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INVESTIGATION OF INFLAMMATORY CELL COUNT BEFORE AND AFTER SURGICAL GINGIVAL MELANIN DEPIGMENTATION (HISTOLOGICAL AND CLINICAL STUDY)

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ABSTRACT

Background: Many individuals with hyperpigmented gingiva encounter esthetic problem which can be solved by surgical depigmentation procedures. As melanin acts as a scavenger for reactive oxygen species that attribute to the inflammation , depigmentation may unfortunately cause increase of inflammatory process.

Aim: To investigate if the surgical melanin depigmentation could cause increased inflammation

Subjects and methods: Seven individuals complaining from increased gingival melanin pigmentation have undergone surgical melanin depigmentation .Dummet Oral Pigmentation Index (DOPI) was the clinical parameter tested to investigate degree of melanin pigmentation. Inflammatory cell count was the histologic parameter to analyze number of inflammatory cells before and after depigmentation. Punch biopsy was taken at baseline and one month after depigmentation.

Results: Both DOPI and inflammatory cell counts have significantly changed one month postoperative but there was no significant relation between these changes.

Conclusion : Depigmentaion could possibly increase inflammation .

Recommendations: More studies are needed to confirm direct proportion between depigmentation and inflammation.

KEYWORDS: depigmentaion , inflammation , melanin

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INTRODUCTION

The countenance of a smile serves as a nuanced reflection of virtues such as benevolence, self-assurance and felicity. The potency of this communicative gesture hinges upon the harmonious interplay of two pivotal elements; teeth and gingiva. The former encompasses a multifaceted array of criteria to be evaluated as shape, position and color whereas the latter has only one predominant parameter which is the gingival color ***(Sepolia et al., 2014).***

Many individuals can have gingival pigmentation with colors ranging from light brown to deep black. Labial gingiva is more commonly affected than palatal gingiva. The most frequent cause of gingival pigmentation is melanin pigments synthesized by melanocytes in oral mucosa. Melanocytes are present in the basal cell layer of the epithelium of oral mucosa even in the sites with no visible signs of melanin pigmentation ***(Feller et al., 2014).***

Melanocytes, being dendritic in nature, undertake a pivotal role in safeguarding neighboring cells from the deleterious impacts of free radicals and reactive oxygen species (ROS). Furthermore, they furnish a shield against ultraviolet radiation through their adept capacity to disperse and assimilate light.

Unfortunately, most of the previous studies have focused on the role of skin melanocytes and their potential protective roles against ultraviolet rays, free radicals and ROS while very few studies have investigated oral melanocytes' role in protecting oral epithelial tissues ***(Nilima and Vandana 2011; Saud. et al., 2022).***

Since gingival brown or black color has been an esthetic problem to many individuals, depigmentation was performed either surgically or non-surgically to overcome this problem but with taking the protective role of melanin in consideration, there was a fear of removing the melanin protective shield from the gingiva and subjecting it to more injuries and infections. So, the current study investigated possible increase of inflammatory cells following surgical depigmentation procedures ***(Mahayni et al., 2023).***

AIM:

To investigate if the decrease of melanin pigmentation measured by Dummett-Gupta Oral Pigmentation Index (DOPI) ***(Dummett and Gupta 1964)*** can cause increased inflammation following surgical depigmentation procedure.

Ethics Approval:

The study was approved by ethics committee of Faculty of Oral and Dental medicine, Future University in Egypt with number FUE-REC(8)/3-2024.

SUBJECTS AND METHODS

PEO format:

P- Population:

As the primary outcome of this study was the change of pigmentation degree (measured by DOPI), seven patients were sufficient to detect a mean difference of 1 unit of DOPI between groups in reducing pigmentation assuming a standard deviation (SD) of 0.1 with 80% power and a 5% level of significance.

Sample size calculation

As the main study outcome (DOPI) is a continuous variable, we used the following equation to calculate the proper sample size.

$$n = \frac{2\,[\,(a + b)^{2}\,\sigma^{2}}{(\mu_1 - \mu_2)^{2}}$$

Where n = Sample size in each of the groups, $\mu 1$ = Population mean in treatment Group 1, $\mu 2$ = Population mean in treatment Group 2, $\mu 1 - \mu 2$ = the difference the investigator wishes to detect, σ = Population variance (SD), a = Conventional multiplier for alpha = 0.05, b = Conventional multiplier for power = 0.80.

With value of a = *1.96,* b = *0.842 with 80% power and a significance level alpha of 0.05. The equation was calculated as following:*

$$n = \frac{2\,[(1.96+0.842)^2 \times 19^2}{(52-24)^2}$$

= 7.2. That means 7 subjects.

Eligibility criteria:

I- Inclusion criteria:

1- Age 18-50 years; ability to understand verbal and written instructions; American Society of Anesthesiology physical status classification class I normally healthy individuals ***(Horvath et al, 2021)***.
2- Individuals free from any systemic disease as evidenced by health questionnaire using modified Cornell Medical Index ***(Abramson 1966)***.
3- Individuals with untreated melanin pigmentation in the anterior portion of the upper or lower gingiva.
4- Both genders.
5- Individuals who were able to return for the follow up visits.
6- Individuals who agreed to sign a written consent after the nature of the study was explained according to Declaration of Helsinki ***(WMA 2013).***

II- Exclusion criteria:

1- Smokers.
2- Patients with periodontal diseases.
3- Pregnant and lactating women.

E- Exposure:

Surgical melanin depigmentation.

O- Outcome measure:

Outcome measure		Measure unit	Measure device
1ry Outcome	DOPI	index	Visual examination
2ry Outcome	Inflammatory cell	Number of cells	Electron microscope

I- Dummett-Gupta Oral Pigmentation Index (DOPI)

- It is the clinical parameter that determines gingival tissue color and it is graded as following:

  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].

  3 = deep brown/blue–black tissue [heavy clinical pigmentation].

- Degree of pigmentation was recorded for each participant at baseline and after 1 month following depigmentation.

2- Inflammatory cell count:

A punch biopsy was taken for each participant before depigmentation and one month after depigmentation for histologic assessment*.

Specimen preparation

***Hematoxylin and Eosin (H&E) staining**

The pigmented gingival tissue specimens were immediately fixed in neutral buffered formalin solution with concentration of 10% then washed by tap water, dehydrated by ethyl alcohol, cleared by xylene and finally embedded in paraffin wax.

Sections were cut with thickness of 4-5 µm and then mounted on clean glass slides. These sections were then deparaffinized in xylene and rehydrated by ethanol ending with distilled water before histological staining with H&E solutions to identify histological details ***(Feldman and Wolfe 2014).***

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Surgical Procedure:

After achieving adequate local anesthesia, the pigmented gingival epithelium along with a layer of the underlying connective tissue was surgically removed by splitting the epithelium with blade no.15. Due care was taken to remove any pigmented remnants which could be source of pigmentation recurrence. After the surgical depigmentation, the depigmented area was covered with a periodontal dressing to decrease pain sensation, allow better healing and prevent surgical site infection.

Statistical methods:

Data were coded and entered using the statistical package for the Social Sciences (SPSS) version 28 (IBM Corp., Armonk, NY, USA). Data were summarized using mean, standard deviation, median, minimum and maximum values. For comparison of serial measurements (baseline to one month after intervention) within each patient paired t-test was used for normally distributed data while the non-parametric Wilcoxon signed rank test was used for non normally distributed data ***(Chan, 2003a)***. Correlations between quantitative variables were done using Spearman correlation coefficient ***(Chan, 2003b)***. P-values less than 0.05 were considered as statistically significant.

RESULTS

1- Comparison between baseline and after 1 month:

DOPI values have significantly decreased one month postoperative while the inflammatory cell counts have significantly increased after one month as shown in table (1) and figure 1 and 2.

2- Correlation between Inflammatory Cell count increase with DOPI decrease:

Interestingly, there was no significant relation between DOPI values' decrease and inflammatory cell increase as shown in table (2).

TABLE (1) DOPI and inflammatory Cell count at base line and 1 month postoperative

	Mean	Standard Deviation	Median	Minimum	Maximum	P value
Baseline DOPI	2.29	0.49	2.00	2.00	3.00	0.014
After 1M DOPI	0.86	0.38	1.00	0.00	1.00	
Baseline Inflammatory Cell count	8.43	2.07	8.00	6.00	12.00	0.001
After 1m Inflammatory Cell count	14.57	3.36	14.00	10.00	20.00	

TABLE (2) Correlation between Inflammatory Cell count increase with DOPI decrease

		Inflammatory Cell count increase
DOPI decrease	**Correlation Coefficient**	0.295
	P value	0.521
	N	7

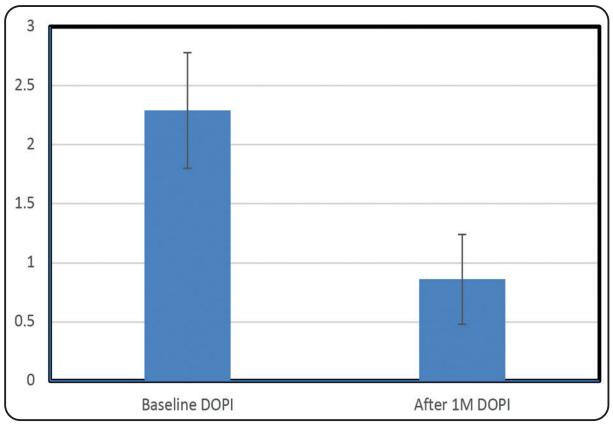

Fig. (1) DOPI Values decreased after 1 month of treatment

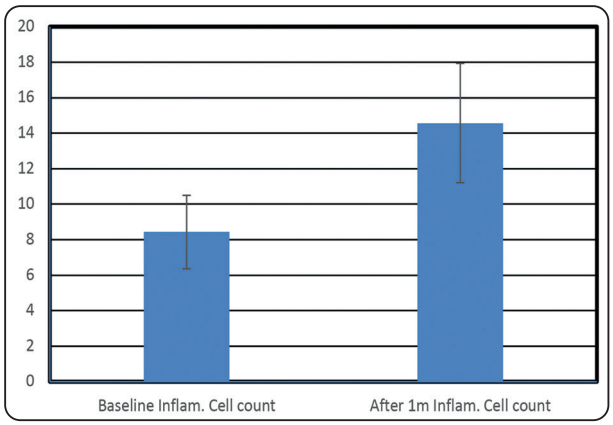

Fig. (2) Inflammatory Cell count after 1 month of treatment.

DISCUSSION

In 2013, Ponnaiyan studied the intensity and distribution of gingival melanin pigmentation and described them as follows:

Class 1: Pigmentation in the attached gingiva only,

Class 2: Pigmentation in the attached gingiva and interdental papilla,

Class 3: Diffuse pigmentation affecting all parts of the gingiva,

Class 4: Pigmentation in the marginal gingiva only,

Class 5: Pigmentation in the interdental papilla only, and

Class 6: Pigmentation in both the marginal and interdental gingiva.

Given the distribution of melanin in the gingiva, gingival pigmentation offers a suitable platform for studying the potential defensive role of melanin in the oral environment. However, the protective role of melanin-pigmented gingiva in the context of plaque-induced inflammation is not fully understood ***(Barrett and Scully 1994)***.

Furthermore, melanin pigmentation may impede the success of dental treatments, including prosthetic restorations. Consequently, oral melanin depigmentation procedures not only address aesthetic concerns but also facilitate accurate diagnosis and optimal treatment outcomes for affected individuals. By elucidating the scientific rationale behind oral melanin depigmentation, these findings underscore its essential role in comprehensive oral healthcare ***(Araújo et al., 2016)***.

The need for oral melanin depigmentation is proved by scientific evidence indicating its multifaceted benefits. Melanin pigmentation in the oral mucosa, particularly when associated with chronic inflammation or trauma, can pose significant challenges in diagnosis and management of oral diseases. Melanin pigmentation often occurs in areas of oral mucosa subjected to chronic inflammation, such as in cases of lichen planus, hindering the identification of pathological lesions ***(Alwi, 2013).***

Although there were many studies that confirmed the protective role of skin melanin and its role against inflammatory processes, there were not many studies discussing the relation between melanin and inflammation in oral mucosa ***(Saud. et al., 2022)***. So, the current study discussed this relation and whether it could be significant or not.

Many epidemiologic studies have confirmed inverse relation between dermal sun-induced cancers and skin pigmentation which consequently confirm photo-defensive role of melanin. These studies also confirmed that dark skinned populations are naturally protected against skin cancers more than light skin populations .The real photo-protection shield against hazardous effects of sunrays lies in the upper epidermis and consequently decrease of melanocytes in this layer is a critical factor in pathogenesis of many cancers ***(Bustamante et al., 1993; Gilchrest et al., 1999).***

In addition to color of skin, oral mucosa, hair and eyes, Melanin is responsible for inhibition of free radicals and reactive oxygen species. It can also counteract enzymes and toxins produced by bacteria. This is specifically important in oral cavity because it is an open media with numerous and various bacterial species. This may be the cause of increased inflammatory cell count after gingival melanin depigmentation observed in the current study similar to these studies ***(Feller et al., 2014; Masilana et al., 2015)***.

An intriguing observation proved that the density of melanophores in the epithelium increased when inflammation in the adjacent connective tissue of the attached gingiva became more severe. This phenomenon might be attributed to the stimulating effect of inflammation on melanocytes, enhancing their melanin production ***(McHugh and Zander, 1965)***.

In agreement with the current study, a former study by ***Nilima and Vandana, 2011*** reported that patients with gingival melanin hyperpigmentation have relatively low levels of IL-1β which indicates a negative correlation between pigmentation with IL-1β levels, although this correlation was not statistically significant. So, more studies are needed to investigate the relation between depigmentation and inflammation.

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