A COMPARATIVE CLINICAL STUDY ON GINGIVAL DEPIGMENTATION TECHNIQUE USING SCALPEL AND DIODE LASER- A 6 MONTH STUDY

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BRANCH II

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CERTIFICATE

This is to certify that this dissertation titled "**A COMPARATIVE CLINICAL STUDY ON GINGIVAL DEPIGMENTATION TECHNIQUES USING SCALPEL AND DIODE LASER**' is a bonafide record of work done by **Dr. W. R. GNANASAGAR.** under my guidance during the study period of **2010-2013.**

This dissertation is submitted to **THE TAMILNADU Dr. MGR MEDICAL UNIVERSITY** in partial fulfillment for the degree of **MASTER OF DENTAL SURGERY, BRANCH II- PERIODONTOLOGY.** It has not been submitted (partial or full) for the award of any other degree or diploma.

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ABSTRACT

BACK GROUND:

This study was undertaken to compare the two different surgical techniques for Gingival depigmentation in 20 patients. And evaluated quantitatively and qualitatively the percentage of Regimentation in 6 months follow up period

MATERIALS AND METHODS:

Totally 20 systemically healthy patients aged between 18- 40 yrs consists of 18 males and 2 females were enrolled in this study patients were randomly divided in to two groups. Each group consist of 10 patients, Group A was treated with conventional scalpel technique and Group B with semiconductor diode laser (Biolase®) was used at 810nm wave length with contact mode. All the patients were recalled on 10 days, 30days, 60days, 90days, and 180days, post operatively. Standardized photographs were taken pre-operatively as well as post-operatively. Patients were evaluated for pain, discomfort and percentage of Repigmentation using image analysis soft ware J 1.42 q (National Institute of Health)

RESULTS:

There is a statistically significant difference in the scalpel group with ($p=0.005$) and there is no statistically significant difference in laser group

(p=0.20) pre and post-operatively. Statistically significant differences noted between scalpel group and laser group.

CONCLUSION:

The percentage of Repigmentation is less in scalpel technique when compared with diode laser technique and hence extending the longevity of the scalpel depigmentation procedure. But the laser technique showed ease of handling and patient comfort.

However drawback of this study is less number of subjects, shorter follow up period, Histological and Histochemical assessment of Melanocytes activity were not carried out.

KEYWORDS:

Gingival Pigmentation, Scalpel technique, Semiconductor diode laser, Image analysis, Percentage of Repigmentation, Visual Analogue Scale.

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INTRODUCTION

Esthetic dentistry has become an important branch of dentistry, and clinicians have to face the challenge of achieving acceptable Ginigival esthetics, along with addressing biological and functional problems to meet the needs of beauty conscious society. The color of the Gingiva plays an important role in overall esthetics.[39]

Facial esthetics involves the interaction of many elements of which the periodontium serves as a backdrop for the teeth which provides the environment for esthetic rehabilitation and Periodontal procedures are considered for the comprehensive esthetic treatment plan with at most importance for the esthetic concerns.

The gingival complex plays a distinct role in the overall beauty of the smile and it is the duty of the dental surgeon to create and maintain the gingival state with resultant beauty of the smile.Common esthetic gingival problems are Excessive Gingival display, Different colored, HyperPigmented gingiva, unequal gingival contours, loss of interdental papilla and exposure of root surface.

Pigmentation is the process of deposition of pigments in the tissues. Melanin is one of the four important pigments, which impart color to the normal skin and mucosa. Melanocytes are highly differentiated cells

responsible for production of melanin. These cells are situated in the basal layer of surface epithelium and oral mucosa. They are of neural crest origin and are only cells, which synthesize the pigment melanin, and are packed, in specialized organelles known as melanosomes. Under normal conditions it is not the numbers of melanocytes determine the degree of pigmentation but their levels of activity.Gingival hyperpigmentation is seen as a genetic trait in some population, and is more appropriately termed physiological or racial gingival pigmentation.[26]

Gingival hyperpigmentation usually does not present as a medical problem, but many patients may consider their black gums to be unaesthetic. This problem is aggravated in patients with a "gummy smile" or excessive gingival display while smiling.[61]

Due to demand for cosmetic therapy, removal of gingival melanin pigmentation by various methods were tried like gingivectomy, gingivectomy with free gingival autografts, electrosurgery, cryosurgery, application of chemical agents such as 90% phenol and 95% alcohol, abrasion with diamond bur, Nd:YAG laser,semiconductor diode laser,and CO2 laser have been used for this purpose.[27,74]These techniques have shown variable results and each technique has its own advantages and disadvantages.

But among these the Nd:YAG lasers and cryosurgical treatment technique achieved satisfactory results with no reported recurrences up to period of 11-20 month follow up period.[5]The drawback of this technique is use of sophisticated, costly equipments which are not commonly available making these techniques less popular[39].

Surgical depigmentation procedure a still popular technique with patients satisfaction and improved esthetics involving the removal of gingival epithelial layer along with the layer of underlying connective tissue allowing the denuded connective tissue to heal by secondary intention so the new epithelium formed is devoid of melanin pigmentation .

But the removal should be performed cautiously and the adjacent teeth should be protected since inappropriate technique may cause gingival recession and damage to underlying periosteum and bone.[57]

Lasers have been used in dentistry since the begining of the 1980s semiconductor diode laser has been used for gingivectomy, frenectomy, incisional and excisional biopsy, soft tissue tuberosity reduction,operculum removal, coagulation of graft donor site and exposure of soft tissue covering osseointegrated implants.[45]

Laser ablation for gingival depigmentation has been recognized as one of the most effective, pleasant, and reliable techniques.[46]

The diode laser is a solid-state semiconductor laser that typically uses a combination of Gallium (Ga), Arsenide (Ar), and other elements, such as Aluminum (Al) and Indium (In), to change electrical energy into light energy.[45]This 810-nm diode laser has energy and wavelength characteristics that specially target the soft tissues. It has an affinity for hemoglobin and melanin, therefore it is more efficient and better equipped to address deeper soft tissue problems.[48]

There is little information on the behavior of melanocytes after surgical injury. Spontaneous repigmentation has been shown to occur and the mechanism suggested is that the active melanocytes from the adjacent pigmented tissues migrate to treated areas .[56]

Large variation in time of repigmentation may be related to the technique used and the race of the patient. Repigmentation may also be attributed to the melanocytes which are left during surgery as these may become active and start synthesizing melanin.[33]

Etiology of pigmentation is elusive and as of now no definite or single etiological factor has been suggested for hyperpigmentation of gingiva and the condition poses a therapeutic challenge.

Many attempts have been made in the past to assure the cosmetic demand with elimination of dark pigmented patches.

Data regarding on depigmentation and Repigmentation following surgical removal of pigmented Gingiva in human is extremely limited. In order to find a better approach for gingival depigmentation, a comparative evaluation of two surgical techniques using conventional scalpel method and 810 nm wavelength (Biolase®) semiconductor diode laser was undertaken in this study.

AIMS AND OBJECTIVES

The aims and objectives of the present study was

- To evaluate quantitatively the percentage of Repigmentation post operatively after using scalpel and diode lasers for depigmentation.
- To compare statistically the treatment outcome of depigmentation after two different surgical techniques with a 6 month follow up period.

REVIEW OF LITERATURE

PIGMENTATION:

Pigmentation is the process of deposition of pigments in the tissues. Melanin is one of the four important pigments, which impart color to the normal skin and mucosa[16]. Melanocytes are highly differentiated cells responsible for production of melanin. These cells are situated in the basal layer of surface epithelium and oral mucosa. They are of neural crest origin and are only cells, which synthesize the pigment melanin, and are packed, in specialized organelles known as melanosomes[27]

CLASSIFICATION OF OROMUCOSAL PIGMENTATION[14]

Endogenous Pigmentation in Oral Mucosal Disease:

Pigment	Color	Disease process
Haemoglobin	blue, red, Purple.	Varix, haemangioma Kaposis sarcoma angiosarcoma, hereditary hemorrhagic telangiectasia.
Haemosiderin	Brown	Ecchymosis, petechiae, thrombosedvarix, haemorrhagic mucocele
Melanin	Brown, black	Melanotic macule, nevus, melanoma, basilar or grey melanosis with incontinence

Exogenous Pigmentation of Oral Mucosa[14]

Source	Color	disease process
Silver amalgam	Grey, black	Tattoo, iatrogenic trauma
Graphite	Grey, black	Tattoo, trauma
Lead, mercury, bismuth	Grey	Ingestion of paint and medicinals
Chromogenic bacteria	Black, brown, green	Superficial colonization

Clinical classification of Oral pigmentation[14]

Color	Focal	Diffuse	Multifocal
Blue/purple	VarixHaemangioma	Haemangioma	Kaposis sarcoma Hereditary haemorrhagic telangiectasia
Brown	Melanotic macule Nevus Melanoma	Ecchymosis Melanoma Drug induced Hairy tongue	Physiologic pigmentation Neurofibromatosis Hemochromatosis Lichen planus Addison's disease Drug induced Petechiae
Gray/black	Amalgam Graphite Nevus	Amalgam Melanoma Hairy tongue	Peutz-Jeghers syndrome Heavy metal ingestion

Dummett and Barens[24] 1971 in their review divided oromucosal pigmentation in following categories.

Local and ethnic pigmentations.

Oral pigmentary manifestations of systemic diseases.

Pigmentary disturbances associated with pharmaceutical and other chemicals.

Benign and malignant neoplasms of pigmentary origin.

Brocheriouet al[11]subdivides pigmented lesions as follows:

Tumoral pigmentations.

Non melanin pigmented tumors or tumor like lesions.

Benign melanin pigmented tumors.

Malignant melanomas.

Pigmentation of oral mucosa mainly composed of five primary pigments-

Melanin

Melanoid

Haemoglobin

Reduced haemoglobin

Carotene

Others-Bilirubin and iron

Melanin:

Melanin, a non Haemoglobin derived brown pigment, is the most common of the endogenous pigments.[56,67] Melanin is produced by the specialized pigment cells called

melanocytes, which are situated in the basal layer of the oral epithelium and the epidermis. These cells are derived embryologically from the neural crest ectoderm and enter the epithelium at about the eleventh week of development. There they divide and maintain themselves as a self-reproducing population. Melanocytes lack Desmosomes and Tonofilaments but possess long dendritic processes that extend between the keratinocytes, often passing through several layers of cells. Melanin pigment is synthesized within the melanocytes as small structures called Melanosomes. These are inoculated or injected into the cytoplasm of adjacent keratinocytes by the dendritic process of the melanocyte. Both lightly and darkly pigmented individuals have the same number of melanocytes in any given region of skin or oral mucosa. Color differences result from the relative activity of the melanocytes in producing melanin and from the rate at which Melanosomes are broken down in the keratinocytes. In individuals with very heavy melanin pigmentation, cells containing melanin may be seen in the connective tissue. These cells are probably macrophages that have taken up Melanosomes produced by Melanocytes in the epithelium and are sometimes termed melanophages.[36]

Melanins are usually classified into two main groups: the black and the brown eumelanins, which are in soluble and the yellow and reddish brown phaeomelanins, which are alkali soluble. Eumelanins arise by oxidative polymerization of 5,6-dihydroxyindoles; phaeomelanins are chemically distinct, in that they contain sulphur in addition to nitrogen and are formed from cystein-S-yl-dopas. Both eumelanins and phaeomelanins are derived from tyrosine. Tyrosine is oxidized to 3,4-dihydroxyphenylalanine (DOPA) by the copper containing enzyme tyrosinase, which also catalyses the future oxidation of dopaquinone. The number of melanocytes in the mucosa corresponds numerically to that of skin; however, in the mucosa their

activity is reduced. Various stimuli can result in an increased production of melanin at the level of mucosa including trauma, hormones, radiation, and medications.[36]

Melanoid

Granules of melanoid pigment are scattered in the stratum lucidum and stratum corneum of the skin. Initially it was assumed that melanoid was a degradation product of melanin, but more recently it has been shown that such a relationship is highly improbable. Melanoid imparts a clear yellow shade to the skin.[53]

Oxyhemoglobin and Reduced Hemoglobin

Oxyhemoglobin and reduced hemoglobin are pigments resulting fromhemosiderin deposits. The skin color is affected by the capillary and venous plexuses shining through the skin. [53]

Carotene:

Carotene is distributed in the lipids of the stratum corneum and stratum lucidum giving a deep yellow color to the skin. It is found in higher concentrations in more women than in men. Melanocytes

Melanocytes possess the metabolic machinery for the synthesis of melanin, which is a major determinant of skin color[65]. They are components of melanin pigmentary system, which when fully developed is made up of melanocytes distributed in various sites: the eye (retinal pigmentary epithelium, uveal tract); the ear (in the striavascularis); the central nervous system (in the leptomeninges); mucous membranes: the hair (in the hair matrix) and the skin (at the dermal epidermal interface) and occasionally in the dermis. They are located in the basal layer of the epithelium or epidermis and project their dendrites into the malphigian layer where they transfer melanosomes to keratinocytes[65].

Under normal conditions it is not the numbers of melanocytesin the skin that determine the degree of pigmentation but their levels of activity. Although there are regional variations inthe density of epidermal melanocytes, their numbers are consistent even in different skin types and ethnic groups[23]. Therefore, constitutive or basal skin pigmentation is considered to depend on the level of melanogenic activity and the transfer of melanin into the neighboring keratinocytes. The presence of centrioles suggests that oral mucosal melanocytes are self-replicating[7]. Using histochemistry it is suggested that oral mucosal melanocytes are present in unipolar, bipolar or multiple dendritic forms with marked variation in cell size and complexity of dendritic systems.

Work on epidermal melanocytes has suggested that actin microfilaments are needed for dendrite extension and microtubules are needed for maintenance of extension.

Mammalian melanin pigments have one of the two chemical compositions [40, 65]

1. Eumelanin, a brown pigment derived from the conversion of amino acid tyrosinase, to all the alkali-insoluble chromophore.
2. Phaeomelanin, a yellow reddish, alkali soluble pigment derived from tyrosine but in which one of the intermediates in the tyrosine-melanin pathway, combines with cysteine to form cysteinyldopa, this leads to yellow pigment phaeomelanin.

Conversion of tyrosine to DOPA and DOPA to dopaquinone is accomplished by an aerobic copper containing oxidizer, tyrosinase.

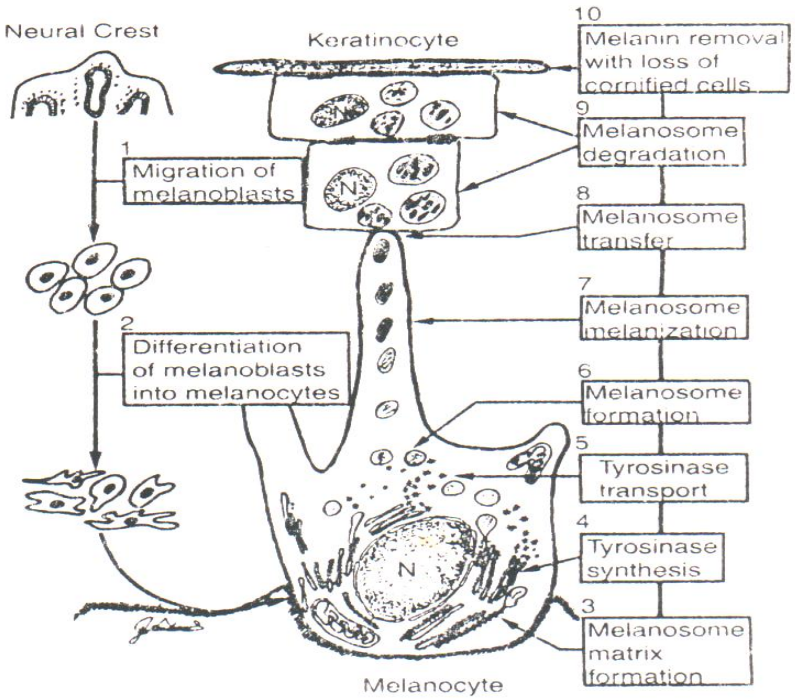
Zelickson AS and Harton JF[76] (1962) first studied the electron microscopic study of normal human non-keratinising oral mucosa and reported the appearance of melanocytes. Since then a number of studies were done to identify the appearance of melanocytes on the gingiva[7].

Becker SW (1967)[8] found gingival melanocytes to be regularly distributed and present at about every 15 basal keratinocytes spread, but do not break the desmosomes between which they permeate. Using histochemistry it has been found that oral mucosal melanocytes are present in unipolar, bipolar and multipolar dendritic forms[7].

Burchill SA, Thody AJ and Ito S[13] (1986) stated that the synthesis of melanin takes place in the melanosome. Melanosomes are highly organized organelles that contain melanin inside a unit membrane and deposit it on an internal filamentous/ microvesicular matrix. This intracellular membrane-coated organelle originates from the endoplasmic reticulum. During its development the melanosome acquires tyrosinase and the Tyrosinase-Related Proteins 1 and 2 (TRP1, TRP2). Tyrosinase is the rate-limiting enzyme for melanogenesis and catalyzes the conversion of L-tyrosine to Dopaquinone, which is required for the synthesis of both eumelanin and Pheomelanin pigments. It may also catalyze later steps specific to the eumelanin pathway and this could explain why eumelanogenesis is especially dependent on tyrosinase. Less is known about the control of pheomelanin synthesis, although it appears to be less dependent on tyrosinase and can proceed even when the levels of tyrosinase activity are virtually undetectable.

Scott GA (1991)[60] worked on epidermal melanocytes structure and suggested that actin microfilaments are needed for dendritic extensions and microtubules are needed for maintenance of extension. They considered that factors produced by keratinocytes might dictate melanocytes morphology to certain degree[7].

MECHANISM OF MELANIN SYNTHESIS:



Once melanin is produced the melanosomes are transferred into the neighboring keratinocytes (approximately 36). The size of these organelles and their numbers are important in determining pigmentation. This partnership of a melanocyte and a neighboring group of keratinocytes is called epidermal melanin unit[23]. The melanosomes in black skin are larger than their counter parts in white skin and are packed as single units rather than in groups. This has the effect of retarding their degradation in the keratinocytes and contributes to a higher level of skin pigmentation.

The ultra structure of the oral mucosal epithelium does not differ from the epidermal melanocytes. They contain a well-developed rough endoplasmic reticulum, mitochondria and Golgi complex, suggesting active protein synthesis, but lack tonofilaments and desmosomal connections to adjacent keratinocytes[7].

There is variation in the number of melanocytes between the individuals and it is different in different parts of the body within same individual. There are about 2000 or more epidermal melanocytes per square mm in the skin of head and forearm. The rest of the body has about 1000 melanocytes in all the races. Racial differences in pigmentation are not due to the differences in melanocyte number but because of the difference in the activity of the cells in the synthesis of melanin[65].

The melanocyte requires specific factors and nutrients to maintain growth. One of the important factors is Basic Fibroblast Growth Factor (bFGF). Other factors are Transforming Growth Factor Alpha (TGFα), Epidermal Growth Factor (EGF), Nerve Growth Factor (NGF), Platelet Derived Growth Factor (PDGF) and hormones like insulin, melanocyte stimulating hormone (MSH) and hydrocortisone[65].

Oral mucosal melanocytes, despite the absence of tonofilaments , anchoring filaments desmosomes remain in its basal location. This is possible because of the melanocyte cell adhesion molecules. Examples of these adhesion molecules are neural cell adhesion molecule (NAM), cadherin and substrate adhesion molecules (SAM). These adhesion molecules are crucial to maintain the normal physiological position of the melanocytes

Seiberg M, Paine C, SharlowE (2000) state that melanocyte dendriticity and contact with keratinocytes is likely to be essential for the transfer of melanin-containing melanosomes. A

recent study showed that activation of the Protease-Activated Receptor 2 (PAR-2), which is expressed only on keratinocytes, increases melanin transfer to keratinocytes.

Epidemiology:

Oral pigmentation occurs in all races of man.[56,67] There were no significant differences in oral pigmentation between males and females[17].The intensity and distribution of racial pigmentation of the oral mucosa is variable, not only between races, but also between different individuals of the same race and within different areas of the same mouth. Physiologic pigmentation is probably genetically determined, but as Dummett suggested, the degree of pigmentation is partially related to mechanical, chemical, and physical stimulation. In darker skinned people oral pigmentation increases, but there is no difference in the number of melanocytes between fair-skinned and dark skinned individuals. The variation is related to differences in the activity of melanocytes. There is some controversy about the relationship between age and oral pigmentation.

Giansanti et al 1971[32] reported a case of oromucosal pigmentation resulting from antimalarial therapy in a 63-year old patient. During the course of a routine oral examination, a slate-gray discoloration of palate was noticed. This appeared to be bilateral, sparing median raphe. Questioning revealed that the patient had lupus erythematosus for more than 13 years. During this time, she had been treated with antimalarial drugs like Arlen or Plquenil. No additional knowledge regarding the nature of the pigments was achieved.

Westbury and Najera 1997[71] reported 5 cases of minocycline induced intra oral pigmentation. Intra orally the pigmentation included "green" roots of erupted teeth,"black" roots of extracted teeth and all the cases had "black" alveolar bone. They reported the incidence of

minocycline staining of alveolar bone is probably 2% of that population taking the drug for 2 months or longer.

La Porta et al 2005[39] reported a 45 years old, Caucasian female presenting with pigmentation of gingiva, lips and nail bed. Past medical history revealed initiation of minocycline therapy 6 months earlier. Histopathologic examination of biopsy specimen of gingiva showed increased evidence of melanocytes in the epithelium and melanophages in the connective tissue. 9 months after cessation of therapy the patient exhibited marked reduction in pigmentation.

Takashi Hanioka et al 2005[65]In his study on relationship between melanin pigmentation in the gingiva of children and passive smoking performed on 59 nonsmoking children selected from patient records of a dental clinic in a rural town in Japan concluded that excessive pigmentation in the gingiva of children was associated with passive smoking.

Clinical Characteristics:

The color of the healthy gingiva is variable, ranging from pale pink to deep, bluish purple. Between these extremes are many color variations which are dependent upon the intensity of melanogenesis, the degree of epithelial cornification, the depth of epithelialisation and the arrangement of gingival vascula.[20]The gingiva are the most frequently pigmented intraoral tissues.[15]Microscopically, melanoblasts are normally present in the basal layers of the laminapropria.[12] The most common location is the attached gingiva (27.5%) followed in decreasing order by the papillary gingiva, the marginal gingiva, and the alveolar mucosa.[36]The total number of melanophores in the attached gingiva was approximately 16 times greater than in the free gingiva.[55] The prevalence of gingival pigmentation was higher on the labial part of the

gingiva than on the buccal and palatal/lingual parts of the arches.[36] The shade of pigment was classified as very dark brown to black, brown, light brown yellow.[53]

Differential Diagnosis[19]

Oral pigmentation has been associated with a variety of lesions and conditions.Differential diagnosis of oral mucous membrane pigmentations are made according to thefollowing situations:

A. **Localized Pigmentations:** Amalgam tattoo, graphite or other tattoos, nevus, melanotic macules, melanoacanthoma, malignant melanoma, Kaposi's sarcoma,epithelioid oligomatosis, verruciform xanthoma.

B. **Multiple or Generalized Pigmentations**

1. **Genetics:** Idiopathic melanin pigmentation (racial or physiologic pigmentation),Peutz-Jegher's syndrome, Laugier-Hunziker syndrome, complex of myxozomas, spotty pigmentation, endocrine overactivity, Carney syndrome, Leopard syndrome, andlentiginosis profuse.

2. **Drugs: A**nti-malarial, antimicrobials, Minocycline, Amiodarone, Clorpromazine,ACTH, Zidovudine, Ketoconazole, methyldopa, busulphan, menthol, contraceptive pills,and heavy metals exposure (gold, bismuth, mercury, silver, lead, copper).

3. **Endocrine:** Addison's disease, Albright's syndrome, Acanthosisnigricans, pregnancy, hyperthyroidism.

4. **Post inflammatory:** Periodontal disease, postsurgical gingival repigmentation.

5. **Others:** Haemochromatosis, generalized neurofibromatosis, incontinentipigmenti,Whipple's disease, Wilson's disease, Gaucher's disease, HIV disease, thalassemia,
pigmented gingival cyst, and nutritional deficiencies.

Management of Gingival Pigmentation

Gingival depigmentation is a periodontal plastic surgical procedure whereby the gingival hyperpigmentation is removed or reduced by various techniques.[50]

Chemical Agents

Hirschfeld&Hirschfeld in 1951[40], used a mixture of phenol (90%) and alcohol (95%)to burn out pigmented gingiva. The growth of new gingiva was prolonged, and repigmentation soon developed in three patients and the rest with the same results in short while. These substances caused tissue necrosis in addition to pain. The treatment was not acceptable to the clinician or the patient.

Gingivectomy Procedure

Dummett and Bolden 1963[25] used gingivectomy to remove pigmented gingiva .Incisions were made so as to remove as much as clinically pigmented tissue as possible and surgical pack placed. They concluded that gingival resective procedures, if performed solely for cosmetic reasons, offer no permanent results. This procedure resulted with prolonged healing by secondary intention, excessive pain and discomfort caused by exposure caused by underlining bone.

De-epithelialization Techniques

The procedure essentially involves surgical removal of gingival epithelium along with a layer of the underlying connective tissue and allowing the denuded connective tissue to heal by secondary intention. The new epithelium that forms is devoid of melanin pigmentation.[50]

Ginwalla et al 1966[33] attempted to remove gingival pigmentation surgically in 6 Indian males. Three different techniques were employed; these were slicing, bone denudation and abrasion. The areas subjected to complete denudation showed no reappearance after 6 months of observation, 50% cases of slicing and abrasion techniques allowed a mild degree of repigmentation after 24 to 56 days. Scraping technique to remove heavy continuous band of gingival pigmentation was performed by **Manchandia1979 .**He observed repigmentation in the form of spots in 42% of the subjects.

A split thickness flap was reflected covering the span of teeth included in the smile window, in this case from 1st premolar to 1st premolar. The cervico-apical height of the flap depends on the extent of pigmented layer. After a vertical incision is given at the extreme end of the span, the flap is carefully peeled away to leave behind the raw denuded surface of the gingiva. It is the mucosal layer which contains the pigmented layer and after healing by secondary intention the gingiva displays a good uniform layer of depigmented gingiva.

Perlmutter and Haim Tal 1986[56] Conducted a study where pigmented keratinized gingiva was removed in two Jewish Yemanite adult males. After surgery, the exposed lamina propria was covered by a periodontal pack for 7 to 10 days. The tissues were then observed periodically for signs of repigmentation. Healing was uneventful and the surgically treated areas in both patients remained depigmented over the first 2 years .After 32 months, some pigmentation was found in one of the patients, and with the exception of two limited sites, the area was completely repigmented after 7 years. The surgically treated area in the second patient remained depigmented over an 8- year followup period. They agreed with previous reports that the gingival repigmentation can occurs spontaneously and suggested that further controlled studies to be undertaken to explore the biologic basis for repigmentation.

Farnoosh A.A 1990[28] used a high speed hand piece and surgical diamond bur to eliminate dark pigmentation in 20 patients. Slight repigmentation was observed in 2 cases after 20 months post surgical follow up. He concluded that since this technique is relatively simple, versatile and requires minimum time and effort, if repigmentation occurs, the procedure can be repeated in the same area without limitation or causing any permanent damage.

Pal T.K et al 1994[54] used the fissure bur at slow speed to eradicate melanin pigmentation from the gingiva, and authors reported that out of 54 cases, 10 cases, (18.51 %) showed repigmentation at end of 90 days and the rest 44 cases (81.48%) remained depigmented during the follow up period of 90 days.

Jaya prasadK 1998[42]described de-epithelialization technique by using a no 15 B.P. Blade and also used diamond bur with a high-speed hand piece. He concluded that the technique was very simple and did not require sophisticated and expensive armamentarium and that if repigmentation occurred later, the procedure could be repeated on the same area.

Almas K et al 2002[3] used scalpel surgical technique for depigmentation of gingiva. His study showed that after surgical epithelial excision technique, healing was uneventful ,patient's acceptance of the procedure was good and the results were excellent. There was no sign of repigmentation upto 6months.

Nandakumar K et al 2005[50] performed periodontal plastic surgery combining gingival depigmentation and esthetic crown lengthening in a single appointment using scalpel surgical technique. Crown lengthening by external bevel gingivectomy was completed initially. The gingivectomy was followed by the depigmentation procedure. Using a number 11 scalpel blade, the entire pigmented epithelium along with a thin layer of connective tissue (split thickness flap) was removed. This incision was carried out from the apical level of external bevel incision to the

apical end of the attached gingiva(mucogingival junction) up to where the pigmentation extended. The area healed well after two weeks. A one-year follow up period did not demonstrate any tendency towards repigmentation of the gingiva.

Cryosurgery:

Tal H. et al 1987[66] conducted a study to test the effectiveness of cryosurgical destruction of gingival epithelium in the removal of gingival melanin pigmentation. A gas expansion cryoprobe, cooled to - 81°C was applied to the gingiva for 10 seconds. The treated gingiva remained depigmented during the follow up period of 20 months. He concluded that cryosurgery may prove to be the treatment of choice when gingival depigmentation is indicated.

Tal H. et al 1987[66] did a study based on 2 to 5 years clinical observation after superficial cryosurgical treatment in seven nonsmoking patients. The site was exposed to a gas expansion cryoprobe cooled to – 810C for 10 seconds. Follow up observations during the first three years after treatment did not show significant change in the 5 patients. In two patients limited areas (3x5mm and 4x4mm) were slightly darker than the surrounding gingiva, but with out any cosmetic significance.

YehC.J.et al 1998[72]Direct application of liquid nitrogen with a cotton swab for 20 to 30 seconds was carried out in twenty patients with dark gingiva and were followed up to 2years. No repigmentation was observed during the follow up period. It was concluded that this was a simple, bloodless procedure for depigmentation of the gingiva requiring no local anesthesia or sophisticated equipment.

Darbandi A. et al 2004[1] in this study, ten patients who had oral mucosal physiologic pigmentation were selected and a questionnaire was filled out for every one of them. The location and extent of every lesion was determined and local anesthesia was obtained by supra

periosteal infiltration. By applying nitrogen oxide (with the temperature of - 89.5°C)and using a suitable probe of equal size of lesions, they were frozen for 20-30 seconds. Then patient were examined on the 2nd and 7th day, 2nd and 4th week post-operatively. After four weeks all pigmented parts were cured and no recurrent lesion was observed in any of patients. They concluded that oral cavity is an ideal environment for cryotherapy and it can be used as an effective method for treating oral pigmentation and some other oral lesions.

Free gingival autograft

Severe physiologic gingival pigmentation was treated by **Tamizi et al in 1996**[67] with an unpigmented free gingival autograft in 10 patients. In all 10 areas in which recipient site received full thickness bed preparation, no evidence of repigmentation was found after4.5 years. Of the 10 areas that received partial thickness bed preparation, only one exhibited repigmentation (after 1 year) and the authors suggested, the use of free gingiva lgraft on denuded bone for the treatment of esthetic problem in patients suffering from severe gingival melanin pigmentation.However this technique requires an additional surgical site and color matching.Furthermore, the presence of a demarcated line commonly observed around the graft in the recipient site may itself pose an esthetic problem.

Novaes AB Jr et al 2002[51] used acellular dermal matrix allograft for the elimination of gingival melanin pigmentation as aesthetic treatment of bilateral gingival melanin pigmentation.

Laser Irradiation:

Lasers were first introduced in 1960 by Maiman and were brought in to general practice by Dr william and Terry myers. although co_2 lasers are used for depigmentation procedure, they can damage tooth structure and the delivery system is very cumbersome. since co_2 laser are used in non contact mode they can also cause loss of tactile sense also , diode is an excellent soft

tissue laser and it is indicated for cutting and coagulating gingival tissue[19] wavelengths of diode laser are highly absorbed by pigmented tissue .lasers have been used in dentistry since the begining of the 1980s.two different lasers such as carbon dioxide laser Nd:YAG laser , semi conductor diode laser, argon laser Er:YAG laser and Er,Cr:YSGG laser have been reported as effective, pleasant and reliable method with minimal post operative discomfort and faster wound healing for depigmentation procedure3 the diode laser is a solid state semiconductor laser that typically uses a combination of gallium(GA) Arsenide (Ar) and other elements, such as aluminum(Al) and indium(In), to change electrical energy in to light energy(4) dental laser energy has an affinity for different tissue components. the 810 nm diode laser has energy and wave length characteristics that specially target the soft tissue. it has an affinity for heamoglobin and melanin therefore it is efficient and better equipped to address deeper soft tissue problem(5) tissue penetration of diode laser is less than that of Nd:YAG laser while the rate of heat generation is higher. the advantages of diode lasers are the smaller size of the units as well as the lower financial costs. diode laser did not produce any deleterious effect on root surface thus it is generally considered that diode laser surgery can be performed safely in close proximity to dental hard tissue [4]

Trelles et al 1993[68] treated melanotic spots in the gingiva with monoline 514 nm greenlight (1.5W, 300 milliseconds, O.5mm spot size) produced by an argon laser. The results reported that the restoration of the mucosa was optimal with excellent esthetic results.

Sharon E et al 2000[62] conducted a study to know the efficacy of carbon-dioxide (Co_2)laser vaporization in ablating gingiva, oral mucosal and cutaneous melanin in dogs. 3dogs with pigmentation of the oral mucosa, gingiva and skin were recruited for the study. The procedure was performed by using 3W continuous-wave carbon-dioxide laser. Clinical and histologic

examination showed carbon-dioxide laser to be effective in eliminating pigmented areas. No recurrence of melanin was detected in either the oral mucosa, or gingiva during the follow up period of 11 weeks. In the skin a small amount of melanin repigmentation was noticeable. It was concluded that carbon-dioxide laser surgery proved an effective tool for obliterating superficial melanin discoloration and It also suggested that, to prevent recurrence of the pigmentation the area must be cleared completely of melanin, directing the laser beam carefully along the visible margin of the area.

Atsawasuwan et al 2000[6] reported the use of Nd: Y AG laser for gingival depigmentation in 4 cases. The Nd : Y AG laser was set at 6 Watts, 60 mill joules per pulse, and 100 pulses per second. They found no recurrence of pigmentation during the follow up period of 11 to 13 months. The authors concluded that Nd: Y AG laser had)shown to be a good option for gingival depigmentation and caution must be exercised indelicate areas such as marginal gingiva while using Nd: YAG laser.

Eldad Sharon etal 2000 tested the efficacy of carbon dioxide(co_2) laser vaporization in ablating gingival, oral mucosal. and cutaneous melanin in dogs. Clinical and histologic examination showed the CO_2 laser to be effective in eliminating the pigmented areas in all tissues treated. No recurrence of melanin was detected in either the oral mucosa or gingiva at any of the follow-up times. in skin a small amount of melanin repigmentation was noticeable and he concluded that CO2 laser surgery proved an effective tool for obliterating superficial melanin discoloration.

To prevent recurrence of the pigmentation, the area must be cleared completely of melanin, directing the laser beam carefully along the visible margins of the area.

Haim Tal 2003[38] in this study 10 patients who requested cosmetic therapy for melanin pigmented gums. Treatment was carried out using an erbium:YAG laser. The laser beam was set at 500 mJ/10 pulses/second. The "brush" technique was applied until the gingival surface appeared clinically free of pigmentation. Patients were observed for 6 months. He concluded that depigmentation of gingival melanin pigmentation by erbium:YAG laser radiation in a defocused mode was a safe and effective procedure. The esthetic results were pleasing and healing was uneventful.

EminEsen, 2004[27]used super pulse mode CO_2 laser for gingival depigmentation and evaluated for clinical parameters like post operative bleeding, pain and rate of repigmentation clinically in a 24 month follow up study in 10 patients and he concluded Two cases of partial repigmentation were observed during 24-month follow-up. And Application of the super pulse mode of CO2 laser appears to be an effective and safe method for the elimination of gingival melanin pigmentation.

Berk, k et al 2005 [10] used Er,Cr:YSGG hydrokinetic system laser set at 20 Hz, 1.75 W to 1.5W, with 20% to 40% air and 12% to 5% water spray for removal of pigmented gingiva in 2 patients. The pigmented areas were treated in noncontact mode, and both cases were completed during one appointment. Both cases were performed without any anesthesia, no intra-operative or postoperative pain or discomfort appeared. After 24 hrs, the lased gingiva was partly covered with a thin layer of fibrin, which exfoliated during the first week following treatment. The ablated wound healed almost completely in 1week. The results pointed out that YSGG laser is a good and safe choice for removal of pigmented gingiva without local anesthesia. The postoperative period is comfortable for the patient and healing is fast and good. No repigmentation occurred in either patient after 6 months .**GBerketal 2005** Treatment of gingival

hyperpigmentation by Er,Cr:YSGG laser radiation in a defocused mode was found to be a safe and effective procedure. post operative patient satisfaction in terms of esthetics and pain was excellent the gingiva healed unevenfully and completely regenerated with no infection, pain, swelling or scarring. No repigmentation occured in either patient after 6 month follow up

Daniel Simo˜es A. Rosa,2007 used Er:YAG laser for gingival depigmentation procedure and Clinical parameters, such as bleeding, swelling, redness, and healing, were evaluated immediately after the surgery and 24 hours, 1 and 4 weeks, and 3 months later in five patients and he concluded Removal of gingival melanin pigmentation can be performed safely by Er:YAG laser resulting in an esthetically significant improvement of gingival discoloration

Manal M. Azzeh 2007[46]used Er:YAG laser for the treatment of gingival hyperpigmentation in 6 patients and evaluated for the clinical parameters like post operative bleeding, pain, wound healing, discomfort and the rate of repigmentation for the follow up period of 18 months. And he concluded that depigmentation of melanin hyperpigmented gingiva by the Er:YAG laser is a reliable and satisfactory procedure. Esthetic results were satisfactory for patients and the operator,and no repigmentation was found during the followup period.

sushmaLagdive 2009[45] in a split mouth design study used a semiconductor diode laser for gingival depigmentation and scalpel surgical technique for gingival depigmentation and compared the effectiveness of the two surgical procedures and clinical parameters like post operative bleeding, pain, wound healing and difficulty of procedure was evaluated in 3 patients for a 3 week follow up period and concluded that the application of diode laser appears to be safe and effective alternative procedure for the treatment of gingival melanin pigmentation

Hyuk-jinko 2010 used Nd: YAG laser for gingival depigmentation procedure and compared with high speed rotator diamond bur abrasion technique in three patients and evaluated

clinical colour changes after four weeks and he concluded that The Nd:YAG laser and the high speed rotary instruments seem to be effective for the esthetic treatment of gingival melanin hyperpigmentation.

Vishal singh 2012[70] used semiconductor diode laser for gingival depigmentation procedure and compared with carbon tetrafluroethylene cryosurgical depigmentation technique in 10 patients and he concluded during the 18-month follow-up, the depigmentation achieved using both the techniques was found equivalent and satisfactory

Different Techniques Employed for Gingival Depigmentation

Methods Aimed at Removing the Pigment Layer and Complications:

A. Abrasion technique: using a large, round diamond bur

Difficult to control depth of deepithelialization, bleeding, and pain

B. Surgical methods of depigmentation

1. Scalpel surgical technique (gingivectomy)- Excessive bleeding, pain,

2. Cryosurgery- Needs high skills

3. Electrosurgery- Need high skills, Eventful healing

C. Chemical methods of depigmentation-Harmful to oral soft tissues

D. Lasers

1. Carbon dioxide (CO2) laser- Delayed wound healing

2. Diode lasers- Deep thermal damage

3.Nd:YAG lasers-Deep thermal damage

4.Er:YAG lasers- Deep penetration

5. Er,Cr:YSGG lasers- Deep penetration

Methods Aimed at Masking the Pigmented Gingiva With Grafts From Less-Pigmented

E. Free gingival graft (FGG)- Two-site procedure, pain,bleeding, and color difference

F. Subepithelial connective tissue graft- Two-site procedure, pain, and bleeding

G. Acellular dermal matrix allografts -Pain, bleeding, and color difference

REPIGMENTATION:

oral repigmentation refers to clinical reappearance of melanin pigment after a period of clinical depigmentation of the oral mucosa[1] in this study the pigmentation started to reappear after 3 months and during the 6 month follow up period, the patchy pigmentation could be a result of the ongoing process of repigmentation. the decreased intensity of pigmentation may be due to less production of pigments. the intensity may increase with time and may reach to a pretreatment level as it depend on the racial background of the patient Kon et al demonstrated that permanent results cannot be offered when gingival depigmentation procedures are performed for cosmetic reasons[44]. Dummet and Bolden[25] observed partial recurrence of hyper pigmentation in 6 out of 8 patients after gingivectomy at 1 to 4 months.

Recurrence of pigmentation in gingiva is a nagging problem. The results of depigmentation are visually so appealing that the patient and the clinician can get very euphoric. The recurrence parameters like intensity, area of pigmentation, time elapsed before re-pigmentation is all to be considered, so that a predictable line of treatment can be encountered.[28]Oral repigmentation refers to the clinical reappearance of melanin pigment following a period of clinical depigmentation of oral mucosa as the result of chemical thermal, surgical, pharmacological or idiopathic factors.[15] The exact mechanism of skin repigmentation is unclear, but the "migration theory" seems to be favored. According to his theory active melanocyctes from normal skin and hair matrix proliferate and migrate into the

depigmented areas, therefore the active melanocycte from the adjacent pigmented, tissue migrate to treated areas causing failure.[56]Post surgical Repigmentation of gingiva has been reported in six out of nine cases. following gingivectomy. Repigmentation occurred in 3 cases as early as 33 days after surgical removal. In the remaining 6 specimens, the time of initial repigmentation varied up to 120 days postsurgically[25].

Perlmutter S. et al 1986[56] conducted a study on Repigmentation of the gingival following surgical injury. Two patients who had moderate to heavily pigmented gingival were treated. Surgically treated areas in both patients remained depigmented over the first 2 years. After 32 months some pigmentation was found in one of the patients and with the exception of two limited sites. They found different degree of Repigmentation after 7 years. The other subject revealed no repigmentation over an 8 years follow up period. The authors concluded that these observations agreed with previous reports that described gingival pigmentation as spontaneous and suggested that further controlled experimental studies be undertaken to explore the biologic basic for repigmentation.

MATERIALS AND METHODS

A total of 20 systemically healthy patients, within the age group of 18-40 years with an esthetic complaint of hyper pigmented gingiva were enrolled in this study from the outpatient department of periodontics of Ragas Dental College. The sample population had 18 males and 2 females. The patients were briefed about the surgical procedure. After obtaining the informed consent they were randomly divided in to 2 groups.

Group A -10 patients - Depigmentation with Scalpel technique.

Group B -10 patients - Depigmentation with Diode lasers.(Biolase®)

INCLUSION CRITERIA:

1.Patients of age group 18-40 years.

2.Both male and female patients.

3.Patients with physiological melanotic pigmented gingiva in relation to maxillary anterior region 13-23.

4.Patient with esthetic concern.

5.Patient with thick gingival phenotype and healthy gingiva.

6. Patient with good oral hygiene.

EXCLUSION CRITERIA:

1.Patient under medication.

2.Chronic Smokers.

3.Systemically compromised patients.

4.Pathological pigmentation other than physiological pigmentation.

5. Pregnant and lactating women.

6. Apprehensive patients

7. Patients with history of periodontal treatment for past 6 months.

8. Patient with history of post surgical keloid.

METHODOLOGY:

The patients who consented for the study underwent thorough oral prophylaxis procedure and oral hygienic instructions were given.

DEPIGMENTATION WITH SCALPEL TECHNIQUE:

After obtaining informed patient consent under local anesthesia with 1:80,000 adrenaline.Pigmented epithelium was excised with split thickness flap using no 15 blade. Care is taken to include the epithelium at the tip of interdental papilla and the mucogingival junction on the other end without disturbing the marginal gingiva. Periodontal pack was placed and post operative instructions were given. Patients were recalled after 10 days, 30days,60days,90days and 180days intervals for assessment.

DEPIGMENTATION WITH DIODE LASER TECHNIQUE:

After obtaining informed patient consent, Depigmentation procedure was done with topical application of local anesthetic gel. Diode laser (BIOLASE®) with Settings of 810 nm 60J/sec in pulsed mode was delivered through a 400 micron fiber optic tip. The pigmented gingival epithelium wasablated using direct contact mode in painting stokes.Care was taken to

include the epithelium at the tip of interdental papilla and the muco gingival junction on the other endwithout disturbing the marginal gingiva. Post operative instructions were given and recalled after 10 days, 30days, 60days, 90days and180 days intervals for assessment. Laser safety protocols were followed during the lasing process.

POST SURGICAL CARE:

Patients were instructed to continue with good oral hygiene and avoid trauma around the surgical site. Patients were prescribed with Paracetamol 500mg TD to be taken incase of pain only and 0.2% chlorhexidine digluconate rinse twice daily for 2 weeks. Periodontal dressing was removed at the end of 10days. And also were put on gentle and soft brushing after pack removal for a week. Patients were recalled at the end of 10 days, 30days, 60days, 90 days and 180 days postoperatively for monitoring and reinforcement of oral hygiene.

.**CLINICAL PARAMETERS ASSESSED:**

A)Oral Hygiene Index:

Patient oral hygiene index scores were recorded before surgical procedure and then thorough oral prophylaxis was done.(ANNEXURE-I)

B**)Bleeding On Probing**:

Full mouth bleeding scores were assessed before the surgical procedure (ANNEXURE-I)

C**) Gingival phenotype:**

The gingival phenotype was assessed by probing the gingival sulcus with Williams probe.The probe transparency test (TRAN technique) was used.The gingival biotype was considered thin when the outline of the periodontal probe showed through the gingival margin from inside the sulcus.The biotype was considered thick if the probe did not show through the gingival margin. [13]

D) **Distribution Of Gingival Pigmetation**:

The pigmentation distribution was clinically notedafter isolating the gingiva with cotton rolls as.

1. Marginal
2. Attached
3. Papillary
4. Diffuse

E) **Pattern Of Distribution:**

The pigmentation pattern of distribution was clinically noted after isolating the gingiva with cotton rolls as.

1. Generalized
2. Patchy

F**) Assessment of Pigmentation:**

The intensity of pigmentation was assessed using Digital image acquisition and Image analysis software. After isolating the gingiva with

cotton rolls digitalized images were recorded with NIKON-D 100 digital SLR camera and ring flash with the following settings at a pre determined distance of 37 cms as indicated by the camera. Pre and post operative photographs were taken at 10days, 30 days, 60days, 90days, and 180days interval. A total of 6 photographs were taken for each patient.

1. Light intensity – auto sensory and auto light intensity adjusted.
2. Shutter speed-1/250.
3. Focal length-37 cms.
4. Depth of focus-1: 2.8D.
5. Lens-macro lens. (AF macro Nikkor lens 105mm)
6. Aperture ring -36 diaphragm.

Image analysis:

The digitalized images were assessed for the intensity at three randomly selected sites from the image. The image was scaled using **Adobe photoshop cs7** once the images were scaled down to 6 megapixels resolution the intensity was measured using **Image J 1.42q software (National Institutes Of Health)**.

G)Pain and discomfort assessment following surgery:

Pain intensity was assessed using a visual analogue scale where the patients wereasked to record and mark the severity of pain based on visual analogue scale along with numbers assigned to each level as 0 to 4 respectively where 0 indicates no pain and 4 indicates severe pain. Pain

intensity ofthe patient was recorded 24 hours post surgically in the questionnaire proforrma (ANNEXURE-I)

H**) Post surgical Bleeding Assessment:**

Post operative Bleeding was assessed based on the questionnaire form which was given to the patient during the day of procedure in which patient has to record as yes or no. (ANNEXURE-I)

I) **Difficulty of the procedure Assessment:**

Difficulty of the procedure was assessed by examiner who performs the procedure on 1st day of the treatment based on the time taken for the surgical technique as easy and difficult.

J) **Assessment of patient satisfaction:**

Patient satisfaction was assessed byusing a visual analogue scale where the patients wereasked to record in a scale of -2 to+2 where -2represent extremely unhappy and +2 represent extremely happy about the outcome.

K) Assessment of Percentage of Repigmentation:

Percentage of Repigmentation was assessed during the follow up appointments, amount of the melanin pigmentation of the gingiva was assessed using the digital photograph which was taken preoperatively as well as on all post operative visits. The intensity value is measured by using image-J 1.42Qsoft ware.[2] and expressed in mean grey scale values of 0-

255.Were 0 represents black in the colour spectrum and 255 represents white in the colour spectrum.

L)Statistical Analysis:

All the data's of 10 patients treated by scalpel method and 10 patients with laser application were analyzed statistically with Wilcoxon signed rank test and Mann whiteny U- test for significance. P value less than 0.05 was considered to be significant.

SUMMARY AND CONCLUSION

The present study was undertaken to compare the efficacy of two different surgical techniques for the depigmentation procedure, split thickness excision technique with a surgical scalpel blade as against semiconductor diode laser.In thisstudy totally 20 patients aged between 18yrs- 40 yrs were enrolled (18 males and 2 female patients) who were esthetically conscious of their hyperpigmented gums and requested treatment for the same were recruited for the study. They were randomly divided in to two groups, each group comprising of 10 patients. Group A of patients were treated with conventional scalpel blade and other Group B with laser surgery using semiconductor diode laser (Biolase®)and recalled on 10 days ,30 days, 60 days, and 180 days, intervals.

For all the patients pre-operative and post operative standardized photographs were taken at each interval period and all the patients were evaluated qualitatively for post operative bleeding , pain wound healing, discomfort and ease of the procedure was evaluated by the operator . Quantitatively for the percentage of Repigmentation and colour intensity was evaluated using a image analysis software pre and post-operatively. Statistical analysis was done using Wilcoxon Signed rank test and Mann whitney U test.

The results showed that there is an statistically significant difference in the scalpel group comparing pre and post operatively with (p=0.005) and there is no statistically significant difference (P=0.20) in the diode laser group . In

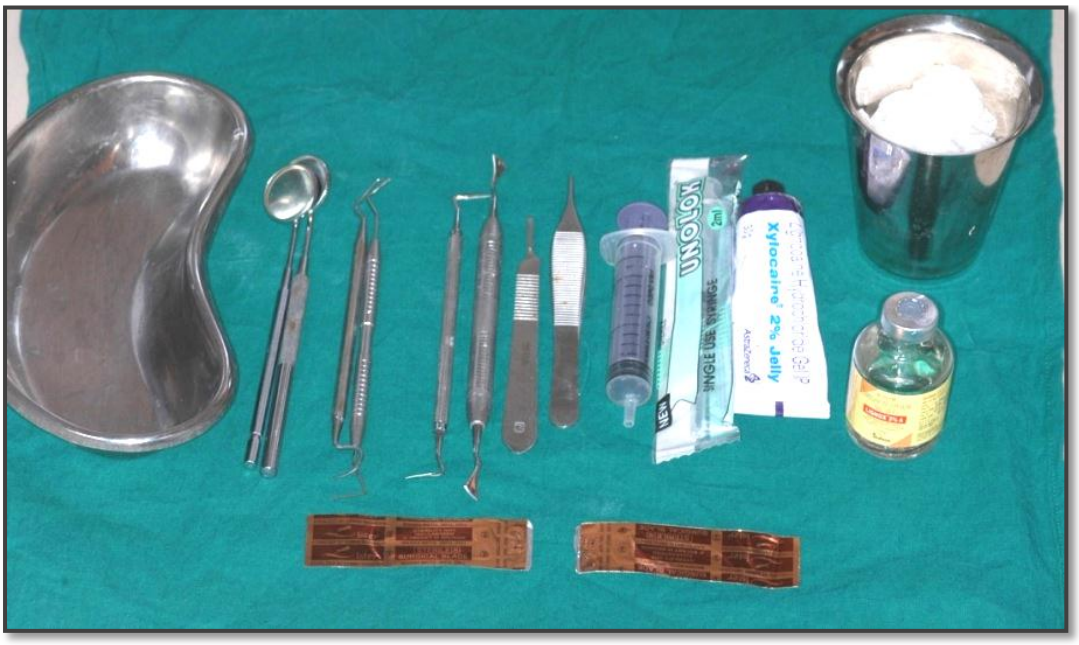
the diode laser settings which was used for the surgical procedure may not be suitable for the study population and the tissue penetration of laser beam also minimal which is not sufficient to clear all melanocytes from the surgical site, Also there is an statistically significant difference between scalpel and laser group post operatively.

The percentage of Repigmentation is also slow in scalpel technique when compared with diode laser technique and hence extending the longevity of the depigmentation procedure. But the laser technique showed ease of handling and patient comfort.

The study conducted would be more validated if Histochemical assessment for the Melanocyte activity and a longer follow up period was carried with increased sample size.

ARMAMENTARIUM

SCALPEL TECHNIQUE



NO.15 BRAD PARKER BLADE WITH HANDLE

KIRKLAND KNIFE

ORBANS KNIFE

MOUTH MIRRORS AND PROBES

STERILE GAUZE PIECES

SURGICAL SCISSOR

TISSUE HOLDING TWEEZERS

LOCAL ANESTHETIC SOLUTION WITH 1:80,000 ADRENALINE - LIGNOX 2% (WARREN)

LOCAL ANESTHETIC GEL-XYLOCAINE R 2% JELLY (ASTRA ZENECA)

SALINE-10ml

PERIO PACK (COE PACK-GC AMERICA)

LASER TECHNIQUE

SEMICONDUCTOR DIODE LASER 810 nm -BIOLASE® 7 W WITH 60J/ SEC.

400 MICRON FIBEROPTIC TIP

PROTECTIVE EYEWEAR GLASSES

TOPICAL ANESTHETIC GEL-XYLOCAINE-2% JELLY (ASTRA ZENECA)

PLASTIC SUCTION TIP

PLASTIC CHEEK RETRACTOR.

SALINE -10 ml

FIGURE -I SCALPEL TECHNIQUE

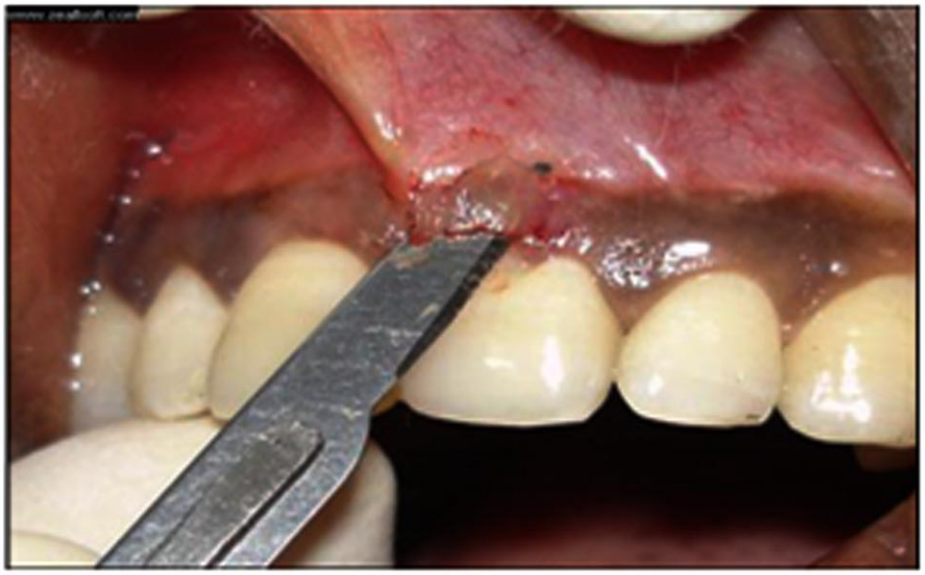
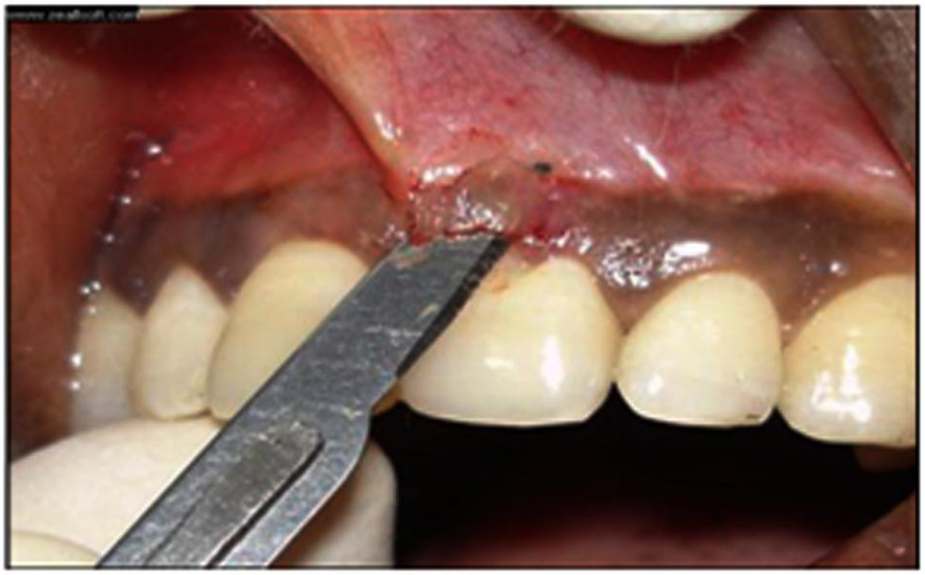
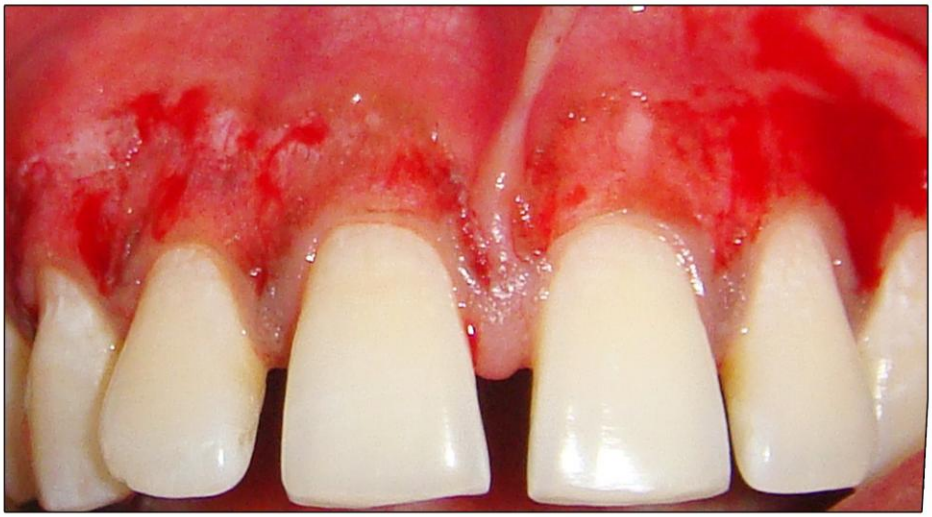


FIGURE -I SCALPEL TECHNIQUE



IMMEDIATE POST OPERATIVE



PERIO PACK PLACED

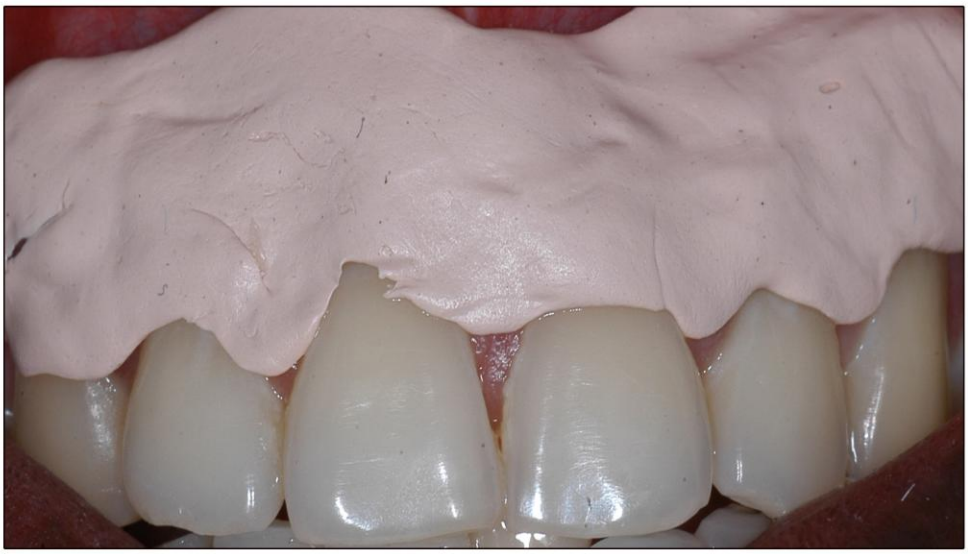
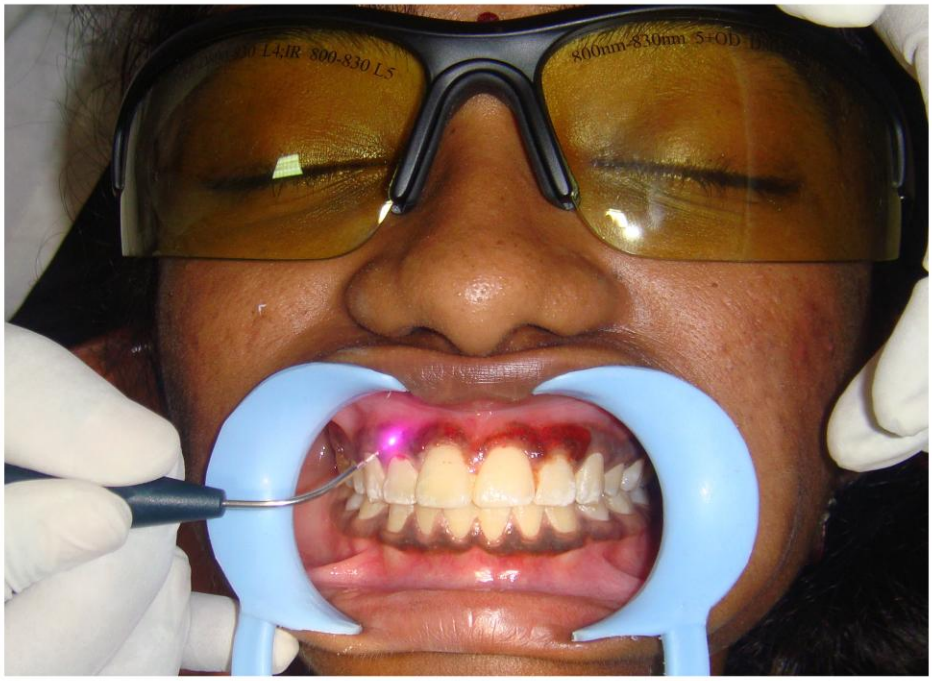


FIGURE -II LASER TECHNIQUE



IMMEDIATE POST OPERATIVE

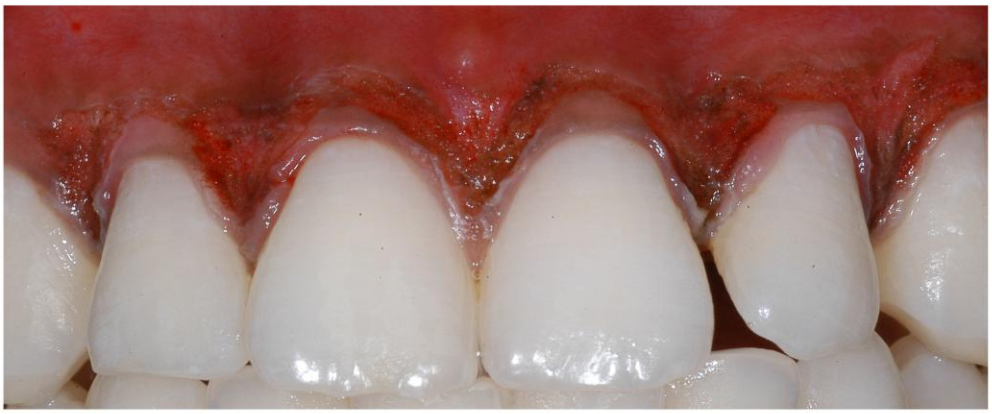
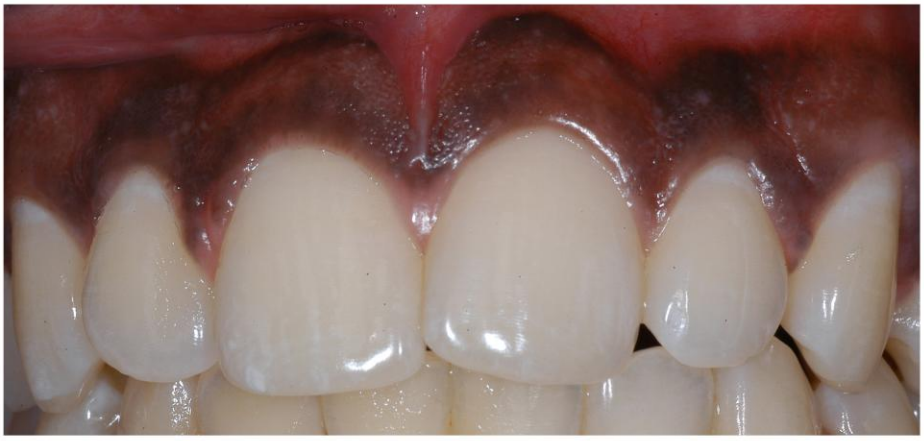


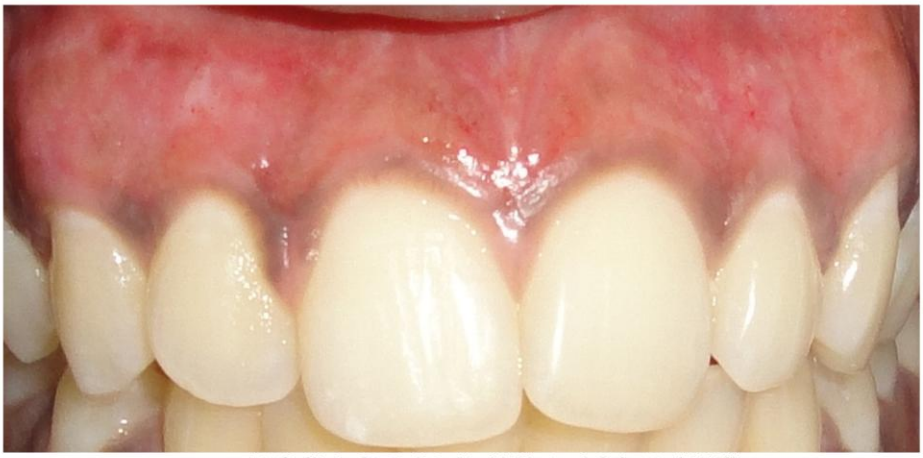
FIGURE III-CASE PHOTOGRAPHS

GROUP-B LASER DEPIGMENTATION

PRE OPERATIVE



POST OPERATIVE-10 DAYS



POST OPERATIVE -180 DAYS

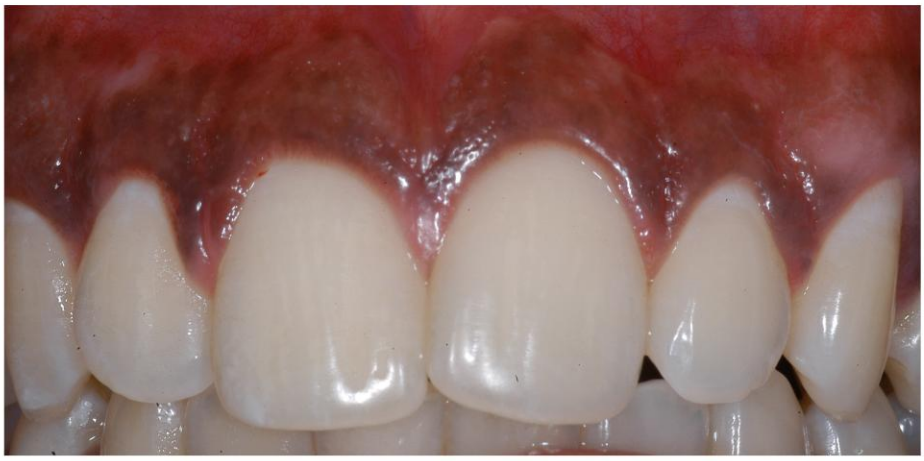
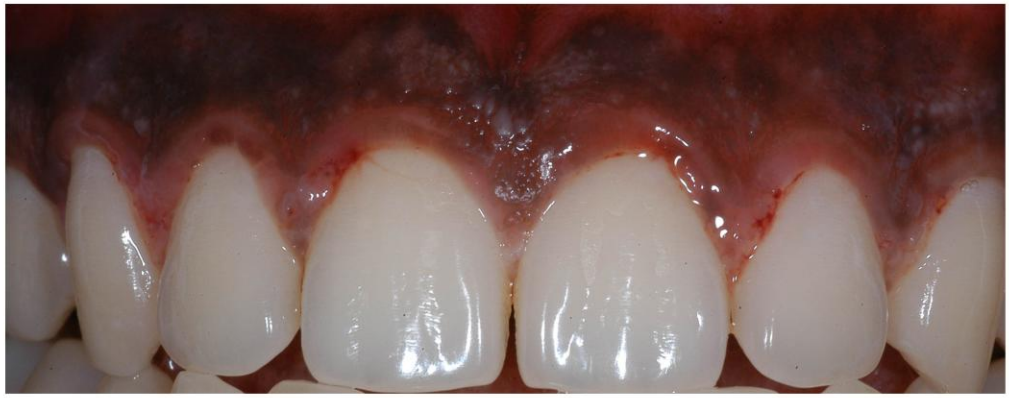


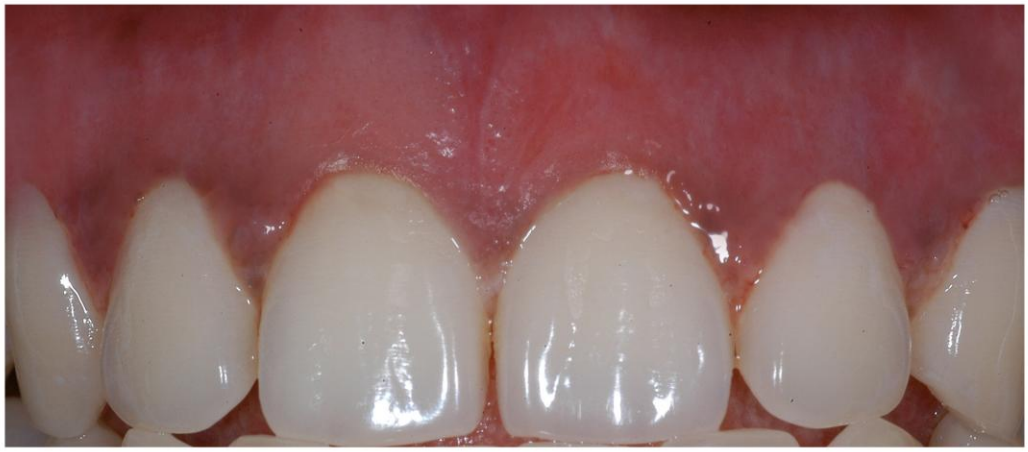
FIGURE-IV CASE PHOTGRAPHS

GROUPA- SCALPEL DEPIGMENTATION

PRE OPERATIVE



POST OPERATIVE-10 DAYS



POST OPERATIVE-180 DAYS

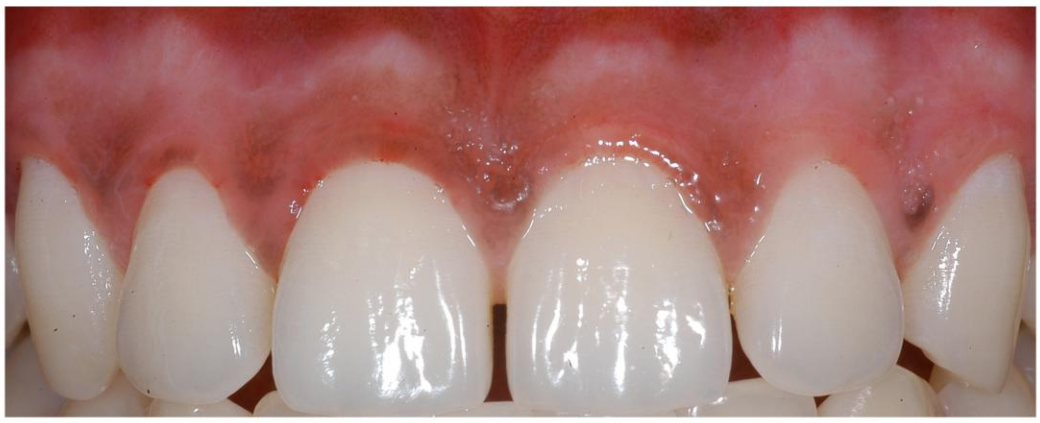
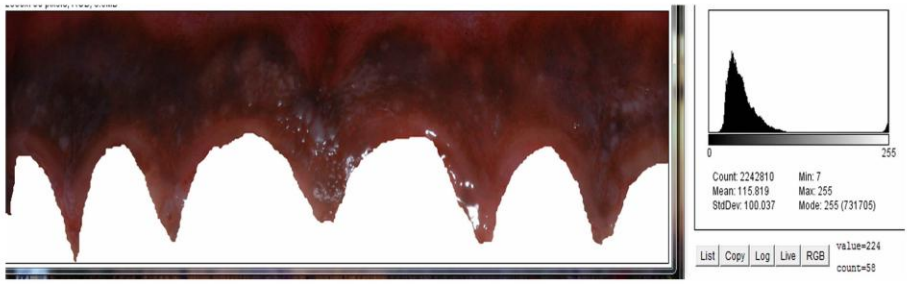


FIGURE-V

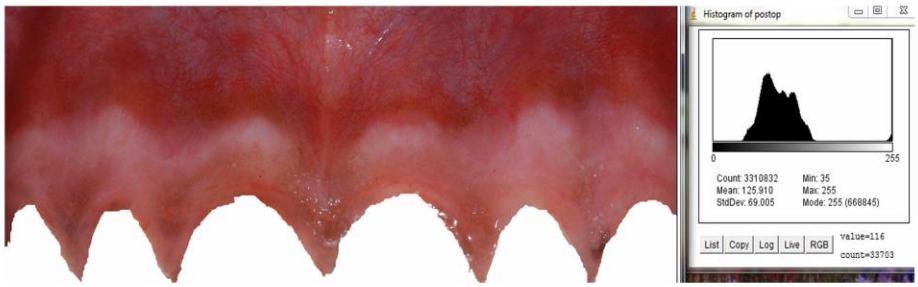
EVALUATION OF INTENSITY OF PIGMENTATION

SCALPEL DEPIGMENTATION

PRE OPERATIVE

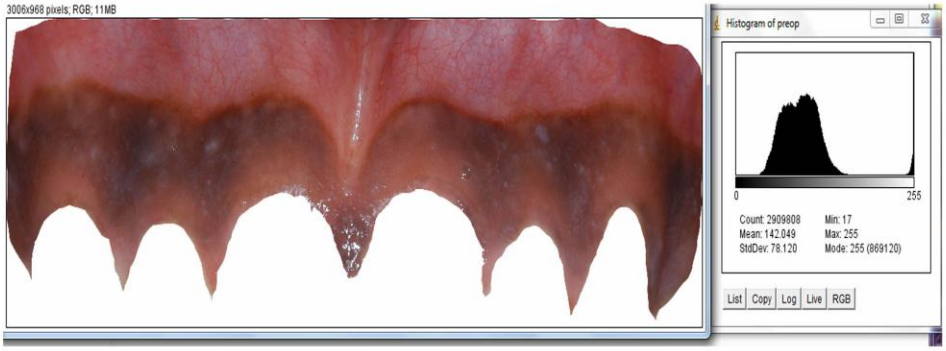


POST OPERATIVE-180DAYS



LASER DEPIGMENTATION

PRE OPERATIVE



POST OPERATIVE-180DAYS

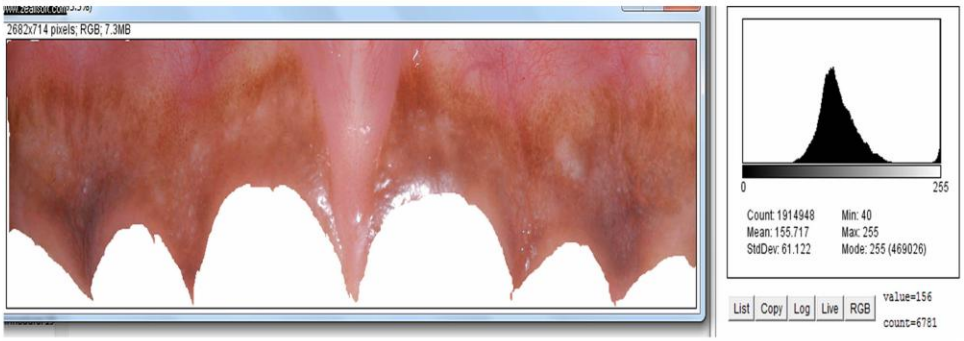
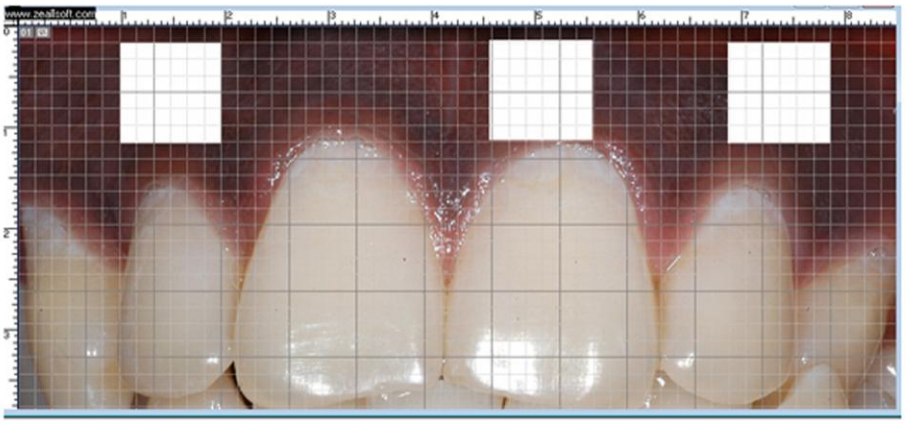
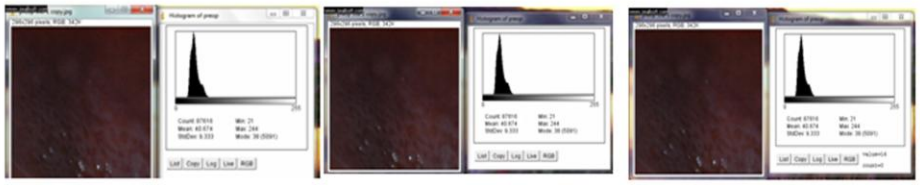
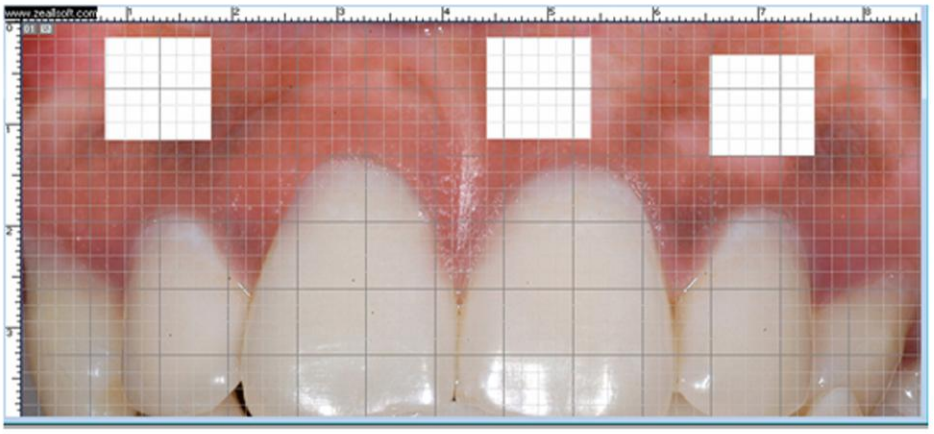


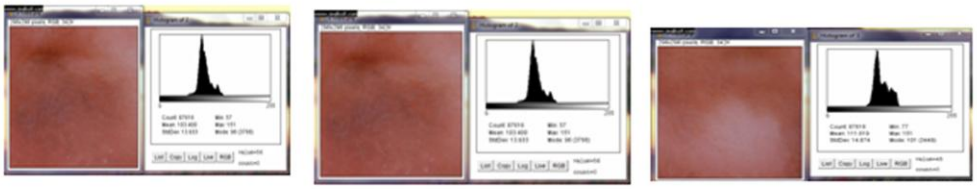
FIGURE - VI EVALUATION OF PERCENTAGE OF REPIGMENTATION

RANDOM SELECTION OF THREE SITES AND ASSESED WITH IMAGE J 1.42q SOFTWARE









RESULTS

A Total of 20 systemically healthy patients of age between 18-40yrs were enrolled in this study they were divided in to two groups each group has 10 patients Group A was treated using surgical technique and Group B was treated using diode laser for gingival hyperpigmentation and all the patients were recalled and evaluated on 10 days 30 days 60 days 90 days 180 days interval for the percentage of repigmentation qualitatively and quantitatively using image analysis software J 1.42 q (National Institute of Health)

Three individual sites were randomly selected measuring 1x1 inch in the anterior region and the intensity of colour was measured using image J software in each individual site and compared with the base line values for all 20 patients and the intensity of colour measured in the total surface area of surgical site from13-23 and the mean values were recorded and compared

The results were mean grey scale value for the Scalpel group (GROUP A) pre operavtively 148 ± 16.44 and post operatively 156.91 ± 26.44. The mean grey scale value for the Laser group (GROUP B) pre operavtively186.82 ± 5.2 and post operatively 193.74 ± 5.71.

Statistical analysis was done using Wilcoxon Signed Rank Test and Mann whiteny U test the results showed statistically significant difference in scalpel group (GROUP A) with (p=0.005) pre and post operatively and no statistically significant difference in laser group (GROUP B) with (p=0.20).

Statistically significant difference in the percentage of Repigmentation between the scalpel and diode laser group at 3 months with ($p = 0.001$).

Table 1: COMPARISON OF TOTAL SURFACE AREA AT PRE-OP VS POST-OP IN LASER GROUP

Time duration	N	Mean	Std. Deviation	Minimum	Maximum	Mean Rank	Z	p value*
Pre-op	10	148.00	16.44	130.52	186.82	5.00	-1.274	**0.20 (NS)**
Post-op	10	156.91	26.44	112.08	193.74	5.71		

Table 2: COMPARISON OF PERCENTAGE OF REPIGMENTATION AT VARIOUS TIME WITH THAT OF THE BASELINE VALUE FOR LASER GROUP

	N	Mean	Std. Deviation	Minimum	Maximum
Baseline	10	92.65	22.73	56.37	126.09
Ten days	10	104.35	27.14	59.51	144.83
One month	10	98.29	26.72	47.67	123.56
Two months	10	85.13	19.56	56.70	117.59
Three months	10	83.77	11.45	67.36	106.27
Six months	10	100.85	24.49	58.64	130.84
Baseline Vs Ten days	z value – 0.96			**p value – 0.33 (NS)**	
Baseline Vs one month	z value – 0.66			**p value – 0.51 (NS)**	
Baseline Vs two months	z value – 0.35			**p value – 0.72 (NS)**	
Baseline Vs three months	z value – 0.86			**p value – 0.38 (NS)**	
Baseline Vs six months	z value – 0.86			**p value – 0.38 (NS)**	

***Wilcoxon Signed rank test**

Table 3: COMPARISON OF TOTAL SURFACE AREA AT PRE-OP VS POST-OP IN SCALPEL GROUP

Time duration	N	Mean	Std. Deviation	Minimum	Maximum	Mean Rank	Z	p value*
Pre-op	10	136.84	16.77	115.82	166.37	0	2.80	0.005
Post-op	10	155.75	13.40	125.91	170.88	5.5		

Table 4: COMPARISON OF PERCENTAGE OF REPIGMENTATION AT VARIOUS TIME WITH THAT OF THE BASELINE VALUE FOR SCALPEL GROUP

Time period	N	Mean	Std. Deviation	Minimum	Maximum
Baseline	10	**69.12**	**17.92**	40.67	94.70
Ten days	10	**110.95**	**17.85**	73.40	134.78
One month	10	**123.17**	**32.59**	76.96	168.95
Two months	10	**117.69**	**19.19**	91.20	149.08
Three months	10	**119.74**	**21.79**	93.48	162.36
Six months	10	**109.80**	**20.88**	91.99	156.89
Baseline Vs Ten days	z value – 2.80			**p value – 0.005 (S)**	
Baseline Vs one month	z value – 2.80			**p value – 0.005 (S)**	
Baseline Vs two months	z value – 2.80			**p value – 0.005 (S)**	
Baseline Vs three months	z value – 2.80			**p value – 0.005 (S)**	
Baseline Vs six months	z value – 2.80			**p value – 0.005 (S)**	

***Wilcoxon Signed rank test**

Table 5: COMPARISON OF PERCENTAGE OF REPIGMENTATION AT VARIOUS TIME INTERVALS BETWEEN THE LASER GROUP AND THE SCALPEL GROUP.

Time period	n	Laser group		Scalpel group		z value	p value*
		Mean	SD	Mean	SD		
Baseline	10	92.65	22.73	69.12	17.92	2.26	0.02
Ten days	10	104.35	27.14	110.95	17.85	0.61	0.57
One month	10	98.29	26.72	123.17	32.59	1.43	0.16
Two months	10	85.13	19.56	117.69	19.19	3.11	0.001
Three months	10	83.77	11.45	119.74	21.79	3.55	0.001
Six months	10	100.85	24.49	109.80	20.88	0.52	0.63

*Mann Whitney test

GRAPH-1- COMPARISON OF PERCENTAGE OF REPIGMENTATION BETWEEN SCALPEL AND LASER DEPIGMENTATION

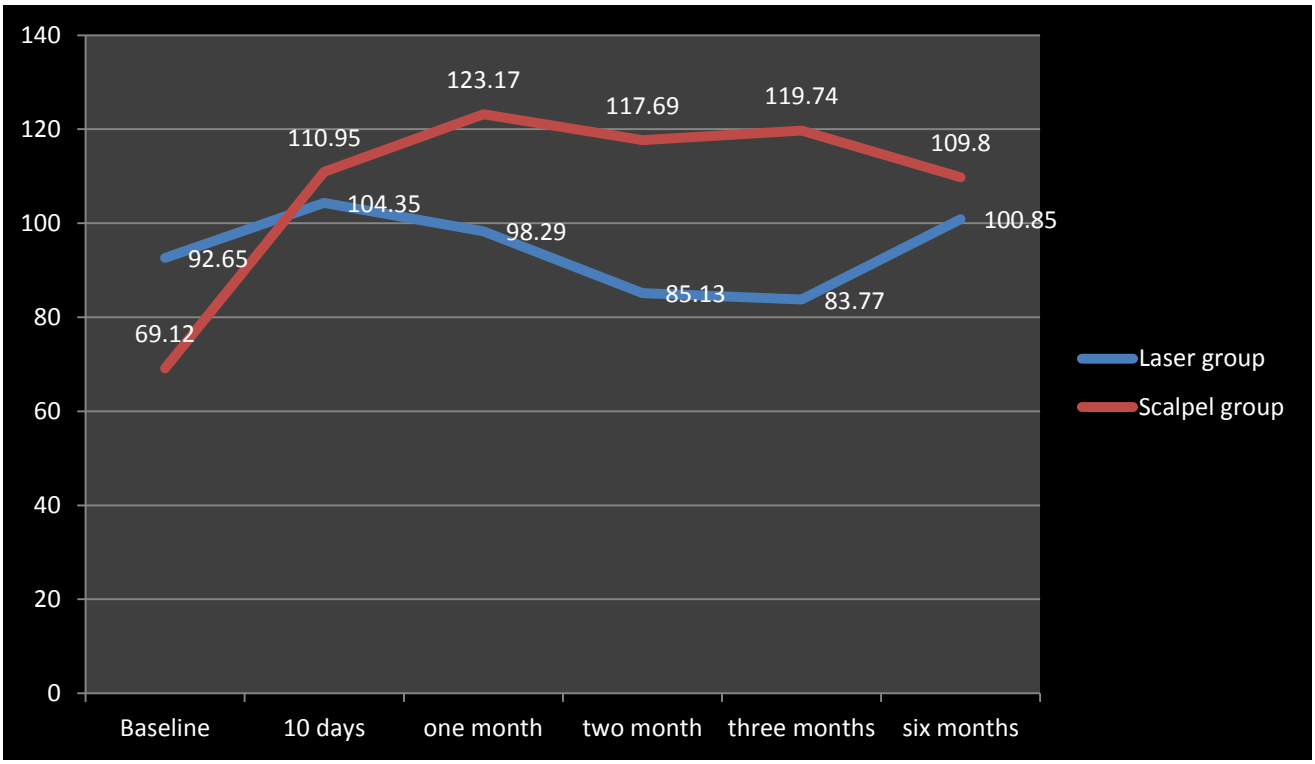
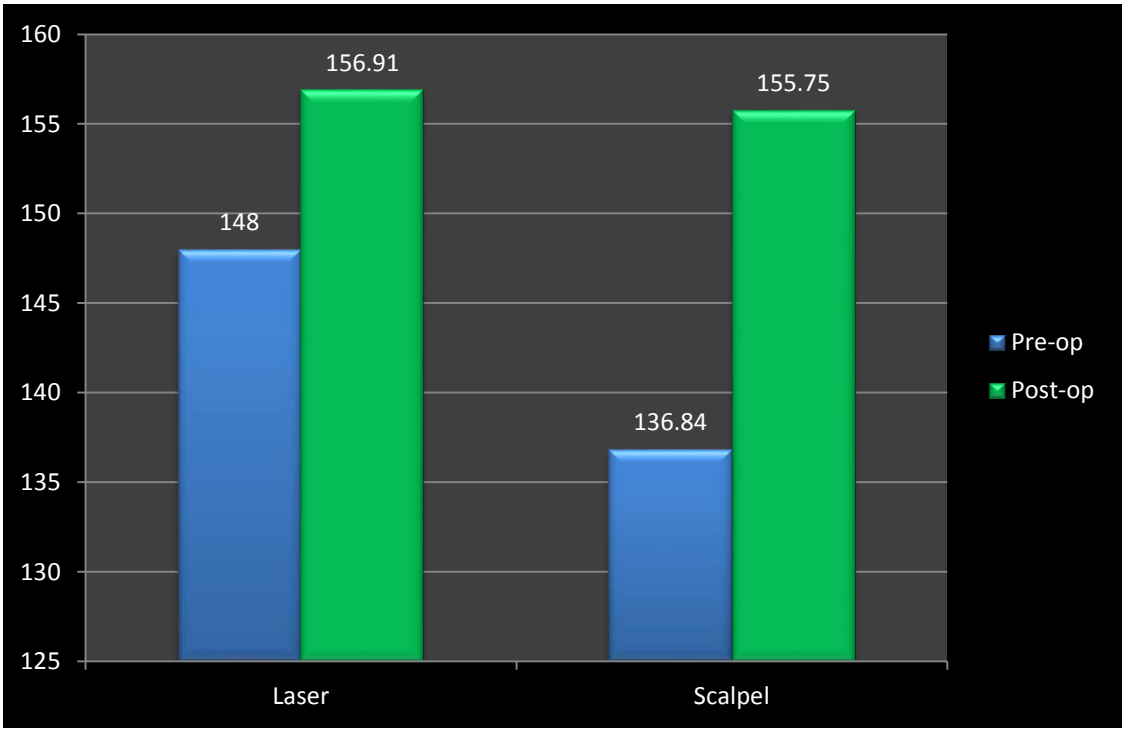


Table 6: COMPARISON OF TOTAL SURFACE AREA BETWEEN THE LASER GROUP AND THE SCALPEL GROUP AT VARIOUS TIME INTERVALS

Time duration	N	Laser group		Scalpel group		Z	p value*
		Mean	SD	Mean	SD		
Pre-op	10	148.00	16.44	136.84	16.77	1.28	0.21
Post-op	10	156.91	26.44	155.75	13.40	0.52	0.63

***Mann Whitney test**

GRAPH-2 COMPARISON OF PERCENTAGE OF REPIGMENTATION BETWEEN SCALPEL AND LASER DEPIGMENTATION



DISCUSSION

Esthetics plays an important role in patients psychological and social well being. Gingival health and appearance are essential components for a pleasant and confident smile. The modern dentistry has not only evolved to address the functional need but also in treating esthetic needs.

Gingiva is the most frequently pigmented intra oral tissue in addition to being the most readily seen during inspection, frequent cause of gingival pigmentation is due to melanin[35]. It is a non heamoglobin derived pigment formed by the cells called melanocytes, which are dendritic cells of neural ectodermal origin located in the basal and spinous layers of the gingival epithelium. Melanin granules are phagocytosed and contained within other cells of epithelium and connective tissue called melanophages or melanophores.[59]It is generally accepted that pigmented areas are present only when melanin granules synthesized by melanocytes are transferred to keratinocytes. This close relationship between melanocyte and keratinocytes was named by Fitzpatrick and Breathnach as **The Epidermal- Melanin Unit.**[9]

Oral melanin pigmentation is well documented in the literature and is considered to be multifactorial, whether physiological or pathological and can be caused by a variety of local and systemic factors.[61]including genetic, tobacco use, prolonged administration of certain drugs especially antimalarial agents and tricyclic antidepressants [37]. Gingival hyperpigmentation is seen as a

genetic trait in some population and termed as physiologic or racial gingival pigmentation[26].It has also been suggested that although pigmentation under normal conditions is genetically determined, its distribution in the mouth may be the result of secondary influences and environmental factors.

High levels of oral pigmentation are normally observed in individuals of African, East-Asian or Hispanic ethnicity.[28]Earlier studies have shown that no significant difference exists in the density of distribution of melanocytes between light-skinned, dark-skinned, and black individuals. However, melanocytes of dark-skinned and black individuals are uniformly highly reactive than in light-skinned individuals[64].Individuals may become more conscious of black or dark patches of pigmentaion and request their removal.[59]Demand for depigmentation is usually made for esthetic reasons, particularly in patients having a very high or high smile line with more of gingival exposure.

Elimination of these melanotic areas are done through surgical excision, lasers.[23 67] cryosurgery through use of a gas expansion system,[66] bur abrasion, scrapping electrocautery[34] and chemicals have been reported by many authors (**Hirschfeld&Hirschfeld 1951[40], Dummet& Bolden 1963[25], Ginwalla et al 1966[33], Manchandia 1979, Tal et al 1987 [66]and Atsawasuwan et al 2000)[6].** Each technique has its own advantages and disadvantages.

Hirschfeld and Hirschfeld (1951) used phenol (90%) and alcohol (95%) to remove areas of oral pigmentation by destroying tissue down to and slightly below the basal layer of the mucous membranes. Repigmentation soon developed in three patients; the rest of the subjects met with the same results a short while later. **Dummett** and **Bolden (1963)**[25] excised pigmented gingiva by gingivectomy procedure in 9 cases. Repigmentation occurred in 67% of the areas, as early as 33 days after surgical removal. **Ginwalla et al (1966)**[33] attempted to remove gingival pigmentation in 6 cases using three different techniques:Slicing, Bone denudation and abrasion**. Tal et al (1987**)[66] described depigmentation of the gingiva by cryosurgery, using gas expansion cryosurgical system based on the Joule-Thomson effect. **Trelles et al (1993**)[68] were the firstto treat patients with pigmented gingiva by argon laser. **Chin-JyhYeh (1998)** described cryosurgical treatment of melanin-pigmented gingiva using direct application of liquid nitrogen with a cotton swab to the pigmented gingiva**. Sameer (2006**)[58] treated 3 cases of gingival hyperpigmentation by abrasion with a high speed hand piece and diamond bur and no repigmentation in an 18 month follow up period but in a similar technique. **Farnoosh(2008)**[28] reported slight repigmentation in 2 cases. A free gingival auto graft can also be used to eliminate pigmented areas, but it is an extensive procedure which requires an additional surgical site and colour matching

Atsawasuwan et al (2000)[6] used Nd :YAG laser for the treatment of hyperpigmented gingiva and reported no recurrence in a period of 11 to 13 months of follow up.

The split thickness Scalpel technique was introduced by **Ginwalla et al (1966)**[33] , the procedure involves surgical removal of the gingival epithelium along with a layer of the underlying connective tissue under adequate local anesthesia and allowing the denuded connective tissue to heal by secondary intention. The new epithelium that forms is devoid of pigmentation. Care has been taken to remove all remnants of the pigmented layer and it was removed in thin sections to avoid exposing the underlying bone. Scalpel de-epithelization is relatively simple and effective, and most economical of all the other techniques available. It requires minimal armamentarium, easy to perform and, most importantly, requires minimum time and effort[44]. Also, the healing period for scalpel wound is faster than other techniques. However, it might result in unpleasant hemorrhage during or after surgery. Hence, it is necessary to cover the lamina propria with periodontal dressing for 7–10 days.[12] It also has chances of infection or recurrence. The wound healing takes place by proliferation of cells present along the periphery of the wound, these cells migrate and help in Reepithelialization of wound[22]

Lasers were first introduced in 1960 by Maiman and were brought in to general practice by Dr William and Terry myers although, co_2 lasers are used for depigmentation procedure, they can damage tooth structure and the

delivery system is very cumbersome. since co_2 laser are used in non contact mode they can also cause loss of tactile sense[7] also , diode is an excellent soft tissue laser and it is indicated for cutting and coagulating gingival tissue.[35] The use of semiconductor diode laser for depigmentation procedure was introduced by **Yousuf A etal 2000[74].** The semiconductor diode laser which is made up of aluminum, garnet and arsenide diode emitting in continuous mode and operated in a contact method using a flexible fiber-optic delivery system. Laser light at 810 nm is poorly absorbed by water But highly absorbed in hemoglobin and other pigments.[13] Since the diode laser basically does not interact with dental hard tissues, the diode laser is an excellent soft tissue surgical laser indicated for cutting and coagulating gingival and oral mucosa. The diode laser exhibits thermal effects using the "Hot-tip" effect caused by heat accumulation at the end of the fiber, and produces a relatively thick coagulation layer on the treated surface. The usage is quiet similar to electrocauterization. As the Tissue penetration of a diode lasers is minimal it does not produce any deleterious effect on the root surface.

Thus it is generally considered that diode laser surgery can be performed safely in close proximity to dental hard tissue.

The usual mechanism of diode laser that lead to ablation or decomposition of biological materials is photochemical, thermal or plasma mediated. Thermal ablation means that the energy delivered by the laser interacts with irradiated material by an absorption process, yielding a

temperature rise there [21]. As the temperature increases at the surgical site, the soft tissue are subjected to warming (37° to 60°C), protein denaturation, coagulation (>60°C). The rapid rise in intracellular temperature and pressure leads to cellular rupture, as well as release of vapour and cellular debris termed the laser plume.[49]**Moritz 2006 et al** found that an extraordinarily high reduction of bacteria could be achieved. It creates locally sterile condition, resulting in a reduction of bacteremia concomitant with operation.

The rapid wound healing after using lasers may be related to the photobiomodulation (PBM). PBM or low level laser therapy (LLLT) is the application of electromagnetic energy in the red and near-infrared region to damaged or diseased tissue. LLLT may occur simultaneously with the high-level laser therapy at the periphery of the target tissue, therefore explaining some of the advantages of lasers in high level laser therapy.[52]

Safety glasses were worn by the operator, patient and assistant. Highly reflective instruments or instruments with mirrored surfaces were avoided as there could be reflection of the laser beam. Care was taken to avoid using laser in presence of explosive gases.

The healing period of scalpel wounds is shorter than with diode laser[12]. However, scalpel surgery causes unpleasant bleeding during and after the operation and it is necessary to cover the exposed lamina propria with a periodontal pack for 10 days.But the diode laser causes minimal damage to the periosteum and bone under the gingiva being treated, and it has the unique

property of being able to remove a thin layer of epithelium. Although healing of laser wound is slower than healing of scalpel wounds, a sterile inflammatory reaction occurs after lasering.[56] Blood vessels in the surrounding tissue up to a diameter of 0.5 mm are sealed thus the primary advantage of hemostasis and a relatively dry operating field.

Patients with smoking habit are excluded from the study, as Smoking provokes the activation of melanocytes to produce melanin upon stimulation. Polycyclic amines, like nicotine and benzpyrenes, which are known to penetrate into the oral mucosa and bind to melanin. The term ‘‘smoker’s melanin’’ has been used to describe this benign melanin pigmentation.[12]

All patients were recalled on 10 days 30 days 60 days 90 days and 180 days post operatively Clinical parameters, such as bleeding, wound healing, gingival colour, pain and difficulty of the procedure were evaluated immediately after and then at 10 days 30 days 60 days 90 days and 180 days intervals. A list of clinical observation and patient responses prepared by Ishii et al and Kawashima et al was used for evaluation each parameter.

The visual analogue scale (VAS) was used to evaluate the subjective pain level experienced by each patient it consist of horizontal line from0-4, starting at the left end with the descriptor ‘no pain’ and ending at the right end with unbearable pain, patients were asked to mark the severity of the pain. The distance of this point, in millimeters, from left end of the scale was recorded and used as VAS score. If the score was 0-no pain ,scores between 1-2 were

recorded as slight pain, 2-3 was considered as moderate pain, and scores 3-4 were recorded as severe pain

The intensity of the pigmentation was measured using an image analysis software called Image-j1.42 q (National Institute of Health)[2] at base line ,10 days ,30 days,60 days, 90 days, and 180 days using standardized photographs. Randomly three individual sites measuring1x1 inch and the total surface area from maxillary canine to canine was measured. The measurement of the intensity of the pigmentation is based on the image histogram by the software expressed in mean grey scale values from 0-255.[2] statistical analysis was done by comparing the pre-operative and post operative mean grey scale values and the difference between the two surgical techniques evaluated.

Oral Repigmentation refers to clinical reappearance of melanin pigment after a period of clinical depigmentation of the oral mucosa[1] in this study the pigmentation started to reappear after 1 months and during the 6 month follow up period, the patchy pigmentation could be a result of the on going process of Repigmentation. The decreased intensity of pigmentation may be due to less production of pigments. the intensity may increase with time and may reach to a pretreatment level as it depend on the racial background of the patient Kon et al demonstrated that permanent results cannot be offered when gingival depigmentation procedures are performed for esthetic reasons.[12]

Recurrence of pigmentation has been attributed to the migration of melanocytes from the adjacent untreated sites or from the remnants of the melanocytes left behind at the surgical site these melanocytes may become activated and start synthesizing [59]

All the depigmentation techniques has shown considerable amount of recurrence rates even at time intervals less than 5 month. Ginwalla et al reported repigmentation in 50% of cases after split thickness excision in 24 and 55 days.Dummet et al reported recurrence of ginginval hyperpigmentation after gingivectomy in 6 of 8 patients in follow up period of 33-120 days.Atsawasuwan et al reported no recurrence after 11-30 months in 4 patients after Nd:YAG laser depigmentation. Ozbayarak et al reported no recurrence after co2 laser ablation of gingival melanin pigmentation in 18 months of follow up .Nakamura et al also reported that despite the lack of recurrence during the first year of follow up there was Repigmentaion in 4 of 7 cases almost equal to the pre operative state at 24 months with use of co_2 laser.Tal et al, and Berk et al, observed no Repigmentation occurring in any of their patients treated with Er:YAG laser after 6 months.

Interestingly most reported data in literature on Depigmentation using diode laser are case reports and one recent study conclude with recurrence in 1 of the 10 cases. None of the above studies has compared the intensity of Repigmentaion quantatively.

To prevent the recurrence, the gingival tissue should be cleared of melanin entirely including free gingiva and interdental papilla since Repigmentation starts as a result of migrating melanocytes from free gingiva.[22] Adequate tissue removal may not be possible at the gingival margins and interdental papillary region due to close proximity of the adjacent teeth which may be damaged by the laser beam, this limitation may result in incomplete vaporization of the pigment in such delicate areas, which tend to promote Repigmentation.

This study was undertaken to determine the efficiency of conventional scalpel surgical method and diode laser technique for depigmentation.

Results of this study shows that there is an statistically significant difference in the pre and post-operative values in scalpel surgical technique with a (p value=0.005) and there is no statistically significant(p value= 0.20) difference in the diode laser technique because the diode laser settings which was used for the surgical procedure may not be suitable for the study population and the tissue penetration of laser beam also minimal which is not sufficient to clear all melanocytes from the surgical site , There is a statistically significant difference in scalpel surgical technique when compared with diode laser technique ,the rate of repigmentation is also low in scalpel technique when compared to laser depigmentation technique. And hence extending the longevity of the depigmentation procedure. Less number of

subjects, shorter follow up period, and no Histological and Histochemical assessment for the activity of melanocytes are the limitations of the study.

SUMMARY AND CONCLUSION

The present study was undertaken to compare the efficacy of two different surgical techniques for the depigmentation procedure, split thickness excision technique with a surgical scalpel blade as against semiconductor diode laser.In thisstudy totally 20 patients aged between 18yrs- 40 yrs were enrolled (18 males and 2 female patients) who were esthetically conscious of their hyperpigmented gums and requested treatment for the same were recruited for the study. They were randomly divided in to two groups, each group comprising of 10 patients. Group A of patients were treated with conventional scalpel blade and other Group B with laser surgery using semiconductor diode laser (Biolase®)and recalled on 10 days ,30 days, 60 days, and 180 days, intervals.

For all the patients pre-operative and post operative standardized photographs were taken at each interval period and all the patients were evaluated qualitatively for post operative bleeding , pain wound healing, discomfort and ease of the procedure was evaluated by the operator . Quantitatively for the percentage of Repigmentation and colour intensity was evaluated using a image analysis software pre and post-operatively. Statistical analysis was done using Wilcoxon Signed rank test and Mann whitney U test.

The results showed that there is an statistically significant difference in the scalpel group comparing pre and post operatively with (p=0.005) and there is no statistically significant difference (P=0.20) in the diode laser group . In

the diode laser settings which was used for the surgical procedure may not be suitable for the study population and the tissue penetration of laser beam also minimal which is not sufficient to clear all melanocytes from the surgical site, Also there is an statistically significant difference between scalpel and laser group post operatively.

The percentage of Repigmentation is also slow in scalpel technique when compared with diode laser technique and hence extending the longevity of the depigmentation procedure. But the laser technique showed ease of handling and patient comfort.

The study conducted would be more validated if Histochemical & Histological assessment for the Melanocyte activity and a longer follow up period was carried with increased sample size.

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