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Original Research Article

Comparative Evaluation of Efficacy of Injectable Vitamin C and Glutathione in the Treatment of Physiological Gingival Melanin Hyperpigmentation– A Randomized Clinical Trial

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Abstract: ***Objectives:*** The present study aimed to comparatively evaluate the efficacy of injectable Vitamin C and Glutathione in the treatment of physiological gingival melanin hyperpigmentation. Secondary objectives included assessment of changes in gingival biotype and evaluation of pain and discomfort associated with both treatment modalities. ***Materials and Methods:*** Forty selected sites in 20 subjects with physiological gingival melanin hyperpigmentation were randomly divided into two groups namely Site A and Site B. In a double blinded study scaling and root planing was performed in all the selected subjects. Prior to the procedure baseline parameters were recorded namely DOPI Index, Gingival Biotype, VAS score. The area was anaesthetized by topical local anesthetic agent. After achieving adequate anesthesia, about 0.2ml of Vitamin C (500mg/ml) was administered at Site A and about 0.2ml of Glutathione (600mg/ml) was administered at Site B; using an insulin syringe. The procedure was repeated at weekly interval for a period of 4 weeks. The gingival tissue biotype was assessed by transgingival method using an endodontic reamer no: 20 with a rubber stopper measured using digital vernier caliper. Subjects were recalled at 1 week, 1month and 3 months for follow up and parameters were recorded. ***Results***: Both injectable Vitamin C and Glutathione demonstrated a statistically significant reduction in gingival melanin pigmentation scores from baseline to follow-up periods. Intergroup comparison revealed that Vitamin C showed comparable or slightly superior depigmenting efficacy compared to Glutathione. Gingival biotype remained unchanged in both groups. Pain and discomfort scores were minimal in both treatment modalities, with no statistically significant difference between groups. ***Conclusion***: Injectable Vitamin C and Glutathione are effective, minimally invasive non-surgical modalities for the management of physiological gingival melanin hyperpigmentation. Vitamin C showed slightly better clinical outcomes, though both agents were well tolerated by patients. These therapies can be considered promising alternatives to conventional surgical depigmentation procedures.

Keywords: Gingival hyperpigmentation, Injectable Vitamin C, Glutathione, Gingival depigmentation, Randomized clinical trial, Periodontal aesthetics.

INTRODUCTION

A beautiful smile not only needs good dental profile but also an appealing gingival display.[1] An aesthetically satisfying smile plays a vital role in psychological well-being and self-confidence[2] According to the principles of visual perception, a harmonic and symmetric composition of teeth, visible gingiva, buccal corridors, and lips is a requirement for an esthetic and pleasing smile.[3] Harmony between facial complexion and gingival health goes hand in hand.[4] Therefore, gingival health and appearance are

essential components of an attractive smile.[5]

A smile line that displays the entire length of the teeth and some gingival tissue is associated with youth. In contrast, a smile line with only a portion of the teeth results in a less youthful smile.[3] The excessive gingival display when a patient smile (4 mm or more), known as a gummy smile, along with a short clinical crown of the anterior maxillary teeth, characterizes esthetic problems.[6] Over the last decade, an exponential request for aesthetic treatments has been increasingly observed, especially in dentistry.[4]

The gingiva consists of melanin pigment producing cells called melanocytes. Melanin (non-hemoglobin-derived pigment) is deposited by melanocytes, mainly located intertwined between the basal and the suprabasal cell layers of epithelium.[7] Deposition of excessive melanin by melanocytes can result in hyperpigmentation.[8] Gingival hyperpigmentation is influenced by exogenous factors such as smoking, heavy metals, medications, and chronic mucosal disorders. Several syndromes have been associated to gingival hyperpigmentation, including Peutz-Jeghers syndrome, Addison's illness, and certain neoplasms.[8]

Gingival depigmentation is a periodontal plastic procedure performed to remove melanin hyperpigmentation. Various modalities have been proposed for management of melanin hyperpigmentation involving surgical scraping, gingival autograft, cryotherapy, electrosurgery, and lasers.[9]

Gingivectomy with free gingival autografting, electro-surgery, and radio-surgery are examples of traditional methods for treating gingival pigmentation. These methods have drawbacks such as pain, high surgical costs, and risks of scarring, gingival recession, bone and periosteal trauma, and delayed wound healing. In place of conventional methods, laser therapy was suggested as a substitute for gingival depigmentation. It is widely used in dentistry and medicine and has no serious negative effects.[10]

The non-surgical methods, also called gingival peeling techniques, includes the use of Vitamin C (ascorbic acid), phenols, salicylic acid, glycolic acid, trichloroacetic acid, and alcohol.[5] The role of several natural agents, such as vitamins, anti-oxidants, etc., in preserving the original skin color through their anti-oxidant potential, induction of collagen production, and enhancement of blood supply was observed by dermatologists.[6]

Vitamin C (ascorbic acid) is synthesized by all plants and most animals. Vitamin C is an essential nutrient for the biosynthesis of collagen, L-carnitine, and the conversion of dopamine to norepinephrine. Vitamin C (ascorbic acid) is a potent anti-oxidant that plays an essential role in skin depigmentation.[11] Vitamin C is used as a treatment modality in depigmentation of hyperpigmented spots on the skin. It can be used topically, transdermally as well as intravenously (IV). It is an important vitamin for cells and a water-soluble antioxidant. Because of a defect in the gene necessary for its production, humans are unable to produce this vitamin, despite its immense importance.[11]

Vitamin C was found to be effective in depigmentation as a result of its direct effect on melanogenesis. Melanin is said to be a reservoir for reactive oxygen species (ROS), copper (cu) and calcium (Ca) within the cells. It attaches itself to melanin once it has entered the target tissue. This results in a lack of ROS, Cu, and Ca, which lowers the formation of melanin.[12] Vitamin C interacts with the copper (Cu) ions at the tyrosinase active site and inhibits action of the enzyme tyrosinase, thereby reducing melanin formation. It also acts on the perifollicular pigment. However, Vitamin C is an unstable compound. Therefore, it is used in combination with soy and liquorice for depigmentation .[12]

Glutathione, a cysteine –Glycine –Glutamate tripeptide which exerts several effects on melanogenesis through different mechanisms involving the functions and cellular transport of tyrosinase, rate limiting step in melanin formation. It is well known that when glutathione or cysteine is added to melanocytes or melanoma cell lines, the melanogenetic pathway is shifted from eumelanin towards pheomelanin formation.[13] Glutathione's potential as a skin-lightening agent has been heavily promoted, following the discovery of its antimelanogenic properties. Key mechanisms proposed for these properties include the inhibition of tyrosinase enzyme, shifting melanogenesis from the production of darker eumelanin to lighter pheomelanin, and scavenging free radicals and peroxidase and modulation of depigmenting abilities of melanocytotoxic agents. Glutathione is available in market as oral, inhaled or topical formulations besides intravenous injectables. Despite the hype and widespread availability of glutathione in various forms, including tablets, capsules, topical preparations, and parenteral formulations, there is a discrepancy between the evidence supporting its efficacy and safety.[14]

Similar to Glutathione, Vitamin C and Alpha Lipoic Acid (ALA) are also potent antioxidants that have numerous attractive features for use in cosmetic and dermatological products. Many clinical investigations have documented the effectiveness of Vitamin C in treating scars and wrinkles, promoting skin rejuvenation, and managing pigmentation disorders. However; there is no evidence of comparative evaluation of efficacy of injectable vitamin C and

Glutathione in the treatment of gingival melanin hyperpigmentation.

Therefore, the present study was conducted to comparatively evaluate efficacy of injectable Vitamin C and Glutathione in the treatment of physiological gingival melanin hyperpigmentation.

MATERIALS AND METHOD:

Sampling technique

The subjects were selected by a simple random sampling technique.

Method of selection

The subjects for the study were selected based on the following inclusion and exclusion criteria

Inclusion criteria

- Systemically healthy cooperative subjects
- Age group of 20-50 years of either sex
- Presence of bilateral gingival melanin hyperpigmentation (DOPI Index score 2 or <2)

Exclusion criteria

- Subjects undergoing orthodontic treatment
- Smokers and tobacco chewers (AHA guidelines)
- Pregnant or lactating females and those on oral contraceptives
- Subjects with known allergies to any medications or dietary substances

Withdrawal criteria

i Non- compliant subjects.
ii Failure to report for follow-up examinations.
iii Those subjects who tested positive for skin allergic test

In the present study no subjects tested positive for skin allergic test to Vitamin C and Glutathione

Blinding proposed:

Double blinded (Study subjects and evaluator was blinded; to reduce the methodology bias, evaluation parameters were recorded by a secondary observer.

Method of Data Collection

Twenty subjects with physiological gingival melanin hyperpigmentation were included in this study. Ethical clearance was obtained from the Institutional Ethics Committee. The subjects participating in this study were informed verbally and subsequently written signed consent was obtained, after they were explained about the nature of the study in detail in a language best understood by them. A detailed case history was recorded. Based on site allocation, skin allergic testing as per standard protocol was done. Subjects who tested negative were subjected to further study protocol.

Forty selected sites were randomly grouped as:

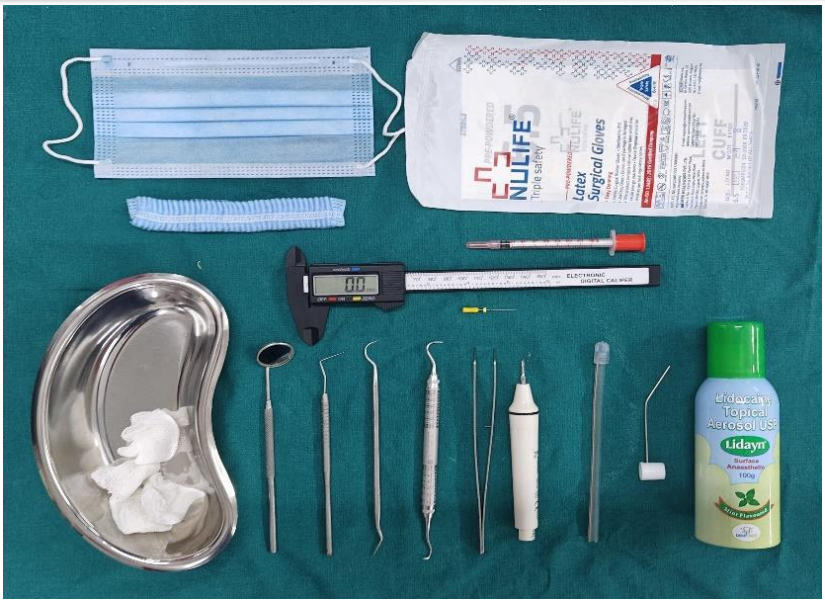
Site - A (n = 20) 0.2ml of Vitamin C (500mg/ml) was administered at Site A after adequate anaesthesia using an insulin syringe

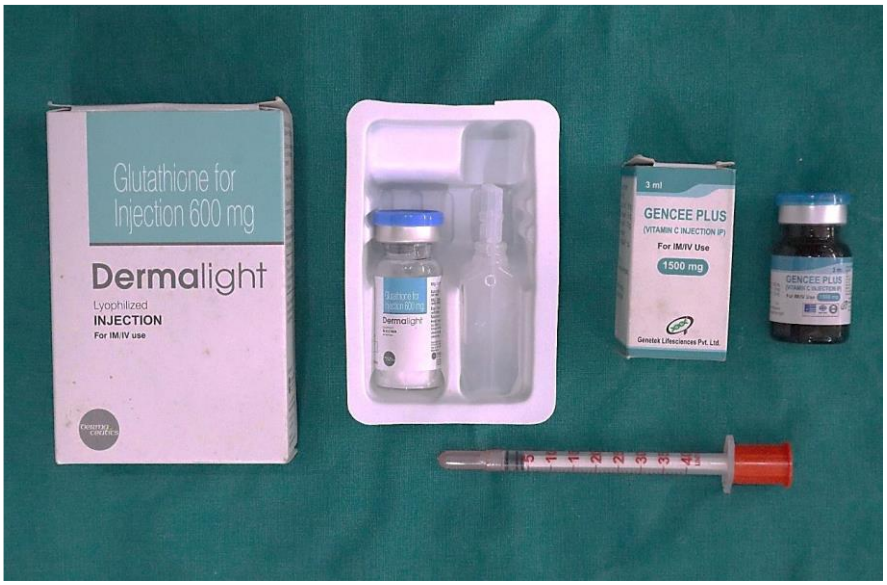
Site - B (n = 20) 0.2ml of Glutathione (600mg/ml) was administered at Site B after adequate anaesthesia using an insulin syringe

Methodology

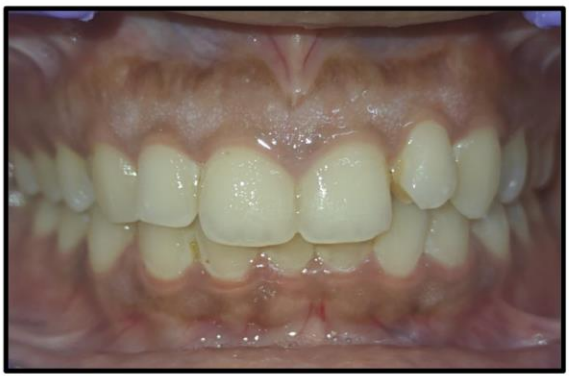
- Scaling and root planing was performed in all the selected subjects.
- Subjects were motivated to maintain good oral hygiene. Modified Bass technique of brushing was explained and demonstrated to them.
- Prior to the procedure baseline parameters were recorded namely DOPI Index, Gingival Biotype, VAS score.
- Photographs were taken for all subjects in the same dental set up with the same position at 11 a.m. with fixed magnification and distance (at baseline,1month and 3months).
- The area was anaesthetized by topical local anesthetic agent (Lidocaine 100g USP).
- After achieving adequate anesthesia, about 0.2ml of Vitamin C (500mg/ml) was administered at Site A and about 0.2ml of Glutathione (600mg/ml) was administered at Site B; using an insulin syringe.
- The procedure was repeated at weekly interval for a period of 4 weeks.
- The gingival tissue biotype was assessed by transgingival method using an endodontic reamer no: 20 with a rubber stopper.
- Subjects were instructed to avoid eating hot and spicy foods for the first 24 hours and were advised to use Chlorhexidine (0.2%) mouthwash twice daily.
- Subjects were recalled at 1 week, 1month and 3 months for follow up and parameters were recorded.

Armamentarium

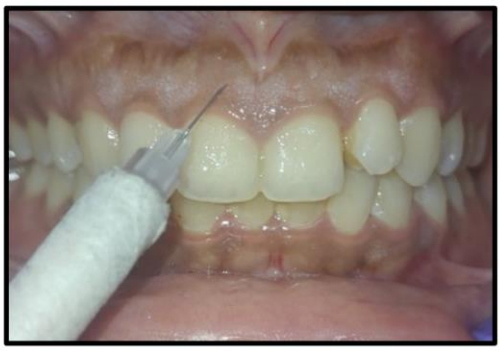


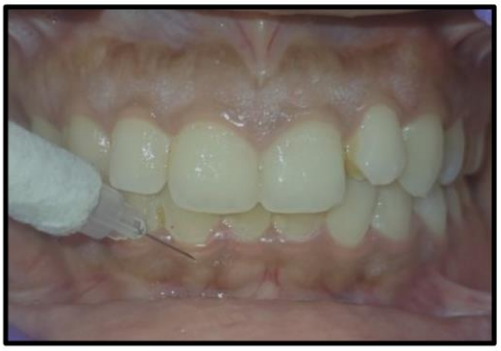


Clinical Photographs - Site A (Vitamin C)

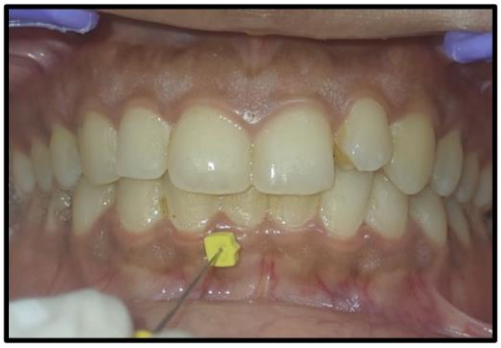


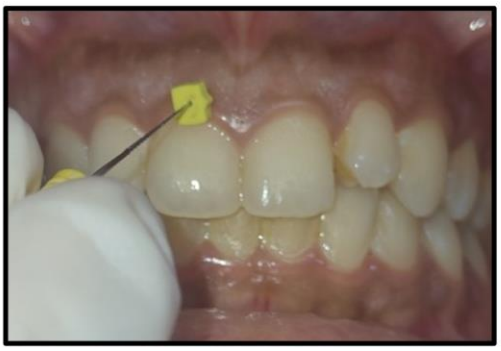
At Baseline





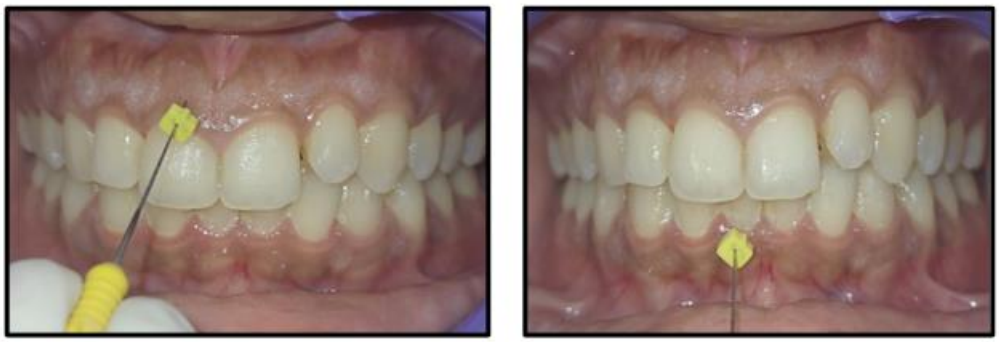
Intraepithelial injection of Vitamin C



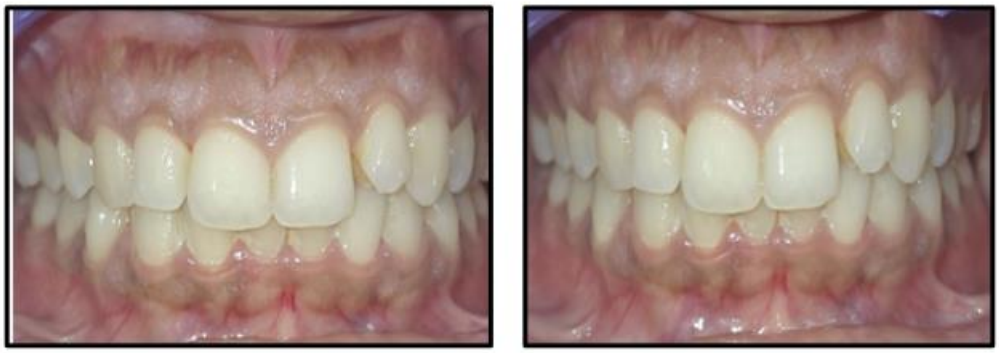


Assessment of Gingival Biotype using Endodontic Reamer at Baseline

Clinical Photographs - Site A (Vitamin C)



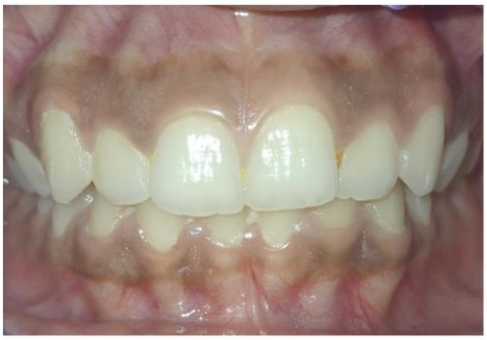
Assessment of Gingival Biotype using Endodontic Reamer at 3months Follow-up



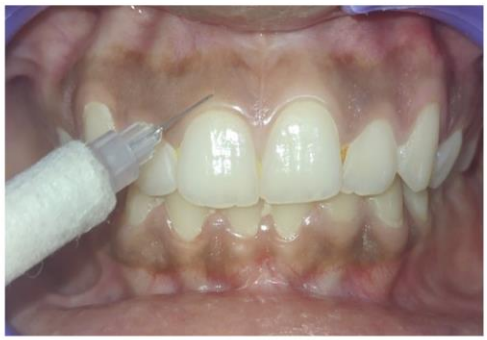
1month Follow-up

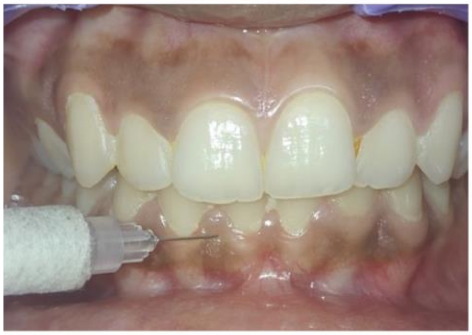
3months Follow-up

Clinical Photographs - Site B (Glutathione)

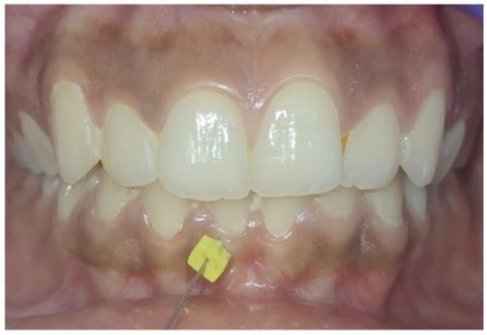


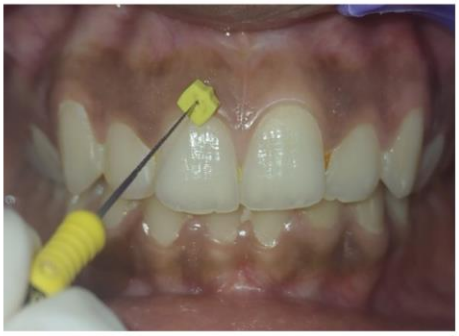
At Baseline





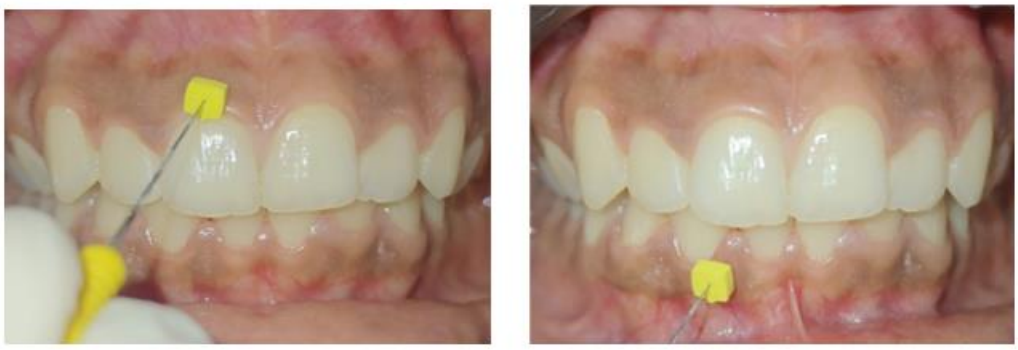
Intraepithelial injection of Glutathione



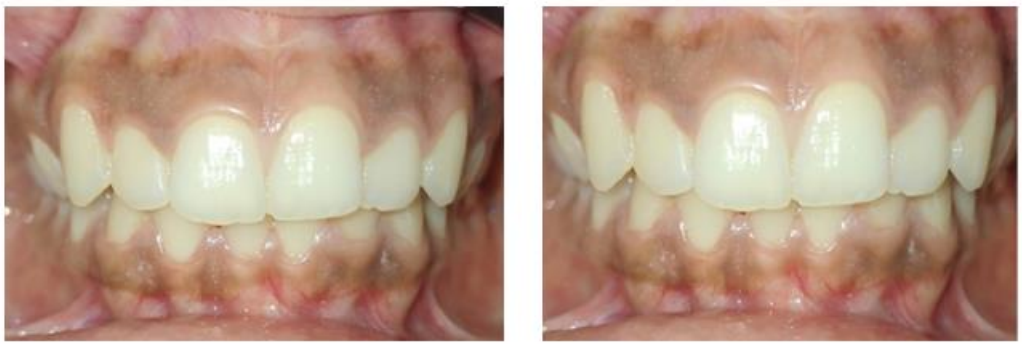


Assessment of Gingival Biotype using Endodontic Reamer at Baseline

Clinical Photographs - Site B (Glutathione)



Assessment of Gingival Biotype using Endodontic Reamer at 3months Follow-up



1month Follow-up 3months Follow-up

RESULTS:

Statistical Analysis

- Data collected was compiled on a MS Office Excel Sheet (v 2019, Microsoft Redmond Campus, Redmond, Washington, United States).
- Data was subjected to statistical analysis using Statistical package for social sciences (SPSS v 26.0, IBM).
- Descriptive statistics like frequencies and percentage for categorical data, Mean & SD for numerical data has been depicted.

Normality of numerical data was checked using Shapiro-Wilk test & was found that the data did not follow a normal curve; or for graded data, hence **non-parametric tests** have been used for comparisons.

1. Inter group comparison (2 groups) was done using Mann Whitney U test.
2. Intra group comparison was done using Wilcoxon Signed rank test (upto 2 observations)
3. Intra group comparison was done using Friedman's (for >2 observations) followed by pair wise comparison using Wilcoxon Signed rank test.

For all the statistical tests, $p<0.05$ was considered to be statistically significant, keeping α error at 5% and β error at 20%, thus giving a power to the study as 80%.
* = statistically significant difference ($p<0.05$)
** = statistically highly significant difference ($p<0.01$)
# = non-significant difference ($p>0.05$) … **for all tables**

Dummett-Gupta Oral Pigmentation Index:

The gingival melanin pigmentation was assessed using the Dummett-Gupta Oral Pigmentation Index (Dummett CO, Gupta OP, 1964)[60] at baseline, 1month, 3 months.

On intragroup comparison, showed there was statistically highly significant ($p<0.01$) reduction in gingival pigmentation at Site A throughout the follow-ups from baseline, 1 month and 3 months with higher values at baseline.(Table 1), (Graph 1) On intragroup comparison showed there was statistically highly significant ($p<0.01$) reduction in gingival pigmentation at Site B throughout the follow-ups from baseline, 1 month and 3 months with higher values at baseline. (Table 1), (Graph 1)

On intergroup comparison of Site A and Site B there was a statistically highly significant reduction in gingival pigmentation 1month and 3months ($p<0.01$). However, the reduction observed in Site B was more significant than Site A.(Table 2), (Graph 2)

There was a statistically non-significant difference seen ($p>0.05$) for gingival pigmentation at baseline.

Gingival Biotype:
The gingival tissue biotype was assessed by the transgingival method using an endodontic reamer no: 20 with a rubber stopper. The reamer was inserted mid buccally halfway between gingival margin and mucogingival junction under local anaesthetic spray and the length of the reamer was measured by digital vernier caliper at baseline, 1 month, 3 months.

The digital reading ≤ 1.5mm was categorized as thin; and ≥ 2mm was categorized as thick gingival biotype. (Claffey & Shanley)[61]

On intragroup comparison, showed there was statistically highly significant improvement in gingival biotype seen at Site A ($p<0.01$) between baseline, 1month and 3 month with higher values at 1 month and 3 months (Table 3), (Graph 3) On intragroup comparison showed there was statistically highly significant improvement in gingival biotype seen at Site B ($p<0.01$) between baseline, 1month and 3 month with higher values at 1 month and 3 months.(Table 3), (Graph 3)

On intergroup comparison of Site A and Site B there was no statistically significant difference seen in gingival biotype ($p>0.05$) at baseline, 1month and 3 month (Table 4), (Graph 4).

VAS Score for Pain:
The pain and discomfort experienced by the subjects were assessed by using the VAS scale (Matthews DC and McCulloch CA 1993)[62] at baseline, 1 month and was recorded by making a handwritten mark on a 10-cm line that represented a continuum between 0 (least pain) to 10 (severe pain) experienced by the patient.

On intragroup comparison for pain experienced by the subjects in Site A, results showed a statistically significant mean decrease in pain seen at follow-up ($p<0.05$) of 1 month (Table 5), (Graph 5). On intragroup comparison for pain experienced by the subjects in Site B, there was a statistically significant mean decrease in pain seen at follow-up ($p<0.05$) of 1 month (Table 5), (Graph 5).

An intergroup comparison showed a statistically highly significant reduction in pain experienced by the subjects at baseline between the Site A and Site B, with more reduction in pain in Site B. There was no statistically significant difference seen ($p>0.05$) for reduction in Pain at 1month (Table 6), (Graph 6).

VAS Score for Discomfort:
On intragroup comparison for discomfort experienced by the subjects in Site A, results showed a statistically significant mean decrease in discomfort seen at follow-up ($p<0.05$) of 1 month (Table 7), (Graph 7). On intragroup comparison for discomfort experienced by the subjects in Site B, there was a statistically significant mean decrease in discomfort seen at follow-up ($p<0.05$) of 1 month (Table 7), (Graph 7).

An intergroup comparison showed a statistically highly significant reduction in discomfort experienced by the subjects at baseline between the Site A and Site B, with more reduction in discomfort in Site B. There was no statistically significant difference seen ($p>0.05$) for reduction in discomfort at 1month (Table 8), (Graph 8). Table 1 **- Intragroup comparison of Dummett-Gupta Oral Pigmentation Index for Site A and Site B**

	Site	N	Mean	Std. Deviation	Chi-Square value	p value of Friedman Test
BL DOPI	A	20	1.73000	0.197617	37.130	0.000**
	B	20	1.72500	0.224488	37.284	0.000**
1M DOPI	A	20	0.74500	0.119097		
	B	20	0.57000	0.117429		
3M DOPI	A	20	0.67500	0.171295		

Friedman Test; **indicates statistically highly significant p<0.01.
There was statistically highly significant difference seen (p<0.01) in DOPI between baseline, 1 month and 3 months at Site A and Site B

Table 2 - Intergroup of comparison of Dummett-Gupta Oral Pigmentation Index

	Site	N	Mean	Std. Deviation	Mann-Whitney U value	Z value	p value of Mann-Whitney U test
BL DOPI	A	20	1.73000	0.197617	177.500	-0.629	0.530#
	B	20	1.72500	0.224488			
1M DOPI	A	20	0.74500	0.119097	53.500	-4.084	0.000**
	B	20	0.57000	0.117429			
3M DOPI	A	20	0.67500	0.171295	97.000	-2.84	0.005**
	B	20	0.50000	0.171679			

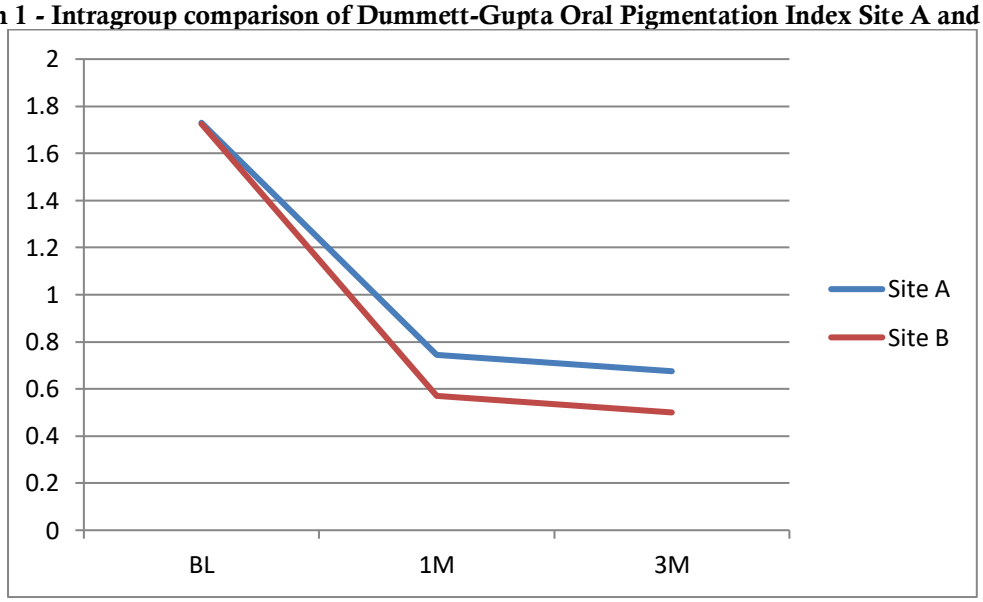
Site A and Site B

Mann-Whitney U test; **indicates statistically highly significant p<0.01; # indicates statistically non significant p>0.05.

There was a statistically highly significant difference seen (p<0.01) for DOPI at 1month between the Site A and Site B, with higher values in Site A, for DOPI at 3 months between the Site A and Site B, with higher values in Site A.

There was a statistically non-significant difference seen (p>0.05) for DOPI at baseline.

Graph 1 - Intragroup comparison of Dummett-Gupta Oral Pigmentation Index Site A and Site B



Graph 2 - Intergroup comparison of Dummett-Gupta Oral Pigmentation Index Site A and Site B

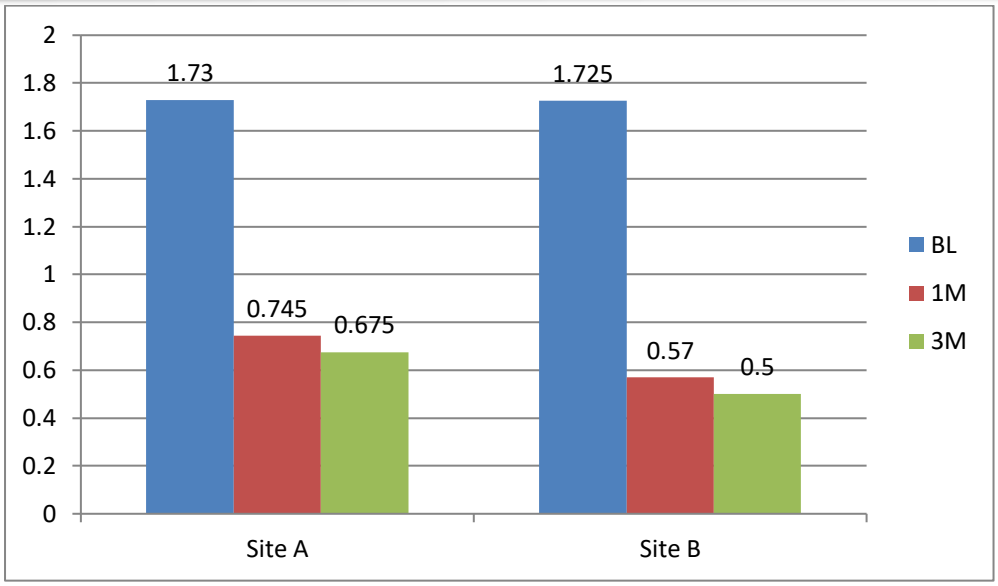


Table 3 - Intragroup comparison of Gingival Biotype at Site A and Site B

	Site	N	Mean	Std. Deviation	Chi-Square value	p value of Friedman Test
BL Gingival Biotype	A	20	1.86500	0.093330	38.000	0.000**
	B	20	1.83000	0.097872	40.000	0.000**
1M Gingival Biotype	A	20	2.03500	0.048936		
	B	20	2.00000	0.085840		
3M Gingival Biotype	A	20	2.03500	0.048936		
	B	20	2.00000	0.085840		

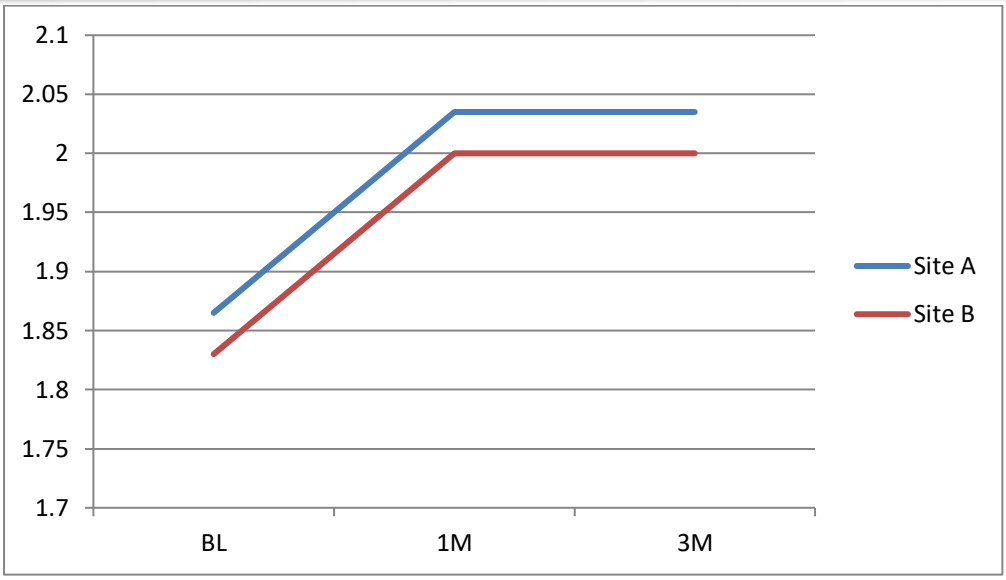
Friedman Test; **indicates statistically highly significant p<0.01.
There was statistically highly significant difference seen (p<0.01) in Gingival Biotype between baseline, 1month and 3 months with higher values at 1month and 3 months at Site A and Site B.

	Site	N	Mean	Std. Deviation	Mann-Whitney U value	Z value	p value of Mann-Whitney U test
BL Gingival Biotype	A	20	1.86500	0.093330	155.000	-1.296	0.195#
	B	20	1.83000	0.097872			
1M Gingival Biotype	A	20	2.03500	0.048936	160.500	-1.251	0.211#
	B	20	2.00000	0.085840			
3M Gingival Biotype	A	20	2.03500	0.048936	160.500	-1.251	0.211#
	B	20	2.00000	0.085840			

Table 4- Intergroup comparison of Gingival Biotype at Site A and Site B

Mann-Whitney U test; # indicates statistically non-significant p>0.05.
There was a statistically non-significant difference seen (p>0.05) for Gingival Biotype at baseline, 1month and 3month.

Graph 3 - Intragroup comparison of Gingival Biotype at Site A and site B



Graph 4 - Intergroup **comparison of Gingival Biotype at Site A and site B**

Table 5- Intragroup comparison of VAS Score for Pain Site A and Site B

	Site	N	Mean	Std. Deviation	Mean diff	SD of diff	Z value	p value of Wilcoxon Signed Ranks Test
BL Pain	A	20	2.25	0.550	2.250	0.550	-4.064	0.000**
	B	20	3.10	0.852	3.100	0.852	-3.974	0.000**
1M Pain	A	20	0.00	0.000				
	B	20	0.00	0.000				

Wilcoxon Signed Ranks Test; **indicates statistically highly significant p<0.01.
There was a statistically significant difference seen for the values between the time intervals (p<0.05) for Pain with higher values at baseline as compared to 1month.

Table 6 - Intergroup VAS Score for Pain Site A and Site B

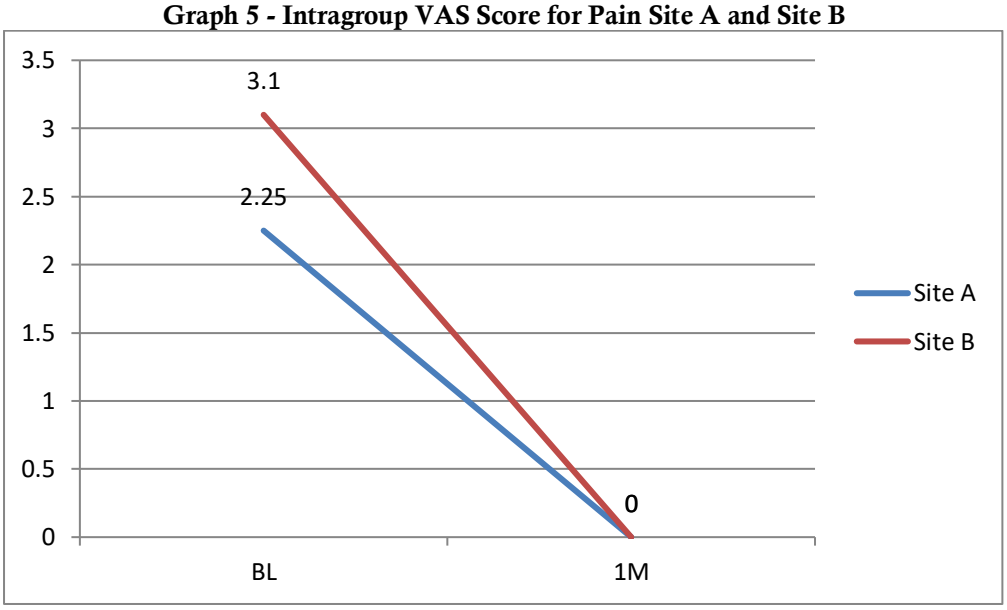
	Site	N	Mean	Std. Deviation	Mann-Whitney U value	Z value	p value of Mann-Whitney U test
BL Pain	A	20	2.25	0.550	93.000	-3.124	0.002.**
	B	20	3.10	0.852			
1M Pain	A	20	0.00	0.000	200.000	0.000	1.000#
	B	20	0.00	0.000			

Mann-Whitney U test; **indicates statistically highly significant $p<0.01$; # indicates statistically non-significant $p>0.05$.

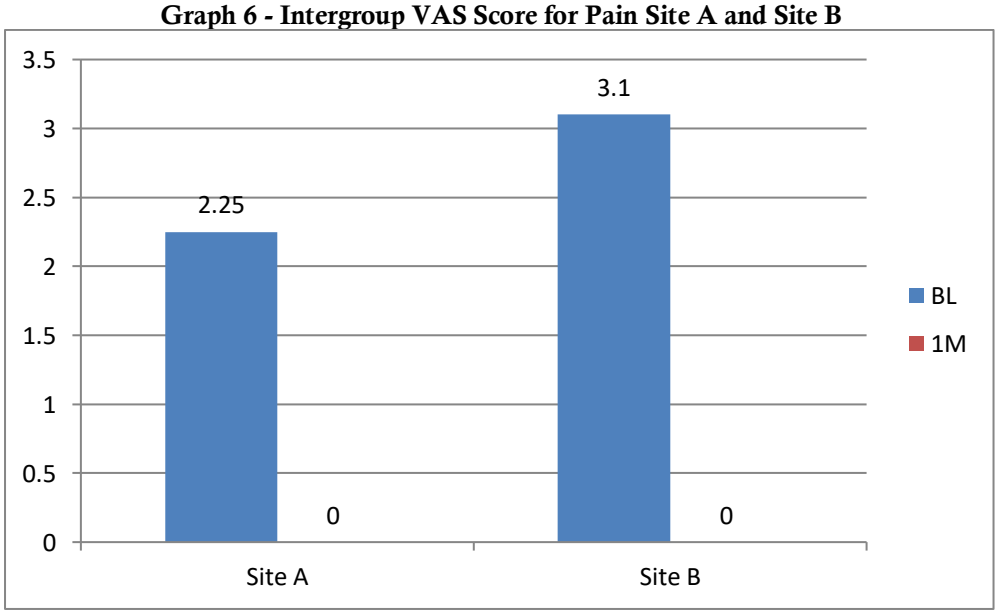
There was a statistically highly significant difference seen ($p<0.01$) for Pain at baseline between the Site A and Site B, with higher values in Site B.

There was a statistically non-significant difference seen ($p>0.05$) for Pain at 1month.

Graph 5 - Intragroup VAS Score for Pain Site A and Site B



Graph 6 - Intergroup VAS Score for Pain Site A and Site B



	Site	N	Mean	Std. Deviation	Mean diff	SD of diff	Z value	p value of Wilcoxon Signed Ranks Test
BL Discomfort	A	20	1.25	0.444	1.250	0.444	-4.134	0.000**
	B	20	3.40	1.095	3.300	1.129	-3.951	0.000**
1M Discomfort	A	20	0.00	0.000				
	B	20	0.10	0.308				

Table 7- Intragroup comparison of VAS Score for Discomfort Site A and Site B

Wilcoxon Signed Ranks Test; **indicates statistically highly significant p<0.01.
There was a statistically significant difference seen for the values between the time intervals (p<0.05) for Discomfort with higher values at baseline as compared to 1month.

Table 8- Intergroup VAS Score for Discomfort Site A and Site B

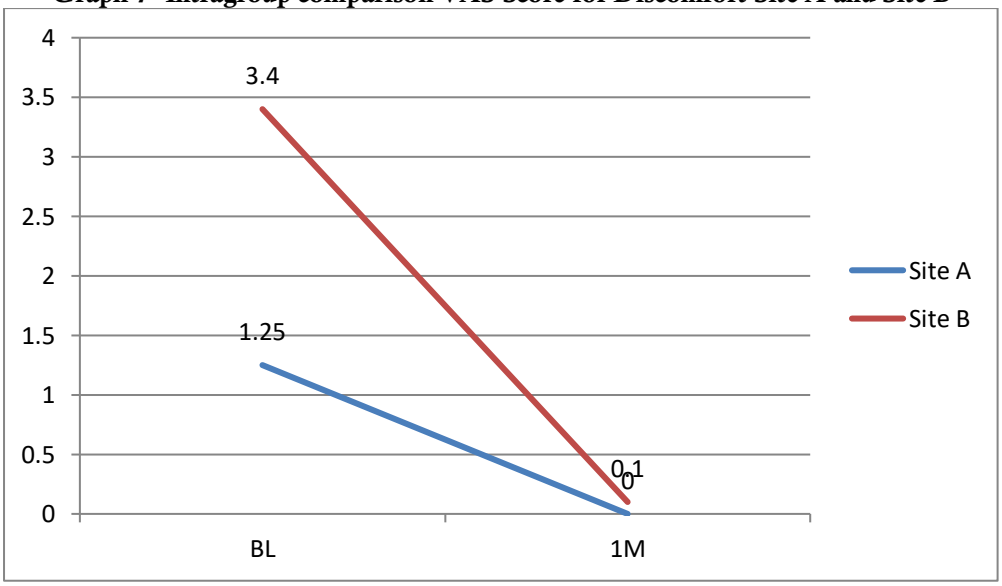
	Site	N	Mean	Std. Deviation	Mann-Whitney U value	Z value	p value of Mann-Whitney U test
BL Discomfort	A	20	1.25	0.444	12.500	-5.271	0.000**
	B	20	3.40	1.095			
1M Discomfort	A	20	0.00	0.000	180.000	-1.433	0.152#
	B	20	0.10	0.308			

Mann-Whitney U test; **indicates statistically highly significant p<0.01; # indicates statistically non-significant p>0.05.

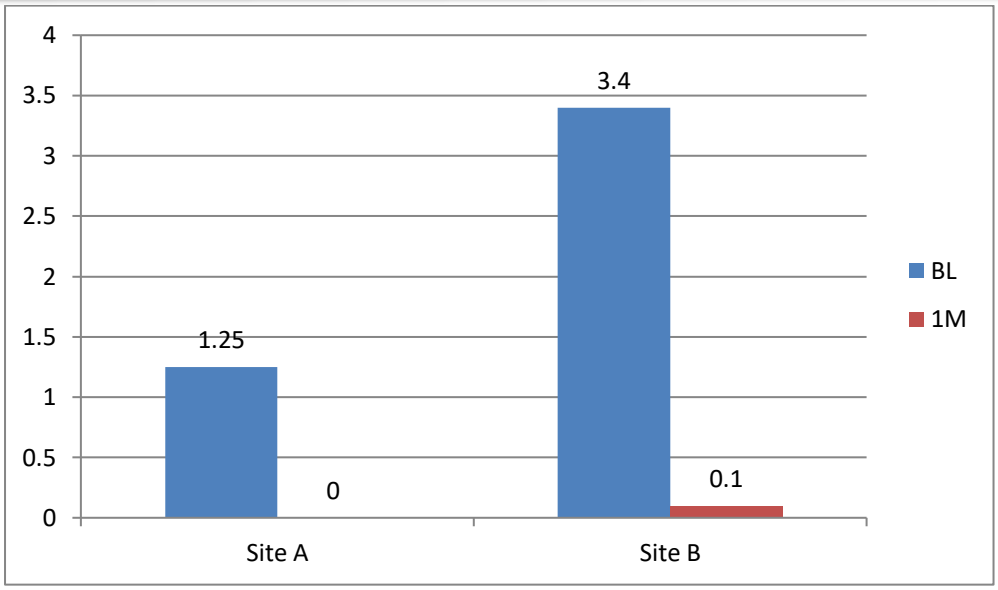
There was a statistically highly significant difference seen (p<0.01) for Discomfort at baseline between the Site A and Site B, with higher values in Site B.

There was a statistically non-significant difference seen (p>0.05) for Discomfort at 1month

Graph 7- Intragroup comparison VAS Score for Discomfort Site A and Site B



Graph 8 - Intergroup comparison VAS Score for Discomfort Site A and Site B



DISCUSSION:

Melanocytes in the basal and supra-basal cell layers of the epithelium produce melanin, a brown pigment that serves as the main natural pigment. Gingiva is the most often pigmented tissue of the oral cavity. However excessive deposition of melanin in gingiva results in gingival melanin hyperpigmentation which may be aesthetically unpleasant.[4]

Gingival depigmentation procedure is used to treat gingival melanin hyperpigmentation caused by melanin. Several surgical and nonsurgical methods are available for gingival depigmentation. Different methods include split thickness epithelial excision/scalpel, electrosurgery, lasers, cryosurgery, gingival abrasion/surgical stripping, or masking using acellular dermal matrix allografts and free gingival autografts. Vitamin C (Ascorbic acid), phenols, salicylic acid, glycolic acid, trichloroacetic acid, and alcohol are among the nonsurgical procedures, also known as gingival peeling techniques.[4]

The decision for the surgical technique for gingival depigmentation is dependent upon the complete thickness of the epithelium being removed as well as the layer of papillary connective tissue (Hedge et al., 2013 and Patil et al., 2015). Despite the general benefits of surgical depigmentation, many individuals who want better aesthetics are apprehensive to the surgical procedures.[42] Therefore, there is a need for other minimally invasive non-surgical therapeutic option in such conditions.

Vitamin C has strong antioxidant properties and has been found effective in depigmenting the gingival and dermal tissues when applied topically or intradermally (Shimada et al., 2009, Yussif NM et al. 2016 and Yussif NM et al., 2017).[42] Proper skin function and appearance are attributed to collagen. Along with type III collagen (15%), which is in charge of providing fibrous support, type I collagen is the most prevalent in skin (80%). Type III collagen is also the primary component of blood vessel walls. Adequate collagen production is directly attributed to vitamin C. When proline is hydroxylated to 4-hydroxyproline, it participates as a co-factor. Moreover, vitamin C application can raise collagen I and III of mRNA levels, according to in vivo research.[63]

Glutathione has shown promise as a skin-whitening agent and has been used in the management of hyperpigmentation conditions like melasma. Glutathione inhibits the enzyme tyrosinase, which is involved in the synthesis of melanin, both directly and indirectly. Furthermore, it alters the synthesis of eumelanin, resulting in skin whitening.[58] Puri evaluated the efficacy of mesotherapy utilizing Glutathione and Vitamin C for the treatment of melasma in forty participants. They observed a reduction in the cutaneous melanin score.[58]

This double blinded randomized clinical trial was conducted to comparatively evaluate the efficacy of injectable Vitamin C and Glutathione as a depigmenting agent in physiological gingival melanin hyperpigmentation. Twenty subjects (forty sites) with physiological gingival melanin hyperpigmentation were included in this study. Prior to the procedure baseline parameters were recorded namely DOPI Index, Gingival Biotype, VAS score. The area was anaesthetized by topical local anesthetic agent (Lidocaine 100g USP). After achieving adequate anesthesia, about 0.2ml of Vitamin C (500mg/ml) was administered at Site A and about 0.2ml of Glutathione (600mg/ml) was administered at Site B; using an insulin syringe. Subjects were recalled at 1month and 3 months for follow up and parameters were recorded.

Dummett-Gupta Oral Pigmentation Index

In this study, it was observed that on intragroup comparison of DOPI scores at Site A (Vitamin C) and Site B (Glutathione) were significantly reduced throughout the follow-ups. However, there was a more significant reduction in gingival pigmentation at the sites where Glutathione was injected. On the intergroup comparison between Site A and Site B, there was significant reduction in gingival pigmentation in both the groups from baseline to 3 months.

The results of present study were similar to that reported by Yussif NM, et al. (2016,2017)[44 45], Kadri KA, et al. (2021)[11], Mostafa D, et al. (2023).[47] They concluded that topical ascorbic acid is a novel, non-invasive dental technique that can effectively treat gingival hyperpigmentation. However, Mounika B (2018)[54] conducted a study on individuals having black gums using Laser in one group and Laser with 0.5% Glutathione gel as an adjunct in the other group. They concluded that during the 9-month follow-up, the depigmentation achieved using both the techniques was effective. Glutathione when used as an adjunct to Laser depigmentation resulted in delaying of repigmentation. Ezzat OM, et al. (2024)[58] comparatively evaluated injectable Glutathione versus Laser and found no significant improvement in the mean DOPI score with the injectable Glutathione as compared to Laser.

Gingival Biotype

In this study, gingival biotype was assessed by transgingival method using an endodontic reamer no: 20 with a rubber stopper under local anaesthesia. The gingival thickness was measured using digital vernier caliper.

On intragroup comparison, there was statistically highly significant improvement in gingival biotype seen at Site A and Site B between baseline, 1month and 3 months with higher values at 1 month and 3 months. The increase in gingival thickness was higher at Site A as compared to Site B. However on intergroup comparison between Site A and Site B, there was no statistically significant difference seen in gingival biotype at baseline, 1 month and 3 months.
The results of present study were in accordance with Yussif NM, et al. (2016,2017)44 45 Yussif NM et al. (2019)42 who stated that following a Vitamin C injection, the gingival thickness improved (going from a thin to a thick biotype). They noticed improvements in tissue color, shape. Kadri KA and Sanadi RM (2021)11 reported that injectable Vitamin C was considerably effective, economical and minimally invasive depigmentation technique, especially in individuals with a thin gingival biotype. However there has been no study conducted comparing the effect of Glutathione on gingival biotype; In present study we observed significant improvement in gingival biotype in Site B (Glutathione) at 1month and 3 months follow-up.

Visual Analogue Scale for Pain (VAS)

In the present study, On intragroup comparison for pain and discomfort experienced by the subjects in Site A and Site B , results showed a statistically significant mean decrease in pain and discomfort seen at follow-up of 1 month. On intergroup comparison there was a statistically highly significant reduction in pain and discomfort experienced by the subjects at baseline between the Site A and Site B, with more reduction in pain and discomfort in Site B. Chaudhary DS, et al. (2023)4 Haque AU, et al. (2024)48 Agrawal R, et al. (2024)49 stated that following a Vitamin C injection, there was a statistically significant reduction in visual analogue scale scores. The follow up time in the present study was 3 months which was the limitation of the study.

Thus, within the limitations of this study, it was observed that Glutathione was a predictable treatment option to use as depigmenting agent in the treatment of physiological gingival melanin hyperpigmentation.

CONCLUSION:

The primary natural pigment of gingiva, the most frequently pigmented tissue of the oral cavity, is melanin, which is produced by melanocytes in the basal and supra-basal cell layers. Vitamin C is potent antioxidant qualities and has ability to effectively depigment gingival and dermal tissues when given topically or intradermally. In the treatment of hyperpigmentation condition like melasma, Glutathione has demonstrated potential as a specific skin-whitening agent. Glutathione inhibits the tyrosinase enzyme, which is involved in the formation of melanin. Hence, this double blinded randomized clinical trial this randomized double-blind clinical trial was conducted to comparatively evaluate the efficacy of injectable Vitamin C and Glutathione as a depigmenting agent in physiological gingival melanin hyperpigmentation.

Twenty subjects (forty sites) with physiological gingival melanin hyperpigmentation were included in this study. Subjects were recalled at 1month and 3 months for follow up and parameters namely DOPI Index, Gingival Biotype, VAS score were recorded. There was a statistically significant improvement in all the clinical parameters from baseline to 3 months follow-up at both the sites, however, a highly significant improvement was seen at Site B (Glutathione).

Thus, within the scope of this study, it can be concluded that both Vitamin C and Glutathione were effective, while injectable Glutathione was more effective in reducing gingival pigmentation. However, Vitamin C was found to have beneficial effect on

improving gingival biotype. Long term clinical trials with a larger sample size are essential to evaluate the results of this study and establish the success of this approach.

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