INTRODUCTION

Normal physiologic color of gingiva is coral or salmon pink, with physiological variations that depend upon the degree of vascularization, epithelial thickness, the thickness of keratinized layer and the amount of melanin pigment. The excessive deposition of melanin by active melanocytes, located mainly in the basal and suprabasal cell layer of the oral epithelium, can cause dark colored areas that occur most frequently in the gingiva, known as melanin hyperpigmentation or black gums ***(Ishii et al., 2002; Rosa et al., 2007; Bakhshi et al., 2015;* Balaji et al., 2019*).***

Melanin hyperpigmentation (MH) is known to be associated with various etiological factors, such as: drugs, heavy metal ingestions/poisonings, genetics, endocrine disturbances (Addison's disease, Albright and Nelson's syndrome, acromegaly), and exposure to ultraviolet (UV) rays, inflammation, benign and malignant lesions, cultural intentional tattooing and smoking ***(Kawashima et al., 2003; Tal et al., 2003; Ishikawa et al., 2004).***

Melanin hyperpigmentation can also be caused by some pathologic conditions, such as malignant melanoma, Kaposi's sarcoma, Peutz Jeghers syndrome, trauma, hemochromatosis, and chronic pulmonary disease. Therefore, a detailed medical history of the patient and further histopathological examination is crucial in determining whether the MH cause is physiological or

pathological. Although clinically MH is not a medical problem or a disease, the demand for cosmetic corrections is increasing, mainly by fair-skinned people and when MH is located on the anterior labial gingiva. This problem is aggravated in patients with a "gummy smile" or excessive gingival display while smiling ***(Ishii et al., 2002; Kawashima et al., 2003; Tal et al., 2003; Ishikawa et al., 2004; Rosa et al., 2007; Sawabe et al., 2015).***

Gingival depigmentation is a periodontal surgical procedure, whereby the gingival hyperpigmentation is removed or reduced with different treatment methods, such as bur abrasion, scraping with scalpel, partial thickness flap, gingivectomy, cryotherapy, electrosurgery, free gingival autografting, chemical methods, subepithelial connective tissue graft, lasers which have been descriped as the most effective, pleasant and reliable technique for depigmentation of gingiva. and Alloderm ***(Ishii et al., 2002; Kawashima et al., 2003; Ishikawa et al., 2004; Bakhshi et al., 2015).***

Scalpel Surgical Technique is also called as split thickness epithelial excision and surgical stripping. Conventional scalpel method involves the surgical excision of gingival epithelium using a scalpel and allowing the denuded connective tissue to heal by secondary intention ***(Dummett, 1946; Dummett and Bolden, 1963).*** It is simple, most economical and convenient to perform with minimum time and efforts**.** Healing with this technique is faster in comparison to other surgical techniques ***(Ozbayrak et al.,***

2000; Kathariya and Pradeep 2011; Verma et al., 2013; El-Shenawy et al., 2017).

Although lower cost and lower rate of recurrence favor the surgical stripping of gingival ***(Bergamaschi et al., 1993)***, it is usually associated with pain, post-operative discomfort, intra- and post-operative bleeding and requires placement of periodontal dressing ***(Mokeem 2006; Prasad et al., 2010; Verma et al., 2013; Senthinathan et al., 2014).*** Thinner gingival biotype and narrow papillary areas contraindicate the use of this technique ***(El-Shenawy et al., 2017).***

Cryosurgery is the most widely accepted method of gingival depigmentation according to ***(Kumar et al., 2013; Lin et al., 2014; Ho et al., 2015; Narayankar et al., 2017).*** It involves freezing of gingiva with the application of different materials, i.e. cryogen such as liquid nitrogen at very low temperatures ***(Moneim et al., 2017).***

The effect of ultra-low temperature of cryogen on gingival tissue causes the epithelium to undergo cryonecrosis, which helps to eliminate gingival pigmentation. It is an inexpensive method with long-term superior esthetic results, rapid healing, low recurrence rate together with lack of bleeding, pain and scar formation, application without regional anesthesia, sutures or drugs, ease of application of cryogen at papillary areas and uncomplicated instruments which prioritize the cryosurgery over other depigmentation methods however,

post-operative swelling and difficulty in controlling the penetration depth constitute the disadvantages of this technique ***(Ahmed et al., 2002; Arikan and Gürkan, 2007; Yu et al., 2009; Lin et al., 2014; Narayankar et al., 2017).***

Laser therapy has optimal efficacy in the treatment of gingival hyperpigmentation. Most commonly used lasers for gingival depigmentation are carbon dioxide (CO2, 10, 600 nm) lasers, neodymium: Yttrium, aluminum, and garnet (Nd: YAG, 1,064 nm) and diode (980 nm) lasers ***(Atsawasuwan et al., 2000; El Shenawy et al., 2015).*** Lasers exhibit enhanced hemostatic activity, good visibility at the surgical site and fewer post-operative complications such as pain, bleeding, edema, infection, and impaired wound healing ***(Chandna and Kedige, 2015; Khalilian et al., 2016; Nagati et al., 2017).***

Laser is considered as an effective and safe treatment modality with ease of access to interdental papilla and low rate of recurrence ***(Prasad et al., 2010; Murthy et al., 2012; Thangavelu et al., 2012)*** Although better esthetic results can be achieved by lasers, it requires sophisticated equipment, occupies large space and is expensive method**.** Inappropriate application may cause damage to gingiva and underlying alveolar bone which, in turn, can cause gingival recession, gingival fenestrations, and delayed wound healing ***(Kumar et al., 2012, 2013; Senthinathan et al., 2014; Khalilian et al., 2016).***

Ascorbic acid/Vitamin C has potential in the treatment of gingival melanin pigmentation**.** It inhibits the melanin formation by suppressing the tyrosine activity which is essential for melanin biosynthesis. Furthermore, ascorbic acid directly downregulates dopaquinone formation, a precursor in melanin synthesis, thus inhibiting the melanin formation ***(Shimada et al., 2009).***

A critical concern in the management of hyperpigmented gingiva is a relapse or gingival repigmentation. Repigmentation refers to the clinical appearance of melanin pigment following a period of clinical depigmentation.As it depends on methodology and follow-up period, the duration of repigmentation mentioned in literature remains controversial from one technique to another. Futhermore, factors such as smoking, sun exposure, and genetic determination of skin color, influence the duration of relapse. However, the majority of the available literature has shown lower recurrence rate for cryosurgery ***(Srivastava et al., 2014; El-Shenawy et al., 2017).***

REVIEW OF LITERATURE

Facial attractiveness and harmony of the facial features play an important role in building or maintaining self-confidence in social life. During a verbal communication, the primary components of a person that are in focus are their eyes and mouth. Thus, the health of oral cavity components and their suitability are important for facial attractiveness. This suitability includes factors such as the shape, position, size, and color of teeth together with the color, contour, and health of the gingival tissue ***(Geld et al., 2007).***

- Normal variations of gingival tissue color:

One of the earliest to investigate gingival pigmentation was ***Dummet (1966)*** who investigated the frequently used description of normal gingiva as 'coral pink' and suggested a more accurate statement of the pattern of normal pigmentation in the following definition; *"The color of healthy gingiva varies from pale pink to bluish purple"*. Between these limits of normality, Color variation of gingiva occurs. It may be uniform, unilateral, bilateral, mottled, macular or speckled and varies from pale pink, coral pink to light and dark brown. This color variation may depend on the amount of physiological melanin pigmentation in the epithelium, the amount of keratinization of the epithelium, the vascularity and the fibrous

nature of the underlying connective tissue. Keratinization of the oral epithelium decreases with age and varies in the following order; palate, gingiva, tongue and cheek with the highest keratinization in the palate and the least in the cheek/alveolar mucosa ***(Dosumu and Dosumu, 2010).***

- New Periodontal Disease classification:

In the new classification scheme for periodontal and peri-implant diseases and conditions by ***Caton et al. (2018),*** Gingival pigmentation was classified among non dental plaque induced gingival diseases beside genetic and developmental disorders, specific infections, inflammatory and immune conditions, neoplasms, endocrinal diseases, nutritional and metabolic diseases, reactive processes and Traumatic lesions as discussed by ***Holmstrup et al. (2018)***.

- Gingival pigmentation:

Gingival health and appearance are essential parts of a charming smile. Gingival pigmentation is a discoloration of the gingiva due to a variety of lesions and conditions associated with several endogenous and exogenous etiologic features. It may range from physiologic reasons (e.g. racial or physiologic pigmentation) to manifestations of systemic illnesses (e.g. Addison's disease) to malignant neoplasms (e.g. melanoma and Kaposi's sarcoma) ***(Jonathan et al., 2003; Kauzman et al., 2004).*** So, gingival pigmentation can be generally classified as or physiologic or pathologic:

- Physiologic Pigmentation:

Physiological melanin pigmentation of the oral mucosa affects males and females equally, as asymptomatic, solitary or multiple brown maculae with well-defined borders. It may include any part of the oral mucosa but most frequently the gingiva ***(Kauzman et al., 2004; Meleti et al., 2008; Lerman et al., 2009; Caldeira et al., 2010).***

Pigmentation has also been reported to be minimal in white people and can be found as brown or blue black areas in Asians or Africans***).*** It was also reported that gingival pigmentation is as prevalent as 60% in blacks. It has been documented that a definite correlation exists between the color tone within the oral cavity and the external skin pigmentation of the individuals in White, Negroes, Asiatic and Indian populations ***(Hedin and Axell, 1991).***

The distribution of oral pigmentation in black individuals is as follows: hard palate = 3%; tongue = 15%; mucous membranes = 22% and gingiva = 60%. Therefore, the gingiva is the most frequently pigmented tissue in the oral cavity. The pigmentation rate is the least at the posterior regions ***(Tamizi and Taheri, 1996).***While; it is the highest at the attached gingiva in the area of incisors ***(Kauzman et al., 2004).***

In a classification scheme suggested by ***Ponnaiyan et al., 2013***, they described the patterns of anatomic distribution of

gingival pigmentation. This classification included 6 classes; Class I: pigmentation in attached gingiva only, Class II: pigmentation in the attached gingiva and interdental papilla, Class III: diffuse pigmentation involving all parts of gingiva, Class IV: pigmentation in marginal gingiva only, Class V: pigmentation in interdental papilla only and Class VI: pigmentation in marginal gingiva and interdental papilla but there was no significant correlation between pigmentation distribution and pigmentation intensity.

The gingival pigmentation is most frequently bilateral, symmetrical, does not extend beyond the mucogingival junction and doesn't involve free gingival ***(McCarthy and Shlkar, 1964; Gaeta et al., 2002).*** Gingival melanin color may appear distinctly as early as three hours after birth or gradually appearing in the first two decades of life**,** but affected subjects may be unaware of it ***(Meleti et al., 2008; Lerman et al., 2009; Chandra et al., 2010).***

Although sources of pigments that contribute to the normal gingival color are variable, the most common source is melanin**.** Melanosomes produced by melanocytes uniquely synthesize and store melanin pigments. Melanocytes located in the epithelial basal cell layer convert tyrosine to melanin by using the enzyme, tyrosinase, which is then stored in basal cells in the form of melanosomes ***(Raposo and Marks, 2007).***

- Melanocytes and melanin sythesis:

Melanocytes are melanin-producing cells originating from the neural crest.During development, melanocyte stem cells travel from the neural crest to the skin and to mucous membranes. Active melanocytes are present in many areas of the brain, and in the heart, where they play a number of indefinite roles ***(Wood et al., 1999; Thomas and Erickson, 2008; Plonka et al., 2009).***

Moreover, Melanocyte stem cells have the capability for self-renewal and for differentiation and thus can maintain the population of mature melanocytes. In the epidermis, the melanocyte stem cells are mainly located in the hair follicles' bulge region ***(Nishimura et al., 2002; Kumano et al., 2008).***

As summarized by ***Moneim et al., (2017),*** the process of pigmentation involves three phases**. The first phase** involves activation of melanocytes when stimulated by factors like stress hormones and sunlight leading to production of chemical messengers like melanocyte stimulating hormone. **The second phase** is the melanin synthesis phase in which the melanocytes form granules called melanosomes. This process occurs when the enzyme tyrosinase converts amino acid tyrosine into a molecule called dehydroxy-phenylalanine (DOPA). Tyrosinase then converts DOPA into secondary chemical dopaquinone. After a series of reactions, dopaquinone is converted into either dark

melanin (eumelanin) or light melanin (pheo-melanin) and **the third** mealnin expression phase is when melanosomes are transferred from the melanocytes to the keratinocytes which are the cells located above melanocytes in the epithelium. After this, melanin color finally becomes visible on the surface. Major determinant of normal human tissue color is the melanogenic activity within the melanocytes and the quantity and quality of melanin production, but not melanocyte density. The degree of clinical melanin pigmentation in human epidermis and in the epithelium of oral mucosa is related to the amount of melanin i.e. the maturation of melanosomes, the number of keratinocytes containing melanosomes and the distribution of melanin loaded keratinocytes throughout the epithelium.

Melanin synthesis involves catalysation of the substrates L-phenylalanine and l-tyrosine to produce L-DOPA via enzymes like phenylalanine hydroxylase (PAH), tyrosinase and partly tyrosinase hydroxylase 1 (TH-1). The pathways are then divided into two main types of polymeric phenolic compounds; large irregular granules of eumelanin which are black-brown in colour and smaller more regular granules of pheomelanin which are yellow-red in colour ***(Mackintosh, 2001)*** Fig (1). The other melanogenic enzymes are tyrosinase-related protein 1 (TRP-1) and tyrosinase-related protein 2 (TRP-2) or (DOPAchrome tautomerase DCT) for eumelanogenesis. No specific enzymes have been found that are involved in pheomelanogenesis so far ***(Ando et al., 2007).***

So, Melanocytes are capable of producing both eumelanin and pheomelanin, and the proportion of the two types of melanin produced by a particular melanocyte depends on availability of tyrosine, of reducing agents, and of the types of pigment enzymes expressed ***(Lin and Fisher, 2007).***

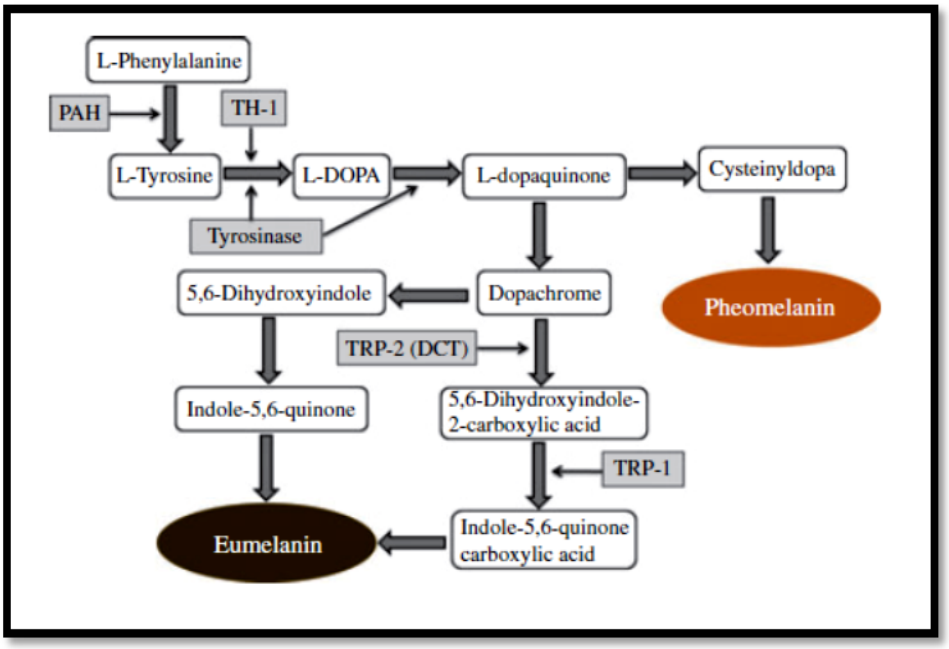

Fig. (1): Melanin synthesis pathway ***(Ando et al., 2007).***

In mammals, melanin formation occurs through a chain of events which facilitates the conversion of undifferentiated vesicles (stage I) to dense (stage IV) heavily pigmented melanocytes ***(Chi et al., 2006).*** Various pigment-cell-specific enzymes are involved in this process, including tyrosinase and tyrosinase-related proteins TYRP1 and 2 Fig (2).

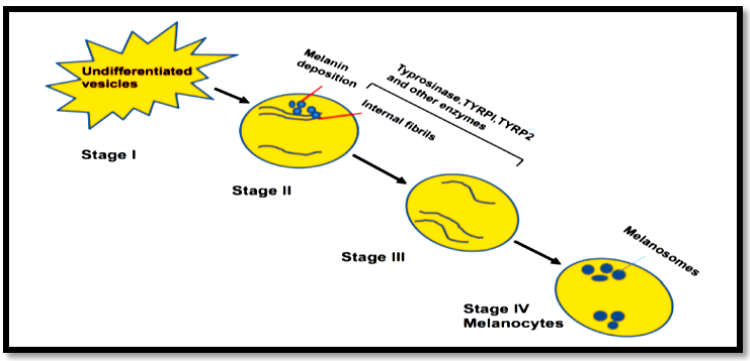

Fig. (2): Cycle showing the maturation of melanocytes and formation of melanin. Enzymes are involved in the differentiation of unstructured vesicles to mature and heavily pigmented melanocytes ***(Jha et al., 2017).***

Inside a 'keratinocyte-melnin unit', harmonious events occur whereby mature melanosomes are transported to the keratinocytes through the long melanocytic dendrites that act as a transport medium Fig (3). Within each keratinocyte, the melanin acts against ultraviolet rays as a shield for the DNA by forming supranuclear cap above nucleus to protect against mutations ***(Marks and Seabra, 2001; Plonka et al., 2009; Felleret al., 2014).*** Because the activity of melanosomes is directly related to that of keratinocytes, the occurrence of pigmentation is strongly influenced by keratinocytes.

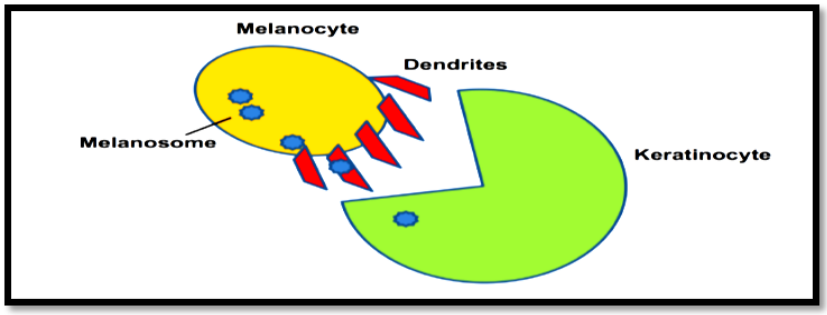

Fig. (3): Keratinocyte-melanin unit showing the transfer of melanosome by the dendritic processes of melanocytes. The microtubules facilitate of dendritic processes facilitate this transfer ***(Jha et al., 2017).***

- Functions of melanocytes:

The functions of melanocytes are not totally understood, but it is clear that the melanin they produce determines the color of skin, hair and eyes and provides protection from stressors such as UV radiation, reactive oxygen species (ROS) and free radicals in the environment**.** It has also been postulated that oral mucosal melanin provides a defense barrier by acting as a binder of toxic products such as free radicals and polycyclic compounds. Therefore, the oral melanocytes may act as antioxidants and prevent oxidative stress by scavenging radicals ***(Hedin and Larsson, 1984; Sturm, 2009)***.

As keratinocytes ascend through the cell layers of the epithelium and shed their melanosomal membranes undergo degradation with release of melanin 'dust' which becomes involved in the keratin filaments of the desquamating surface cells. This melanin dust inactivates pathogenic chemicals, microbial toxins and other biologically active molecules ***(Wood et al., 1999).***

It was reported that markers of gingival inflammation are decreased in subjects with pigmented gingiva compared to subjects with non-pigmented gingiva, despite similar dentogingival plaque levels in both groups of subjects. It is also interesting to know that the sulcular and junctional epithelium, in contrast to the keratinized epithelium usually does not have any

melanocytes. Melanosomes contain lysosomal enzymes including α-mannosidase, acid phosphatase, β-N acetylglycosaminidase, β-galactosidase, and acid lipase that can destroy pathogenic bacteria ***(Mackintosh, 2001; Nilima and Vandana 2011).***

Melanin itself can neutralize bacteria-derived enzymes and toxins, and since it has strong binding properties, it can also act as a physical barrier against microorganisms. Furthermore, melanocytes can act as antigen presenting cells, can stimulate T-cell proliferation, and can phagocytose microorganisms ***(Mackintosh, 2001; Tolleson, 2005).***

Melanin also have the capacity to sequester metal ions and to bind certain drugs and organic. As melanin synthesis is an oxygen dependent process, paradoxically, it also generates ROS that may accumulate in the melanocytes and cause DNA damage, and in fact UV radiation exaggerates the production of ROS during the biosynthesis of melanin and more particularly pheomelanin. Thus, melanin possesses both antioxidant and ROS-dependent cytotoxic properties ***(Wood et al., 1999; Smit et al., 2008).***

It was also assumed that the primary role of melanocytes is not to produce melanin as it does not offer an advantage to the organisms, but that melanin production is only a secondary specialization and the melanocytes must have important functions to perform other than melanin synthesis. Melanocytes in skin, and perhaps in oral mucosa express genes encoding