Effect of Vitamin E V/S Vitamin C application on wound healing in the gingival depigmentation procedure: A split-mouth clinical comparative study

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Article Info

ISSN (online): 2582-8940
Volume: 05
Issue: 02
April-June 2024
Received: 01-04-2024;
Accepted: 03-05-2024
Page No: 38-42

Abstract
Aim: To evaluate the clinical efficacy of vitamin E and C application on healing after the gingival depigmentation procedure.
Materials and Methods: A Split mouth study was planned for subjects with gingival melanin hyperpigmentation in maxillary and/or mandibular arches. Each site was randomly divided into two groups. Scalpel Surgical Technique for depigmentation was performed under local anesthesia. After the depigmentation procedure vitamin E was applied in group 1 and vitamin C was applied in group 2. The Healing Index and Visual Analog Scale for pain were recorded on the 15th day and the 45th day postoperatively.
Results: There was a statistically significant reduction in visual analogue scale scores and improvement in healing in both groups on the 15th day. Subjects in the vitamin E showed better healing and reduced pain on the 15th day as compared to vitamin C but at the 45th day, there was no significant difference in both groups.
Conclusion: Subjects would show faster healing with the use of vitamin E application after the surgical procedure of gingival depigmentation.

DOI: https://doi.org/10.54660/IJMBHR.2023.5.2.38-42

Keywords: Gingival hyperpigmentation, gingival depigmentation, vitamin E, vitamin C, ascorbic acid, tocopherol

Introduction

A perfect smile is determined not only by the shape and position of the teeth or lips but also by the appearance of the gingival tissues. The color of gingiva depends on several factors including the degree of vascularization, the thickness of the gingival epithelium, keratinization of the stratum layer, and the amount of melanin pigments within the epithelium [1].

Gingival hyperpigmentation (GHP) or black gum is due to the excessive release of melanin pigments in the gingival epithelium [2]. Gingival depigmentation (GD) is a surgical procedure used to remove or reduce excessive pigmentation [3]. This procedure leads to the formation of the surgical wound that heals by secondary intention [4].

Vitamins undoubtedly play an important role in the wound-healing process. Vitamin E has a scavenging and antioxidant activity that protects cell membranes and polyunsaturated lipids from reactive oxygen species (ROS) attack by inducing the activation of various signal transduction pathways [5].

Vitamin C, commonly known as ascorbic acid (AA), plays pivotal roles in cell migration and transformation, collagen synthesis, antioxidant response, and angiogenesis. In the inflammatory phase, it facilitates the recruitment of cells to the wound and their transformation into macrophages [6]. During collagen synthesis, vitamin C forms extra-bounds between collagen fibers that enhance the collagen matrix's stability and strength [7].

However, to date, to the best of the authors' knowledge, there has been no study that has directly compared healing by vitamin

E and vitamin C application after the gingival depigmentation procedure. The aim of our study was to evaluate the clinical efficacy of vitamin C and vitamin E on the healing and the degree of postoperative pain after a surgical depigmentation.

Materials and methods

The study was conducted in the Department of Periodontology and Oral Implantology. As determined by the following inclusion and exclusion criteria, 15 sites of the patient with 20–50 years of age were divided into two groups using a split-mouth design.

Inclusion criteria

1. Gingival melanin hyperpigmentation in maxillary and/or mandibular arches.
2. Systemically healthy.
3. Cooperative subjects.

Exclusion Criteria

1. Habit of smoking and tobacco chewing.
2. Pregnant or lactating women.
3. Subjects undergoing orthodontic therapy.
4. Subjects with a history of use of medications or nutritional supplements in the past six
5. Months.
6. Hypersensitivity to vitamin C or vitamin E

Study design

Our study design used a split-mouth approach with different quadrants selected for each patient. The evaluation of gingival pigmentation was performed in the similar examination room under consistent lighting circumstances by a reliable and calibrated examiner. The patients were positioned at a distance of one arm's length from the clinician, with their faces oriented upright so that the clinician's eyes were level with their mouths [8]. The gingival color was scored using the Dummett-Gupta Oral pigmentation Index (DOPI) [9]. The physiological gingival pigmentation score 2 and 3 were selected for the study.

Before the procedure, each quadrant was randomly assigned by the toss of a coin. After obtaining the patient's consent, subjects were assigned into two groups, A and B (figure: 1).

Fig 1: Pre-operative view

One surgeon performed depigmentation procedures through Scalpel Surgical Technique[10] for each patient under 2% lignocaine local anesthesia in the anterior maxillary and mandibular region. The complete deepithelialization and a layer of connective tissue were removed until all visible pigment was eradicated from marginal gingiva to the mucogingival junction by using surgical scalpel blade No. 15. A pressure pack was given till the hemostasis of surgical site was achieved.

- In group A: After the depigmentation procedure, vitamin E capsule (Capsule Evion 400 mg has Tocopheryl Acetate- 400mg) was directly applied tropically on the surgical site (figure: 2).

Fig 2: Vitamin E application on the right side

- In group B: After the depigmentation procedure, vitamin C (Curevit C 100 mg contains Ascorbic Acid-100mg) was applied topically with the help of the syringe on the surgical site (figure: 3).

Fig 3: Vitamin C application on the left side

Once the procedure was completed, a periodontal pack was placed. The patients were instructed to maintain oral hygiene, avoid eating spicy food and avoid brushing over the operated site till the next visit. The periodontal dressing was removed after a week. The patients were evaluated on the 15th day (figure: 4, 5) and 45th day post-operatively (figure: 6) and clinical parameters were recorded.

Fig 4: 15th day follow-up of the maxillary arch

Fig 5: 15th day follow-up of the mandibular arch

Fig 6: 45th day follow-up

Assessment of clinical parameters

1. Visual analogue scale (VAS) for pain[11]
2. Healing index (Landry *et al*. 1988)[11]

(at postoperative 15th day and 45th day)

Statistical Analysis

The observations were statistically analyzed by using the software IBM SPSS 22. Differences between the groups in terms of scores were explored using a student t-test for inter-group comparison and a Paired t-test for intra-group comparison. Mean, standard deviation (SD) and median are also calculated and P value ≤ 0.05 was taken as statistically significant.

Tables and graphs

Table 1: Visual Analogue Index

Group	Days	N	Mean±SD	Std. Error Mean	Mean Difference	Paired t-test	p value
Vitamin E (Group I)	Day 15th	15	3.600±0.828	0.214	3.533±0.834	16.412	0.001 (HS)
	Day 45th	15	0.067±0.258	0.067			
Vitamin C (Group II)	Day 15th	15	4.800±0.775	0.200	4.733±0.799	22.949	0.001 (HS)
	Day 45th	15	0.067±0.258	0.067			

HS- highly significant

Table 2: Healing Index

Group	Days	N	Mean±SD	Std. Error Mean	Mean Difference	Paired t-test	p value
Vitamin E (Group I)	Day 15th	15	4.200±0.676	0.175	0.800±0.676	4.583	0.001 (HS)
	Day 45th	15	5.000±0.000	0.000			
Vitamin C (Group II)	Day 15th	15	3.600±0.507	0.131	1.333±0.488	10.583	0.001 (HS)
	Day 45th	15	4.933±0.258	0.067			

HS- highly significant

Table 3: Visual Analogue Index

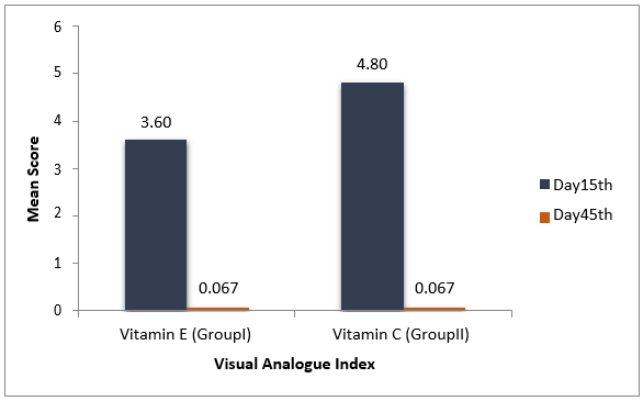
Days	N	Vitamin E (Group I)	Vitamin C (Group II)	t-test	p value
Day 15th	15	3.60±0.83	4.80±0.78	4.099	0.001 (HS)
Day 45th	15	0.07±0.26	0.07±0.26	0.000	1.000 (NS)

HS- highly significant, NS- non-significant

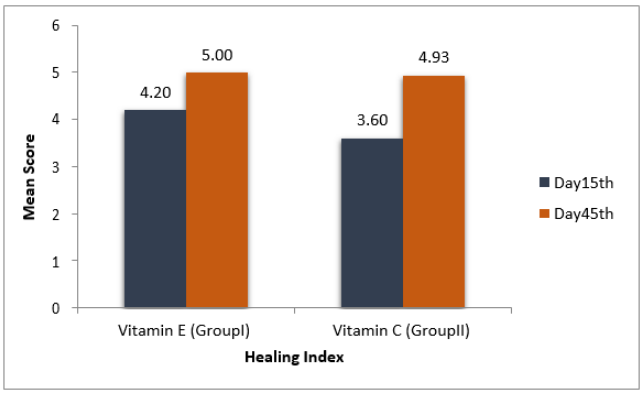
Table 4: Healing Index.

Days	N	Vitamin E (Group I)	Vitamin C (Group II)	t-test	p value
Day 15th	15	4.20±0.68	3.60±0.51	2.750	0.011 (S)
Day 45th	15	5.00±0.00	4.93±0.26	1.000	0.326 (NS)

S- significant, NS- non-significant



Graph 1: Visual analogue index



Graph 2: Healing index

Results

There was a statistically significant reduction in the Visual Analogue Scale and an improvement in healing in both groups. But when a comparison was done between the 2 groups subjects in the vitamin E group showed faster healing and reduced pain on the 15th day compared to subjects in the vitamin C group. Subjects of both groups on the 45th day showed similar healing and pain reduction.

Discussion

Gingival hyperpigmentation is an aesthetic issue that concerns many people. One frequent approach to manage this problem is surgical depigmentation using the Scalpel Surgical Technique. The excision the gingival epithelium and a layer of the connective tissue is done. The surgical site will heal by secondary intention. The newly formed epithelium will not contain melanin pigmentation [4]. However, the healing of the surgical wound plays a crucial role in achieving desirable aesthetic outcomes and patient comfort.

The study used a split-mouth design, ensuring a balanced comparison between vitamin E and vitamin C applications in the same patient. Both vitamins were applied topically after the surgical procedure to know the potential role of vitamins E and C in wound healing. Vitamin E is known for its antioxidant properties, protecting cell membranes from oxidative stress, while vitamin C (ascorbic acid) plays a key role in collagen synthesis, cell migration, and angiogenesis.

The findings of this study align with previous research that has demonstrated the beneficial effects of vitamin E on wound healing. Earlier studies have shown that the administration of a tocopherol could promote the proliferation and migration of human gingival fibroblasts (HGFs), MG-63 osteoblast-like cells, and periodontal ligament fibroblasts (PDLFs) in the presence or absence of a stimulus [12, 13, 14].

In addition, a-tocopherol has been shown to increase the expression of basic fibroblast growth factor (bFGF) and the production of collagen type I [14]. bFGF is a potent angiogenic factor. It is widely expressed during cell differentiation and wound healing. bFGF enhances the proliferation of various cells, including fibroblasts, osteoblasts, and endothelial cells, which are important for periodontal wound healing and tissue regeneration [15]. Sanadi R and Singh U. (2018) [16] reported that the applications of vitamin E showed significant improvement in healing with better VAS scores. Vitamin E can exert its anti-nociceptive effects because of its antioxidant properties [17]. Nitric oxide (NO) is one of the free radicals that has been implicated in central pain processing. The interaction of vitamin E with Nitric Oxide is a mechanism by which it could exert its analgesic effect [18].

Vitamin C also promotes collagen biosynthesis [19], scavenges free radicals, and enhances the immune system [20]. Vitamin C was chosen due to its potent antioxidant effect and its great efficiency in depigmenting the dermal and gingival tissues either topically (Shimada *et al*., 2009) [21]. Or intradermally (Yussif *et al*. 2019) [22]. Sheel *et al*. [23]. Conducted a study to assess the effectiveness of Vitamin C as a depigmenting agent following surgical scalpel depigmentation on the gingiva. They recorded satisfactory aesthetic results with low subjective levels of pain, and throughout a 9-month monitoring period, there were no occurrences of recurrence. In this study, vitamin E appeared to have a more significant impact on early healing outcomes compared to vitamin C on the 15th day and reduced VAS. The results are encouraging and provide evidence for the prospective utilisation of vitamin E as a supplementary treatment to enhance the outcomes of gingival depigmentation. Overall, this study opens the door for continued investigation into the role of vitamins in optimizing the aesthetic outcomes of gingival depigmentation procedures.

The gingival repigmentation defined as a clinical reappearance of melanin pigment after a period of clinical depigmentation of the oral mucosa [24]. Perlmutter and Tal [25]. documented a case where a patient experienced gingival repigmentation seven years after the excision of gingival tissues. The process of repigmentation is currently not fully comprehended. However, based on the migration theory, it is believed that melanocytes, which are the cells responsible for producing pigment, become active in the surrounding pigmented tissues and migrate towards the treated areas, resulting in unsuccessful outcomes. There is a scarcity of reports regarding the process of repigmentation, and the available reports show a wide range of outcomes. Ginwalla *et al*. [26] conducted studies that demonstrated a 50% repigmentation rate in patients employing the abrasion approach within a period of 24-56 days [27]. Pal *et al*. [28] demonstrated that surgical bur gingival depigmentation resulted in repigmentation in 19% of the instances. However, in this study occurrence of repigmentation of gingiva has been reported at 45th day in few cases and difference can't be made between group 1 and group 2.

The study has certain limitations, such as a small sample size and a relatively short follow-up period. Vitamin E is in gelatin consistency it might be retained more on surgical sites than vitamin C which may influence the result. Further research exploring the molecular mechanisms through which vitamin E and vitamin C impact gingival wound healing could offer more comprehensive insights into their clinical potential.

Conclusion

The findings suggest that the application of Vitamin E and Vitamin C shows promising results however the application of vitamin E shows faster healing and reduced pain following the surgical depigmentation procedure compared to vitamin C.

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