Mann-Whitney U-test.

Results: A total of 16 subjects, with a mean age of 27.64±7.64 years and of both genders, were included in the study. Statistically significant differences (p=0.001) were reported in pain perception, aesthetic appraisal, and the rate of repigmentation at 1, 3, 6, 9 months, and one year, respectively. Intergroup comparison revealed an increase in the mean amount of repigmentation at three months in the Denlase group, indicating a recurrence of pigmentation after three months.

Conclusion: The Bluelase diode laser (470 nm) demonstrated superior results compared to the Denlase diode laser (810 nm) in terms of pain intensity, aesthetic appraisal, and repigmentation.

Keywords: Aesthetics, Low-power laser therapy, Pigmentation, Semiconductor diode

INTRODUCTION

Of late, pink aesthetics are considered to play a major role in enhancing an individual's overall smile, unlike in the past when smile designing was restricted to only white aesthetics [1,2]. Hyperpigmentation of the gingiva, which is a major aesthetic concern, especially among younger generations who have an increasing desire to look more beautiful and attractive, has become a primary concern [2]. This hyperpigmentation, resulting from excessive melanin production in the basal and suprabasal layers of the epithelium, is usually physiological and does not manifest any medical problems [2,3]. However, certain conditions and syndromes such as Peutz-Jeghers syndrome, Albright syndrome, melasma, and Graves' disease can cause increased melanin secretion [1]. Although it is a physiological condition, patients are more concerned about the cosmetic and aesthetic aspects [3,4].

Various techniques have been used for gingival depigmentation, including gingivectomy, mucosal excision by scalpel, abrasion technique, free gingival grafts, and chemical methods such as electrosurgery, cryosurgery, and lasers [3-8]. However, these techniques have drawbacks such as the need for local anaesthesia, bleeding, and postoperative pain [5-7]. Laser technology, a recent innovation, has emerged as a boon for patients as it eliminates the need for local anaesthesia and reduces bleeding and postoperative pain, ensuring minimal discomfort and increasing patient acceptance due to shorter surgical time [1,5]. Different types of lasers, such as Carbon Dioxide (CO_2) lasers, ND:YAG lasers, semiconductor diode lasers, argon lasers, and Er:YAG lasers, have been used and have shown acceptable results in treating hyperpigmentation [2,9-14]. Diode lasers, in particular, are indicated due to their high absorption capacity and limited penetration rate, thus showing promising results for both clinicians and patients [3,4]. Diode lasers have flexible wavelengths, including 810 nm, 940 nm, and 980 nm, and the literature has shown positive results with the use of diode lasers in various applications [5]. Bluelase, a new form of laser with a lower wavelength of 470 nm, has various applications such as crown lengthening, frenectomy, and biostimulation [4]. In the field of laser dentistry, it can be applied for treating gingival depigmentation, as it has an increased scattering effect and higher absorption of haemoglobin and melanin compared to other diode lasers [4]. This unique feature of Bluelase led us to evaluate the effectiveness of different diode wavelengths, specifically Denlase (810 nm) and Bluelase (470 nm).

The main objective of present study was to compare the conventional 810 nm laser with the low-level 470 nm Blue laser, as there is a lack of literature on this topic, with only one recent case report published [7]. To date, no study has compared these two diode lasers (Denlase and Bluelase) with wavelengths of 810 nm and 470 nm, respectively, in a split-mouth design for the treatment of gingival pigmentation. Therefore, present study aimed to provide insights into the efficiency of different wavelengths in treating gingival depigmentation.

MATERIALS AND METHODS

This was a randomised clinical trial with a split-mouth design. A total of 16 participants, aged between 18 and 30 years and from both

genders, with complaints of hyperpigmented gingiva on the facial aspect, who reported to the Department of Periodontics at Vishnu Dental College, Andhra Pradesh, India, were selected for the study. The trial was conducted from August 2021 to August 2022, with a duration of one year follow-up. The participants were assigned based on Dummett's criteria [6] for scoring hyperpigmentation. Ethical approval was obtained from the Institutional Ethics Committee (IECVDC/20/PG01/PI/IVV/10) and CTRI (CTRI/2021/08/035599). The participants were provided with detailed explanations of the procedure and the chances of recurrence, and written informed consent was obtained. Scattering effect was taken into consideration at dual levels, from both the patients' and clinicians' perspectives, and protective eyewear was judiciously used. Utmost care was taken to prevent scattering to adjacent quadrants by proper isolation with cotton rolls.

Sample size calculation: The sample size calculation was performed to determine the sample size for the change in gingival depigmentation as the primary outcome using G*Power 3.1.9.4 software. The calculations were based on an effect size of 0.82, an alpha level of 0.05, and a desired power of 80% for a split-mouth study. The estimated sample size was 14. Considering a 10% loss of follow-up, 16 subjects were included in the study [1].

Inclusion criteria: Patients with the chief complaint of hyperpigmented gingiva on the facial aspect and a desire for aesthetic improvement and patients with moderate to severe physiological hyperpigmentation in the aesthetic zones were included in the study.

Exclusion criteria: Patients with a history of hyperpigmentation due to drug usage and patients with diseases that could compromise healing (uncontrolled diabetes, autoimmune diseases) and with acute gingival and periodontal diseases such as necrotising lesions, and abscesses, pregnant or lactating women, with pathologic hyperpigmentation such as heavy metal pigmentation, haemangioma, graphite tattoo, and amalgam tattoo and smokers were excluded from the study.

Study Procedure

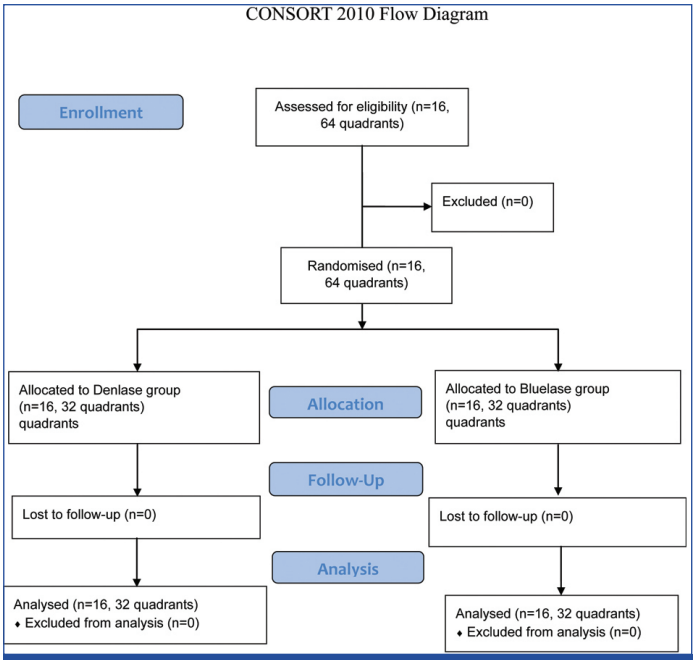
Thorough clinical examinations and detailed medical histories were recorded to rule out any pathological hyperpigmentation (heavy metal pigmentation, haemangioma, graphite tattoo, and amalgam tattoo). Oral prophylaxis was subsequently performed to ensure that the gingiva was disease-free. Sixteen patients were included in present study, with the right and left quadrants (both maxilla and mandible) randomly assigned to two groups using the coin toss method.

Laser equipment: Two laser equipments with different wavelengths were used in present study:

1. Denlase with 810 nm (FONA, China).
2. Bluelaser with 470 nm (PIOON, China).

Depigmentation with laser was performed using 810 nm in the Denlase group and 470 nm in the Bluelase group. Therefore, a total of 64 quadrants were treated with diode lasers of Varying wavelengths. The Consolidated Standards of Reporting Trials (CONSORT) has been depicted in [Table/Fig-1]. Preoperative images were taken using a digital camera (Canon 350D, Tokyo, Japan). FDA laser safety guidelines were followed, and safety eyeglasses were worn by both the patient and the operator. The entire procedure was performed under topical anaesthesia (two sprays of 10% lidocaine). Even though the laser technique is a painless approach, topical anaesthesia was used to minimise the discomfort caused by the heat waves generated during the procedure.

After preparing the patient, the laser procedure was performed on the same day, depending on the smile line. The first and fourth



[Table/Fig-1]: The CONSORT flow diagram.

quadrants were treated with the 810 nm diode laser (FONA, China), and the second and third quadrants were treated with the 470 nm Bluelase (PIOON, China). The lasers were operated in contact mode with continuous wave for approximately 60 seconds, using a 400 nm fibre optic cylindrical handpiece in a sweeping brush technique. Care was taken to avoid excess heat and charring of the tissues.

After the procedure, patients were asked to record their pain intensity levels using the Visual Analogue Scale (VAS) immediately after treatment. The VAS for pain is a straight line with one end representing no pain and the other end representing the worst pain imaginable. Aesthetic appraisal was also assessed at one week using a specially designed questionnaire consisting of three grades (excellent, satisfactory, and unsatisfactory) [5].

The rate of repigmentation at 1 month, 3 months, 6 months, 9 months, and 1 year, using the Dummett oral pigmentation index, was also evaluated [6]. The index is as follows:

0- No clinical pigmentation (pink-coloured gingiva)

1- Mild clinical pigmentation (mild light brown colour)

2- Moderate clinical pigmentation (medium brown or mixed pink and brown colour)

3- Heavy clinical pigmentation (deep brown or bluish-black colour)

Patients were advised not to consume hot or spicy foods and were instructed to avoid brushing in the treated area for one week. They were also instructed to rinse with warm water twice daily. No analgesics were prescribed, and patients were advised to take diclofenac 100 mg if they experienced severe pain.

STATISTICAL ANALYSIS

Intergroup analysis was conducted using the Mann-Whitney U test, and intragroup analysis was performed using Friedman analysis with Statistical Packages for the Social Sciences (SPSS) version 21.0. A p-value <0.05 was considered statistically significant.

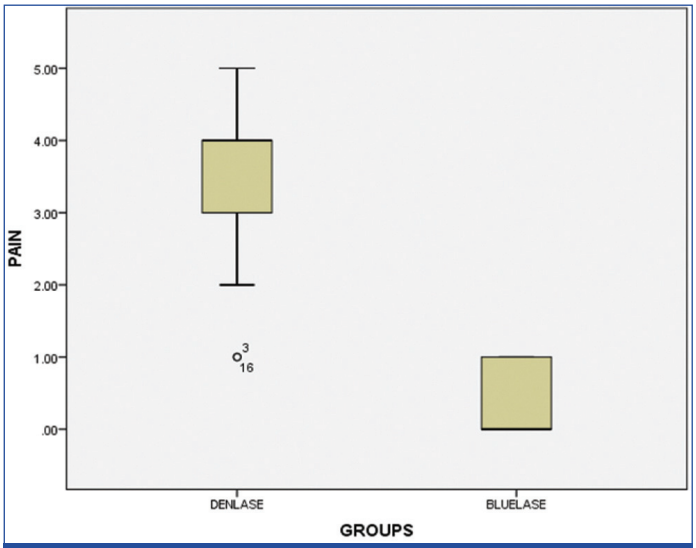
RESULTS

The mean age of the subjects was 27.64±7.64 years. A total of 16 subjects of both genders were included in present study. A total of 64 quadrants were treated, with 32 treated with Denlase and 32 treated with Bluelase. No adverse effects were observed in any of the cases during the study.

Pain perception: The pain score was 3.4±1.2 with Denlase and 0.3±0.5 with Bluelase, with a statistically significant difference in pain perception at baseline between the two groups (p<0.001**), as shown in [Table/Fig-2,3]. This indicates that patients treated with Bluelase (470 nm) reported less pain and minimal discomfort compared to treatment with Denlase (810 nm).

Group	Mean	Median	Standard Deviation (SD)	Mann-Whitney U value	p-value
Denlase	3.5000	4.000	1.21106	6.000	<0.001**
Bluelase	0.3750	0.000	.50000		

[Table/Fig-2]: Intergroup comparison of patients' pain perception between Denlase (810 nm) and Bluelase (470 nm).
*statistically significant, Mann-Whitney U test

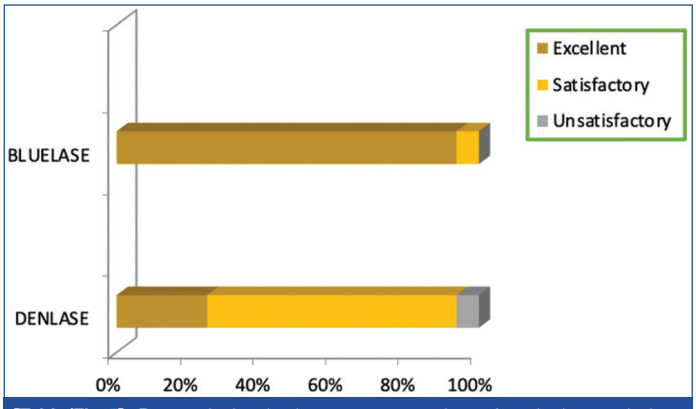


[Table/Fig-3]: Box plot showing intergroup comparison of patients' pain perception between Denlase (810 nm) and Bluelase (470 nm).

Aesthetic appraisal: In terms of aesthetic appraisal, approximately 93.8% of participants reported excellent patient satisfaction levels with Bluelase, while treatment under Denlase (810 nm) was reported to be satisfactory by 68.8% of the sample. Furthermore, 1% of the participants reported unsatisfactory outcomes with Denlase. A statistically significant difference was observed between the groups in terms of aesthetic appraisal (p<0.001**), as shown in [Table/Fig-4,5].

Group	Excellent	Satisfactory	Unsatisfactory	Chi-square value	p-value
Denlase	4 (25)	11 (68.8)	1 (6.2)	15.707	<0.001**
Bluelase	15 (93.8)	1 (6.2)	0		

[Table/Fig-4]: Intergroup comparison of aesthetic appraisals between Denlase and Bluelase groups.



[Table/Fig-5]: Bar graph showing intergroup comparison of aesthetic appraisal.

Rate of repigmentation: Repigmentation was evaluated at different time intervals (1 week, 3 months, 6 months, 9 months, and 1 year) in both groups. In the Denlase group, the mean value at one week was 0.0±0.0, which eventually increased to 0.06±0.25 at three months. However, there was a significant change in the score at one year, with a mean value of 0.5±0.6. Repigmentation started to occur after three months in this group (p<0.001**).

In the Bluelase group (470 nm), the mean value at one week was 0.0±0.0, and interestingly, no traces of repigmentation were seen until nine months. Slight repigmentation started at one year, with mean values showing 0.18±0.40. This indicates that repigmentation was delayed and less prominent in the Bluelase group compared to the Denlase group [Table/Fig-6].

Group	Time	Mean±SD	p-value
Denlase	1 week	0.0000±0.00000	<0.001**
	3 months	0.0625±0.25000	
	6 months	0.0625±0.25000	
	9 months	0.2500±0.44721	
	1 year	0.5000±0.63246	
Bluelase	1 week	0.0000±0.00000	0.017*
	3 months	0.0000±0.00000	
	6 months	0.0000±0.00000	
	9 months	0.0000±0.00000	
	1 year	0.1875±0.40311	

[Table/Fig-6]: Intragroup comparison of repigmentation at different time intervals.
*statistically significant, Friedman's analysis

On Intergroup analysis, no significant difference in repigmentation was found between the two groups during all the time intervals [Table/Fig-7]. Depigmentation using both Denlase (810 nm) and Bluelase (470 nm) is illustrated in [Table/Fig-8].

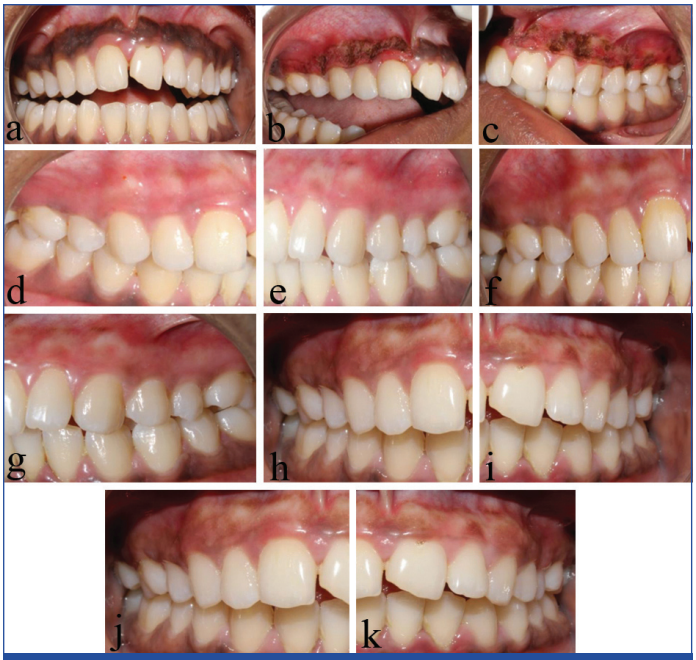
Time	Groups	Mean±SD	p-value
1 week	Denlase	0.000±0.0	1.000
	Bluelase	0.000±0.0	
3 months	Denlase	0.062±0.25	0.780
	Bluelase	0.000±0.0	
6 months	Denlase	0.062±0.25	0.780
	Bluelase	0.000±0.0	
9 months	Denlase	0.250±0.44	0.239
	Bluelase	0.000±0.0	
1 year	Denlase	0.500±0.63	0.210
	Bluelase	0.187±0.40	

[Table/Fig-7]: Intergroup comparison of repigmentation at different time intervals.
*statistically significant, Mann-Whitney U test

DISCUSSION

According to the findings of present study, a statistically significant difference (p<0.001**) was observed between the two groups in terms of pain perception. The Bluelase group showed lower pain levels compared to the diode laser (Denlase), which may be attributed to the reduced thermal impact on tissues when using the Bluelase laser, as opposed to the infrared diode laser [15-18]. These findings are consistent with a study conducted by Cercadillo-Ibarguren et al., where they histologically evaluated thermal damage produced by different lasers and assessed pain levels, showing reduced thermal damage and pain with the diode laser [19].

Another interesting finding in this study was a statistically significant difference in aesthetic appraisal for the Bluelase group (p<0.001**). This may be due to the higher absorption levels of the Bluelase laser in melanin and haemoglobin [12]. Additionally, the increased scattering effect of the Bluelase laser compared to the infrared diode



[Table/Fig-8]: Clinical images showing depigmentation areas after using Denlase (810 nm) and Bluelase (470 nm). a) Preoperative view showing diffuse hyperpigmentation. b) Immediate postoperative view (Denlase 810 nm) c) Immediate postoperative view (Bluelase 470 nm). d) Three months re-evaluation (Denlase 810 nm). e) Three months re-evaluation (Bluelase 470 nm). f) Six months re-evaluation (Denlase 810 nm). g) Six months re-evaluation (Bluelase 470 nm). h) Nine months re-evaluation (Denlase 810 nm). i) Nine months re-evaluation (Bluelase 470 nm). j) One year re-evaluation (Denlase 810 nm). k) One year re-evaluation (Bluelase 470 nm).

laser may have improved the treatment efficiency at similar power settings [15,17]. Frentzen M et al., explained in their study that the increased absorption of blue light diode laser helps in reducing thermal side-effects. The high absorption levels in haemoglobin and melanin, with minimal absorption in water, allow for complete absorption of radiation energy by the tissues, leading to immediate cutting without the need for initiation [15]. Despite the use of a lower wavelength, the increased absorption levels compared to the infrared diode laser eliminate the need for initialisation [15] and due to less thermal impact on tissues aesthetic appraisal is excellent with the Bluelase group.

Repigmentation is a common finding that can be influenced by various factors, such as the technique used and race/ethnicity [17,18]. However, laser therapy has been found to result in lower rates of repigmentation compared to other alternative methods [1-3,17,18]. In present study, the most interesting finding regarding repigmentation was that repigmentation was observed at the end of one year in the Bluelase group, whereas in the Denlase group, repigmentation occurred at three months in three participants. This finding aligns with a study conducted by Rao PV et al., where patients treated with cryosurgery and diode laser (980 nm) showed recurrence from the third month [5]. In present study, authors observed minimal repigmentation at the end of one year in the Bluelase group. This may be attributed to the higher absorption and scattering effects of the blue light diode laser, which increase the treatment efficiency by improving tissue cutting and reducing the likelihood of repigmentation [14,17].

Additionally, lasers have been found to have antimicrobial effects, which is an added advantage. Studies have shown that blue light diode lasers exhibit similar antimicrobial effects to other lasers [14,15,20]. Another noteworthy fact is that due to the strong absorption of Blue laser light by haemoglobin, coagulation effects are more pronounced [14,15]. This property allows clinicians to use Bluelase for excision or incision procedures even in patients receiving anticoagulation therapy [15]. To standardise the use of diode laser technology, this study compared two different wavelengths of diode lasers. The efficacy of the shorter wavelength was validated for the first time in a depigmentation procedure.

Limitation(s)

However, present study is not without limitations. The use of multiple protocols instead of a single protocol could provide better insights. Additionally, as present study is the first of its kind, the findings cannot be generalised. In the future, further clinical trials need to be conducted to assess the efficacy of Bluelase in depigmentation techniques.

CONCLUSION(S)

The study demonstrated differences in pain perception and aesthetic appraisal between the Denlase and Bluelase groups, with lower pain levels and excellent aesthetic appraisal observed in the Bluelase group. Repigmentation was observed at the end of one year in the Bluelase group and at three months in the Denlase group. Therefore, it can be concluded that treatment with Bluelase of 470 nm yielded superior results compared to Denlase. The study clearly indicates that repigmentation was not evident in the Bluelase group, which is a positive outcome. This encourages the use of low-level lasers in depigmentation procedures.

REFERENCES

[1] Taher Agha M, Polenik P. Laser treatment for melanin gingival pigmentations: A comparison study for 3 laser wavelengths 2780, 940, and 445 nm. Int J Dent. 2020;2020:3896386.
[2] El Shenawy HM, Nasry SA, Zaky AA, Quriba MA. Treatment of gingival hyperpigmentation by diode laser for esthetical purposes. Open Access Maced J Med Sci. 2015;3(3):447-54.
[3] Mojahedi SM, Bakhshi M, Babaei S, Mehdipour A, Asayesh H. Effect of 810 nm diode laser on physiologic gingival pigmentation. Laser Ther. 2018;27(2):99-104.
[4] Reichelt J, Winter J, Meister J, Frentzen M, Kraus D. A novel blue light aser system for surgical applications in dentistry: Evaluation of specific laser-tissue interactions in monolayer cultures. Clin Oral Investig. 2017;21(4):985-94.
[5] Rao PV, Penmetsa GS, Dwarakanath CD. Gingival depigmentation by cryosurgery and laser application-a comparative clinical study. J Adv Med Med Res. 2015;5(11):1403-12.
[6] Dummett CO, Gupta OP. Estimating the epidemiology of oral pigmentation. J Natl Med Assoc. 1964;56(5):419-20.
[7] Ramadan RE, Segaan L, Abdel-Hamid AM, Khamis MM. Gingival melanin depigmentation with 4 different laser wavelengths 445, 940, 1064 and 2940 nm: A case report. Alexandria Dental Journal. 2022;47(4):27-27. Doi: 10.21608/adjalexu.2023.273862.
[8] 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. J Periodontol. 2013;84(6):738-48.
[9] Gholami L, Moghaddam SA, Ladiz MA, Manesh ZM, Hashemzehi H, Fallah A, et al. Comparison of gingival depigmentation with Er, Cr: YSGG laser and surgical stripping, a 12-month follow-up. Lasers Med Sci. 2018;33:1647-56.
[10] Elavarasu S, Thangavelu A, Alex S. Comparative evaluation of depigmentation techniques in split-mouth design with electrocautery and laser. J Pharm Bioallied Sci. 2015;7(Suppl 2):S786-90.
[11] Jha N, Ryu JJ, Wahab R, Al-Khedhairy AA, Choi EH, Kaushik NK. Treatment of oral hyperpigmentation and gummy smile using lasers and role of plasma as a novel treatment technique in dentistry: An introductory review. Oncotarget. 2017;8(12):20496-509.
[12] Singh V, Giliyar SB, Kumar S, Bhat M. Comparative evaluation of gingival depigmentation by diode laser and cryosurgery using tetrafluoroethane: 18-month follow-up. Clin Adv Periodontics. 2012;2:129-34.
[13] Elemek E. Gingival melanin depigmentation by 810 nm diode laser. Eur J Dent. 2018;12(1):149-52.
[14] Pang P, Andreana S, Aoki A, Coluzzi D, Obeidi A, Olivi G, et al. Laser energy in oral soft tissue applications. J Laser Dent. 2010;18:123-31.
[15] Frentzen M, Kraus D, Reichelt J, Engelbach C, Dehn C, Meister J. A novel blue light diode laser (445 nm) for dental application. Laser. 2016;8:06-13.
[16] Parker S, Cronshaw M, Anagnostaki E, Mylona V, Lynch E, Grootveld M. Current concepts of laser-oral tissue interaction. Dent J 2020;8(3):61.
[17] Braun A, Kettner M, Berthold M, Wenzler JS, Heymann PG, Frankenberger R. Efficiency of soft tissue incision with a novel 445-nm semiconductor laser. Lasers Med Sci. 2018;33(1):27-33.
[18] Jokar L, Bayani M, Hamid Hamidi MK, Azari-Marhabi S. A comparison of 940 nm diode laser and cryosurgery with liquid nitrogen in the treatment of gingival physiologic hyperpigmentation using split mouth technique: 12 months follow up. J Lasers Med Sci. 2019;10(2):131-38.

[19] Cercadillo-Ibarguren I, España Tost AJ, Arnabat Domínguez J, Valmaseda Castellón E, Berini Aytés L, Gay Escoda C. Histologic evaluation of thermal damage produced on soft tissues by CO2, Er, Cr: YSGG and diode lasers. Med Oral Patol Oral Cir Bucal. 2010;15(1):912-18.

[20] Gupta G. Management of gingival hyperpigmentation by semiconductor diode laser. J Cutan Aesthet Surg. 2011;4(3):208-10.

PARTICULARS OF CONTRIBUTORS:
1. Professor and Head, Department of Periodontics and Implantology, Vishnu Dental College, Bhimavaram, Andhra Pradesh, India.
2. Postgraduate Student, Department of Periodontics and Implantology, Vishnu Dental College, Bhimavaram, Andhra Pradesh, India.
3. Professor, Department of Periodontics and Implantology, Vishnu Dental College, Bhimavaram, Andhra Pradesh, India.
4. Professor, Department of Prosthodontics, Vishnu Dental College, Bhimavaram, Andhra Pradesh, India.
5. Postgraduate Student, Department of Periodontics and Implantology, Vishnu Dental College, Bhimavaram, Andhra Pradesh, India.
6. Postgraduate Student, Department of Periodontics and Implantology, Vishnu Dental College, Bhimavaram, Andhra Pradesh, India.
7. Postgraduate Student, Department of Periodontics and Implantology, Vishnu Dental College, Bhimavaram, Andhra Pradesh, India.
8. Postgraduate Student, Department of Periodontics and Implantology, Vishnu Dental College, Bhimavaram, Andhra Pradesh, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Gautami S Penmetsa,
Professor and Head, Department of Periodontics and Implantology, Block 2,
Vishnu Dental College, Vishnupur, Bhimavaram-534202, Andhra Pradesh, India.
E-mail: gautamipenmetsa@gmail.com

PLAGIARISM CHECKING METHODS: [Jain H et al.]
- Plagiarism X-checker: Mar 28, 2023
- Manual Googling: Jun 23, 2023
- iThenticate Software: Oct 21, 2023 (8%)

ETYMOLOGY: Author Origin

EMENDATIONS: 8

AUTHOR DECLARATION:
- Financial or Other Competing Interests: None
- Was Ethics Committee Approval obtained for this study? Yes
- Was informed consent obtained from the subjects involved in the study? Yes
- For any images presented appropriate consent has been obtained from the subjects. Yes

Date of Submission: **Mar 24, 2023**
Date of Peer Review: **Jun 01, 2023**
Date of Acceptance: **Jul 31, 2023**
Date of Publishing: **Oct 01, 2023**