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Glutathione Injection versus Laser in the Treatment of Gingival Hyperpigmentation (A randomized clinical controlled trial with histological assessment)

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Aim: To assess the effect of intra –mucosal injection glutathione and diode laser for treatment gingival hyperpigmentation concerning: Evaluate Clinical outcome and patient satisfaction. as a primary objective. Assess histological responses of gingival tissues as secondary objective.

Materials and Methods: This research involved 10 individuals seeking treatment for gingival hyperpigmentation for an esthetic concern (10 quadrant per group). Patients were recruited between January 2022 to December 2023, and then were randomly and equally allocated to each of the tested group; Group I (Glutath.) was considered the study group, Group II (laser) was considered the control group. All patients were committed to treatment protocol, none of the cases reported serious side effects or adverse outcomes for the interventions used.

Results: There was no improvement in the mean DOPI sore with the injectable GLU (Group I) while with laser (Group II), there was a significant reduction in the mean of DOPI from baseline (2.66±0.72) to (0.79±0.40) 3 months after treatment. The mean % of Surface Area decreased insignificantly from baseline (5.99±2.79) to 3 months (4.62±2.40) after treatment in Group I (GLU). While in Group II, there was a significant decrease from baseline (5.90±1.74) to 3 months (2.23±1.41) in Group II (LASER). There was a significant difference in MAF on comparing Group I & II to being (1.62±0.56) (0.73±0.30) respectively after a three-month follow up there was a higher percentage of reduction among Group II than Group I. There was also a reduction in MAF among Group I from baseline (1.76±0.43) to (1.62±0.56) after 3 months of treatment but it was non-significant.

Conclusion: Intra-mucosal injection of 1ml Glutathione (repeated 3 times with 1-week intervals) had no detectable effect on gingival pigmentation score and surface area compared to rapid initial response and more patient satisfaction with Diode Laser gingival depigmentation. Histologically, Diode Laser treatment showed a greater and significant reduction in MAF of melanosomes after three months post-treatment than Glutathione.

Keywords: Glutathione injection, gingival hyperpigmentation

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Introduction

Currently, esthetic dentistry focuses on the crucial role of the gingival tissue, as well as the color, form, and position of the teeth, in order to generate a visually pleasing and balanced smile. [1]

Physiological gingival hyperpigmentation is caused by enhanced melanocyte activity rather than an increased number of melanocytes. As a result, the gingiva in these individuals displays a higher concentration of melanophores.[2] Physiological gingival hyperpigmentation is caused by several pigments, with melanin being the primary pigment produced by melanocytes. Melanocytes synthesize melanosomes granules by converting the amino acid tyrosine into a compound called dehydroxyphenylalanine (DOPA) through a hydroxylation process facilitated by the enzyme tyrosinase. [3]

Both surgical and non-surgical methods have been used to treat gingival hyperpigmentation. The primary principle behind most surgical procedures is the removal of the epithelium in the target area. This can be achieved through several approaches such as employing a surgical blade [4] or bur abrasion [5], cryosurgery [6], or laser.[7]

In accordance with the existing literature, gingival melanin pigmentation might differ based on whether it is caused by normal bodily processes or by a disease or abnormal condition. The esthetic significance of gingival melanin pigmentation is contingent upon the patient's skin color, which is a crucial determinant in selecting the appropriate therapy. Choosing a proper procedure for treating gingival hyperpigmentation is essential. The treatment should produce minimum discomfort and have long-lasting effectiveness. [8]

The discovery of glutathione as a skin-lightening agent was unintentional, since it was seen that excessive dosages of glutathione resulted in the side effect of skin lightening. This finding provided valuable insights into the properties of this tiny thiol compound. Several mechanisms have been suggested to explain the hypopigmentary action of glutathione, with the most significant one being the inhibition of tyrosinase. Glutathione has the ability to decrease tyrosinase activity through three distinct mechanisms. The thiol group directly inhibits tyrosinase by chelating the copper site. Furthermore, glutathione hinders the cellular transportation of tyrosinase to premelanosomes, which is a necessary step for the production of melanin. Furthermore, the inhibition of tyrosinase occurs indirectly through its antioxidant properties. Glutathione alters the process of melanin production, causing a change from the production of eumelanin to phaeomelanin. This shift occurs through interactions between thiol groups and dopaquinone, resulting in the development of sulfhydryl-dopa conjugates. [9]

So based on the previous studies describing the biological effect of glutathione we hypothesized that glutathione injection in the gingival tissues would be effective in gingival depigmentation technique procedure in compare with diode laser.

The objective of this research is to evaluate the effect of intra –mucosal injection glutathione in comparison with diode laser for treatment gingival hyperpigmentation in terms of: Evaluate Clinical outcome and patient satisfaction. as a primary objective. Assess histological responses of gingival tissues as secondary objective.

Materials and methods

I-Patients selection

Patients attending the outpatient clinic of the Oral Medicine, Periodontology and Oral Diagnosis Department, seeking gingival depigmentation for esthetic reasons at Faculty of Dentistry Ain Shams University

were recruited for this randomized controlled clinical research in the period between January 2022 to December 2023.

A power analysis was conducted to provide sufficient statistical power for the implementation of a two-sided test of the research hypothesis. Given an alpha (α) level of 0.05 (5%), a Beta (β) level of 0.20 (20%) which corresponds to a power of 80%, and an effect size (d) of 1.79 for the DOPI score based on the findings of Chandna & Kedige[10]. The anticipated sample size (n) was 18 samples in total, with 9 samples allocated to each group. Sample size computation was conducted using G*Power (version 3.1.9.22). The sample size was augmented to 20, with 10 participants assigned to each group, in order to account for any dropouts. This study was a randomized controlled clinical trial conducted on 10 cases who were seeking gingival depigmentation for aesthetic reasons. The experiment used a split-mouth design, with two parallel arms and a single center.

Demographic and medical data, such as age, gender, medical history, drug history, dental history, and family history, were recorded.

Patients underwent clinical examination utilizing a mirror and a periodontal probe under the illumination of a flashlight. The state of the gums and surrounding tissues, as well as the distribution of pigmentation in the oral cavity, were documented.

At the first visit before initiation of research; all cases were assessed for eligibility in accordance with the following criteria; the participants were enrolled into the research based on the inclusion criteria as follow: Both genders aged from 18-50 years. Clinically diagnosed gingival diffuse melanin pigmentation on the maxillary keratinized gingiva with minimum mean DOPI score of 2 (medium brown or mixed brown and pink tissues).[11] Patient with thick gingival Biotype (>1mm).[12] The individual is determined to be completely free from any ailment based on the health questionnaire conducted using the modified Cornell medical index. [13] The exclusion criteria was as follows: Pregnant or lactating females. Known sensitivity to Glutathione or any of its derivatives as documented in heath questionnaire. Patients taking any drug that may cause gingival pigmentation (as minocycline, chloroquine, ketconazoleand contraceptives). Previous treatment of gingival depigmentation. Patients diagnosed with periodontal disease according to workshop classification 2017.[14] Vulnerable groups (prisoners, handicapped, orphans).

The study received an ethical clearance from the Research Ethical committee of Ain Shams University, Faculty of Dentistry ethical approval FDASU-ReclM 012017 that the study is following ethical guidelines of research. The patient was clearly understood the purpose of this research and signed a written informed consent.

II- Study Design and Randomization.

The trail was designed as a prospective two parallel arms randomized clinical trials, single center and in split mouth design study.

Eligible patients had total number of 20 areas of gingival hyperpigmentation (Two in each individual) treated, so that each patient will receive the two different treatment modalities in split mouth technique (Right and Left).

Group I (Glutath.): included 10 pigmented quadrants that were treated with intra mucosal field injection of Glutathione and was considered the study group.

Group II (Laser): involved 10 pigmented quadrants that were treated with Diode laser technique for gingival depigmentation and was considered the control group.

Pre-operative preparation:

All patients received full arches professional scaling using ultrasound scalers

and given oral hygiene instruction at initial visit.[1]

Biopsy procedure:

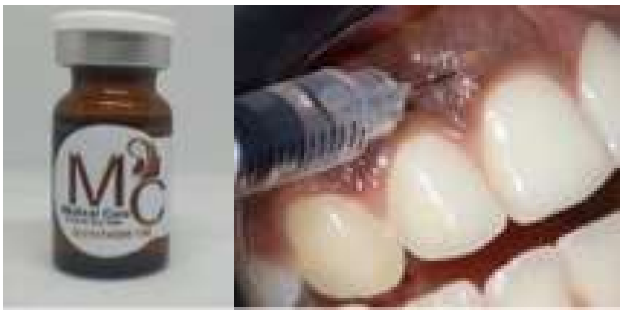
A week before the treatment process, tiny samples of gingival soft tissue measuring 2 mm in width and 1 mm in depth were extracted from the furthest site of the hyperpigmented area utilizing a biopsy punch[1]. The purpose of this extraction was to verify the clinical diagnosis of benign melanin pigmentation [figure (1)]. Utilizing local anesthetic, a punch was carefully introduced into the mucosa with a rotating motion to effectively cut the tissue, including the surface epithelium and the underlying lamina propria. Care was taken to avoid exposing the periosteum. A biopsy was performed on each patient on two occasions: the first prior to initiating treatment (baseline), and the second after a period of 3 months. In the case of conventional histology, the samples were immersed in a solution containing 10% formaldehyde (weight/volume) in 0.2 M phosphate-buffered saline (pH 7.4) to fix them. Then, they were dehydrated using a series of ethanol solutions with increasing concentration and finally embedded in paraffin. The slides were treated with Fontana Masson stain. [2] For each section, microscopic fields were selected, and photomicrographs were captured at magnification of X200. A light microscope[3] was utilized to mount a digital camera[4], which was utilized to capture every image. The images were subsequently uploaded to the computer system for examination. The experiment was conducted in the Precision Measurement Unit of the Oral Pathology Department at the Faculty of Dentistry, Ain Shams University. The assessment process involved the utilization of picture J[5], a software specifically designed for picture analysis. The analysis involved measuring the average area fraction of pigmentation for each sample by examining all the photos. The data was organized and recorded in a Microsoft Excel sheet.

Figure (1): Showing biopsy soft tissue biopsy from the most distal part of pigmented quadrant using Tissue Punch.

IV- Interventions:

Group I- (Glutath.)

The procedure involved an intra-mucosal injection (G1. Mesotherapy) with local anesthesia[6] achieved using the field block technique. This was followed by injecting one milliliter of glutathione[7] (1 mg/5 ml) into the local area. The injection was done in a way that the needle[8] was inserted no more than one millimeter underneath the epithelial surface, parallel to the junction between the epithelium and connective tissue. The bevel of the needle was facing upwards, and the injection was repeated every 2-3 mm apart, with 0.1 ml of glutathione injected at each point until the tissues blanched. [Figure 2]. The treatment was repeated 3 times with 1 week interval for each patient El-Mofty [15], and all the injection procedures were performed by the same operator. No post-operative instructions were needed.



A B

Figure (2): A) Glutathione solution uploaded in Insulin syringe B) injection technique of glutathione

Group II- (LASER)

Laser Depigmentation

A soft tissue diode surgical laser unit was utilized in a contact approach employing a flexible fiber optic hand piece with an activated tip. The laser irradiation parameters were modified according to the following specifications: The wavelength utilized is 940 ± 10 nm, the irradiation mode is contact continuous wave, the power is 2W, and the diameter of the fiber tip is 300 µm [16]. Depigmentation was done in one session. Local anesthesia was achieved using infiltration technique. Avoidance of highly reflective devices with mirrored surfaces was necessary to prevent any potential reflection of the laser beam. Safety glasses were worn to assure eye protection for the patient, assistant, and operator. The depigmentation treatment commenced at the free gingival border and progressed towards the mucogingival junction, encompassing the interdental papilla. During the operation, the fiber tip moved in a circular manner, creating overlapping circles. The laser progressively removed the pigments from the gingival and mucosal epithelial surfaces without producing any bleeding.

Post-operative instructions: In order to facilitate re-epithelialization and avoid mechanical damage, patients were advised not to clean their teeth on the day following surgery. Patients were also advised to stay away from spicy and hot foods for the first day. Starting on day 2, it was strongly advised to practice regular dental care using a toothbrush and interproximal devices.

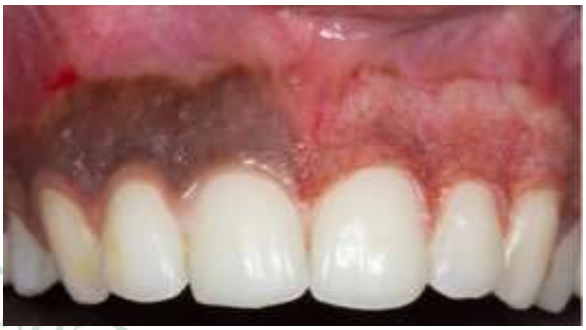


Figure (3): Showing gingival tissue immediately following LASER depigmentation (left quadrant)

V- Assessment:

A. Clinical Assessment:

Clinical assessment, digital photographs were obtained for all of the patients on the same dental unit with the same position (45-degree position with head supported), light exposure and distance were fixed and standardized. Photos were obtained using digital camera[4] adjusted to the following settings, focal stop (22-29), exposure time 1/200, focal length100mm.

The clinical parameters were evaluated 3 times for each patient; at baseline (immediately before surgical depigmentation), 1, 3 months post operatively. The measured parameters included;

1) Dummet oral pigmentation index (DOPI), The level of pigmentation of the gingival was determined as: 0 indicates pink tissue, 1 light brown tissue, 2 moderate brown or pink and brown tissue, or 3 heavy brown/blue-black tissue, all indicating different levels of clinical pigmentation [11].

DOPI was assessed in the daylight and from digital photographs taken in daylight with the same camera parameters and settings in each visit. DOPI at specific marked sites was determined and the mean of DOPI at these marked sites was calculated. These sites were recorded for all photos for each patient

at baseline. (Figure 4) showing mean of 3 areas of DOPI

Figure (4): DOPI at specific marked sites was recorded to the group I (G) and group II (L).

2. Assessment of pigment surface area (SA) Photometric assessment of the pigment intensity using Image J analyzer software: Standardized photos for the area of interest were obtained then transformed into JPEG format, then introduced into image analyzing software[1] for tracing and calculation of existing pigment within gingiva Figure (5)

Figure (5): Showing steps Calculation of surface area (SA) of existing gingival pigment. A) Cropped interest area. B) Color thresholding and tracing of existing pigment. C) Traced pigment and all data analyesd to measure the mean surface area.

3. Satisfaction questionnaire was used to score the degree of experienced pain during and after treatment and the degree of patient satisfaction with the cosmetic results of the procedure .[7]

The majority of this questionnaire is made up of three major categories of word descriptors: sensory, emotional, and evaluative. Patients utilize these word descriptors to reflect their subjective experience during the course of their treatment.It evaluates three primary aspects: first, the pain rating index; second, the cosmetic alterations that happened at various times (3) pleasure of the individuals. It provides data and quantitative information that can be sufficiently sensitive to detect differences among the two different treatment modalities.

B. Histopathological assessment:

The slides were stained then with Fontana Masson stain to assess average Melanin Area Fraction (MAF).

Four microscopic fields displaying the highest concentration of black/brownish black staining, which is indicative of melanin granules, were chosen from each Fontana-Masson-stained segment. Photomicrographs were then taken at an original magnification of x200.

The photographs were obtained using a digital camera that was attached to a light microscope. The images were subsequently uploaded to the computer system for examination. The experiment was conducted in the Precision Measurement Unit of the Oral Pathology Department at the Faculty of Dentistry, Ain Shams University. The assessment process involved the utilization of image J analysis software for all the necessary procedures.

The initial step was adjusting the brightness and contrast of the images. Corrected images were then converted into 8-bit grayscale type[9].

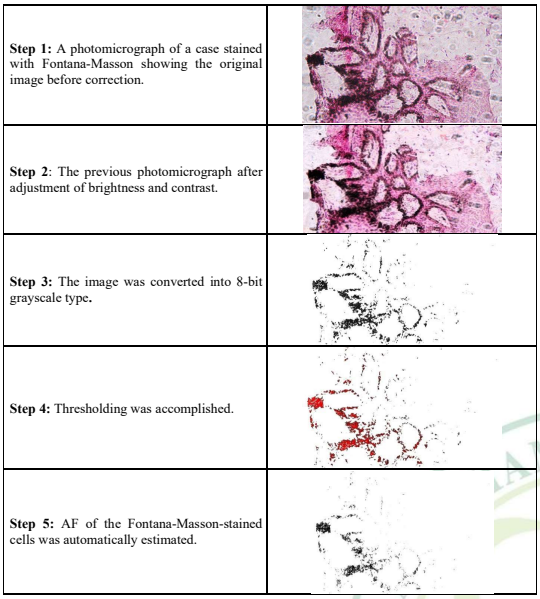


Figure (6): Plate showing steps for the histopathological assessment of MAF of melanosphores in a Fontana Masson-stained microscopic field.

V- Statistical analysis:

The recorded data were analyzed utilizing the statistical software package for social sciences, version 23.0 (SPSS Inc., Chicago, Illinois, USA). The quantitative data were displayed as the mean value plus or minus the standard deviation, along with the range. Furthermore, the qualitative factors were expressed in numerical values and percentages.

The following tests were done: The independent-samples t-test was utilized to compare the significance between two means. The statistical technique of repeated-measures analysis of variance (ANOVA) was employed to compare numerous measures taken within the same group. The Bonferroni adjustment was employed to correct the p-value for multiple comparisons conducted within the same group. A paired sample t-test was employed to compare related samples for significance. The comparison of groups with qualitative data was conducted using both the Chi-square test and Fisher's exact test. The Fisher's exact test was used in cases where the predicted count in any cell was less than 5, instead of relying solely on the Chi-square test. The confidence interval was established at a level of 95% and the accepted margin of error was set at 5%. The p-value was deemed significant for the following reasons:

Probability (P-value): A p-value below 0.05 was deemed statistically significant. A P-value of below 0.001 was deemed to be extremely significant. A P-value above 0.05 was deemed to be statistically insignificant.

Results

Table (1): Descriptive statistics and test of significance of mean dummett oral pigmentation index "DOPI" between the groups and within the same group at different time intervals.

Time intervals	Group I	Group II	t-test	p-value
	Mean±SD	Mean±SD		
Baseline	2.88±0.35	2.66±0.72^{A}	0.754	0.464ns
1 month	2.75±0.46	0.90±0.19^{B}	10.495	<0.001**
3 months	2.88±0.35	0.79±0.40^{B}	11.036	<0.001**
RMANOVA	***1.583***	***5.386***		
p-value	***0.362ns***	***<0.001****		

Using: t-Independent Sample t-test for Mean±SD
RMANOVA: Repeated-measures analysis of variance
p-value >0.05 is insignificant; *p-value <0.05 is significant; **p-value <0.001 is highly significant

Table (2): Descriptive statistics and test of significance of mean surface area% between the groups and within the same group at different time intervals.

Time intervals	Group I	Group II	t-test	p-value
	Mean±SD	Mean±SD		
Baseline	5.99±2.79	5.90±1.74	0.074	0.942ns
1 month	4.70±2.13	1.76±1.15	3.434	0.004*
3 months	4.62±2.40	2.23±1.41	2.424	0.029*
RMANOVA	***2.052***	***5.334***		
p-value	***0.125***	***<0.001****		

Table (3): Descriptive statistics and test of significance of mean area fraction between the groups and within the same group at different time intervals.

Time intervals	Group I	Group II	t-test	p-value
	Mean±SD	Mean±SD		
Baseline	1.76±0.43	1.72±0.46	0.198	0.846ns
3 month	1.62±0.56	0.73±0.30	3.992	<0.001**
Paired sample t-test	***0.883***	***3.779***		
p-value	***0.572***	***<0.001****		

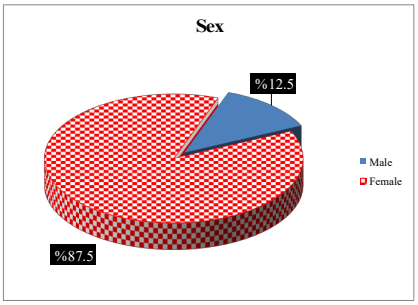


Fig. (7): Sex distribution among study group.

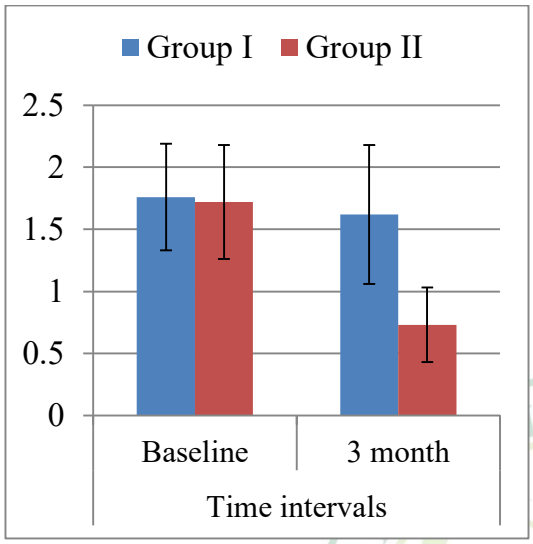


Fig. (8): Descriptive statistics and test of significance of mean area fraction between the groups and within the same group at different time intervals.

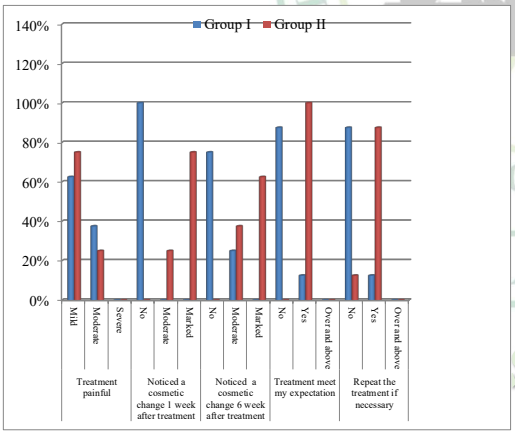


Fig. (9): Comparison among Group I & Group II according to patient satisfaction questionnaire.

Discussion

Physiological gingival hyperpigmentation is regarded as a problem of esthetic concern among individuals. Despite the variable available treatment modalities of depigmentation, the most common concern after treatment is the recurrence of the pigment. The reappearance of gingival pigmentation was found to occur irrespective of the used treatment modality for depigmentation. [17] Moreover, studies showed great variability in the duration of gingival re-pigmentation which may be attributed to various factors. [18] As a result, all recent research hypotheses have centered on various drugs that act directly to disrupt crucial phases of melanogenesis.

Both as a specific skin-whitening agent and in the treatment of hyperpigmentation disorders like melasma, glutathione proved to be an effective tool. [19] The enzyme tyrosinase is involved in the production of melanin, and glutathione inhibits it in two ways: directly and indirectly. Additionally, it changes the way eumelanin is made, which is responsible for whitening the skin [20,21]. The recommended systemic dosage for skin whitening was 20-40 mg/kg, which is equivalent to 1–2 g divided into two doses; noticeable results were achieved after three months. Because of the increased risk of hazardous overdose or other additions in glutathione injection, the recognized adverse effects of intravenous doses (600-1200 mg once or twice weekly) are more prominent .[22]

In 2019, a systematic review by Dhippayom[23] showed that Topical application of 2.0% oxidized glutathione appears to be more effective than oral intake of 250 to 500 mg/day glutathione in enhancing skin brightness and improving skin problems. Topical glutathione has been proposed as a potentially efficacious product for enhancing skin tone and addressing other associated skin disorders, with its effectiveness being influenced by the dosage and duration of use. [23] Puri conducted research on forty individuals to assess the effectiveness of mesotherapy using glutathione & vitamin C for treating melasma. The findings revealed a decrease in cutaneous melanin score. [24]

The first and only clinical trial that assessed the effect of glutathione on the treatment of gingival hyperpigmentation reported that glutathione was effective in treating gingival pigmentation as an adjuvant to laser depigmentation, and it was non-toxic,

safe, and stable. It was also a less invasive and non-surgical depigmenting method. [25]Therefore, our study was performed to address a gap in knowledge regarding the efficiency of intramucosal injection of glutathione on gingival physiological depigmentation.

The key advantages of the intramucosal injection delivery method of glutathione that have been used in the current study were the extent of the pharmacological effects at a local level[26], without the need for large systemic concentrations, and minimize the required dose.[27].The protocol for intra-mucosal injections of gingival depigmenting agent was adapted from El-Mofty [15] for Vitamin C as 1 ml of the medication (1mg / 5ml) locally injected and repeated 3 times with 1-week intervals for each patient as it was assumed to be effective dose and duration to cause a reduction in epithelial melanin score.

In the current randomized controlled clinical trial, the treatment modality that has been selected as a control treatment was diode laser assuming the following advantages of controlled bleeding during procedure and more acceptance by the patients. However, systemic reviews revealed that the incidence of recurrence of pigment is more with laser than with other treatment modalities of pigment removal. This might be attributed that laser bio-modulates the tissues stimulating the migration of adjacent residual melanocytes, resulting in faster recurrence. [4] The wavelength of the diode laser used was (800-980nm) which falls within the absorption spectrum of the melanin pigment (351-1064 nm) so it is highly absorbed by the pigmented tissue that contains melanin chromophores and also it is less absorbed by hard dental tissues based on the principle of selective photo thermolysis [28]

As far as the author knows, this is the first randomized, clinical trial that compares the effectiveness of glutathione intra-mucosal injection with diode laser in treating gingival melanin hyper-pigmentation. The research evaluates patient satisfaction, clinical efficacy, as well as histological changes.

This research was a single-center randomized controlled clinical trial and two parallel arms in a split-mouth design to eliminate any factors that may contribute to bias; however, blinding was not applicable for both patients and investigators due to differences in the mode of delivery of intervention strategies. Moreover, depending on certain predetermined inclusion and exclusion criteria; few patients were excluded depending on factors that might create a bias as smokers [29] or periodontitis cases, pregnant and lactating females, or history of reported allergy to glutathione.

DOPI is one of the most common methods applied for oral pigmentation assessment. It is regarded as an epidemiological gold standard index for oral pigmentation assessment [11]. Moreover, the calculation for the surface area of treated pigment using Image analyzer software from digital standardized photographs contributed to the assessment of pigment in terms of quantity and quality. Digital images provide a sensitive, reproducible, accurate, and objective method.[30] Furthermore, to evaluate the pain and cosmetic perception as patient-oriented outcomes; the patients' satisfaction questionnaire modified from the McGill pain Questionnaire, was utilized.[7]

For histological assessment, a special stain was used for specific staining of the melanosomes (Fontana Masson stain) to assess the impact of glutathione intra-mucosal treatment on melanocytes and melanosomes. It reflected melanocyte function rather than number. A histological estimation of melanin analysis of the MAF melanin pigment from digital melanin analysis histological slides was done. This method was considered more accurate reproducible and objective in comparison to the visual grading which was usually made qualitatively based on the visual grading of each of the affected areas evaluated.[31]

In this study, all patients were followed up clinically and histologically for three months as an average follow-up time according to other reviewed studies conducted for treating gingival hyperpigmentation using diode laser or intramucosal vitamin C as a depigmented agent.

There was no improvement in the mean DOPI sore with the injectable GLU (Group I) while with laser (Group II), there was a significant decline in the mean of DOPI from baseline (2.66±0.72) to (0.79±0.40) 3 months after treatment.

The DOPI significant reduction in Group II may be due to instant eradication or ablation of melanin pigment, these results follow what was reported by "*Giannelli (2014)*". [7] when using a diode laser for treating gingival hyperpigmentation. The mean DOPI showed significant enhancement one month after the treatment.

The insignificant decrease in DOPI score In Group I may be attributed to the dose-dependent benefits of GLU, and 2% dose we used might not have been sufficient to decrease gingival pigmentation. Our result was inconsistent with a study by Watanabe et al., who discovered that applying 2% w/w GLU lotion to sun-exposed areas may significantly reduce the skin's melanin index. The disagreement may have originated from the fact that the oral mucosa has a faster metabolic rate and turnover than the skin. Moreover, Wiraguna and colleagues discovered no significant variations in the skin melanin index when administering a dosage of 250 mg/day of glutathione. In contrast, Arjinpatana and colleagues. detected significant variations in the skin melanin index while administering a dosage of 500 mg/day of glutathione [32]. So, the hypothesized advantage of glutathione for skin tone is strongly related to the elevation of glutathione levels within the body. [33]

The mean % of Surface Area decreased insignificantly from baseline (5.99±2.79) to 3 months (4.62±2.40) after treatment in Group I (GLU). While in Group II, there was a significant decrease from baseline (5.90±1.74) to 3 months (2.23±1.41) in Group II (LASER). The slight decrease in mean surface area observed in Group I (GLU) may be attributed to GLU's ability to modulate the intensity of melanin pigment rather than eliminating preexisting pigment through increased cysteine levels. This shifts melanogenesis from eumelanin to pheomelanin synthesis, resulting in gingival lightening [34]. As a result, the depigmented impact of GLU might be time-dependent, necessitating multiple administrations over a minimum of 6 months as reported by Richie et al.[35]

Our finding was inconsistent with Mounika et al. [25] who observed a significant reduction in mucosa pigment surface area when GLU was employed in conjunction with laser. [25] This diversity could be attributed to glutathione's ability to prevent the development of new melanophores or the lighting of existing melanin pigment. So, when paired with laser, the outcomes are better.

According to the satisfaction questionnaire, neither of the treatment modalities caused any pain over the course of the procedure. In Group II, the laser's bio-modulating effect and coagulation reduce pain, whereas Group I undergoes less invasive GLU intradermal injection. This outcome was per the findings of Azma and Safavi [36], who stated that the benefits of using a diode laser during surgery included a comparatively bloodless procedure and less discomfort and swelling subsequently [36]. Additionally, it is likely that the pain scores reported immediately following treatment in Group I were solely a result of the discomfort linked to the several injections that were necessary for that particular treatment procedure. On the other hand, the protein coagulum that forms on the treated surface may explain why Group II felt less pain. It acts as a biological barrier that covers sensory nerve terminals. Laser therapy's photobiomodulation effects can also help alleviate pain.

All patients in Group II were satisfied with cosmetic changes and it met their expectations, although 12.5% only in the Group I were satisfied with the results, these could be related to the immediate effect of laser but a minimal and delayed depigmented effect of GLU due to the inhibition of tyrosinase enzyme was supposed to prevent melanin production in mucosal epithelium as was proved in previous studies. [20, 37]

The histopathological assessment revealed and confirmed that laser treatment modality caused a significant reduction in MAF after three months post-treatment. There was a significant variation in MAF on comparing Group I & II to being (1.62±0.56) (0.73±0.30) respectively after a three-month follow up there was a higher percentage of reduction among Group II than Group I. There was also a reduction in MAF among Group I from baseline (1.76±0.43) to (1.62±0.56) after 3 months of treatment but it was non-significant.

The overall reported insignificant results of GLU in the current study as the depigmenting agent of gingiva when compared to the results that have been reported in skin depigmentation could be explained by the possible more effectiveness of GLU in sun-exposed skin areas but not in sun-protected skin areas (as oral tissue) because it may require active melanocytes to act on [23]; UV radiation controls skin pigmentation by enhancing melanocyte proliferation, oxidizing pre-existing melanin, redistributing melanosomes to the epidermal upper layers, and increasing tyrosinase activity and GLU blocks this activation cycle while oral mucosa is not exposed to UV. [38]

The present findings were consistent with the research carried out by Zubair [39] where that research GSH Detox forte 1200 mg injection (sodium chloride, ascorbic acid, hydrolyzed collagen 35 mg, aqua, and glutathione 1200 mg) was used and given twice a week for six weeks, for a total of twelve injections, it was shown that glutathione was not very effective at lightening skin tone, and that treatment becomes less effective with time. And the treatment side effects were common. Conversely, a trial that employed glutathione as a depigmenting agent with a higher dose in solution form (2400 mg) was conducted; each patient had six sessions spread over three months, every two weeks. demonstrated a noteworthy and substantial reduction in the cutaneous melanin score index.[40]

Within the limitation of a relatively small sample size and short follow-up period. In this research, we concluded that glutathione was not effective in comparison with diode laser in treating gingival pigmentation, despite the effectiveness of glutathione in dermal depigmentation in previous studies andthis might be attributed to improper dose and protocol of injection in gingival mucosa, which might necessitate future research in this field to elaborate possibilities of further use of glutathione as gingival depigmenting agent.

Conclusion

Intra-mucosal injection of 1ml Glutathione (repeated 3 times with 1-week intervals) had no detectable effect on gingival pigmentation score and surface area compared to rapid initial response and more patient satisfaction with Diode Laser gingival depigmentation. Histologically, Diode Laser treatment showed a greater and significant reduction in MAF of melanosomes after three months post-treatment than Glutathione

Declarations

Competing interests

No conflict of interest

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