The adjunctive effect of hyaluronic acid gel on healing and patient satisfaction following conventional surgical gingival depigmentation: A Randomized Clinical Trial with Histological Analysis

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Abstract

Aim: The aim of this study was to evaluate the effect of hyaluronic acid gel following conventional surgical gingival depigmentation technique on the healing period and assessment of patient satisfaction.
Materials and methods: 16 healthy non-smokers with hyperpigmented gingiva aged from 20-30 years were randomly assigned for conventional surgical gingival depigmentation using abrasive burs (Group I) (study); HA-gel was applied topically to the wound surface postoperatively for one week, and no application was made to (Group II) (control). Pigmentation index (DOPI) was evaluated regularly at baseline,3days,1 week,2 weeks,1 month and 3 months. While wound Healing Score and Visual analogue scale (VAS) were evaluated at 1day,3days,1 week and 2 weeks, also patient satisfaction questionnaire was done. Histological analysis was performed after 1 week.
Results: In both groups, the DOPI decreased significantly from baseline and maintained 0 score through the 3 months of follow up. Regarding wound healing, there was a statistically significant difference between the values at 1 week (P=0.0014) and 2 weeks (P=0.0256) where reduction of wound healing score was significantly higher in group I. Pain decreased gradually through the follow up periods in both groups but there was statistically significant difference between both groups. After 1week of treatment, Epithelial thickness and number of blood vessels mean value in study group had a significantly higher mean value than control group and were statistically significant. There was a significantly strong negative correlation between the epithelial thickness and clinical wound healing (CWH), and VAS at 1 week, r = -0.861, -0.903 (P-value =0.0002, < 0.0001) respectively.
Conclusion: HA was observed to be useful in inducing better wound healing, reducing pain while no effect on depigmentation when comparing the two groups. All patients were satisfied with the pain and esthetic outcomes. H.A also induced better epithelium, vascular and less inflammatory cells formation.
Keywords: Conventional surgical; Depigmentation; Gel; Hyaluronic acid; Healing.

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Introduction

Esthetic needs are increasing in recent years with a greater demand on pleasant appearance of the smile. Gingiva is a vital component of the smile along with lips and a healthy set of dentitions .[1,2]

Gingiva is the part of the oral mucosa which covers the alveolar processes, surrounds the necks of the teeth, and is divided anatomically into marginal, attached, and interdental areas .[3]

The normal gingival color is pink (coral pink). It is determined by the degree of keratinization, epithelial thickness, the size and number of underlying blood vessels and melanocytes function .[4]

Melanocytes are responsible for the production of melanin which causes the oral pigmentation. The presence of melanocytes in the oral epithelium is a well-established fact. It provides protection from environmental stress such as ultraviolet radiation and reactive oxygen species .[5]

Excessive deposition of melanin in the basal and supra-basal cell layers of the epithelium causes excessive discoloration of the gingival mucosa which is called **gingival hyperpigmentation.** It can be physiologic or pathologic. Pathologic pigmentation can be classified according to the reason into endogenous and exogenous .[6]

Gingival hyperpigmentation is one of the factors which affects smile esthetics, especially when it is located in the anterior labial region and for individuals with high-smile line .[7]

Gingival depigmentation is a periodontal plastic surgical procedure by which gingival hyperpigmentation is removed or reduced by different treatment modalities which can be classified into: methods that remove pigments and methods that mask the pigment .[8]

Removal of pigment can be done by surgical and chemical methods. Surgical methods mainly include conventional method which usually refers to scalpel blade techniques or bur abrasion or using advanced electrosurgery, cryosurgery, lasers and radiosurgery technique .[9]

In bur abrasion method a medium grit flame-shaped diamond bur is used at high speeds to denude the epithelium. Controlling the speed and pressure of handpiece to avoid undesirable pitting of the tissue is mandatory. It is relatively a non-invasive, simple, cost-effective technique, however, is a sensitive technique, its wound is left for healing by secondary intention, associated with, post-treatment pain and greater risk of bacterial contamination in these types of wounds .[10]

In this respect, there is a need for therapeutic agents to improve the clinical results of wound healing, increase patient comfort, shorten the healing process, and obtain the formation of healthy tissue .[11]

Several different types of natural and

commercially available wound care products have been used, including platelet rich fibrin, collagen, cyanoacrylate, eugenol–containing and non-eugenol periodontal dressings and hyaluronic acid (HA) .[12]

Hyaluronic acid (HA) is a naturally occurring linear polysaccharide of the extracellular matrix of connective tissue, synovial fluid, and other tissues. It retains various physiological and structural functions that include cellular and extracellular interactions, interactions with growth factors and regulation of the osmotic pressure, and tissue lubrication .[13]

HA provides very important functions in wound healing. The degradation product of HA is reported to have a proangiogenic effect. It makes a local environment with a water binding effect, which may provide a chance to produce a matrix for wound healing. HA has been reported to be a substance with viscoelastic properties, and by forming a protective layer on wound surfaces.[14]

The hyaluronic acid gel has anti-inflammatory properties and has been found to induce more epithelial tissue formation. Also, it has anti-oedematous effect. All these properties may be useful in accelerating tissue healing .[15]

Materials and Methods:

Study design and Settings:

All procedures in the current trial were carried out in accordance withthe ethical standards of the Research Ethics Committee of the Faculty of Dentistry , Ain Shams University approval no.1053. written consent was signed by patients, agreeing to the clinical procedures. The current investigation is a randomized clinical trial using a parallel study design, a 1:1 allocation ratio. The current study was carried out in the Department of oral Medicine, Periodontology and Oral Diagnosis, Faculty of Dentistry at Ain Shams University.

Sample size calculation:

Sample size calculation was done by an expert statistician. A power analysis was designed to have adequate power to apply a two-sided statistical test of the null hypothesis that there is no effect of hyaluronic acid gel following conventional surgical gingival depigmentation technique on the healing period, pain perception and patient satisfaction so, no difference would be found between tested groups. By adopting an alpha level of (0.05) a beta of (0.2) i.e., power=80% and an effect size (d) of (1.506) calculated based on the results of a previous study; the predicted sample size (n) was a total of (16) samples (i.e., 8 patients per group). Sample size calculation was performed using G*Power version 3.1.9.7 .[16]

Eligibility criteria:

Inclusion criteria:

1. Male and female with age range from 18 to 50 years old.
2. Systemically free from any disease as

evidenced by the health questionnaire guided by modified Cornell medical index.[17]

3. Gingival hyperpigmentation on maxillary or mandibular labial keratinized gingiva.

Exclusion criteria:

1. Smokers.

2. Pregnant and lactating women.

3. Gingival pigmentation associated with occupational Hazards.

4. Previous treatment of gingival hyperpigmentation.

5. History of being allergic to hyaluronic acid.

6. Patients with missing anterior teeth.

Recruitment:

Patient attending the outpatient clinic of the Oral Medicine, Periodontology, Oral Diagnosis and Oral Radiology Department, at Faculty of Dentistry Ain Shams University seeking gingival depigmentation for esthetic reason were assessed for the eligibility for this study, at the first visit before the initiation of research; Information including age, gender, medical history, drug history, dental history, and family history was documented. Patients were examined clinically by a mirror, periodontal probe using spotlight. Gingival and periodontal condition and distribution of the oral gingival pigmentation were recorded.

Allocation of participants:

In this randomized controlled clinical trial, a total number of 16 patients with gingival hyperpigmentation were selected. All of them met the eligibility criteria. The patients were randomly assigned for 2 different treatment modalities using computer generated random tables by non-involved investigator.

Group I (study group): Included eight patients that were treated with conventional surgical gingival depigmentation by abrasive burs, followed by topical application of hyaluronic acid gel on surgical field.

Group II (control group): Included eight patients that were treated with conventional surgical gingival depigmentation by abrasive burs.

Interventions:

Pre-treatment phase:

All patients received professional non-surgical periodontal debridement using ultrasound scalers and hand instrumentation and received personalized oral hygiene instructions.

Treatment phase

Study group:

Conventional surgical gingival depigmentation by abrasive burs with hyaluronic acid gel application:

• A mouthwash containing 0.2% chlorhexidine was used preoperatively for 1 minute.

• Local anesthesia (Mepivacaine 2% with levonordefrine 1:20000, Memphis pharmaceutical and chemical industries, Cairo,

Egypt) was achieved using field block technique.

• Gingival depigmentation was planned using a high-speed hand-piece and pear-shaped diamond bur with copious water irrigation.

• The largest diamond bur was used, as the small bur does not easily smooth the surface and has a tendency to make small holes in the healing area.[18]

• Feather-like light strokes were used to remove the pigmented epithelium area without holding bur in one place .[18]

• The clinically visible pigmentation was removed while maintaining gingival contours and leaving a thin layer of connective tissue over the alveolar bone preserving the periosteum, avoiding bone exposure or damaging teeth.

• Tissue hydration was maintained by copious irrigation with water .[19]

• Hemostasis was achieved using direct pressure with normal saline-soaked gauze at the surgical site.[19]

Hyaluronic acid gel application

• Commercially available HA gel (Gengigel R) (Ricerfarma S.r.l., Milano, Italy) was applied immediately post-surgery and gentle massaging of it was done for 1-2 minutes to complete covering of the surgical field.

Post treatment instructions

• Patients were instructed to apply commercially available HA gel (GENGIGEL) on the surgical field and massaging gently with clean fingers for 1-2 minutes to aid its correct distribution and absorption.

• They were instructed to apply it three to five times a day (After main meals) for one week.

• It was advisable not to eat or drink for at least 30 minutes after application. (According to manufacture instructions)

• Patients were instructed to avoid tooth brushing for 3 days to prevent mechanical trauma and allow re-epithelialization and use a soft brush for plaque control after that.

• Chlorhexidine 0.2% was prescribed to the patients twice a day for 1 week.

• The patients were also asked to avoid spicy food for several days.[18]

Control group:

Conventional surgical gingival depigmentation by abrasive bur:

• Local anesthesia was achieved using the field block

technique and depigmentation was performed as sites in study group.

Post-treatment instructions

• Patients were instructed to avoid tooth brushing for 3 days to prevent mechanical trauma and allow re-epithelialization and use a soft brush for plaque control after that.

• Chlorhexidine 0.2% was prescribed to the patients twice a day for 1 week.

• The patients were also asked to avoid spicy food for several days. [18]

All patients in both groups were recalled on the 3rd day,7thday, 2 weeks,1 month, and 3 months

postoperatively.

Methods of evaluation:

Clinical assessment:

a) The following outcomes were evaluated for each patient; **1. Dummet oral pigmentation index (DOPI),** the degree of gingival pigmentation were scored as: 0 = pink tissue [no clinical pigmentation]; 1 = mild light brown tissue [mild clinical pigmentation]; 2 = medium brown or mixed brown and pink tissue [moderate clinical pigmentation]; or 3 = deep brown/blue–black tissue [heavy clinical pigmentation]. [20]

This index was assessed at baseline (pre-operative), day 3, 1week, 2 weeks, one month and 3 months post-operatively.

2. Wound Healing Score were recorded as follows: 1) Complete re-epithelialization,2) Incomplete re-epithelialization, 3) Ulcer and 4) Tissue defect or necrosis at day 3, 1 week and 2 weeks. [21]

3. Satisfaction questionnaire: Satisfaction questionnaire modified from McGill Pain Questionnaire to score degree of pain experienced during and after treatment and the degree of patient satisfaction with the cosmetic results of the procedure was used . Table 1 [22]

4. Visual analogue scale (VAS) score for pain assessment. Pain assessment was performed at day 1, day 3, 1week, 2 weeks using 10-cm (100 mm) horizontal continuous scale marked “no pain” on the left and “maximum pain” on the right side of the scale. The scores were as follows: no pain (0), slight pain (0.1–3.0 mm), moderate pain (3.1–6.0 mm), and severe pain (6.1–10 mm).[23]

b) Histological assessment of healing: Biopsy procedure one week after the treatment procedure, a gingival specimen of 2 mm width and 1 mm depth taken from the most distal site of hyperpigmented area using biopsy punch (Kai industries co., Japan) under local anesthesia the punch was gently inserted into the mucosa with a rotating motion to facilitate cutting the tissue including surface epithelium and the underlying lamina propria paying attention not to expose the periosteum.

For conventional histology, samples were fixed by immersion in 10% (weight/volume) formaldehyde in 0.2 M phosphate-buffered saline (pH 7.4), dehydrated in graded ethanol and embedded in paraffin. The slides were stained with H&E. For each section, microscopic fields were selected, and photomicrographs were captured at magnification of 10X. All images were captured using MIC-W16 digital camera installed on MEIJI MX5200L microscope (MEIJI TECHNO, Japan). Images were then transferred to the computer system for analysis. This was performed in the Precision Measurement Unit, Oral Pathology Department. All the steps of assessment were carried out on Intel® core I7® based computer using Fiji ImageJ (version 1.51r; NIH, Maryland, USA) software. The mean of epithelial thickness for

each case was calculated. Measurement of the thickness at 5 different areas were taken for each section. The Number of blood vessels for each case was calculated. The area fraction of hematoxylin stain in H&E-stained slides was measured automatically by the color deconvolution 2 plugin followed by selecting the five random fields from the connective tissue as the ROI. The area fraction of hematoxylin represented the number of inflammatory cells, fibroblasts, and endothelial cells. This number was used as indication for abundance of inflammatory cells. The collected data was tabulated in Microsoft Excel sheet.

Data Management and Analysis:

The collected data were revised, coded, tabulated, and data was fed to the computer and analyzed using GraphPad Prism 8 (GraphPad Software). Numerical data were analyzed for normality by using tests of normality (Kolmogorov-Smirnov and ShapiroWilk tests).

Age and histological analysis data showed normal (parametric) distribution, while DOPI, VAS, and Clinical Wound Healing showed non-normal (non-parametric) distribution. Parametric data were presented as mean, standard deviation (SD) values, while non-parametric data were presented as median and range values.

For parametric data, the Student's t-test was used to compare between the two groups. For non-parametric data, the Mann-Whitney U test was used between the two groups, and Friedman's test was used to study the changes by time within each group followed by Dunn's test for pair-wise comparisons.

Qualitative data (questionnaire answers, gender, and operation site) were presented as frequencies and percentages. Fisher's Exact test was used for comparisons between the groups with two by two variables and the Freeman-Halton extension of Fisher's exact test for two by three variables. To measure the strength of a linear association between variables Spearman's rank correlation coefficient was used where the value $r = -1$ means a perfect negative correlation and the value $r = 1$ means a perfect positive one. The significance of the obtained results was judged at the (0.05) level as $P>0.05$: Non significant (NS) while $P\leq 0.05$: Significant (S).

Results:

Demographic data:

Patients' age: This study included sixteen patients, all of them completed the healing period without any complications and no patients dropped out from the study. They were with age ranged from 20 to 30 years with a mean age 24.06 years. Study group patients had mean age of 22.75± 3.33 years with minimum 20 year and maximum 28 years while the control group patients had mean age of 25.38±3.42 years with minimum 21 year and maximum 30 years. There was no statistical significant difference between the age of the study and control group (P= 0.25).

In each group, study and control, of eight

patients there were 2 males (25%) and 6 females (75%) making the total male participants 4 (25%) and the total female participants 12 (75%). There was no statistical significant difference between the gender of the study and control group (P= 1).

Clinical Data:

Dummet Oral Pigmentation Index (DOPI): Comparison between DOPI in the two groups and the changes by time within each group. In study and control groups, the median of DOPI decreased significantly (P=0.0201) from baseline (3.00) to 3 days, 1 week, 2 weeks, 1 month and 3 months after treatment (0.00) with intergroup statistical significant difference (P0.9999). On comparing the two groups, it was demonstrated that there was no statistically significant difference regarding DOPI at baseline to 3 months postoperatively (P>0.9999).

Visual Analogue Scale (VAS): Comparison between VAS in the two groups and the changes by time within each group. In study group, the median of VAS at baseline (2.00) was not statistically different from 3 days (1.00) (P>0.9999), but significantly different from 1 week (0.00), and 2 weeks (0.00) after treatment (P=0.0042) with intergroup statistical significant difference (P 0.728), but significantly different from 1 week (2.00), and 2 weeks (0.00) after treatment (P= 0.0084, 0.9999).Table 2

Wound Healing Score: Comparison between Clinical Wound Healing in the two groups and the changes by time within each group. Table 3 demonstrate that in study group, median of wound healing score recorded at 3 days after treatment (2.00) was significantly higher than median value recorded after 1 week and 2 weeks (1.00) (p= 0.0081) with intergroup statistical significant difference (P=0.0005).

In control group, the median value recorded at 3 days after treatment (2.5) was also significantly higher than the median value recorded after 2 weeks only (2.00) (p= 0.026) with intergroup statistical significant difference (P=0.0027). On comparing the two groups, it was found that there was no statistically significant difference regarding wound healing score at baseline (P=0.0769). However, there was a statistical significant difference between the values at 1 week (P=0.0014) and 2 weeks (P=0.0256).

Satisfaction level: Majority of patients in study and control group (87.5%) experienced no pain during treatment with no significant difference between both groups (P=1). At the day of treatment, most of the patients in the study group experienced mild pain (87.5%) and (12.5%) reported no pain at all where the patients in the control group were equally divided between suffering from mild pain (50%) or sever pain (50%). There was no significant difference between both groups (P=0.0769).

One week after the treatment, all patients of study group experienced no pain (100%) but in the other hand, few of the control group had no

pain during the first week after treatment (12.5%) and the majority reported pain (87.5%) with a statistical significant difference between the two groups (P=0.0014).

All the patients in both groups noticed a marked cosmetic change after one week and 3 months (100%) (p=1). Also, all patients of both groups agreed that the treatment met their expectation (100%) (p=1) and all the patients in both groups agreed to repeat the procedure if necessary with majority of study group (87.5%) highly agreed and (12.5%) agreed while the patients in the control group were equally divided (50%) between agreeing and highly agreeing with no statistical significant difference between the groups (p=0.282). Table 4

Histological evaluation: Comparison between groups regarding mean values of epithelial thickness (μm), number of blood vessels and inflammatory cells (percentage of hematoxylin stain) are demonstrated in Table 5. Epithelial thickness in study group (488.07±200.3) had a significantly higher mean value than control group (218.75±28.27) (P=0.0021).

Number of blood vessels mean value after 1 week of treatment in study group (19.25±4.33) was higher than that of control group (12±2.83) and was statistically significant (p=0.0014). Regarding inflammatory cells; after 1 week the percentage of hematoxylin stain in the connective tissue, representing the nuclei of inflammatory cells, were significant statistically lower in the study group with mean of (12.63±1.86) compared to the control group mean (24.66±3.26) (P<0.0001).

Correlations: The findings of this study have shown that there was a nonsignificant weak positive correlation between the patient's age and gender, r = 0.379 (P-value = 0.154), a significant moderate negative correlation between the patient's age and epithelium thickness, r = -0.619 (P-value = 0.012), a non-significant weak positive correlation between the patient's age and inflammatory cells, r = 0.425 (P-value = 0.102), a non-significant weak positive correlation between the patient's age and clinical wound healing after 1 week, r = 0.358 (P-value = 0.179), and a significant moderate positive correlation between the patient's age and VAS after 1 week, r = 0.51 (P-value = 0.048).

In this study, we also found a significant moderate positive correlation between the epithelial thickness and blood vessels number, r = 0.684 (P-value = 0.004), a significantly strong negative correlation between the epithelial thickness and inflammatory cells, CWH, and VAS at 1 week, r = -0.762, -0.861, -0.903 (P-value = 0.001, =0.0002, < 0.0001) respectively. There was a significant strong negative correlation between the number of blood vessels and inflammatory cells, r = -0.757 (P-value = 0.001), and a significant weak negative

correlation between the number of blood vessels and CWH, VAS at 1 week, r = -0.516, -0.504 (P-value = 0.048, 0.048) respectively. Figure1&2

Computerized analysis of the hematoxylin stain area percentage representing the number of inflammatory cells in the connective tissue showed a significant moderate positive correlation between inflammatory cells and CWH, VAS at 1 week, r = 0.67, 0.59 (P-value = 0.008, 0.018). There was a significant very strong positive correlation between CWH and VAS after 1 week, r = 0.953 (P-value < 0.0001). However, there was a non-significant very weak correlation between the other assessed parameters. Figures 3

Discussion

Melanin hyperpigmentation of gingiva is an esthetic problem for many subjects, mainly if this hyperpigmentation is visible during smiling so gingival depigmentation is applied to treat it. Bur abrasion method was chosen for depigmentation in current study as it is relatively a non-invasive, simple, cost-effective technique and need a less of treatment chair time.[14,19]

Wound healing is a process involving different molecular and cellular mechanisms, including stages of clotting formation, accumulation of inflammatory cells, formation of granulation tissue, angiogenesis, wound contraction, and reshaping of the newly formed tissue. Factors such as difficulty in mechanical cleaning post-operatively and flora in the mouth have negative effects on the normal healing process of wounds left to heal after periodontal surgery .[11]

Wound of gingival depigmentation is left for healing by secondary intention similar to free gingival graft wound. There is a pain and greater risk of bacterial contamination in these types of wounds. In this respect, there is a need for therapeutic agents to improve the clinical results of periodontal wound healing, increase patient comfort, shorten the healing process, and obtain the formation of healthy tissue .[24]

HA provides very important functions in wound healing. The degradation product of HA is reported to have a pro-angiogenic effect. It makes a local environment with a water-binding effect, which may provide a chance to produce a matrix for wound healing. HA has been reported to be a substance with viscoelastic properties, and by forming a protective layer on wound surfaces, it is helpful in protecting these areas in periodontal regenerative treatments.[14]

The hyaluronic acid gel has anti-inflammatory properties and has been found to induce more epithelial tissue formation. Also, it has anti-oedematous effect which may be related to the osmotic activity. All these properties may be useful in accelerating tissue healing .[25]

We hypothesized that topical application of H.A on the surgical field after bur abrasion depigmentation technique would enhance healing time and reduce the pain perception after the procedure; there are no published studies of the effect of H.A on the gingival tissues after bur

abrasion technique.

Clinically, the present study evaluated the effect of treatment using different parameters; Dummett oral pigmentation index (DOPI) which is one of the most common and commonly used methods used for oral pigmentation assessment.[20]

Visual Analog Scales (VAS) was a reliable measuring instruments designed to document the pain severity in individual patients.[23]

While, to evaluate the pain and cosmetic changes, patients satisfaction questionnaire modified from McGill Pain Questionnaire [22]was used to fulfill all the criteria of evaluation including pain experienced during and post treatment, cosmetic changes during the follow up period and how the treatment met their expectations.

Wound healing assessment score used in our study was convenient for clinicians and researchers, safe for patients, applicable to all types of wounds, proven reliable, accurate and reflecting the progression of the whole healing spectrum with time. Moreover, no special or expensive equipment was needed [21].

The oral biopsy was considered essential for proper assessment of wound changes on histological level, a punch biopsy was easy with a low incidence of postsurgical morbidity. Suturing was not required because the surgical wounds heal rapidly by secondary intention with minimal or no scar formation and maximum esthetic results .[26]

The results of this study revealed that there were no statistically significant differences between study group and control group regarding DOPI; at baseline, 1 day,3 days, 1 week, 2 weeks, 1 month and 3 months after treatment. In both Study group and control group; the DOPI decreased significantly from baseline to 1 day and maintained 0 score at 3days, 1 week,2 weeks and 1 month and 3 months after treatment.

In a study by Roshannia and Nourellahi to compare bur abrasion and CO2 laser methods in removing gingival pigmentation, reported that there was no statistically significant difference between the two treatment modalities before the procedure, 1 month and 6 months after the procedure regarding DOPI.[18]

Also, in both methods, the DOPI improved and was statistically significant difference in the follow-up period 1 month and 6 months after treatment compared to before. Regarding pain perception; It decreased gradually through the follow up periods in both groups but there was statistical significant difference between the two groups at first day, 3 days and 1 week.

One week after the treatment, all patients of Group(I) (study group) experienced no pain (100%) but in the other hand, few of the Group (II) (control group) had no pain during the first week after treatment (12.5%) and the majority reported mild pain (87.5%) with a statistical significant difference between the two groups

(P=0.0014).

The absence of pain after one week was achieved in study group which used H.A after bur abrasion, is similar to the result was achieved when used diode laser in the study applied by Hariati and Sunarto to Compare between diamond bur and diode laser to treat gingival hyperpigmentation. Both groups showed significant reduction in median value of wound healing score from 3 days to 1 week and 2 weeks with intergroup statistical significant difference.[27]

On comparing the two groups, there was a statistical significant difference between the values at 1 week (P=0.0014) and 2 weeks (P=0.0256) where reduction of wound healing score was significantly higher in group I (study group) which supported the hypothesis of the present study. However, since there is no published data regarding the use of H.A following depigmentation, the results could not be compared to others.

Majority of patients in study and control group (87.5%) experienced no pain during treatment with no significant difference between both groups (P=1) which may be due to using the same method of gingival depigmentation in the both groups.At the day of treatment, most of the patients in the study group experienced mild pain (87.5%) and (12.5%) reported no pain at all where the patients in the control group were equally divided between suffering from mild pain (50%) or sever pain (50%) which supported the hypothesis of the current study that the H.A reduced pain.

There was no significant difference between both groups (P=0.0769). All the patients in both groups noticed a marked cosmetic change after one week and 3 months (100%) (p=1). Also, all patients of both groups agreed that the treatment met their expectation (100%) (p=1) and all the patients in both groups agreed to repeat the procedure if necessary with majority of study group (87.5%) highly agreed and (12.5%) agreed while the patients in the control group were equally divided (50%) between agreeing and highly agreeing with no statistical significant difference between the groups (p=0.282).In both groups the degree of pain perception and cosmetic change were concomitant with patient acceptance to repeate the procedure if necessary.

Regarding histological analysis; After 1week of treatment, Epithelial thickness in study group (488.07±200.3) had a significantly higher mean value than control group (218.75±28.27) (P=0.0021) and number of blood vessels mean value in study group (19.25±4.33) was also higher than that of control group (12±2.83) and was statistically significant (p=0.0014). Regarding inflammatory cells; the percentage of hematoxylin stain in the connective tissue, representing the nuclei of inflammatory cells, were significant statistically lower in the study group with mean of (12.63±1.86) compared to the control group mean (24.66±3.26) (P<0.0001).

Although the exact results cannot be compared to similar published data However, can be explained by the fact that H.A enhances gingival thickness and keratinized tissue and could be effective in the soft tissue wound healing process because it stimulates angiogenesis, granulation tissue formation, and epithelial migration in addition to its viscoelastic, anti-inflammatory and anti-edematous features.[28,29]

The histological findings confirm the clinical results of the present study as we found a significant moderate positive correlation between the epithelial thickness and blood vessels number, a significantly strong negative correlation between the epithelial thickness and inflammatory cells, CWH, and VAS at 1 week. There was also a significant strong negative correlation between the number of blood vessels and inflammatory cells, and a significant weak negative correlation between the number of blood vessels and CWH, VAS at 1 week. Also, it showed a significant moderate positive correlation between inflammatory cells and CWH, VAS at 1 week. There was a significant very strong positive correlation between CWH and VAS after 1 week. This is conducted with a study by Yıldırım and Kuru et al in which topically applied HA alongside periodontal dressing of palatal donor sites after free gingival graft (FGG) surgery is a useful and supportive method for reducing postoperative discomfort and accelerating epithelization of wound sites compared with coverage with periodontal dressing only.[30]

Based on results of the current study; topical application of hyaluronic acid gel following gingival depigmentation using bur abrasion technique although it did not influence the pigmentation score but enhanced the normal healing process, reduced patient discomfort and pain feeling thus had positive effect on patient's tendency to this technique.

Limitations:

Limitations of our study remain the small sample size and short duration of follow up period.

Conclusions:

1. H.A induced significantly better wound healing compared to Group II (control group) bur abrasion alone.

2. H.A reduced pain (VAS) in comparison to Group II bur abrasion alone.

3. Clinically H.A had no adjunctive effect on depigmentation.

4. Histologically, H.A induced better epithelium, vascular and less inflammatory cells.

5. The Majority of patients in both groups experienced satisfaction with the treatment outcome in terms of pain and esthetic.

Clinical recommendations:

1. Further clinical trials are recommended to compare the effect of different ways of administration of H.A (gel, hydrogel, spray and so on).

2. Future studies on the use of H.A in wound healing following diode laser depigmentation are recommended.

3. Future studies on the use of medical plants traditionally used in wound healing following conventional surgical gingival depigmentation are recommended.

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Patient declaration of consent statement:
The patients understand that their names and initials will not be published, and due efforts will be made to conceal their identity.

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Table (1): Patient satisfaction questionnaire

Question	Scoring
Was the treatment painful?	1, no pain; 2, mild pain; 3, severe pain
Did you experience pain on the day of the treatment?	1, no pain; 2, mild pain; 3, severe pain
Did you experience pain during the first week after the treatment?	1, no pain; 2, mild pain; 3, severe pain
Did you notice a cosmetic change 1 week after the treatment?	1, not at all; 2, moderate; 3, marked
Did you notice a cosmetic change 3 months after the treatment?	1, not at all; 2, moderate; 3, marked
Did the treatment meet your expectations?	1, no; 2, yes; 3, over and above
Would you repeat the treatment if necessary?	1, no; 2, yes; 3, over and above

Table (2): Comparison between VAS in the two groups and the changes by time within each group.

Time	Study (n = 8)		Control (n = 8)		P value
	Median	Range	Median	Range	
Baseline	2	3	6	3	0.0002*
3 Days	1	1	4	2	0.0002*
1 Week	$0^{\$}$	0	$2^{\$}$	3	0.0014*
2 weeks	$0^{\$}$	0	$0^{\$\#}$	0	>0.9999 ns
P value	0.0001*		<0.0001*		

Date is presented as median and range

Used test: Friedman's test followed by Dunn's multiple comparisons test intrageroup and Mann Whitney test intergroup.

*: significant at $p < 0.05$. ns: non-significant.

$^{\$}$: significant at $p < 0.05$ Vs Baeline.

$^{\#}$: significant at $p < 0.05$Vs 3 days.

Table (3): Comparison between Clinical Wound Healing in the two groups and the changes by time within each group:

Time	Study (n = 8)		Control (n = 8)		P value
	Median	Range	Median	Range	
3 Days	2	0	2.5	1	0.0769 ns
1 Week	1$^{\$}$	0	2	1	0.0014*
2 weeks	1$^{\$}$	0	2$^{\$}$	1	0.0256*
P value	0.0005*		0.0027*		

Date is presented as median and range

Used test: Friedman's test followed by Dunn's multiple comparisons test intrageroup and Mann Whitney test intergroup.

*: significant at $p < 0.05$. ns: non-significant.

$^{\$}$: significant at $p < 0.05$ Vs Baeline.

Table (4): Comparison between groups in frequencies and percentages (%) of responses for satisfaction questionnaire:

Questionnaire Questions		Group I (No=8)	Group II (No=8)	*P*-value
Was the treatment painful?				
	No	7 (87.5%)	7 (87.5%)	
	Mild	1 (12.5%)	1 (12.5%)	1 ns
	Severe	0 (0%)	0 (0%)	
Did you experience pain on the day of the treatment?				
	Not	1(12.5%)	0 (0%)	
	Mild	7(87.5%)	4 (50%)	0.0769 ns
	Severe	0 (0%)	4 (50%)	
Did you experience pain during the first week of the treatment?				
	No	8 (100%)	1 (12.5%)	0.0014*
	Yes	0 (0%)	7 (87.5%)	
Did you notice a cosmetic change 1 week after the treatment?				
	No	0 (0%)	0 (0%)	
	Moderate	0 (0%)	0 (0%)	1 ns
	Marked	8 (100%)	8 (100%)	
Did you notice a cosmetic change 3 months after the treatment?				
	No	0 (0%)	0 (0%)	
	Moderate	0 (0%)	0 (0%)	1 ns
	Marked	8(100%)	8 (100%)	
Did the treatment meet your expectations?				
	No	0 (0%)	0 (0%)	
	Yes	0 (0%)	0 (0%)	1 ns
	Over and above	8(100%)	8(100%)	
Would you repeat the treatment if necessary?				
	No	0 (0%)	0 (0%)	
	Yes	1(12.5%)	4 (50%)	0.282 ns
	Over and above	7(87.5%)	4 (50%)	

Date is presented as frequencies (percentages)

Used test: Freeman-Halton extension of Fisher's exact test and Fisher's exact test.

*: significant at $p < 0.05$. ns: non-significant.

Table (5): Comparison between the two groups regarding mean of epithelial thickness number of inflammatory cells and blood vessels after 1 week of treatment.,

	Study	Control	P value
Epithelium Thickness (µm)	488.07±200.3	218.75±28.27	0.0021*
Blood vessels (N)	19.25±4.33	12±2.83	0.0014*
Inflammatory cells (Percentage of hematoxylin stain)	12.63±1.86	24.66±3.26	<0.0001*

Data are presented as mean ± standard deviation.

Used test: Unpaired t test.

*: significant at $p < 0.05$.

Figure (1): Heat-map of Spearman's rank correlation coefficient matrix between demographic data, CWH, and VAS after 1 week.

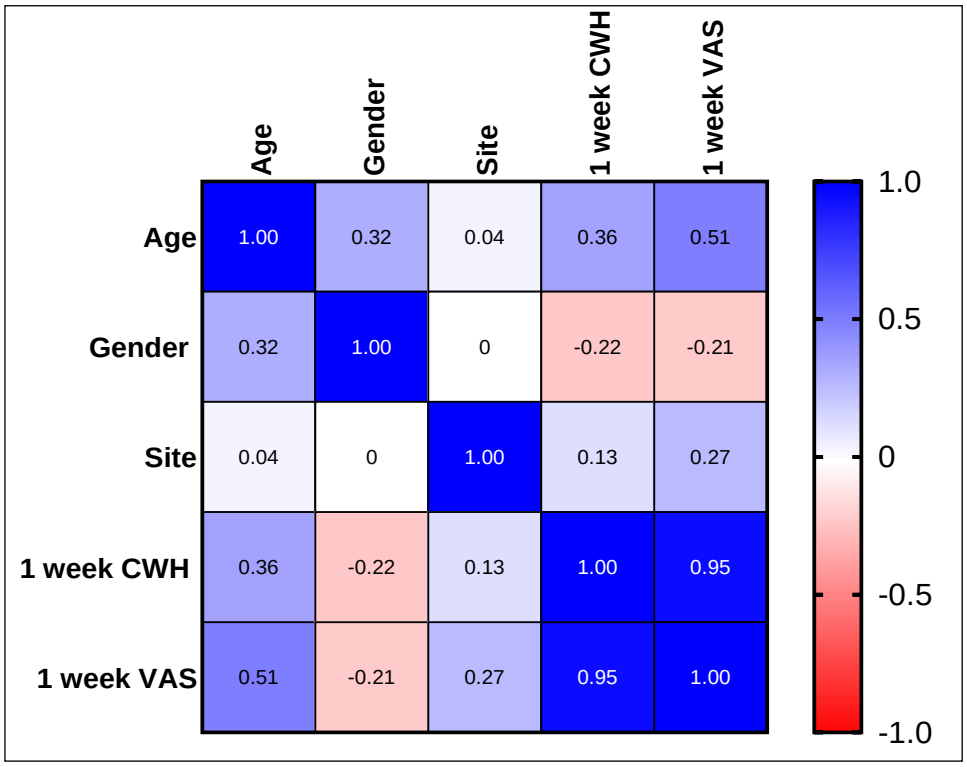


Figure (2): Heat-map of Spearman's rank correlation coefficient matrix between demographic data and histological analysis after 1 week.

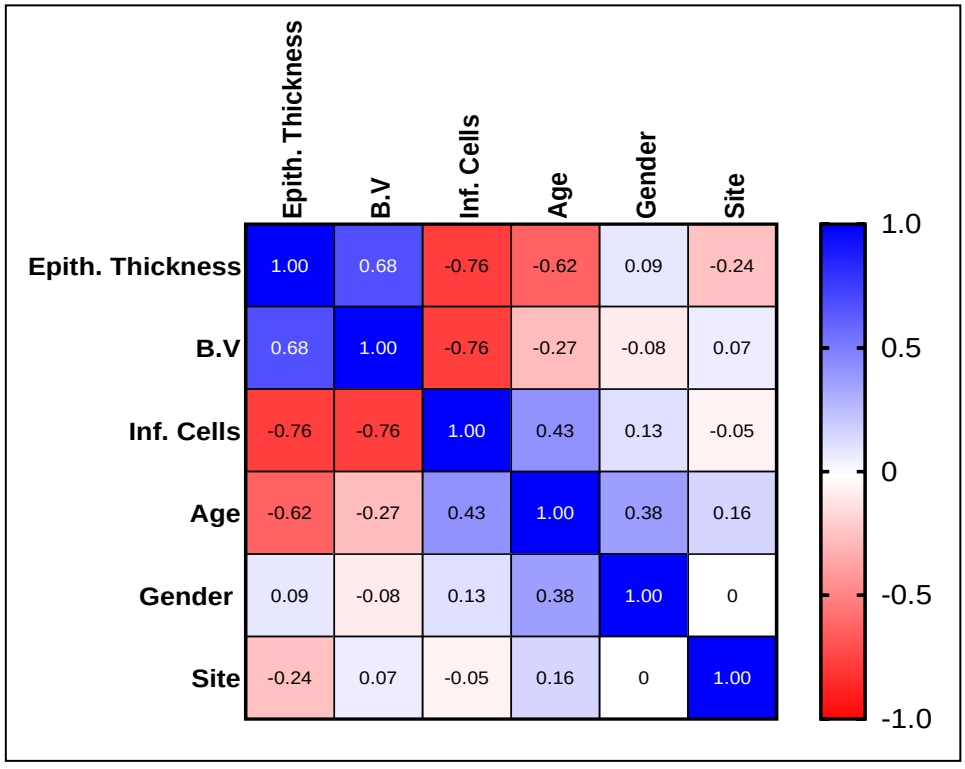
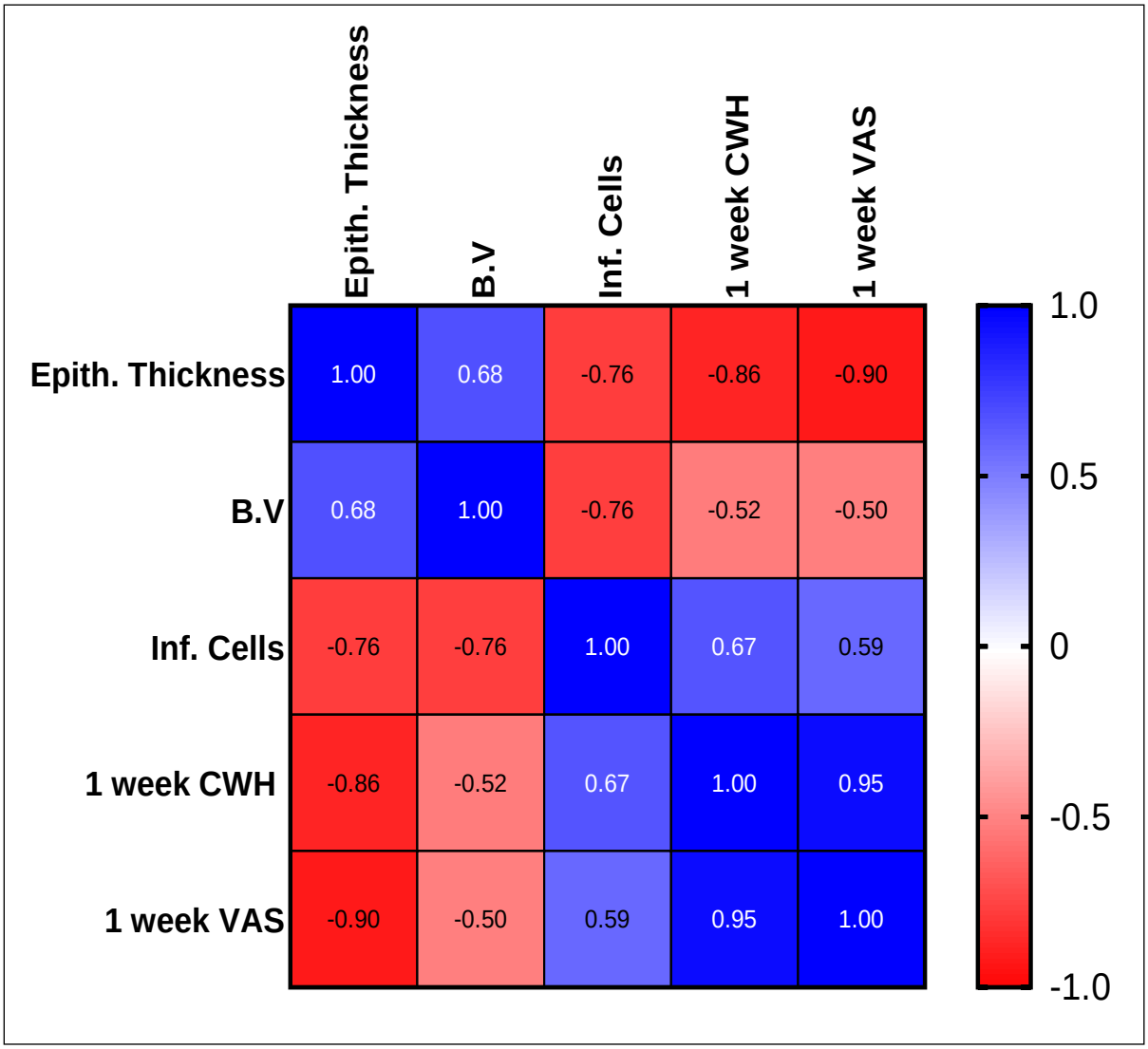


Figure (3): Heat-map of Spearman's rank correlation coefficient matrix between histological analysis CWH, and VAS after 1 week.



Presentation of cases

Figure(4): Pretreatment intraoral photograph in Study group

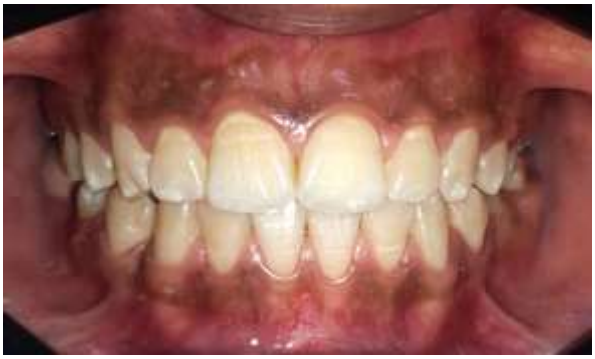
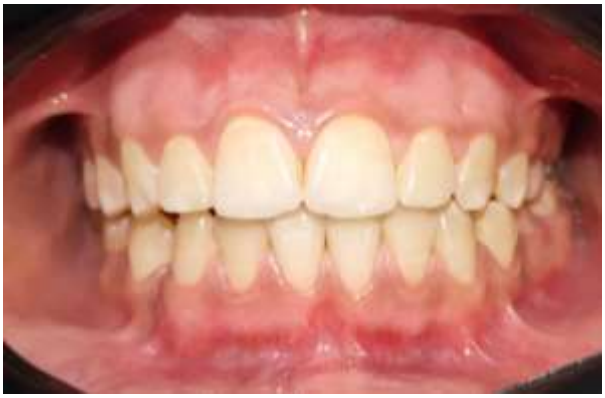


Figure (5): Three month follow up intraoral photograph in study group



Figure(6): Pretreatment intraoral photograph in control group.

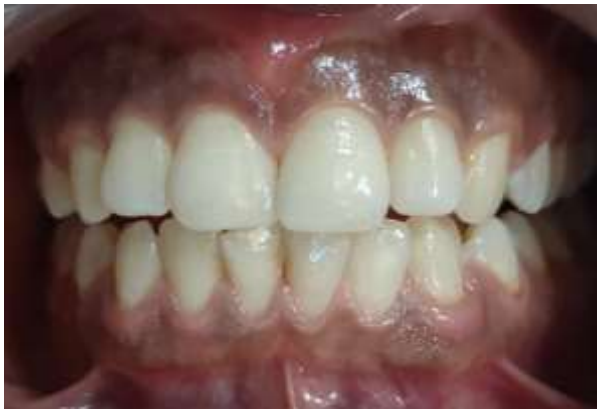


Figure (7): Three month follow up intraoral photograph in control group