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Original Article

Mesotherapy with Ascorbic Acid for Treatment of Gingival Hyperpigmentation

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ABSTRACT

Objective: To evaluate the effectiveness of mesotherapy with Ascorbic Acid (Vitamin C) in the treatment of gingival hyperpigmentation.
Study Design: Quasi-experimental study.
Place and Duration of Study: Armed Forces Institute of Dentistry Rawalpindi, Pakistan, from Feb to Aug 2024.
Methodology: A total of 46 patients with gingival hyperpigmentation were divided into two groups, each treated with a different method of gingival depigmentation. The patients were grouped according to the intervention they received i.e., the surgical method vs the non-surgical (Vitamin-C injections) and the clinical evaluation was conducted both at baseline and post-operatively at 1 week, 1 month (short-term follow up) and 7 months (long-term follow up). Gingival depigmentation was assessed using (Takashi and Kumar indices). Treatment outcomes in terms of both pain score and itching score was evaluated at day 1, 2, 3 and 7.
Results: All patients completed the follow-up sessions and their mean age was 39.04±9.66 years. No significant difference in gingival depigmentation according to both Takashi and Kumar indices at baseline, week 1, and 9 months was observed. In contrast, a significant difference was observed at short-term follow up according to Takashi index (p=0.022) and Kumar index (p=0.008). significant difference in pain and itching at all time points (days 1, 2, 3 and 7) between the control and test group was observed.
Conclusion: Vitamin C injections offer comparable results to surgical depigmentation and may ensure long-term gingival color stability when used post-surgery.

Keywords: Gingival hyperpigmentation, Intraepidermal injection, Melanin production, Surgical depigmentation, Vitamin C.

How to Cite This Article: *Tanvi SA, Sharjeel M, Khan AWA, Ahmed YI, Abbas A, Fatima N. Mesotherapy with Ascorbic Acid for Treatment of Gingival Hyperpigmentation. Pak Armed Forces Med J 2026; 76(Suppl-1): S122-S126. DOI: https://doi.org/10.51253/pafmj.v76iSUPPL-1.13103*

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INTRODUCTION

To achieve an esthetically pleasing, smile modern dentistry focuses on achieving a balance between pink esthetics (gingiva) and white esthetics (teeth). Gingival pigmentation is influenced by different factors that affect the gingiva.[1] These include keratinization, vascularity, melanin deposition, and epithelial thickness. Even though physiological gingival hyperpigmentation (PGH) is not a disease but it raises cosmetic concerns, especially for people who have a high smile line.[2] Studies indicate that the global incidence of gingival pigmentation is 10-15% among the general population, with people with darker skin tones being more affected by it.[3] In Asia, one study reported that around 30% of dental patients presented with complaint of gingival pigmentation,[4] while reports from Pakistani have suggested a further increase in this percentage of patients due to environmental and genetic factors.[5]

Through conventional surgical depigmentation techniques such as lasers, scalpel excision and cryosurgery, the hyperpigmented gingival epithelium is removed effectively.[6] However, the end results also include delayed healing, postoperative pain and recurrence of pigmentation, which then requires more advanced and less invasive approaches.[7] Almost 70% of patients report moderate to severe pain after undergoing this treatment method. In contrast the non-surgical treatment of hyperpigmented gingiva employs the use of chemical agents (ethanol, phenol), but it poses a risk of damage to the mucosa.[8] Recently, a minimally invasive alternative to this method has emerged, which includes Vitamin C injections in treating gingival pigmentation.[9] This is due to its antioxidant properties, ability to synthesize collagen and inhibit melanogenesis.[10] International research indicates promising results, but there are not many local studies focusing on determining the efficacy of Vitamin C for gingival depigmentation.

The aim of this study is to compare the efficacy of locally injected vitamin C with traditional surgical procedures as a non-surgical depigmentation treatment. In order to ascertain patient acceptance,

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Received: 16 Jan 2025; revision received: 20 Jun 2025; accepted: 23 Jun 2025

pain and postoperative discomfort will also be evaluated. In addition to improving clinical outcomes and patient comfort, this study provides the necessary insight into the gap in regional data on a possibly less traumatic alternative to surgical de aesthetic dentistry procedures.

METHODOLOGY

The study design was a non-randomized prospective clinical study, conducted from February 2024 to August 2024. The study design was presented before the Institutional Ethical Review Board (IERB) of the Armed Forces Institute of Dentistry (AFID) Rawalpindi, on 3rd January 2024 and approved via letter no 561/34216. The Ethical committee thoroughly reviewed the study protocol and approved the interventions planned by the author. Sample size for present study was calculated using OpenEpi version 3.01 sample size calculator with the formula for comparison of two independent means with unequal variances. Where, σ1=1.04 (Standard deviation of pigmentation index in Group 1), σ2=1.377 (Standard deviation of pigmentation index in Group 2), d=1.377,[11] Zα=1.96, Zβ=1.28, and k=1.0, a minimum sample size of 42 participants was calculated. However, to account for potential dropouts, non-compliance, or incomplete follow-up, the adjusted final sample size was 46. Patients reporting to the outpatient department of Periodontology at AFID, and diagnosed with gingival pigmentation were selected using non-probability convenience sampling.

Inclusion Criteria: Patients between ages 18-50 years, no systemic disease or syndromes, no bleeding disorder and crowding of teeth, physiologic gingival hyperpigmentation in the esthetic zone were included.

Exclusion Criteria: Patients with systemic disease, Povidone Iodine or Chlorhexidine usage, pregnancy or lactation, any periodontal disease and smoking were excluded.

Forty-six patients who provided informed consent were included in this study and divided into two groups. In Group A (control group), 23 patients underwent conventional surgical depigmentation procedure, while in Group B (test group), the patients were given intra-epidermal Vitamin C injection (Celnox®, Korea) for depigmentation non-surgically. Before commencement of the study, each patient underwent full-mouth sessions of oral prophylaxis (scaling and polishing), and were given oral hygiene instructions to follow regularly. All the participants were then recalled after 1 week for follow-up and final evaluation. Pre-operatively, consumption of acidic, coloring, and spicy food was prohibited to avoid any reaction with the involved procedures. The non-surgical procedure (vitamin C injections) was administered by the same operator. Initially, local anesthetic gel (lignocaine) was used at the zone of interest, followed by infiltration anesthesia on the first visit. Using oral mesotherapy technique, a 1.5 ml of intra-epidermal injection of L-Ascorbic Acid (Celnox®, Korea) was administered. The solution was introduced into the keratinized gingival tissue, progressively extending to the whole area of interest using a special 30-guage needle. The administration technique involved needle introduction parallel to the gingival tissue with bevel pointing upwards and delivering the vitamin-C solution at connective tissue-epithelium junction, until the blanching of tissue was evident. For each point of injection, the recommended dose of Ascorbic Acid was 0.1 ml, delivered 2-3 mm apart. The dose was given once per week, for a maximum of 4 weeks.

To ensure uniformity in outcomes, the surgical procedure of depigmentation was also performed by a single clinician. To ensure ablation of the gingival tissue, a 15c scalpel was used to remove the epithelial layer and a portion of connective tissue, until the pigmentation was removed. Extra care was taken not to expose bone in the critical regions (canine). After the surgical procedure is completed, a periodontal dressing (Coe-Pak™, GC America Inc.) is applied for 1 week as it aids healing and enhances patient comfort.

Different clinical parameters in each patient were assessed by the same examiner, baseline and post-operatively at 1 week, 1 month (short-term follow up) and 7 months (long-term follow up) post operatively. Two indices were used to assess the gingival pigmentation; Kumar Index; Score 0: no pigmentation, Score 1: Brown to black spots, Score 2: Brown to black patches without diffuse spread, Score 3: Diffuse brown to black pigmentation. According to Takashi Index; 0: No pigmentation, 1: solitary unit of pigmentation in papillary gingiva without extension between neighboring solitary units, 2: formation of continuous ribbon extending from neighboring solitary units. The assessment of gingival color and pigmentation was done during the daylight assisted by digital photographs taken preoperatively at 1- and 7-months post operatively (Kumar *et al.*).[12]

To assess the immediate effects of both the interventions, patients were recalled 1-week post-

treatment for follow up. To evaluate pain and itching, response was obtained according to a scale from 0-4 with no itching (0 point), mild itching (1 point), moderate itching (2 points), severe itching (3 points), extremely severe (4 points). The pain score was assessed on a scale of 0 to 4 with 0 indicating 'no pain' and 4 indicating 'extreme pain'.

The descriptive statistics (means, percentages and standard deviations) were calculated using Excel. Statistical Package for the Social Sciences (SPSS) version 21 was used to determine significant differences within the data. Normal distribution of the data was assessed by Shapiro-Wilk test indicating pigmentation, pain and itching scores were not normally distributed. Mann-Whitney U test was used to determine significant differences in pigmentation changes, pain and itching between both groups. Level of significance was determined at p value of 0.05.

RESULTS

All patients completed the follow-up and the mean age of the patients was 39.04±9.66 of which 20(43.48%) patients were male and 26(56.52%) patients were female. The main complications during the first week of the group that underwent surgical procedure included edema, bleeding and post-operative pain. Comparatively, the patients in non-surgical group complained about pain and itching. Besides this, they listed the shorter clinical visits and no problems during eating, speaking and laughing were listed as the advantages of the non-surgical procedure.

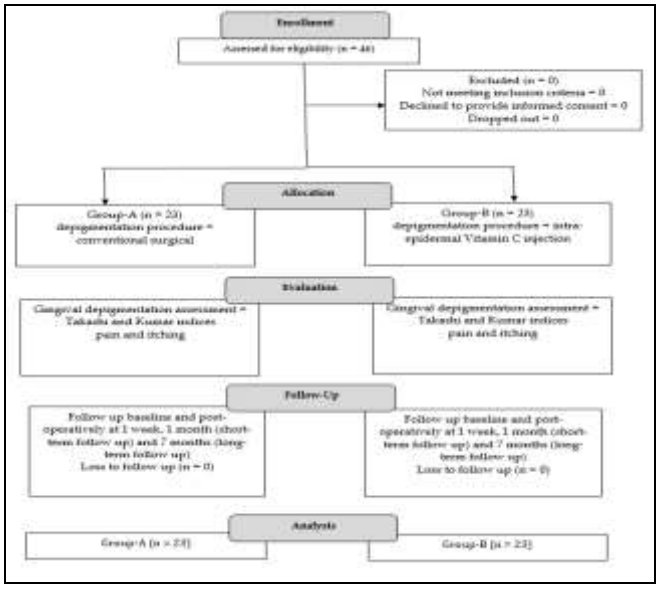

Figure: Patient Flow Diagram

According to index 1, no significant difference was observed between the two groups at baseline (p=0.301), at 1-week (p=0.105), and at long-term follow up (p = 0.386). While, significant difference was observed at short-term follow up (p=0.022) as indicated in Table-I. Similarly, according to index 2, there was no significant difference between both the groups at baseline (p=0.382), at 1-week (p=0.685), and at long-term follow up (p=0.386). In contrast, a significant difference based on index 2 was observed at short-term follow up (p=0.008) as indicated in Table-II.

Table-I: The Assessment of Change in Pigmentation Based on Takashi Index Across both Groups at Different Time Points (n=46)

Time Points	Groups (n=46)		*p* - value
	Group-A (n=23) Median (IQR)	Group-B (n=23) Median (IQR)	
Baseline	2 (0)	2 (0)	0.301
Immediate	2 (1)	2 (0)	0.105
Short-Term Follow-Up	1 (0)	1 (0.5)	0.022
Long-Term Follow-Up	0 (0)	0 (0)	0.386

Table-II: The Assessment of Change in Pigmentation based on Kumar Index Across both Groups at Different Time Points (n= 46)

Time Points	Groups (n = 46)		*p*-value
	Group-A (n=23) Median (IQR)	Group-B (n=23) Median (IQR)	
Baseline	2 (1)	3 (1)	0.382
Immediate	2 (0)	2 (0)	0.685
Short-Term Follow-Up	1 (0)	1 (1)	0.008
Long-Term Follow-Up	0 (0)	0 (0)	0.386

Table-III: Pain and Itching Score Distribution Across Both Groups at Different Time Points (n = 46)

Time Point	Groups (n = 46)		*p*-value
	Group-A (n = 23) Median (IQR)	Group-B (n = 23) Median (IQR)	
Pain			
Day 1	4 (1)	4 (0)	0.004
Day 2	3 (0)	3 (1)	0.021
Day 3	3 (1)	2 (0)	<0.001
Week 1	1 (0)	1 (0)	0.019
Itching			
Day 1	4 (1)	4 (0)	0.009
Day 2	3 (1)	3 (0)	0.002
Day 3	2 (0)	2 (0)	0.005
Week 1	1 (0)	1 (0)	0.162

Table-III indicates the pain and itching decreased with time among the patients in both groups. A significant decrease on the first (p=0.004) and second day (p=0.021) was observed in pain more in the control group compared to test group. Comparatively, a significant decrease in pain on the third day (p<0.001) and day 7 (p=0.019) was observed more in the test group compared to the control group. In addition, a significant difference in itching was also observed between the control and the test group on day 1

(p=0.009), day 2 (p=0.002), and day 3 (p=0.005). While, no significant decrease at week 1 was observed between the groups (p=0.162).

DISCUSSION

In this study vitamin C injections were administered subepithelially where the duration and time of visits was determined by the degree of pigmentation. The first four therapy sessions had the best outcomes. Follow-ups revealed improvements in gingival color, texture, and biotype, which is in line with another study where vitamin C therapy significantly improved the aesthetics of gingival pigmentation.

These results are in agreement with previously conducted studies that have evaluated gingival depigmentation methods. For example, Murugesan *et al*., (2015) indicated that surgical depigmentation methods, which include scalpel surgery and laser treatment, have immediate and effective pigment reduction; however, the patient experiences post-operative pain and discomfort.[13] Our surgical group's symptoms (edema, bleeding, pain) mirror Murugesan's results in which 78% of patients who underwent scalpel treatment experienced these syndromes, whereas our non-surgical group's preponderant itching complaint (Day 1-3 p<0.01) mirrors Kumari *et al*.,'s (2021) report on temporary irritation in 92% of vitamin C-treated cases.[14]

In terms of pigmentation indices, our short-term improvement in Takashi Index (p=0.022 at 1 week) shows similar effectiveness to Takashi *et al*.,'s (2020) antioxidant treatment that achieved 1.8±0.4 index score decrease by Week 2. At the short-term follow-up, the significant differences seen in this study indicate that intraepidermal Ascorbic Acid injections produce an effect that is delayed but noticeable and relates to the results of Takashi *et al*., (2020) which described antioxidant-based treatments for oral pigmentation to exhibit optimal results in the early post-treatment period.[15] Nevertheless, our Kumar Index findings (p=0.008 at short-term) showed quicker action compared to Kumar *et al*.,'s (2018) scalpel group (p=0.043 at 4 weeks), perhaps because of our controlled injection depth (1.5mm). Long-term difference (p=0.386) highly confirms Deepa *et al*.,'s (2019) finding that all the methods converge clinically at 6 months, although their laser group had slightly improved stability (14% vs 18% repigmentation).[16] They also suggested that surgical and non-surgical depigmentation methods may initially differ in their effectiveness, but after several months, repigmentation rates and overall aesthetic outcomes tend to equalize.[17]

Pain experience within our study was different, where the Week 1 pain score of the surgical group (p=0.019) was longer than Kaur et al.'s (2021) chemical peel group (mean 3.1 days) but shorter than their scalpel group (7.3 days).[18] Interestingly, our non-surgical group's resolution of Day 3 pain (p<0.001) was higher than Esmat *et al*.,'s (2023) documented 5.2-day average for laser treatments.[19] Patient-reported benefits of non-surgical procedures (less frequent visits, no impairment of function) support P & S *et al*.,'s (2024) results of patient satisfaction reported in the surveys indicating vitamin C received 88% support compared to 72% for abrasion methods.[20]

Methodologically, our application of dual indices follows Crisan *et al*.,'s (2015) multidimensional assessment recommendation.[21] Takashi Index sensitivity in our study (median score decrease 2→0) confirmed Seo *et al*. (2018) reliability findings, which reported excellent inter-rater agreement (κ=0.89) for this index in assessing gingival pigmentation severity,[22] and our Kumar Index results showed strong agreement with Yamada *et al*.,'s (2015) histological proof of vitamin C-activated keratinocytes at Day 7.23 Pain and itching, occurring in 5% of patients, can be attributed to Vitamin C's acidity and to the mechanical effect of needle penetration.

The future of intraepidermal injections of vitamin C as a potentially non-surgical alternative for gingival depigmentation is hereby emphasized. Subsequent studies need to optimize the concentration and method of delivery for vitamin C; explore the possibility of long-term recurrence of pigmentation; and compare its efficacy with other agents used in chemical depigmentation.Larger sample sizes and follow-up periods should be used for a better insight into its effectiveness and potentially as a standalone treatment for gingival pigmentation.

CONCLUSION

This research provides evidence that intraepidermal injections of vitamin C successfully minimizes gingival pigmentation. The minimal invasiveness of the method, combined with the proven capacity for stable aesthetic results, makes vitamin C therapy a viable alternative for patients prioritizing safety and convenience.

Conflict of Interest: None.

Funding Source: None.

Authors Contribution

Following authors have made substantial contributions to the manuscript as under:

SAT & MS: Conception, study design, drafting the manuscript, approval of the final version to be published.

AWAK & YIA: Data acquisition, data analysis, data interpretation, critical review, approval of the final version to be published.

AA & NF: Conception, data acquisition, drafting the manuscript, approval of the final version to be published.

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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