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Methodology

Minimally invasive non-surgical locally injected vitamin C versus the conventional surgical depigmentation in treatment of gingival hyperpigmentation of the anterior esthetic zone: A prospective comparative study

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SUMMARY

Objective: The purpose of the current study is to evaluate the efficacy of intra-epidermal vitamin C injection in comparison to the conventional surgical technique in order to manage patients with gingival hyperpigmentation.
Design: Thirty patients were enrolled seeking for gingival depigmentation with mild to severe hyperpigmented gingival tissues. Clinical evaluation was performed pre and post-operatively. Double evaluation of the depigmenting effect was performed using two different color assessment indices (Takashi and Kumar indices). The pain and itching grades were reported using VAS scale.
Results: at baseline and 9 months, there was no statistical significant difference between both groups in index 1 and 2. At one month, statistical significant difference was reported in index 1 (p value = 0.003) and index 2 (p value = 0.002). Regarding pain score, statistical significant reduction in scores starting from day 1 (0.004), day 2 (0.0001) to 7 days (0.0015). Both surgical and non-surgical techniques revealed nearly equivalent results. Although non-surgical technique is less traumatic, it has several disadvantages such as time consumption - at least four weeks to achieve

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homogenous gingival color and needs tactile sensation. In addition, critical zones for injection have to be avoided.

Conclusions: The current study examines the points of strength and weakness of both techniques in depth. The surgical technique is considered as the gold standard for management of gingival hyperpigmentation. The usage of vitamin C injection for depigmentation showed comparative results in comparison to the conventional technique. Furthermore, the usage of vitamin C could provide a long term stability of the gingival color if used after the surgical procedure. Finally, we suggest that using both techniques has to be pre-determined according to the clinical examination and the patient's desire. Further researches are recommended to study the efficacy of using booster doses for long term color stability.

1. Introduction

Although physiologic gingival hyperpigmentation is not a pathologic condition, it is considered one of the main esthetic problems in dentistry. It was found that the attached gingiva is the most frequently pigmented intraoral tissues followed by the papillary gingiva and the alveolar mucosa [1,2] *(Prasad et al., 2010; Javali et al., 2011)*.

Melanocytes are the main cells that are responsible for melanin production. They are mainly found in the basal layers of oral epithelium. They are profoundly particular somatic cells portrayed by peculiar behavior [3,4] (*Nanci, 2008 and* Almeida-Paes *et al., 2012)*. Melanocytes have several functions that are of noteworthy significance than melanin production as stress-sensor, immune-regulator and neuroendocrine cell [5,6] *(Thomas & Erickson, 2008 and Feller et al., 2014)*. Nevertheless, there is a type of melanocytes that could not produce melanin called amelanotic melanocytes. The amelanotic cells are physiologically found all over the body especially the oral cavity and other sites. They differ in shape from previously described melanotic cells. They are small, round and contain melanin free premelanosomes. The number of amelanotic cells exceeds that of melanotic cells in tissues. Amelanotic melanocytes explains why no visible pigmentation is detected, inspite of presence of melanocytes in the whole oral mucosa [7,8] *(Busam et al., 2001 and Zhang et al., 2007)*. Among human beings, oral cavity is one of the non-classical sites for melanin in the human body [6,7,9] *(Busam et al., 2001; Colombo et al., 2011 and Feller et al., 2014)*.

Despite its predetermined number, melanocytes have lower proliferation rate with longer life expectancy. The produced melanin provides great protection to both melanocytes and the surrounding keratinocytes [10] *(Plonka et al., 2009)*. Melanocytes can undoubtedly defeat its limited number either by migration or relocation starting from one site to another or donation of their products to the surrounding cells through different communicating techniques [11] *(Boissy, 2003)*. The dendrites' length is related to the quantity of the surrounding keratinocytes. Melanin could be delivered to the surrounding cells in the form of melanosomes. The thicker the gingival biotype is, the longer and higher the number of the menlanocyte's dendrites which mirrors the role of keratinocytes in stimulating melanogenesis [12,13] *(Kippenberger et al., 1998 and Letort et al., 2010)*.

During inflammation, melanocytes relocate to the inflammatory site carrying E-cadherins on its surface. Once melanocytes achieve the chose locale, switch from the E-cadherin into N-Cadherin takes place so as to communicate the surrounding keratinocytes and start proliferation [12,14] *(Kippenberger et al., 1998 and Tolleson, 2005)*.

The color of the dermal and oral tissues commonly varies according to melanin type. In case of high cellular content of cysteine, the produced melanin is predominantly pheomelanin (yellow/red color).

On the other hand, eumelanin (brown/black) is commonly produced in case of cysteine deficiency [15,16] *(Prota, 2000 and Lin & Fisher, 2007)*. Calcium, copper and iron are essential elements for eumelanin formation [17] *(Joshi et al., 2007)*.

Different treatment modalities have been utilized as a part to overcome the gingival hyperpigmentation. The selection of the appropriate depigmenting technique ought to be based on the type of presenting pigment, gingival biotype, clinical experience, patient's financial status and individual preferences [18,19] *(Doshi et al., 2012 and Patil et al., 2015)*.

Non-surgical approaches as well as surgical intervention have been proposed for the management of melanin pigmentation. The surgical approaches depend mainly on the de-epithelization of the target region. It could be performed utilizing various techniques as free gingival grafts, gingivectomy or de-epithelialization using bur abrasion [20] *(Lee et al., 2011)*, surgical blade [21] *(Hegde et al., 2013)*, laser [22] *(Giannelli et al., 2013)* and cryosurgery [23] *(Kumer et al., 2013)*.

The mechanism of the non-surgical approach used previously was the chemical de-epithelization which depends predominantly on the chemical burn and sloughing of the epithelial layer containing melanocytes. The pharmacological agents commonly used were 90% phenol or 95% ethanol solutions which are highly traumatic to the oral tissues [24] *(Hirschfeld & Hirschfeld, 1951)*.

A nearly equal range of pigment recurrence was recognized with the previously mentioned different techniques. The recurrence rate is uncontrolled and unpredictable due to the obliviousness of the three dimensions of the epithelial retepegs [19] *(Patil et al., 2015)*. On the other hand, some researches justified this recurrence by the migration or relocation hypothesis of melanocytes [25] *(Oringer, 1975)*.

Vitamin C (ascorbate, ascorbic acid, AA) is one of the approaches involved in skin depigmentation whether via topical or transdermal or intravenous approaches [26] *(Ngamratanapaiboon et al., 2012)*. Vitamin C is a water-soluble antioxidant and essential nutrient for immune cells and host cells [27] *(Velisek & Cejpek, 2007)*. Despite the great significance of vitamin C, it cannot be synthesized in the human body due to mutation of the gene needed for its synthesis [28] *(Nishikimi et al., 1994)*. L-Ascorbic acid (vitamin C) also promotes collagen biosynthesis [29] *(Urban et al., 2012)*, provides photoprotection [30] *(Panich et al., 2011*), strengthening skin layers, reduces the melanin [31,32] (*Huh et al., 2003 and Choi et al., 2009)*, scavenging of free radical [33] *(Weidinger & Kozlov, 2015)* and enhancing of the immune system [34] (*Diaz et al., 2015)*.

In addition to all of the previously mentioned actions of vitamin C, it was found to be involved in depigmentation due to several factors that not only depend on its direct effect on melanin and melanocytes but also due to the overall effect on the applied tissues. Melanin is one of the main reservoirs for ROS, copper and calcium in the tissue cells [17,35,36] *(Morganti et al., 1999; Joshi et al., 2007 and Tsai et al., 2014)*. Once vitamin C is introduced to the target tissue, it binds efficiently to melanin due to the reactive oxygen species (ROS), calcium and copper content which causes intracellular deficiency of these items and the inability of the cells to produce melanin. Calcium deficiency causes failure of melanocytes to perform cellular adhesion to keratinocytes as calcium is essential to form cadherins [12,14] *(Kippenberger et al., 1998 and Tolleson, 2005)*. Adhesion to keratinocytes is important stimulator to melanocytes in order to produce melanin, format dendrites and transfer the produced melanin to neighboring cells [12,13] *(Kippenberger et al., 1998 and Letort et al., 2010)*. Shortage of the intercellular copper limits the formation of tyrosin, tyrosinase enzyme and peroxidase enzyme which in turn stops the melanin production [37] (*Chang, 2009)*.

Therefore, the aim of the present study was to compare the clinical efficiency of the non-surgical intraepidermal injection of vitamin C in comparison to the gold standard surgical technique (scalpel technique) for gingival depigmentation.

2. Methodology

2.1. Ethical aspects

The study was conducted after the approval of the Ethical Committee of the Faculty of Dentistry, Cairo University prior to conducting the research. The Ethical Committee confirmed that the study was

performed according to the established ethical guidelines. All participants signed an informed detailed consent form before participation, where benefits, steps and side effects of the treatment protocol were fully explained.

2.2. The study design

The study was designed as non-randomized prospective clinical study. Following examination, the enrolled patients were divided equally to the study groups.

2.3. Screening procedures

Patients were recruited among those diagnosed with plaque induced gingivitis in the post-graduate periodontology clinic at Cairo University between May 2016 and October 2016. An initial evaluation, including medical and dental history, clinical examination, and radiographic examination, was conducted to determine patient eligibility for the study. Thirty examined patients who met the following inclusion criteria: 1) above 18 y; 2) systemically free; 3) physiologic gingival hyperpigmentation related to esthetic region were selected. All the reasons that could provoke an inflammatory reaction were excluded such as: 1) systemic diseases (especially auto-immune diseases and chemotherapy uptake); 2) pregnant and lactating mothers; 3) usage of chlorohexidine or povidone iodine; 4) Local causes (smoking) 5) Free of periodontal diseases (plaque and non-plaque induced gingivitis or periodontitis).

The included patients were divided into 2 groups: group 1 (control group) of 15 patients treated with conventional scalpel surgical depigmentation and group 2 (test group) of 15 patients treated with non-surgical intra-epidermal injection of vitamin C depigmentation.

2.4. Patient preparation

Each patient received full-mouth sessions of supragingival scaling using ultrasonic and hand instrumentation and received personalized oral hygiene instructions. In addition, quaternary ammonium mouth rinsing (Citrolin mouth wash, Cid Company, Egypt) was recommended to be used before procedure. All patients were placed on a 2 weeks maintenance recall appointments. Oral hygiene measurements with modification of the patient habits were performed prior to treatment. Stoppage of spicy, acidic, coloring and hard food was recommended pre-operatively.

2.5. Non-surgical procedure

The same operator (N.Y) performed all the injection procedures. The site of interest was anesthetized using topical anesthetic agent (lignocaine gel or xylocaine gel). Infiltration anesthesia was recommended during the first injection visit. Intraepidermal injection (oral mesotherapy technique) of 1–1.5 ml (200–300 mg concentration) of L-ascorbic acid (Cevarol ampoule- Memphis company- Egypt) was done. It was locally introduced in relation to the keratinized gingival tissues with extension to the whole target region successively using special syringes (30 gauge). The needle was introduced parallel to the gingival tissues with the bevel facing upwards. Vitamin C was then delivered through the attached gingival tissues at the epithelium-connective tissue junction (equivalent to epidermal- dermal junction) till the tissues blanch. Maximum 0.1 ml of ascorbic acid was recommended for each point with 2–3 mm apart. The same dose was repeated once per week for maximum 4 visits till no further color improvement gained [38,39] (*Matarasso & Pfeifer, 2009 and Latha &Vandana, 2011*).

2.6. Surgical depigmentation

The same operator (N.Y) performed all the surgical procedures. For gingival ablation, epithelial layer and part of the connective tissue layer were removed using 15c blades till the pigment disappeared. Caution was taken at the canine region to avoid bone exposure.

Thick gingival biotype is considered the most suitable condition for performing the surgical technique. Removal of adequate thickness of the gingival tissues is mandatory in order to reduce the recurrence rate. The marginal and interdental papillary tissues remain critical regions even in thick gingival biotypes. The biotype of these regions is not usually equivalent to the biotype of the remaining parts of the gingival tissues. In case of thin gingival biotype, papilla spare technique is recommended (being away from the gingival margin and interdental papilla with 1–2 mm). Thus, papillary depigmentation is not recommended in case of interproximal bone loss. Fortunately, these regions are not deeply pigmented which may be due to the thin epithelial layer and thus causing; minimal induction to the pigment cells. In case of thin gingival biotype and deeply located pigmentation, surgical depigmentation is not highly recommended. Periodontal dressing is recommended after treatment for 7–10 days. Healing occurs by secondary intention. Up till now, surgical depigmentation is considered one of the simplest periodontal surgical techniques as it requires minimal time, less effort and armamentarium [21,40] *(Bhusari & Kasat, 2011 and Hegde et al., 2013)*.

2.7. Post-operative instructions

The postoperative instructions differed between both groups. In the surgical group, periodontal pack was currently placed following the surgical procedure in order to provide high protection to the surgical field till healing occurs. Patients were instructed to avoid mechanical oral hygiene during the first week after surgery so as to avoid the mechanical trauma to the treated sites. Patients were prescribed a rescue analgesic (Ibuprofen 400 mg) to be used as needed. While following each injection visit, the patients were asked to abstain from mechanical oral hygiene procedures in relation to the target region for the day of procedure only. Plain tooth pastes (Colgate tooth paste) with anti-inflammatory mouth wash (Citrolin mouth, Pharco Company, Egypt) were recommended. Analgesics were recommended if pain or itching was detected in the first day.

2.8. Clinical parameters

Pre and post each procedure, the following clinical measurements were recorded by the same examiner (A.R.A). The clinical assessment of the degree of gingival pigmentation was performed using two different indices; Takashi index [41] *(Hanioka et al., 2005)* 0: no pigmentation; 1: solitary unit (s) of pigmentation in papillary gingiva without extension between neighboring solitary units, 2: formation of continuous ribbon extending from neighboring solitary units and Gingival pigmentation index [23] *(Kumar et al., 2013)*; *Score 0:* absence, *Score 1:* spots of brown to black, *Score 2:* brown to black patches but not diffuse and *Score 3:* diffuse brown to black pigmentation. The color assessment was evaluated in the day light and by examining the regular digital photographs which were taken pre-operative, and post-operatively after 1 and 9 months.

The immediate effect of both treatments was detected after one week of the completion of the procedure. The recurrence rate was measured after 9 months post-operatively. Pain and itching were reported by the patient after the surgical procedure and following each injection visit using visual analogue scale (VAS). Patient satisfaction was performed by using a 5- graded self-assessment analysis; excellent (grade 4: improved over 75%) good (grade 3: improved 50–75%), moderate (grade 2: improved 25–50%); fair (grade 1: improved less than 25%); no change or worse (grade 0: not improved or darkened) [31] *(Huh et al. 2003)*.

The visual analogue scale is divided into vertical and horizontal parts. The horizontal part (numerical) consists of 10 cm line with two end points represents "no pain" to "severe pain". While the vertical scale (verbal) measured by 5 points: no itching (zero), mild itching (1 point), moderate itching (2 points), severe itching (3 points), extremely severe (4 points) [42] *(Reich et al., 2012)*.

2.9. Statistical analysis

Data collection and analysis was performed by one author (A. E. B). *Numerical data were presented as mean, median, standard deviation (SD), minimum, maximum and 95% Confidence Interval (95% CI) values. Data were explored for normality using Kolmogorov-Smimov and Shapiro–Wilk tests. * Index 1,

index 2 and pain score data showed non parametric distribution. * For non-parametric data, Mann–Whitney U test with Bonferroni's adjustment was used for pair-wise comparison between the groups when Kruskal–Wallis test is significant. Wilcoxon signed-rank test was used to compare between test and control sides as well as to study the changes after treatment. * For previous tests a probability value (P value) $\leq$ 0.005 was considered statistically significant. *Statistical analysis was performed with IBM (® IBM Corporation, NY, USA) ® SPSS® (SPSS, Inc., an IBM Company) Statistics Version 20 for Windows. The power analysis was performed in accordance to Yussif et al., 2016 [43].

3. Results

All the enrolled patients completed the entire follow up period. The degree of gingival color, patient satisfaction, pain and itching score were measured in both groups.

The treatment period between the used treatment protocols, the enrolled patients showed great satisfaction about the end results of both groups. Bleeding, edema and post-operative pain were the main complications of the surgical group during the first week following the procedure. First day itching was the main complaint of the non-surgical group as well as the surgical group. The main advantages of the non-surgical technique from the patient's point of view were the short clinical visits and such technique does not interfere with the normal functions as speaking, eating and laughing.

Stages of color improvement during vitamin C injection:

1-Immediately following injection; the gingival tissues fainted and then darkening of the pigmented areas occurred.

2-Stage 1 (1st week): fainting of the whole color of the gingival tissue and the tissues became glossy and stretched. The areas of least pigmentation turned pink

3-Stage 2 (2nd week): the gingival biotype began to improve. More fainting action appeared in relation to the whole gingival tissues and the pinkish color began to spread. A whitish coat covering the gingival tissues appeared clearly, which could be rubbed off easily using cotton. This coat resembled material Alba. On rubbing, the underlying tissues appeared totally healthy and intact.

4-Stage 3 (3rd week): further improvement of the gingival biotype followed. Further fainting with the pink color predominated. The gingival tissues became glossier and highly stippled.

5-Stage 4 (4th week): almost pinkish gingival tissue detected.

The tissues treated with vitamin C depigmentation usually took longer time for remodeling than the surgical one. Better color was usually detected 1 month after stoppage the injection (Fig. 1).

During and after the intra-epidermal injection, itching was the predominant unpleasant sensation which enforced the patient's desire to scratch. Its evaluation ranges between verbal questionnaire and severity assessment (using visual analogue scale).

After the first visit of vitamin C injection, the patient was asked about the intensity and the duration of itching. According to the patient's answer, the dose is adjusted. If itching lasted for the whole night or to the next day with the same intensity, this is an indicator for higher dose usage or deeper introduction of the vitamin during injection. Lower dose or shallower injection was done in the following visits. In general, there is no definite dosage to be used as it differs from one to another but we proposed guidelines or range of the proposed efficient one.

During the first visit, avoidance of the critical areas (the dangerous zones) is preferred. The mucogingival line remains the most resistant region because of the probable escape of the watery vitamin C into the alveolar mucosa which differs from the attached gingiva as it cannot keep the liquid intercellularly (Fig. 2).

3.1. Comparison between test and control groups (intergroup comparison) (degree of pigmentation according to index 1, Takashi index)

At the base-line, there was no statistically significant difference (p value = 0.9) in pigmentation area according to index 1 value between test and control groups. After one month follow up, there was a statistically significant difference (p value = 0.003) in pigmentation area according to index 1 value between test and control groups while there was no significance (p value = 0.46) after 9 months follow up, Fig. 4.

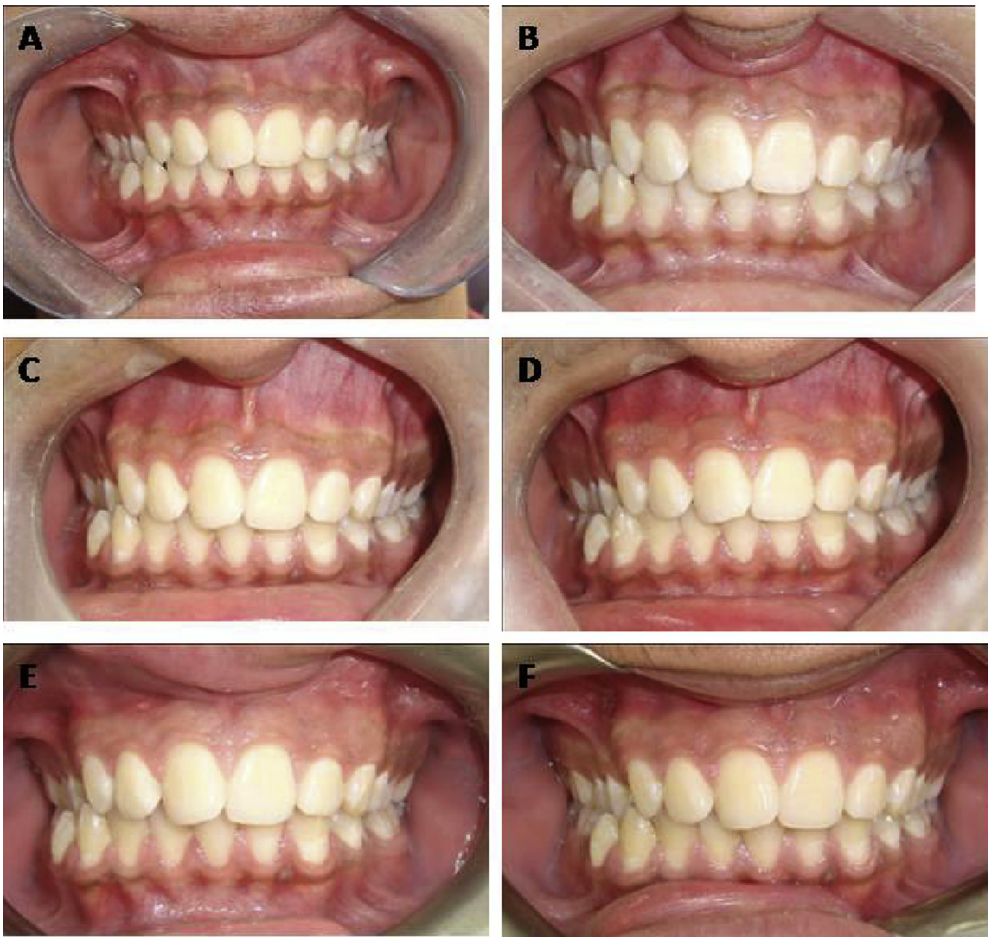

Fig. 1. The stages of vitamin C depigmentation; A: pre-operative, B: after 1st visit, C: after 2nd visit, D: after 3rd visit, E: after 1 month and F: after 9 months follow up.

3.1.2. Comparison between test and control groups (degree of pigmentation according to index 2, Kumar index)

At the base-line, there was no statistically significant difference (p value = 0.14) in pigmentation area according to index 2 value between test and control groups. After one month follow up, there was statistically significant difference (p value = 0.002) in pigmentation area according to index 2 values between test and control groups while there was no significance (p value = 0.39) after 9 months follow up, Fig. 5.

3.1.3. Degree of pain or itching in both groups

In the 1st day there was statistically significant decrease (p value = 0.004) in pain or itching score in the control group more than that in the test group. Pain score statistically and significantly decreased in the test group in the 2nd (p value = 0.0001), 3rd (p value = 0.0001) and after 1 week (p value = 0.0015) more than that in the control group Fig. 6.

4. Discussion

Gingival melanin hyperpigmentation is an aesthetic problem which concerns many individuals. Although, it is not a medical problem, demands for cosmetic therapy of gingival melanin

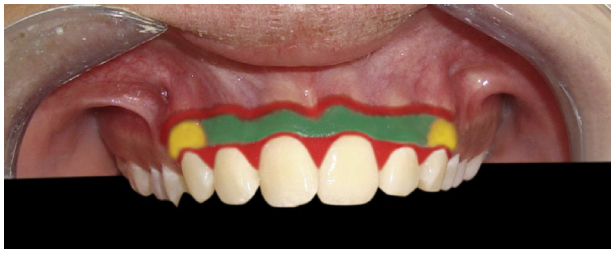

Fig. 2. The red color represents the critical zone; the green color represents areas to be safely injected while the yellow color represents the areas to be injected with caution due to canine eminence (thin gingival and bone biotype).

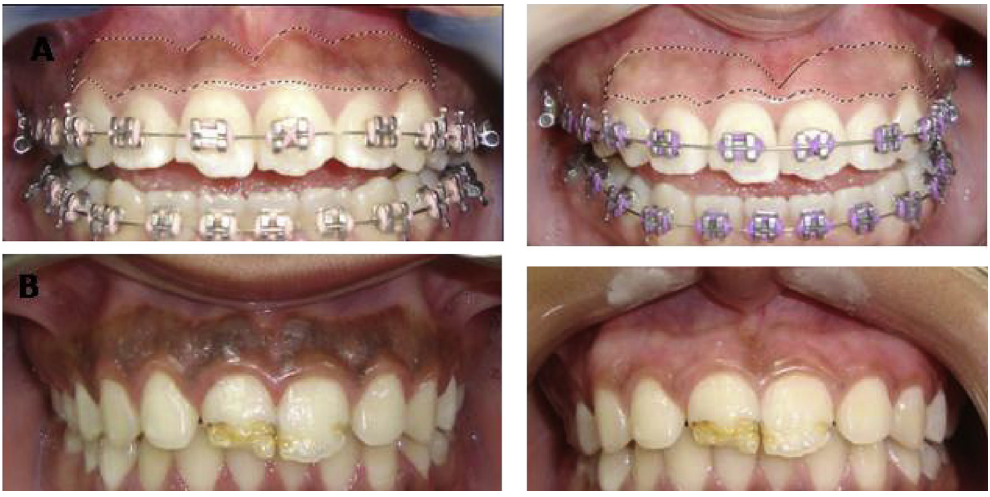

Fig. 3. A) pre- and post-operative photos of vitamin C depigmentation, B) represents pre and post-operative photos of surgical depigmenation.

hyperpigmentation have been increasingly common [1,2] (Prasad et al., 2010 and Javali et al., 2011). It was found that melanin pigment is essential for protection of the nuclei of the dermal keratinocytes against ultraviolet radiation and other irritating factors [10] (Plonka et al., 2009). While in the gingival tissues, there is minimal need to such protection.

Due to high esthetic demands, the proposed technique should be simple, easy and based on careful analysis of pigmented gingival tissues. The surgical intervention is most commonly used to treat physiologic gingival hyperpigmentation. It depends on removal of the full thickness of the epithelial and the papillary connective tissue layer [19,21] (Hedge et al., 2013 and Patil et al., 2015). In spite of, the overall advantages of the surgical depigmentation, it remains a source of fear to patients asking for better esthetics. Bleeding, pain, large postoperative wound and recurrence are the main patient relevant disadvantages [19] (Patil et al., 2015) (Fig. 3). In thin gingival biotype, higher possibility of recurrence was detected. Recurrence also depends on the character and shape of basement membrane, melanocyte activity and phagocytic ability of the keratinocytes [25] (Oringer, 1975).

Therefore, there is a need to other minimally invasive non-surgical therapeutic option in order to depigment such conditions. The aim of the current study was to compare the efficiency of vitamin C versus the traditional surgical technique in the gingival depigmentation.

Thirty patients were enrolled, divided into two groups and finally evaluated at the baseline (after 4 visits) and in the recall visit (after 1 and 9 months of the last intraepidermic vitamin C injection).

Vitamin C was chosen due to its potent antioxidant effect and its great efficiency in depigmenting the dermal and gingival tissues either topically [44] (Shimada et al., 2009) or intradermally [43,45] (Yussif et al. 2016 and Yussif et al., 2017).

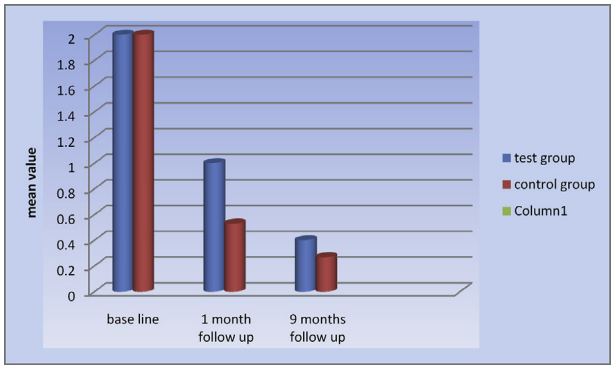

Fig. 4. Showing the mean values of changes in pigmentation in both groups according index 1.

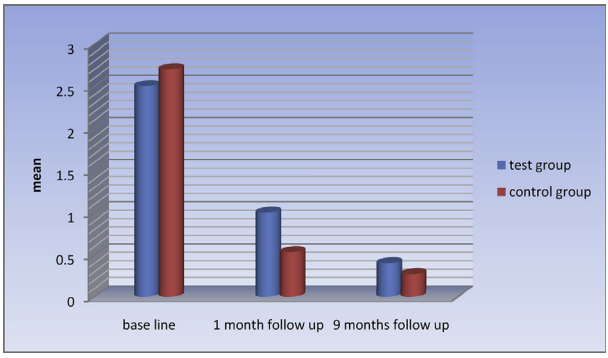

Fig. 5. Showing the mean values of changes in pigmentation in both groups according index 2.

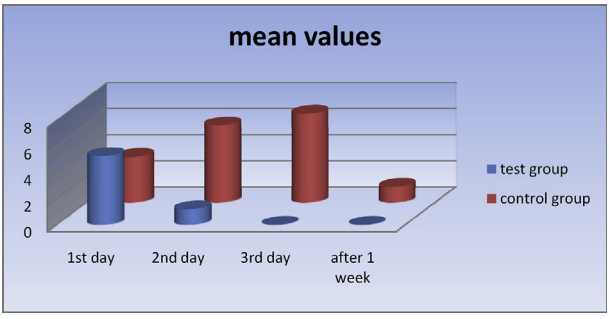

Fig. 6. Showing the mean values of changes in pain and itching in both groups.

In the current study, Vitamin C was injected at the sub epithelial level. There was no limit of the number of the injection visits regards to its safety. Best Results were detected during the first four visits. The number of visits was determined according to the severity of the condition. Improvement of the tissue color, form and biotype was clearly observed in the recall visits.

Our Results were in agreement with [44] *Shimada et al. (2009)* and [46] *Sheel et al. (2015)*. *Shimada et al. (2009)* [44] confirmed the potential role of ascorbic acid in treatment of gingival melanin pigmentation by their placebo-controlled clinical trial. *Sheel et al. (2015)* [46] reported the ancillary role of vitamin C along with surgical scalpel excision in their case report study.

Although vitamin C is safe, it remains a weak acidic product which may result in tissue necrosis if given in extra dose. Tissue capacity differs according to the thickness, and relation to the underlying bone. The interproximal papillary region, the mucogingival junction, areas of thin gingival biotype and gingival tissues without underlying bone loss are the most critical regions. Therefore, tactile sensation and avoidance of injection (if possible) is quite important.

The immediate changes occurred as an effect of the intraepidermal injection caused by the rapid introduction of vitamin C into keratinocytes [43,47] *(Kimura et al 1981 and Yussif et al., 2016)*. Attraction, most probably, occurs between vitamin C molecules and melanin due to its high ROS content.

In the second visit, the gingival biotype began to improve and the whole texture of the gingival tissues changed. The injection of the interproximal regions began with minimal amounts. White layer covering the gingival tissues might be detected after a week which may be due to the high turnover of the epithelial layer.

The efficiency of vitamin C in the management of physiologic gingival melanin hyperpigmentation was evaluated in the present study by two pigmentation indices (Takashi Melanin pigmentation index and Kumar index). Following injection, the resulted color of the gingival tissues was heterogonous. Faint demarcation lines were detected between the injected areas. Higher degree of heterogeneity could be detected with deeper pigmentation. After 1 month, total remodeling of the gingival tissues was achieved.

By screening the pigmentation scores of both indices, no statistical significant difference was found between mean scores using both indices at baseline and 9 months follow up. On the other hand, statistical significant difference was obtained between test and control groups at one month follow up. This was in accordance with [44] *Shimada et al. (2009)* who evaluated the depigmenting effect of locally applied vitamin C. They revealed significant improvement of the gingival color at 4 weeks but no significant difference was apparent between 8 and 12 weeks.

Pain and itching were regarded as painful stimuli. Itching may transit to pain due to increased discharge frequency of nociceptors (intensity theory) [48] *(Schmelz, 2010)*. Pain and itching are mainly caused by acidity and/or needle prick. Acidity is an essential property for ideal tissue penetration depth [49] *(Crisan et al., 2015)*. About 6% of the patients complained from mild itching and local discomfort for about 30 min after the introduction of a 5% concentration of topical vitamin C. If itching persisted till night or to the next day, it was considered an indicator for higher dose or deeper penetration. Regarding the intergroup comparison, statistical significant difference was detected between group at the first day treatment revealing higher itching of the test group over the control group. On the other hand, such relation was completely reversed at the days after. Furthermore, the intragroup comparison in the test group revealed clinically and significant itching during the first day of the first injection visit in comparison to other injection visits.

4.1. Recommendations for future studies

Using booster doses of vitamin C injection following both techniques is highly recommended. Prospective studies with long term follow up are also recommended.

Disclosure

This paper's contents are solely the responsibility of the authors

Conflict of Interest

The study is self funded and none of the authors have any competing interests with respect to this paper.

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