

Clinical Comparison of PRF Membrane And Microneedling-Assisted Vitamin C As Adjuncts To Laser Therapy in The Management of Gingival Hyperpigmentation

Dr. Sherin Azhar Khan¹, Dr. Pragya Tripathi², Dr. Preeti Upadhyay³, Dr. Nikita Jain⁴, Dr. Ananya Sharma⁵, Dr. Shama Parveen⁶

¹MDS, Consultant Periodontist, Department Of Periodontology and Implantology, Inderprastha Dental College and Hospital, Sahibabad, Ghaziabad, Uttar Pradesh, India.

²MDS, Professor, Department Of Periodontology and Implantology, Inderprastha Dental College and Hospital, Sahibabad, Ghaziabad, Uttar Pradesh, India.

³MDS, Professor and Head of Department, Department Of Periodontology and Implantology, Inderprastha Dental College and Hospital, Sahibabad, Ghaziabad, Uttar Pradesh, India.

⁴MDS, Senior Lecturer, Department Of Periodontology and Implantology, Inderprastha Dental College And Hospital, Sahibabad, Ghaziabad, Uttar Pradesh, India.

⁵MDS Resident, Department Of Periodontology and Implantology, Inderprastha Dental College and Hospital, Sahibabad, Ghaziabad, Uttar Pradesh, India.

⁶MDS, Consultant Periodontist, Department Of Periodontology and Implantology, Inderprastha Dental College and Hospital, Sahibabad, Ghaziabad, Uttar Pradesh, India.

CORRESPONDING AUTHOR: DR. Sherin Azhar Khan

Abstract

Background: Physiologic gingival hyperpigmentation is a common esthetic concern caused by excessive melanin deposition within the gingival epithelium. Laser-assisted gingival depigmentation is an effective treatment modality, and adjuncts such as Platelet-Rich Fibrin (PRF) and microneedling-assisted Vitamin C may further improve healing and clinical outcomes.

Objectives: This study aimed to evaluate and compare the effectiveness of laser-assisted gingival depigmentation combined with PRF membrane and laser-assisted depigmentation combined with microneedling-assisted Vitamin C in the management of physiologic gingival hyperpigmentation.

Methods: A randomized controlled clinical trial was conducted in 30 sites with physiologic gingival hyperpigmentation. Sites were randomly allocated to Group A (laser + PRF membrane) and Group B (laser + microneedling-assisted Vitamin C). Pain perception, pigmentation index, epithelialization, and patient-reported outcomes were assessed at baseline, 4th day, 7th day, 14th day, and 6 months. **Results:** Both groups showed significant reductions in pain and pigmentation scores. Complete depigmentation was achieved by the 4th postoperative day and remained stable at 6 months. Group B demonstrated lower early postoperative pain scores, whereas no significant intergroup differences were observed for pigmentation reduction, epithelialization, or patient satisfaction.

Conclusions: Both PRF membrane and microneedling-assisted Vitamin C were effective adjuncts to laser-assisted gingival depigmentation. The two treatment approaches produced comparable clinical and esthetic outcomes in the management of physiologic gingival hyperpigmentation.

Keywords: Gingival hyperpigmentation, platelet-rich fibrin, vitamin C, microneedling, diode laser.

INTRODUCTION

Gingival appearance plays a significant role in smile esthetics and overall facial attractiveness¹. In recent years, increasing emphasis on esthetic dentistry has led to greater patient awareness regarding the appearance of gingival tissues. Among the various esthetic concerns affecting the gingiva, physiologic gingival hyperpigmentation is a common condition characterized by excessive melanin deposition within the basal and suprabasal layers of the gingival epithelium². Although physiologic pigmentation is benign and does not represent a pathologic condition, it may adversely affect smile esthetics, particularly in individuals with a high smile line or excessive gingival display. Consequently, many patients seek treatment for cosmetic improvement.

Melanin is the primary endogenous pigment responsible for gingival coloration and is synthesized by melanocytes through a tyrosinase-mediated pathway¹. The degree of gingival pigmentation is influenced by genetic predisposition, racial characteristics, hormonal factors, smoking habits, systemic conditions, and chronic inflammatory changes. While pigmentation may vary among individuals, excessive gingival discoloration frequently becomes a source of esthetic dissatisfaction and reduced self-confidence³.

Various treatment modalities have been proposed for the management of gingival hyperpigmentation⁴. Conventional approaches include scalpel surgery, rotary abrasion, free gingival grafting, cryosurgery, and electrosurgery⁵⁻⁷. Although these techniques are effective in removing pigmented tissues, they are often associated with postoperative pain, bleeding, delayed healing, patient discomfort, and the possibility of repigmentation. As a result, less invasive and more predictable treatment options have gained increasing attention in periodontal practice⁸.

Laser-assisted gingival depigmentation has emerged as a widely accepted treatment modality because of its precision, excellent hemostasis, minimal intraoperative bleeding, and improved patient comfort⁹. Various laser systems, including diode, CO₂, Nd:YAG, and Er:YAG lasers, have demonstrated favorable clinical outcomes in

the treatment of gingival pigmentation¹⁰⁻¹¹. Among these, diode lasers are particularly advantageous because of their affinity for melanin and hemoglobin, allowing selective ablation of pigmented epithelial tissues while minimizing damage to adjacent structures. Furthermore, laser therapy is associated with reduced postoperative discomfort and satisfactory esthetic outcomes.

Recent advances in periodontal regenerative therapy have focused on the use of biologic adjuncts to enhance wound healing and improve clinical outcomes following surgical procedures. Platelet-Rich Fibrin (PRF) is a second-generation platelet concentrate that acts as a biologic scaffold and serves as a reservoir of growth factors¹. The sustained release of platelet-derived growth factor, transforming growth factor- β , and vascular endothelial growth factor promotes angiogenesis, fibroblast proliferation, collagen synthesis, and epithelial regeneration¹². Consequently, PRF has been used successfully to accelerate healing and reduce postoperative morbidity in various periodontal procedures.

Vitamin C has also gained considerable interest because of its antioxidant, anti-inflammatory, and depigmenting properties³. It inhibits tyrosinase activity, thereby reducing melanin synthesis, while simultaneously promoting collagen formation and tissue repair. The clinical efficacy of Vitamin C may be enhanced through microneedling, a technique that creates controlled microchannels within the tissue and facilitates deeper penetration of therapeutic agents¹³. In addition, microneedling stimulates angiogenesis and tissue remodeling, which may contribute to improved healing and patient comfort.

Although both PRF and microneedling-assisted Vitamin C have shown promising results as adjuncts to gingival depigmentation procedures, comparative clinical evidence regarding their effectiveness remains limited¹⁴⁻¹⁵. Therefore, the present randomized controlled clinical trial was undertaken to evaluate and compare the clinical effectiveness of PRF membrane and microneedling-assisted Vitamin C when used in conjunction with laser-assisted gingival depigmentation for the management of physiologic gingival hyperpigmentation¹⁶.

Objective

To compare the effectiveness of laser-assisted gingival depigmentation combined with Platelet-Rich Fibrin (PRF) membrane and microneedling-assisted Vitamin C in reducing physiologic gingival hyperpigmentation and promoting epithelial healing in adults. Research Hypothesis does the adjunctive use of microneedling-assisted Vitamin C with laser-assisted gingival depigmentation more effectively reduce physiologic gingival hyperpigmentation and enhance epithelial healing compared with PRF membrane application.

Methods

This study was designed as a single-blind randomized controlled clinical trial with two parallel arms and was conducted in the Department of Periodontology, Inderprastha Dental College and Hospital, Ghaziabad. The study included systemically healthy individuals aged 18–40 years presenting with physiologic gingival hyperpigmentation in the anterior esthetic region. Participants with smoking habits, pregnancy, systemic conditions affecting healing, active periodontal disease, medication-induced pigmentation, or hypersensitivity to Vitamin C were excluded. Eligible participants provided written informed consent before enrollment.

The sample size was calculated from a previous study and estimated at 13 participants per group. To compensate for possible attrition, the final sample size was increased to 15 participants per group, resulting in a total of 30 study sites. Participants were randomly allocated into two intervention groups in a 1:1 ratio. Group A received diode laser-assisted gingival depigmentation followed by application of a Platelet-Rich Fibrin (PRF) membrane, whereas Group B received diode laser-assisted gingival depigmentation followed by microneedling-assisted topical Vitamin C application.

All participants underwent Phase I periodontal therapy before the intervention. Following reevaluation, baseline clinical measurements were recorded. Gingival depigmentation was performed using a diode laser under standardized clinical conditions. In Group A, an autologous PRF membrane was prepared and adapted over the treated site immediately after laser ablation. In Group B, microneedling was performed using a dermapen device, followed by topical application of Vitamin C over the depigmented area.

The primary outcomes of the study were pain perception, pigmentation reduction, and epithelial healing. Pain was assessed using the Visual Analog Scale (VAS), while pigmentation was evaluated using the pigmentation index. Epithelialization and patient-reported outcomes were also recorded. Clinical assessments were performed at baseline and at the 4th day, 7th day, 14th day, and 6-month follow-up visits.

Data were analyzed using SPSS software version 27.0. Appropriate statistical tests were applied to compare changes within and between the groups. A p-value of <0.05 was considered statistically significant.

Intervention

The intervention regimen consisted of diode laser-assisted gingival depigmentation followed by either PRF membrane application or microneedling-assisted Vitamin C administration. At the baseline visit, clinical assessment of gingival pigmentation was performed for all participants.

Following administration of local anesthesia, laser-assisted depigmentation was carried out by removing the pigmented gingival epithelium from the affected esthetic area. In Group A, an autologous PRF membrane was prepared and placed over the treated site immediately after laser therapy. In Group B, microneedling was performed over the depigmented gingival surface, followed by topical application of Vitamin C.

The primary outcomes of the study were pain perception, pigmentation reduction, and epithelial healing. Outcome assessments were conducted at 4 days, 7 days, 14 days, and 6 months following treatment. Pain was assessed using the Visual Analog Scale, while pigmentation reduction and epithelialization were evaluated using standardized clinical criteria. Patient-reported outcomes were also recorded during the follow-up period.

Results

A total of 30 sites with physiologic gingival hyperpigmentation were included in the study and randomly allocated into two treatment groups, with 15 sites in each group. Group A received laser-assisted gingival depigmentation followed by PRF membrane application, whereas Group B received laser-assisted gingival depigmentation combined with microneedling-assisted Vitamin C. All participants completed the 6-month follow-up period, and no adverse events, postoperative complications, allergic reactions, or delayed healing were observed during the study period.

Pain perception, pigmentation index, degree of epithelialization, and patient-reported outcomes were evaluated at baseline (D0), Day 4 (D4), Day 7 (D7), Day 14 (D14), and 6 months. Both treatment groups demonstrated progressive improvement in all clinical parameters throughout the follow-up period. The mean VAS score decreased from 3.47 ± 0.52 at baseline to 0.00 ± 0.00 at 6 months in Group A and from 3.00 ± 0.76 to 0.00 ± 0.00 in Group B. Intragroup analysis revealed a statistically significant reduction in pain scores over time in both groups ($p < 0.001$). Intergroup comparison showed significantly lower pain scores in Group B on the 4th postoperative day ($p < 0.001$), whereas no statistically significant differences were observed between the groups at baseline, Day 7, Day 14, or 6 months (Tables 1–3).

Both treatment modalities resulted in substantial reduction of gingival pigmentation. At baseline, the majority of sites exhibited moderate-to-severe pigmentation scores. By Day 4, complete depigmentation (Score 1) was observed in all sites in both groups. This improvement remained stable throughout the study period, with only minimal recurrence observed at the 6-month follow-up. Pairwise comparisons demonstrated statistically significant differences between baseline and all postoperative evaluations in both groups ($p < 0.05$). However, no statistically significant differences were observed between the two treatment groups at any follow-up interval (Tables 4–6).

Progressive epithelial healing was observed in both groups during the postoperative period. At Day 4, all treated sites exhibited incomplete epithelialization. The proportion of incompletely epithelialized sites gradually decreased by Day 7 and Day 14 in both groups, indicating continuous maturation of the healing tissue. Intragroup analysis demonstrated statistically significant improvement in epithelialization over time in both treatment groups. Nevertheless, intergroup comparison did not reveal any statistically significant differences between Group A and Group B at any evaluation period (Tables 7–9).

Patient-reported outcomes assessed at the 6-month follow-up demonstrated high levels of treatment acceptance and satisfaction in both groups. More than half of the participants reported marked cosmetic improvement following treatment, while the remaining participants reported moderate cosmetic improvement. Most participants stated that the treatment met or exceeded their expectations. Absence of repigmentation was reported by 73.3% of participants in Group A and 80% of participants in Group B, whereas the remaining participants reported only mild-to-moderate repigmentation. Similar findings were observed for treatment satisfaction, confidence level, and willingness to undergo retreatment if required. No statistically significant differences were observed between the groups for any patient-reported outcome measure.

Overall, both laser-assisted depigmentation with PRF membrane and laser-assisted depigmentation combined with microneedling-assisted Vitamin C were effective in reducing gingival pigmentation and improving postoperative healing. Although microneedling-assisted Vitamin C demonstrated superior early postoperative pain reduction, both treatment modalities produced comparable clinical, esthetic, and patient-centered outcomes at the 6-month follow-up.

Discussion

This randomized clinical trial aimed to evaluate and compare the effectiveness of PRF membrane and microneedling-assisted Vitamin C as adjuncts to diode laser-assisted gingival depigmentation in patients with physiologic gingival hyperpigmentation during a 6-month follow-up period. Both treatment modalities demonstrated significant reductions in pigmentation and pain scores, together with progressive epithelial healing and high patient satisfaction. However, microneedling-assisted Vitamin C was associated with significantly lower pain scores during the early postoperative period.

Several studies have demonstrated the effectiveness of diode laser-assisted depigmentation in the management of physiologic gingival hyperpigmentation and have compared it with scalpel surgery, electrosurgery, cryosurgery, and other treatment modalities^{7,20}. These studies consistently reported significant reductions in pigmentation scores together with favorable healing and patient acceptance. The present findings are in agreement with those of El Shenawy et al.¹¹, Jagannathan et al.¹⁷, Yadav et al.¹⁸, and Mikhail et al.¹⁹, who reported successful elimination of gingival pigmentation following diode laser therapy with minimal postoperative complications.

The present study demonstrated complete depigmentation in both groups by the fourth postoperative day, which remained stable throughout the follow-up period. Earlier studies evaluating diode laser depigmentation have similarly reported stable clinical outcomes and delayed recurrence following treatment. The favorable outcomes observed in the present study may be attributed to the selective absorption of diode laser energy by melanin-containing tissues, resulting in effective elimination of pigmented epithelial cells while minimizing injury to adjacent tissues.

Pain perception is an important determinant of patient acceptance following esthetic periodontal procedures. Both treatment groups demonstrated significant reductions in pain scores throughout the study period. However, participants receiving microneedling-assisted Vitamin C experienced significantly lower pain scores during the early postoperative phase. Similar findings were reported by Maheshwari et al.²¹, Mostafa Sr et al., and Mansuri et al., who observed improved patient comfort and favorable healing following Vitamin C-assisted depigmentation procedures. The beneficial effects of Vitamin C may be related to its antioxidant activity and its role in collagen synthesis and wound repair.

Progressive epithelial healing was observed in both groups throughout the study period. Although slightly better healing was observed in the microneedling-assisted Vitamin C group, the difference was not statistically significant. The absence of significant intergroup differences in epithelialization in the present study may be related to the favorable wound healing characteristics associated with diode laser therapy itself.

Patient-reported outcomes demonstrated high levels of cosmetic improvement and treatment satisfaction in both groups. Most participants reported that treatment outcomes met or exceeded their expectations, and only minimal repigmentation was observed during the follow-up period. These findings are consistent with previous reports evaluating laser-assisted depigmentation procedures, which have demonstrated high patient acceptance and favorable esthetic outcomes.

The present study employed a randomized controlled design and evaluated both clinical and patient-centered outcomes following laser-assisted gingival depigmentation. However, certain limitations should be considered while interpreting the findings. The sample size was relatively small, the follow-up period was limited to 6 months, and histologic evaluation of epithelial healing and melanocyte activity was not performed. Future studies with larger sample sizes and longer follow-up periods are required to further evaluate the long-term stability of treatment outcomes and recurrence patterns.

Conclusion

The present study demonstrated that both PRF membrane and microneedling-assisted Vitamin C are effective adjuncts to diode laser-assisted gingival depigmentation in the management of physiologic gingival hyperpigmentation. Although microneedling-assisted Vitamin C provided superior early postoperative pain reduction, both treatment modalities produced comparable outcomes with respect to pigmentation reduction, epithelial healing, patient satisfaction, and recurrence control. These findings suggest that both approaches are safe, predictable, and effective treatment options for achieving favorable clinical and esthetic outcomes following gingival depigmentation.

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Table 1 : Intragroup comparison of VAS score

Time point	Group A		Group B	
	Mean	SD	Mean	SD
D0	3.47	0.52	3.00	0.76
D4	2.33	0.49	1.33	0.49
D7	1.00	0.66	1.00	0.66
D14	0.20	0.41	0.13	0.35

6 months	0.00	0.00	0.00	0.00
χ^2 value	57.068		55.455	
p-value	<0.001*		<0.001*	

Friedman's test; significant $p < 0.05$; highly significant $p < 0.01$; very highly significant $p < 0.001$; not significant $p > 0.05$

Table 2 : Pairwise comparison of change in VAS score within each group

Pairwise comparison	Group A	Group B
D0 vs D4	0.833	0.153
D0 vs D7	0.001*	0.012*
D0 vs D14	<0.001*	<0.001*
D0 vs 6 months	<0.001*	<0.001*
D4 vs D7	0.327	1.000
D4 vs D14	0.001*	0.012*
D4 vs 6 months	<0.001*	0.003*
D7 vs D14	0.735	0.153
D7 vs 6 months	0.282	0.056
D14 vs 6 months	1.000	1.000

Post hoc Bonferroni test; significant $p < 0.05$; highly significant $p < 0.01$; very highly significant $p < 0.001$; not significant $p > 0.05$

Table 3 : Intergroup comparison of VAS score

Time point	Group A		Group B		z-value	p-value
	Mean	SD	Mean	SD		
D0	3.47	0.52	2.47	0.52	-1.759	0.116
D4	2.33	0.49	1.33	0.49	-3.974	<0.001*
D7	1.00	0.66	1.00	0.66	0.000	1.000
D14	0.20	0.41	0.13	0.35	-0.482	0.775
6 months	0.00	0.00	0.00	0.00	0.000	1.000

Mann Whitney test; significant $p < 0.05$; highly significant $p < 0.01$; very highly significant $p < 0.001$; not significant $p > 0.05$

Table 4 : Intragroup comparison of change in pigmentation index

Time point	Score 1	Score 2	Score 3	Score 4	Mean rank	χ^2 value	p-value
Group A							
D0	0 (0%)	4 (26.7%)	5 (33.3%)	6 (40%)	4.93	49.667	<0.001*
D4	15 (100%)	0 (0%)	0 (0%)	0 (0%)	2.20		
D7	14 (93.3%)	1 (6.7%)	0 (0%)	0 (0%)	2.30		
D14	11 (73.3%)	4 (26.7%)	0 (0%)	0 (0%)	2.73		
6 months	11 (73.3%)	3 (20%)	1 (6.7%)	0 (0%)	2.83		
Group B							
D0	0 (0%)	7 (46.7%)	5 (33.3%)	3 (20%)	4.93	51.290	<0.001*
D4	15 (100%)	0 (0%)	0 (0%)	0 (0%)	2.33		
D7	15 (100%)	0 (0%)	0 (0%)	0 (0%)	2.33		
D14	13 (86.7%)	2 (13.3%)	0 (0%)	0 (0%)	2.63		
6 months	12 (80%)	2 (13.3%)	1 (6.7%)	0 (0%)	2.77		

Friedman's test; significant $p < 0.05$; highly significant $p < 0.01$; very highly significant $p < 0.001$; not significant $p > 0.05$

Table 5 : Pairwise comparison of change in pigmentation index score within each group

Pairwise comparison	Group A	Group B
D0 vs D4	<0.001*	<0.001*
D0 vs D7	<0.001*	<0.001*
D0 vs D14	0.001*	0.001*
D0 vs 6 months	0.003*	0.002*
D4 vs D7	1.000	1.000
D4 vs D14	1.000	1.000
D4 vs 6 months	1.000	1.000
D7 vs D14	1.000	1.000
D7 vs 6 months	1.000	1.000
D14 vs 6 months	1.000	1.000

Post hoc Bonferroni test; significant $p < 0.05$; highly significant $p < 0.01$; very highly significant $p < 0.001$; not significant $p > 0.05$

Table 6 : Intergroup comparison of pigmentation index

Time point	Group A	Group B	z-value	p-value
D0	3.47	0.52	-1.321	0.217
D4	2.33	0.49	0.000	1.000
D7	1.00	0.66	-1.000	0.775
D14	0.20	0.41	-0.898	0.539
6 months	0.00	0.00	-0.393	0.775

Mann Whitney test; significant $p < 0.05$; highly significant $p < 0.01$; very highly significant $p < 0.001$; not significant $p > 0.05$

Table 7 : Intra- and Intergroup Comparison of Degree of Epithelialization

Time point	Group A (PRF) Positive n (%)	Negative n (%)	Group B (Vitamin C) Positive n (%)	Negative n (%)	Intergroup p-value	Within-group significance
D4	15 (100)	0 (0)	15 (100)	0 (0)	—	Group A: Cochrane Q=15.200, $p=0.001^*$ Group B: Cochrane Q=9.333, $p=0.009^*$
D7	11 (73.3)	4 (26.7)	13 (86.7)	2 (13.3)	0.651	Pairwise: A (D4 vs D7=0.125); B (D4 vs D7=0.500)
D14	5 (33.3)	10 (66.7)	6 (40.0)	9 (60.0)	0.272	Pairwise: A (D4 vs D14=0.002*; D7 vs D14=0.031*);

						B (D4 vs D14=0.031*; D7 vs D14=0.125)
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Intragroup comparison was performed using the Cochran Q test followed by Post hoc Bonferroni test. Intergroup comparison was performed using the Chi-square test. $p < 0.05$ was considered statistically significant.

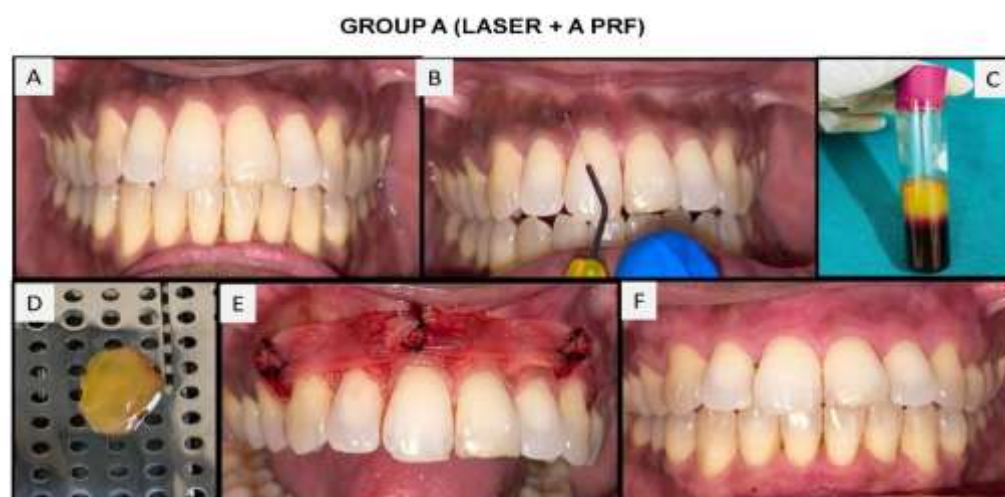


Figure 1 : (A) Immediate pre operative view (B) Laser assisted gingival depigmentation (C) Centrifuged blood sample (D) Isolation of A PRF (E) A PRF membrane placement at surgical site (F) 6 months postoperative view

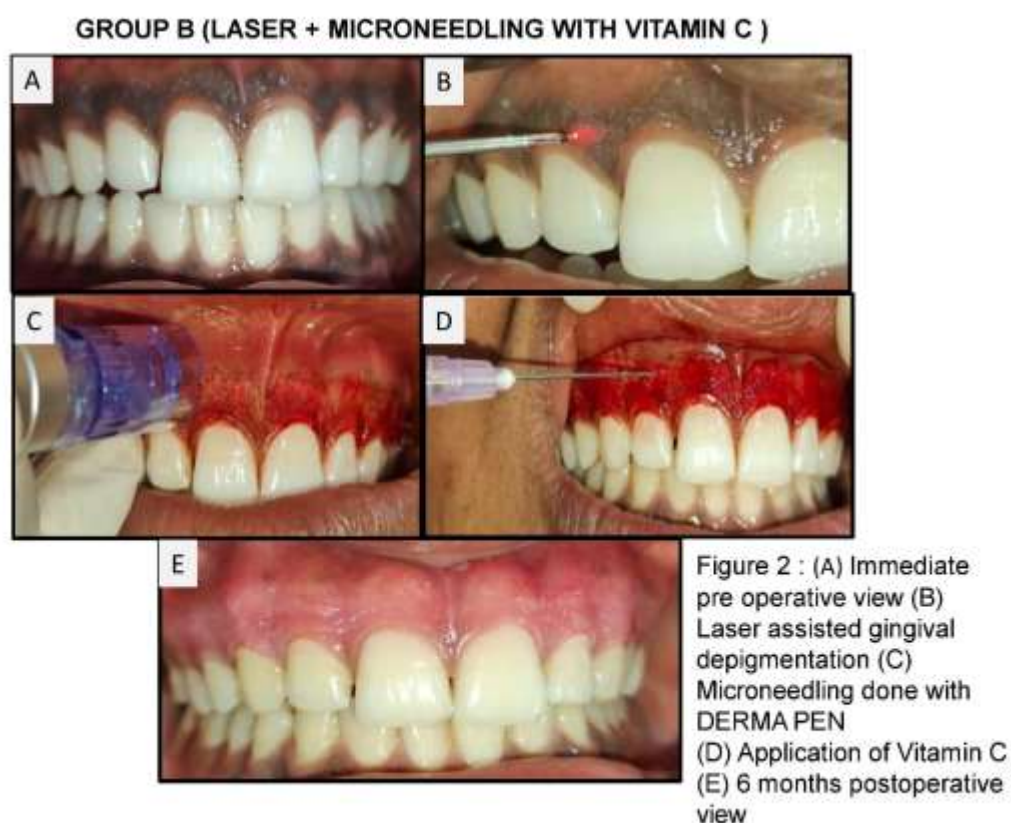


Figure 2 : (A) Immediate pre operative view (B) Laser assisted gingival depigmentation (C) Microneedling done with DERMAPEN (D) Application of Vitamin C (E) 6 months postoperative view