Original Article

Expression of tyrosinase gene in gingiva: A pilot study

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Abstract

Background: Melanin is the predominant pigment responsible for the color of skin, hair, iris of eyes, and oral mucosa. Tyrosinase (TYR) is the key enzyme involved in melanin synthesis. Studies in dermatology have shown a positive correlation between TYR enzyme levels and melanin pigmentation of the skin. However, no study has been conducted to assess TYR levels in the gingiva. Hence the present study was conducted to assess TYR levels in gingival melanin hyperpigmentation.

Aim: To assess the TYR gene expression in gingiva in individuals with moderate to severe gingival melanin hyperpigmentation.

Methodology: Subjects with a chief complaint of blackish appearance of gums with an unesthetic smile were included in the study. Informed consent was obtained. Scaling and root planning were done and subjects were recalled after 2 weeks. The gingival depigmentation procedure was performed using the conventional scalpel technique under adequate local anesthesia. The selected sites underwent conventional gingival depigmentation technique using Bard-Parker handle no: 3 and blade no: 11. The excised layer of epithelium along with a thin layer of underlying connective was sent to the laboratory to assess the TYR gene expression by real-time polymerase chain reaction technique.

Results: The levels of the TYR enzyme activity in the gingival tissues from the selected sites were assessed. Table 1 and Graph 1 show the levels of TYR enzyme gene expression in the gingival tissue.

Conclusion: TYR gene expression and the degree of gingival melanin hyperpigmentation are positively correlated. Hence the assessment of TYR enzyme activity in gingiva could be of great value in today's cosmetologically conscious individuals.

Keywords: Gingival melanin hyperpigmentation, melanin, melanin pigmentation, melanocytes, melanogenesis, tyrosinase, depigmentation

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INTRODUCTION

Melanocytes are a heterogeneous group of cells derived from neural crest cells[1] that synthesize melanin. Melanin is responsible for the color of skin, hair, iris of eyes, and oral mucosa including the gingiva.[2] The presence of oral mucosal melanocytes (OMMC) in gingiva was first identified by Laidlaw and Cahn in 1932.[3] The capacity to produce melanin by these Melanocytes (MCs) varies among individuals.[1] The large brown-black granules

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of eumelanin give the skin a darker shade, while the much smaller yellowish pheomelanin imparts lighter skin. Apart from the pigmentation of the skin, melanins have much broader functions like photoprotection by absorbing Ultraviolet radiation (UVR).[1] The melanin pigment globules transferred to the keratinocytes reside in the nucleus of these cells forming "supranuclear caps" that protect the genetic material of epithelial cells from UVR.[4]

Oral mucosal pigmentation can be physiologic or pathologic.[5] Physiological/racial melanin pigmentation of the oral mucosa is common in black persons and is more frequent in darker skinned whites (Caucasians) than in lighter skinned whites. It can range from light brown to almost black.[6] Several factors such as smoking, hormones, and systemic medications can affect the color intensity of pigmentation.[7] Pathologic pigmentation is associated with endocrine disorders, chronic irritation, reactive or neoplastic lesions, drugs, smoking or tobacco use, etc.[3] Among these, long-term use of certain drugs, such as nonsteroidal anti-inflammatory drugs, antimalarials, psychotropic drugs, tetracyclines, and oral contraceptives, is the most common cause of oral and perioral pigmentation.[8,9]

Melanins are produced in melanocytes, cells that are capable of synthesizing the enzyme tyrosinase (TYR), which, when incorporated into specialized organelles called melanosomes, promotes a series of events leading to the synthesis and the accumulation of the pigments.[10] In humans, TYR is the key enzyme involved in the biosynthesis of melanin, the primary determinant of the color of the skin, hair, and iris of the eye.[11]

TYR is an 80 kD melanosomal membrane-bound glycoenzyme comprising 529 amino acids. TYR, encoded by the gene TYR (11q14-21, MIM606933), is expressed in epidermal, follicular, and ocular melanocytes (Hearing 2011).[12] TYR is the critical and rate-limiting enzyme; it catalyzes the hydroxylation and subsequent oxidation of tyrosine.[11] TYR is a glycoprotein located in the melanosomal membrane, with an internal, a transmembrane, and a cytoplasmic domain.[13] It is synthesized on the ribosomes of the Rough endoplasmic reticulum (RER) and transported to the Golgi complex where it undergoes glycosylation, which is a process essential for its normal structure and function.[1]

The process of melanin biosynthesis (eumelanin and pheomelanin) requires TYR to convert tyrosine as the precursor of melanin. TYR is an enzyme that dependent on copper and plays a crucial role in the initial catalysis process to convert tyrosine to L-3,4-dihydroxyphenylalanine (DOPA) and subsequently oxidize it to DOPA quinone (DQ).[2]

Epidermal melanocytes have complex biology and protect against UVR. However, the role of oral melanocytes in protecting against UVR is unclear. Also, the gingiva (part of oral mucosa) is covered by the lips and not exposed to the UVR.[14] Hence, melanin does not seem to play a role in protecting against UVR in the gingiva.

Melanin hyperpigmentation of gingiva is a completely benign condition. However, it could be of esthetic concern in individuals with a gummy smile or high smile line or short upper lip with excessive display of gums.[15] Hence gingival depigmentation is performed as a periodontal plastic surgical procedure to eliminate the pigmented gingival epithelium either by surgical or non-surgical techniques.

Studies have been conducted assessing the role of dermal melanocytes in melanin synthesis and its correlation with TYR enzyme activity. However, there has been no study conducted on the assessment of TYR enzyme activity in the gingival tissue and its correlation with the degree of melanin hyperpigmentation of gingiva. Hence the present study was conducted to assess the TYR enzyme activity in individuals who underwent a gingival depigmentation procedure for an esthetic smile.

METHODOLOGY

The subjects with the chief complaint of black-looking gums or blackish discoloration of gums and desiring treatment for the same were selected for the study. On intraoral examination of the gingiva, gingival melanin hyperpigmentation was observed as assessed by Dummett Gupta oral pigmentation index [(DOPI) Score ≥2 (DOPI Score 2—moderate pigmentation and Score 3—severe pigmentation)].

Gingival depigmentation procedure using conventional scalpel technique was planned. The subjects were explained the procedure and informed consent was obtained. A thorough scaling and root planning was performed and oral hygiene instructions were given. The subjects were recalled after 2 weeks for performing gingival depigmentation. Laboratory blood investigations were carried out before the surgical procedure. All the blood parameters were in the normal range.

Local anesthesia was achieved using 2% lignocaine hydrochloride with 1:80,000 dilution adrenaline at the selected surgical sites. Conventional gingival depigmentation

was performed using a Bard-Parker (BP) blade no: 11 mounted in a BP handle no: 3. The melanin pigmented epithelial layer along with a thin layer of connective was excised. The excised tissue was sent to the laboratory for analysis of TYR enzyme gene expression.

The surgical sites were thoroughly cleaned with saline irrigation and any remnants of the pigmented layer were removed. The periodontal dressing was placed and postoperative instructions were given. The subjects were recalled after 1 week for pack removal and follow up examination. On 2 weeks follow up the healing was uneventful.

The estimation of TYR enzyme gene expression was performed by real-time polymerase chain reaction (RT-PCR). The excised tissues were minced into small pieces and digested with a collagenase–dispase enzymatic cocktail at 37°C of temperature. Ribonucleic acid was isolated using the triazole method according to the manufacturer's guidelines (Thermofisher). The SuperScript® III Cells Direct cDNA Synthesis Kit (Invitrogen, Carlsbad, CA, USA) was used to isolate complementary DNA from SW982 cell as per the manufacturer's instructions. RT-PCR analysis was performed using an Applied Biosystems QuantStudio™ 5 (QS5) Real-Time PCR system (ThermoFisher Scientific, USA). Human TYR [F-5′-(GTC TTT ATG CAA TGG TT) and R-5′-(GCT ATC CCA GTA AGT GGA CT)] gene expression was analyzed.

RESULTS

The levels of the TYR enzyme activity in the gingival tissues from the selected sites were assessed and tabulated. Table 1 and Graph 1 show the levels of TYR enzyme gene expression in the gingival tissue. It was observed that the individuals with higher DOPI score had higher levels of TYR gene expression.

DISCUSSION

Melanin pigmentation is a physiologic process and is responsible for the normal color of skin, hair, iris of eyes, and oral mucosa.[11] Melanin hyperpigmentation is associated with excessive deposition of melanin. Gingival melanin hyperpigmentation results in a blackish appearance or blackish discoloration of gums. This results in an unesthetic or unpleasant smile, especially in individuals with a gummy smile.[15]

Table 1: TYR gene expression levels

Sites	TYR levels
Site 1 (DOPI >2)	0.649
Site 2 (DOPI >2)	0.543
Site 3 (DOPI >2)	0.616
Site 4 (DOPI >2)	0.593
Site 5 (DOPI <2)	0.478
Site 6 (DOPI <2)	0.512
Site 7 (DOPI <2)	0.498
Site 8 (DOPI <2)	0.465

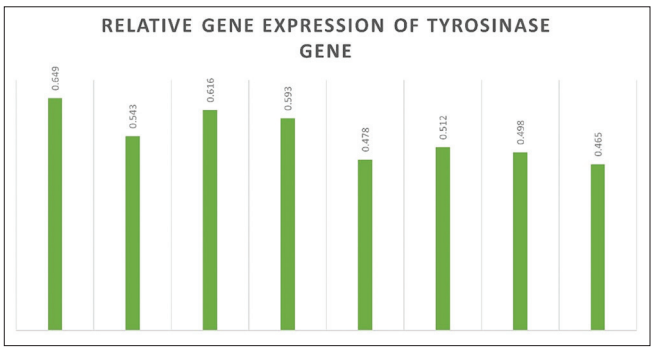
Melanocytes are highly differentiated cells situated in the basal stratum of surface epithelia. They are of neural crest origin and are the only cells that synthesize the pigment melanin, which is packaged in specialized organelles: melanosomes.[16] Melanin has a critical role in photoprotection due to its ability to absorb UVR.[13]

The role of OMMC in health and disease has been unclear.[14] OMMC is indeed present in oral mucosa, but only a proportion are actively engaged in melanin synthesis. This is possibly explained by the fact that TYR is more active at the cooler temperatures found on the skin surface.[14]

The oral cavity is not exposed to UVR. It is subjected to microbial challenges. The defense mechanisms acting in the oral cavity include intact epithelial barrier, saliva along with salivary enzymes, gingival crevicular fluid along the antimicrobial substances present in it, oral granulocytes (polymorphonuclear neutrophils), lymphocytes, mast cells, plasma cells, etc.[17]

TYR is an enzyme that is dependent on copper and plays a crucial role in the initial catalysis process to convert tyrosine to DOPA and subsequently oxidize it to DQ.[2] It seems likely that racial differences in human skin color may be due primarily to differences in TYR activity in melanocytes from varying skin types.[16] The level of TYR enzyme expression has been correlated with the degree of melanin pigmentation of the skin.

TYR is exclusively produced by melanocytes. In dermatology, skin-whitening/lightening agents have been developed for the management of melanin hyperpigmentation. These agents contain TYR inhibitors, which may specifically



Graph 1: TYR gene expression levels

inhibit melanogenesis in cells without side effects on other cells and tissues. Many TYR inhibitors such as hydroquinone, arbutin, kojic acid, azelaic acid, L-ascorbic acid, ellagic acid, and tranexamic acid have been used as skin-whitening agents.[18]

However, no study has been conducted to correlate the degree of gingival melanin hyperpigmentation with TYR enzyme gene expression. Hence the present study was conducted to assess the levels of TYR gene expression in gingival melanin hyperpigmentation by RT-PCR technique.

Studies have been conducted assessing the efficacy of TYR inhibitors in dermatology. However, no such study has been conducted in the gingiva. This study will help us in developing therapeutic measures for the management of gingival melanin hyperpigmentation, thereby providing an esthetic smile to individuals having esthetic concerns.

The observation of the present study suggests a positive correlation between the levels of TYR gene expression and the degree of gingival melanin hyperpigmentation. These observations could help us to develop treatment modalities for reducing the TYR enzyme levels for the management of gingival melanin hyperpigmentation.

CONCLUSION

From the observation of the present study, it can be concluded that there exists a positive correlation between the levels of TYR gene expression and the degree of gingival melanin hyperpigmentation. Hence the assessment of TYR enzyme activity in gingiva could be of great value in today's cosmetologically conscious individuals.

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Conflicts of interest
There are no conflicts of interest.

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